Epidemiology and Clinical Manifestation of Rotavirus and Norwalk-Like Viruses in Thai Children

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Acute gastroenteritis is one of the most common diseases affecting children and rotavirus is the major etiological agent worldwide. Although the role of rotavirus as a causal agent of gastroenteritis has been previously established in Thailand, little is known about the epidemiology of Norwalk-like viruses.The present study was designed to determine the epidemiology and clinical manifestation of Norwalk-like viruses in comparison with rotavirus in Thailand.One hundred and one children with watery diarrhea between November 2002 and October 2003 were recruited into the study. The clinical data and stool specimens were collected for clinical analysis and rotavirus and Norwalk-like virus detection. Rotavirus and Norwalk-like viruses were detected viral agent by RT-PCR method. Of 101 stool samples obtained, rotavirus was detected in 25/101(24.7%). Norwalk-like viruses were detected in 23/101(22.7%). Dual infections were detected in 6/101(5.9%). Rotavirus and Norwalk-like viruses peaked in the winter time. Clinical manifestations of rotavirus and Norwalk-like viruses were watery stool, nausea, vomiting, abdominal pain and fever. Norwalk-like viruses and rotavirus were the most common etiologic agents in children with acute gastroenteritis in Thailand. Epidemiology and clinical manifestations of rotavirus and Norwalk-like viruses were not different.

Keywords : *Rotavirus, Norwalk-like virus, Acute gastroenteritis*

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Acute gastroenteritis is a major cause of childhood morbidity and mortality worldwide especially in developing countries. Causes of acute gastroenteritis in children are bacteria, viruses and parasites. Viral gastroenteritis has been found to be the second most common viral clinical entity in developed countries, following behind viral upper respiratory tract illness. Rotavirus has been identified as the most common cause of gastroenteritis in children and Norwalk-like viruses are the commonest cause of epidemic gastroenteritis outbreaks in adults and children.

Rotavirus is a member of the *Reovirus* family. The virus was first discovered in 1973 [1]. This double stranded RNA virus can be transmitted by fecal-oral route. Rotaviral gastroenteritis is common in the infant and childhood and may result in mortality for infants. Rotavirus can be detected by electron microscopy, enzyme immunoassay and reverse transcriptase polymerase chain reaction.

The Norwalk group of viruses is in the *Calicivirus* family. Its structure is a single stranded RNA. The virus was first discovered in 1968 in stool samples of an outbreak in Norwalk, Ohio, USA [2]. Norwalk-like viruses can be detected by electron microscopy, enzyme immunoassay, reverse transcriptase polymerase chain reaction and nucleic acid sequence-based amplification.

According to the National Surveillance System of the Ministry of Public Health, the rate of gastroenteritis in children younger than 5 years of age during 1995 was 1.33 times/person/year. While the role of rotavirus as a causal agent of gastroenteritis has been previously established in the country (Louisiri-rotchanakul S *et al*, 1984; Maneekarn N *et al*, 2000; Noppornpanth S *et al*, 1999); however, little is known about the frequency of other gastrointestinal viruses such as Norwalk-like viruses. An accurate understanding of the relative prevalence of these agents would help design strategies to control the disease. Therefore, the authors' primary goal was to determine the incidence of rotavirus and Norwalk-like viruses in Thailand. In addition the authors' subsidiary goal was to examine the association of the incidence of viral gastroenteritis in relationship to sex, season, age and to compare the clinical manifestations of subjects in both groups

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Material and Method

Specimens and patients

The protocol of the study was approved by the Ethics Committee for Research in Humans, Faculty of Medicine, Chulalongkorn University. The subjects of the study were informed and written consent forms were obtained from their parents. Stool samples from 101 children who attended the Inpatient Department of King Chulalongkorn Memorial Hospital in Bangkok, with the diagnosis of acute watery diarrhea, were collected from November 2002 to October 2003. Watery diarrhea was defined as three or more semi-liquid or liquid stools per day for less than 7 days. All samples were stored at 4 C until they were sent to the laboratory. Fecal samples were diluted 1: 3 in phosphate-buffered saline. After thorough mixing, each fecal suspension was centrifuged (1500 round/min, 10 min). The supernatant solutions were stored at -70 C until undergoing further analysis.

Viral RNA extraction

RNA was extracted from small (100 L) subsamples of the supernatant solutions, using the guanidinium-isothiocyanate method (Theamboonlers *et al*, 2002).

Norwalk-like viruses detection

Using the RT-PCR method, viral RNA was reverse-transcribed into cDNA using primer Y2 (5' TCA GAM AGK GCA CAS AGA GT-3'). The cDNA was then amplified in a PCR, using primer Y1 (5' TGG GAC TCA ACA CAR CAG AG-3') (Hiroyoshi Kawamoto et al, 2001)as the sense primer and primer Y2 as antisense primer. Amplification reaction was performed in a thermocycler set to run for 5 min at 94 C, 30 cycles of 1 min at 94 C, 1 second at 55 C and 1 second at 72 C, with a final extension step at 72 C for 10 min. After electrophoresis in 2% agarose and staining with ethidium bromide, an ultra-violet transilluminator was used to check for the expected 233-bp band.

Rotavirus detection

Using the same RT-PCR method as Norwalk-like viruses, primer A (5' CCG TCT GGC TAA CGG TAA GCT-3') and primer B(5' GGT CAC ATC GAA CAA TTC TAA-3') (Govea et al, 1993) were used. An ultraviolet transilluminator was used to check for the expected1062-bp band.

DNA sequencing and BLAST

The target PCR products within agarose gel were purified for sequencing using a gel extraction kit (QIAquick, Germany) according to the manufacturer's specifications and then sequenced in both senses (Genetic Analyzer 310, Perkin Elmer, USA). Each sequence was BLAST to compare sequence with database in Genbank by BLAST program. In the present study, the authors used sequence and BLAST for confirmation and used it as positive control.

Statistical analysis

Analysis was performed by the use of the Mann-Whitney test for continuous factors, and either Chi-square test or Fisher's exact test for discontinuous factors, depending on the sample size. Analyses were performed using SPSS software, version 9.0. *p*-value <0.05 was considered statistically significant.

Results

Of the 101 stool samples obtained, rotavirus was detected in 25/101(24.7%). Norwalk-like viruses were detected in 23/101(22.7%). Dual infections were detected in 6/101(5.9%) (Table 1). All rotaviruses that the authors detected were confirmed by sequencing and BLAST that the author's accession number AY456226-31, AY 456232-44, AY 662513-15, AY 660559-61 which similar to Human rotavirus 99%. Six Norwalk-like viruses that the authors detected were also confirmed by sequencing and BLAST that the authors accession number AY653301-3, AY655734-6 like Norwalk-like virus pol gene for RNA-dependent RNA polymerase 99-100%.

The seasonal distribution of viral gastroenteritis in percentage of each pathogen isolated is displayed in Fig. 1. Rotavirus peaked in the months of February and January, while Norwalk-like viruses peaked in August and March.

The proportional age and sex distribution of patients with gastroenteritis is depicted in Table 2. Rotavirus occurred most frequently in the age group of 0-1 year old and in the1-5 years old, with a reported prevalence of 29.1% and 21.7%, respectively.

Rotavirus infection was identified in 25.7% of the male and 22.8% of the female gastroenteritis patients examined. Norwalk-like viruses were detected

Table 1. Detection of viral agents of gastroenteritis in 101 stool specimens from patients with gastroenteritis

Viral enteric pathogens	No. of patients positive (%)
Rotavirus	25 (24.7%)
Norwalk-like viruses	23 (22.7%)
Dual infection	6 (5.9%)

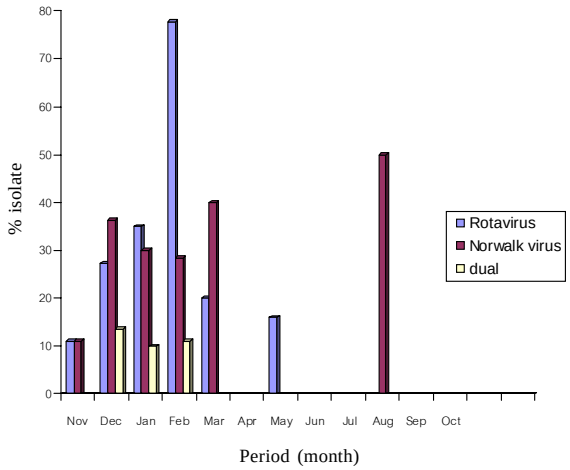

Fig. 1 Seasonal distribution of Rotavirus and Norwalk-like viruses in Bangkok, Thailand

predominantly in the age group of 5-12 years old with 57.1%, followed by 20.8% in the age group of 0-1 year old and 19.5% in the age group of 1-5 years old. A higher rate of infection among the female patients was found for Norwalk-like viruses (Table 2).

The major clinical symptoms related to viral gastroenteritis in this study are shown in Table 3. Symptoms associated with rotavirus infection were mainly watery stool(100%), vomiting(80%), fever(76%) and abdominal pain(75%). Similarly, patients with NLV infection displayed symptoms including watery stool (100%), fever (86.6%), vomiting(82.6%) and abdominal pain(50%).

Table 4 shows the clinical characteristics of the children in the study, according to the type of infection. The age range in patients with gastroenteritis due to rotavirus is similar to those with gastroenteritis due to Norwalk-like viruses. Length of hospital stay, vomiting, diarrhea, dehydration and respiratory tract infection in patients with gastroenteritis due to rotavirus were not statistically different from those with gastroenteritis due to Norwalk-like virus.

Table 2. Detection of viral gastroenteritis by sex and age in patients with gastroenteritis

Pathogen	Male	Female	0-1 year	1-5 year	5-12 year	All sex/ age
Rotavirus (percent)	17 (25.7) n=66	8 (22.8) n=35	14 (29.1) n=48	10 (21.7) n=46	1 (14.2) n=7	25 (24.7) n=101
NLV (percent)	12 (18.2)	11 (31.4)	10 (20.8)	9 (19.5)	4 (57.1)	23 (22.7)

* NLV = Norwalk like virus

Table 3. Clinical symptoms associated with viral-related diarrhea disease

Pathogens	No. of cases	Watery/ loose stool	Mucus in stool	Nausea/ Vomiting	Abdominal Pain*	Fever
Rotavirus (percent)	25	25 (100)	3 (12)	20 (80)	3 (75) n=4	19 (76)
NLV (percent)	23	23 (100)	3 (13)	19 (82.6)	3 (50) n=6	20 (86.6)

***** Abdominal pain can be evaluated in children older than 4 years old

Discussion

This report was an attempt to complete a comprehensive study of the viral etiology of the gastroenteritis in cases attending King Chulalongkorn Memorial Hospital. The study was based on low to middle socioeconomic status of an urban population in Bangkok. While rotavirus has been extensively studied in Thailand, little is known about the frequency of Norwalk-like viruses that cause gastroenteritis.

Rotavirus was detected in 24.7% of patients with acute gastroenteritis. Peak seasonal distribution

Table 4. Infecting virus and relationship to demographic characteristics of 101 children with acute gastroenteritis

Characteristic	Type of infection		*P*
	Rotavirus	NLV	
No.(%) of children infected	25(24.7)	23(22.7)	
Age, (years)			
Mean	1.62	2.62	0.51
Range	2 mo-11 yrs	1 mo-10 yrs 9 mo	
Length of hospital stay,(days)			
Mean 3.52	3.74	0.392	
Range	1-12	1-12	
Length of fever, (days)			
Mean	2.92	2.70	0.925
Range	0-9	0-9	
Vomiting, (times per day)			
Mean	4.68	5.52	0.653
Range	0-10	0-20	
Diarrhea, (times per day)			
Mean	8.68	6.09	0.092
Range	2-40	1-10	
No.(%) of dehydrated children	88	79	0.696
Percentage of children with respiratory tract infection	36	30.4	0.686

of rotavirus was observed in the winter months (December and January). The authors' findings for rotavirus infection are in agreement with previous studies in Thailand [12], which showed a 27-34% rate of rotavirus in diarrhea patients that presented the peak in winter. Norwalk-like virus was detected in 22.7% of patients with acute gastroenteritis. Previous studies in other countries, such as Indonesia [16] and France [17], showed that Norwalk-like viruses were also found in 21% and 7.3% of the patients, respectively. Seasonality for Norwalk-like virus was observed in March and August when Norwalk-like virus incidence peaked. This finding is in agreement with a previous study done in Indonesia[16], which showed the Norwalk-like virus peaking in August and September.

Rotavirus was detected most frequently among the age group of 0-1 year. This finding is in agreement with previous studies done in Thailand and Indonesia[10,12,13,16]. Among the age group of 5-12 years, Norwalk-like viruses were most frequently detected. This finding is in agreement with previous studies[3] that Norwalk-like viruses were detected in children and adults.

The clinical symptoms associated with rotavirus infection in the present study showed a strong percentage associated with watery stool(100%), vomiting(80%), fever(76%) and abdominal pain(75%). This finding was similar to studies conducted in Indonesia and Egypt[16,19]. The clinical symptoms associated with Norwalk-like viruses infection were similar to those with rotavirus infection. There was no statistical difference between clinical manifestations of rotavirus and Norwalk-like virus.

Finally, the present study shows a high frequency of viral agents, mainly rotavirus and Norwalk-like viruses, in children admitted for the treatment of acute gastroenteritis in King Chulalongkorn Memorial Hospital. The data supports the need to develop rapid and sensitive tests for the diagnosis of Norwalk-like virus. The introduction of affordable viral diagnosis in pediatric hospitals will improve patient care by reducing the unnecessary use of antibiotics. Rotavirus and Norwalk-like viruses may be prevented by vaccines. The authors' data suggest that a significant proportion of the diarrhea disease burden in Thailand could be prevented in the near future.

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References

1. CDC. Rotavirus. http://www.cdc.gov/ncidod/dvrd/revb/gastro/rotavirus.htm.
2. Xi JN, Graham DY, Wang KN, Estes MK. Norwalk virus genome cloning and characterization. Science. 1990; 250: 1580-3.
3. O' Neill HJ, McCaughey C, Coyle PV, Wyatt DE, Mitchell F. Clinical utility of multiplex RT-PCR for group F adenovirus, rotavirus and norwalk-like viruses in acute viral gastroenteritis in children and adults. J Clin Virol 2002; 25: 335-43.
4. Vinje J, Green J, Lewis DC, Gallimore CI, Brown DW, Koopmans MP. Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses". Arch Virol 2000; 145: 223-41.
5. Gotz H, Ekdahl K, Lindback J, et al. Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden. Clin Infect Dis 2001; 33: 622-8.
6. Hiroyoshi Kawamoto, Kenji Yamazaki, Etsuko Utagawa et al. Nucleotide Sequence Analysis and Development of Consensus Primers of RT-PCR for Detection of Norwalk-Like Viruses Prevailing in Japan. J Med Virol 2001; 64: 569-76.
7. Katayama K, Shirato-Horikoshi H, Kojima S, et al. Phylogenetic Analysis of the complete Genome of 18 Norwalk-like Viruses. Virology 2002; 299: 225-39.
8. Richards AF, Lopman B, Gunn A, et al. Evaluation of a commercial ELISA for detecting Norwalk-like virus antigen in faeces. J Clin Virol 2003; 26: 109-15.
9. Greene SR, Moe CL, Jaykus LA, et al. Evaluation of the NucliSens Basic Kit assay for detection of Norwalk-virus RNA in stool specimens. J Virol Methods 2003; 108: 123-31.
10. Louisirirotcnanakul S, Wasi C, Satrasook S, Surakhaka H, Thongcharoen P. Rotavirus in Pediatric diarrhoea at Siriraj Hospital, Bangkok, Thailand. Southeast Asian J trop Med Public Health 1984; 15: 348-53.
11. Gouvea V, Ramirez C, Li B, et al. Restriction endonuclease analysis of the vp7 genes of human and animal rotaviruses. J Clin Microbiol 1993; 31: 917-23.
12. Maneekarn N, Ushijima H. Epidemiology of rotavirus infection in Thailand. Paediatr Int 2000; 42: 415-21.
13. Noppornpanth S, Poovorawan Y. Comparison between RT-PCR and rapid agglutination test for diagnosis of human rotavirus infection. Southeast Asian J Trop Med Public health 1999; 30: 707-9.
14. Lopman BA, Brown DW, Koopman SM. Human caliciviruses in Europe. J Clin Virol 2002; 24: 137-60.
15. Reuter G, Farkas T, Berke T, Jiang X, Matson DO, Szues G. Molecular epidemiology of human calicivirus gastroenteritis outbreaks in Hungary. J Med Virol 2002; 68: 390-8.
16. Subekti D, Lesmana M, Tjaniadi P, Safari N, Frazier E, Simanjuntak C, et al. Incidence of Norwalk-like viruses,

rotavirus and adenovirus infection in patients with acute gastroenteritis in Jakarta, Indonesia. FEMS Immunol Med Microbiol 2002; 33: 27-33.
17. Marie-cardine A, Gourlain K, Mouterde O, et al. Epidemiology of acute viral gastroenteritis in children hospitalized in Rouen, France. CID 2002; 34: 1170-8.
18. Theamboonlers A, Poovorawan Y, et al. Molecular characterization of hepatitis-A-virus infections, in the context of two outbreaks in southern Thailand. Ann Trop Med Parasitol. 2002; 96: 727-34.
19. Naficy AB, Abu-Elyazeed R, Holmes JL, et al. Epidemiology of rotavirus diarrhea in egyptian children and implications for disease control. Am J Epidemiol 1999; 150: 770-7.

ระบาดวิทยาและอาการทางคลินิกของโรตาไวรัสและนอร์วอร์คไวรัสในเด็กไทย

มณฑิดา วีรวิกรม, อภิรดี เทียมบุญเลิศ, ยง ภู่วรวรรณ

โรคอุจจาระร่วงเป็นโรคที่พบได้บ่อยในเด็กและโรตาไวรัสเป็นสาเหตุส่วนใหญ่ของโรคอุจจาระร่วงในเด็กทั่วโลก มีผลงานวิจัยรายงานว่าโรตาไวรัสเป็นเชื้อไวรัสที่เป็นสาเหตุสำคัญของโรคอุจจาระร่วงในประเทศไทย แต่ความรู้เกี่ยวกับระบาดวิทยาของเชื้อนอร์วอร์คไวรัสในประเทศไทยยังมีน้อยมากงานวิจัยนี้จึงออกแบบเพื่อศึกษาเกี่ยวกับระบาดวิทยา และอาการทางคลินิกของเชื้อนอร์วอร์คไวรัสโดยเปรียบเทียบกับเชื้อโรตาไวรัส ผู้วิจัยทำการศึกษาในเด็กโรคอุจจาระร่วงจำนวน 101 คน ระหว่างเดือนพฤศจิกายน พ.ศ.2545 ถึงเดือนตุลาคม พ.ศ. 2546 โดยรวบรวมข้อมูลทางคลินิก และอุจจาระเพื่อทำการวิเคราะห์ข้อมูลและตรวจหานอร์วอร์คไวรัสและโรตาไวรัสโดยวิธี RT-PCR จากอุจจาระ 101 ตัวอย่าง ตรวจพบโรตาไวรัส 25 ราย(24.7%) ตรวจพบนอร์วอร์คไวรัส 23 ราย(22.7%) มีการติดเชื้อทั้ง 2 ชนิด 6 ราย (5.9%) โรตาไวรัสและนอร์วอร์คไวรัสพบมากในฤดูหนาว อาการทางคลินิกของโรตาไวรัสและนอร์วอร์คไวรัสคือ ถ่ายเหลวเป็นน้ำ, คลื่นไส้อาเจียน, ปวดท้องและไข้

***สรุป** : นอร์วอร์คไวรัสและโรตาไวรัสเป็นเชื้อที่พบบ่อยในโรคอุจจาระร่วงในเด็กในประเทศไทย ระบาดวิทยาและอาการทางคลินิกของเชื้อนอร์วอร์คไวรัสและโรตาไวรัสไม่แตกต่างกัน*