ROTAVIRUS INFECTION IN CHILDREN AND ADULTS WITH ACUTE GASTROENTERITIS IN THAILAND

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Abstract. Rotaviruses are the most important cause of severe diarrhea in infants and young children, but rotavirus gastroenteritis in adults is uncommon. In this study, 260 stool samples collected in Thailand from January 2006 to February 2007 from patients of all ages with acute gastroenteritis, were tested for group A rotavirus and compared with rotavirus infections in children and adults. Rotavirus was detected in 42% of the patients' samples, but children (< 18 years old) have a significantly higher prevalence (57%) of rotavirus infection than adults (≥ 18 years old) (27%) (OR 3.55; 95% CI: 2.11-5.96; $p < 0.001$). The highest attack rate was found in the age group of < 2 years old (14%), followed by 2-4 years of age (9%), 18-59 years of age (8%), 5-17 years of age (6%) and ≥ 60 years of age (5%). The dominant genotype was G1P[8] (27%), followed by G2P[4] (7%), G3P[8] (1%), and G9P[8] (1%). The rare genotypes identified were G1P[4], G1P[6], G2P[6], G2P[8], and G3P[6]. Mixed infections mostly occurred in children, comprising G1P[4]/P[8], G1P[4]/P[6], G1P[6]/P[8], G1/G2P[4], G1/G3P[4], and G1/G3P[4]/P[8]. Rotaviruses G3, G9, and P[4] were found only in children and genotype P[6] was found in adults (75%) at a higher frequency than in children (25%) ($p < 0.001$). The number of rotavirus in children was 1.99×10^8/ml and in adult patients was 7.32×10^6/ml. The present study highlights the higher prevalence of rotavirus infection in children compared to adults and rotavirus genetic heterogeneity.

Keywords: acute gastroenteritis, adults, children, genotype, rotavirus, Thailand

INTRODUCTION

Rotaviruses are an important cause of acute diarrhea in infants and young children worldwide. The viruses affect an estimated 453,000 deaths (in the range of 420,000-494,000) in 2008, in children younger than 5 years old, mostly in developing countries (Tate *et al*, 2012). Although acute gastroenteritis, caused by rotaviruses, in adults is uncommon, outbreaks of gastroenteritis associated with rotaviruses among adults and elderly persons have been reported (Griffin *et al*, 2002; Cardemil *et al*, 2012; Gunn *et al*, 2012).

Rotaviruses belong to the genus *Rotavirus* in the family Reoviridae, and contain 11 segments of double-stranded RNA (Matthijnssens *et al*, 2012). Eight distinct groups (A to H) of rotaviruses have been classified based on their antigenic and genetic properties. Group A rotaviruses

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are the most common cause of acute gastroenteritis in humans, and within group A different serotypes are identified according to the glycoprotein VP7 (G types) and the protease-sensitive protein VP4 (P types) that surround the outer capsid of the virus particles and elicit neutralizing antibody response (Estes and Kapikian, 2007). Molecular characterization of the VP7 and VP4 genes has identified 27 G and 37 P genotypes in humans and animals (Matthijnssens *et al*, 2011; Trojnar *et al*, 2013). The most prevalent rotavirus strains recovered from infected patients are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] (Bishop, 2009). Uncommon rotavirus strains in humans include G5, G6, G8, G10, G11, G12, and G20 of G types and P[1], P[3], P[6], P[9], P[10], P[11], P[13], P[14], P[19], P[25], and P[28] of P types (Matthijnssens *et al*, 2011).

In Asia the highest incidence rates of rotavirus-related hospitalizations in children under 5 years of age have been reported in Bangladesh, South Korea, Taiwan, Thailand, and Vietnam (Kawai *et al*, 2012). In Thailand, rotaviruses are the major causative agents responsible for 28-50% of children admitted to hospital with acute diarrhea, with rotavirus genotype G1P[8] being the most prevalent, but G2, G3, G4, and G9 also are found in children with rotaviral diarrhea (Khamrin *et al*, 2007; Theamboonlers *et al*, 2008; Khananurak *et al*, 2010; Maiklang *et al*, 2012). Although rotaviruses can cause gastroenteritis in adults (Anderson and Weber, 2004), data concerning rotavirus infection among adults are limited.

The objectives of this study were to determine the prevalence of rotavirus infections in patients of all ages with acute gastroenteritis, to identify rotavirus G and P genotypes, and to quantify the numbers of rotavirus in stool samples of rotavirus-infected patients.

MATERIALS AND METHODS

Stool samples

A total of 260 stool samples were collected from patients with acute gastroenteritis admitted to Lop Buri Hospital, Lop Buri Province, located in the central region of Thailand, from January 2006 to February 2007. Ten percent stool suspensions in 0.05 M phosphate-buffered saline, pH 7.2 were clarified by centrifugation at 3,000*g* for 10 minutes and supernatants were stored at -70ºC until used. The study was approved by the Ethics Committee for Human Rights Related to Human Experimentation, Mahidol University (COA. no. MU-IRB 2008/254.2312).

Nested multiplex RT-PCR

Virus double-stranded RNA was extracted from 140 μl of stool supernatant using QIAamp® viral RNA extraction kit (QIAGEN, Hilden, Germany). In order to detect the rotavirus and determine its G and P genotypes, the extracted RNA was analyzed by nested multiplex RT-PCR to amplify VP7 and VP4 genes using separate reactions. For the first-round PCR, primers RV1 and RV2 were employed to amplify a 1,059-bp region of VP7 gene (Gilgen *et al*, 1997), and primers Con3 and Con2 to amplify an 877-bp fragment of VP4 gene (Gentsch *et al*, 1992). For the second-round PCR to genotype VP7, the consensus primer 9Con1 and the G-type-specific primers 9T1-1, 9T1-2, 9T-3P, 9T-4, and 9T-9B were used for detection of G1, G2, G3, G4, and G9, respectively (Das *et al*, 1994). For the second-round PCR genotyping of VP4, the consensus primer Con3mod and the P-type-specific primer 2T-1, 3T-1, and 1T-1 were used for detection of P[4], P[6], and P[8], respectively (Gentsch *et al*, 1992; Maunula *et al*, 1998).

The nested multiplex RT-PCR assays were carried out as described by Asmah *et al* (2001) and Phan *et al* (2007) with some modifications. In brief, a 5 µl aliquot of RNA was heated to 97ºC for 5 minutes, and placed on ice for 10 minutes. The RT-PCR was performed in a final volume of 50 µl and amplified for 35 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 60 seconds. For nested PCR amplification, 1 µl aliquot of the primary PCR solution was added to 49 µl of reaction mixture and amplified for 25 cycles of 94ºC for 60 seconds, 50ºC for 60 seconds, 72ºC for 60 seconds, followed by a final incubation at 72ºC for 7 minutes. The amplicons were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. The expected amplicon size was 159, 245, 467, 404, and 111 bp for G1, G2, G3, G4, and G9, respectively. For P[4], P[6] and P[8] the expected amplicon was 484, 268 and 346 bp, respectively.

Quantitative real-time RT-PCR

A Rotavirus Real Time RT-PCR kit containing rotavirus specific primers, Taqman probes and reagents for the detection and quantification of rotavirus genome was used according to Guidelines issued by the European Authorized Representative SA Obelis, Brussels, Belgium (Shanghai ZJ Bio-Tech, Shanghai, China). Rotavirus amplification was performed according to the manufacturer's instructions in a LightCycler 1.5 Real Time PCR System (Roche Diagnostics, Mannheim, Germany). Rotaviruses in the stool samples were quantified from the threshold cycle (Ct) values and compared with a standard curve of rotavirus positive control.

Statistical analysis

Statistical parameters were calculated using the Statistical Package for the Social Sciences (SPSS) computer software program version 17.0 (SPSS, Chicago, IL). A chi-square test was used to analyze the associations among age, sex, department attended and rotavirus infection. The Mann-Whitney *U* test was used to compare differences between age group and rotavirus copy numbers. A $p < 0.05$ is considered statistically significant and a $p < 0.001$ as highly significant.

RESULTS

Rotavirus prevalence

Of the 260 stool samples from children and adults with acute gastroenteritis, 110 (42%) tested positive for group A rotavirus by nested multiplex RT-PCR. The virus could be detected in samples taken on days 1-9 after the onset of the illness. The detection rate of rotavirus infection in children (< 18 years old, 57%) was higher than that in adults (≥ 18 years old, 27%) (OR 3.55; 95% CI: 2.11-5.96; $p < 0.001$). Rotavirus infection occurred in both genders, but the frequency was greater in males (47%) than in females (37%). The rate of rotavirus infection in patients attending the Inpatient Department (46%) was higher than that found in patients attending the Outpatient Department (31%) ($p < 0.05$) (Table 1). The age distribution of rotavirus infection in patients with acute gastroenteritis was from 2 months to 86 years. The attack rate of rotavirus infection was highest in the <2 years of age group (14%), followed by the 2-4 years of age (9%), 18-59 years of age (8%), 5-17 years of age (6%) and ≥ 60 years of age (5%) (Table 2).

Distributions of G and P genotypes

Of 110 rotavirus-positive samples, 63 (57%) were typed completely for both G and P genotypes by nested multiplex RT-PCR, with single infection at 46% and

Table 1
Association between characteristics of 260 patients with acute gastroenteritis and rotavirus infection as determined by nested multiplex RT-PCR.

Characteristics	Total no. of cases	Rotavirus infection (%)		OR[a]	95% CI[b]	*p*-value
		Positive	Negative			
Age (years)						<0.001
<18	129	57	43	3.55	2.11-5.96	
≥18	131	27	73	1.00		
Sex						0.129
Male	137	47	53	1.47	0.89-2.41	
Female	123	37	63	1.00		
Department attended						0.028
Inpatient	187	46	54	1.89	1.07-3.35	
Outpatient	73	31	69	1.00		

[a]Odds ratio; [b]95% confidence interval.

Table 2
Distribution by age of patients with acute gastroenteritis caused by rotavirus.

Age group (years)	Number (n = 260)	Rotavirus infection		
		Number (n = 110)	Percent of each age group	Percent of 260 cases
<2	70	36	51	14
2-4	36	23	64	9
5-17	23	15	65	6
18-59	70	22	31	8
≥60	61	14	23	5

mixed infections at 11% (Table 3). Ninety (82%) of the positive stool samples were identified as VP7 and 87 (79%) revealed a single G type, with G1 being the most prevalent (69%), followed by G2 (28%), G3 (2%) and G9 (1%). Rotavirus G4 was not found in this study. Among mixed infections of G genotypes, G1/G2 (1 sample) and G1/G3 (2 samples) were identified. Eighty-three (75%) samples were identified as VP4 and 71 (64%) showed a single P type, with P[8] being predominant (51%), followed by P[6] (28%) and P[4] (21%). Among mixed infections of P genotypes, P[4]/P[8] (8 samples), P[6]/P[8] (3 samples) and P[4]/P[6] (1 sample) were identified. Rotaviruses were typed partially in 47/110 (43%) samples. Ungenotyped G (G?) (where a sample could not generate a full length VP7 and/or G genotype-specific amplicon but could generate a P genotype specific PCR product) and ungenotyped P (P?) (where a sample could not generate a full length VP4 and/or P genotype-specific amplicon but could generate a G genotype specific PCR product) was found in 20 (18%) and 27 (24%), samples, respectively. Overall, G and P genotype combinations

Table 3
Rotavirus genotypes identified by nested multiplex RT-PCR of 110 stool samples from patients with acute gastroenteritis.

G and P genotypes	No. of samples (%)
Completely typed rotavirus: single infection	51 (46)
G1P[4]	2 (2)
G1P[6]	4 (4)
G1P[8]	30 (27)
G2P[4]	8 (7)
G2P[6]	3 (3)
G2P[8] or G3P[6] or G3P[8] or G9P[8]	1 (1)[a]
Completely typed rotavirus: mixed infection	12 (11)
G1P[4]/P[8]	7 (6)
G1P[4]/P[6] or G1P[6]/P[8] or G1/G2P[4] or G1/G3P[4] or G1/G3/P[4]/P[8]	1 (1)[a]
Partially typed rotavirus	47 (43)
G1P?[b]	15 (14)
G2P? or G?P[6]	12 (11)[a]
G?P[4] or G?P[8]	3 (3)[a]
G?P[6]/P[8]	2 (2)

[a]Percent of each typed rotavirus; [b]nested multiplex RT-PCR produced no identifiable band.

of rotaviruses were G1P[8] (27%), G2P[4] (7%), G3P[8] (1%), and G9P[8] (1%). Other genotypes, including G1P[4], G1P[6], G2P[6], G2P[8], and G3P[6] were detected at a relatively low frequency of occurrence. Of the mixed rotavirus infections (11%), G1P[4]/P[8] was the most frequently detected. One stool sample each was positive for G1P[4]/P[6], G1P[6]/P[8], G1/G2P[4], G1/G3P[4] and G1/G3P[4]/P[8]. Partially typed rotaviruses with a high frequency of occurrence were G1P? (14%), G2P? (11%) and G?P[6] (11%).

As regards G or P genotypes of group A rotavirus associated with age, sex, and hospital department attended, rotaviruses G3, G9, G combination and P[4] were found only in children (< 18 years of age) attending Inpatient Department. The frequency of rotavirus P[6] infection found in adults (15/20 samples) was higher than that found in children (5/20 samples). In addition patients attending the Outpatient Department (12/20 samples) scored higher than patients attending the Inpatient Department (8/20 samples) ($p < 0.001$).

Rotavirus quantification

Of the 110 stool samples positive for rotavirus by nested multiplex RT-PCR, 22 (20%) were found to be positive by quantitative RT-PCR [19/74 (26%) and 3/36 (8%) of rotavirus-infected children and adults, respectively]. Quantitative RT-PCR revealed a narrow range (6.69×10^6-1.95×10^7 copies/ml) of rotavirus genome content in infected adult stool samples compared with that found in infected children samples (3.99×10^5-5.10×10^{10} copies/ml), but there is no significant difference between the two median values (1.99×10^8

copies/ml) in children and $7.32x10^6$ copies/ml in adults, $p = 0.094$).

DISCUSSION

Acute gastroenteritis caused by rotaviruses remains an important public health problem and is ranked first in viral diarrhea in children admitted to hospital, but rotavirus can also cause acute gastroenteritis in adults (Anderson and Weber, 2004). This study, employing nested multiplex RT-PCR to detect and genotype rotavirus in stool samples from patients with acute gastroenteritis, showed that rotavirus was the most significant cause of acute diarrhea both in children and adult patients. Previous studies showed the occurrence of rotavirus infections in both adults and elderly patients (del Refugio González-Losa *et al*, 2001; Griffin *et al*, 2002; Cardemil *et al*, 2012; Gunn *et al*, 2012). In the present study, all children with rotavirus infection attended the Inpatient Department, whereas the majority of rotavirus-infected adults attended the Outpatient Department. These adult patients had a milder form of the disease probably because of protective immunity obtained from previous infections. Young children less than 2 years of age were the most affected in agreement with reports from other countries (Lou *et al*, 2010; Morris *et al*, 2012).

The predominant rotavirus genotype was G1, followed by G3 and, finally, G9 in agreement with a previous report (Kittigul *et al*, 2009). The nested multiplex RT-PCR method was 7-fold more sensitive in detecting rotavirus in stool samples than the conventional RT-multiplex PCR method. This increased sensitivity may be due to the additional re-amplification step of the nested PCR technique. Four different rotavirus G genotypes (G1, G2, G3, and G9) and 3 different P genotypes (P[4], P[6], and P[8]) were identified. Within the same year of this study (2007), a prevalent G1P[8] rotavirus genotype was also reported in children under 5 years of age admitted to a hospital in Chiang Mai Province, located in the north of Thailand (Chaimongkol *et al*, 2012). The predominance of G1P[8] genotype has been of concern globally (Kirkwood *et al*, 2010). Other common rotavirus genotypes including G2P[4], G3P[8] and G9P[8], and rare genotypes: G1P[4], G1P[6], G2P[6], G2P[8], and G3P[6] found in this study also have been reported in other countries (Lee *et al*, 2009; Kirkwood *et al*, 2010; Hull *et al*, 2011). Interestingly the rare P[6] genotype was found mainly in rotavirus-infected adults, 80% of whom attended the Outpatient Department. Although the pattern of rotavirus G type distribution (G1, G2, G3, and G9) does not change much among different geographical regions, unusual human rotavirus strains have emerged as global strains, which have important implications for effective vaccine development. Rotarix®, a monovalent live-attenuated human rotavirus G1P[8] vaccine and Rotateq®, pentavalent human bovine reassortant (G1, G2, G3, G4, and P[8]) vaccine might not be efficient enough in protecting individuals from rare strains with unusual G-P combinations. Licensed rotavirus vaccines have been available for use in Thailand but are not yet included as part of the national immunization program (Muangchana *et al*, 2012).

Various rotavirus genotypes and mixtures of genotypes were found in children with acute gastroenteritis. The ability of rotaviruses to undergo genetic reassortment leads to the generation of new strains through interspecies transmission between human and animal strains. This event is likely to occur in developing countries, where children become infected

frequently with multiple strains of rotavirus at the same time (Kirkwood, 2010). As regards the partially typed rotaviruses, the stool samples might contain too few rotaviruses to be detected by nested multiplex RT-PCR assay or contain other rotavirus genotypes not specific to the primers currently employed (Fischer *et al*, 2005).

The viral load of rotavirus in stool samples of infected children was higher than in adults. A previous study showed that the rotavirus load correlates with clinical severity of diarrhea in children (Kang *et al*, 2004). The findings in the current study imply that children have more severe diarrhea than adults and both groups of patients can potentially transmit rotavirus. Thus, continuous epidemiological study of rotavirus infection is essential not only to develop effective strategies for prevention and control of rotavirus gastroenteritis but also for monitoring of rotavirus strains required for implementation of an effective vaccine.

ACKNOWLEDGEMENTS

This study was supported for publication by the China Medical Board (CMB), Faculty of Public Health, Mahidol University. We thank Mr Kevin Kirk, The Language Center, Faculty of Graduate Studies, Mahidol University for editorial assistance.

REFERENCES

Anderson EJ, Weber SG. Rotavirus infection in adults. *Lancet Infect Dis* 2004; 4: 91-9.

Asmah RH, Green J, Armah GE, *et al*. Rotavirus G and P genotypes in rural Ghana. *J Clin Microbiol* 2001; 39: 1981-4.

Bishop R. Discovery of rotavirus: Implications for child health. *J Gastroenterol Hepatol* 2009; 24: S81-5.

Cardemil CV, Cortese MM, Medina-Marino A, *et al*. Two rotavirus outbreaks caused by genotype G2P[4] at large retirement communities: cohort studies. *Ann Intern Med* 2012; 157: 621-31.

Chaimongkol N, Khamrin P, Malasao R, *et al*. Genotypic linkages of gene segments of rotaviruses circulating in pediatric patients with acute gastroenteritis in Thailand. *Infect Genet Evol* 2012; 12: 1381-91.

Das BK, Gentsch JR, Cicirello HG, *et al*. Characterization of rotavirus strains from newborns in New Delhi, India. *J Clin Microbiol* 1994; 32: 1820-2.

del Refugio González-Losa M, Polanco-Marín GG, Manzano-Cabrera L, Puerto-Solís M. Acute gastroenteritis associated with rotavirus in adults. *Arch Med Res* 2001; 32: 164-7.

Estes MK, Kapikian AZ. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Straus SE, Martin MA, Roizman B, eds. Fields virology. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2007: 1917-74.

Fischer TK, Eugen-Olsen J, Pedersen AG, *et al*. Characterization of rotavirus strains in a Danish population: high frequency of mixed infections and diversity within the VP4 gene of P[8] strains. *J Clin Microbiol* 2005; 43: 1099-104.

Gentsch JR, Glass RI, Woods P, *et al*. Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 1365-73.

Gilgen M, Germann D, Lüthy J, Hübner P. Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus, and small round structured viruses in water samples. *Int J Food Microbiol* 1997; 37: 189-99.

Griffin DD, Fletcher M, Levy ME, *et al*. Outbreaks of adult gastroenteritis traced to a single genotype of rotavirus. *J Infect Dis* 2002; 185: 1502-5.

Gunn L, Feeney SA, Cashman O, Collins PJ, Coyle PV, O'Shea H. Molecular charac-

terization of group A rotavirus found in elderly patients in Ireland; predominance of G1P[8], continued presence of G9P[8], and emergence of G2P[4]. *J Med Virol* 2012; 84: 2008-17.

Hull JJ, Teel EN, Kerin TK, *et al*. United States rotavirus strain surveillance from 2005 to 2008: genotype prevalence before and after vaccine introduction. *Pediatr Infect Dis J* 2011; 30: S42-7.

Kang G, Iturriza-Gomara M, Wheeler JG, *et al*. Quantitation of group A rotavirus by real-time reverse-transcription-polymerase chain reaction: correlation with clinical severity in children in South India. *J Med Virol* 2004; 73: 118-22.

Kawai K, O'Brien MA, Goveia MG, Mast TC, El Khoury AC. Burden of rotavirus gastroenteritis and distribution of rotavirus strains in Asia: a systematic review. *Vaccine* 2012; 30: 1244-54.

Khamrin P, Peerakome S, Tonusin S, *et al*. Changing pattern of rotavirus G genotype distribution in Chiang Mai, Thailand from 2002 to 2004: decline of G9 and reemergence of G1 and G2. *J Med Virol* 2007; 79: 1775-82.

Khananurak K, Vutithanachot V, Simakachorn N, Theamboonlers A, Chongsrisawat V, Poovorawan Y. Prevalence and phylogenetic analysis of rotavirus genotypes in Thailand between 2007 and 2009. *Infect Genet Evol* 2010; 10: 537-45.

Kirkwood CD. Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. *J Infect Dis* 2010; 202: S43-8.

Kirkwood CD, Boniface K, Bishop RF, Barnes GL. Australian Rotavirus Surveillance Program: annual report, 2009/2010. *Commun Dis Intell* 2010; 34: 427-34.

Kittigul L, Pombubpa K, Taweekate Y, Yeephoo T, Khamrin P, Ushijima H. Molecular characterization of rotaviruses, noroviruses, sapovirus, and adenoviruses in patients with acute gastroenteritis in Thailand. *J Med Virol* 2009; 81: 345-53.

Lee SY, Hong SK, Lee SG, *et al*. Human rotavirus genotypes in hospitalized children, South Korea, April 2005 to March 2007. *Vaccine* 2009; 27: F97-101.

Lou JT, Xu XJ, Wu YD, Tao R, Tong MQ. Epidemiology and burden of rotavirus infection among children in Hangzhou, China. *J Clin Virol* 2010; 50: 84-7.

Maiklang O, Vutithanachot V, Vutithanachot C, Hacharoen P, Chieochansin T, Poovorawan Y. Prevalence of group A genotype human rotavirus among children with dirarrhea in Thailand, 2009-2011. *Southeast Asian J Trop Med Public Health* 2012; 43: 904-16.

Matthijnssens J, Ciarlet M, McDonald SM, *et al*. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch Virol* 2011; 156: 1397-413.

Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol* 2012; 157: 1177-82.

Maunula L, von Bonsdorff C. Short sequences define genetic lineages: phylogenetic analysis of group A rotaviruses based on partial sequences of genome segments 4 and 9. *J Gen Virol* 1998; 79: 321-32.

Morris SK, Awasthi S, Khera A, *et al*. Rotavirus mortality in India: estimates based on a nationally representative survey of diarrhoeal deaths. *Bull World Health Organ* 2012; 90: 720-7.

Muangchana C, Riewpaiboon A, Jiamsiri S, Thamapornpilas P, Warinsatian P. Economic analysis for evidence-based policy-making on a national immunization program: a case of rotavirus vaccine in Thailand. *Vaccine* 2012; 30: 2839-47.

Phan TG, Khamrin P, Trinh DQ, *et al*. Genetic characterization of group A rotavirus strains circulating among children with acute gastroenteritis in Japan in 2004-2005. *Infect Genet Evol* 2007; 7: 247-53.

Tate JE, Burton AH, Boschi-Pinto C, *et al*. 2008 estimate of worldwide rotavirus-

associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; 12: 136-41.

Theamboonlers A, Bhattarakosol P, Chongsrisawat V, Sungkapalee T, Wutthirattanakowit N, Poovorawan Y. Molecular characterization of group A human rotaviruses in Bangkok and Buriram, Thailand during 2004-2006 reveals the predominance of G1P[8], G9P[8] and a rare G3P[19] strain. *Virus Genes* 2008; 36: 289-98.

Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. *J Gen Virol* 2013; 94: 13642.