Molecular Characterization and Zoonotic Potential Analysis of Rotaviruses Detected in Pediatric Patients with Acute Gastroenteritis

การตรวจหาคุณลักษณะเฉพาะของเชื้อไวรัสโรตาที่พบในผู้ป่วยเด็กที่มีอาการกระเพาะอาหารและลำไส้อักเสบเฉียบพลันและการวิเคราะห์ความเป็นไปได้ของการติดเชื้อจากสุกรสู่คน

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ABSTRACT

Rotaviruses are the main cause of severe gastroenteritis in infants and young children worldwide. The aims of this study were to investigate the epidemiology and molecular characteristics of rotaviruses in children with diarrhea in Thailand during January to November 2013. From a total of 246 fecal specimens tested, 58 (23.6%) were positive for group A rotavirus. Among these, wide variety of G-P combinations were detected, G1P[8] (36.2%), G2P[4] (31.0%), G1P[4] (8.6%), G3P[8] (6.9%), G2P[8] (5.2%), G8P[8] (5.2%), G9P[8] and G9P[19] each of 1.7%. Interestingly, an uncommon human rotavirus strain G9P[19] was detected and the nucleotide sequences of VP4 and VP7 genes of this strain were closely related to these of porcine rotaviruses reported previously. The data imply that interspecies transmission among human and porcine rotaviruses have been occurred in nature. In conclusion, this study provides the epidemiological information and molecular characteristics of rotaviruses circulated in children with diarrhea in Chiang Mai, Thailand.

บทคัดย่อ

เชื้อไวรัสโรตาเป็นสาเหตุสำคัญของโรคกระเพาะอาหารและลำไส้อักเสบอย่างรุนแรงในเด็กทารกและเด็กเล็กทั่วโลก วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาระบาดวิทยาและคุณลักษณะเฉพาะในระดับโมเลกุลของเชื้อไวรัสโรตาในเด็กเล็กที่มีอาการอุจจาระร่วงในประเทศไทย ในช่วงเดือนมกราคมถึงพฤศจิกายน ปี พ.ศ. 2556 จากตัวอย่างอุจจาระที่ทดสอบทั้งหมด 246 ตัวอย่าง พบว่ามีอยู่ 58 ตัวอย่าง (ร้อยละ 23.6) ให้ผลบวกต่อ group A rotavirus และจากการตรวจจำแนก G และ P genotypes พบว่ามีความหลากหลายของ G-P combinations ที่ตรวจพบในการศึกษาครั้งนี้ได้แก่ G1P[8] (ร้อยละ 36.2), G2P[4] (ร้อยละ 31.0), G1P[4] (ร้อยละ 8.6), G3P[8] (ร้อยละ 6.9), G2P[8] (ร้อยละ 5.2), G8P[8] (ร้อยละ 5.2), G9P[8] (ร้อยละ 1.7) และ G9P[19] (ร้อยละ 1.7) สิ่งที่น่าสนใจในการศึกษานี้คือ สามารถตรวจพบเชื้อไวรัสโรตาสายพันธุ์ G9P[19] ซึ่งเป็นสายพันธุ์ที่ไม่ค่อยพบในคน และผลการศึกษาลำดับนิวคลีโอไทด์ของยีน VP4 และ VP7 ของเชื้อสายพันธุ์นี้พบว่ามีความเหมือนอย่างมากกับยีน VP4 และ VP7 ของไวรัสโรตาในสุกรที่มีการรายงานก่อนหน้านี้ จากข้อมูลดังกล่าวแสดงให้เห็นว่าในธรรมชาติมีการติดเชื้อข้ามสายพันธุ์ระหว่างไวรัสโรตาในคนและสุกร โดยสรุปการศึกษาในครั้งนี้ได้ให้ข้อมูลทางระบาดวิทยาและคุณลักษณะเฉพาะในระดับโมเลกุลของเชื้อไวรัสโรตาในเด็กเล็กที่มีอาการอุจจาระร่วงในจังหวัดเชียงใหม่ ประเทศไทย

Key Words: Rotavirus, Epidemiology, Molecular characterization

คำสำคัญ: ไวรัสโรตา ระบาดวิทยา คุณลักษณะเฉพาะในระดับโมเลกุล

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Introduction

Rotavirus (RV) is a major pathogen associated with severe diarrhea or acute gastroenteritis in infants and young children at the age of under 5 years worldwide (Cox & Christenson, 2012; Kapikian, 2001). Each year, rotavirus causes approximately 2 million hospitalizations and 450,000 deaths in children worldwide, with the majority of deaths occurring in developing countries in Asia and Africa (Parashar et al., 2003; Tate et al., 2012).

RVs belong to the genus *Rotavirus* within the *Reoviridae* family. RVs is a non-enveloped triple-layered icosahedral capsid of about 70-75 nm in diameter. The viral genome consists of 11 segments of double-stranded RNA, encoding 6 structural viral proteins (VP1–4, VP6, and VP7) and 6 nonstructural proteins (NSP1–6) (Estes & Kapikian, 2007). The RVs are classified into seven groups (A–G), based on the group specific antigen of the VP6 capsid protein (Estes & Kapikian, 2007). Groups A, B, and C rotaviruses have been identified in humans and animals while group E-G are found only in animals (Matthijnssens & Desselberger, 2012). Of these, group A rotavirus (RVA) is the major cause of severe acute viral gastroenteritis in humans and animals. RVs have been classified into G (glycoprotein) and P (protease-sensitive protein) genotypes (Estes & Kapikian, 2007). So far, at least 27 G types (VP7) and 37 P types (VP4) of RVs have been detected in humans and animal species with various G-P combinations (Matthijnssens et al., 2011; Trojnar et al., 2013).

The epidemiological studies worldwide have demonstrated that rotavirus strains G1P[8], G2P[4], G3P[8], G4P[8], G9P [8] and G9P[6] are responsible of most rotavirus infections (Santos & Hoshino, 2005). The uncommon strains were found in animals and found to be cross-infected to humans, such as G9P[8] genotype, which is closely related to the genotype in pigs (Khamrin et al., 2006). Since rotavirus genome contains 11 double-stranded RNA segments, the generation of unusual rotavirus strains may occur through interspecies transmission and reassortment among rotaviruses that infect the same cell (Dunn et al., 1993; Urasawa et al., 1992; Varghese et al., 2004). Epidemiological studies of rotaviruses have been performed extensively worldwide, and novel genotypes, animal-like strains, or animal-human rotavirus reassortants have been increasingly detected (Martella et al., 2010; Matthijnssens et al., 2011). These findings indicate that interspecies transmission of rotaviruses between humans and animals or animals and animals might be taken place in nature (Cook et al., 2004; Gouvea & Brantly, 1995; Nakagomi O & Nakagomi T, 1993; Palombo, 2002).

Recently, in Chiang Mai, Thailand, the prevalence of group A rotaviruses causing severe diarrhea in infants and young children was reported at 29.4% (Chaimongkol et al., 2012). However, the surveillance of rotavirus infection and genotype distribution in infants and young children with diarrhea has not been conducted continuously in northern, Thailand. Therefore, it is of interest to determine the prevalence of human rotaviruses and to perform molecular characterization of rotavirus genotypes circulating in this area. The objective of this study is to screening of RVA in stool samples of infants and children with diarrhea during January to November, 2013 in Chiang Mai, Thailand. The human rotavirus strains detected will be analyzed

further for their genetic background by G and P genotyping, and genome sequence analysis.

Materials and methods

Specimen collection

A total of 246 fecal specimens were collected from children hospitalized with diarrhea in two hospitals in Chiang Mai, Thailand. The age of the patients ranged from neonate up to 14 years old. The specimen collection period was from January through November 2013. All fecal sample materials were stored at -20°C until used.

Viral RNA extraction and reverse transcription (RT) reaction

For viral RNA extraction, fecal sample was prepared as 10% suspension in phosphate-buffered saline (PBS), pH 7.2, and then clarified by centrifugation at 5000 rpm for 5 min at room temperature. The RNA genome of rotavirus was extracted from the supernatant by using Geneaid Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan), according to the manufacturer's protocol. The total viral RNA was eluted by 50 µL of RNase-free water and reverse transcription (RT) was then performed according to the manufacturer's protocol (Fermentas, MD, USA). Briefly, 10 µL of viral genome were added to 1 µL of 50% dimethyl sulfoxide (DMSO) at 95°C for 5 minutes. Then, RT reaction using random hexamer primers (Takara, Shiga, Japan) was carried out at 42°C for 1 hr, followed by 72°C for 10 min and then immediately chilled on ice.

Detection of group A rotaviruses

The presence of group A rotaviruses in fecal specimens was detected by polymerase chain reaction (PCR) using a protocol described previously (Yan et al., 2004). PCR product were detected by electrophoresis on 1.5% agarose gel, stained with ethedium bromide, and visualized under UV light. The sizes of PCR products were identified by comparing with 100 bp Ladder marker (Fermentas, MD, USA). The expected fragment length of RVA was 395 bp. All of RVA positive samples were analyzed further for their G and P genotypes by multiplex PCR genotyping method. For the uncommon RV strains detected, the viruses were subjected for nucleotide sequencing and sequence analysis.

Multiplex PCR for G and P genotyping

The G genotypes of RVAs were identified using the method described previously by Gouvea et al. (1990) with minor modifications. The amplification of VP7 gene was performed using a pool of forward primer (BT1, CT2, ET3, DT4, AT8, FT9, G12F) in combination with a reverse primers (End9(s)) for identification of G1, G2, G3, G4, G8, G9 and G12 genotypes, respectively (Gouvea et al., 1990; Khamrin et al., 2011). However, the viruses of which their G genotypes could not be identified with this primer set were subjected further to multiplex PCR using alternative sets of primers reported by Gouvea et al. (1990, 1994). The P genotypes were identified using the method described previously by Gentsch et al. (1992) with slight modifications. The amplification of VP4 gene was performed using a forward primer (Humcom5) in combination with a pool of reverse primers (2T-1, 1T-1mo) for identification of P[4] and

P[8] genotypes, respectively (Gentsch et al., 1992). The samples of which the P genotypes could not be identified by this primer set, the alternative sets of primers reported previously by Gentsch et al. (1992), Maneekarn et al. (2006), and Winiarczyk et al. (2002) were used. The rotavirus isolates of which their G and P genotypes could not be identified by these multiplex PCR methods, the virus strains were analyzed further to identity their G and P genotypes by nucleotide sequencing, and sequence analysis.

Results

Prevalence of group A rotaviruses in children with diarrhea

A total of 246 fecal samples collected from pediatric patients with acute gastroenteritis were included in this study and 58 out of 246 (23.6%) were positive for RVA.

Identification of G and P genotypes by RT-multiplex PCR

A total of 58 group A rotavirus detected in this study were subjected further for identification of their G and P genotypes by RT-multiplex PCR. For RVAs that could not be typed by RT-multiplex PCR, they were subjected to nucleotide sequencing. Five different G genotypes were identified, including G1, G2, G3, G8, and G9. Of these, two strains of the G genotypes remained unidentified. For P genotype, three different P genotypes were detected, comprising P[8], P[4], and P[19]. The G1 genotype was identified as the most prevalent genotype at 44.8% (26 of 58), followed by G2, G3, G8, and G9 at 36.2% (21 of 58), 6.9% (4 of 58), 5.2% (3 of 58), and 3.4% (2 of 58), respectively, as shown in Fig 1. Of the P genotypes, P[8] was the most predominant genotype with the prevalence of 56.9% (33 of 58) while P[4] and P[19] were detected with lower frequency of 41.4% (24 of 58), and 1.7% (1 of 58), respectively, as shown in Fig 2.

Most of G1 (21 of 26 strains) were found in combination with P[8] and five strains were found to combine with P[4], while the majority of G2 (18 of 21 strains) were found in combination with P[4] and three strains were found to combine with P[8]. Four G3 and three G8 were found in combination with P[8]. One each of G9 genotype was found in combination with P[8] and P[19], respectively. Furthermore, one each of P[4] and P[8] genotype was found in combination with G-nontypeable as shown in Table 1.

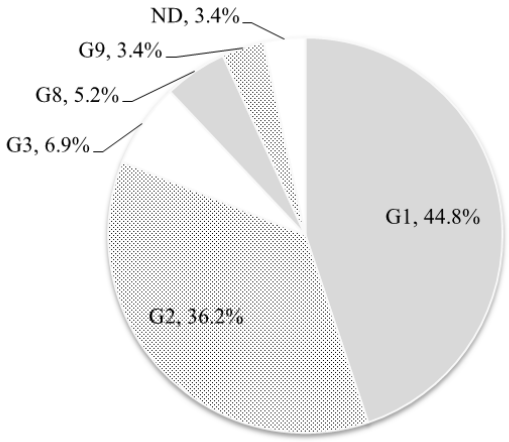

Fig. 1 The prevalence of G genotype of group A rotaviruses. Data are shown as the percentage. (ND; G genotype could not be identified).

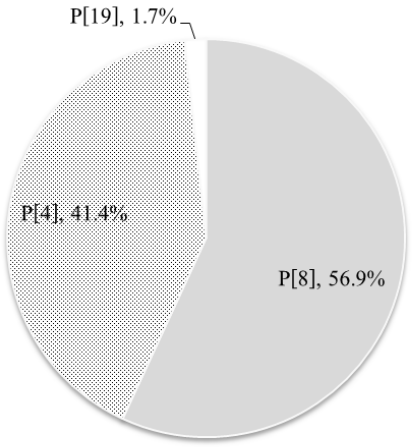

Fig. 2 The prevalence of P genotype of group A rotaviruses. Data are shown as the percentage.

Discussion and Conclusions

The present study has investigated the epidemiology and genotype characteristics of group A rotavirus infection among infants and young children hospitalized with diarrhea in Chiang Mai, Thailand during January to November 2013. A total of 246 stool specimens were tested for Group A rotavirus using RT-PCR and 23.6% were positive. The prevalence of RVA infection reported in this study is consistent with previous reports in Chiang Mai, Thailand in 2005 (Khamrin et al., 2010) and 2007 (Chaimongkol et al., 2012) which were reported the prevalence rate of 29.3% and 29.4%, respectively.

Previously, epidemiological studies of group A rotavirus in Chiang Mai from 2000-2001 indicated that RVA G9, G3, and G2 genotypes with P[8], P[4], and P[3] were circulated in children hospitalized with diarrhea (Khamrin et al., 2006). Then, epidemiological surveillance of group A rotavirus infection in 2005 revealed that G1P[8] was identified as the most prevalent genotype, followed by G2P[4], G9P[8], G3P[8], and G3P[10] respectively, as well as the studies in 2007 reported the G1P[8] with high prevalence followed by G2P[4] and G3P[8] respectively (Khamrin et al., 2010; Chaimongkol et al.,2012). In the present study five different G genotypes, including G1, G2, G3, G8, and G9, and three different P genotypes, including P[8], P[4], and P[19] were detected. In addition, eight different G and P genotype combinations were found, G1P[8] genotype continues to be the most common genotype circulated in Chiang Mai, followed by G2P[4], G1P[4], G3P[8], G2P[8], G8P[8], G9P[8], and G9P[19]. Moreover, sporadic cases of G8P[8] and

Table 1 Distribution and relative frequencies of G and P genotype combinations of group A rotaviruses.

G genotypes (%)	P genotypes (%)			Total (%)
	P[4]	P[8]	P[19]	
G1	5 (8.6)	21 (36.2)	-	26 (44.8)
G2	18 (31.0)	3 (5.2)	-	21 (36.2)
G3	-	4 (6.9)	-	4 (6.9)
G8	-	3 (5.2)	-	3 (5.2)
G9	-	1 (1.7)	1 (1.7)	2 (3.4)
ND[a]	1 (1.7)	1 (1.7)	-	2 (3.4)
Total (%)	24 (41.3)	33 (56.9)	1 (1.7)	58 (100)

[a]ND; G genotype could not be identified.

G9P[19] were also detected in this surveillance study. Our study clearly demonstrates that group A rotaviruses circulating in Chiang Mai Thailand are genetically diverse and various G-P genotype combinations are identified. Previous studies demonstrated that G9P[19] is detected originally from pigs (Chan-It et al., 2008; Khamrin et al., 2007; Maneekarn et al., 2006), while in human rotavirus G9P[19] was occasionally found and have been described as porcine-like human rotaviruses (Chitambar et al., 2009; Ghosh et al., 2012; Maneekarn et al., 2006; Okada et al., 2000; Wu et al., 2011). In addition, the VP7 nucleotide sequences of G9 detected in the present study were closely related to those of porcine rotaviruses previously reported in Chiang Mai, Thailand (CMP066/09 and CMP054/10), while P[19] nucleotide sequence was most closely related to those of porcine P[19] which were reported previously in Chiang Mai, Thailand as well (CMP138/10 & CMP139/10). Nucleotide sequence analysis demonstrated clearly that VP7 and VP4 genes of human rotavirus G9P[19] strain may derive from rotavirus of porcine origin (Ghosh et al., 2012; Maneekarn et al., 2006; Okada et al., 2000). The data also imply that interspecies transmission of rotaviruses between pigs and humans could be occurred in nature.

In conclusion**,** the present study revealed that a wide variety of rotavirus genotypes were circulating in infants and young children with diarrhea in Chiang Mai, Thailand between January to November, 2013. The results demonstrated that G1, G2, G3, G8, G9, P[8], P[4] and P[19] were circulating in this area and the co-predominance of G1P[8] and G2P[4] was detected at 36.2% and 31.0%, respectively. Some of human rotavirus strains carry the VP7 and VP4 genes highly similar to those of porcine rotaviruses. The data imply that human rotaviruses circulating in this area are highly genetically diverse and provide evidence that reassortment may occur in nature among rotaviruses of human and porcine origins. Therefore, the epidemiological study of rotavirus should be conducted continuously in order to gain the overview updated data of RVA circulating in this area.

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