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Short communication

The prevalence and genotype diversity of Human Rotavirus A circulating in Thailand, 2011–2014

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ABSTRACT

Human rotavirus A (RVA) is the major infectious virus causing acute watery diarrhea in children, especially those younger than 5 years of age, and is a major public health problem in Thailand. Outbreaks of this virus have been reported worldwide. Besides the common genotypes, unusual genotypes providing evidence of inter-species transmission have also been described. Therefore, the aim of this study was to investigate the prevalence and genotypes of RVA in Thailand. A total of 688 samples were collected from children who were hospitalized with acute diarrhea in Chumphae Hospital in Khon Kaen and Chulalongkorn Hospital in Bangkok. RVA was detected using one-step RT-PCR and the genotypes were evaluated by sequencing. Overall, 204 of the 688 samples (30%) were positive for RVA. Nine genotypes were identified: three common in humans (G1P[8] [53%], G2P[4] [18%], G3P[8] [12%]), one feline-like (G3P[9] [1%]), four porcine-like (G4P[6] [0.5%], G5P[6] [0.5%], G9P[8] [0.5%], G12P[6] [1.5%]), and one bovine-like (G8P[8] [13%]). The variation in virus genotypes and the animal-like genotypes detected in this study suggested that a high diversity of RVA types is circulating in the Thai population. Therefore, continuous molecular epidemiological monitoring of RVA is essential and has implications for the national vaccination program.

1. Introductions

Rotaviruses belong to the *Reoviridae* family, genus *Rotavirus*. Based on their antigenic properties, rotaviruses are classified into eight species designated A through H. Rotavirus A (RVA) is commonly found in humans and causes acute watery diarrhea worldwide, especially in children younger than 5 years of age (Parashar et al., 2006). The virus contains 11 segmented double-stranded RNAs, which are translated into six structural (viral proteins [VPs]) and six non-structural proteins (NSP). The glycoprotein (G) VP7 and protease-sensitive protein (P) VP4 genes are used to classify rotaviruses into different genotypes (Desselberger, 2014). To date, at least 27 G and 35 P (Matthijnssens et al., 2011) genotypes have been identified for RVA. G1 to G4, G9 and G12 are commonly found genotypes reported worldwide (Jain et al., 2014).

In Thailand, outbreaks among children were reported over the past few years (Jiraphongsa et al., 2005; Maiklang et al., 2012; Nelson et al., 2008; Theamboonlers et al., 2008). Interestingly, different viral genotypes played major roles in each outbreak (Maiklang et al., 2012; Nelson et al., 2008; Theamboonlers et al., 2008). Moreover, evidence of inter-species transmission from porcine (Mladenova et al., 2012), bovine (Steyer et al., 2007), or feline (Mijatovic-Rustempasic et al., 2014; Mladenova et al., 2015; Theamboonlers et al., 2014) sources to humans had also been reported.

Therefore, the objective of this study was to identify the prevalence and genotype distribution of human RVA in Thailand between 2011 and 2014.

2. Materials and methods

2.1. Study population

A total of 688 stool samples were collected over a 3-year period (26 May 2011 to 9 August 2014). Samples were obtained from individuals presented with acute diarrhea defined by three or more abnormally loose or watery stools within 24 h. A total of 157 samples were collected

Abbreviations: BLAST, Basic Local Alignment Search Tool; G, glycoprotein; RVA, human rotavirus A; P, protease-sensitive protein; RT-PCR, reverse transcription polymerase chain reaction; VP, viral protein.

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from the Chulalongkorn Memorial Hospital in Bangkok and 531 samples from the Chumphae Hospital in Khon Kaen. Patients included in this study were between the ages of 1 day and 15 years. Samples were initially tested using the SD BIOLINE Rota/Adeno Rapid test (Standard Diagnostics, Kyonggi, Korea) as previously described (Chieochansin et al., 2014) following the manufacturer's instructions. To further test the stool samples by molecular methods, the samples were diluted with PBS (ratio 1:10), vortexed, and centrifuged at 1500 rpm for 10 min. The supernatants were kept at −70 °C.

2.2. Viral genome extraction and virus detection

Total viral nucleic acids were extracted using Ribo-Spin vRD II (GeneAll, Seoul, Korea) according to the manufacturer's instructions. The RVA nucleic acids were detected using one-step reverse transcription polymerase chain reaction (RT-PCR) as previously described (Theamboonlers et al., 2008), but with some modifications. Two sets of primers were used to amplify two RVA genes in which primers BEG9 and END9 (Gouvea et al., 1990) were specific for VP7 gene and primers CON3 and CON2 were specific for VP4 gene (Gentsch et al., 1992). Human housekeeping gene GAPDH, which served as an internal control, was successfully amplified in all samples (data not shown). SuperScript III One-step RT-PCR System with Platinum *Taq* (Invitrogen, Paisley, UK) was used for viral genome amplification. The reaction mixture consisted of 2 μL RNA, 1 μL *Taq*Mix, 1 × reaction buffer, 0.25 mM of each forward and reverse primer, 7% *v*/*v* DMSO, 1.25 mM $MnSO_4$, and up to 20 μL nuclease-free water. The RT condition comprised 50 °C for 30 min and an inactivation step at 95 °C for 5 min, followed by an activation of the hot-start *Taq* for the PCR reaction. Amplification cycles for both VP7 and VP4 were 94 °C for 30 s, 50 °C for 30 s, 68 °C for 1 min, and a final extension at 68 °C for 10 min. The expected amplicon was 1036 bp for VP7 and 876 bp for VP4.

2.3. Direct sequencing and phylogenetic tree analysis

PCR amplicons were purified using GeneAll Expin Gel SV. Direct Sanger DNA sequencing in both the forward and reverse directions was performed by 1st BASE Laboratories (Seri Kembangan, Selangor, Malaysia). Sequencing results were annotated, aligned, and managed using a combination of software, including BLAST (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov./Blast.cgi), Chromas Lite (http://www.technelysium.com.au/chromas_lite.html), and BioEdit

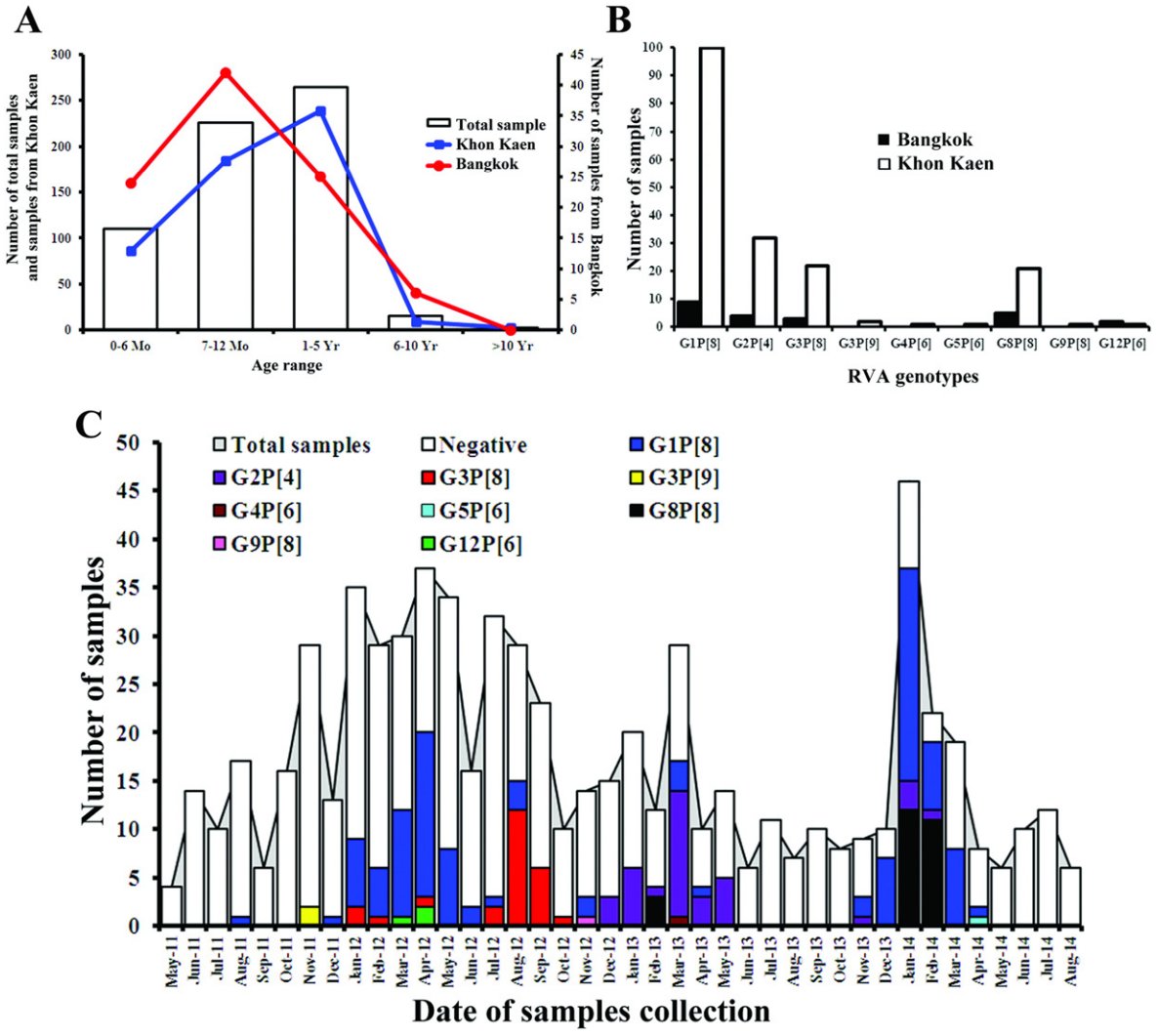

Fig. 1. Analysis of the patient age and genotype distribution of RVA identified in this study. (A) Bar graph denotes the total number of samples, while the line graphs denote samples from Khon Kaen (blue) and Bangkok (red). (B) Different RVA genotypes identified in the samples. (C) Distribution of RVA genotypes found each month between 2011 and 2014.