Title of dissertation			
High prevalence of equine-like G3P[8] rotavirus in children and adults with acute gastroenteritis in Thailand			
RONPAKU Fellow			
Name	**Ratana Tacharoenmuang**		
Position	Medical Scientist, Professional Level	ID No.	R11720
Department	Enteric Viruses Section, National Institute of Health		
Institution	Department of Medical Sciences, Ministry of Public Health	Nationality	Thailand
Japanese Advisor			
Name	Tetsushi Yoshikawa		
Position	Professor	Institution	Fujita Health University

1. Introduction

Group A rotaviruses (RVAs) are a leading cause of acute gastroenteritis in infants and young children worldwide. It is estimated that RVA gastroenteritis is associated with 215,000 deaths in children younger than 5 years, especially in developing countries in Asia and Africa. The RVA genome consists of 11 segments of double-stranded RNA (dsRNA) encoding six structural proteins (VP1-VP4, VP6, and VP7) and six nonstructural proteins (NSP1-NSP6). A binary genotype system based on outer capsid proteins VP7 and VP4 had been used for RVA classification. Currently, RVAs are grouped into 36 G types and 51 P types, and the predominant RVA genotypes in humans worldwide are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8]. Recently, a whole genome-based genotyping system for all 11 genes employing the convention Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx was introduced, x indicating the number of corresponding genotypes. Most human RVA strains show either Wa-like genogroup (G1/3/4/9/12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) or DS-1-like genogroup (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2). This study aims to clarify the distribution of G/P types and genetic characteristics of RVAs circulating in Thailand.

2. Materials and Methods

In total, 1,867 stool specimens were collected from children and adults with acute gastroenteritis admitted to six hospitals in Thailand. The hospitals are located in the Bangkok, Udonthani, Beung Kan, Phuket, Tak, and Chanthaburi provinces. The stool specimens were collected between January 2014 and September 2016. The RVA dsRNA was extracted from stool specimens and the extracted dsRNA was subjected to genotyping of the VP7 and VP4 genes by semi-nested reverse transcription-polymerase chain reaction (RT-PCR). The dsRNAs were electrophoresed in 10% polyacrylamide gels, followed by silver staining to determine the electropherotypes. Illumina MiSeq sequencing and sequence analysis were carried out on two representative Thai equine-like G3P[8] strains, MS2014-0134 and DBM2016-096. Nearly full-length nucleotide sequences of the VP7 genes of 96 Thai equine-like G3P[8] were determined by direct sequencing of amplified RT-PCR products with specific primers.

3. Results and Discussion

Five hundred and fourteen (27.5%) of the 1,867 samples were found to be positive for RVA. In 2014 to 2016, G1P[8] (44.7%) was the most predominant type, followed by G3P[8] (33.7%), G2P[4] (11.5%), G8P[8] (7.0%), and

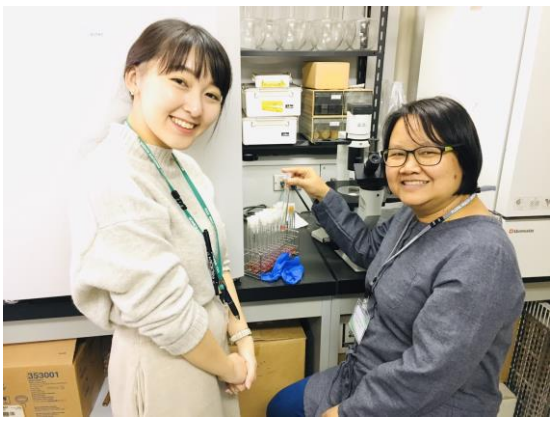
G9P[8] (1.3%). Unusual G3P[9] (0.8%), G3P[10] (0.4%), G4P[6] (0.4%), and G10P[14] (0.2%) strains were also detected at low frequencies. The predominant genotype, G1P[8] (64.4%), in 2014 decreased to 6.1% in 2016. In contrast, the frequency of G3P[8] markedly increased from 5.5% in 2014 to 65.3% in 2015 and 89.8% in 2016. On polyacrylamide gel electrophoresis, most (135/140; 96.4%) of the G3P[8] strains exhibited a short RNA profile, suggesting a DS-1-like backbone. Successful determination of the nucleotide sequences of the VP7 genes of 98 G3P[8] strains with a short RNA profile showed that they are all equine-like G3P[8] strains. On phylogenetic analysis of genome segments of two representative Thai equine-like G3P[8] strains, it was noteworthy that they possessed distinct NSP4 genes, one bovine-like and the other human-like.

4. Conclusion

In this study, it was found that the predominant genotype, G1P[8], decreased markedly in 2015 and 2016, and that equine-like G3P[8] strains with a short RNA profile increased drastically in Thailand. Thus, it can be concluded that equine-like G3P[8] strains are prevailing widely in Thailand. The equine-like G3P[8] strains detected previously in Thailand had only bovine-like NSP4 genes. In this study, equine-like G3P[8] strains with an NSP4 gene of human origin ware first detected in Thailand, indicating the increase of diversity of the equine-like G3P[8] strains in Thailand.

Photos

Operating the Illumina MiSeq next-generation sequencer



Propagation of RVAs in cell culture using the roller-tube technique