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Short communication

High incidence of group A rotaviruses G4P[6] strains among children in Gyeonggi province of South Korea, from 2009 to 2012

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ABSTRACT

The genotype distribution of group A rotaviruses (RVAs) circulating in Gyeonggi province, South Korea between 2009 and 2012 was investigated. A total of 2619 stool specimens from sporadic acute gastroenteritis cases and 117 acute gastroenteritis outbreaks were analyzed. Among them, RVAs were detected from 263 (10.0%) sporadic cases and 3 (2.6%) outbreaks. The G4P[6] strains predominated (29.7%), followed by G1P[8] (19.4%), G2P[4] (15.6%), G3P[8] (13.3%) and G9P[8] (6.5%) strain. Especially 96.2% of the genotype G4P[6] strains were isolated from children < 1 year of age. Phylogenetic analysis revealed that genotype G4P[6] strains were members of sub-lineage Ie(G4) and Ia(P[6]). Intensified monitoring of RVAs, especially G4P[6] strains among young children, is essential to control RVA infections.

1. Introduction

Group A rotaviruses (RVAs) are the most common causes of acute gastroenteritis (AGE) in young children. Annually, they are associated with >500,000 deaths of children under 5 years of age worldwide (Parashar et al., 2009). The spike protease-sensitive attachment protein VP4(P) and the glycoprotein VP7(G) in the viral capsid outer layer carry the major antigenic determinants. Because VP4(P) and VP7(G) are independently segregated, RVAs can generate novel G-P antigen combinations via reassortment during mixed infections (Gentsch et al., 2005). The G-P genotype combinations of RVA, G1P[8], G2P[4], G3P[8], G4P[8] and recent G9P[8], are the common causes of RVAs infection in the world including South Korea (Gentsch et al., 2005; Jeong et al., 2011). However, RVA G4P[6] strains were steadily circulating in South Korea compared with global surveillance data (Han et al., 2010; Huh et al., 2009; Kang et al., 2005; Shim et al., 2010). Genotype G4P[6] (Gottfried; P2B serotype) was originally isolated from pigs, but was later identified (P2A serotype) in symptomatic and asymptomatic infections in human beings (Bohl et al., 1984). P[6] strains combined with various G genotypes have been occasionally detected in RVAs infections worldwide (Gentsch et al., 2005; Heylen et al., 2013; Nordgren et al., 2012).

Two live oral attenuated vaccines, RotaTeq (Merck & Co., Inc., USA) and Rotarix (GlaxoSmithKline Biologicals, Belgium) were introduced in South Korea in 2007 and 2008, respectively (Jeong et al., 2011). RotaTeq is a pentavalent vaccine, which contains five human-bovine reassortant RVA strains (G1, G2, G3, G4 and P[8]) (Matthijnssens et al., 2010). Rotarix is a live, monovalent vaccine comprising an attenuated human G1P[8] RVA strain (Ward and Bernstein, 2009). It is meaningful to monitor the changes of RVAs genotype after introduction of two vaccines, which inevitably affect the prevalence of G and P genotype of RVAs. Therefore, we investigated prevalence level and genotype distribution of RVAs in Gyeonggi province of South Korea between 2009 and 2012 rotavirus seasons after introduction of two RVA vaccines.

2. Materials and methods

2.1. Ethics statement

All procedures of sample collections and experiments were approved by the IRB of Korea National Institute of Health (KNIH) and Gyeonggi Province Institute of Health and Environment (GIHE) (approval number: KNIH-2008-03CON-2-P). There was no human rights abuse or ethical issues during the process of this study. Fecal samples were collected by physicians during the medical treatment of patients with AGE. A written informed consent was signed by each patient's

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parent. The information was analyzed anonymously at GIHE. The patients ranged in age from neonate to five years for the sporadic cases, but were of any age for the AGE outbreaks.

2.2. Sample collection

The laboratory surveillance system for enteric pathogens in sporadic AGE has been established since 2000 as a part of national surveillance system, Enter-Net Korea, which was described in previous report (Huh et al., 2009; Jeong et al., 2011). A sporadic AGE case was defined as an individual had a clinical diarrhea (defined as >2 episodes of watery stools within a 24-hour period) which may be accompanied with abdominal pain, loss of appetite, nausea, vomiting and discomfort. An AGE outbreak was defined as an incident, apparently associated with a common event or location, in which five or more individuals had symptoms of AGE. A season was defined as the period from August of one year to July of the next year. For three seasons (August 2009 to July 2012), stool specimens (n = 2619) from sporadic cases of AGE from three general hospitals (Korea University Ansan hospital, Bundang Cha hospital and St. Vincent's hospital) and stool specimens (n = 1792) from 117 AGE outbreaks sent from 34 local health centers were collected in Gyeonggi province located near Seoul, the capital city of South Korea, where approximately 12.5 million people (24% of the country's population) live in this province.

2.3. Virus detection

The fecal specimens were diluted to be 10% (w/v) suspension with phosphate-buffered saline (PBS), vortexed and centrifuged at 800 ×*g* for 15 min. RVA and adenovirus antigens were detected from the stool supernatants using an enzyme-linked immunosorbent assay (ELISA) with BioTracer™ Group A Rotavirus (Group A) kit and Adenovirus kit (Bio Focus Co., Uiwang, South Korea), respectively, according to the manufacturer's instructions. Norovirus, astrovirus and sapovirus were detected by reverse transcription-polymerase chain reaction (RT-PCR).

Viral RNA was extracted from the supernatants using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). RT-PCR assay was performed to amplify the VP7- and VP8* of VP4, which then were sequenced to determine the genotypes of the strains. The VP7-F (5′-ATGTATGGTATTGA ATATACCAC-3′) and VP7-R (5′-AACTTGCCACCATT TTTTCC-3′) were used to amplify an 881 bp product from the VP7(G) gene (Gentsch et al., 1992). The VP4(P) gene was amplified with the primers Con-3 (5′-TGGCTTCGCCATTTTATAGACA-3′) and Con-2 (5′-ATTTCGGACCATTTATAACC-3′), generating an 876 bp product (Gomara et al., 2001). Among 263 RVA detected by ELISA, the 228 amplified RT-PCR products of the VP7(G) and VP8* of VP4(P) genes were sequenced using an ABI 3730XL analyzer (Applied Biosystems, Foster City, CA). RotaC$^{2.0}$ automated genotyping tool (http://rotac.regatools.be/) was used to determine RVA genotype (Maes et al., 2009).

2.4. Phylogenetic analysis

Phylogenetic analysis was performed to identify the origin of RVA G4P[6] strains using neighbor-joining analysis and bootstrap analysis (n = 1000; MEGA (version 5.0) Software, Tempe, AZ, USA). All nucleotide sequences of VP7 of G4 strains and VP4 of P[6] strains were submitted to the GenBank database with following accession numbers: KF650074–KF650095 (Supplementary Table S1).

3. Results

3.1. RVA G4P[6] strain in sporadic cases

Of the total number of AGE sporadic cases (n = 2619) and outbreaks (n = 117), 263 (10.0%) and 3 (2.6%) were RVA-ELISA-positives, respectively. In sporadic AGE cases, the seasonal distribution of RVAs was high during winter to spring (Supplementary Fig. S1). During the study period, the major viral agents for sporadic AGE in children were RVA (10.0%, 263/2619) and norovirus (10.2%, 266/2619), followed by enteric adenovirus (1.1%, 28/2619) and astrovirus (0.5%, 13/2619), and the rest of the AGE cases were of non-viral infections. Ten G-P combinations of RVAs were identified (Table 1). G4P[6] (29.7%) was the most predominant genotype, followed by G1P[8] (19.4%), G2P[4] (15.6%), G3P[8] (13.3%) and G9P[8] (6.5%). The other G-P genotypes, G1P[4], G3P[4], G2P[6], G2P[8] and G4P[8], were detected by <1.0%. The frequency of genotype G4P[6] strains fluctuated annually. The frequency was 36.3% in 2009–2010, decreased to 16.5% in 2010–2011, and then sharply increased to 42.9% in 2011–2012 (Table 1). G1P[8] strains steadily decreased from 23.1% in 2009–2010 to 15.9% in 2011–2012, whereas G3P[8] strains dramatically decreased from 22.0% in 2009–2010 to 6.3% in 2011–2012 (Table 1). On the other hand, G9P[8] strains increased from 4.6% in 2010–2011 to 15.9% in 2011–2012, and interestingly, G2P[4] strains showed a considerable increase (29.4%) in 2010–2011 compared with 2009–2010 (6.6%) and 2011–2012 (4.8%).

3.2. RVA G4P[6] strain in outbreak cases

Among 117 AGE outbreaks, 3 (2.6%) were related with RVA infections (Table 1) and norovirus were detected in 28 (23.9%) outbreaks. Other enteric viruses, including enteric adenovirus, astrovirus and sapovirus, were not found from AGE outbreaks. The rest of AGE outbreaks (n = 86) were non-viral infections. A G2P[4] rotavirus-associated outbreak was reported from a nursery school (mean age of 3.5 years old) in February 2011. The other two of G4P[6] strains-associated outbreaks were identified from neonatal care centers (below 1 year of age) in December 2011 and June 2012.

3.3. Age distribution of RVA G4P[6] strains

Most of the G4P[6] strains (96.2%; n = 75) were detected in young children under one year of age; 3.7% (n = 3) were detected in children between 1 and 2 years old (Fig. 1). The other major strains, G1P[8] (52.9%), G2P[4] (60.9%), G3P[8] (60.5%) and G9P[8] (70.5%), were prevalent in young children between 1 and 2 years old and showed a similar distribution at each age.

3.4. Phylogenetic analysis of G4/VP7 gene and P[6]/VP4 gene

The total 78 VP7(G) and VP8*(P) of VP4(hereafter referred to as VP8*) genes of G4P[6] strains were sequenced as shown in Table 1. The strains used in the phylogenetic analysis were selected based on seasons and sequence diversity of at least >1%. Phylogenetic analysis revealed that two outbreak-associated G4P[6] strains were clustered with other G4P[6] strains from sporadic AGE cases (Fig. 2). For the phylogenetic analysis, nucleotide and deduced amino acid sequences of 10 G4P[6] and 2 G4P[8] strains were compared with the VP7(G) gene of 19 G4 strains and RotaTeq-BrB-9 G4P[5] strain (Fig. 2A). The analysis revealed that 10 G4P[6] strains were clustered with the sub-lineage Ie of the G4 genotype which includes the strains previously reported as Korean strains (Fig. 2A). The sequence identity between Korean G4 strains was >97.8% in nucleotides and 97.6% in amino acids, and 95.8% in nucleotides and 95.9% in amino acids compared to prototype ST3 strain and 95.0% in nucleotides and 94.5% in amino acids compared to RVA vaccine RotaTeq-BrB-9 G4P[5] strain. As shown in Fig. 2A, 2 G4P[8] strains were differently grouped with sub-lineage Ic of the G4 genotype.

Nine G4P[6] and 1 G2P[6] strains were compared with the VP8* of 21 P[6] RVA strains (Fig. 2B). All G4P[6] strains were clustered with sub-lineage Ia of genotype P[6] (represented by the M37 strain) which includes the strains previously reported as Korean and Taiwanese, TE56 and CH, strains (Fig. 2B). The sequence identity between Korean P[6] strains was >96.5% in nucleotides and 97.1% in amino acids, and 94.7% in nucleotides and 89.4% in amino acids compared to prototype M37