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# Detection of Unusual Rotavirus Genotypes G8P[8] and G12P[6] in South Korea

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Five hundred four fecal specimens, collected between 2004 and 2006 from young children with acute diarrhea, were screened for rotavirus by ELISA with VP6-specific antibody. Of these samples, 394 (78.2%) were confirmed as group A rotavirus and they underwent G- and P typing using a combination of ELISA, RT-PCR, and sequence analysis methods. The dominant circulating G serotype was G1 (35.6%) followed by G3 (26.4%), G4 (14.7%), and G2 (11.9%). There was a low prevalence of G9 (1.0%) and of unusual G type rotavirus, in particular, G12 (0.5%) and G8 (0.3%). Of the P genotype rotavirus in circulation, P[8] (53.0%) was most common followed by P[6] (15.5%), P[4] (15.2%), and P[9] (2.3%). Determination of G- and P type combinations revealed that G1P[8] strains were most prevalent (25.4%), amid G3P[8] (16.8%), G2P[4] (6.3%), and G4P[6] (6.1%) strains. Unusual or rare combinations such as G2P[6], G2P[8], G3P[4], G2P[9], G1P[9], G3P[9], G12P[6], G1P[4], G3P[6], and G8P[8] were also found. Owing to the recent emergence of G8 and G12 rotavirus, the findings from this study are important since they provide new information concerning the local and global spread of rotavirus genotypes. ***J. Med. Virol. 80:175–182, 2008.*** 

**KEY WORDS:** diarrhea; ELISA; RT-PCR; G type; P type

## INTRODUCTION

Rotaviruses are the most common etiological agent of severe diarrhea in infants and young children [Kapikian et al., 2001] and are responsible, worldwide, for an estimated 454,000–705,000 deaths annually [Parashar et al., 2006]. The virus is a member of family *Reoviridae* and its genome is composed of 11 segments of double-stranded RNA that encode for six structural and six nonstructural proteins. The outer capsid is composed of two proteins, glycoprotein VP7 and protease-sensitive VP4 that define G- and P types of rotavirus as well as confer protective immunity [Estes, 2001].

To date, 16 G serotypes [Gulati et al., 2007] and at least 27 P genotypes have been reported [Khamrin et al., 2007; Martella et al., 2007; Steyer et al., 2007a].

Of these variants, epidemiological studies have shown that four G (G1–G4) and three P (P[4], P[6], and P[8]) are the most frequent VP7 and VP4 types associated with global human rotavirus infection [Santos and Hoshino, 2005].

Despite the high prevalence of G1, G2, G3, and G4, a fifth, new genotype, G9, has emerged as a causative agent of diarrhea in children from the United States, Canada, Australia, United Kingdom, Europe, Latin America, Africa and many Asian countries including

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South Korea [Iturriza-Gomara et al., 2000; Palombo et al., 2000; Doan et al., 2003; Kostouros et al., 2003; Steele and Ivanoff, 2003; Clark et al., 2004; Kim et al., 2005; Banerjee et al., 2006; Hung et al., 2006; Khamrin et al., 2006; Linhares et al., 2006; Yoshinaga et al., 2006; Chen et al., 2007; Van Damme et al., 2007; Wang et al., 2007]. Another rotavirus genotype, G8, was first detected in Indonesian children [Matsuno et al., 1985] and an increase in its occurrence has been reported recently in studies worldwide [Gerna et al., 1990; Holmes et al., 1999; Cunliffe et al., 2000; Adah et al., 2001; Matthijnssens et al., 2006; Pietruchinski et al., 2006; Uchida et al., 2006; Volotao et al., 2006; Steyer et al., 2007b]. The unusual human strain, G12 (L26 and L27) was first identified in 1987 from strains causing gastroenteritis in children from the Philippines [Taniguchi et al., 1990; Urasawa et al., 1990]. Since then, G12 has appeared in the United States and Thailand, and shortly thereafter, in India and Japan [Griffin et al., 2002; Pongsuwanna et al., 2002; Das et al., 2003; Shinozaki et al., 2004] such that today, G12 rotavirus has been detected in many countries across the world [Rahman et al., 2007a]. Even though G9 was first detected in the rural provinces of South Korea [Kim et al., 2005], G8 strains have not yet appeared and only a few reports are available on the occurrence of G12 [Kang et al., 2005; Santos and Hoshino, 2005].

Socioeconomic status and environmental conditions have improved but rotavirus is still the most common viral agent of acute diarrhea in young children aged between 6 and 24 months in South Korea [Kim et al., 1990; Seo and Sim, 2000]. South Korean surveillance studies reveal significantly different changes in rotavirus types across recent years. G4, G2, and G9 strains were most frequent during the period from 1998 to 2004 [Kim et al., 2005; Song et al., 2003; Min et al., 2004; Kang et al., 2005; Moon et al., 2007] while previous epidemiological reports spanning 1987–1999, indicate that G1–G4 were common—G1 being the predominant strain [Kim et al., 1993, 1999; Seo and Sim, 2000].

These distinct changes in the prevalence of G- and P type rotavirus make it essential that a thorough understanding is gained of the relative importance of rotavirus strains circulating locally. Surveillance studies are necessary since they allow a comprehensive evaluation of evolving rotavirus and the resultant data can be used, in part, for assessing the capacity of vaccines to provide heterotypic protection. Furthermore, they provide the basis for any argument that may be advanced in support of new vaccination programs.

This study is an extension of an earlier molecular epidemiological investigation [Song et al., 2003] into the distribution of G- and P type strains of rotavirus in Seoul, South Korea. Conducted between 2004 and 2006, a combination of enzyme-linked immunosorbent assay (ELISA), multiplex reverse transcription polymerase chain reaction (RT-PCR) and sequence analysis methods were employed. An update on the most prevalent rotavirus strains currently circulating South Korea is reported.

## MATERIALS AND METHODS

### Stool Specimens

A total of 504 fecal specimens were obtained from young children less than 5 years old who presented with acute diarrhea in five general hospitals located in Seoul during the period between January 2004 and February 2006. All specimens were diluted 10-fold with phosphate buffered saline (PBS; pH 7.4) and clarified by centrifugation 10,000*g* for 10 min. The supernatants were tested for group A rotavirus antigen by ELISA with VP6-group-specific antibody (Dako Diagnostics, Cambridgeshire, UK).

### VP7 (G) Serotyping by ELISA

Fecal specimens were used for serotyping by ELISA with serotype-specific monoclonal antibodies (mAb) following the manufacturer's protocol (rotaMA; Serotec Company, Sapporo, Japan). G1- to G4-specific neutralizing mAbs to HRV included G1-specific KU, G2-specific S2, G3-specific YO, and G4-specific ST3. When the A492 value of a specimen was greater than 0.2, and 2 times greater than those of other serotypes, the specimen was determined as positive.

### RNA Extraction for Genotyping

Rotavirus dsRNA was extracted using Trizol reagent (Life Technologies, Grand Island, NY). In brief, 0.3 ml of supernatant of a fecal suspension in PBS was mixed with 0.7 ml of Trizol reagent and 0.2 ml of chloroform/isoamylalcohol (24:1). After centrifugation at 12,000*g* for 10 min, the RNA in the aqueous solution was precipitated by adding an equal volume of isopropanol. The RNA precipitate was collected by centrifugation at 12,000*g* for 10 min, washed with 70% ethanol and finally dissolved in 20 μl of RNase-free water.

### VP7 (G) Genotyping by RT-PCR

RT-PCR of the *VP7* gene was performed using primers specific for genotypes G1–G6, G8–G10, and G11 [Gouvea et al., 1990, 1994; Das et al., 1994]. Also, three different sets of G-typing primers (H1, C and A pools) [Santos et al., 2003] were used to analyze specimens that were untyped by ELISA and to confirm those that were typed by ELISA. The reaction was carried out with one cycle of reverse transcription at 45°C for 30 min, followed by 35 cycles of amplification (30 sec at 94°C, 30 sec at 48°C, 1 min at 72°C), and a final extension of 7 min at 72°C in a GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA). Electrophoresis of each PCR product in 1.2% SeaKem LE agarose gel (FMC Bioproducts, Rockland, ME) was performed and following ethidium bromide staining, the results were viewed under the GelDoc 2000 image-analysis system (BioRad, Hercules, CA).

### VP4 (P) Genotyping by RT-PCR

PCR typing for the *VP4* gene was performed using step-amplification as in the VP7 genotyping. The first

step amplified gene 4 with con3 and con2 primers, and the second amplification was performed with P type-specific primers (1T-1, 2T-1, 3T-1, and 4T-1) and con3. The result was confirmed with an alternative set of type-specific primer pairs (1C-1 and 1C-2; 2C-1 and 2C-2; 3C-1 and 3C-2; and 4C-1 and 4C-2) [Gentsch et al., 1992].

### Nucleotide Sequencing and Phylogenetic Analysis

The *VP7* genes untyped by RT-PCR genotyping were examined using sequence analysis. Each amplified product was inserted into a pCR 2.1. cloning vector, transformed to *E. coli* TOP 10F' (Invitrogen, Carlsbad, CA), and sequenced using the BigDye terminator cycle sequencing kit (Applied Biosystems) and ABI PRISM 310 automated DNA sequencer. The resultant *VP7* gene sequences were aligned using the CLUSTAL X program [Thompson et al., 1997] against corresponding sequences of representative rotavirus G types from the NCBI GenBank. An unrooted phylogenetic tree based on nucleotide sequences was constructed using neighbor-joining algorithms [Saitou and Nei, 1987] from the PHYLIP suite of programs [Felsenstein, 1993]. Evolutionary distance matrices were generated by the neighbor-joining method described by Jukes and Cantor [1969] and tree topology was evaluated using a bootstrap analysis [Felsenstein, 1985] of the neighbor-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The nucleotide sequences obtained in this study were deposited in the NCBI GenBank under accession numbers EF059916 and EF059917.

## RESULTS

### Determination of G Serotypes

The distribution of human rotavirus G types by ELISA and RT-PCR is presented in Table I. Of a total of 504 fecal specimens obtained from young children with acute diarrhea, 394 (78.2%) samples were positive for group A rotavirus and were subjected to serotyping using G1–G4 specific mAbs and the RT-PCR method. As shown in Table I, all four major G types are represented. G1 (35.6%, n = 140) was the dominant circulating serotype, followed by G3 (26.4%, n = 104), G4 (14.7%, n = 58), and G2 (11.9%, n = 47). Minor or unusual serotypes, G9 (1.0%, n = 4) and G8 (0.3%, n = 1) were detected as well as mixed serotypes such as G1/3 (1.0%, n = 4), G1/4 (0.5%. n = 2), G2/9 (0.5%, n = 2), and G3/4 (0.5%, n = 2). Twenty-eight samples (7.1%) that underwent full-length *VP7* gene amplification with G serotype-specific primers were negative but were positive with VP4-specific primers.

### Determination and Phylogenetic Analysis of G12 Serotypes

The complete *VP7* gene sequences of 1,062 nucleotides from two isolates, CAU 195 and CAU 214, could not be typed by ELISA and RT-PCR methods. These sequences were analyzed and compared against the corresponding sequences of representative serotypes from the genus Rotavirus in the NCBI GenBank database. A phylogenetic tree based on nucleotide sequences was constructed using neighbor-joining algorithms and the tree topology, supported by high bootstrap values, shows the positions of CAU 195 and CAU 214 (Fig. 1). Figure 1 clearly indicates that CAU 195 and CAU 214 isolates form a tight group with the prototype G12 human rotavirus, L26 (M58290) as their closest phylogenetic relative with nucleotide sequence similarities of 90.7% and 90.3%, respectively (Table II). In addition, this cluster can be separated distinctly from clusters of other G serotype rotaviruses. *VP7* gene nucleotide sequence similarities of CAU 195 and CAU 214 with other G serotype clusters were much lower, ranging between 47.7% (G5 porcine rotavirus OSU, X06722) and 77.9% (G9 human rotavirus L169, DQ873674). It is evident, therefore, that isolates CAU 195 and CAU 214 belong to the G12 serotype cluster.

### Determination of P Genotypes

The distribution of VP4 genotypes is presented in Table I and all four major human rotavirus P genotypes are represented. Table I shows that the most common circulating genotype was P[8] (53.0%, n = 209) followed by P[6] (15.5%, n = 61), P[4] (15.2%, n = 60), and P[9] (2.3%, n = 9). The mixed genotypes of P[4]/[8] and P[6]/

TABLE I. Distribution of Human Group A Rotavirus G- and P Types From Young Children Between 2004 and 2006 in Seoul, South Korea

| | G serotype | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P genotype | G1 | G2 | G3 | G4 | G8 | G9 | G12 | G1/3 | G1/4 | G2/9 | G3/4 | NT | Total (%) |
| P[4] | 7 | 25 | 6 | 14 | — | — | — | 1 | — | 2 | — | 5 | 60 (15.2) |
| P[6] | 16 | 10 | 1 | 24 | — | — | 2 | — | — | — | 1 | 7 | 61 (15.5) |
| P[8] | 100 | 7 | 66 | 17 | 1 | 2 | — | 1 | 2 | — | — | 13 | 209 (53.0) |
| P[9] | 2 | 3 | 2 | — | — | 1 | — | — | — | — | — | 1 | 9 (2.3) |
| P[4]/[8] | 6 | — | 13 | 1 | — | — | — | 1 | — | — | — | 2 | 23 (5.8) |
| P[6]/[8] | — | — | — | 1 | — | 1 | — | — | — | — | 1 | — | 3 (0.8) |
| NT | 9 | 2 | 16 | 1 | — | — | — | 1 | — | — | — | — | 29 (7.4) |
| Total (%) | 140 (35.6) | 47 (11.9) | 104 (26.4) | 58 (14.7) | 1 (0.3) | 4 (1.0) | 2 (0.5) | 4 (1.0) | 2 (0.5) | 2 (0.5) | 2 (0.5) | 28 (7.1) | 394 (100) |

NT: nontypeable, samples were not amplified in first RT-PCR using primers detecting the VP4 or VP7 gene.

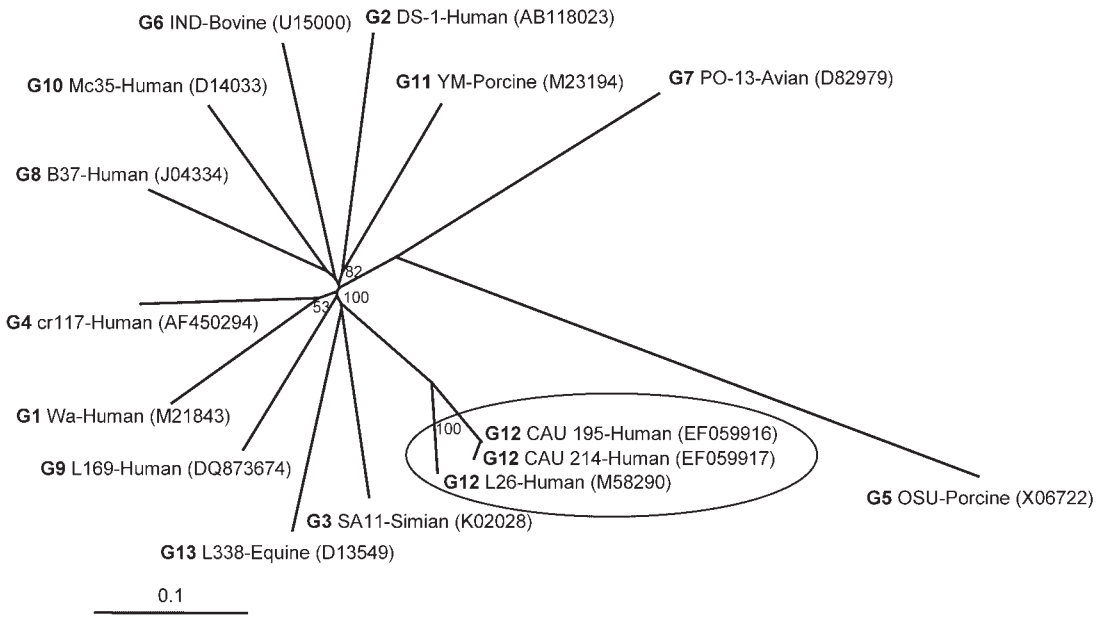


Fig. 1. Unrooted phylogenetic tree based on *VP7* gene nucleotide sequences shows relationships between two Korean isolates CAU 195, CAU 214 and representative serotypes of rotavirus. Numbers at nodes indicate the level of bootstrap support (%) based on the neighbor-joining analysis of 1,000 re-sampled datasets; only values above 50% are given. Bar represents 0.1 substitutions per nucleotide position. Porcine rotavirus OSU (X06722) was used as the out-group.

[8] occurred with a prevalence of 5.8% (n = 23) and 0.8% (n = 3), respectively. Twenty-nine samples (7.4%) that underwent full-length *VP4* gene amplification with P genotype-specific primers were negative but were positive with VP7-specific primers.

### Distribution of G- and P Types

The distribution of G- and P type combinations is shown in Table I. The results indicate that G1P[8] strains are most prevalent (25.4%, n = 100) followed by strains of G3P[8] (16.8%, n = 66), G2P[4] (6.3%, n = 25), G4P[6] (6.1%, n = 24), and G4P[8] (4.3%, n = 17). Unusual or rare combinations such as G1P[6] (4.1%, n = 16), G4P[4] (3.6%, n = 14), G2P[6] (2.5%, n = 10), G1P[4] (1.8%, n = 7), G2P[8] (1.8%, n = 7), G3P[4] (1.5%, n = 6), G2P[9] (0.8%, n = 3), G1P[9] (0.5%, n = 2), G3P[9] (0.5%, n = 2), G12P[6] (0.5%, n = 2), G3P[6] (0.3%, n = 1), and G8P[8] (0.3%, n = 1) were also detected.

## DISCUSSION

The development of new, efficient, and better rotavirus vaccines depends on a library of good surveillance data that monitors change in the populations of circulating rotavirus. Even though current epidemiological data have been collected from nation-wide or individual studies from provinces in South Korea [Min et al., 2004; Kang et al., 2005; Kim et al., 2005; Moon et al., 2007], the continuous study in Seoul, is partic-

TABLE II. Percent Similarity of the Nucleotide Sequence Encoding Gene VP7 in Korean Isolates CAU 195 and CAU 214 With the Corresponding Nucleotide Sequence of Other Rotavirus Serotypes

| | CAU 195 | CAU 214 | Wa | DS-1 | SA11 | cr117 | OSU | IND | PO-13 | B37 | L169 | Mc35 | YM | L26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CAU 214 | 99.6 | | | | | | | | | | | | | |
| Wa | 75.8 | 75.5 | | | | | | | | | | | | |
| DS-1 | 74.1 | 73.8 | 74.9 | | | | | | | | | | | |
| SA11 | 77.8 | 77.6 | 76.3 | 73.9 | | | | | | | | | | |
| cr117 | 73.9 | 73.5 | 77.1 | 71.3 | 75.3 | | | | | | | | | |
| OSU | 47.7 | 49.0 | 49.8 | 48.8 | 47.1 | 49.9 | | | | | | | | |
| IND | 73.0 | 72.7 | 74.0 | 73.4 | 76.4 | 74.6 | 49.5 | | | | | | | |
| PO-13 | 64.9 | 64.6 | 65.6 | 62.7 | 65.9 | 64.6 | 44.7 | 64.1 | | | | | | |
| B37 | 73.4 | 73.2 | 72.5 | 73.9 | 74.7 | 71.4 | 50.3 | 74.5 | 64.2 | | | | | |
| L169 | 77.9 | 77.6 | 77.1 | 75.1 | 79.0 | 77.0 | 50.0 | 74.9 | 66.2 | 75.7 | | | | |
| Mc35 | 74.6 | 74.4 | 74.5 | 73.7 | 76.8 | 74.4 | 47.7 | 74.3 | 64.3 | 75.8 | 77.3 | | | |
| YM | 74.9 | 74.5 | 74.4 | 76.0 | 77.7 | 75.9 | 46.9 | 74.5 | 63.7 | 76.1 | 78.7 | 74.8 | | |
| L26 | 90.7 | 90.3 | 75.1 | 73.8 | 77.1 | 74.2 | 49.6 | 73.5 | 66.3 | 73.5 | 76.8 | 72.9 | 74.2 | |
| L338 | 75.7 | 75.7 | 74.1 | 73.4 | 77.6 | 73.4 | 48.7 | 73.9 | 63.8 | 73.4 | 76.2 | 74.0 | 74.4 | 75.2 |

ularly significant because this capital city has a very high density of inhabitants. Approximately 10 million people reside here—nearly a quarter of the entire population of South Korea. In this study, fecal samples from children with acute infectious diarrhea were collected and tested in the same hospitals in Seoul as those participating in a previous surveillance study conducted between 1998 and 2000 [Song et al., 2003].

Four major G serotypes (G1–4) have been documented worldwide [Beards et al., 1989; Santos and Hoshino, 2005] and until 1996, G1 appeared to be the most prevalent strain followed by strains G4, G2, and G3 [Gentsch et al., 1996]. A similar trend in the prevalence of G serotype strains is evident from studies conducted over the past 10 years in South Korea. Until 1997, G1 was also the most prevalent strain (45–81%) regardless of geographical area or season [Kim et al., 1990, 1999; Kim, 1993; Seo and Sim, 2000]. Since then, the predominant G type strain became G4 (28.0–40.9%) [Kim et al., 2002; Song et al., 2003; Kang et al., 2005], then G2 (40.9–50.6%) [Min et al., 2004; Moon et al., 2007] and more recently, G9 (39%) [Kim et al., 2005].

Study data from this 2-year investigation indicates that the G1 strain (35.6%) is again, the most prevalent serotype replacing the G4 (40.5–40.9%) strain that was dominant in the previous Seoul survey [Kim et al., 2002; Song et al., 2003]. The prevalence of G1 is slightly higher than that currently recorded in other nation-wide studies, 30.1% [Min et al., 2004] and 18% [Kang et al., 2005], and in other rural surveys, 25% [Kim et al., 2005] and 27.8% [Moon et al., 2007].

Minor G serotypes documented as common in developing countries include G5, G8, G9, G10, and G12 [Pietruchinski et al., 2006; Duan et al., 2007; Gulati et al., 2007; Wang et al., 2007; Steyer et al., 2007b] and in several countries around the world, the G9 serotype is most prevalent [Kostouros et al., 2003; Steele and Ivanoff, 2003; Clark et al., 2004; Linhares et al., 2006; Van Damme et al., 2007]. In this study, the G9 prevalence rate of 1.0% is much lower than that reported for some Asian countries: 54.8–91.6% in Thailand [Jiraphongsa et al., 2005; Khamrin et al., 2006], 78.3% in Malaysia [Hung et al., 2006], 24.1% in Taiwan [Chen et al., 2007], and 19.1% in South India [Banerjee et al., 2006]. This 1.0% prevalence rate is, however, similar to the rates found in Vietnam, 0.5% [Doan et al., 2003], in China, 0.9–4.0% [Fang et al., 2002, 2005], in Japan, 1.0–5.9% [Zhou et al., 2003; Yoshinaga et al., 2006], in eastern India, 2.1% [Samajdar et al., 2006], and in Hong Kong China, 5.1% [Lo et al., 2005]. This difference in prevalence may be accounted for by factors such as study population demographics, periods of the study, seasonal initiation times, and analytical methods. Concerning the latter, it has been observed that some G9 strains can be mistyped as G3 [Santos et al., 2003] owing to the type-specific primers used [Gouvea et al., 1990]. Therefore, all study samples identified as G3 and G4 strains in this investigation, were confirmed as these serotypes using an alternative set of G-typing primers [Das et al., 1994].

Table III presents the prevalence of G9 strains in South Korea. Although G9 was not detected until 2002 [Song et al., 2003; Min et al., 2004; Moon et al., 2007], its prevalence, especially in rural provinces, has increased from 11% to 39% [Kang et al., 2005; Kim et al., 2005]. In urban areas, however, G9 occurs with far lower prevalence, 1.2% [Kim et al., 2002; Song et al., 2003; Min et al., 2004; Kang et al., 2006; Moon et al., 2007], a finding consistent with the observation in this study, that G9 strains were found in only four samples from Seoul (1.0%). One possible explanation for the higher prevalence in small rural areas, 39% in the Jeongeub District [Kim et al., 2005], may be the occurrence of outbreaks of infection. Alternatively, geographical area and season may play a role.

To date, several reports have been made on the emergence of rare G type strains, G8 and G12. Since rotavirus G8 was first detected in Indonesian children [Matsuno et al., 1985], it has been found sporadically all over the world. Its appearance in the human population is thought to be a possible consequence of bovine-human rotavirus genome re-assortment [Browning et al., 1992; Adah et al., 2003; Matthijnssens et al., 2006; Steyer et al., 2007b]. Combinations of G8 with P[4] and P[6] are most frequently reported [Cunliffe et al., 2000; Adah et al., 2001; Steele et al., 2002; Fischer et al., 2003] and in this South Korean study, one strain, G8[P8] was identified for the first time.

G12 rotaviruses were first detected in 1987 among children with diarrhea from the Philippines [Taniguchi et al., 1990], but no further cases were reported until 1998. Nonetheless, G12 rotaviruses have spread globally and have been detected in the United States [Griffin et al., 2002], Thailand [Pongsuwanna et al., 2002], India [Das et al., 2003], Japan [Shinozaki et al., 2004], Italy [Grassi et al., 2006], Argentina [Castello et al., 2006], Brazil [Pietruchinski et al., 2006], Nepal [Uchida et al., 2006], Slovenia [Steyer et al., 2007b], Hungary [Banyai,

TABLE III. Prevalence of G9 Serotypes in South Korea

| Authors | Year Published | Period of sample collection | No. of G9/no. of total samples | Percentage (%) | Province |
|---|---|---|---|---|---|
| Kim et al. | 2002 | 2001–2002 | 0/89 | 0 | Seoul/Urban |
| Song et al. | 2003 | 1999–2000 | 0/205 | 0 | Seoul/Urban |
| Min et al. | 2004 | 2000–2001 | 1/322 | 0.3 | 6 large cities/Urban |
| Kim et al. | 2005 | 2002–2003 | 79/203 | 38.9 | Jeongeub District/Rural |
| Kang et al. | 2005 | 2002–2003 | 49/461 | 11.0 | Urban and Rural |
| Kang et al. | 2006 | 2005–2006 | 1/81 | 1.2 | Cheju/Urban |
| Moon et al. | 2007 | 1999–2002 | 0/115 | 0 | Seoul suburb/Urban |

2007], and Bangladesh [Rahman et al., 2007b]. A striking increase in the prevalence of G12 from 4.2% (2003) to 30% (2005) was reported in India [Samajdar et al., 2006], mirroring the rise of G9 in the late 1990s [Iturriza-Gomara et al., 2000]. As a result, G12 is now the sixth most important genotype in the world [Rahman et al., 2007a].

In this study, the phylogenetic analysis of nucleotide sequences from two G12 isolates, CAU 195 and CAU 214 showed that they clustered tightly with the G12 serotype with similarities in excess of 90%. This article is the first to report on the comprehensive validation of G12 strains in South Korea, an analysis that included relevant type strains from the NCBI GenBank. Although an earlier report exists of a sample suspected of being G12 [Kang et al., 2005], the data from this report are inconclusive because the G12 serotype cannot be detected using the multiplex RT-PCR employed [Santos et al., 2003]. Furthermore, there is no evidence of sequence analysis, or of an immunological assay that uses G12-specific antibody. A description of four other G12 strains has been presented [Santos and Hoshino, 2005] but evidence related to P-specificity status is lacking and sequence information on the *VP7* gene is not available on public databases. The findings discussed here, provide important information concerning the spread of G8 and G12 type rotavirus and they may either represent the early emergence of extant Korean strains or be isolates newly introduced to South Korea from abroad.

The prevalence of mixed infections (G type, 2.5%; P type, 6.6%) was similar to reports from the developed countries [Gentsch et al., 1996; Griffin et al., 2000; Iturriza-Gomara et al., 2000] but was lower than the rates seen in developing countries such as India, Bangladesh and Brazil [Timenetsky et al., 1994; Unicomb et al., 1999; Jain et al., 2001]. A global rotavirus surveillance study indicated that the G/P combinations most frequently reported in humans were G1P[8], G3P[8], G4P[8], G2P[4], G9P[8], and G9P[6] [Santos and Hoshino, 2005]. Recent Korean studies uncovered a temporal change in the most common G type combinations of rotavirus that is dependent on season and geography. The dominant combination shifts from G1P[8] (25.4%) to G4P[8] (7.8%; 1999–2000) [Song et al., 2003] to G2P[4] (52.4; 1999–2002) [Moon et al., 2007], (45.7%; 2000–2001) [Min et al., 2004], (28.1%; 2001–2002) [Kim et al., 2002] to G4P[6] (27%; 2002–2003) [Kang et al., 2005], and then, G9P[8] (39%; 2002–2004) [Kim et al., 2005]. In this Seoul study, 210 (53.3%) samples comprised these common G/P combinations and the G1P[8] combination (25.4%) was dominant.

Although G4P[6] is considered to be rare globally, P[6] strains combined with G1, G2, G3, or G4 have been reported as a cause of outbreaks of nosocomial rotavirus infection in hospitals and newborn nurseries [Steele et al., 1995; Kilgore et al., 1996; Lee et al., 2001; Linhares et al., 2002]. The implication is that the prevalence of formerly common G/P combinations is falling in South Korea while that of uncommon G/P combinations is on the increase. Of note, is the first appearance in South Korea of rare combinations that include G8P[8] (0.3%, n = 1), G12P[6] (0.5%, n = 2), and G2P[9] (0.8%, n = 3).

In this study, co-infections with two different G types and a single P type (5.6%, n = 22), or with two different P types and a single G type (1.8%, n = 7) were detected but their occurrence was limited. In addition, the finding that 28 samples (7.1%) that were negative upon full-length *VP7* gene amplification but positive with *VP4* gene-specific primers was unexpected. Similarly, the 29 samples (7.4%) that were negative upon full-length *VP4* gene amplification but positive with *VP7* gene-specific primers were unusual. Further investigation of these findings is needed to confirm whether the rotavirus is undergoing genetic diversification.

The data from this study demonstrate that in Seoul, South Korea, there is a high level of diversity among G- and P type rotavirus, and that the prevalence of well-established or newly, emerging serotypes such as G8P[8] and G12P[6] changes relatively quickly. More surveillance studies are vital for amassing information on the molecular epidemiology of unusual serotypes, and the further characterization of G8 and G12 rotavirus is essential. Only by collating large volumes of stringent data, can an efficient, evidence-based program for vaccine development be progressed.

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