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부산지역에서 유행하는 Rotavirus 유전자형 분포에 관한 연구

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미생물과

Distribution of the Rotavirus Genotypes Prevalent in Busan Area

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Abstract

The distribution of the prevalent human rotavirus genotypes was determined by analyses of 224 rotavirus isolates from diarrheal patients during January 2005 to October 2006 at the local 7 hospitals in Busan. The analyses were performed with the use of reverse transcriptase polymerase chain reaction (RT-PCR) and multiplex polymerase chain reaction. Gastroenteritis caused by rotavirus was most prevalent in children less than 5 years of age (83.5%) and in male (mail : female, 1.47 to 1.55: 1). During the 2-year period, the epidemiological trends of the rotavirus infection by month showed that the rotavirus season began in December-January, and ended in April-May. Genotypes of G2 (30.4%) and G1 (29.5%) were predominant, followed by G3 (15.6%), G4 (10.3%), and G9 (3.6%). The G8 genotype was found in only G1G8 mixed infection (2.5%). The P[4] was the most prevalent genotype (33.0%), followed by P[8] (26.8%), P[6] (14.7%), P[9] (3.1%), and P[10] (1.8%).

We found significant variability in the prevalence of different G and P types of rotavirus by year. Predominant G genotypes were shifted G2 in 2005 to G1 in 2006. Predominant P genotypes did not be changed but their distribution differed by year. We also found that uncommon genotypes were highly detected among prevalent strains (25%). The incidence of G-P type combinations was as followed: P[4]G2 (22%), P[8]G1 (13.4%), P[8]G3 (10.3%), and P[6]G4 (6.7%). But P[8]G4, the common worldwide strains, was not detected. The low incidence of the novel types (P[4]G1, P[4]G4, P[6]G2, P[6]G4, and P[6]G1) and mixed infections designated the extraordinary diversity of rotaviruses circulating in Busan.

Key Words : rotavirus, genotype, RT-PCR, muitiplex PCR

INTRODUCTION

Diarrhea, especially acute diarrhea, remains a major public health problem in the world. In developing countries, approximately 4.6 million pediatric deaths annually, about 25% to 30% of all deaths among children less than age 5 years, can be attributed to acute diarrhea[1,2]. Acute diarrhea also contributes considerably to morbidity and medical expenses in developed countries. In the United States, approximately 16.5 million children under 5 years of age develop 1.3 to 2.3 diarrheal episodes per year. This accounts for up to $ 1 billion of direct and indirect expenses[3,4].

Many different agents including viruses, bacteria, and parasites, of which viruses have been intensively studied in recent years, can cause acute diarrhea. The most notable viral agents causing acute diarrhea are rotaviruses, adenoviruses, astroviruses, and noroviruses[5]. Rotaviruses are the leading cause of infantile gastroenteritis worldwide and are responsible for approximately 20% of diarrhea-associated deaths in children under 5 years of age[2].

Bishop *et al.*[6] first identified rotaviruses in humans in 1973 when they observed characteristic particles in the cytoplasm of duodenal epithelial cells from young children admitted to the hospital for treatment for acute diarrhea by use of electron microscopy.

Rotaviruses, members of the Reoviridae family, are triple-layered icosahedral particles, and characterized

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by their segmented (11 segments), double-stranded RNA genomes. Each segment codes for one protein, with the exception of segment 11 which codes for two proteins. Out of a total of 12 proteins, six proteins are structural proteins and six are non-structural proteins. The outer capsid layer is made by VP7 and VP4; their proteins are associated with attachment and entry of virus into the cells. The intermediate layer is composed of VP6, which distinguishes seven serogroups specific antigens A to G. However, only groups A to C have been shown to infect humans and most animals, with rotavirus disease mainly being caused by group A[7,12,14]. Group B caused outbreaks of severe diarrhea among adults in China and India, and the viruses are therefore known as adults diarrhea rotaviruses. During 2000-2001, group B rotavirus caused severe diarrhea among adults and children in Bangladesh[10]. Group C rotaviruses have been shown to cause sporadic diarrhea outbreaks. In Japan, outbreaks of diarrhea caused by group C rotavirus were reported during april to june almost every year[11].

Rotaviruses are classified according to the genetic and antigenic diversity of the two outer capsid proteins VP4 and VP7, carrying distinct epitopes. These proteins independently induce type-specific neutralizing antibodiese and form the basis of the present dual classification of group A rotaviruses into 20 P subtypes (P stands for protease-sensitive protein) and 14 G subtypes (G stands for glycoprotein), respectively[8,12,13,14]. As the VP4 genes and VP7 genes segregated independently, various combinations P and G types have been observed in natural isolates[15].

For G types, serotypes have been fully coincident with genotype, and RT-PCR for G serogroup classification is conducted by analyzing virus RNA. But all P genotypes have not yet been confirmed with P serotypes[16].

The relative incidence and distribution of rotavirus types varies according to geographical areas during a rotavirus season and from one season to the next season in one area.

Global reviews of the main P and G type combinations encountered in human infections have identified P1A[8]G1(where P1A is the serotype designation and P[8] is the genotypic designation), P1B[4]G2, P1A[8]G3, and P1A[8]G4 as the most frequent genotypes[17], however, since the introduction and wide use of molecular biology-based typing methods about 10 years ago, other G types have increasingly been reported in different parts of world, such as G5 (found in pigs and horses) in Brazil[18], G8 (found in cattle) in various parts of Africa[19,20,21], and G10 (found in cattle) in India[22]. Since 1995, the G9 strain (found in pigs) has infected humans in most continents[23], suggesting a possible emergences of a fifth common G type worldwide. Some of these serotypes are more commonly found in animals, so their emergence in humans is seen as evidence for inter-species transmission of rotaviruses. Unusual combinations of VP7 and VP4 suggest that reassortment between common and less common genotypes or between human and animal viruses enhances the diversity of circulating strains[18].

Public health interventions aimed at improving water, food and sanitation are unlikely adequately to control the rotavirus disease[52]. Vaccination is the current strategy for control and prevention of severe rotavirus infections. The medical, social, and economic burdens associated with rotavirus disease make it imperative to develop and implement a safe and effective rotavirus vaccine. Studies with vaccines against group A rotavirus began in 1982. The first vaccine developed was the tetravalent human-rhesus reassortant vaccine, which induces protection against the four main rotavirus serotypes, G1-G4. Efficacy studies of the first vaccine showed a reduction in the appearance of severe gastroenteritis caused by rotavirus of between 69% and 91% in vaccinated children[24]. The vaccine was licensed by the US Food and Drug Administration in August, 1998, and was immediately recommended for all children in the USA25). However, the detection of an increase in the risk of intussusception after vaccination led to its suspension[26,27]. At present, plasmic DNA (DNA vaccine), antigenic vaccines, which code for specific viral proteins and virus-like particles produced by genetic engineering are being investigated[28]. The next generation of rotavirus vaccine may incorporate a VP4 component in the formulation, because knowledge of the genetic and antigenic diversity of epidemiologically important VP4s may be important[54].

Accelerated development and introduction of new generation of rotavirus vaccines into global

immunization programs has been a high priority for many international agencies, including WHO and the Global Alliance for Vaccines and Immunizations. Vaccines have been developed that could prevent the enormous morbidity and mortality from rotavirus and their effect should be measurable within 2-3 years[66].

For development of a safe and highly effective vaccine against rotavirus, appropriate rotavirus strain surveillance in a community is essential before the introduction of a vaccine, during a vaccine campaign, and at follow-up to monitor the prevalence of the different G and P types circulating in an area and to detect uncommon and novel types which might help explain vaccine failure. Because serotype-specific protection appears to play an important role against rotavirus disease[8,9,14], an important issue to be addressed in future trails is the level of protection provided against severe diarrheal disease in countries with high prevalences of uncommom strains and mixed infection.

Rotavirus vaccines are now expected to be widely used globally, within a couple of years. Informations are urgently required about the genotype distribution of rotaviruses isolated in Korean children, and clinical characteristics of rotavirus infection before introduction of licensed rotavirus vaccine in Korea. There are few data available for rotavirus types circulating in Korea, especially in Busan area. So this study designed to monitor the antigenic variation of rotaviruses and describe the frequency and temporal distribution of human group A rotavirus types among children admitted to seven hospitals located in Busan, during a 2-year period.

MATERIALS AND METHODS

Specimens

Fecal specimens from patients with history of watery diarrhea were included in this study. The specimens were collected during the period between January 2005 and October 2006 at the 7 hospitals in Busan, Korea. Each sample was 10-fold diluted in 0.2 M phosphate-buffered saline (pH 7.4) with shaking vigorously by vortex for 5-10 min and clarified by centrifugation for 20 min at 3,000 rpm in tabletop centrifuge. 10% viral suspension was stored at 4℃ until processing and store them into -70℃ or below for backup.

Enzyme Immuno-Assay

10% fecal suspension were tested for group A rotavirus antigens by an enzyme immuno-assay (EIA) with Amplified IDEATM Rotavirus (DakoCytomation Ltd., Denmark House, Angel Drove, Cambridgeshire, UK) and Viro-Capture™ Rotavirus (Bioincell Houston, TX, USA) kit.

Isolation of viral ds RNA

RNA was extracted from rotavirus positive fecal suspension with a Trizol reagent (Invitrogen, Carlsbad, CA) as described below. For experiment 200 μL of the supernatant of fecal suspension was mixed with 600 μL Trizol reagent, and the sample was vortexed well for 20 sec, and incubated for 10 min at room temperature. Spin downed the sample for 5 sec and add 200 μL chloroform (sigma C-2432) to completely mix supernatant and Trizol reagent and chloroform, and stand 10~20 min. at room temperature. Each sample was then centrifuged at 4℃, 11,000 rpm for 10 min. The supernatant (about 500 μL) transfered to new microcentrifuge tube and add 550 μL isopropyl alcohol (sigma I-9516) and shake vigorously by vortex. The mixture stored at -20℃ overnight or 4℃ for 6 hour and the sample were centrifuged at 4℃, 14,000 rpm for 30 min, and the supernatant was removed by aspiration with separate filtered micropipette for each sample. The pellets were washed with 800 μL cold 70% ethanol and centrifuged at 4℃, 14,000 rpm for 15 min. The pellets were air dried for 10 min, resuspended in 20 μL DMSO (dimethyl sulfoxide) treated DEPC (diethyl pyrocarbonate) distilled water for rotavirus and stored at -20℃ until they were used.

Primers

RT-PCR primers were selected on the basis of sequence data of gene 4, gene 9, which are highly conserved among all strains. Genotype-specific primers were synthesized, complementary to RNA strand by using each of variable regions as the blueprint for a distinct genotype. The sequences of primers and their location are shown in Table 1 and Fig. 1.

Table 1. Sequences of oligonucleotide primers used for rotavirus gene amplification and genotyping

Primer	Sequence (5'→3')	Detection	nt Position	Amplicon (bp)	Reference
G-typing(a)					
Full-length VP7 gene amplification					
Beg9	GGC TTT AAA AGA GAG AAT TTC CGT CTG G		1~28	1062	1~28
End9	GGT CAC ATC ATA CAA TTC TAA TCT AAG		1062~1036		1062~1036
G-typing PCR					
aBT1	CAA GTA CTC AAA TCA ATG AGG	G1	314~335	749	
aCT2	CAA TGA TAT TAA CAC ATT TTC TGT G	G2	411~435	652	Gouvea et
aET3	CGT TTG AAG AAG TTG CAA CAG	G3	689~709	374	al. (1990)
aDT4	CGT TTC TGG TGA GGA GTT G	G4	480~498	583	
aAT8	GTC ACA CCA TTT GTA AAT TCG	G8	178~198	885	
aFT9	CTA GAT GTA ACT ACA ACT AC	G9	757~776	306	
RVG9	GGT CAC ATC ATA CAA TTC T		1062~1044		
P-typing(b)					
VP4 gene amplification					
Con-3	TGG CTT CGC CAT TTT ATA GAC A		11~32	876	
Con-2	ATT CGG ACC ATT TAT AAC C		887~868		
P-typing PCR					
1T-1	TCT ACT TGG ATA ACG TGC	P[8]	356~339	345	Gentsch et
2T-1	CTA TTG TTA GAG GTT AGA GTC	P[4]	494~474	483	al. (1992)
3T-1	TGT TGA TTA GTT GGA TTC AA	P[6]	278~259	267	
4T-1	TGA GAC ATG CAA TTG GAC	P[9]	402~385	391	
5T-1	ATC ATA GTT AGT AGT CGG	P[10]	594~575	583	
Con-3	As above				

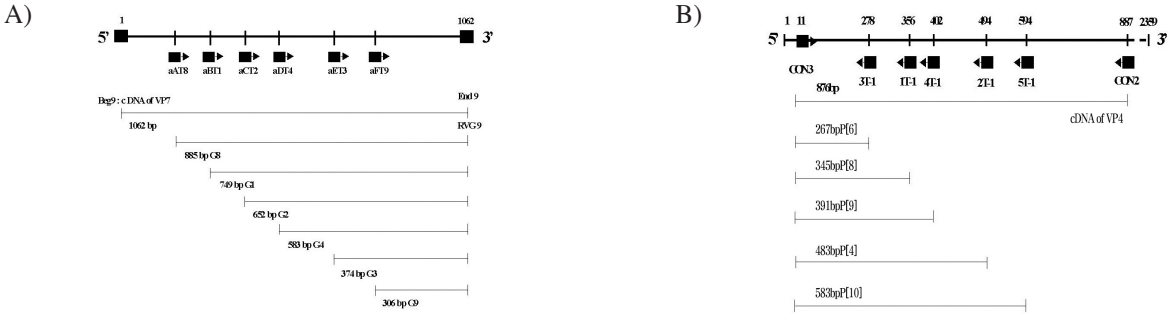

Fig. 1. (A) VP7 PCR typing of human rotavirus, with the positions and directions of amplification relative to those for the plus (mRNA) sense genomic strand for the consensus primers Beg9 and End9 and for the gene 9 type-specific primers aAT8, aBT1, aCT2, aDT4, aET3, and aFT9 shown. The sizes of the expected products of amplification from Beg9, End9 (first amplification), and RVG9 plus aAT8, aBT1, aCT2, aDT4, aET3, and aFT9 (second amplification, gene 9 typing PCR) are also shown. (B) VP4 PCR typing of human rotavirus, with the positions and directions of amplification relative to those for the plus (mRNA) sense genomic strand for the consensus primers Con3 and Con2 and for the gene 4 type-specific primers 1T-1 to 5T-1. The sizes of the expected products of amplification from Con3, Con2 (first amplification), and Con3 plus 1T-1 through 5T-1 (second amplification, gene 4 typing PCR) are also shown.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Portions of rotavirus dsRNA were used as templates for reverse transcriptase to synthesize cDNA copies from both viral strands. And then cDNAs were amplified by the PCR. Immediately before use for reverse transcription, the suspended RNA were predenaturated at 95℃ for 5 min and stored on ice for 2 min. The 10 μL denaturated RNA was added to the reaction mixture consisting of 8 μL of RNase-free

distilled water, 2.5 μL of 5 RT M-MLV buffer, 3 μL of 2.5mM dNTP(dATP, dGTP, dCTP, and dTTP), 0.5 μL of reverse and forward primer(Con2 and Con3 for VP4, Beg9 and End9 for VP7), and 0.5 μL of 200 U/μL M-MLV RT (reverse transcriptase, Promega M1701, Madison, USA). The reverse transcriptase-polymerase chain reaction was carried out with an initial reverse transcription step at 20℃ 10 min, 42℃ 60 min, followed by 95℃ 5 min.

VP7 (G) Genotyping by Polymerase Chain Reaction (PCR)

PCR conducted for VP7 (G) genotyping was undertaken in two steps based on the system employed by Gouvea et al.(1990)[29]. First VP7 full-length gene from dsRNA genome was amplified with Beg9 and End9 primers. Each samples were preincubated in a thermal cycler(ABI 2720) at 94℃ for 2 min, and amplified for 35 cycles of 1 min at 94 ℃, 1 min at 52℃, 1 min at 72℃, followed by a final incubation 7 min at 72℃. The second PCR was performed using first product as a template with pooled genotype-specific primers (aBT1, aCT2, aET3, aDT4, aAT8, and aFT9) and RVG9. For genotyping multiplex PCR, each samples were preincubated in a thermal cycler at 94℃ for 4 min, and amplified for 30 cycles of 1 min at 94℃, 2 min at 42℃, 1 min at 72℃, followed by a final incubation 7 min at 72 . Both the first and second PCR products were electrophoresed in 1.5% agarose gel (Takara LO3, Japan) with ethidium bromide (Bioneer C-9009) staining. When a mixed infection was recognized, the second PCR was repeated with specific primer known for each genotype and RVG9 for confirmation.

VP4 (P) Genotyping by Polymerase Chain Reaction (PCR)

PCR conducted for VP4 (P) genotyping was undertaken in two steps based on the system employed by Gentsch et al(1992)[30]. First VP4 partial gene from dsRNA genome was amplified with Con2 and Con3 primers. Amplication was carried out the following profile: 94℃ for 2 min, 35 cycles of 94℃ for 1 min, 50℃ for 1min, 72℃ for 1min, followed by a final extension 7 min at 72℃. The second PCR was performed using first product as a template with pooled genotype-specific primers(1T-1, 2T-1, 3T-1, 4T-1, and 5T-1) and Con3. For genotyping multiplex PCR, each samples were preincubated in a thermal cycler at 94℃ for 4 min, and amplified for 30 cycles of 1 min at 94℃, 2 min at 45℃, 1 min at 72℃, followed by a final incubation 7 min at 72℃. Both the first and second PCR products were electrophoresed in 1.5% agarose gel(Takara LO3, Japan) with ethidium bromide(Bioneer C-9009) staining. When a mixed infection was recognized, the second PCR was repeated with specific primer known for each serotype and Con3 for confirmation.

RESULTS AND DISCUSSIONS

Incidence, Age & Sex distribution and Peak seasons of Rotavirus infection

In this study, 224 rotavirus-positive samples collected from 1,970 diarrheal stool samples during a 2-year period (January 2005 to October 2006) at the local 7 hospitals in Busan. Samples were collected from inpatients and outpatients diagnosed with gastroenteritis and were positive for rotavirus by antigen detection (enzyme immuno-assay[EIA]). The age distribution of rotavirus gastroenteritis was most prevalent among children less than 5 years of age (83.5%), and especially 74.6% of all cases was identified in children between 0 to 24 months of age. In this Study, the results showed that the number of cases were higher than female cases (Table 2). During the 2-year period, the epidemiological trends of the rotavirus infection showed that the rotavirus season began in December-January, and ended in April-May

Table 2. Age & sex distribution of cases of rotavirus gastroenteritis detected in Busan, during 2005-2006

Sex	Age						
	Total	<30days	1~12months	13~24months	25~36months	37~60months	60months>
Male	134	31	50	21	6	5	21
Female	90	12	34	22	8	1	13

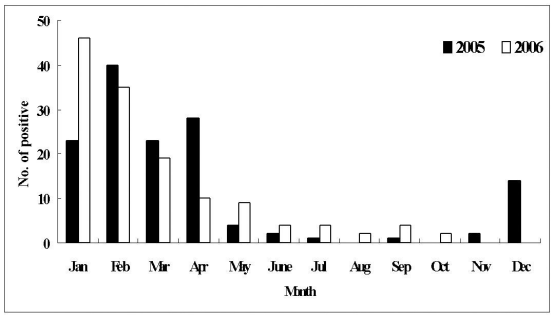

Fig. 2. Seasonal distribution of cases of rotavirus gastroenteritis in Busan, during 2005-2006.

(Fig. 2). After rotavirus was identified as a major cause of nationwide outbreaks in Korea, outbreaks of the documented rotavirus gastroenteritis were founded to occur October, November and December (late autumn and early winter in Korea) in the early 1990s[31,32,33,34,35,36]. Over the recent years, annual epidemic peaks have been gradually delayed[37,39,50,51]. Our study also revealed that the epidemic peaks occured at January-April. The autumn epidemic of the rotavirus has declined and the peak season for rotavirus has been moving toward late winter and early spring in Korea.

This findings are similar to other studies that rotavirus was most prevalent viral agent of acute diarrhea among younger children aged 6-24 months (84% of all cases), particularly in the winter season, and most of the papers published in Korea show a male predominance; male: female, 1.5: 1[33,34,35,39].

Distribution of G typing of Rotavirus Strains

G genotypes were successfully determined for 210 of the 224 (93.7%) rotavirus positive samples: the VP7 gene of 14 strains (6.3%) could not be amplified. The distribution of G types detected in this study was shown in Table 3. In Fig. 2, A) showed the patterns of the amplified G types obtained in agarose gels in different clinical samples, compared to molecular weigh markers.

Genotypes G2 (30.4%) and G1 (29.5%) were predominant, followed by G3 (15.6%), G4 (10.3%), and G9 (3.6%). But the G8 genotype was detected only G1G8 mixed infection (2.5%). In total, 14 strains (6.3%) remained untypeable. Information derived from multiple studies in various regions of the world indicates that genoype G1 (61%) was the most prevalent type, while genotype G2, G3, and G4 were evenly distributed (10-16%)[40].

Although in most years and regions, G1 was the predominant serotype, considerable G-type fluctuations were found from year to year. For example, G4 represented 41% of all rotavirus specimens isolated in Seoul, between 1998 and 2000[38] and G1 was predominant genotype (46%) in 2000[39]. G2 was the predominant genotype in Seoul[55] and Kyoungsangnamdo, during 2000-2001[47] and in Busan, 2005. In this study, 85.7% of rotavirus isolates belonged to the common genotype G1, G2, G3, or G4. However, dominant G type has shifted from G2 to G1 year by year. Mixed infections that might be permissive of reassortment were found in 10 samples (4.5%). This is higher compared to other countries: 0.6-3.5% in Japan[41], 3% in China[42].

Three cases of G9 were found in 2005. G9 strain that was documented first in India was common in developing countries and then continually reported in several other countries around the world, including Asian countries such as Japan and Thailand[42,43]. Reports along with Japan from 1996 to 2000 showed that the G9 serotype virus spreaded quickly. In India, the importance of high frequency of G9 strains became clear, vaccine developers initiated steps to produce a vaccine with the G9 specificity. The response to the apparent emergence of new strains is an example of how rotavirus surveillance data can guide vaccine development[44].

Table 3. G genotyping results from cases of rotavirus gastroenteritis during 2005~2006 in Busan

Year	No. of samples (%)										
	G1	G2	G3	G4	G9	G1/G2	G1/G3	G1/G4	G1/G8	Untypeable	Total
2005	27(26.2)	38(36.9)	18(17.5)	5(4.9)	6(5.8)	2(1.9)	-	1(1.0)	-	6(5.8)	103(100)
2006	39(32.2)	30(24.8)	17(14.0)	18(14.9)	2(1.7)	-	4(3.3)	-	3(2.5)	8(6.6)	121(100)
Total	66(29.5)	68(30.4)	35(15.6)	23(10.3)	8(3.6)	2(0.9)	4(1.8)	1(0.4)	3(1.3)	14(6.3)	224(100)

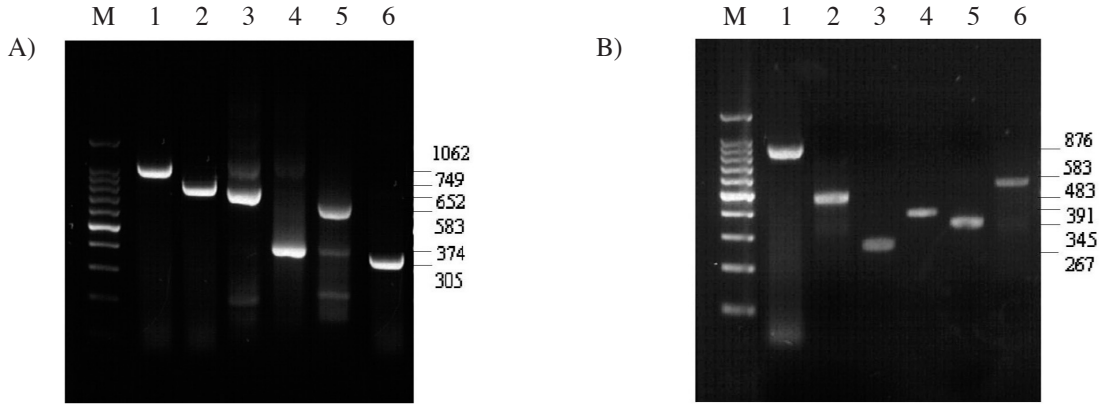

Fig. 3 Agarose gel electrophoresis of human group A Rotavirus VP7 and VP4 gene by multiplex PCR (A) Amplification products of the VP7 genes in stool samples for G typing, lane: M, molecular weigh markers (100bp ladder); lane 1, VP7 full-length gene (1062bp); lane 2, G1 (749bp); lane 3, G2 (652bp); lane 4, G3 (374bp); lane 5, G4 (583bp); lane 6, G9 (305bp) (B) Amplification product of the VP4 genes in stool samples for P typing, M, molecular weigh markers (100bp ladder); lane 1, VP4 partial gene (876bp); lane2, P[4] (483bp); lane 3, P[6] (267bp); lane 4, P[9] (391bp); lane 5, P[8] (345bp); lane 6, P[10] (583bp).

Distribution of P typing of Rotavirus Strains

P genotypes were determined for 190 of the 224 (84.8%) rotavirus positive samples which showed high diversity. Five P genotypes (P[8], P[4], P[6], P[9], and P[10]) were identified, with P[8] and P[4] being the most common and accounting for 60% of strains, but distribution rate are smaller than that have seen worldwide (83.5%). 34 samples (15.2%) were untypeable by use of genotype-specific primers to five human rotavirus genotypes, suggesting that other P genotypes might be present or that variants of known genotypes could not be amplified with these primers. The distribution of P types detected is shown in Table 4. In Fig. 2, B) showed the patterns of the amplified P types obtained in agarose gels, compared to molecular weigh markers.

Overall, P[4] was the most prevalent genotype (33.0%), followed by P[8] (26.8%), P[6] (14.7%), P[9] (3.1%), and P[10] (1.8%). These distributions were differed year by year. P[4] types were more decreased in 2006 than 2005, but P[6] types are increased in 2006. Although genotype P[6] strains were originally isolated from neonates with no symptoms of diarrhea, in recent years they were identified to be virulent and common causes of gastroenteritis globally[4,9].

In 12 mixed infections, eleven P[4]/[6] types (9.1%) were detected in 2006, but one P[4]/[8] type was detected in 2005. There are several explanations for mixed infections: some are related to the primers, such as interprimer suppression or competition during the standard multiplex PCR approach, and/or silent mutation of primer binding site, others are double infection[21]. These results need further confirmation since they probably represent coinfections with two rotavirus strains. Four of P[10], that is uncommon strain, were detectd in 2006.

Distribution of Human Group A Rotavirus G and P type Combinations

Various combinations of G and P genotypes were observed. The incidence of G-P type combinations was as follows: P[4]G2 (22%), P[8]G1 (13.4%), P[8]G3

Table 4. P Genotyping results from cases of rotavirus gastroenteritis during 2005~2006 in Busan

Year	No. of samples (%)								
	P[4]	P[6]	P[8]	P[9]	P[10]	P[4]/[6]	P[4]/[8]	Untypeable	Total
2005	43(41.7)	11(10.7)	30(29.1)	2(1.9)	-	-	1(1.0)	16(15.5)	103(100)
2006	31(25.6)	22(18.2)	30(24.8)	5(4.1)	4(3.3)	11(9.1)		-18(14.9)	121(100)
Total	74(33.0)	33(14.7)	60(26.8)	7(3.1)	4(1.8)	11(4.9)	1(0.4)	34(15.2)	224(100

(10.3%), and P[6]G4 (6.7%). Three types (P[4]G2, P[8]G1, and P[8]G3) of the four common worldwide strains G-P type combinations accounted for the majority (46.0%) of the all typeable strains, but P[8]G4 was not detected (Table 5). The predominant genotype P[4]G2 was similar results to seoul in 2001 and 2002[49], Kysangnamdo in 2000 and 2001[49]. Even though the others showed different and a significant genotype shifts was not observed[48,51]. The detected uncommon strains (P[4]G1, P[4]G4, P[6]G1, P[6]G2, P[6]G4 and P[8]G9) have been reported in high frequency in India[44] and Brazil[18].

However, when G and P combinations were examined, the diversity of strains was much greater: P[8]G1 was the most common G1 strain (45.2%) but P[6]G1 (13.5%), P[4]G1 (10.5%), and P[9]G1 (3.0%) were also present. Similarly, genotype P[4]G2 was the most common G2 strain (72.9%), followed by P[6]G2 (5.8%) and P[10]G2 (2.9%). P[8]G3 was the most common G3 strain (68.1%), followed by P[9]G3 (5.7%), P[6]G3 (2.8%), and P[10]G3 (2.8%). P[6]G4 was the most common G4 strain (64.8%) which virulent strain caused an outbreak of neonatal diarrhea in an obstetric ward at a hospital in Beijing in November 1998[42]. P[4]G4 (4.1%), was also present in G4 strain. Of G9 strains, four were P[8]G9 and one was P[9]G9, and three were untypeable.

In our study, P[8] strains were mainly in combination with G1 (50.8%), G3 (40%), G9 (6.8%) and accounted for 26.3% of all P-typed specimens. P[4] strains were mainly in combination with G2 (67.6%), G1 (9.5%), G4 (9.5%), mixed types (2.2%), untypeable (2.2%) and accounted for 33% of all P-typed strains. P[6] strains were identified in combination with G4 (46.9%), G1 (28.2%), G2 (12.5%), and G3 (3.1%) genotypes and accounted for 14.3% of all P-typed specimens. Overall, Many different G-P combinations were identified. The three common worldwide strains accounted for 46.0% of the total, but the presence of uncommon strains, including the novel types P[4]G1, P[4]G4, P[6]G2, P[6]G4, and P[6]G1, and mixed infections highlighted the extraordinary diversity of rotaviruses circulating in Busan. Unusual G-P combinations could emerge as the result of mixed rotavirus infections. The impact of the those uncommon strains on rotavirus evolution is yet to be determined, however, such strains could provide the opportunity for introduction of novel P or G genes into human population via reassortment events[53]. Some of the uncommon G-P combinations such as P[4]G1 and P[4]G4 have been detected at relatively high frequency in different parts of the world[54] which may suggest their genetic stability and potential capability of spreading among the populations.

Occurrence of non-typeable rotavirus strains has been reported in almost every epidemiological survey around the world. Because rotaviruses have been demonstrated to undergo constant genetic variation via sequential point mutations or 'antigenic drift', genetic reassortment or antigenic shift', genomic rearrangement or intragenic recombination[54], the emergence of strains cannot be typed by the currently available methodologies. This work is the first study in Busan to document the distribution of rotavirus G and P types and the diversity of the G-P type combinations among children with acute diarrhea. Of note, rotavirus was detected in 57.6% of all children screened for viral gastroenteritis at 7 hospitals, underscoring its importance as the most common cause of severe diarrhea in Busan. It was estimated that rotavirus gastroenteritis was extremely prevalent in Korea long before the discovery of this virus. Nationwide epidemics of so called 'pseudocholera infantum' have been reported every year since early 1910 with a peak in November. In contrast to bacterial gastroenteritis, the incidence of rotavirus infection has not decreased over the past few years in Korea. It is mainly due to the fact that rotavirus infection is less dependent on socioeconomic status or environmental conditions and no specific antiviral therapy is available. So introduction of vaccine is the answer of rotavius infection.

Results of this study and prior knowledge of rotavirus strain diversity can provide information important for considering the introduction of vaccines, determining the background of strains in circulation, and predicting changes that might occur when a licensed vaccine is introduced. Our results indicate that vaccines targeting rotavirus diarrhea in Busan need to protect primarily against the common genotypes, and, if effective, they could decrease the number of hospitalizations of children due to rotavirus gastroenteritis. At the same time, continuous monitoring of rotavirus strains is

Table 5. Distribution of Rotavirus genotypes among 224 samples of human group A rotaviruses in Busan, during 2005~2006

Rotavirus strains		No of Samples(%)		
		2005	2006	Total
Common strains	P[4]G2	32(31.1)	18(14.9)	50(22.3)
	P[8]G1	16(15.5)	14(11.6)	30(13.4)
	P[8]G3	9 (9.7)	14(11.6)	23(10.3)
	Subtotal	57(55.3)	46(38.0)	103(46.0)
Uncommon strains	P[4]G1	4	3	7 (3.1)
	P[4]G4	2	5	7 (3.1)
	P[6]G1	2	7 (5.8)	9 (4.0)
	P[6]G2	3	1	4
	P[6]G3	1	–	1
	P[6]G4	2	13(10.7)	15 (6.7)
	P[8]G9	3	1	4
	P[9]G1	–	2	2
	P[9]G3	2	–	2
	P[9]G9	–	1	1
	P[10]G1	–	1	1
	P[10]G2	–	2	2
	P[10]G3	–	1	1
	Subtotal	19(18.4)	37(30.6)	56(25.0)
Mixed infections	P[4]G1G2	2	–	2
	P[4]G1G3	–	2	2
	P[4]G1G8	–	1	1
	P[4]/[6]G1	–	4	4
	P[4]/[6]G2	–	7(5.8)	7(3.1)
	P[4]/[8]G1	1	–	1
	P[6]G1G4	1	–	1
	P[8]G1G8		1	1
	Subtotal	4(3.9)	15(12.4)	19(8.5)
Partly typed stains	P[4]	3	2	5
	P[6]	2	1	3
	P[8]	1	–	1
	P[9]	–	2	2
	G1	4	8	1 (5.4)
	G2	3	2	5
	G3	5	2	7 (3.1)
	G4	1	–	1
	G9	3	–	3
	G1G3	–	2	2
	G1G8	–	1	1
	Subtotal	22 (21.4)	20 (16.5)	42 (18.8)
	Subtotal	1 (1.0)	3 (2.5)	4 (1.8)
Total		103 (100.0)	121 (100.0)	224 (100.0)

necessary before and after the implementation of vaccine against rotavirus, because the proportion of uncommon genotypes and untypable strains may fluctuate from year to year.

국문초록

2005년 1월부터 2006년 10월까지 부산시내 7개병원에서 급성장염증상의 환자로부터 분리된 224주의 로타바이러스를 Reverse transcriptase polymerase chain reaction(RT-PCR)과 Multiplex polymerase chain reaction

(Multiplex PCR)을 이용하여 유전자형 분포를 조사하였다. 로타바이러스성 장염은 주로 5세 이하의 어린이(83.5%)와 남성(남:여 1.47~1.55:1)에게서 주로 발생하였다. 2년간의 조사기간을 통해 로타바이러스는 12월과 1월에 시작되어 4월과 5월에 소멸되는 계절적 분포를 나타내었다. 유전자형 분포조사에서는 G 유전자형은 G2(30.4%)와 G1(29.5%)이 가장 많이 검출되었고 G3(15.6%), G4(10.3%), G9(3.6%) 순으로 분포하였으며, G8형은 G1G8 혼합 감염형만 검출되었다. P 유전자형은 P[4](33.0%)가 주요 유행형으로 나타났으며, P[8](26.8%), P[6](14.7%), P[9] (3.1%), P[10](1.8%) 순의 분포를 보였다. G와 P 유전자 조합형은 P[4]G2(22%), P[8]G1(13.4%), P[8]G3(10.3%), P[6]G4(6.7%) 순으로 분포하였으나, 전 세계적으로 유행하는 유전자형의 하나인 P[8]G4형은 검출되지 않았다. 연도별 G와 P 유전자형의 유행양상은 달리 나타났다. 2005년에는 G2가 주요 유행형이었으나 2006년에 G1형으로 바뀌었다. P 유전자형은 주요 유행형이 바뀌지는 않았으나 분포비율은 달리 나타났다. 주요 유행형으로 여겨지지 않는 유전자형의 분포가 비교적 높게(25%) 나타나는 결과를 보였다. P[4]G1, P[4]G4, P[6]G2, P[6]G4, P[6]G1 같은 낮은 분포도의 여러 유전자형과 혼합감염형의 출현은 부산지역에서 유행하는 로타바이러스 유전자형의 높은 다양성을 나타내었다.

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