Journal of Medical Virology 78:1113–1118 (2006)

Detection of Group A Human Rotavirus G9 Genotype Circulating in Córdoba, Argentina, as Early as 1980

P.A. Barril,[1,4] L.C. Martinez,[1] M.O. Giordano,[1] A.A. Castello,[2] R.P. Rota,[2,3] M.B. Isa,[1] G. Masachessi,[1,4] L.J. Ferreyra,[1] G. Glikmann,[2] and S.V. Nates[1]*

[1]*Instituto de Virología "Dr. J.M. Vanella," Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Enfermera Gordillo Gómez s/n Agencia 4—Ciudad Universitaria, Córdoba, Argentina*
[2]*Laboratorio de Inmunología y Virología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña, Buenos Aires, Argentina*
[3]*Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET), Buenos Aires, Argentina*
[4]*Agencia Córdoba Ciencia/CONICET, Buenos Aires, Argentina*

The incidence of human rotavirus G types was determined over a 25-year period (1979–2003) by using reverse transcription-PCR (RT-PCR) to examine 519 stool specimens found to be positive for rotavirus by enzyme linked immunosorbent assay (ELISA) or polyacrylamide gel electrophoresis (PAGE). These stool samples were obtained from children under 3 years old who had been treated for acute diarrhea at public hospitals in Córdoba, Argentina. The present study describes the continued circulation of the common human G types G1 (53.8%), G2 (10.2%), G3 (4.4%), and G4 (27%), and also the detection of the unusual types G8 (0.5%) and G9 (4.2%). Genotype G9 was detected during the 1980–1988 and 1997–2003 periods at relatively low rates. Rotavirus G types distribution was independent of age (1–18 months), gender or outpatient or in-patient status. Unexpectedly, 44.6% of mixed infections were detected, involving common and unusual genotypes. Overall, 95.4% of the typed strains belonged to the most prevalent human serotypes (G1–G4) but the detection of G9 infection throughout this study period highlights the importance of this serotype as a human pathogen. ***J. Med. Virol. 78:1113–1118, 2006.***

KEY WORDS: rotavirus; genotype; common and atypical G-types; emerging genotype; RT-PCR

INTRODUCTION

Group A rotaviruses have been recognized as the major etiologic agents of acute gastroenteritis in infants and young children worldwide [Glass et al., 1996; Parashar et al., 1998]. These infections are associated with high rates of mortality in developing countries, accounting for about 608,400 deaths annually among children under 5 years of age [Parashar, 2004].

Rotaviruses are non-enveloped, icosahedral viruses of the family *Reoviridae*, which possess a genome of 11 double-stranded RNA (dsRNA) segments coding for 12 viral proteins, enclosed in a triple-layered protein capsid [Estes, 2001]. The surface proteins, VP4 and VP7, are involved in virus neutralization following natural infection and are thought to be important for the development of protective immunity. The antigenic specificities of VP7 and VP4 are termed G (for glycoprotein) and P (for protease-sensitive protein), respectively [Bishop, 1984; Estes and Cohen, 1989]. At least 14 G types and 20 P types have been identified among human and animal strains. Since the severity of disease can be reduced by vaccination, several vaccines are under development to provide specific protection against the most prevalent rotavirus G types, G1–G4 [Bishop, 1993; Gentsch et al., 1996; Bernstein et al., 1999; Clements-Mann et al., 1999]. However, previous studies have shown that patterns of G type distribution appear to have regional and local particularities, and 'unusual' G serotypes are now detected throughout the world, especially in developing countries, including G9, G5, and G10 in Brazil [Leite et al., 1996; Santos et al., 1998],

Grant sponsor: Council of Science and Technology of the National University of Cordoba, Argentina; Grant number: 2003-2004.

*Correspondence to: S.V. Nates, PhD, Instituto de Virología "Dr. J.M. Vanella," Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Enfermera Gordillo Gómez s/n Agencia 4—Ciudad Universitaria, 5000 Córdoba, Argentina.
E-mail: snates@cmefcm.uncor.edu

Accepted 6 February 2006
DOI 10.1002/jmv.20670
Published online in Wiley InterScience (www.interscience.wiley.com)

G9 and G12 in Argentina [Bok et al., 2001a; Castello et al., 2004a], and G9 in India [Ramachandran et al., 1996].

A retrospective study was undertaken in order to obtain an insight on the human rotaviruses G types circulating in Córdoba, Argentina, between 1979 and 2003.

MATERIALS AND METHODS

Viruses

Human rotavirus strains Wa (G1P[8]), DS-1 (G2P[4]), P (G3P[8]), and VA70 (G4P[8]), propagated in cultures of MA_{104} cells, were used in this study as reference rotavirus G types. These prototype strains were kindly provided by Dr. G. Glikmann, Virology lab, Universidad Nacional de Quilmes, Buenos Aires, Argentina.

Clinical Samples

Between 1979 and 2003, a total of 2,047 stool samples were collected from children under 3 years of age who had been treated at different Public Hospitals in Córdoba city, Argentina. Five hundred and nineteen fecal samples were found to be positive for rotavirus infection by enzyme linked immunosorbent assay (ELISA) or polyacrylamide gel electrophoresis (PAGE) analysis of dsRNA. Stool specimens were prepared as 10% homogenates in 0.02 M Tris-HCl (pH 7.2) and were stored at −20°C until they were typed. The records of the time between the onset of the illness and collection of the samples was available for 114 of 519 (22%) samples examined, the patient ages were provided for 325 of 519 (62.62%) and the gender was provided for 438 of 519 (84.4%) patients. Details of the patients' attendance at a general practice or admission to a hospital were available for 325 of 519 (62.62%) of the patients included in this study.

RNA Extraction

Viral dsRNA was extracted from fecal suspensions by the phenol-chloroform method followed by alcohol precipitation according to standard procedures [Herring et al., 1982]. After drying, the pellets were diluted in 20 μl of sterile distilled water for reverse transcription-PCR (RT-PCR).

RT-PCR

Extracted RNA in volumes of 3 μl was reverse-transcribed into gene 9 (VP7) full-length cDNA with the generic primers Beg9 (RA1) [Gouvea et al., 1990; Das et al., 1994] and End9 (RA4) [Gouvea et al., 1990] (0.5 μM each). This solution was heated 5 min at 95°C to denature the RNA, and quickly chilled on ice for 2–5 min. A reverse-transcriptase (RT) mixture was then added containing 1 mM of each dNTP (Invitrogen, Carlsbad, California), 6% dimethyl sulfoxide (DMSO), 3 U of cloned AMV RT (Invitrogen), 2 mM dithiothreitol (DTT), and AMV buffer (Invitrogen): 50 mM Tris-acetate (pH 8.4), 75 mM potassium acetate, 8 mM magnesium acetate and stabilizer, in a total volume of 10 μl and incubated 2 hr at 45°C and 15 min at 85°C. One microliter of cDNA was mixed with a PCR reaction mixture containing 0.25 μM of each primer (Beg9, End9), 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen) and the buffer provided by the manufacturer (Invitrogen): 20 mM Tris-HCl (pH 8.4), 50 mM KCl, in a volume of 10 μl. Amplification was performed under PCR condition of one cycle at 94°C for 1 min, 30 cycles at 92°C for 10 sec, 45°C for 15 sec and 72°C for 1 min, and 1 cycle at 72°C for 5 min, using a Biometra UNO II thermo-cycler.

As templates for a second amplification round, 0.4 μl of the PCR products from the above RT-PCR were used in combination with two cocktails of type-specific forward primers. The cocktails were as follows: G1 (aBT1), G2 (aCT2), and G3 (aET3), in one mixture, and G4 (aDT4), G8 (aAT8), and G9 (aFT9), in the second one and a generic reverse primer, End9, whose sequence is conserved among G types. These primers combinations amplify variable regions of the VP7 gene. The 10 μl reaction mixture consisted of 0.25 μM of each type-specific primer, 0.5 μM of primer End9 (RA4), 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen), and a buffer provided by the manufacturer (Invitrogen). Conditions for the multiplex PCR were otherwise the same as those for the first amplification round. Five microliters of the final product was analyzed by electrophoresis on 10% polyacrylamide gel. In this system, the sizes of the type-specific PCR products were 749bp (G1), 652bp (G2), 374bp (G3), 583bp (G4), 885bp (G8), and 306bp (G9).

PAGE

Equal volumes of 5 μl of the nested-PCR products and Phyndia buffer (0.02 M Tris-HCl pH 7.4, 0.3 M NaCl, 0.01 M $MgCl_2$, 0.1% SDS, 5 mM EDTA, 4% sucrose, 0.04% bromophenol blue) [Laemmli, 1990] were mixed and electrophoresed on 10% polyacrylamide gel in running buffer pH 8.9 (0.3% Tris, 1.44% Glicine, 0.1% SDS) along with a 100 bp DNA ladder (PB-L Productos BioLogicos, Buenos Aires, Argentina). Fragments were visualized after silver staining according to data published elsewhere [Pereira et al., 1986].

RESULTS

Distribution of G Types

From the 519 rotavirus-positive stool samples, 386 (74.4%) were genotyped. The specimens that could not be genotyped did not produce amplification products of the VP7 gene. In order to test whether these samples were VP7 gene negative (i.e., no first amplification product visualized) because of the presence of contaminating inhibitors of RT-PCR enzymes or viral dsRNA degradation, approximately 15% of them (n = 20) were analyzed by PAGE. Of the total, four samples (20%) were positive by PAGE, which indicates the presence of contaminating inhibitors of RT-PCR enzymes in these samples.

Rotavirus types circulating in Córdoba, Argentina, from 1979 to 2003 are shown in Table I. Mixed G types

TABLE I. Rotavirus G Types in Samples Over 25 Years (1979–2003) in Córdoba, Argentina

Years analyzed	No. of stool samples	No. (%)[a] of VP7 (−) samples	No. (%)[b] of infection type			No. of typeable strains	No. (%)[d] of strains with the indicated G types					
				Mixed infections[c]								
			Simple	Dual	Triple		G1	G2	G3	G4	G8	G9
1979	25	1 (4)	11 (44)	13 (52)	—	37	23 (62)	—	1 (3)	13 (35)	—	—
1980	14	5 (36)	5 (36)	4 (28)	—	13	8 (62)	1 (8)	—	2 (15)	—	2 (15)
1981	8	1 (12.5)	—	5 (62.5)	2 (25)	16	6 (37.5)	6 (37.5)	—	3 (19)	—	1 (6)
1982	4	1 (25)	2 (50)	—	1 (25)	5	2 (40)	—	—	1 (20)	—	2 (40)
1983	1	1 (100)	—	—	—	—	—	—	—	—	—	—
1984	13	—	10 (77)	2 (15)	1 (8)	17	9 (52.9)	2 (11.8)	2 (11.8)	3 (17.6)	—	1 (5.9)
1985	12	3 (25)	3 (25)	4 (33)	2 (17)	17	8 (47)	1 (6)	1 (6)	7 (41)	—	—
1986	12	—	6 (50)	2 (17)	4 (33)	22	12 (54.55)	2 (9.1)	1 (4.5)	6 (27.3)	—	1 (4.55)
1987	10	4 (40)	4 (40)	1 (10)	1 (10)	9	4 (44.4)	3 (33.3)	—	2 (22.2)	—	—
1988	3	—	1 (33.3)	1 (33.3)	1 (33.3)	6	3 (50)	1 (17)	—	1 (17)	—	1 (17)
1989	14	1 (7)	11	2	—	15	10 (66.7)	—	1 (6.7)	4 (26.7)	—	—
1992	1	—	—	1 (100)	—	2	1 (50)	—	—	1 (50)	—	—
1993	1	1 (100)	—	—	—	—	—	—	—	—	—	—
1995	3	—	3 (100)	—	—	3	2 (67)	—	—	1 (33)	—	—
1996	20	11 (55)	7 (35)	2 (10)	—	11	6 (54.5)	3 (27.3)	—	2 (18.2)	—	—
1997	121	35 (29)	40 (33)	35 (29)	11 (9)	143	68 (47.5)	23 (16)	7 (5)	39 (27)	1 (1)	5 (3.5)
1998	128	32 (25)	52 (41)	36 (28)	8 (6)	148	89 (60)	4 (3)	5 (3)	44 (30)	1 (1)	5 (3)
1999	11	6 (55)	2 (18)	3 (27)	—	8	4 (50)	—	1 (12.5)	3 (37.5)	—	—
2000	40	10 (25)	23 (57.5)	7 (17.5)	—	37	24 (65)	5 (13.5)	2 (5)	5 (13.5)	—	1 (3)
2001	38	10 (26)	11 (29)	11 (29)	6 (16)	51	22 (43)	3 (6)	3 (6)	20 (39)	—	3 (6)
2002	23	5 (22)	13 (56)	3 (13)	2 (9)	25	16 (64)	2 (8)	2 (8)	3 (12)	1 (4)	1 (4)
2003	17	6 (35)	10 (59)	1 (6)	—	12	4 (33)	5 (42)	—	1 (8)	—	2 (17)
Total	519	133 (25.6)	214 (55.4)	133 (34.5)	39 (10.1)	597	321 (53.8)	61 (10.2)	26 (4.4)	161 (27)	3 (0.5)	25 (4.2)
				172 (44.6)								

[a]Based on the number of total samples (n = 519).
[b]Based on the number of positive samples (n = 386).
[c]The genotypes included in these samples are numbered in each specific G type.
[d]Based on the number of typeable strains (n = 597).

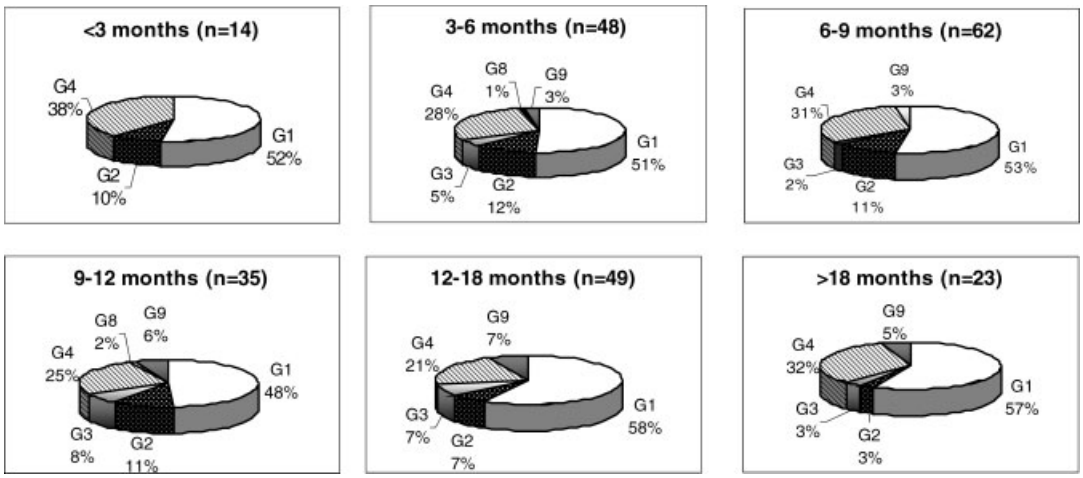

Fig. 1. Age distribution of patients with rotavirus gastroenteritis detected in Córdoba, Argentina, during 1979–2003.

reflecting multiple infections were detected in 44.6% of the samples; most of the cases were infections with two rotavirus genotypes (77.3%), but some were infections with three genotypes (22.7%). Due to the high rate of mixed infections, 10% of these samples were submitted to individual RT-PCR reactions using primers specific for each genotype involved in the co-infection. The results obtained in all the tests confirm the genotypes originally stated with the cocktail of primers: G1 (aBT1), G2 (aCT2), and G3 (aET3), in one mixture, and G4 (aDT4), G8 (aAT8), and G9 (aFT9), in the second one.

In order to examine the rotavirus genotype distribution, these mixed infections were considered as independent (each genotype added separately), so that the total number of genotypes was 597. The percentage figures in Table I refer to this total number.

As shown by the results in Table I, the four major human G types were represented in this survey. Overall, G1 was the main type detected and was responsible for 53.8% of the rotavirus infections. G4 was the second most common being responsible for 27% of the infections and, the G2 and G3 rotavirus types were detected in 10.2% and 4.4% of the infections, respectively. Unexpectedly, a significant number of G9 strains was found since 1980 with a total rate of 4.2% similar to that of G3 (4.4%).

The temporal distribution showed that prevalence of rotavirus types differed from year to year (Table I). G1 was the most frequent rotavirus type throughout the 25-year study, followed by G4 and G2. The genotypes G3 and G9 were uncommon since 1979 and 1980, respectively, and occurred sporadically year-to-year. The uncommon strain G8 was only detected in three samples (0.5%) during 1997, 1998, and 2002.

Rotavirus Genotypes Distribution by Age and Gender

Most of the rotavirus infections (98.6%) were in children under 18 months old. The age distribution of G types among this cohort did not show data of significant interest, since every genotype was almost equally represented in each of these age groups (Fig. 1). Rotavirus G types were neither significantly different among male nor female patients.

Mixed Infections in Patients Attending at General Practice Compared With Those Admitted to a Hospital

The number of out-patients who were infected with more than one rotavirus strain was slightly lower than the number of patients who required hospitalization (40% vs. 48%), but this difference was not significant ($P > 0.05$).

DISCUSSION

Rotavirus G-types circulating in a specific geographical area (Córdoba) in a well-defined target population of children under 3 years old are described.

The G typing results showed that more than 95% of the genotypes corresponded to common G types (G1–G4) distributed worldwide [Santos and Hoshino, 2005]. Consistent with findings from numerous other studies, G1 (53.8%) was by far the most common genotype during the sampling period, followed by G4 (27%), G2 (10.2%), and G3 (4.4%). Although the total rate of the type G4 was higher than that of G2, sample fluctuations in both frequencies were observed over the 25 years of study and G2 or G4 were alternatively the second most prevalent genotype during several years (Table I). Studies on the distribution of rotavirus types in several countries have identified important regional variations and temporal changes in G and P types [Leite et al., 1996; Ramachandran et al., 1996; Unicomb et al., 1999; Argüelles et al., 2000]. The present study, in agreement with others [Bishop et al., 1991; Noel et al., 1991; Buesa et al., 2000; Kolsen Fischer et al., 2000; Bok et al., 2001b; Castello et al., 2004b; Sánchez-Fauquier et al., 2004], shows that major changes in the predominant rotavirus types can occur like the rise of G2 in 2003.

Uncommon genotypes were found during the period examined including, G9 detected since 1980, being almost as common as G3 type, and G8, which was rarely found. Since the introduction and wider use of molecular biology-based typing methods over the last 10 years, unusual rotavirus types have been reported increasingly in different parts of the world. G9 rotaviruses have also been described in Australia [Palombo et al., 2000], India [Ramachandran et al., 1996; Jain et al., 2001], Bangladesh [Unicomb et al., 1999], Malawi [Cunliffe et al., 1999], the United States [Ramachandran et al., 1998], Brazil [Santos et al., 2003], United Kingdom [Cubitt et al., 1996; Iturriza-Gómara et al., 2000], Spain [Sánchez-Fauquier et al., 2004], France [Gault et al., 1999], and Argentina [Bok et al., 2001a,b] at rates of detection between 0.4% and 28%. Nevertheless, some retrospective studies failed to find any evidence of G9 type prior to the mid 1990s in Argentina, although using the G9 primers. This study defined that G9 strains have been circulating in Córdoba, Argentina, since at least 1980, long before the increase in prevalence described by the National Surveillance Network in 1999 [Bok et al., 2001a]. Since three different sub-lineages were defined for the G9 genotype [Ramachandran et al., 2000; Bok et al., 2001a] which have a rough correspondence with year of isolation, it will be extremely interesting to sequence the VP7 gene of representative samples from our collection.

Two modifications were introduced to the genotyping methods used commonly: First, the type-specific reactions with similar molecular weight products (G2/G4 and G3/G9) were separated into two independent *multiplex*-PCR reactions and second, PAGE followed by silver staining was used for PCR-product analysis, which has a higher resolution and sensitivity level than agarose gel electrophoresis and ethidium bromide. These modifications could have improved at some point our capabilities for detection and discrimination of genotypes. This may suggest that unusual genotypes, such as G9, are more common than thought previously and were probably misdiagnosed in other studies. Although every sample found positive for VP7 gene could be genotyped, it cannot be ruled out that atypical G types that have not been included in this study, like G12, could be co-circulating with the G types detected here.

The great majority of rotavirus mixed infections was co-infection with the most frequent G types, G1 and G4 (72%), detected during the period of this study. These mixed infections occurred at high rates in the present survey as described also in other developing countries like India (11%) [Jain et al., 2001] and Brazil (29%) [Timenetsky et al., 1994]. Conversely, the prevalence of mixed infections found in the United States was considerably lower (2.3%) [Ramachandran et al., 1998]. High prevalence of mixed infections may provide a good environment for this segmented virus to form reassortants and thus evolve. It is likely that in Córdoba, virus evolution will be related more closely to that of regions with similar epidemiological characteristics like India and Brazil, where mixed infections are common [Timenetsky et al., 1994; Leite et al., 1996; Ramachandran et al., 1996; Jain et al., 2001].

No significant differences in age distribution or gender were found among the children suffering from acute diarrhea caused by different genotypes. Although the number of patients with mixed infections in children admitted to hospital was slightly higher than the number of ambulatory cases, no significant differences were detected between these two group of patients ($P > 0.05$) suggesting that mixed infections are not responsible for more severe forms of the illness.

Since Córdoba and Buenos Aires are the most densely populated cities in Argentina, the epidemiological data from these provinces are representative at both local and regional levels. In this context, the detection of G12 serotype in Buenos Aires between 1999 and 2002 [Castello et al., 2004a], and the circulation of G9 in Córdoba since 1980, imply that there are at least two unusual genotypes circulating in the region. The detection of rotavirus G9 in almost all periods of study in Córdoba and in several other countries during different years of study [Ramachandran et al., 1996; Unicomb et al., 1999; Bok et al., 2001a], suggests that this type should be incorporated into candidate rotavirus vaccines and/or G9 type should be used to measure heterotypic immunity.

In view of the results obtained in this study, it will be of particular interest to examine the P-types (VP4 analysis) of the strains in this collection. In addition, in order to further study rotavirus evolution, sequencing and phylogenetic analyses of the genome of the positive strains will be carried out.

REFERENCES

Argüelles MH, Villegas GA, Castello AA, Abrami A, Ghiringhelli PD, Semorile L, Glikmann G. 2000. VP7 and VP4 genotyping of human group A rotavirus in Buenos Aires, Argentina. J Clin Microbiol 38:252–259.

Bernstein DI, Sack DA, Rothstein E, Reisinger K, Smith VE, O'Sullivan D, Spriggs DR, Ward RL. 1999. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: A randomised placebo-controlled trial. Lancet 354:287–290.

Bishop RF. 1984. Natural history of human rotavirus infections, In: Kapikian AZ, editor. Viral infections of the gastrointestinal tract, 2nd edition. New York, N.Y: Marcel Dekker, Inc. pp 131–167.

Bishop RF. 1993. Development of candidate rotavirus vaccines. Vaccine 11:247–254.

Bishop RF, Unicomb LE, Barnes GL. 1991. Epidemiology of rotavirus serotypes in Melbourne, Australia, from 1973 to 1989. J Clin Microbiol 29:862–868.

Bok K, Palacios G, Sijvarger K, Matson D, Gomez J. 2001a. Emergence of G9 P[6] human rotaviruses in Argentina: Phylogenetic relationships among G9 strains. J Clin Microbiol 39:4020–4025.

Bok K, Castagnaro N, Borsa A, Nates S, Espul C, Fay O, Fabri A, Grinstein S, Miceli I, Matson D, Gómez J. 2001b. Surveillance for rotavirus in Argentina. J Med Virol 65:190–198.

Buesa J, de Souza CO, Asensi M, Martinez C, Prat J, Gil MT. 2000. VP7 and VP4 genotypes among rotavirus strains recovered from children with gastroenteritis over a 3-year period in Valencia, Spain. Eur J Epidemiol 16:501–506.

Castello AA, Argüelles MH, Rota R, Gentsch J, Jiang B, Glass RI, Glikmann G. 2004a. Molecular epidemiology of rotavirus diarrea among children in Buenos Aires, Argentina: Emergence of the genotype G12. Presented at: International Conference on Emerging Infectious Diseases, CDC, February 29-March 3, 2004, Atlanta.

Castello AA, Arvay ML, Glass RI, Gentsch J. 2004b. Rotavirus strain surveillance in Latin America. A review of the last nine years. Pediatric Infect Dis J 23:S168–S172.

Clements-Mann ML, Makhene MK, Mrukowics J, Wright PF, Hoshino Y, Midthun K, Sperber E, Karron R, Kapikian AZ. 1999. Safety and immunogenicity of live attenuated human-bovine (UK) reassortant rotavirus vaccines with VP7-specificity for serotypes 1, 2, 3 or 4 in adults, children and infants. Vaccine 17:2715–2725.

Cubitt WD, Steele AD, Iturriza M. 1996. Characterization of rotaviruses from children treated at a London hospital during 1996: Emergence of strains G9P2A[6] and G3P2A[6]. J Med Virol 61:150–154.

Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME, Woods PA, Bresee JS, Glass RI, Gentsch JR, Hart CA. 1999. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malaei, from 1997 to 1998: Predominance of novel P[6]G8 strains. J Med Virol 57:308–312.

Das BK, Gentsch JR, Cicirello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI. 1994. Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol 32:1820–1822.

Estes M. 2001. Rotaviruses and their replication, In: Fields BN, Knipe DM, Howley PM, editors. Fields virology, 4th edition, vol. 2. Philadelphia, Pa: Lippincott Williams & Wilkins. pp 1747–1786.

Estes MK, Cohen J. 1989. Rotavirus gene structure and function. Microbiol Rev 53:410–449.

Gault E, Chikhi-Brachet R, Sandrine D, Schnepf N, Albiges L, Grimprel E, Girardet JP, Begue P, Garbarg-Chenon A. 1999. Distribution of human rotavirus G types circulating in Paris, France, during the 1997-1998 epidemic: High prevalence of type G4. J Clin Microbiol 37:2373–2375.

Gentsch JR, Woods PA, Ramachandran M, Das BK, Leite JP, Alfieri A, Kumar R, Bhan MK, Glass RI. 1996. Review of G and P typing results from a global collection of rotavirus strains: Implications for vaccine development. J Infect Dis 174:S30–S36.

Glass RI, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA, Clarke MJ, Ho MS, Gentsch JR. 1996. The epidemiology of rotavirus diarrhea in the United States: Surveillance and estimates of disease burden. J Infect Dis 174:S5–S11.

Gouvea V, Glass RI, Woods PA, Taniguichi K, Clark HF, Forrester B, Fang ZY. 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 28:276–282.

Herring AJ, Inglis NF, Ojeh CK, Snodgrass DR, Menzies JD. 1982. Rapid diagnosis of rotaviral infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J Clin Microbiol 16:473–477.

Iturriza-Gómara M, Green J, Brown D, Ramsay M, Desselberger U, Gray J. 2000. Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. J Clin Microbiol 38:4394–4401.

Jain V, Das BK, Bhan MK, Glass RI, Gentsch JR. 2001. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infections in India. J Clin Microbiol 39:3524–3529.

Kolsen Fischer T, Steinsland H, Molbak K, Ca R, Gentsch JR, Valentiner-Branth P, Aaby P, Sommerfelt H. 2000. Genotype profiles of rotavirus strains from children in a suburban community in Guinea-Bissau, Western Africa. J Clin Microbiol 38:264–267.

Laemmli UK. 1990. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.

Leite JP, Alfieri AA, Woods PA, Glass RI, Gentsch JR. 1996. Rotavirus G and P types circulating in Brazil: Characterization by RT-PCR, probe hybridization, and sequence analysis. Arch Virol 141:2365–2374.

Noel JS, Beards GM, Cubitt WD. 1991. Epidemiological survey of human rotavirus serotypes and electropherotypes in young children admitted to two children's hospitals in northeast London from 1984 to 1990. J Clin Microbiol 29:2213–2219.

Palombo EA, Masendycz PJ, Bugg HC, Bogdanovic-Sakran N, Barnes GL, Bishop RF. 2000. Emergence of serotype G9 human rotavirus in Australia. J Clin Microbiol 38:1305–1306.

Parashar U. 2004. Proceeding of the 6th International Rotavirus Symposium, Mexico City, July 7-9

Parashar UD, Bresee JS, Gentsch JR, Glass RI. 1998. Rotavirus Emerg. Infect Dis 4:561–570.

Pereira HG, Gouvea V, Fialho AM. 1986. A comparison of simian rotavirus SA11 preparations maintained in different laboratories. Mem Inst Oswaldo Cruz Rio J 81:389–393.

Ramachandran M, Das BK, Vij A, Kumar R, Bhambal SS, Kesari N, Rawat H, Bahl L, Thakur S, Woods PA, Glass RI, Bhan MK, Gentsch JR. 1996. Unusual diversity of human rotavirus G and P genotypes in India. J Clin Microbiol 34:436–439.

Ramachandran M, Gentsch JR, Parashar UD, Jin S, Woods PA, Holmes JL, Kirkwood CD, Bishop RF, Greenberg HB, Urasawa S, Gerna G, Coulson BS, Taniguchi K, Bresee JS, Glass RI. 1998. Detection and characterization of novel rotavirus strains in the United States. J Clin Microbiol 36:3223–3229.

Ramachandran M, Kirkwood CD, Unicomb L, Cunliffe NA, Ward RL, Bhan MK, Clark HF, Glass RI, Gentsch JR. 2000. Molecular characterization of serotype G9 rotavirus strains from a global collection. Virol 278:436–444.

Santos N, Hoshino Y. 2005. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 15:29–56.

Santos N, Lima RC, Pereira CF, Gouvea V. 1998. Detection of rotavirus types G8 and G10 among Brazilian children with diarrhea. J Clin Microbiol 36:2727–2729.

Santos N, Soares CC, Volotao EM, Albuquerque MC, Hoshino Y. 2003. Surveillance of rotavirus strains in Rio de Janeiro, Brazil, from 1997 to 1999. J Clin Microbiol 41:3399–3402.

Sánchez-Fauquier A, Wilhelmi I, Colomina J, Cubero E, Roman E. 2004. Diversity of group A human rotavirus types circulating over a 4-year period in Madrid, Spain. J Clin Microbiol 42:1609–1613.

Timenetsky M, do C, Santos N, Gouvea V. 1994. Survey of rotavirus G and P types associated with human gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992. J Clin Microbiol 32:2622–2624.

Unicomb LE, Podder G, Gentsch JR, Woods PA, Hasan KZ, Faruque AS, Albert MJ, Glass RI. 1999. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: Emergence of type G9 in 1995. J Clin Microbiol 37:1885–1891.