Characterization and Molecular Epidemiology of Rotavirus Strains Recovered in Northern Pretoria, South Africa during 2003–2006

L. M. Seheri,[1] N. Page,[1,2] J. B. Dewar,[1] A. Geyer,[1] A. L. Nemarude,[1] P. Bos,[1] M. Esona,[1] and A. D. Steele[1,3]

[1]MRC Diarrhoeal Pathogens Research Unit, University of Limpopo (Medunsa Campus), Pretoria, and [2]Viral Gastroenteritis Unit, National Institute for Communicable Diseases, Sandringham, Johannesburg, South Africa; and [3]Vaccines and Immunizations, PATH, Seattle, Washington

Rotavirus infection is the most common cause of severe dehydrating gastroenteritis in infants and young children and remains a significant clinical problem worldwide. The severity and the burden of rotavirus disease could be reduced through the implementation of an effective vaccine. The aim of this study was to characterize rotavirus strains circulating in the local community as part of an ongoing hospital burden of disease study when a G1P[8] rotavirus vaccine candidate was being evaluated in the same community. From 2003 through 2006, 729 rotavirus-positive stool specimens were collected from children <5 years of age who were treated for diarrhea at Dr George Mukhari Hospital, Ga-Rankuwa, South Africa. Molecular characterization of the strains was performed by polyacrylamide gel electrophoresis and genotyping of the VP4 and VP7 alleles using well-established seminested multiplex reverse-transcription polymerase chain reaction methods. In 2003, 62% of strains exhibited the short rotavirus electropherotype, and the most common rotavirus strain was G2P[4]. In subsequent years, predominant rotavirus strains included G1P[8] and G1P[6] in 2004, G3P[8] and G3P[6] in 2005, and G1P[8] in 2006. For the 4 years of the study, rotavirus strains with P[6] genotype were detected in 25% of all rotavirus-positive specimens. In addition, unusual G12P[6] and G8 strains were detected at a low frequency. These results reflect the diversity of rotavirus strains circulating in South African communities.

Rotavirus infection is the most common cause of severe dehydrating gastroenteritis in infants and young children and remains a significant clinical problem throughout the world. Recently, it was estimated that rotavirus disease is responsible for ~527,000 deaths annually among children <5 years of age, primarily in developing countries [1]. In sub-Saharan Africa alone, the virus is estimated to account for ~230,000 deaths annually among children <5 years of age (A.D.S., personal communication).

The genetic and antigenic diversity of the outer capsid proteins VP7 and VP4 allow the classification of rotavirus strains into G and P types, respectively. Both VP4 and VP7 are important targets for vaccine development, because these proteins independently elicit protective neutralizing antibodies [2, 3]. Epidemiological studies of rotavirus infection have shown that there is great diversity of rotavirus strains circulating throughout the world. Currently, there are 20 G genotypes and 28 P genotypes identified in humans and animals [4–9]. Human group A rotavirus strains of 5 G/P combinations are frequently detected worldwide, including G1, G2, G3, G4, and G9 strains in association with P[8] and P[4] genotypes [3, 4]. However, in developing countries, alternate strains may predominate, including P[6] and G8 genotypes [10–13].

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Reprints or correspondence: Dr Nicola Page, Viral Gastroenteritis Unit, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131, South Africa (nicolap@nicd.ac.za).

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The introduction of new live attenuated oral rotavirus vaccines has led to the prospect of substantial reductions of childhood morbidity and mortality worldwide. The 2 new rotavirus vaccines licensed for use are RotaTeq (Merck) and Rotarix (GlaxoSmithKline Biologicals). RotaTeq, based on homotypic immunity, consists of bovine-human monoreassortant strains with G1P[7], G2P[7], G3P[7], G4P[7], and G6P[8] specificity [14]. In a large cohort of 70,301 participants from 11 developed countries, RotaTeq demonstrated high efficacy rates against severe rotavirus diarrhea and was not associated with an increased risk of intussusception [15].

Rotarix is a human attenuated monovalent G1P1A[8] rotavirus vaccine with a proposed efficacy based on heterotypic immunity [16]. Results from phase III Rotarix efficacy studies performed in Latin America and Finland demonstrated high efficacy rates against severe rotavirus diarrhea associated with G1P[8], G3P[8], G4P[8], and G9P[8] strains and that the vaccine was not associated with an increased risk of intussusception [17]. However, the vaccine appeared to be less effective against G2P[4] rotavirus strains causing any disease severity but was effective against severe G2 rotavirus infection [18]. The World Health Organization (WHO) recommended that the safety and immunogenicity of rotavirus vaccines should be demonstrated in developing countries in Africa and Asia. Thus, Rotarix was evaluated in the Madibeng and Ga-Rankuwa Districts in South Africa from 2001 onwards to determine (1) noninterference of the rotavirus vaccine with oral polio vaccine [19], (2) the appropriate dosage and regimen for rotavirus vaccine administration, (3) rotavirus vaccine safety in children with HIV infection, and (4) the efficacy of the rotavirus vaccine in an African setting. The vaccine trials for oral polio vaccine interference and vaccine regimen were conducted from 2001 through 2004, and trials for safety and efficacy were conducted from 2005 through 2008.

Epidemiological studies of rotavirus infection have shown that there is great diversity of rotavirus strains circulating throughout the world. The ability of the currently available vaccines to provide protection against unusual or uncommon rotavirus strains circulating in developing countries cannot be predicted. Therefore, the effectiveness of rotavirus vaccines against diverse strains should be monitored over a long period. The aim of this study was to describe the genetic diversity of rotavirus strains circulating in the community before universal mass vaccination against rotavirus disease. Continued surveillance of rotavirus strain diversity in the human population is also required to monitor the emergence of new or previously uncommon strains.

MATERIALS AND METHODS

Stool sample collection. From 2003 through 2006, stool samples were collected from children <5 years of age who were treated for gastroenteritis at both the pediatric inpatient and outpatient departments at Dr George Mukhari Hospital (formerly Ga-Rankuwa Hospital), North of Pretoria, South Africa. Infants in the neonatal ward were excluded from the study. A single stool specimen was obtained within 48 h after admission to exclude nosocomial infections. Informed consent was obtained verbally from the parent or caretaker accompanying the child. The research protocol for this study received approval from the Ethics Committee of the University of Limpopo (Medunsa Campus). A 10% fecal suspension of 0.5 g of stool was made in 5 mL of distilled water and stored at 4°C until analysis.

Detection of group A rotavirus. All stool samples collected were analyzed for the presence of rotavirus antigens with use of the commercially available DAKO Rotavirus IDEIA enzyme immunoassay (EIA), according to the manufacturer's instructions. The assay uses a polyclonal antibody in a solid phase sandwich EIA to detect group A rotavirus in fecal suspensions.

Rotavirus genotyping. The viral double-stranded RNA (dsRNA) was extracted from 10% rotavirus-positive fecal suspensions with use of Tri-Reagents-LS (Molecular Research Centre) or the QIAamp viral RNA extraction kit (Qiagen), according to the manufacturers' instructions. The primer pairs sBeg/End9 and Con2/Con3 were used to reverse transcribe full-length copies of the VP7 gene (1062 base pairs [bp]) and a fragment of the VP4 gene (876 bp), respectively [20, 21]. The G and P typing was performed using a nested multiplex polymerase chain reaction (PCR) and previously described G-specific and P-specific primers [22–26]. Nontypeable G and P genotypes were further analyzed using animal G and P primers described by Gouvea et al [27, 28]. PCR was performed at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 42°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min, before being stored at 4°C. The first round VP7 amplicons that could not be identified by either human or animal primers were subjected to sequencing.

Sequencing of VP7 genes. The VP7 genes of various selected rotavirus strains were sequenced to determine or confirm the VP7 genotypes. Samples were randomly selected for sequencing based on genotype results and year of isolation. Rotavirus PCR products of the VP7 gene (1062 bp) were excised from a 2% agarose gel and purified with the QIAquick Gel extraction kit (Qiagen), according to the manufacturer's instructions. Purified PCR products were sequenced directly by Gene Care Molecular Genetics, Stellenbosch, with sBeg and End9 primers [20]. Sequences of 917–930 bp were obtained starting between nucleotides 75–80 and ending between nucleotides 995–1015. The nucleotide and deduced amino acid sequences of the VP7 genes of the South African strains were compared with the corresponding VP7 sequences of representative strains of human rotavirus group A strains available from the PubMed EMBL/GenBank data libraries.

Sequence alignment and phylogenetic analysis. For accurate alignment, the consensus sequences of the VP7 genes from South African and international rotavirus strains were aligned and analyzed manually using Chromaslite [29] and BioEdit [30] software packages. The genetic relatedness of the VP7 genes of South African rotavirus strains was determined by constructing a phylogenetic tree using the neighbor joining and clustering methods. The phylogenetic tree was constructed using the TreeView Program [31], with genetic distances generated on DNAMan computer software. Genetic distances in genotypes were calculated using replicate data sets of 1000.

Nucleotide sequence accession numbers. The DNA sequences for the VP7 genes of the South African G1, G3, G8, G9, and G12 strains were submitted directly to Genbank and assigned the following accession numbers: G1 (SA648DGM/03; GQ338876, SA1199DGM/04; GQ338878, SA2107DGM/04; GQ338877, SA4799DGM/04; and GQ338879), G3 (SA1198DGM/04; GQ338880, SA1304DGM/04; GQ338881, SA1306DGM/04; GQ338882, SA1380DGM/04; GQ338883, SA1401DGM/04; GQ338884, SA188DGM/05; and GQ338885), G8 (SA1396DGM/04 and GQ344620), G9 (SA2125DGM/03; GQ338886, SA2144DGM/03; GQ338887, SA2171DGM/03; and GQ338888), and G12 (SA1365DGM/04; EU284727, SA4747DGM/04; GQ338889, SA4727DGM/04; EU284738, SA3426DGM/04; EU284736, SA4792DGM/04; EU284741, SA193DGM/05; GQ338890, SA731DGM/05; and GQ338891).

RESULTS

Samples. During the 4 years of surveillance, a total of 3191 diarrheal stool samples were collected from children aged <5 years. Of these, 729 were positive for group A rotavirus strains and 648 were available for further genotyping. Ninety percent of children with rotavirus diarrhea were <2 years of age. A marked seasonal trend of rotavirus infection was noted with bimodal peaks during the cooler, dry months of the year [32].

G and P rotavirus genotypes. The G and P types of rotavirus strains collected during 2003–2006 from children with acute gastroenteritis or diarrhea are summarized in Table 1. The most common human G- and P-genotype combinations detected were G2P[4] ($n = 62$) in 2003, G1P[8] and G1P[6] ($n = 69$) in 2004, G3P[8] and G3P[6] ($n = 128$) in 2005, and G1P[8] ($n = 74$) in 2006. In 2003, the serotypes G2P[4] and G2P[6] were detected in 67 specimens (47%), with G1P[8] and G9P[6] noted in 24% and 8% of specimens, respectively. The majority of the strains (56%) detected during the 2004 rotavirus season were G1P[8] and G1P[6]. Serotypes G3P[8] (13%), G3P[6] (9%), G12P[6] (7%) G2P[4] (2%), and G8P[6] (2%) were also detected during this time. During the 2005 rotavirus season, the predominant G1 strains found during 2004 were replaced by serotype G3P[8] (51%) and G3P[6] (17%) strains. In addition, G1P[8], G12P[6], G1P[6], and G8P[6] strains were detected at relatively low prevalences of 6%, 5%, 4%, and 3%, respectively. During the 2006 season, G1P[8] (39%) was again the predominant genotype, although G8 was detected in 10% and G9 in 14% of strains associated with P[4], P[6], and P[8] genotypes.

The human P[8] genotype was detected throughout the study period and was predominant in 2004 (61%) and 2005 (59%; data not shown). The P[4] genotype was the major VP4 type observed during 2003 (46%), coinciding with the circulation of increased numbers of G2 strains. A significant proportion (25%) of rotavirus strains had a P[6] genotype during the study period.

Mixed infections of G and P genotype and nontypeable strains were also observed (Tables 1 and 2). Varied mixed in-

Table 1. Summary of Usual, Unusual, Mixed, and Nontypeable (NT) Rotavirus Strains Circulating at Dr George Mukhari Hospital (South Africa) during 2003–2006

	No. (%) of strains				
Genotype	2003	2004	2005	2006	Total
Usual					
G1P[8]	35 (24)	55 (44)	12 (6)	74 (39)	176
G2P[4]	62 (43)	3 (2)	0 (0)	5 (3)	70
G3P[8]	0 (0)	16 (13)	96 (51)	8 (4)	120
G9P[8]	3 (2)	0 (0)	2 (1)	16 (8)	21
Total	100 (70)	74 (60)	110 (58)	103 (54)	387
Unusual					
G1P[6]	12 (8)	14 (11)	7 (4)	7 (4)	40
G1P[4]	1 (>1)	0 (0)	0 (0)	0 (0)	1
G2P[6]	5 (3)	0 (0)	0 (0)	7 (4)	12
G3P[6]	0 (0)	11 (9)	32 (17)	1 (>1)	44
G3P[4]	0 (0)	0 (0)	0 (0)	2 (1)	2
G8P[6]	1 (>1)	2 (2)	5 (3)	1 (>1)	9
G8P[8]	0 (0)	2 (2)	3 (2)	10 (5)	15
G8P[4]	0 (0)	0 (0)	2 (1)	8 (4)	10
G9P[6]	12 (8)	0 (0)	3 (2)	8 (4)	23
G9P[4]	0 (0)	0 (0)	0 (0)	3 (2)	3
G12P[6]	0 (0)	9 (7)	10 (5)	1 (>1)	20
Total	31 (22)	38 (31)	62 (33)	48 (25)	179
Mixed and NT					
Mixed	6 (4)	6 (5)	10 (5)	30 (16)	52
G1P[NT]	2 (1)	0 (0)	0 (0)	1 (>1)	3
G2P[NT]	2 (1)	0 (0)	0 (0)	0 (0)	2
G3P[NT]	0 (0)	2 (2)	2 (1)	1 (>1)	5
G3,1P[NT]	0 (0)	0 (0)	1 (>1)	0 (0)	1
G9P[NT]	0 (0)	0 (0)	1 (>1)	1 (>1)	2
G[NT]P[4,6]	1 (>1)	0 (0)	0 (0)	0 (0)	1
G[NT]P[4,8]	0 (0)	0 (0)	0 (0)	1 (>1)	1
G[NT]P[6]	0 (0)	3 (2)	3 (2)	1 (>1)	7
G[NT]P[6,8]	1 (>1)	1 (>1)	0 (0)	0 (0)	2
G[NT]P[8]	0 (0)	0 (0)	1 (>1)	5 (3)	6
Total	12 (8)	12 (10)	18 (9)	40 (21)	82
Total	143 (100)	124 (100)	190 (100)	191 (100)	648

fections were observed when a single P genotype was associated with 2 or 3 G genotypes or when 2 P genotypes were associated with 1 or 2 G genotypes. The P genotype mixed infections were G2 associated with P[4] and P[6] or G2 with P[4] and P[8], with the predominant P[4] genotype associated with short RNA electropherotypes (data not shown). Additional mixed infections included G1 with P[8] and P[6] with long RNA patterns (data not shown). The percentage of mixed infections increased from relatively stable levels of 4%–5% during 2003–2005 to 16% during 2006 (Table 1). During the 4-year study period, a G or P genotype could not be established in ~5% of the rotavirus-positive samples analyzed.

Sequence analysis of VP7 genes. The results from sequence alignment of the VP7 genes of 21 rotavirus strains detected in South Africa showed nucleotide and deduced amino acid sequence homology and identity of 97%–99%, when compared with corresponding international G1, G3, G8, G9, and G12 strains (data not shown). These results confirmed the genotyping results obtained by seminested PCR.

The South African G1 strains DGM648/03, DGM1199/04, and DGM2107/04 exhibited 97% and 99% identity to Japanese and Italian G1 strains (JP-7265 [EF079066] and PA5/03 [DQ377595]), respectively. An additional G1 strain (DGM4799/04) exhibited 99% identity to G1 strains from India (ISO-4/02) and Thailand (1604-Thai and CMH036/04). From our data sets, the nucleotide sequences of South African G3 strains recovered in 2004 and 2005 were closely related to those of G3 strains from China, Russia, and Thailand, with 99% identity. At the nucleotide level, the South African G8 strains were closely related to a G8 strain identified in the Republic of Congo (DRC86/04 [DQ005120]), with 99% identity. The South African G9 strains were identical (99%) to strains from India and Thailand (S23 [AJ491187] and 97CM113 [AY866505]) and also had 99% homology to a southern African G9 strain detected during a previous rotavirus season in Botswana (BS1414/02 [DQ822599]). All the G12 strains detected were closely related to eastern Indian G12 strains, with 99% identity at the nucleotide level (ISO23 [DQ099753]).

Phylogenetic analysis. The evolutionary distances between South African strains and the reference strains were investigated using pairwise comparison from multiple sequence alignments. The G1 strains fell into 2 different lineages, with 1 strain collected in 2004 in lineage I and 3 strains collected in 2003 and 2004 in lineage II (Figure 1). The phylogenetic trees for G3, G8, G9, and G12 sequences are shown in Figures 2–4 , which appear only in the electronic version of the *Journal*. All South African G3 strains clustered with a G3 strain from Ireland (R472 [EU033979]) collected during 2003–2006, a G3 strain from Japan (5290/Japan [EF088832]) collected during 2001–2003, and a G3 strain from Madrid, Spain (115GMadrid [DQ440616]) collected in 2004 (Figure 2, which appears only in the electronic version of the *Journal*). The South African G8 strain clustered with all human African G8 strains detected after 2001 (data not shown). The South African G9 strains clustered in lineage IIId [33]; however, strain DGM2144/03 clustered with strains from Botswana, and strains DGM2171/03 and DGM2125/03 formed a separate sublineage (albeit with a low bootstrap value) and were more closely related to G9 strains from India, the United States, Malawi, and South Africa (Figure 3, which appears only in the electronic version of the *Journal*). The additional G12 strains identified and sequenced in this study clustered with similar G12 strains detected in South Africa during the same period (Figure 4, which appears only in the electronic version of the *Journal*) [34].

Table 2. Summary of Mixed Rotavirus Infections Circulating at Dr George Mukhari Hospital (South Africa) during 2003–2006

	No. of strains				
Genotype	2003	2004	2005	2006	Total
G1,2P[4]	2	0	0	0	2
G1,2P[8]	0	0	0	1	1
G1,2P[6,8]	0	1	0	1	2
G1,3P[8]	0	2	0	2	4
G1,8P[6]	0	0	0	1	1
G1,8P[8]	0	0	0	4	4
G1,8P[8,4]	0	0	0	1	1
G1,8P[8,10]	0	0	0	1	1
G1,9P[6]	0	0	0	2	2
G1,9P[8]	0	0	0	2	2
G1,2,8P[6]	0	0	1	0	1
G1,3,8P[8]	0	0	1	0	1
G1,3,12P[8]	0	0	0	1	1
G2,3P[4]	0	0	1	0	1
G2,3P[8]	0	0	1	0	1
G2,8P[8]	0	0	0	1	1
G2,9P[8]	0	0	0	1	1
G8,9P[8]	0	0	0	1	1
G8,9P[8,10]	0	0	0	1	1
G9,12P[6]	0	0	2	0	2
G1P[6,8]	1	2	1	3	7
G1P[4,6,8]	0	0	0	1	1
G2P[4,6]	1	0	0	0	1
G2P[4,8]	1	0	0	1	2
G3P[4,8]	0	0	0	1	1
G3P[6,8]	0	1	3	0	4
G8P[6,8]	0	0	0	1	1
G9P[4,6]	0	0	0	1	1
G9P[6,8]	1	0	0	1	2
G10P[8,4]	0	0	0	1	1
Total	6	6	10	30	52

DISCUSSION

This study complements both an ongoing rotavirus-associated burden of disease study at Dr George Mukhari Hospital and

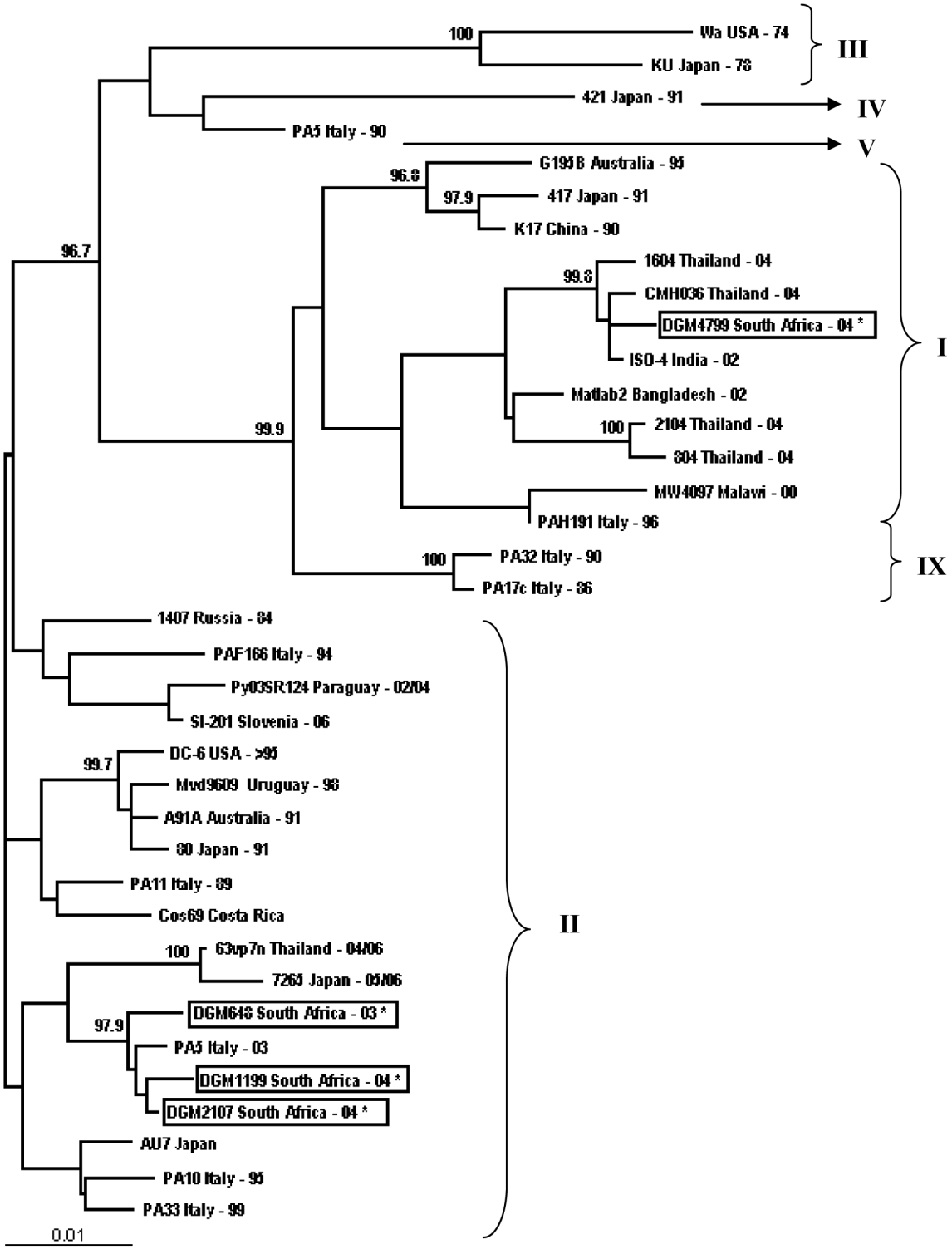

Figure 1. Phylogenetic analysis of the partial VP7 nucleotide sequences of South African and selected international G1 strains. The tree was constructed using the Kimura 2-parameter and neighbor-joining methods. The length of the abscissa to the connecting node is proportional to the genetic distance between sequences and is indicated by the scale bar. Bootstrap values >95% are indicated at the appropriate nodes. Serotype G1 lineages defined by Phan et al [45] are indicated where applicable. *Strains sequenced in this study.

This figure is available in its entirety in the online version of the *Journal of Infectious Diseases.*

Figure 2. Phylogenetic analysis of the partial VP7 nucleotide sequences of South African and selected international G3 strains.

the Rotarix trials that have been conducted in South Africa since 2001. Results from these studies emphasize the immense impact that rotavirus infection has on South African children and the South African health care system. A marked seasonal pattern of rotavirus infection was observed during the cooler and drier months of the year, with peak prevalence among children <5 years of age of 56%–59% [32].

Rotavirus strain surveillance is necessary before and during the introduction of rotavirus vaccines in Africa. Our study provides knowledge on or confirms previous findings of rotavirus strains cocirculating in South Africa. The current study indicates variations in the common rotavirus strain serotypes and genotypes over a 4-year period and also the variety shown in these rotavirus strains, as indicated by the presence of uncommon genotypes and mixed rotavirus infections in communities. This study complements and adds to previous molecular epidemiological studies performed in the Ga-Rankuwa area of South Africa [35–37].

From 2003 through 2006, as reported in this article, serotype G1 and G3 rotavirus strains were detected at a mean prevalence of 36% and 27%, respectively, followed by strains with the G2 serotype (14%). The balance of rotavirus infections were caused by serotype G9 (8%) and G8 (5%) strains. As expected, the predominant annual rotavirus strain profile varied considerably. In 2003, rotavirus strains with the G2 serotype were the most prevalent (50%), followed by strains with the G1 serotype (36%). By 2004, G1 was reestablished as the most predominant serotype. The incidence of strains with the G3 serotype significantly increased, and G3 was the second-most common serotype, closely followed by G12. By 2005, the rotavirus strain profile showed a massive predominance of strains with the G3 serotype (67%), followed by strains with the G1 serotype (12%), and G2 strains were absent. In 2006, G1 was the most common serotype, detected in 42% of cases, followed by G9 and G8.

During the 1980s, rotavirus strain surveillance in the same community showed that serotype G1 was the most frequently detected serotype (44%), followed by G4 (24%) and G2 (23%). During the same time, serotype G4 strains were also fairly common in the adjoining areas of Pretoria and Johannesburg [35]. Serotype G8 rotavirus strains, previously thought to be more common in cattle, were detected in humans in the mid-1980s in the same area [36]. Mixed infections were also detected in ~2% of cases [37, 38].

Compared with the serotypes circulating during the preceding decade, during the 1998–1999 season, serotype G1 predominated (67%), divided between G1P[8] and, to a lesser extent, G1P[6]. Serotype G2 was detected at comparable levels (20%), and serotype G3 was more frequently detected during this time than during the 10 previous years [38]. However, the G4 strains were detected at a very low frequency. G4 rotavirus strains have not been observed in South Africa and the rest of Africa for nearly 20 years (African Rotavirus Network Workshop, unpublished data) [38, 39].

Early studies indicated that the P[6] genotype was associated with asymptomatic, less virulent, neonatal infection [40]. However, the results from this study are consistent with previously published articles from Africa and Asia that report a high prevalence of the P[6] genotype in association with symptomatic infections and with a wide variety of G genotypes [11, 35, 40–42]. Strains with the P[6] genotype were in the minority during 1988–1989 and were most frequently associated with G4 strains. However, by the 1998–1999 season, P[6] strains were regularly detected with both G1 and G3 specificity. From 2003 through 2006, the prevalence of P[6] was still increasing, and P[6] was not only associated with G3 and G1 serotypes but also, to a lesser extent, with G9, G8, and G12 serotypes. These results seem to indicate that P[6] strains are common in newly introduced G types and emerge during interspecies transmission or by genetic reassortment between human and animal rotavirus strains [43, 44]. The epidemiological significance of the P[6] VP4 protein on rotavirus transmission and reassortment dynamics, therefore, requires additional investigation, and additional studies involving full genome sequencing will be required.

Serotype G1 strains are considered to be the most common strain in circulation worldwide. However, 11 genetic lineages have been described in G1 strains [45]. Analysis of South African G1 strains (2 from the 2003 season and 2 from the 2004 season) revealed that 3 were clustered in the G1 genetic lineage II, sublineage IIB [45] and were closely related to the Italian strain PA5/03. The single strain detected in 2004 clustered in G1 genetic lineage I, sublineage IA [46] and was closely related to G1 strains from Thailand and India. Continuous monitoring of G1 molecular epidemiology over time will aid in identifying multiple G1 lineages circulating in a population and any changes in these lineages from one season to the next.

In the Ga-Rankuwa area, cyclic emergences of serotype G2 strains have been reported with regularity, with high prevalence every 3–4 years. Thus, the high prevalence of G2 strains in 2003 may reflect the normal cyclical pattern previously noted [46],

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Figure 3. Phylogenetic analysis of the partial VP7 nucleotide sequences of South African and selected international G9 strains.

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Figure 4. Phylogenetic analysis of the partial VP7 nucleotide sequences of South African and selected international G12 strains.

and the significance of G2 or any other serotype emergence needs to be carefully monitored and analyzed after vaccine introduction.

Similar to G2 strains, G3 strains have also demonstrated cyclic patterns of emergence and disappearance and, in South Africa, were responsible for the majority of rotavirus infections during 2005. Phylogenetic analysis of South African serotype G3 strains from 2004 and 2005 revealed clustering with G3 strains from Ireland, Japan, and Spain in lineage 3 S4 (Figure 2, which appears only in the electronic version of the *Journal*) [47]. Phan et al [48] and Wang et al [47] suggested that G3 strains in lineage 3 S4 are part of a new G3 variant, and the detection and predominance in South Africa supports the emergence of this variant in Africa. The relationship between rotavirus strains from Africa and those from Asia and Europe was seen in the phylogenetic analyses of both G1 and G3 strains. Further monitoring of strains from Asia and Europe in Africa should be conducted to investigate the effects of the movement of persons and/or other reservoirs on the epidemiology of rotavirus stains in African populations.

In South Africa, the first G9P[6] strain was isolated from a child in the Pretoria area in October 1997 [33]. Subsequently, additional G9 strains were detected during the 1998 and 1999 rotavirus seasons. Serotype G9 strains were also detected during the latter part of 1998 from the neonatal ward of the Ga-Rankuwa Hospital when G9P[6] was predominant in the ward. These G9P[6] strains persisted in the ward during 1999, despite the introduction of G1P[8] and G1P[6] strains into the ward in early 1999 [33]. Despite periodical identification in South Africa, G9 strains have never been identified as the predominant circulating serotype in the general population, such as G9-predominant seasons seen in Ghana, Mauritius, and Kenya [33]. Phylogenetic analysis of the 2003 South African G9 strains revealed 2 sublineage clusters (Figure 3, which appears only in the electronic version of the *Journal*). The first was related to G9 strains detected in Botswana in 2002, and the second was related to strains from India, Malawi, the United States, and South Africa during the 1998 and 1999 seasons, but forming a distinct group. These results suggest that G9 strains continue to circulate at low levels in the South African population and are introduced from a pool of related strains and from neighboring countries.

Unusual rotavirus G12P[6] strains with long electropherotypes were detected for the first time in South Africa in late 2004 and early 2005 before the rotavirus season [34]. The G12 serotype has been reported in various areas of the world, including Thailand, Japan, India, Bangladesh, and Argentina [49, –53]. The G12 strains identified were almost identical to the amino acid sequences from strain ISO23 [DQ099753] from eastern India, with 99% homology. Recent strain surveillance studies have demonstrated the G12 serotype in at least 3 locations in South Africa and indicated that these strains appear to be a recent introduction to South Africa (Figure 4, which appears only in the electronic version of the *Journal*) [34]. The 3 additional G12 strains sequenced in this study also support the ideas that South African G12 strains emerged from multiple infection sources and are circulating at low levels in the South African population. On the basis of these observations, G12 rotavirus strains may be next important genotype to emerge, and current vaccine candidates will have to demonstrate efficacy against these strains.

In addition to G12 strains, serotype G8 strains were detected in 5% of cases and exhibited either short or long electropherotypic migration patterns (data not shown). Sequence analysis revealed that G8 strains associated with short electropherotypes were closely related to strains from the Republic of Congo (DRC86 [DQ005120]) and Malawi (MW23 [AJ278254]), with homology of 97% and 96%, respectively (data not shown). The high frequency of human G8 strains in Africa implies a continuous circulation of this strain in African populations, probably in part because of the close contact and living conditions between humans and bovines [10–12, 36, 54, 55].

At present, the rotavirus vaccines candidates RotaTeq and Rotarix have been designed to protect against the globally most common rotavirus strains G1–G4. In Africa, there is marked diversity of circulating, unusual rotavirus strains (African Rotavirus Network Workshop, unpublished data), and these findings are supported by the current study. Thus, improvements in vaccine development may require the addition of G8 and/or P[6] antigens for rotavirus vaccine formulation destined for use in Africa.

The increasing number of reports from different countries about the emergence of unusual G and P combinations raises concerns for the effectiveness of rotavirus vaccine candidates. Unusual P and G combinations observed in the current study were G1P[6], G1P[4], G2P[6], G3P[6], G3P[4], G8P[8], G8P[6], G8P[4], G9P[6], G9P[4], and G12P[6], which were responsible for 22%–33% of rotavirus infections. In a study by Iturriza-Gómara et al [56] on rotavirus strains in the United Kingdom during 1995–1999, reassortants of the most common circulating strains were detected in only 2% of isolates. The results from this study suggest a relatively high degree of natural reassortment and may be partially related to the poorer socioeconomic conditions of the surrounding communities served by the Dr George Mukhari Hospital.

Developing countries may show a higher frequency of mixed

infection. Mixed rotavirus infections were detected frequently in the Ga-Rankuwa study population, together with the annual predominant strain. From 2003 through 2005, mixed infections were detected in 4%–5% of cases, and in 2006, the number of mixed infections increased to 16%. More than half of the mixed infections ($n = 18$) detected in 2006 involved G8 or G9 strains (Table 2) and may account for the increase in mixed infections in 2006. These results may partially explain the promiscuous nature of G8 and G9 strains, because coinfections with these genotypes seem to be common, providing more opportunity for reassortment.

Populations in developing countries with constant exposure to many enteric pathogens may provide fertile ground for the generation of novel rotavirus strains. In the present study, 4.5% of the samples could only be partially typed, possibly indicating atypical rotavirus strains. The emergence of novel rotavirus strains in developing regions of the world needs to be taken into consideration where vaccine efficacy is concerned. It is therefore important to continue surveillance studies to monitor the rotavirus strains associated with severe gastroenteritis in hospital settings after the introduction of a rotavirus vaccine in countries with a high burden of rotavirus disease.

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