

Characterization of Group A Rotavirus Infections in Adolescents and Adults From Pune, India: 1993–1996 and 2004–2007

Vaishali S. Tatte,¹ Jon R. Gentsch,² and Shobha D. Chitambar^{1*}

¹Rotavirus Group, National Institute of Virology, Pune, India

²National Center for Immunization and Respiratory Diseases, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

A total of 1,591 fecal specimens were collected in 1993–1996 and 2004–2007 from adolescents and adults with acute gastroenteritis in Pune, India for detection and characterization of rotavirus. At the two time points, group A rotavirus was detected in 8.6% and 16.2% of the adolescents and 5.2% and 17.2% of the adults, respectively. Reverse transcription-PCR with consensus primers followed by multiplex genotyping PCR detected common strains G1P[8], G2P[4], G3P[8], and G4P[8] in a total of 53.1% of the samples from 1993 to 1996, while the only prevalent strain identified in 2004–2007 was G2P[4] (23.5% of total). Uncommon rotavirus strains (G1P[4], G2P[8] G9P[6]/P[4]) increased from 7.8% (1993–1996) to 41.2% (2004–2007), while the prevalence of mixed rotavirus infections was high (39%/35%) at both time points. Mixed infections detected by multiplex PCR were confirmed by sequencing two or more individual genotype-specific PCR products of the VP7 and VP4 genes from the same sample. Phylogenetic analysis of the sequences showed circulation of a heterogeneous rotavirus strain population comprising genotypes G1 (lineages I and IIb), G2 (lineages I and IIb), G4 (lineage Ia), P[4] (lineages P[4]-5 and P[4]-1), P[8] (lineages P[8]-II and P[8]-III), and P[6] (M37-like lineage). The VP6 gene sequences of the nontypeable strains were most homologous to animal strains. This study documents the molecular epidemiology of rotavirus strains in adolescents and adults in India, and suggests that it may be important to monitor these strains over time for the potential impact on rotavirus vaccines under development for use in the Indian population. **J. Med. Virol. 82:519–527, 2010.** © 2010 Wiley-Liss, Inc.

KEY WORDS: epidemiology; adult diarrhea; G and P types; rotavirus

INTRODUCTION

Group A rotaviruses are the most important etiologic agents of acute gastroenteritis in infants and young children worldwide. Annually, global mortality from rotavirus gastroenteritis has been estimated to be 611,000 with a majority of deaths occurring in low-income countries [Parashar et al., 2006].

Rotaviruses belong to the family Reoviridae, and are classified in seven antigenic groups (A–G), of which groups A, B, and C are known to infect humans. Group A rotaviruses infect all children in their first few years of life and account for the vast majority of rotavirus infections worldwide, while B and C rotaviruses are often found to be associated with outbreaks and sporadic cases. Morphologically, rotavirus is an icosahedral particle consisting of 11 segments of double stranded (ds) RNA encased within a triple layered capsid composed of VP4, VP6, and VP7 proteins. The VP6 protein carries antigens that determine group and subgroup specificities, while VP4 (P-protease sensitive) and VP7 (G-glycoprotein), the outer layer proteins carry epitopes for specific neutralizing antibodies and define different serotypes [Estes and Kapikian, 2007]. The two serotype antigens are encoded by separate genome segments that segregate independently of one another during mixed infections in vivo. As a result, a dual classification system to track both serotypes VP7 (G) and VP4 (P) serotypes has been established for rotaviruses.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention

*Correspondence to: Shobha D. Chitambar, Deputy Director & Group Leader, Rotavirus Department, National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001, Maharashtra, India. E-mail: chitambar@hotmail.com

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To date, 20 G and 28 P genotypes have been reported [Solberg et al., 2009], of which G1–G4, G9, P[4], P[6], and P[8] represent common strains detected among children worldwide [Gentsch et al., 2005; Santos and Hoshino, 2005]. Reassortment in vivo among rotaviruses of the known genotypes has led to the isolation of strains with more than 50 different G–P combinations. However, only the five most common types (G1–G4, P[8]) have been targeted in rotavirus vaccines. Countries considering use of these vaccines conduct surveillance to identify the most common strains in circulation so that subsequent impact of vaccines on circulating strains can be assessed. Virtually, all such studies are conducted in infants.

Group A rotaviruses are also known to cause gastroenteritis among adults in a variety of settings including epidemic outbreaks, travel-related gastroenteritis, infections transmitted from children to adults and endemic disease [Hrdy, 1987]. Only a few studies document the genotypic diversity of rotaviruses and occurrence of unusual rotavirus strains in adults that may pose challenges to new rotavirus vaccine programs [Anderson and Weber, 2004; Fischer et al., 2005; Wang et al., 2007].

The aim of this study was to investigate the molecular epidemiology of rotaviruses causing sporadic acute gastroenteritis in both adolescents and adults from Pune, western India at two different time points.

MATERIALS AND METHODS

Specimens

A total of 1,591 stool specimens were collected from adolescent (10–18 years, $n = 211$) and adult (>18 years, $n = 1,380$) cases admitted to or visiting out-patient departments in local hospitals from the city of Pune for acute gastroenteritis at two time points, 1993–1996 ($n = 1,338$) and 2004–2007 ($n = 253$). The small number of samples analyzed at the later time point was due to less number of admissions in the hospitals that were routinely visited by National Institute of Virology, Pune. The ethical committee of National Institute of Virology approved the methods for specimen collection. Epidemiologic data inclusive of age, date of diarrhea onset, date of specimen collection were available from a majority of the patients. Ten percent (w/v) stool suspension of each of the specimens was prepared in 0.01M phosphate buffered saline (PBS) pH 7.2 containing 0.01M CaCl_2 . The suspensions were centrifuged at 805g for 15 min to remove debris. The supernatants were stored at -70°C until tested for rotavirus antigen and genotype.

Detection of Group A Rotavirus Antigen

All specimens were tested for the presence of rotavirus by an in-house antigen capture ELISA [Kelkar et al., 2004]. Stool specimens known to be negative and positive for rotavirus and culture supernatants of normal and human rotavirus serotype G1 infected MA-104 cells were included as controls. Specimens having optical density (OD) values above the cut off

value ($2.0 \times$ OD of negative control wells) were considered positive for rotavirus antigen.

RNA Extraction, RT-PCR and Genotyping

Rotavirus ds RNA was extracted from stool specimens by using TRIZOL reagent (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol. The VP7 and VP4 genes were genotyped by multiplex reverse transcription (RT)-PCR according to the method described earlier [Gouvea et al., 1990; Gentsch et al., 1992] with a minor modification in the thermal cycling programme [Chitambar et al., 2008]. The rotavirus strains that remained nontypeable (in this protocol) were subjected to one step RT-PCR (Qiagen, Hilden, Germany) using following sets of primers: 9Con1-L/VP7-Rdeg [Freeman et al., 2008] and Con 3 5'-TGG CTT CGC TCA TTT ATA GAC A-3'/Con 2 provided by Centers for Disease Control and Prevention, Atlanta, USA [Gentsch et al., 1992]. Briefly, 4 μl of extracted rotavirus ds RNA was used for RT by denaturing at 95°C for 5 min and then chilling in ice for 2 min. A reaction mix of 46 μl containing $5 \times$ buffer, dNTPs, Rnase-free water, primers 9Con1-L/Con3 and VP7-Rdeg/Con2 and 2 μl of enzyme mix was added to make a final volume of 50 μl . The reaction tubes were placed in a thermocycler for 30 min at 45 and 95°C for 15 min followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, 70°C for 2.5 min with a final extension at 70°C for 7 min. One step RT-PCR based on a target region of 220 bp in gene segment 6 was also performed on all nontypeable specimens [Wilde et al., 1990].

All PCR products, including that from the first-round and multiplex PCRs, were analyzed by electrophoresis using $1 \times$ Tris acetate EDTA (TAE) buffer pH 8.3 on 2% agarose gels, containing ethidium bromide (0.5 $\mu\text{g}/\text{ml}$) and visualized under UV illumination.

Nucleotide Sequencing and Phylogenetic Analysis

The PCR products obtained for gene 6 (220 bp), and multiplex PCR products of genes 7 and 4 were purified on minicolumns (QIAquick; Qiagen, Valencia, CA) and sequencing was carried out using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster city, CA) and a ABI-PRISM 310 Genetic Analyzer (Applied Biosystems).

The sequences of genes 6, 7, and 4 were aligned with the sequences of rotavirus strains available in the GenBank by using Clustal X version 1.83 [Thompson et al., 1997]. The phylogenetic analyzes were carried out in MEGA 3 by using Jukes-Cantor (JC) distance and Neighbour Joining (NJ) algorithm [Kumar et al., 2004]. The reliability of different phylogenetic groupings was confirmed by using the bootstrap test (1,000 bootstrap replications) available in MEGA 3.

Accession Numbers

The sequences of VP7, VP4, and VP6 genes of this study have been submitted to the GenBank under the accession numbers FJ623188–FJ623262.

TABLE I. Rotavirus Prevalence Among Adolescent and Adult Cases of Acute Gastroenteritis by ELISA in 1993–1996 and 2004–2007

Patients with age	No. of positive/no. of tested (%)		Total
	1993–1996	2004–2007	
Adolescents	15/174 (8.6)	6/37 (16.2)	21/211 (10)
Adults	60/1,164 (5.2) a	37/216 (17.2) b	97/1,380 (7.0)

a versus b: $P < 0.01$.

Statistical Analysis

The proportions across two different periods were compared using the chi-squared test with Yates's correction and the proportions within the same period were compared by Binomial test. P -values < 0.05 were considered statistically significant.

RESULTS

Rotavirus Detection

In total, rotavirus was detected in 10.0% (21/211) and 7% (97/1,380) of the specimens collected from adolescents and adults, respectively. Overall, the combined prevalence values obtained for the two time points (1993–1996 and 2004–2007) were not different ($P > 0.05$) in two groups examined in the study (Table I). Age distribution analysis showed that most rotavirus infections occurred in the 10–14 years age group for adolescents and in the 19–40 years group of adults (Fig. 1). Significantly higher prevalence of rotavirus was observed during 2007 for adults ($P < 0.01$) but not for adolescents ($P > 0.05$; Fig. 2). Rotavirus infections occurred year round for both time points (Fig. 3) with peak activity in March during 1993–1996 and in August for 2004–2007.

Genotyping

VP7 and VP4 typing was conducted for all 118 rotavirus positive samples from adolescent ($n = 21$) and adult ($n = 97$) cases of acute gastroenteritis. Both genes were successfully genotyped in 86.7% and 85% of the specimens, respectively, during 1993–1996 but only 50% and 37.8% of the specimens could be typed in 2004–2007. Though the number of nontypeable rotavirus

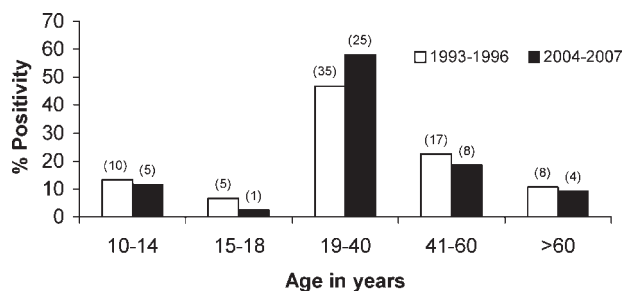


Fig. 1. Age distribution of rotaviruses among adolescent and adult cases of acute gastroenteritis in 1993–1996 and 2004–2007. The figures in parentheses indicate total number of specimens tested.

strains was higher in 2004–2007 for both groups of patients, it was not statistically different from that of the 1993–1996 ($P > 0.05$; Table II).

G-Typing

Among the strains typeable for VP7 or VP7 and VP4 ($n = 75$) recovered during 1993–1996, G1 and G2 were predominant genotypes at 26.7% of total followed by G3 (20.0%), G4 (13.3%), and G9 (6.7%) in adolescents. However, in adults there was predominance of G1 (30.0%) followed by G2 (28.3%), G4 (10.0%), G9 (8.3%), and G3 (1.7%) in adults. Mixed infections caused by different genotypes were 6.7% and 8.3%, in adolescents and adults, respectively. Nontypeable specimens (10.0%) made up the remainder. Among 43 specimens analyzed in 2004–2007, nontypeable strains were predominant in both groups [50% (3/6) in adolescents and 64.9% (24/37) in adults] followed by mixed infections (33.3%) and G2 (16.7%) strains in adolescents, and by G9 (13.5%), G2 (10.8%), G1 (2.7%), G3 (2.7%), G4 (2.7%), and mixed infections (2.7%) in adults.

P-Typing

At both time points, for rotavirus strains (15 in 1993–1996 and 6 in 2004–2007) in adolescents, the most frequently detected genotype was P[8] (33.3%/33.3%) followed by mixed infections (26.6%/16.7%), nontypeables (13.3%/33.3%), P[4] (20.0%/16.7%), and P[6] (6.7%/0.0%). In adults, during 1993–1996 ($n = 60$), mixed infections were the most common (33.3%) followed by P[4] (28.3%), P[8] (25.0%), P[6] (8.3%), and nontypeable (5.0%) strains, while in 2004–2007 ($n = 37$), P[4] was predominant (40.5%) followed by mixed infections (32.4%), P[8] (16.2%), and nontypeable (10.8%) strains.

G–P Combinations

A total of 32 different G–P combinations were detected in 81 rotavirus strains that were typed for both genes (Table III). Overall, globally common G1 P[8], G2P[4], G3P[8], and G4P[8] rotavirus genotypes were detected in 53.1% (34/64) of the strains from adolescents (12.5%) and adults (40.6%) during the years 1993–1996. In contrast, G2P[4] was the only common strain detected in 2004–2007 (23.5%, [4/17]). Thus, uncommon types predominated in 2004–2007 (Table III). Unusual G–P combinations—G1P[4], G2P[8], G3P[4], G4P[4], G9P[4], and G9P[6] were found at both time points, but only in adults. Twenty-one different mixed infections with diverse combinations of G–P types were detected in adolescents and adults (Table III). Triple infections with either G or P types were detected in individual patients in 1993–1996.

Sequencing and Phylogenetic Analysis of VP7 and VP4 Genes of Rotavirus Strains Detected in Multiple Infections

Analysis of VP7 gene sequences of 20 rotavirus strains detected in 9 of 31 total mixed infections confirmed that

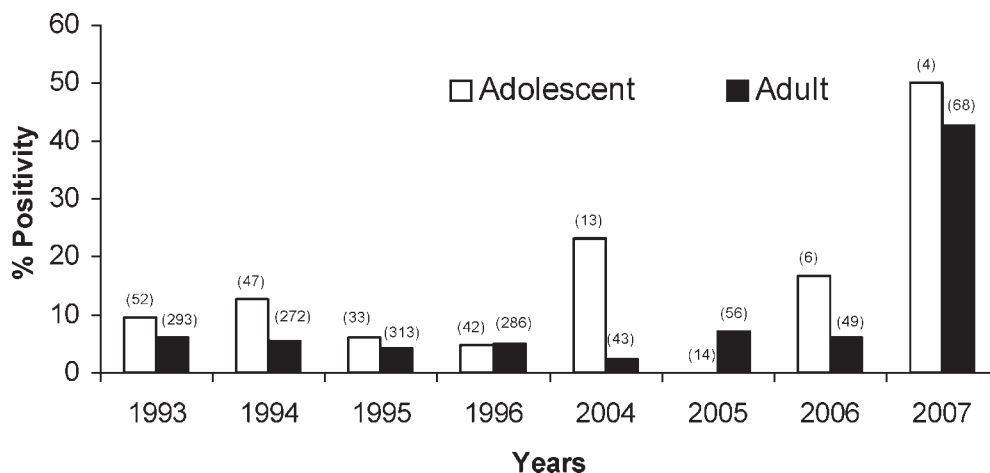


Fig. 2. Yearly distribution of rotaviruses among adolescent and adult cases of acute gastroenteritis in 1993–1996 and 2004–2007. The figures in parentheses indicate total number of specimens tested.

the multiplex assay detected true mixed infections. The 20 genes sequenced came from specimens with the following genotypes; 4 G1/G2, 1 G1/G4, 2 G2/G4, and 2 G1/G2/G4. Thus, in total 7 G1, 8 G2, and 5 G4 genotypes were detected in the mixed infections. All G1 strains clustered in lineage I of G1 genotypes with nucleotide divergence of 6.3–8.4% from the prototype strains KU and Wa of lineage III (Fig. 4). The G2 strains clustered in lineages II b (5/8) and I (3/8) with nucleotide divergence of 5.3–6.1% from the prototype strain DS-1 of lineage I. The G4 strains showed 0.3–1.3% nucleotide divergence from the prototype strain Hocht (Fig. 4).

Analysis of the VP4 gene sequences of 50 rotavirus strains detected in a total of 31 mixed infections confirmed the presence of genotypes P[4](19), P[8](18), and P[6](13). Most of the P[4] strains (17/19) clustered in the lineage P[4]-5, while two strains clustered in lineage P[4]-1, with divergence of 2.8–10% from the prototype lineage P[4]-1 strain DS-1. Analysis of the P[8] strains revealed clustering of 8, 9, and 1 strains in lineages P[8]-2, P[8]-3, and P[8]-4, respectively, with 0–8.6% nucleotide divergence from the prototype strain, KU of lineage P[8]-1. Most of the P[6] strains clustered in the M37 like lineage with 1.1–4.6% divergence (Fig. 5).

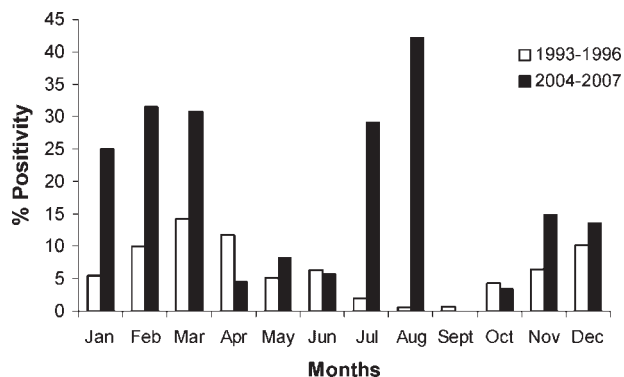


Fig. 3. Monthly distribution of rotaviruses among adolescent and adult cases of acute gastroenteritis in 1993–1996 and 2004–2007.

Characterization of Untypeable Specimens

VP6 genes of rotavirus strains recovered in 2004–2007, that were nontypeable for both the VP7 and VP4 genes had 99.5–100% nucleotide identity with their counter parts in bovine/simian strains (Table IV).

DISCUSSION

In India, extensive studies have been carried out on characterization of the rotavirus strains causing acute gastroenteritis among children [Ramachandran et al., 1996; Jain et al., 2001; Kang et al., 2005; Ramani and Kang, 2007], while studies in adults with acute gastroenteritis were limited to only serologic aspects of rotavirus infections [Kelkar and Ayachit, 2000; Ray and Kelkar, 2004]. This study reports, for the first time, the epidemiologic and molecular characteristics of rotavirus infections in adolescent and adult cases of acute gastroenteritis from India. The studies conducted in countries from Europe, America, Asia, and Australia have reported 2–40% rates of rotavirus prevalence in adults with gastroenteritis [del Refugio Gonzalez-Losa et al., 2001; Anderson and Weber, 2004; Faruque et al., 2004; Rubliar-Abreu et al., 2005]. However, there is no comparative data available for different time points in the same country or region. In the study presented here, the observed prevalence of rotavirus infection in 2004–2007 (16.2%/17.2%) was higher than that in 1993–1996 (5.2%/8.6%) demonstrating a rise in rotavirus infections in Indian adolescents and adults.

The occurrence of rotavirus infections was noted throughout the year with increased rotavirus activity in the winter (November–February) and rainy (June–August) months (Fig. 3). This is distinct from seasonal rotavirus infections in temperate climates [Cook et al., 1990], but similar to previous studies reporting a lack of seasonality with a deviation of infections towards warmer months in children and adults from tropical countries [Maldonado and Yolken, 1990; Carraro et al., 2008].

TABLE II. VP7(G)-VP4(P) Type Distribution in Rotavirus Positive Specimens From Adolescent and Adult Cases of Acute Gastroenteritis in 1993–1996 and 2004–2007

Category of specimens	No. of positive/no. of tested (%)			
	1993–1996		2004–2007	
	Adolescents	Adults	Adolescents	Adults
Typed for both G and P	13/15 (86.7)	51/61 (85.0)	3/6 (50)	14/37 (37.8)
Typed only for G	2/2 (100)	3/9 (33.3)	0/3 (0)	1/23 (4.3)
Typed only for P	0/2 (0)	6/9 (66.6)	1/3 (33.3)	19/23 (82.6)
Nontyped for both	0/2 (0)	0/9 (0)	2/3 (66.7)	3/23 (13.0)

In the study presented here, the contribution of common G–P types and mixed infections (59/64) to diarrhea was significantly higher than the uncommon types (5/64) in adolescents and adults in 1993–1996 ($P < 0.01$ by Binomial test, Table III). Interestingly, this pattern was not retained in 2004–2007 when significantly higher numbers of infections were due to uncommon and mixed types (13/17) ($P < 0.05$ by

TABLE III. Distribution of G and P Typed Rotavirus Strains From Adolescent and Adult Cases of Acute Gastroenteritis in 1993–1996 and 2004–2007

G–P types	No. of positive (%)	
	1993–1996 (n = 64)	2004–2007 (n = 17)
Common types (total)	34 (53.1)	4 (23.5)
G1P[8]	11 (17.2)	0 (0.0)
G2P[4]	17 (26.5)	4 (23.5)
G3P[8]	3 (4.7)	0 (0.0)
G4P[8]	3 (4.7)	0 (0.0)
G9P[8]	0 (0.0)	0 (0.0)
Uncommon types (total)	5 (7.8)	7 (41.2)
G1 P[4]	0 (0.0)	3 (17.6)
G2 P[8]	1 (1.5)	0 (0.0)
G3 P[4]	0 (0.0)	1 (5.9)
G4 P[4]	0 (0.0)	1 (5.9)
G9 P[4]	0 (0.0)	2 (11.8)
G9 P[6]	4 (6.3)	0 (0.0)
Mixed types ^a (total)	25 (39.0)	6 (35.3)
G1, P[6], P[8]	2 (3.1)	0 (0.0)
G1, P[4], P[6]	1 (1.6)	0 (0.0)
G1, P[4], P[8]	2 (3.1)	0 (0.0)
G1, P[4], P[6], P[8]	3 (4.7)	0 (0.0)
G1, G2, P[8]	1 (1.6)	1 (5.9)
G1, G2, P[4], P[6]	1 (1.6)	0 (0.0)
G1, G4, P[6]	1 (1.6)	0 (0.0)
G1, G2, P[4]	1 (1.6)	1 (5.9)
G2, P[4], P[8]	0 (0.0)	1 (5.9)
G2, P[4], P[6]	1 (1.6)	0 (0.0)
G2, G4, P[4]	1 (1.6)	0 (0.0)
G2, G4, P[4], P[8]	0 (0.0)	1 (5.9)
G3, P[4], P[6], P[8]	1 (1.6)	0 (0.0)
G4, P[4], P[8]	2 (3.1)	0 (0.0)
G4, P[4], P[6], P[8]	2 (3.1)	0 (0.0)
G9, P[4], P[8]	0 (0.0)	1 (5.9)
G9, P[4], P[6]	2 (3.1)	1 (5.9)
G2, G3, P[4], P[6], P[8]	1 (1.6)	0 (0.0)
G1, G2, P[4], P[6], P[8]	1 (1.6)	0 (0.0)
G1, G2, G4, P[4], P[8]	1 (1.6)	0 (0.0)
G1, G2, G4, P[4], P[6], P[8]	1 (1.6)	0 (0.0)

^aConfirmed by sequencing as shown in the Figures 4 and 5.

Binomial test). While most G–P combinations were of the uncommon variety during 2007, all of the individual G and P genotypes except P[6] were those recognized as globally common. These results are in contrast to those reported for children with rotavirus gastroenteritis from western India where common genotypes were more prevalent than uncommon types [Zade et al., 2009], but similar to those from southern and eastern India where uncommon G–P combinations were detected frequently among children [Ramchandran et al., 1996; Das et al., 2002]. In this study, uncommon G–P serotype combinations were detected in Indian adults for the first time. Thus, as is the case in children, a wide variety of reassortant rotavirus strains cause diarrhea in adults. In addition, circulation of such strains, for example, G9P[4] which do not share serotype antigens with the vaccine could influence success of vaccination programs, if protective immunity to such strains is lower.

In the present study, the proportion of mixed infections (39.0%/35.3%) detected among adolescents and adults at both time points (Table III), was relatively high compared to that reported for children from India [Ramchandran et al., 1996; Sharma et al., 2008], Brazil [Timenetsky et al., 1996], Bangladesh [Unicomb et al., 1999], UK [Iturriza-Gomara et al., 2000a], Tunisia [Chouikha et al., 2007], and Denmark [Fisher et al., 2005]. In order to confirm that these represented true mixed infections and identify VP7/VP4 lineages present in infected adolescents and adults, the DNA products obtained in multiplex PCR were sequenced. It is of note that the distribution of rotavirus strains (G1, G2, G4, P[4], P[8], and P[6]) in the lineages/sublineages (Figs. 4 and 5) was similar to that reported for children from different countries [Xin et al., 1993; Iturriza-Gomara et al., 2000b; Kudo et al., 2001; Cunliffe et al., 2001; Bok et al., 2002; Banyai et al., 2004; Page and Steele, 2004; Arista et al., 2006; Mascarenhas et al., 2006; Ansaldi et al., 2007; Araujo et al., 2007; Espinola et al., 2008]. Interestingly, cocirculation of two P[4] lineages was observed for the first time. Unlike the VP7 and VP4 genes of adult strains that were homologous to common human strains, some VP6 genes of these strains were most homologous to bovine and simian rotaviruses (Table IV). This also contrasted with the VP6 genes of strains from children that are typically closely related to other common human rotavirus strains

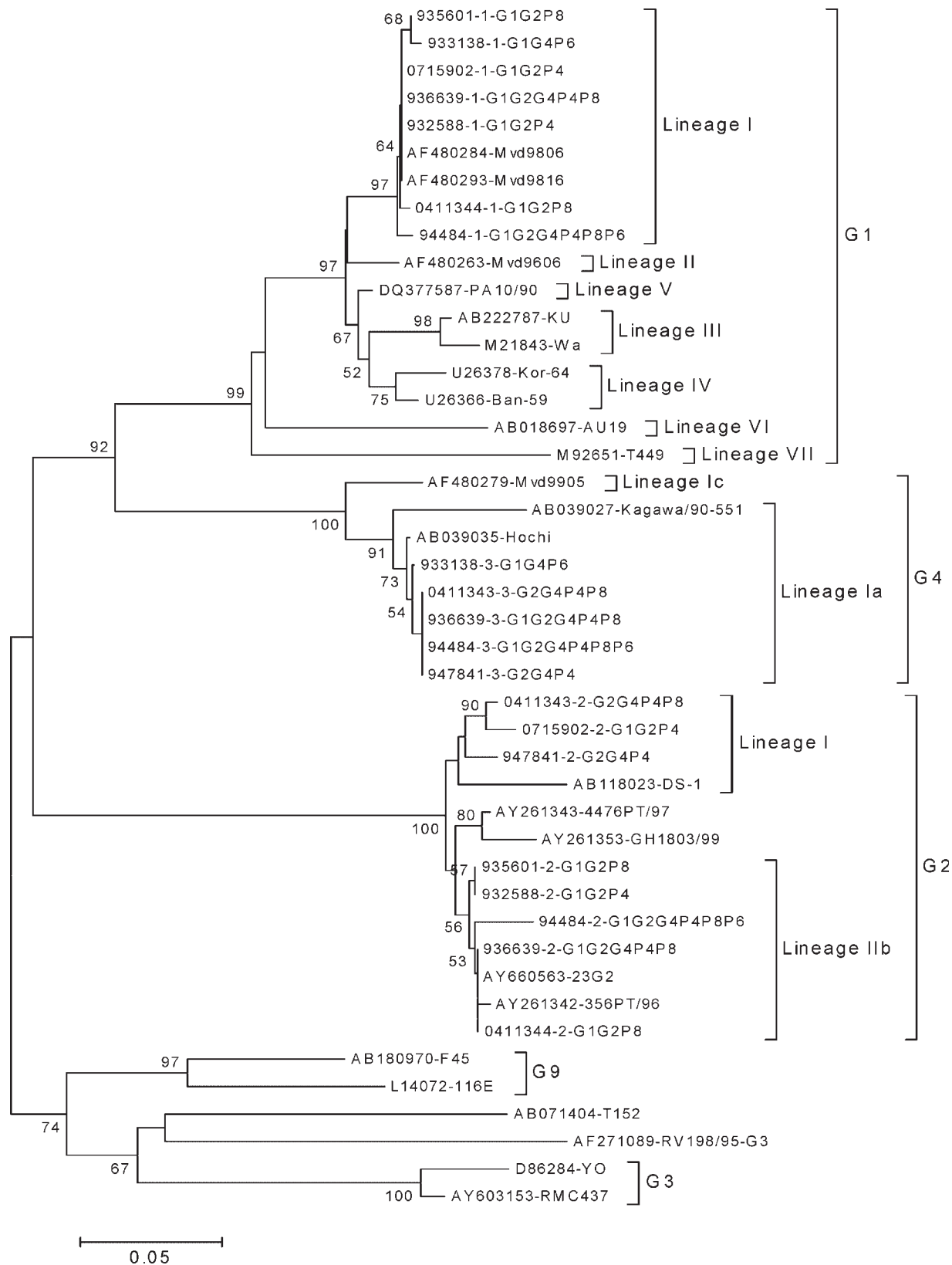


Fig. 4. Phylogenetic tree based on the partial nucleotide sequences of VP7 gene (nt 344–904 for G1, nt 448–903 for G2, and nt 514–899 for G4 strains) from mixed infections. The strains of the present study are identified by the specimen number, number in order of detection in genotyping followed by genotypes identified in mixed infections. The reference strains included for comparison are indicated by accession numbers and strain names.

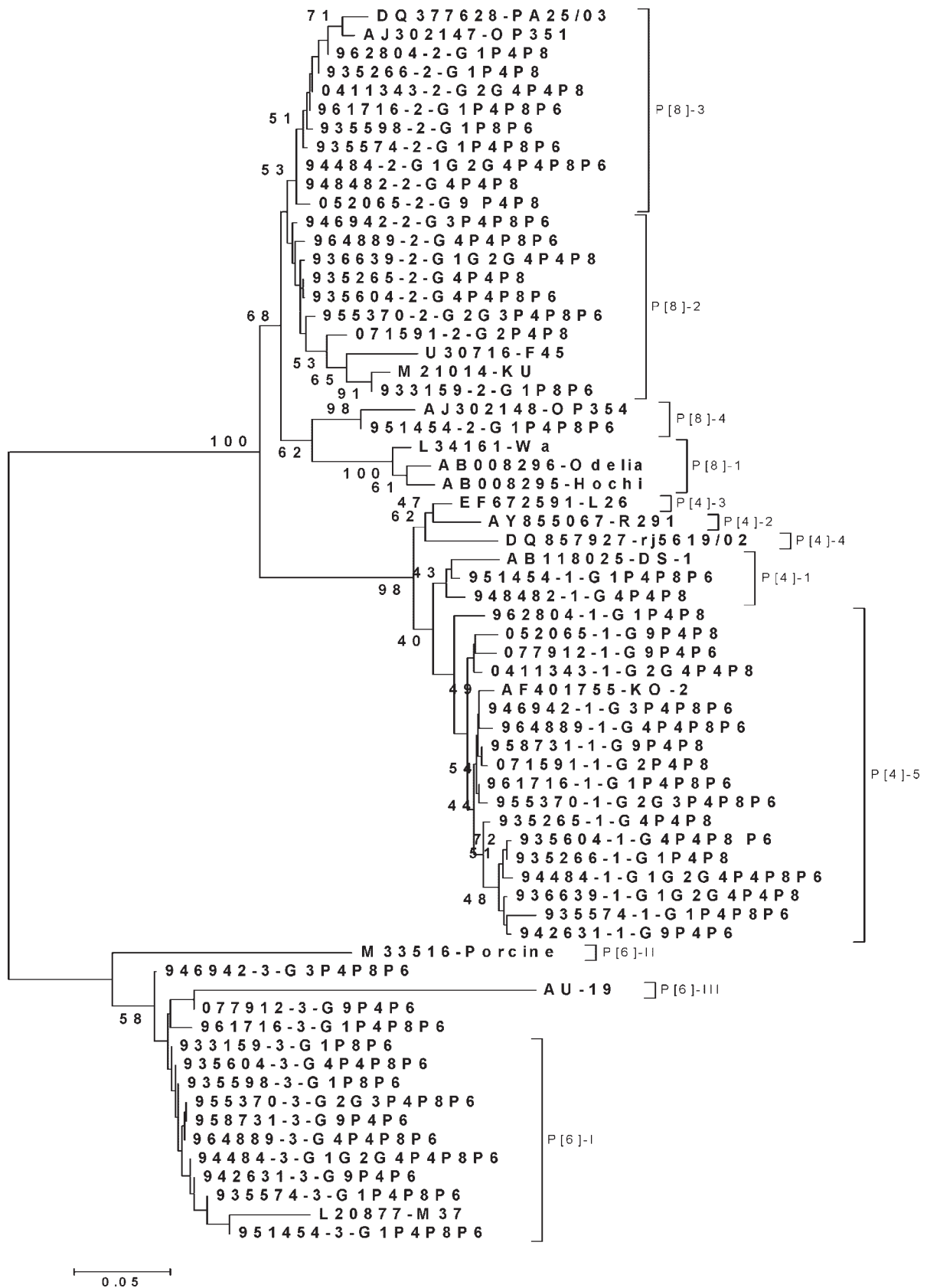


Fig. 5. Phylogenetic tree based on the partial nucleotide sequences of VP4 gene (nt 41–460 for P[4], nt 41–310 for P[8], and nt 41–250 for P[6] strains) from mixed infections. The strains of the present study are identified by the specimen number, number in order of detection in genotyping, followed by genotypes identified in mixed infections. The reference strains included for the comparison are indicated by accession numbers and strain names.

TABLE IV. Characterization of VP6 Gene of Nontypeable Rotavirus Strains

Specimen no.	Age (years)	VP6 gene identity (%)/ animal species/type	VP7 gene	VP4 gene
071586	15	99.5/bovine/G6 P[1]	NT	NT
079232	40	99.5/bovine/G6, P[14]	NT	NT
079566	60	100/simian/SA-11H-96	NT	NT
077916	24	100/simian/SA-11H-96	NT	NT
079228	10	100/simian/SA-11H-96	NT	NT

NT, nontypeable.

[Iturriza-Gomara et al., 2002; Kerin et al., 2007]. These findings suggest that strains causing infections in adolescents and adults need to be studied further to examine the extent of reassortment in other genes to determine their origin.

Although rotaviruses have been recognized for several decades as the most common cause of gastroenteritis in infants and children, their role as a pathogen in adults has not been investigated sufficiently. Comparative analysis of rotavirus strains/genotypes in different age groups is needed for better understanding of the spread of rotavirus in the community. It is to be noted that the studies conducted in cattle to determine the prevalence of enteric virus shedding have suggested the possibility of adult animal as a source of rota or coronavirus infection in calves [Crouch and Acres, 1984]. Since natural infections with rotavirus do not confer life-long immunity, adolescents and adults may repeatedly undergo infections, which can vary from asymptomatic status to severe sustained infections depending on the immune status of the host. Under such circumstances, asymptomatic adults infected with rotavirus could potentially serve as a reservoir to maintain rotavirus circulation in the population [Pietruchinski et al., 2006]. Further, adult infections may serve as another source of rotavirus genomic diversity, which may limit the beneficial use of rotavirus vaccines being introduced in children from India.

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