DISTRIBUTION OF HUMAN ROTAVIRUS G AND P GENOTYPES IN A HOSPITAL SETTING FROM NORTHERN INDIA

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Abstract. Rotavirus gastroenteritis is a major cause of severe dehydrating diarrhea in children worldwide. Rotavirus G and P genotyping is essential for epidemiological surveillance and for better formulation of candidate rotavirus vaccines. Out of 862 diarrheal stool samples collected from hospitalized children aged < 2 years during February 2005 - March 2007, 318 (36.9%) were positive for rotavirus by ELISA. G and P genotyping was performed on 100 randomly selected positive samples using a seminested multiplex RT-PCR assay. The result of G genotyping indicates G1 (60%) was the most predominant VP7 type, followed by G2 (16%), G9 (8%) and G3 (3%). Two cases of G12 genotype were also observed. P genotypes identified were P[8] (40%) followed by P[4] (26%) and P[6] (17%). The most common G-P combinations were G1P[8] (26%), followed by G1P[4] and G1P[6]. Mixed infection involved 28% of strains. In this study the G1 and P[8] genotypes were the leading G and P types. Two cases with G12 genotype were also observed during the study.

Key words: rotavirus, G type, P type, India

INTRODUCTION

Group A rotaviruses are the major etiological agents of severe infantile diarrhea, which accounts for 48-78% of childhood diarrhea cases worldwide. Each year more than 125 million infants and young children develop rotavirus-associated diarrhea, resulting in 440,000 infant and child deaths, mostly in developing countries (Parashar *et al,* 2006). Improvements in hygiene and sanitation alone are not sufficient to decrease or eliminate the burden of rotavirus associated morbidity and mortality (Cunliffe and Nakagomi, 2005). Vaccination is the only tool for preventing the morbidity and mortality caused by rotavirus. Recently, two new live oral attenuated rotavirus vaccines (Rotarix and RotaTeq) from the multinational manufacturers GlaxoSmithKline and Merck, respectively, were licensed. The Rotarix vaccine is a monovalent vaccine containing a single P1A[8] genotype. However, RotaTeq is a pentavalent bovine-human reassortment vaccine (Dennehy, 2008).

Rotavirus is a member of the family Reoviridae and consists of 11 segments of double stranded RNA surrounded by a triple layered viral particle. On the basis

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of antigenic specificities of capsid proteins and the electrophoretic mobility patterns of 11 RNA segments of the viral genome, rotaviruses are classified into 7 different sero groups (A to G). Of these 7 sero groups, only 3, A-C, are known to infect humans. For group A rotavirus, typing schemes were introduced based on antigenic epitopes on the proteins that form the inner capsid (VP6; subgroups I and II) (Greenberg *et al,* 1983). Rotavirus can also be classified into several serotypes using neutralization assays with panels of antisera and genotyped on the basis of two outer capsid proteins, the glycoprotein VP7 (defining G genotypes) and the spike protein VP4 (defining P genotypes) (Gentsch *et al,* 1992). These two structural proteins elicit neutralizing antibodies in the host and encoded by VP7 and VP4 genes of rotavirus, respectively. So far, more than 15 G and 26 P genotypes have been described in humans and in a variety of animals (Martella *et al,* 2006). The incidence and distribution of G and P genotypes that cause disease in humans may vary by geographical location and by year. The major human G types are G1, G2, G3, G4, and G9, which combined with the P types P[8], P[4], and P[6], account for >80% of rotavirus associated gastroenteritis episodes worldwide (Gentsch *et al,* 2005; Santos *et al*, 2005).

Knowledge about the diversity and distribution of G and P types circulating in the population is critical for the formulation of an adequate vaccine as well as for the evaluation of protection after vaccination. Therefore the aim of the present study was to determine the prevalence of rotavirus G and P genotypes present in fecal samples obtained from northern Indian children less than 2 years of age who were suffering from acute diarrhea.

MATERIALS AND METHODS

A total of 862 stool samples were collected from children aged < 2 years, admitted to the Pediatrics Department of LNJP Hospital, New Delhi, due to acute diarrhea, during February 2005-March 2007. All stool samples collected in the hospitals were transported within 2 hours to the testing laboratory (Microbiology Department of Maulana Azad Medical College, New Delhi). Samples were tested for the presence of human rotavirus VP6 antigen by solid phase sandwich type Enzyme Immuno Assay using a Premier™ Rotaclone ELISA kit (Meridian Bioscience, OH). Of 862 samples, 318 (36.9%) were positive for VP6 antigen. Stool samples were stored at -70ºC until further processed. One hundred samples were randomly selected from the 318 VP6 antigen positive stool samples and genotyped into their respective G and P types by RT-PCR.

RNA extraction

PBS suspensions of fecal samples 10% were clarified by centrifugation at 10,000*g* for 10 minutes. Genomic RNA was extracted from 140 µl of 10% stool suspensions using a spin column technique according to the manufacture's instructions. (QIAamp Viral RNA mini kit from QIAGEN Gmbh, Hilden, Germany).

RT-PCR

Reverse transcription was carried out by heating 10 µl of extracted viral genome at 95ºC for 5 minutes and chilling on ice for 5 minutes. Fifteen microliters of RT reaction mixture was added, yielding a total reaction volume of 25 µl. It consisted of 50 mM Tris-HCl (pH 8.3), 4 mM $MgCl_2$, 10 mM DTT, 50 mM KCl, a 0.4 mM concentration of each dNTP (Fermentas Life Sciences), 0.4 µM each of consensus primers (9 Con1

and 9 Con2 for G typing and Con3 and Con2 for P typing), and 100 units of RevertAid H Minus M-MuLV Reverse Transcriptase (MBI Fermentas, Life Sciences). The RT step was carried out at 42ºC for 1 hour, and was stopped by heating the reaction mixture to 70ºC for 15 minutes. The RT product was then maintained at 4ºC.

Rotavirus G genotyping

G genotyping was performed by seminested multiplex PCR. In the first round of PCR, consensus primers 9 Con1 and 9 Con2 (complementary to the conserved region of the VP7 gene) were used to amplify a 905 bp region. The second round of typing PCR incorporated the 9 Con1 consensus primer and G type specific primers for the G1, G2, G3, G4, G9 and G12 types.

The first round PCR mixture consisted of 18 mM Tris-HCl (pH 8.3) 45 mM KCl, 0.4 mM each of dNTP, 1U of Taq DNA polymerase (Fermentas Life Sciences) and a 0.4 µM concentration of each consensus primer. The first round PCR was performed by adding 6 µl of cDNA to 19 µl of the PCR mix. After denaturation at 94ºC for 3 minutes, 35 cycles at 94ºC for 30 seconds, 45ºC for 30 seconds, 72ºC for 60 seconds, and a final extension of 7 minutes at 72ºC were carried out in a thermal cycler.

The PCR mixture for the second round of amplification consisted of 18 mM Tris-HCl (pH 8.3), 45 mM KCl, 0.4 mM of dNTP, 1 U of Taq DNA polymerase (Fermentas Life Sciences) and 0.4 µM of consensus primer 9 Con1 and 0.2 µM concentration of each G type specific primer. The second round of PCR was performed by adding 1 µl of the first PCR product to 24 µl of the PCR mix. This was denatured at 94ºC for 3 minutes. followed by 35 cycles of 94ºC for 30 seconds, 48ºC for 30 seconds, 72ºC for 60 seconds, and a final extension at 72ºC for 7 minutes.

Rotavirus P genotyping

A seminested multiplex type specific PCR was used for P typing. In the first round of PCR, consensus primers Con3 and Con2 (complementary to the conserved region of the VP4 gene) were used to amplify a 877 bp region. The consensus Con3 and the P type specific primers for P4, P6, P8 and P11 were used during the second round of PCR. The PCR mixture composition and thermal conditions for the first and second rounds of amplification were the same for the G typing, except for the primers used for amplification.

Agarose gel electrophoresis

All amplified PCR products after the first and second rounds of PCR were subjected to electrophoresis on 2% agarose gel containing 0.5 µg/ml of ethidium bromide and observed under ultraviolet light. Specific segment sizes for different G and P genotypes were observed.

Nucleotide sequencing

For group A rotavirus isolates that were G and P nontypeable by the second round of semi nested multiplex RT-PCR and which were amplified during the first round of PCR, typing was accomplished by direct sequencing of the first round PCR products. Some typed rotavirus isolates were also sequenced to validate the semi nested multiplex RT-PCR result. A total of 41 PCR products comprised of non-typeable (19), typeable (9) and mixed types (13) were purified and sequenced.

PCR product purification was performed with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequencing was performed. The consensus forward primer 9 Con1 and Con3 were

Table 1
Oligonucleotide primers used in the study of PCR amplification.

Primer (Polarity)	Type	Position (nt)	Sequence (5'-3')	Size of amplicon (bp)
9con1 (+)	VP7	37-56	TAGCTCCTTTTAATGTATGG	
9con2 (-)	VP7	922-941	GTATAAAATACTTGCCACCA	905
9T-1 (-)	G1	176-195	TCTTGTCAAAGCAAATAATG	159
9T-2 (-)	G2	262-281	GTTAGAAATGATTCTCAACT	245
9T-3 (-)	G3	484-503	GTCCAGTTGCAGTGTTAG	467
9T-4 (-)	G4	423-440	GGGTCGATGGAAAATTCT	404
9T-9B (-)	G9	131-147	TATAAAGTCCATTGCAC	111
G12R (-)	G12	471-490	AGTACAGTACCAAATTTCAT	454
Con3 (+)	VP4	11-32	TGGCTTCGCTCATTTATAGAC	
Con2 (-)	VP4	868-887	ATTTCGGACCATTTATAACC	877
1T-1	P8	339-356	TCTACTTGGATAACGTGC	346
2T-1	P4	474-494	CTATGGTTAGAGGTTAGAGTC	484
3T-1	P6	259-278	TGTTGATTAGTTGGATTCAA	268
ND-2	P11	116-133	AGCGAACTCACCAATCTG	123

used to amplify and sequence the VP7 and VP4 genes, respectively. G and P typing was done by comparing the nucleotide sequence with reference rotavirus strains available in the GenBank.

The results of semi-nested multiplex PCR were evaluated by confirmation with direct sequencing of some of the typeable strains. No bias was observed between the results obtained by the different methods. This supports the semi-nested multiplex PCR as a technique for molecular epidemiological studies.

RESULTS

Detection of rotavirus antigen

From February 2005 to March 2007, a total of 862 stool samples was tested for group A rotavirus VP6 antigen, 318 (36.9%) were positive by ELISA.

Age of the rotavirus positive patients

The age range of the 100 rotavirus diarrhea patients (2005-2007) was from 1 month to 24 months, with a mean age of 13.2 months. Most rotavirus positive patients (91%) were < 12 months of age.

Seasonal pattern of rotavirus infection

Table 2 shows the monthly distribution of rotavirus diarrhea in Delhi.

G and P typing

The distribution of the G and P types is shown in Table 3. Five different G types were detected: G1, G2, G3, G9 and G12. Of these, G1 was the most prevalent genotype (60%), followed by G2 (16%), G9 (8%), G3 (3%) and G12 (2%). The P types identified were P[8] (40%), P[4] (26%), and P[6] (17%).

The most common G-P combinations seen were: G1P[8] (26%), G1P[4] (12%), G1P[6] (11%), G2P[4] (8%), G9P[8] (5%), G2P[8] (3%), G2P[6] (3%), G3P[4] (1%), G3P[8] (1%), G9P[6] (1%) and G12P[4] (1%).

Table 2
Temporal distribution of rotavirus cases.

Month (Year)	Total no. of samples	Samples positive	%
February (2005)	33	14	42.4
March (2005)	42	25	59.5
April (2005)	26	8	30.7
May (2005)	17	6	35.2
June (2005)	67	21	31.3
July (2005)	63	11	17.4
August (2005)	60	14	23.3
September (2005)	67	16	23.8
October (2005)	35	15	42.8
November (2005)	29	25	86.2
December (2005)	20	13	65.0
January (2006)	14	4	28.5
February (2006)	22	8	36.3
March (2006)	39	8	20.5
April (2006)	64	10	15.6
May (2006)	18	5	27.7
June (2006)	35	9	25.7
July (2006)	42	14	33.3
August (2006)	23	10	43.4
September (2006)	23	9	39.1
October (2006)	32	19	59.3
November (2006)	20	11	55.0
December (2006)	37	22	59.4
January (2007)	17	13	76.4
February (2007)	16	8	50.0
March (2007)	1	0	0.00
Total no. of samples	862	318	36.9

Mixed infection with more than one G type was in 11% of cases, which were G1-G2 (6%) and G2-G9 (5%). Mixed P type infections were found in 17% of cases: P[4]-P[6] (7%), P[6]-P[8] (5%) and P[4]-P[8] (3%). Two instances of triple P infection were observed: P[4]-P[6]-P[8] (2%).

DISCUSSION

In the US and Europe, rotavirus infection occurs primarily during the winter. Some studies from India suggested the disease occurs year-round there. The peak of infection occurs during the winter (Chakravarti *et al,* 1992), while one study found 2 peaks per year (Singh *et al,* 1989) and another study found no seasonal pattern (Yachha *et al,* 1994). During our study, temporal distribution of rotavirus incidence was observed throughout the year with higher incidences during the colder season (October- January).

The semi nested multiplex RT-PCR strategies used in this work allowed characterization of 87.0% of the strains. One Indian study found G genotyping was more successful than P genotyping (Kang *et al,* 2005a). Our results are also comparable with previously published studies (Fruhwirt *et al,* 2000; Estes, 2001). The strains which could not be typed by semi

Table 3
Genotypic characterization of rotavirus strains into G and P types.

P type	% of children with indicated genotype							
	G1	G2	G3	G4	G9	G12	Mixed	Total
P[4]	12	8	1	-	-	1	4	26
P[6]	11	3	-	-	1	-	2	17
P[8]	26	3	1	-	5	-	5	40
P[11]	-	-	-	-	-	-	-	-
Mixed	11	2	1	-	2	1	-	17
Total	60	16	3	-	8	2	11	100

nested multiplex RT-PCR were further sequenced and their nucleotide sequences were compared with reference strains available at the NCBI in order to know their respective G and P types. A possible reason behind the failure of the second round of semi nested multiplex RT-PCR could be the presence of a mutation at primer binding sites. This was observed in the case of G12 which was untypeable during the second round of semi nested multiplex RT-PCR. On analyzing the nucleotide sequence of both G12 strains, two mismatches were observed at the primer-binding site of the type specific primer. It is possible the nucleotide sequence differences (mismatches) between the target region of the gene and the primer sequences used for typing led to the genotyping failure. This result supports the idea that genotyping methods require close monitoring and updating of oligonucleotide primers in order to minimize the percentage of untypeable strains during rotavirus surveillance studies (Rahman *et al,* 2005).

The prevalence of rotavirus G and P types have been monitored worldwide. The most common G types are G1, G2, G3, G4 and G9 (Arguelles *et al,* 2000; Estes, 2001; Van Man *et al,* 2005). In our study similar G genotypes were detected, except G4, which was not identified during the study. G1 and G2 accounted for 76% of G genotypes. G1 was the predominant genotype comprising 60% of rotavirus cases falling in this type. A previous study reported 44% of the strains to be G1 type from this same geographical area (Bahl *et al,* 2005). The predominance of G1 strains indicates the this region is at higher risk of severe rotaviral diarrhea due to this type (Bahl *et al,* 2005). G9 has been recognized as the most widespread of the emerging genotypes. It was first reported in the United States in the early 1980s. Soon after its detection, it disappeared for more than a decade; then reemerged in the mid-1990s (Clark *et al,* 1987); today it comprises 4.1% of global rotavirus infections, and accounts for as high as 70% of rotavirus infections in some recently published data. (Kirkwood *et al,* 2003; Santos and Hoshino, 2005). In the present study, G9 accounted for 8% of rotaviral diarrhea cases. This finding is similar to a previous report (Banerjee *et al,* 2006). In our study G3 was detected in only 3% of diarrheal cases, which is in concordance with other epidemiological studies indicating G3 is a rare infection (1.9%) in northern India (Kang *et al,* 2005a). A notable finding is the detection of G12 strains in two diarrheal cases. It is an emerging G genotype. Since its first identification in the Philippines in 1990 (Taniguchi *et al,* 1990), it has spread worldwide (Kang *et al,* 2005b; Pietruchinski *et al,* 2006). In India it was first time detected in Kolkata in 2003. The identification of G12 strains in the children younger the 2 years old indicates G12 is circulating in younger children, in contrast to the findings of another study (Bahl *et al*, 2005) which found G12 was confined to older children.

Various epidemiological studies worldwide have shown the most common P genotypes are P4, P6 and P8 (Arguelles *et al,* 2000; Das *et al,* 2002; Fang *et al,* 2005). The same P types were detected in the present study. The P8 genotype was found in 40% of cases, followed by P4 (26%) and P6 (17%). These findings are in concordance with previous published data, which showed the predominance of the P8 genotype in New Delhi (Bahl *et al,* 2005).

Studies in many countries have found G1[P8], G2[P4] and G3[P8] are the most common G-P combinations worldwide (Das *et al,* 2002; Fang *et al,* 2005). In the

present study, G1[P8] was found in 26% of combined cases. Notable findings are the detection of G3[P8] and the emergence of G1[P4] in New Delhi. Earlier reports found G3[P8] was not commonly detected in New Delhi (Bahl *et al,* 2005). G3[P8] is a common circulating genotype combination in the community, but studies have found its identification in hospitalized children is limited (Kang *et al,* 2002). In Argentina, G1P[4] had an incidence of 14%; this may be an emerging natural reassortmant strain (Arguelles *et al,* 2000).

In this study mixed infection occurred in 28% of cases, this finding is similar to previous percentages found in India (21.0 and 23.0 % by Jain *et al* (2001) and Das *et al* (2002), respectively, 24.0% in Bangladesh (Unicomb *et al,* 1999), 18.8% in Ireland (O'Halloran *et al,* 2000) and 16.0% in Brazil (Santos *et al,* 1998).

The present work provides basic information about the prevalence of rotavirus infection and describes circulating G and P types during 2005-2007 in New Delhi. We found a predominance of G1 genotypes, emergence of G12 in younger children and emergence of the G3[P8] combination in hospitalized children. It is hoped these results will help to provide relevant information for designing future rotavirus vaccines.

REFERENCES

Arguelles MH, Vielagas GA, Castello Ae, *et al.* VP7 and VP4 genotyping of human group A rotavirus in Buenos Aires, Argentina. *J Clin Microbiol* 2000; 38: 252-9.

Bahl R, Ray P, Subodh S, *et al.* Incidence of severe rotavirus diarrhea in New Delhi, India, and G and P types of the infecting rotavirus strains. *J Infect Dis* 2005; 192: S114-9.

Banerjee I, Ramani S, Primrose B, *et al.* Comparative study of the epidemiology of rotavirus in children from a community-based birth cohort and a hospital in South India. *J Clin Microbiol* 2006; 44: 2468-74.

Chakravarti A, Kumar S, Mittal SK, Broor S. Clinical and epidemiological features of acute gastroenteritis caused by human rotavirus subgroups. *J Diarrhoeal Dis Res* 1992; 10: 21-4.

Clark HF, Hoshino Y, Bell LM, *et al.* Rotavirus isolate WI61 representing new human serotype. *J Clin Microbiol* 1987; 25: 1757-62.

Cunliffe NA, Nakagomi O. A critical time for rotavirus vaccines: a review. *Expert Rev Vaccines* 2005; 4: 521-32.

Das S, Sen A, Uma G, *et al.* Genomic diversity of group A rotavirus strains infecting humans in eastern India. *J Clin Microbiol* 2002; 40: 146-9.

Dennehy PH. Rotavirus vaccines: An overview. *Clin Microbiol Rev* 2008; 21: 198-208.

Estes MK. Rotaviruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al.* eds. Fields virology. 4th ed. Philadelphia: Lippincott Williams and Wilkins, 2001: 1747-58.

Fang ZY, Wang B, Kilgore PE, *et al.* Sentinel hospital surveillance for rotavirus diarrhea in the People's Republic of China, August 2001-July 2003. *J Infect Dis* 2005; 192: S94-99.

Fruhwirt M, Brosl S, Ellemunter H, Moll-Schuler I, Rohwedder A, Mutz I. Distribution of rotavirus VP4 genotypes and VP7 serotypes among nonhospitalized and hospitalized patients with gastroenteritis and patients with nosocomially acquired gastroenteritis in Austria. *J Clin Microbiol* 2000; 38: 1804-6.

Gentsch JR, Glass RI, Woods P, *et al.* Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 1365-73.

Gentsch JR, Laird AR, Bielfelt B, *et al.* Serotype diversity and reassortment between human and animal rotavirus strain: Impli-

cations for rotavirus vaccine programs. *J Infect Dis* 2005; 192: S146-59.

Greenberg HB, Flores J, Kalica AR, Wyatt RG, Jones R. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. *J Gen Virol* 1983; 64: 313-20.

Jain V, Parashar UD, Glass RI, Gentsch RI, Bhan MK. Epidemiology of rotavirus in India. *Indian J Pediatr* 2001; 68: 855-62.

Kang G, Green J, Gallimore CI, Brown DW. Molecular epidemiology of rotaviral infection in South Indian children with acute diarrhea from 1995-1996 to 1998-1999. *J Med Virol* 2002; 67: 101-5.

Kang G, Kelkar SD, Chitambar SD, Ray P, Naik T. Epidemiological profile of rotaviral infection in India: challenges for the 21st century. *J Infect Dis* 2005a; 192: S120-6.

Kang JO, Kilgore P, Kim JS, *et al.* Molecular epidemiological profile of rotavirus in South Korea, July 2002 through June 2003: emergence of G4P[6] and G9P[8] strains. *J Infect Dis* 2005b; 192: S57-63.

Kirkwood C, Bogdanovic-Sakran N, Palombo E, *et al.* Genetic and antigenic characterization of rotavirus serotype G9 strains isolated in Australia between 1997 and 2001. *J Clin Microbiol* 2003; 41: 3649-54.

Martella V, Ciarlet M, Banyai K, *et al.* Identification of a novel VP4 genotype carried by a serotype G5 porcine rotavirus strain. *Virology* 2006; 346: 301-11.

O'Halloran F, Lynch M, Cryann B, O'Shea H, Fanning S. Molecular characterization of rotavirus in Ireland: detection of novel strains circulating in the population. *J Clin Microbiol* 2000; 38: 3370-74.

Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis* 2006; 12: 304-6.

Pietruchinski E, Benati F, Lauretti F, *et al.* Rotavirus diarrhea in children and adults in a southern city of Brazil in 2003: distribution of G/P types and finding of a rare G12 strain. *J Med Virol* 2006; 78: 1241-9.

Rahman M, Sultana R, Podder G, *et al.* Typing of human rotaviruses: Nucleotide mismatches between the VP7 gene and primer are associated with genotyping failure. *Virology* 2005; 2: 24.

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

Santos N, Lima RC, Pereira CFA, Gouvea V. Detection of rotavirus types G8 and G10 among Brazilian children with diarrhea. *J Clin Microbiol* 1998; 36: 2727-9.

Singh V, Broor S, Mehta S, Mehta SK. Clinical and epidemiological features of acute gastroenteritis associated with human rotavirus subgroups 1 and 2 in northern India. *Indian J Gastroenterol* 1989; 8: 23-5.

Taniguchi K, Urasawa T, Kobayashi N, Gorziglia M, Urasawa S. Nucleotide sequence of VP4 and VP7 genes of human rotaviruses with subgroup I specificity and long RNA pattern: implication for new G serotype specificity. *J Virol* 1990; 64: 5640-4.

Unicomb LE, Podder G, Gentsch JR, *et al.* Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. *J Clin Microbiol* 1999; 37: 1885-91.

Van Man N, Luan Le T, Trach DD, *et al.* Epidemiological profile and burden of rotavirus diarrhea in Vietnam: 5 years of sentinel hospital surveillance, 1998–2003. *J Infect Dis* 2005; 192: S127-32.

Yachha SK, Singh V, Kanwar SS, Mehta S. Epidemiology, subgroups and serotypes of rotavirus diarrhea in north Indian communities. *Indian Pediatr* 1994; 31: 27-33.