

Circulation of Group A Rotavirus Subgroups and Serotypes in Pune, India, 1990-1997

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ABSTRACT

Group A rotavirus-positive stool specimens, collected from 432 hospitalized patients of all age groups with diarrhoea during 1990-1997 from Pune, India, were characterized for subgroups (SGs) and G serotypes (1, 2, 3, 4, 6, 8, and 10). ELISA for subgrouping was carried out by employing subgroup I and II-specific monoclonal antibodies (MAbs). For serotyping, MAbs against G1 (Ku), G2 (S2), G3 (Yo), and G4 (ST-3) were used. In addition, MAbs against G3 (RV-3), G8 (B37), G6 (bovine U.K.), and G10 (B223) were also employed. Of the 432 specimens, 174 (40.27%) belonged to subgroup I, 187 (43.29%) to subgroup II, 15 (3.47%) to subgroup I and II, and 56 (12.96%) did not react to MAbs specific to subgroup I and subgroup II MAbs. Of the 432 specimens, 111 (25.69%) reacted to one of the MAbs used. Thirty-five of the 111 specimens were serotyped as G1, 34 as G2, and 42 as G3, G4, G6, G8, and G10. Sixty-seven (21%) specimens gave dual reaction mainly to MAbs against G6, G10; G2, and G4, and in several other combinations. Forty-seven specimens (10.88%) showed multireactivities. A large number of specimens (47.92%) did not show any reactivity with MAbs employed in this study, and remained non-serotypeable. Subgroup I was found to be more common in Pune, and most specimens negative for subgroup I and II were non-serotypeable. The results implicate the need for characterization of unusual and non-typeable strains before undertaking any rotaviral vaccine studies in India.

Key words: Rotavirus; Diarrhoea, Infantile; Serotyping

INTRODUCTION

Group A rotaviruses are classified according to 3 antigenic specificities, such as subgroup, G serotype, and P serotype. Subgroup I and II of human rotaviruses are now identified by distinctive epitopes on VP6 using subgroup-specific monoclonal antibodies (1). Besides, subgroup I and II and non-I-non-II have also been identified (2). Subgroup specificity carried by VP2 has also been reported (3). G serotype is associated with VP7, and 14 different G serotypes are presently known. P serotype is defined by VP4, but it is difficult to differentiate the P serotype serologically. The term "VP4

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genotype", based on the VP4 sequence, has been proposed for VP4 differentiation (4).

Results of studies in many countries suggest that G1, G2, G3, and G4 are the most common group A rotavirus serotypes (1). Epidemiological studies have shown that strains belonging to serotype 1 are predominantly obtained from children with diarrhoea in the majority of countries (5). However, besides G1-G4, other rotavirus serotypes, viz. G5, G6, G8, G9, G10, and G12, have been found in humans (1). Numerous strains, which carry previously unknown combinations of antigenic specificities, have also been found from India and other developing countries (6-14).

It is essential to understand the nature of rotaviruses circulating in different parts of India before currently available rotavirus vaccines are tried in India. In this communication, we report the results of subgrouping and serotyping of rotavirus-positive faecal specimens collected from hospitalized children from 1990 to 1997.

METHODS AND MATERIALS

Specimens

In total, 3,064 faecal specimens were collected from hospitalized patients of all age groups from 1990 to 1997. Of these specimens, 1,272 (41.51%) were collected from children aged less than 5 years and 1,792 (58.48%) from patients aged over 5 years, with a mean age of 34 years and 18 days.

All the specimens were tested by indigenously-developed ELISA for the diagnosis of rotavirus (15). Besides, MA b Yo-156 reacting with the group A common antigenic epitope on inner-capsid protein VP6 was used for confirming group A rotavirus in faecal specimens.

Subgrouping and serotyping of rotavirus-positive specimens

Rotavirus-positive specimens were further subjected to ELISA for characterization of subgroups (SGs) and serotypes. The following human and animal rotaviruses were used as reference strains for subgrouping and serotyping: Ku (G1, SG II), S2 (G2, SG I), Yo (G3, SG II), ST3 (G4, SG II), 69 M (G8, SG I), SA-II (G3, SG I), NCDV (G6, SG I), and B 223 (G10, SG I). All the reference strains were grown in MA 104 cells in culture. Subgrouping was carried out using an ELISA with subgroup I (S2-37) and II (Yo-5)-specific monoclonal antibodies (MAbs).

For serotyping of faecal specimens, MA b Ku-6BG, S2-2 G-10, Yo-IE2 and ST3-2 G7 each of which recognizes specific serotype G1, G2, G3, and G4 neutralization epitopes in outer-capsid protein VP7 were kindly supplied by Dr. S. Urasawa, Sapporo, Japan; MAbs to bovine serotype G6 (U.K.) and G10 (B 223) and MAbs against human serotype G3 (RV-3) and G8 (B37) by Dr. D.R. Snodgrass, Moredun Research Institute, Edinburgh, U.K.; and MAbs against human rotavirus serotype G3 (RV-3) and G8 (B37) by Dr. Ruth Bishop, WHO Collaborating Centre for Research on Human Rotaviruses, Victoria, Australia. The procedure reported by Taniguchi *et al.* was followed (16).

Electropherotyping of faecal specimens

Genomic RNA was extracted with phenol and precipitated with ethanol from specimens which showed multireactivities. Polyacrylamide gel electrophoresis (PAGE) of genomic

RNA was performed (17) with slight modifications. 7.5% (w/v) polyacrylamide slab gels with 4% stacking gel was used.

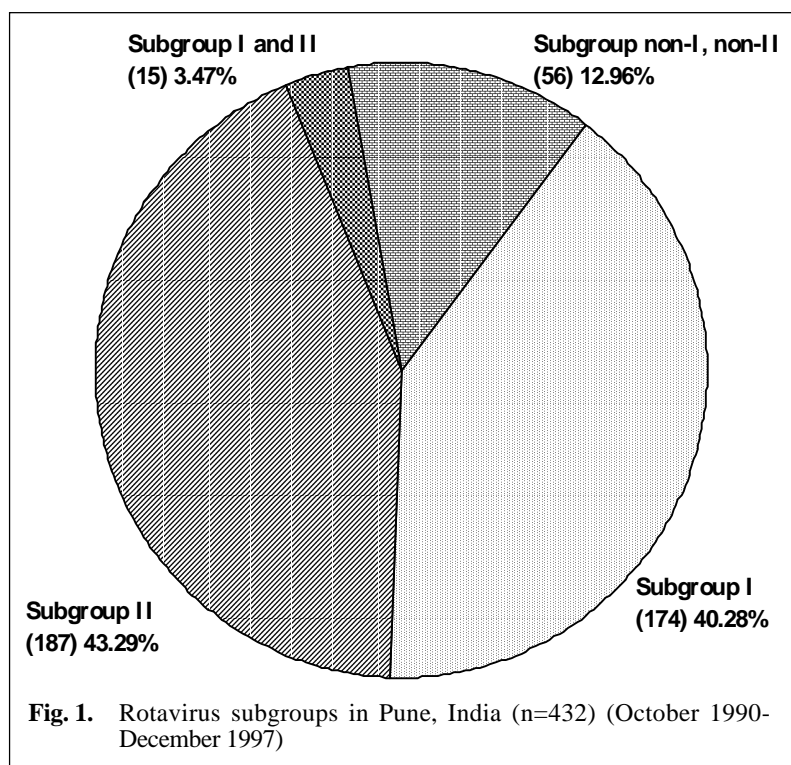
RESULTS

Rotavirus was detected in 432 (14.10%) of the 3,064 specimens collected from all age groups. Of the 1,272 specimens, 334 (26.25%) were positive among <5 years age group, and of the 1,792 specimens, 98 (5.47%) were positive among ≥5 years age group.

Subgrouping of faecal specimens

Results on subgrouping of rotavirus are shown in Fig. 1. A worldwide survey reported that SG I strains have been found much less frequently than SG II strains (4). Whereas in our study, we observed that SG I and II occurred almost in equal numbers. A considerable number (12.96%) of specimens did not react to either subgroup I or II MAbs. We could detect 15 strains (3.47%) having dual SG I and II specificity, which is a characteristic of animal rotaviruses.

The details regarding circulation of rotavirus SGs in Pune, India, are shown in Fig. 2. SG I and SG II strains normally circulated simultaneously. During November 1992-April 1993, the SG II strains predominated. However, from May 1993, the SG I strains started replacing the SG II strains. There was an unusual rise in SG I strains in December 1993. During March 1994-



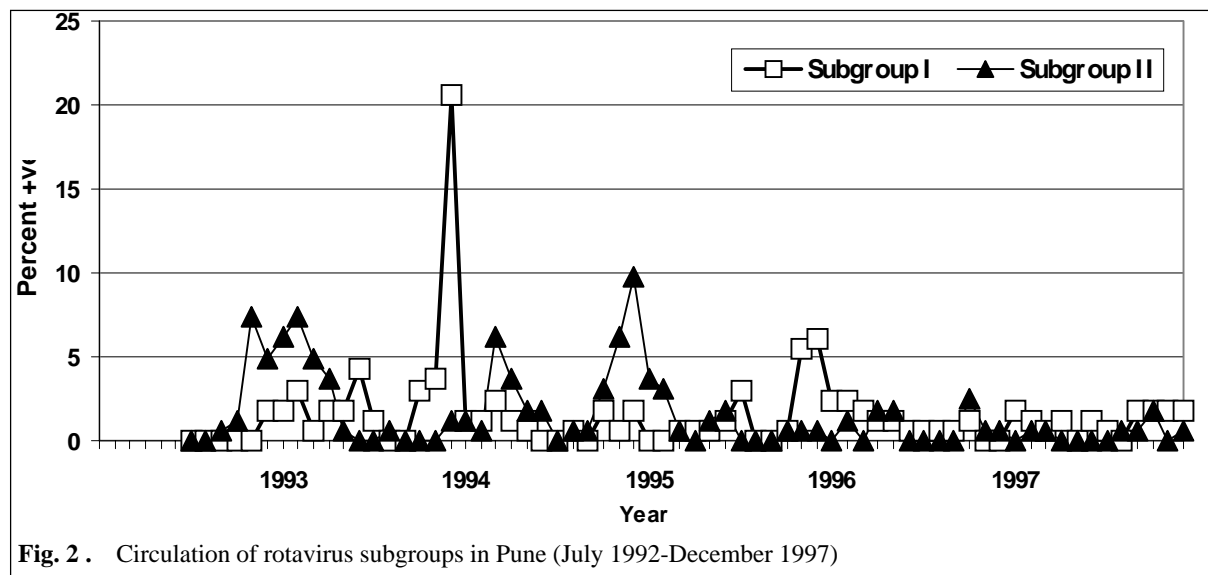


Fig. 2. Circulation of rotavirus subgroups in Pune (July 1992-December 1997)

February 1995, the SG II strains predominated, followed by another slight increase in SG I strains in November and December 1995.

Serotyping of rotavirus-positive faecal specimens

Initially, 205 specimens, collected during 1990-1993, were tested by ELISA for serotyping using MAbs as capture antibodies (16). The capture of rotavirus from the specimens was probed by polyclonal antibody against G1-G4, G6, G8, and G10 (method A). All the specimens were also tested by the procedure in which ELISA plates were coated with polyclonal antibody against G1-G4, G6, G8, and G10 serotypes, and rotavirus in faecal specimens was detected by MAbs against respective rotavirus serotype (method B).

Results of comparison between method A and method B are shown in Table 1. To compare method A with method B, McNemar's test, which allows comparison of 2 proportions in paired cases, was applied (18). It was found that proportions of positive/negative results generated by the 2 methods, did not differ significantly. It was observed that the method B could serotype an increased number of specimens (10), whereas the method A could detect an increased number

of bovine serotypes. Moreover, the method A is as per the guidelines of the manufacturer of the serotyping kit. Therefore, it was decided to serotype the remaining 228 rotavirus-positive faecal specimens by the method A.

Results of serotyping of all the 432 specimens collected during 1990-1997 by the method A for the entire period are shown in Fig. 3. A large number (26.4%) of specimens showed dual and multiple reactivities with MAbs, and 47.92% of the specimens remained non-typeable. The year and seasonwise distribution of single G serotypes and that of dual/multiple/non-typeable rotavirus serotypes are presented in Table 2 and in Fig. 4 respectively. The results of our study showed that the number of rotavirus-positive specimens was maximum during October-February, followed by March to July, and the least number in August and September. The introduction of new rotavirus serotype normally occurred during the winter.

Serotype G1, G2, G3, and G4 represented only 22.45% of the total rotavirus specimens (Table 2). On the whole, the frequency of rotavirus serotype G1-G4 circulating in India appeared to be less compared to developed countries (1). Of 67 dual reactive specimens,

Table 1. Comparison of 2 methods (A and B) for serotyping of rotavirus-positive specimens

Year	1990	1991	1992	1993	Total
Negative for A and B (n=66)	10	2	0	54	66
Positive for A and B, and A=B (n=27)	3	1	1	22	27
Positive for A and B, and A≠B (n=49)	4	4	19	22	49
Negative for A Positive for B (n=37)	1	7	15	14	37
Positive for A Negative for B (n=25)	2	0	1	22	25
Total	20	14	36	134	204

25 reacted to G6, G10 MABs; 19 to G2, G4, and the rest of the specimens reacted in different combinations to MABs against G1-G4, G6, G8, and G10. Similarly, 47 multiple reactive specimens also reacted in different combinations to MABs employed in serotyping.

Non-typeable strains

As shown in Fig. 3, 47.92% of the specimens remained non-typeable. Of the total 432 specimens, 47 (10.88%) showed multireactivities to MABs.

Multireactive specimens

In none of the samples with multiple G serotype reactivities could we identify existence of mixed infections as was evident from their single RNA electropherotypes (Fig. 5).

Unusual specimen

Results of analysis of correlation of subgroup and serotype are presented in Table 3. In our study, about 30% of the specimens belonging to G1-G4 serotypes were unusual specimens. Other unusual combinations of subgroup and serotype in this study

were 6 strains, detected as serotype I, subgroup I and 5 strains as serotype 2, subgroup II. Moreover, in the present study, 69 strains reacted with serotype G2-specific MAb as well as with MABs, specific to other serotypes.

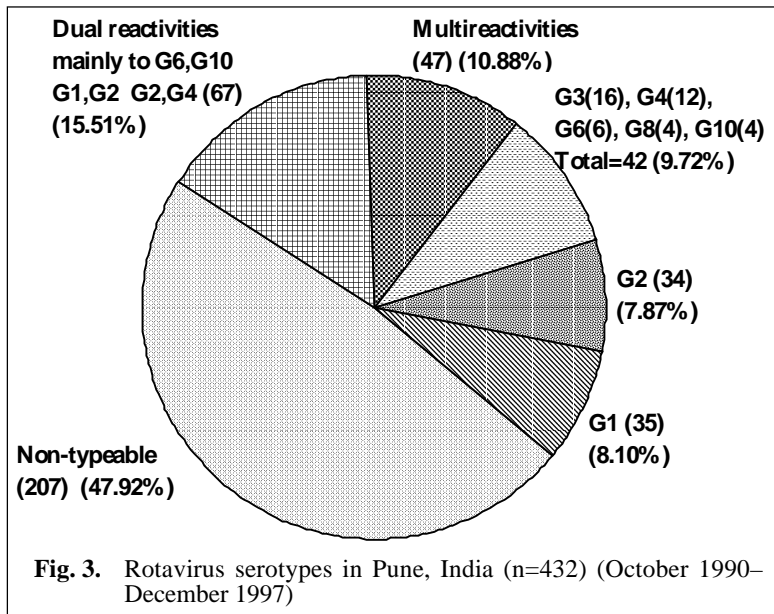


Fig. 3. Rotavirus serotypes in Pune, India (n=432) (October 1990–December 1997)

Year	Single							
	G1	G2	G3	G3*	G4	G6	G8	G10
October 1990-February 1991	-	-	-	-	6	1	-	-
March 1991-July 1991	-	-	-	-	-	1	-	-
August 1992-September 1992	1	-	-	-	-	-	-	-
October 1992-February 1993	8	1	-	-	-	1	-	-
March 1993-July 1993	5	-	-	-	-	1	-	1
August 1993-September 1993	1	-	-	-	-	-	-	-
October 1993-February 1994	1	3	-	-	2	-	-	-
March 1994-July 1994	1	11	4	1	-	-	-	-
August 1994-September 1994	1	-	1	-	-	-	-	-
October 1994-February 1995	5	-	-	6	1	-	4	-
March 1995-July 1995	3	1	-	1	2	-	-	-
August 1995-September 1995	1	-	-	-	-	-	-	-
October 1995-February 1996	7	8	1	-	-	-	-	-
March 1996-July 1996	-	1	-	-	1	-	-	-
August 1996-September 1996	-	-	-	-	-	-	-	-
October 1996-February 1997	-	2	-	-	-	2	-	-
March 1997-July 1997	-	1	-	-	-	-	-	3
August 1997-September 1997	1	2	-	-	-	-	-	-
October 1997-December 1997	-	4	-	2	-	-	-	-
Total	35	34	6	10	12	6	4	4

* MAb against RV-3 strain

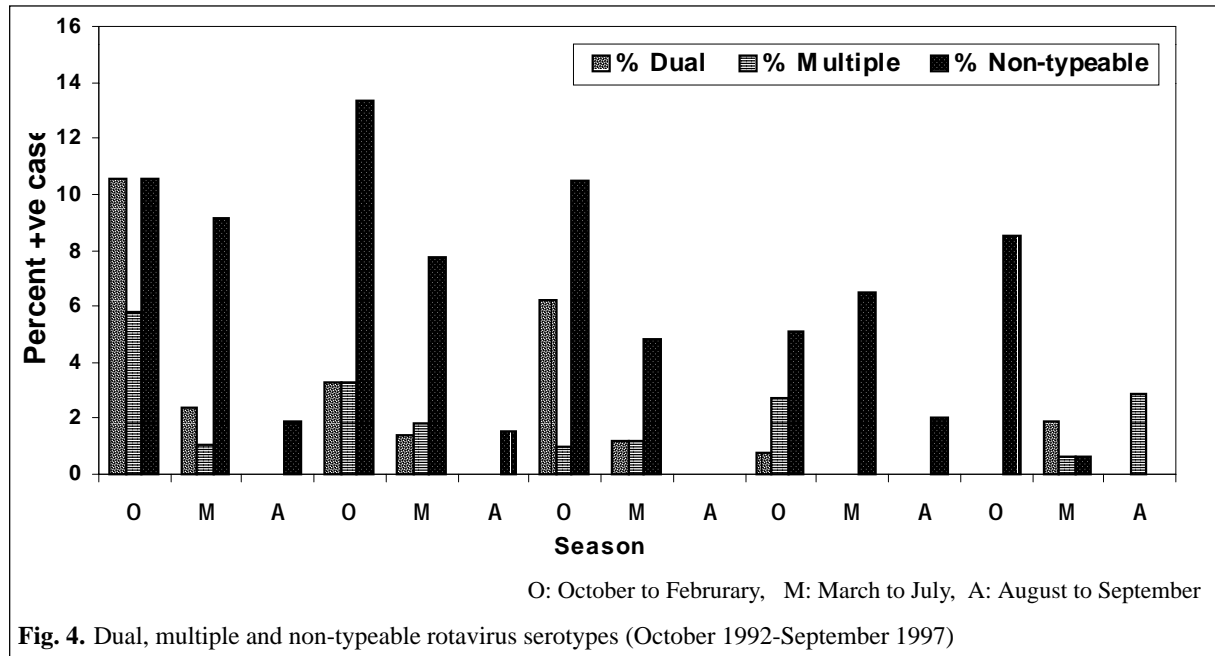


Fig. 4. Dual, multiple and non-typeable rotavirus serotypes (October 1992-September 1997)

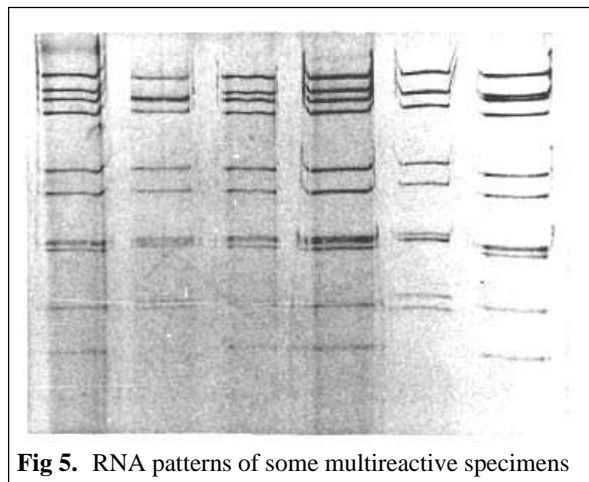


Fig 5. RNA patterns of some multireactive specimens

In addition to above, many specimens reacting with MABs against G3 (RV 3 strain) G4, G6, and G10 showed unusual combinations of subgroup and serotypes.

DISCUSSION

In other parts of India, SG II has been reported in a higher frequency compared to SG I; strains belonging to SG II were 2-5 times more prevalent than SG I strains (7,19-22). However, a higher incidence of SG I compared to SG II strains has been reported from Chennai, India (23). Climatological factors, mainly directions of winds, may be playing a vital role in circulation of rotavirus serotypes in the atmosphere (24).

In our studies, G1 and G2 serotypes were detected at an equal proportion in contrast to other studies in India and Bangladesh (7,19-22,25). Some specimens reacted to MAB raised against Yo strains, and some specimens reacted to MAB raised against RV3. This could be due to difference in the antigenic/epitope make-up or conformation of epitopes. This finding shows the importance of using MABs raised against different strains in serotyping (26,27). G4 serotype, followed by G6, G8, and G10, occurred at a lower frequency. Specimens reacting to MABs against G6, G8 and G10 serotypes could be of bovine origin, because these G serotypes occur mostly among bovines.

Using MABs specific for VP7, serotype G8 was identified in Indonesia and Europe (28), and G9 was identified in the USA and Japan (29,30). In our epidemiological study, we could detect neutralizing ('N') antibodies to G9 in a number of sera collected from patients with diarrhoea due to rotaviruses, and 'N' antibodies to G5 (OSU) rotavirus strain were also detected in young mothers (data not shown). The presence of 'N' antibodies against G5 and G9 could be due to heterologous-neutralizing antibody response to cross-reactive epitopes. Exposure to multiple cycles of infection of gnotobiotic calves with one rotavirus serotype (G6) has been demonstrated to induce homologous and heterologous-neutralizing antibodies (31). Similarly, a single oral exposure of rotavirus-seropositive cows with G6 rotavirus induced neutralizing antibodies to homotypic G6 virus and to a wide range of heterotypic G serotypes (32). However, this may also

Serotype	Subgroup				Total	% correlation
	I	II	Non-I, non-II	I, II		
-ve	91	80	35	1	207	—
1	6	27	1	1	35	77.14
2	19	5	6	4	34	55.88
3	0	5	1	0	6	83.33
3*	4	5	1	0	10	50.00
4	3	8	1	0	12	66.66
6	4	1	1	0	6	66.66
8	0	3	1	0	4	—
10	2	1	1	0	4	50.00
Total	129	135	48	6	318	—

* MAb against RV-3 strain

occur since rotavirus serotype G5 and G9 have been infecting humans in India (Kelkar *et al.*, unpublished data).

The percentage of non-typeable specimens has always been shown to be higher in developing countries (13,20, 33) than in developed countries. The analysis of electropherotype patterns usually showed non-typeable strains to be similar to typeable strains co-existing in the same communities (4). Further work on PCR amplification may enhance the ability to identify the non-typeable strains, and may detect newer serotypes. About 36% of the isolates from Bangalore and 30% of the isolates from Mysore could not be assigned to any serotype (19).

Specimens reacting with MAbs specific for more than one serotype have earlier been reported, but such reactivities have been attributed to mixed infection (13). Reactivities of these strains can be attributed to the presence of shared neutralization epitopes on VP7 (34).

Normally, there is a definite correlation among subgroup and G serotype. Generally, all human rotaviruses having 'short' or 'supershort' RNA electropherotype exhibit subgroup I specificity and those having long RNA electropherotype pattern have subgroup II specificity. Thus, rotavirus strains belonging to serotype G2 and G8 strains belong to subgroup I, and serotype G1, G3, G4, and G9 strains belong to subgroup II. On the other hand, among most animal strains, SG I specificity is associated with long RNA pattern (1). If there is no correlation between subgroup and serotypes, the strains are considered unusual.

There was no correlation between subgroups and serotype probably because of reassortant formation or interspecies transmission, e.g. animal rotaviruses infecting humans. During the investigations on rotavirus strains responsible for severe rotavirus infection among hospitalized children, unusual strains having unexpected

combinations of subgroup, electropherotype and serotype identities were observed in several countries. Subgroup I strain, possessing a long RNA pattern by PAGE, was reported from India (20), Japan in 1982 (35), South Africa (36), Kuwait (37), Italy (38), and Israel (39). We could also detect 3 strains with subgroup I and long RNA pattern in the present study.

Subgroup I-associated long RNA patterns were identified as serotype 3 in Japan (40,41). It has been documented that these strains could be examples of animal strains infecting children (40). Similarly, subgroup I with long RNA pattern described in Manila appeared to possess a new VP7 type (42) and those reported from Manipur, India, to be serotype 2 (43).

Such combinations have been reported from South Africa (36), Brazil (44), and Italy (45). Isolation of similar type of strains was reported earlier by Aijaz *et al.* from Bangalore, India (19). Three strains reacting with MAb against serotype 8 having subgroup II specificity were also detected. Isolation of similar strains was reported from Italy and Finland (28).

The unusual strains represent the possibility of genetic reassortment *in vivo* (46,47). The ability of rotaviruses to form stable reassortants would be likely to enhance the ability of rotaviruses to persist in humans (5).

Thus, the overall picture of detection of rotavirus subgroups is different in Pune, India, compared to that of developed countries and also the one in other places in India. However, it does match, to some extent, with that of Madras, India. A large number of specimens showed unusual combinations of subgroup and serotypes. Besides, major rotavirus serotypes, G1-G4, and other serotypes, *viz.* G6, G8, and G10, were also prevalent. Serotype G5 and G9 may be circulating to a great extent in Pune, India. Non-typeable specimens and specimens showing dual and multiple reactivities need to be studied in depth.

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