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Evidence of High-Frequency Genomic Reassortment of Group A Rotavirus Strains in Bangladesh: Emergence of Type G9 in 1995

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We characterized 1,534 rotavirus (RV) strains collected in Bangladesh from 1992 to 1997 to assess temporal changes in G type and to study the most common G and P types using reverse transcription-PCR, oligonucleotide probe hybridization, and monoclonal antibody-based enzyme immunoassay. Results from this study combined with our previous findings from 1987 to 1991 (F. Bingnan et al., J. Clin. Microbiol. 29:862–868, 1991, and L. E. Unicomb et al., Arch. Virol. 132:201–208, 1993) (n = 2,515 fecal specimens) demonstrated that the distribution of the four major G types varied from year to year, types G1 to G4 constituted 51% of all strains tested (n = 1,364), and type G4 was the most prevalent type (22%), followed by type G2 (17%). Of 351 strains tested for both G and P types, three globally common types, type P[8], G1, type P[4], G2, and type P[8], G4, comprised 45% (n = 159) of the strains, although eight other strains were circulating during the study period. Mixed G and/or P types were found in 23% (n = 79) of the samples tested. Type G9 RVs that were genotype P[6] and P[8] with both long and short electrophoretic patterns emerged in 1995. The finding of five different genotypes among G9 strains, of which three were frequently detected, suggests that they may have an unusual propensity for reassortment that exceeds that found among the common G types. We also detected antigenic changes in serotypes G2 and G4 over time, as indicated by the loss of reactivity with standard typing monoclonal antibodies. Our data suggest that a vaccine must provide protection against type G9 RVs as well as against the four major G types because G9 strains constituted 16% (n = 56) of the typeable RV strains and have predominated since 1996.

Group A rotavirus (RV) is the most important cause of severe diarrhea in children worldwide (12, 34), and RV infection is associated with extensive morbidity and mortality in Bangladeshi children. Almost 25% of children <2 years of age who present with diarrhea to the Clinical Research and Service Center of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), are infected with RV, and an estimated 1 in 200 Bangladeshi children die from RV diarrhea by 5 years of age (66). Vaccination is thought to be the most effective way to reduce this marked morbidity and mortality, and an RV vaccine based on rhesus RV and three recombinant strains (RRV-TV) was recently licensed for use in children (35) and was recommended for routine use in the United States (8a, 30). This vaccine has been formulated to provide serotype-specific protection against four common human serotypes, types G1 to G4, because data for patients with natural infections and from field trials suggest that homotypic (i.e., serotype-specific) protection may be greater than heterotypic protection (9, 21).

Studies in a number of countries (e.g., India and Brazil) have identified large, regional variations in the serotypes of RV strains, including the identification of serotypes not targeted by the RRV-TV vaccine (26, 39, 51). Consequently, before the RV vaccine is field tested, it seems prudent to determine the common RV types to assess whether efficacy might be altered in settings where these uncommon serotypes are prevalent. Subsequent determination of the types of the RV strains that cause diarrhea among recipients of vaccine and placebo would allow investigators to ascertain the efficacy of a vaccine against the circulating types.

Rotavirus has two outer capsid proteins that are capable of producing neutralizing antibodies following natural infection, and both play important roles in protective immunity (32, 47). Serotypes have been defined according to outer capsid glycoprotein VP7, which determines G serotypes, and protease-cleaved protein VP4, which determines P types (19). Fourteen RV G serotypes, including 10 that infect humans, and 10 P serotypes, with 8 that infect humans, have been identified to date by cross-neutralization studies (19, 55, 62). A variety of methods are available for determination of G and P types (13, 19, 23, 27, 33, 61): enzyme immunoassay with VP7-specific monoclonal antibodies (MAbs) is a simple, inexpensive method for G typing that can identify the types in up to 70% of samples, and the nontypeable strains can be resolved by probe hybridization or reverse transcription-PCR (RT-PCR). Nucleic acid-based methods have been used for P typing because antibody-based methods have been difficult to develop and only recently have been tested extensively with fecal specimens (14, 23, 38, 42, 49, 50). Many studies of the globally common types G1 to G4 (4, 8, 25, 43, 48, 64, 68, 71) and, more recently, studies that have examined both G and P types have been conducted (for a review, see reference 24). The addition of VP4 typing has identified a greater diversity of P-G neutral-

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ization antigen combinations in field strains than was previously appreciated (39, 51, 58, 59, 63). Molecular characterization has demonstrated that some of these strains and those from other studies probably arose by reassortment with animal rotaviruses (2, 18, 22, 40, 45, 46, 57).

In Bangladesh, studies of the distribution of RV G serotypes appeared to confirm that the common serotypes G1 to G4, which are targeted by the vaccine, were indeed the predominant strains (1, 7, 70), although nontypeable strains have sometimes been identified. Subsequent studies in neighboring India and Thailand by an MAb-based enzyme immunoassay (EIA; MEIA) and molecular methods (RT-PCR) for determination of both G and P types documented the presence of many variant strains, including a high prevalence of serotype G9 strains in Indian children with diarrhea (51), and an unusual serotype G9 RV strain in Thailand with genetic relationships to animal RVs (67). A subset of samples from Bangladesh subsequently tested for the G and P types of the infecting strains suggested that unusual combinations were circulating there as well (6).

We therefore examined the distribution of the G and P types of RV strains infecting Bangladeshi children using a combination of methods to detect the full diversity of G and P types. We also examined these trends in strain distribution over time and for strains collected from sentinel sites around Bangladesh.

MATERIALS AND METHODS

Study sites and specimens. In preparation for RV vaccine trials, we examined strains that would identify (i) the distribution of G and P types from a subset of 351 strains, (ii) temporal changes in the G serotypes of 1,534 strains collected from 1987 to 1997, and (iii) the prevalence of G and P types of strains collected from six sentinel sites around Bangladesh.

The RV-positive stool samples tested in this study were from children less than 5 years of age who presented to the Clinical Research and Service Centre of ICDDR,B in Dhaka, Bangladesh, from 1992 to 1997 (n = 1,028); the government Medical College Hospitals of Rangpur in northern Bangladesh (n = 57), Sylhet in northeastern Bangladesh (n = 42), and Rajshahi in northwestern Bangladesh (n = 14) from November 1996 to June 1997, the Matlab Clinical Research Centre in southeastern Bangladesh (n = 271) from February 1996 to April 1997; and children enrolled from birth in a longitudinal study of diarrhea in Mirzapur in northern Bangladesh (n = 122) from November 1993 to November 1996.

Laboratory methods. (i) RV detection. RV was detected at all sites by an enzyme immunoassay described previously (7). Positive specimens were frozen at −20°C until further testing.

(ii) RV typing. Between 1992 and 1997, G typing was performed with 718 samples by three different techniques: oligonucleotide probe hybridization as described previously (56), an MEIA, and RT-PCR. The G types of strains in early samples from 1992 were determined by oligonucleotide probe hybridization. MEIA was performed with MAbs from Silenus Laboratories, Melbourne, Australia (MAbs RV4:2, RV5:3, RV3:1, and ST3:1, which are type G1 to G4 specific, respectively) (13), and from Serotec, Tokyo, Japan (MAbs KU-4, S2-2G10, YO-1E2, and ST2G7, which are types G1 to G4 specific, respectively) (61), including group-specific MAb YO-156, by following the protocol given by the manufacturers with hyperimmune antisera prepared in our laboratory, Maxisorb plates (Nunc, Roskilde, Denmark), and horseradish peroxidase-conjugated anti-rabbit immunoglobulins (Dakopatts, Glostrup, Denmark). The origin of MAb F45:8 has been described previously (36). P and G typing was performed by RT-PCR with glass powder-extracted RNA as described previously (17, 23, 27) by using P- and G-specific primers generated by the Molecular Biology Core Facility, Centers for Disease Control and Prevention, Atlanta, Ga. The nomenclature used here for P and G types has been described by Estes (19), in which strains identified only by genetic characterization without serologic testing are given designations such as genotype P[8], G1 or P[4], G2, even though such strains could be closely related antigenically to prototype strains such as DS-1 (serotype P1B[4], G2) or Wa (serotype P1A[8], G1). The designation of P serotypes is reserved for strains that have been serologically characterized by neutralization (19).

(iii) RV electropherotyping. RV-positive samples were tested for electropherotype (E type) by polyacrylamide gel electrophoresis (PAGE) as described by Herring et al. (31).

(iv) Subgrouping. Subgrouping was performed with selected stool samples by an EIA as described previously (28).

(v) Southern hybridization. Samples infected with type P[4+6], G2 were tested with P[4]- and P[6]-specific probes as described elsewhere (3, 51).

(vi) Typing strategy. It was not possible to test all RV-positive samples for the G and P types of the infecting strains, so we developed a typing scheme to select a subset of fecal samples. RV-positive samples were tested by PAGE, and the G types of the strains in positive samples were determined by MEIA. On the basis of the E type, reactivity with VP7-specific MAb YO-4C2 (60), and reactivity with G type-specific MAbs, we selected samples for P and/or G typing by RT-PCR. Samples that were highly positive with MAb YO-4C2 but negative with type G1- to G4-specific MAbs or samples for which the E type did not correlate with the MAb reactivity (e.g., short E-type strain reacting with the type G4-specific MAb), and representative strains whose G types had been determined were selected for P typing.

RESULTS

Temporal distribution of G types. We studied the yearly distribution of G types and selectively determined P types among 2,515 RV-infected specimens collected from 1987 to 1997 (Table 1). Considerable G-type fluctuations were found from year to year. From 1987 to 1992, types G1 to G4 were accompanied by many nontypeable strains when oligonucleotide hybridization was the only technique used and rectal swabs instead of bulk stool specimens were often tested. Overall, type G4 was the most common type (21.5%; n = 540), followed by type G2 (16.8%; n = 423). In 1995, the novel type G9 RVs emerged. These RVs had short E types and commonly (39 of 54 strains) reacted with the G4-specific MAb ST3:1 but were classified as type G9 by RT-PCR. Between 1992 and 1995 (when strains of type G9 were first detected), 147 strains with short E types were identified; 21 reacted with type G4-specific MAb ST3:1, of which 10 were determined by RT-PCR to be type G2, 2 were type G4, 3 were of mixed G types (with type G4), 1 was type G9 (in 1995), and 5 were not tested by RT-PCR. Despite the variety of typing methods used, strains in 42.5% of the specimens were untypeable.

Distribution of serotypes by location. The strains in samples from five sentinel sites and Dhaka were also typed. Among the strains collected during the two time periods studied, we found little variation in the distribution of types between the sentinel areas and Dhaka (data not shown). G9 strains were found at all sites but Sylhet, the city where only five RV-positive samples were detected.

Prevalence of RV P and G types. Of the 351 RV-infected samples that were collected between 1992 and 1997 and that were tested for their G and P types, 66% (n = 232) had a single strain that was fully typeable, 23% (n = 79) were infected with mixtures of strains, and 13% (n = 47) were infected with a strain whose full G and P types could not be determined (Table 2). Four strains that were typed as P[4], G4 were found to have both long and short E types in the same specimen and so were not included in the total of fully typeable strains. Of the single strains that caused infections and that were G typed (n = 257), type G4 was the most common (47%; n = 122), followed by type G2 (28%; n = 72), type G9 (22%; n = 56), and type G1 (21%; n = 55). Among the single strains that caused infections and that were P typed (n = 236), type P[8] was the most common (58%; n = 136), followed by type P[6] (24%; n = 56) and type P[4] (19%; n = 44). The four most common strains found worldwide (P[8], G1; P[4], G2; and P[8], G4) constituted 67% (159 of 236) of all strains that were typed and that were responsible for infections caused by single strains. The diversity of the strains was great, and eight other less common variants were identified (Table 2), not including the strains that were not fully typeable (n = 47). Among the unusual variants, P[6], G9 represented the fourth most common P-G combination overall (11%; n = 37). Among the 79 samples infected with mixtures of strains (Table 3), the infections were caused by a collection of strains representative of the strains responsible for infections caused by single strains but with a predom-

TABLE 1. Distribution of RV types from 1987 to 1997

G type	P type	No. of strains of the indicated type in the following year(s):								No. (%) of strains[a]
		1987–1989	1990–1991[b]	1992	1993	1994	1995	1996	1997	
G1	P[ND][c]	160	54	51						265 (10.5)
G1	P[4]						1	1		2 (0.1)
G1	P[6]				3		2			5 (0.2)
G1	P[8]			1	2		4	11	19	37 (1.5)
G1	P[other][d]			1	2	4		5	3	15 (0.6)
G2	P[ND]	228	96	27						351 (14.0)
G2	P[4]			2	3	4	22	6	1	38 (1.5)
G2	P[6]					1	1		1	3 (0.1)
G2	P[8]			1		1		1		3 (0.1)
G2	P[other]			4	1	6	14	2	1	28 (1.1)
G3	P[ND]	42	31	4						77 (3.1)
G4	P[ND]	126	175	117						418 (16.6)
G4	P[4]					1	2			3 (0.1)
G4	P[6]					2	5	2		9 (0.4)
G4	P[8]			3	12	21	43	6		85 (3.4)
G4	P[other]			1		2	16	4	2	25 (1.0)
G9	P[6]						1	17	19	37 (1.5)
G9	P[8]							3	7	10 (0.4)
G9	P[other]							1	8	9 (0.4)
GNT[e]	P[ND]	278	590	201						1,069 (42.5)
GNT	P[4]						1	1		2 (0.1)
GNT	P[6]			1						1 (0.1)
GNT	P[8]					1				1 (0.1)
GNT	P[other]			5	4	3	6	1	3	22 (0.9)
Total[f]		834	946	419	27	46	118	61	64	2,515

[a] The percentage of the total is >100% (100.3%) because each number was rounded off to the nearest 1/10 of 1%.
[b] Rectal swabs were used for RV testing (65).
[c] P[ND], P genotyping was not done.
[d] Strains were not typeable for P genotype or the samples were infected with strains with different P genotypes.
[e] GNT, G nontypeable.
[f] Total number of strains analyzed.

inance of P[4+6], G2 strains with short E types (30%; 24 of 79), which were confirmed by Southern hybridization with P[4]- and P[6]-specific probes.

RV evolution. Among the 56 G9 strains detected (including 47 that were both P and G typeable), 5 distinct reassortants were detected by a combination of methods (PAGE, MEIA, and RT-PCR) (Table 4) and were found to have differences in four gene segments: segment 7/8/9, which determines the G type; segment 4, which specifies the P type; segment 11 (as determined by PAGE, which identifies a short or long E type), which encodes NSP5, and segment 6, which encodes the subgroup antigen VP6. Among the five reassortants, three were relatively common: P[6], G9 strains with a short pattern (57%; $n = 32$), followed by P[8], G9 with a short pattern (16%; $n = 9$) and P[6], G9 with a long pattern (9%; $n = 5$). Of the five

TABLE 2. Frequency of RV G and P types in specimens from Bangladeshi children, 1992 to 1997

P type	No. of specimens of the following type:						
	G type[a]					NT[b]	Total
	1	2	4	9	Mixed		
[8]	36	3	85	10	9	2	145
[4]	2	38	3		4	1	48
[6]	5	3	10	37	5	1	61
Mixed	7	25	11	5	6	5	59
NT	5	3	13	4	2	11	38
Total	55	72	122	56	26	20	351

[a] No strain was type G3.
[b] NT, nontypeable.

TABLE 3. Frequency of mixed G and P types infecting Bangladeshi children, 1992 to 1997

P type(s)	No. of specimens with the following G type(s):											Total
	1	2	4	9	1 and 2	1 and 4	2 and 4	2 and 9	4 and 9	1, 2, and 4	NT[a]	
4					1		2	1				4
6					1	3			1			5
8					1	8						9
4 and 6	2	24	4	1	1	1	1				5	39
4 and 8		1	3				1			1		6
6 and 8	5		4	4			1					14
NT					1	1						2
Total	7	25	11	5	5	13	5	1	1	1	5	79

[a] NT, nontypeable.

TABLE 4. Distribution of natural RV type G9 reassortants

No. of strains	Type by the following means of recognition (gene segment):				% Strains reactive with the following MAbs[a]:	
	G type (7/8/9)	P type (4)	Subgroup (6)	PAGE pattern (11)	F45:8	ST3:1
1[b]	9	8	II[c]	Long	0 (0/1)	100 (1/1)
32	9	6	I[d]	Short	32 (6/19)	74 (14/19)
5	9	6	I, II[e]	Long	0 (0/2)	50 (1/2)
9	9	8	I[f]	Short	56 (5/9)	75 (6/8)
6	9	0	ND[g]	Short	ND	89 (16/18)
3	9	0	ND	Long	ND	33 (1/3)

[a] Not all strains were available for testing. None of the tested specimens reacted with the serotype-specific MAbs KU-4 (type G1 specific), S2-2G10 (type G2 specific), YO-1E2 (type G3 specific), and ST-2G7 (type G4 specific) (61). The numbers in parentheses indicate number of strains of that type/number of strains tested.

[b] The genotype is identical to that of prototype strain WI61 (11).

[c] One strain was tested.

[d] Eighteen strains were subgroup I, two were not subgroupable, and two were not tested.

[e] One strain was subgroup I and one strain was subgroup II.

[f] Seven strains were subgroup I, and two strains were not subgroupable.

[g] ND, not done.

P[6], G9 long E-type strains, two were tested for their subgroups, and one each belonged to subgroups I and II. The type G9 strains reacted with type G4-specific MAb ST3:1 (76%; 39 of 51), and a subset of these strains reacted weakly with type G9-specific MAb F45:8 (35%; 11 of 31) but did not react with the type G4-specific MAb ST-2G7 (0%; 0 of 31). All short E-type strains that could be grouped belonged to subgroup I, and all except one of the subgroup-reactive long E-type strains were subgroup II (Table 4).

Antigenic change of G2 and G4 RVs with time. While testing for RV G type by MEIA, we used two sets of RV VP7-specific MAbs. Of the 45 specimens with short E types that were identified as type G2 by RT-PCR typing, only 1 reacted with G2-specific MAbs RV5:3 or S2-2G10. To determine whether this represented a change in reactivity with some type G2-specific MAbs, we simultaneously tested 36 of the 45 specimens with an additional type G2-specific MAb, MAb IC10 (48), in addition to MAbs RV5:3 and S2-2G10 and subgroup-specific MAbs 255/60 (subgroup I) and 631/9 (subgroup II) (28). Of 25 specimens that had an optical density of >1.0 with the subgroup I-specific MAb, 11 (44%) reacted with MAb IC10, suggesting that loss of the virus outer capsid could not explain the lack of reactivity with the other two type G2-specific MAbs. Finally, we investigated the reactivities of 127 specimens that were positive with type G4-specific MAb ST3:1 and found that a low proportion ($n = 52$; 41%) reacted with a second type G4-specific MAb, MAb ST-2G7, and when we specifically examined a subset of these 127 specimens that gave an optical density of >1.0 with VP7-specific MAb YO-4C2, only 27 of 86 (31%) reacted MAb ST-2G7 (data not shown).

DISCUSSION

Using a combination of typing methods, we identified a great diversity of RV strains circulating in Bangladesh during the last 5 years. This diversity was similar to the strain diversity found in other developing countries, such as India and Brazil (26, 39, 51, 63). Our findings are in marked contrast to the results of early studies in Bangladesh (1, 7, 70), which suggested that only the four common strains were prevalent. Nonetheless, in combination with typing data from an earlier study from our laboratory (7), type G4 was the most prevalent serotype and the majority of typeable strains were of types G1 to G4, the G types represented in the RRV-TV vaccine. Over the decade of 1987 to 1997, temporal changes in G types occurred, with an uncommon serotype, type G9, emerging in 1995 at several sites to become the predominant type in both 1996 and 1997. Because of the potential impact on RV vaccination programs, future vaccine studies need to assess whether the tetravalent vaccine will effectively prevent severe disease caused by serotype G9.

The type G9 strains are of particular interest in terms of virus evolution because they exhibited a high level of reassortment. In a recent review of studies in which both the G and the P types were determined (24), each G type was frequently associated with a single P type, an observation contradicted by the findings for the G9 strains and, to a minor extent, the G1, G2, and G4 strains in this study (Table 2) and by the findings presented in another recent report on Bangladeshi rotavirus strains (6). By using methods that permitted assessment of features present on four distinct genomic segments, we detected type G9 reassortants with a variety of distinct P types (P[6] or P[8]), subgroup specificities (subgroup I or II), and PAGE profiles (long or short). One hypothesis that explains these results is that the high frequency of mixed infections in Bangladeshi children with diarrhea (Table 3) may provide a good environment for this segmented virus to form reassortants. Our results also suggest that the type G9 VP7 gene may be unusual in its propensity to form viable reassortants within both major human RV genogroups, represented by types P[6], G9 and P[8], G9 with long E types and subgroup II specificity, and by types P[6], G9 and P[8], G9 with short E types and subgroup I specificity. Since we found only one reassortant between segments 6 and 11, it is tempting to speculate that reassortant strains with uncommon G and/or P types may arise because they can escape neutralizing antibodies elicited to the G and P types of common RV strains. We recently reported that the infection of newborn infants in India with unusual P[11], G9 strains may occur through a similar mechanism (54). It would be interesting to test these hypotheses through studies of RV neutralizing antibodies against common P and G types in different populations in which unusual strains are present.

The most prevalent type G9 strain in this study belonged to genotype P[6] and had a subgroup I VP6 antigen and a short E type (Table 4), characteristics which it shared with strains commonly detected during the 1996 and 1997 rotavirus season in the United States (52). More recently, analysis of an archival collection from the United States demonstrated that P[6], G9 strains closely related to those previously identified U.S. isolates (52) were present at least as early as 1995 in the United States (10), the same year that these strains were first detected in Bangladesh. In addition, P[6], G9 strains with short E types whose subgroup has not been determined were detected in children with diarrhea from Malawi, Africa, during 1997 (16). On the basis of the combination of these results, it is possible to speculate that these previously unreported RV strains may have emerged as a cause of RV diarrhea during the mid-1990s and may have a broad or even global distribution.

The finding that these novel strains are widely distributed raises interesting questions about their origins. All of the P[6], G9 strains with short E types that have been studied to date by Northern hybridization apparently have close homology to members of the DS-1 genogroup (37, 53), suggesting that they could have arisen by reassortment with strains from another RV genogroup. Although the donor strains for the type G9 and P[6] genes are unknown, one possible origin for their VP7 genes could be from strains related to the first serotype G9

strain to be described, WI61 (P1A[8], G9), which was isolated in the United States in 1983 (11). Work is in progress to examine this hypothesis by sequencing the VP7 genes of P[8], G9 strains identified between 1996 and 1998 in the United States (29, 53). These strains are not closely related genetically to type G9 strains that were isolated in Thailand and that share genetic homology with animal RV genogroups (67). Serotype G9 strains have also been described in cattle and pigs, but so far a detailed characterization of the genetic or antigenic relationships to other G9 strains has not been reported (20, 41, 44), so that the relationship of P[6], G9 strains to these animal G9 strains is unknown.

For the performance of a typing survey of the nature of the one described here, in which 2,515 specimens were analyzed, RT-PCR is too expensive and time-consuming for the testing of all RV strains. We used MEIA as the first typing step and then chose nontypeable strains with sufficient VP7 (reactivity with MAb YO-4C2), representative typeable strains, and strains with anomalous PAGE patterns and G types for PCR analysis. Using this scheme, we found that several MAbs gave few positive reactivities or unexpected reactivities, making it difficult to interpret the results in the absence of RT-PCR findings. For example, of 58 short E-type strains that reacted with G4-specific MAb ST3:1, 27 were either P[6], G9 or P[8], G9 by RT-PCR typing. Since some of these same strains also react weakly with a G9-specific MAb but do not react with another G4-specific MAb, MAb ST-2G7 (Table 4) (61), it suggests that MAb ST3:1 is cross-reactive with some type G9 strains. The results of another recent study of type G9 strains from India demonstrated cross-reactivity between several type G9 strains and MAb ST3:1 (15). Thus, it will be important to confirm these results through a systematic study, since ST3:1 has been widely used as a MAb for the typing of type G4 strains (5, 8, 13, 64, 69).

An example of a low level of reactivity was with type G2-specific MAb RV5:3, which has been routinely used in other typing surveys (5, 8, 13, 64, 69) and which reacted with 86% of Bangladeshi type G2 strains in a study by Ward and coworkers (69) but which reacted with few type G2 strains in this study. Reduced MAb reactivity with time was also found with type G4 strains with MAb ST-2G7, in contrast to the study by Ward et al. (69), in which 98 to 100% of the Bangladeshi type G4 strains were reactive with MAbs ST3:1 and ST-2G7 (69). Since some of these type G2 strains reacted with at least one type G2-specific MAb (MAb IC10) and many type G4 strains reacted with a VP7-specific MAb, it suggests that they may represent antigenic variants in VP7, an observation that needs confirmation by molecular analysis. If confirmed, these results represent another indicator of a high frequency of change among RV strains in a developing country as well as the reassortment of several genes that may typify the situation in other developing countries of the Indian subcontinent and elsewhere. Finally, these data confirm the work of other investigators (48, 69), suggesting that due to the variable reactivities of local RV strains, several different MAb panels or careful pretesting of MAb panels may be required for different geographic locations.

We have demonstrated that at least 10 other strains (Tables 2 and 4), in addition to those with the 4 major G- and P-type combinations, including 5 distinct G9 strains, infect Bangladeshi children. This may have relevance to vaccine composition because the efficacy of the vaccine against type G9 RV has not been determined. Our study demonstrates the need to incorporate a wider variety of reagents and detection methods in attempting to identify the full variety of strains in circulation. It should be noted, however, that current vaccines do not include human rotavirus P-serotype antigens, so the diversity of P genotypes in Bangladeshi RV strains may not be directly relevant to the success of current vaccines against RV. Finally, this study further illustrates the importance of characterizing nontypeable RV strains since some may emerge as predominant strains in the future as type G9 RVs have in 1996 and 1997. In spite of the variety of methods used, many untypeable strains remained, raising the possibility that additional serotypes may be prevalent in Bangladesh.

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