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Rotavirus Diarrhea in Bangladeshi Children: Correlation of Disease Severity with Serotypes

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To improve the understanding of the relative importance of serotypes of rotavirus in dehydrating diarrhea, we examined the correlation of clinical characteristics and disease severity with serotype in 2,441 diarrheal episodes among children younger than 2 years of age in rural Bangladesh. Of 764 rotavirus-associated episodes, a single G type (serotype 1, 2, 3, or 4) was determined by oligonucleotide probe in 485 (63%), while 233 episodes were nontypeable. Episodes with G types 2 and 3 were associated with more-severe dehydration than episodes associated with G type 1 or 4 or with nontypeable rotavirus. Episodes did not differ by G type in prevalence of vomiting, copious diarrhea, fever, abdominal pain, or length of treatment center stay. Rotavirus reinfections were detected in seven children, with homologous reinfection (G type 2) in one. Twelve children with diarrhea who died had rotavirus detected in stool specimens within 30 days of death. Children who died were more likely to be malnourished than were surviving children with rotavirus diarrhea. Of 40 specimens tested by polymerase chain reaction, 29 (72.5%) were P type 1, 9 (22.5%) were P type 2, 1 (2.5%) was P type 3, and 1 (2.5%) was nontypeable. One severely symptomatic diarrheal episode was associated with P type 3 rotavirus, a serotype usually found in asymptomatic nursery infections. Although G types 2 and 3 were associated with more-severe dehydration than other serotypes, the differences do not appear to be of major clinical importance. Effective vaccines should protect against all four major G types.

Rotavirus is the most important etiologic agent of serious dehydrating diarrhea among infants and young children (19), causing an estimated 9 million cases of severe disease and 873,000 deaths per year worldwide (17). A rotavirus vaccine would greatly decrease this burden of disease, but to date the efficacy of candidate vaccines has varied widely (2). Most human rotaviruses belong to one of four major serotypes, and although early vaccine trials demonstrated good heterotypic protection (29), subsequent studies have suggested that homotypic protection may also be important (8, 15). The strategy for vaccine development would therefore be enhanced by knowledge of patterns of circulating virus serotypes and of the relative importance of these serotypes in causing clinical illness.

Rotavirus has two outer capsid proteins, VP7 and VP4, that independently induce neutralizing antibodies (16). Thus, complete serotype designation requires the specification of both the G type (VP7 or G serotype) and P type (VP4 or P serotype). G types have been defined by a 20-fold difference in serum neutralization titers between the homologous and heterologous strains, while P types do not always meet these standard criteria; however, it is clear that major antigenic differences exist between P types. The laboratory determination of G type has been greatly simplified by newer techniques, such as enzyme immunoassays with monoclonal antibodies (27), polymerase chain reaction (13a), and hybridization with oligonucleotide probes (7, 25). By contrast, determination of P type is difficult, although probe hybridization and polymerase chain reaction techniques have recently become available (12, 20).

Many studies have examined the distribution of rotavirus G types in the circulation (1, 11, 14, 30), but few have linked G types with clinical information concerning disease severity (23, 26, 28). These latter studies have been generally inconclusive, perhaps because the number of rotavirus diarrheal episodes examined was small and few children had severe or fatal disease. The purpose of this study was to examine the relationship between epidemiologic and clinical features of rotavirus gastroenteritis and G type. On the basis of studies suggesting that VP4 plays a major role in rotavirus virulence (21), we hypothesized that clinical differences would likely involve G type 2, which is usually associated with P type 2, whereas symptomatic G type 1, 3, or 4 rotaviruses are generally associated with P type 1. In addition, for a subset of specimens, we were able to determine P type, adding to the small but growing body of data regarding P-type distribution and frequency.

MATERIALS AND METHODS

The study was conducted at two diarrhea treatment centers maintained by the International Centre for Diarrhoeal Disease Research, Bangladesh, in the Matlab area from July 1987 to June 1989. Data and stool specimens were collected from patients presenting for therapy of gastroenteritis, as part of passive surveillance instituted after a cholera vaccine trial initiated in 1985 (3). A total of 5,811 patients who sought treatment during this period contributed stool specimens and completed a surveillance questionnaire. However, only the

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2,441 specimens and corresponding data from children younger than 24 months of age are included in this analysis.

Laboratory methods. Laboratory methods, including routine culture for vibrios, salmonellae, and shigellae, have been described previously (11). Group A rotavirus was detected by using a commercial enzyme immunoassay kit (DAKOPATTS), and G types were determined by hybridization with synthetic oligonucleotides constructed from the nucleotide sequences of seven separate VP7 segments representing five human group A rotavirus G serotypes (1 to 4 and 8), and two of animal origin (3 and 6) (25). Enteric adenovirus was detected by using an enzyme immunoassay based on monoclonal antibody to types 40 and 41 (18).

Rotavirus P types (gene 4 types) were determined for a subset of 40 specimens by a recently developed polymerase chain reaction method and numbered according to a previously described classification scheme (26a). The 40 specimens were chosen to provide an approximately equal distribution of each G type and of specimens associated with the full range of severity of dehydration. Double-stranded RNA was extracted from stool specimens with glass powder and complementary DNA prepared by reverse transcription. The product was then subjected to 40 to 50 cycles of polymerase chain reaction by using step cycles of 1 min at 94°C, 2 min at 42°C, and 3 min at 72°C, with the buffer system, enzyme concentrations, and the type-specific primers described previously (12). All unusual strains and discordant samples were reextracted with glass powder from the original stool sample and retested at least twice for P type and G type by polymerase chain reaction, and gave reproducible results in two consecutive experiments.

Epidemiologic methods. The following demographic and epidemiologic data were collected at the time of the visit: type of diarrhea (i.e., watery, bloody, or mucoid), number of loose motions per day, history of emesis, abdominal pain, temperature, weight and height, treatment with oral rehydration therapy, and treatment with intravenous therapy.

Five parameters of dehydration were scored as indicated in parentheses: sunken fontanelle (0 or 1), sunken eyes (0, 1, or 2), skin turgor (0, 1, or 2), weak or absent pulse (0, 1, or 2), and dry mucous membranes (0 or 1). A composite score for severity of dehydration was derived from the sum of these five parameters (maximum score, 8; mild, 0 to 1; moderate, 2 to 3; severe, 4 to 8). A score of severe was significantly associated with receipt of intravenous therapy. Differences in severity among serotypes were analyzed by examining the score as a continuous variable from 0 to 8 by the Wilcoxon rank-sum test, but severity of dehydration is presented as the categorical variable in the tables because it better approximates clinical thinking. For all other variables, testing for significance of comparisons was done by the chi-square test unless otherwise indicated.

Three measures of nutritional status, weight-for-age, weight-for-height, and height-for-age Z scores, were calculated by using the anthropometry software package EPINUT version 1.0 (5).

If two visits for diarrhea occurred within 7 days of each other, these were taken to represent a single episode. For the comparison between rotavirus G types, episodes associated with enteric adenovirus, vibrios, salmonellae, or shigellae were excluded.

In addition, deaths were identified through the International Centre for Diarrhoeal Disease Research, Bangladesh, Demographic Surveillance System in Matlab (6). For deaths associated with detection of rotavirus in specimens within 30 days of death, the Demographic Surveillance System verbal autopsies were reviewed to determine the cause of death and the duration of illness prior to death.

RESULTS

Of 2,441 specimens from episodes of diarrhea among children younger than 24 months, 487 (20%) specimens were associated with a bacterial pathogen or with enteric adenovirus. A total of 764 (31%) episodes of gastroenteritis were associated with rotavirus detection by enzyme immunoassay and no other pathogen, while for 1,190 (49%) episodes no enteric pathogen was detected. Episodes with rotavirus were significantly more likely than those with no detected pathogen to be associated with watery diarrhea (98% versus 78%; $P < 0.01$), vomiting (93% versus 67%; $P < 0.01$), temperature higher than 102°F (12% versus 9%; $P < 0.01$), and more-severe dehydration (mean severity score, 1.7 versus 1.5; $P < 0.001$). Children with rotavirus episodes did not differ from those with no detected pathogen in terms of age, sex distribution, or measures of nutritional status.

Among the 764 rotavirus episodes, a single G type (1, 2, 3, or 4) was determined by oligonucleotide probe in 485, while 233 were nontypeable by probe hybridization (Table 1). Episodes did not differ by G type in prevalence of vomiting, copious diarrhea, temperature higher than 102°F, abdominal pain, or length of treatment center stay. Episodes associated with G types 2 and 3 were associated with more-severe dehydration than episodes associated with G types 1 or 4 or with nontypeable rotavirus (mean severity scores, 1.9 and 2.0 versus 1.7, 1.6, and 1.6, respectively; $P < 0.05$ for the two-way comparisons of type 2 versus 4, 2 versus nontypeable, 3 versus 4, and 3 versus nontypeable; $P = 0.06$ for 2 versus 1; $P = 0.12$ for 3 versus 1). Episodes with G type 2 were more likely to require intravenous therapy than those with G type 4 or nontypeable rotavirus ($P < 0.05$ for each two-way comparison). There were no differences in age distribution for episodes associated with the various G types.

Regardless of G type, children younger than 18 months had more-severe dehydration associated with rotavirus episodes than children between 18 and 23 months of age (mean severity scores, 1.7 versus 1.5; $P < 0.05$). For children between 12 and 17 months of age, a weight-for-age Z score or weight-for-height Z score of less than -3 was associated with more-severe dehydration (mean severity scores, 1.9 versus 1.5 and 2.4 versus 1.6, respectively; $P < 0.01$ for each comparison). No difference in severity of dehydration was associated with nutritional status for other age groups or with lesser degrees of malnutrition in any age group.

Seven children who presented to one of the treatment centers with two episodes of rotavirus-associated diarrhea were identified. The median age was 8 months at first infection (range, 2 to 14 months) and 15 months at second infection (9 to 27 months). One child experienced two illnesses associated with G type 2 rotavirus; no other homologous reinfections were detected. Of note, three of seven first infections were associated with moderate or severe dehydration, whereas all second infections caused mild dehydration.

Twelve children who died with diarrhea as a major symptom had rotavirus detected in stool specimens within 30 days of death (Table 2). Their median age was 7.5 months (2 to 15 months), and 7 of 12 (58%) were male. No seasonality was apparent. Seven of the 12 children had had acute watery diarrhea, while five had diarrhea lasting more than 14 days, and at least three had malnutrition reported in the verbal

TABLE 1. Clinical characteristics of children with rotavirus diarrhea and G type detected by oligonucleotide probe hybridization[a]

Characteristic	No. (%) of episodes associated with G type or nontypeable rotavirus:				
	1 (*n*, 146)	2 (*n*, 198)	3 (*n*, 36)	4 (*n*, 105)	Nontypeable (*n*, 233)
Acute malnutrition[b]					
Severe	11 (3)	14 (7)	1 (3)	7 (7)	16 (7)
Moderate	58 (40)	93 (47)	17 (47)	41 (39)	98 (42)
Breast fed	146 (100)	197 (99)	35 (97)	104 (99)	228 (98)
Vomiting	140 (96)	184 (93)	33 (92)	96 (91)	212 (91)
>10 stools/day	53 (36)	79 (40)	11 (31)	38 (36)	98 (42)
Temperature >102°F	15 (10)	21 (11)	2 (6)	12 (11)	39 (17)
Abdominal pain	89 (61)	131 (66)	26 (72)	67 (64)	138 (59)
Severity of dehydration[c]					
Mild	79 (54)	90 (46)	16 (44)	65 (62)	136 (58)
Moderate	49 (34)	79 (40)	13 (36)	27 (26)	80 (34)
Severe	18 (12)	29 (15)	7 (19)	13 (12)	17 (7)
Mean score	1.7	1.9[d]	2.0[d]	1.6	1.6
Received IV	9 (6)	23 (12)[d]	3 (8)	4 (4)	14 (6)
Median treatment center stay (range) [days]	2 (0–8)	2 (0–9)	2 (1–5)	2 (0–5)	2 (0–7)

[a] Episodes associated with other pathogens were excluded from analysis. *n*, total number of episodes tested.
[b] Severe malnutrition corresponds to a weight-for-height Z score of <−3; moderate malnutrition corresponds to a weight-for-height Z score of <−2.
[c] Based on composite score: range, 0 to 8; mild, 0 to 1; moderate, 2 to 3; severe, 4 to 8.
[d] Two-way comparisons were statistically significant (see the text).

autopsy. In addition, rotavirus-infected children 2 to 15 months of age who died were more likely to have a weight-for-age Z score of less than −3 than those who survived (73% versus 32%; $P < 0.01$). Five of eleven specimens for which G typing was attempted were nontypeable, a higher proportion than that for the specimens overall (45% versus 30%), while two hybridized with more than one probe.

Of 40 specimens tested by polymerase chain reaction for P type, 29 (72.5%) were P type 1, 9 (22.5%) were P type 2, 1 (2.5%) was P type 3, and 1 (2.5%) was untypeable. P type 1 specimens were predominantly G type 1, 3, or 4, while P type 2 specimens were predominantly G type 2. One G type 1 specimen was P type 3, a type usually associated with asymptomatic neonatal rotavirus infection (9); this specimen was collected from a child with severely dehydrating diarrhea. Episodes associated with P types 1 and 2 did not differ in severity of dehydration or other clinical features.

DISCUSSION

Six studies have previously examined clinical features associated with G type or subgroup. One early study found G type 2 rotavirus more strongly associated with dehydrating diarrhea than G type 1 (31). Two more recent studies reported no differences in clinical severity or symptomatic-to-asymptomatic ratio (13, 23), while three others found minor clinical differences in prevalence of vomiting, high fever, or days ill, but with no consistent pattern (10, 26, 28). However, only one of the recent studies (10) examined more than 170 specimens, and the small numbers of each G type or subgroup identified may have precluded the detection of small differences in clinical features.

Our study is the largest one which has examined the relationship between G type and clinical features. Our findings support the hypothesis that G type 2, and perhaps G

TABLE 2. Deaths associated with rotavirus-positive specimen within 30 days prior to death

Child no.	Age (months)	Sex	Month/year of death	Days ill prior to death	Days between specimen and death	Serotype	Cause of death[a]
1	10	M	8/87	25	10	1	PD
2	15	F	8/87	15–20	3	2	PD
3	9	M	9/87	10	10	2	AWD
4	6	M	10/87	8	4	Nontypeable	AWD
5	8	M	12/87	8	1	Nontypeable	AWD
6	2	F	1/88	30+	23	Nontypeable	MAL, PD
7	7	M	2/88	30	30	1, 3	PD, possible measles
8	8	M	2/88	10	7	Nontypeable	AWD, ARI
9	4	F	5/88	30	24	2	MAL, PD
10	6	F	6/88	10–15	9	1, 2[b]	MAL, AWD, ARI
11	10	M	9/88	5	4	Nontypeable	AWD
12	7	F	11/88		3	ND[c]	AWD

[a] AWD, acute watery diarrhea; PD, persistent diarrhea; ARI, acute respiratory infection; MAL, malnutrition.
[b] *Vibrio cholerae* was also detected.
[c] ND, typing not done.

type 3, rotavirus may be associated with more severe dehydration than other G types, although the differences do not appear to be of major clinical importance. Among all four G types, the mean severity score ranged from 1.6 to 2.0, a difference which is probably not clinically distinguishable. While we hypothesized that these differences are related to the association of G type 2 with P type 2, we were not able to demonstrate a difference in severity of dehydration associated with P type; however, P typing was conducted for only 40 specimens, limiting our ability to detect differences. Nonetheless, our analysis suggests that in this study population, a rotavirus vaccine must protect against G type 2 rotavirus to be effective: although 27% of rotavirus specimens were G type 2, 40% of episodes associated with severe dehydration were due to G type 2 rotavirus.

Despite the large number of episodes examined, we were not able to demonstrate clinically important differences in severity by G type. The data in this study are facility based, precluding the inclusion of episodes too mild to prompt a visit to the health facility. Although this factor limited the range of disease severity that we were able to study, facility-based studies have been used successfully to examine differences in severity for other enteric pathogens (4).

The other major limitation of our study is the lack of microbiologic data concerning enterotoxigenic *Escherichia coli*. Many of the episodes with no detected pathogen may have been due to this organism. However, this limitation should not greatly affect serotype comparisons for rotavirus-associated episodes, as mixed infections should represent an equal proportion for each serotype.

We detected a number of rotavirus reinfections and, as was found in previous studies (22, 24), at least one reinfection with the same G type. Because this study was based on passive surveillance data, these reinfections represent a small proportion of true reinfections, since all illnesses detected were severe enough to bring the child to a treatment center. Repeat illnesses were milder than first ones, but this may have been due to the older age of the child, as well as to acquired immunity, factors which cannot be separated in this study.

Finally, 12 children died, with rotavirus-associated diarrhea as a sole or contributing cause of death. Several of these children had persistent diarrhea, a diagnosis not previously associated with rotavirus. No single G type was predominant among the children who died, and other diagnoses, such as malnutrition, acute respiratory infection, and measles, were common, suggesting that virulence sufficient to lead to death is not limited to one G type and is potentiated by other factors.

Our data suggest that, although G types 2 and 3 were associated with more-severe dehydration than were other serotypes, the differences are not of major clinical importance. Other factors, such as the age of the child and severe malnutrition, also affect the severity of illness associated with rotavirus. While coverage for G type 2 rotavirus will be essential, rotavirus vaccines will have to protect against all four major G types to be effective.

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