Rotavirus Gastroenteritis in Italian Children: Can Severity of Symptoms Be Related to the Infecting Virus?

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The aim of our study was to determine whether the severity of rotavirus gastroenteritis may be related to the different characteristics of infecting viral strains. The severity of clinical symptoms in 401 children with acute rotavirus gastroenteritis was assessed using a scoring system for frequency and duration of vomiting, diarrhea, and fever, as well as the patients' requirements for intravenous rehydration. Rotavirus strains were characterized by determining the electropherotype of their double-stranded RNA, the G type and subgroup by a panel of monoclonal antibodies, and the P type by reverse transcription–polymerase chain reaction. Strains with a short electropherotype, G2P[4] type, and subgroup I were associated with more-severe gastroenteritis and affected children older than those infected with strains with a long electropherotype, G1P[8] or G4P[8] type, and subgroup II. Minor differences in clinical symptoms were also detected in children infected with different long electropherotypes and with G1P[8] and G4P[8] specificities.

Worldwide, rotaviruses are the single most important cause of severe acute diarrhea in young children. Rotaviruses are responsible for high morbidity in developed countries and high mortality in developing countries (estimated at ~1,000,000 deaths annually) [1]. Classically, the characterization of human rotavirus (HRV) strains has been carried out by revealing the migration pattern of the 11 segments of the viral genomic double-stranded RNA by PAGE (electropherotype); furthermore, with EIA, it is possible to determine the subgroup I (SG I) and II (SG II) specificities (associated with the major outer capsid protein VP6) and G serotype specificities (associated with the major outer capsid protein VP7). More recently, P serotype specificities (associated with the minor outer capsid protein VP4) were predicted by a typing method based on reverse transcription–PCR analysis (RT-PCR) [2, 3].

Electropherotype, subgroup, and G and P type usually are linked. In fact, HRV strains with long electropherotypes (faster-moving gene segment 11) have been found to be associated with SG II specificity and G1P[8], G3P[8], G4P[8], or G9P[8] type, whereas HRV strains with short electropherotypes (slower-moving gene segment 11) usually exhibit SG I specificity and G2P[4] type [2]. So far, the few studies carried out to attempt to correlate electropherotype, subgroup, and G specificities with clinical information concerning disease severity have generally been inconclusive [4–8], or, at best, they have shown that differences do not appear to be of major clinical importance [9–12].

The aim of our study was to examine the relationship between clinical features of HRV enteritis and electropherotypes, subgroups, and G and P types of the virus.
MATERIALS AND METHODS

Study design. All children who presented to the G. Di Cristina Children’s Hospital in Palermo, Italy, from January 1993 to May 1997 with diarrhea were assessed by the medical staff of the emergency department; those admitted to the hospital were considered eligible for the study if they had a primary diagnosis of acute diarrhea—defined as ≥3 watery stools in a period of 24 h with a sudden onset, with or without vomiting, of ≤7 days’ duration—with no identifiable cause for the symptoms other than infective gastroenteritis. Diarrhea of >7 days’ duration, antibiotic treatment 7 days before presentation, other gastroenteric diseases, and immunodeficiency were considered exclusion criteria for the study.

For all children, feeding was interrupted for 8 h, and orally administered rehydration solution with reduced osmolarity was provided [13]. Milk without lactose was used to refeed children administered rehydration solution with reduced osmolarity was gastroenteric diseases, and immunodeficiency were considered.

The medical staff of the infectious diseases department interviewed the adults accompanying the children, examined the children, and filled out a form with demographic aspects (sex and age) and clinical data (duration of diarrhea and number of bowel movements per day, occurrence and duration of vomiting and fever, and presence of mucus or blood in stools); the criterion for starting iv rehydration was an estimated dehydration of ≥5% in children refusing or unable to ingest the orally administered rehydration solution or to consume the rice and apples was provided to the older children.

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A 14-point scoring system slightly modified from that of Flores et al. [14] was used to classify the severity of the cases. Five clinical parameters were scored: duration of the diarrhea (<2 days, 1; 2–4 days, 2; >4 days, 3), maximum number of bowel movements per day (3 bowel movements, 1; 4–5 bowel movements, 2; >5 bowel movements, 3), duration of vomiting (no vomiting, 0; 1–2 days, 1; >2 days, 3), occurrence of fever (no, 0; yes, 2), and necessity of iv rehydration (no, 0; yes, 3).

Stool specimens were collected within 12 h after each patient’s admission to the hospital, to avoid inclusion of nosocomial cases. Specimens were divided into aliquots, depending on the quantity of available stool, and were stored at −20°C until assay could be performed.

PAGE. Electropherotyping of viral RNA was performed on 10% polyacrylamide gels, as described elsewhere [15]. Electropherotypes were classified by dividing the 11 RNA segments into 4 groups, according to Loureno et al. [16].

EIA with monoclonal antibodies. G typing was performed by EIA using type 1-, 2-, 3-, 4-, 6-, and 9-specific neutralizing monoclonal antibodies reactive with viral protein VP7, and subtyping was performed by an EIA with SG I– and SG II–specific monoclonal antibodies reactive with viral protein VP6, as described elsewhere [15].

RT-PCR. P typing was performed by RT-PCR with glass powder–extracted RNA, as described elsewhere [17], by use of P[4]–, P[6]–, P[8]–, P[9]–, and P[14]–specific primers (Life Technologies).

Bacteriology. All the specimens were plated on MacConkey, Salmonella-Shigella, and thiosulfate-citrate– bile salts– sucrose agars, incubated at 37°C for 18 h, and examined for the presence of Salmonella, Shigella, vibrios, and other enteropathogens by standard methods.

Statistical analysis. Analysis of the data was carried out with Statistica (StatSoft) and Epi Info (Centers for Disease Control and Prevention) software. All the variables were analyzed by means of the Shapiro-Wilk W test (a powerful normality test) [18], and, because none had a normal distribution, only nonparametric tests were subsequently used. Initially, the Kruskal-Wallis analysis of variance was used to understand whether there were differences by variable (e.g., age or duration of vomiting) among the different groups of patients, and then, in the case of positive results, data were analyzed 2 by 2 using the Mann-Whitney U test. Categorical variables were analyzed by multeway frequency tables, and Pearson’s χ² value was calculated. In the case of positive results, the groups were analyzed 2 by 2 using frequency tables. Spearman’s rank order coefficient was computed to verify the existence of correlations between variables. A 2-sided level of P < .05 was used for all analyses.

RESULTS

From January 1993 to May 1997, 1166 stool specimens were obtained and examined by PAGE. A total of 401 (34.4%) of the specimens were positive for group A rotavirus. In 7 cases, a Salmonella-HRV coinfection was documented. Non–group A rotavirus was not detected. The age of the HRV-infected children ranged from 15 days to 12 years (median, 17 months). A total of 231 (57.8%) patients were boys.

Fourteen different electropherotypes were identified by PAGE (figure 1), but only 5 (bbee, bbfb, bcea, cbea, and ccea) were detected in 95.5% of the samples. According to the monoclonal antibodies used, 84 (20.9%) strains belonged to SG I and 300 (74.8%) to SG II; 17 (4.2%) strains were not groupable.

With regard to G specificities, 203 (50.6%) HRV strains belonged to G1 type, 42 (10.4%) to G2, 55 (13.7%) to G4, 10 (2.5%) to G3, 3 (0.4%) to G6, and 2 (0.7%) to G9, whereas 86 (21.4%) were not typeable. Thirty-three of the nontypeable strains constituted 39.3% of all the strains with short electropherotypes.

In table 1, the associations among electropherotype length (long and short), subgroup, and G type of all the HRV strains are shown. We observed that the majority of the strains with long electropherotypes belonged to SG II and to the G1 or G4 types (79.8%), whereas strains with short electropherotypes be-
Longed to SG I and to the G2 type (48.8%). An anomalous association between electropherotype and G type was observed for only 7 strains, all belonging to SG I: 4 had a long pattern (1 cafa, 2 caga, and 1 aafa) and G3 type; 2 had a short pattern (bbfb) and G6 type; and 1 had a short pattern (bbeb) and G1 type.

Despite the fact that an initial analysis made separately for subgroup, G type, and electropherotype exhibited statistically sig-

ificant differences (data not shown), we decided to analyze data for gastroenteritis caused by the 9 prevalent electropherotype–G type associations: bbea-G1, bbea-G4, cbea-G1, cbea-G4, cbea–G not typeable (GNT), bbea-G4, ccea-G1, bbfb-G2, and bbfb-GNT. P typing was carried out on 26 (49.5%) of the HRV strains exhibiting bbea-G1 and bbea-G4 patterns and on all HRV strains with bbfb-G2 and bbfb-GNT patterns. All strains with long elec-

Table 1. Associations among electropherotype, subgroup, and G type of 401 human rotavirus strains found in children in Palermo, Italy.

<table>
<thead>
<tr>
<th>Electropherotype</th>
<th>Subgroup I</th>
<th>Subgroup II</th>
<th>Subgroup NT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>Long (n = 317)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aafa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bbea</td>
<td>39</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>bbga</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bceaa</td>
<td>3</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>cafa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>caga</td>
<td>3</td>
<td>145</td>
<td>9</td>
</tr>
<tr>
<td>ccceae</td>
<td>11</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Short (n = 84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bbfb</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bbeb</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bbfb</td>
<td>38</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>cbfb</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** NT, not typeable.

* Strains with anomalous associations.
tropherotypes showed a P[8] type. All but 1 with short patterns were P[4] type; the different one exhibited a P[8] type.

The overall median score (figure 2) of enteritis caused by strains with the short electropherotype bbea (G2 or GNT) was 2 points higher than that of enteritis caused by strains with the long electropherotypes bbea-G1 (P<.01), bbea-G4 (P<.05), and cbea-GNT (P<.0004); 3 points higher than for strains with bbea-G4 (P<.0004), cbea-G1 (P<.00001), and ccea-G1 (P<.004); and 4 points higher than for strains with cbea-G4 (P<.003). The median score of the enteritis caused by strains with bbea-G1 was 1 point higher than that for strains with cbea-G1 (P<.03) and 2 points higher than that for strains with cbea-G4 (P<.05). The median score of the enteritis caused by strains with bbea-G4 was 2 points higher than that for strains with cbea-G1 (P<.04).

The median duration of vomiting by children infected with HRV strains with bbea-GNT (3 days) was 24 h longer than the duration of vomiting by children shedding strains with bbea-G4 (P<.02) and 48 h longer than the duration of vomiting by children shedding strains with bbea-G4 (P<.006), cbea-G1 (P<.002), cbea-G4 (P<.05), or cbea-GNT (P<.006).

The median duration of fever in the children who shed HRV strains with bbea-GNT (3 days) was 23 h longer than that for infections caused by strains with bbea-G1 (P<.018), bbea-G4 (P<.05), cbea-G1 (P<.04), or cbea-GNT (P<.02) and 48 h longer than that for infections caused by strains with bbea-G4 (P<.05).

The median duration of diarrhea in children who shed HRV strains with bbea-GNT (6 days) was 48 h longer than that for infections caused by strains with cbea-G1 (P<.00002), cbea-GNT (P<.0003), bbea-G4 (P<.0006), cbea-G1 (P<.05), or bbea-G2 (P<.0003) and 60 h longer than that for infections caused by strains with bbea-G4 (P<.0004). The median duration of diarrhea in children who shed HRV strains with bbea-G1 was 24 h longer than that for infections caused by strains with cbea-G1 (P<.004), cbea-GNT (P<.03), or bbea-G2 (P<.05) and 36 h longer than that for infections caused by strains with bbea-G4 (P<.02).

Intravenous rehydration was required by 52% of the children shedding strains with bbea-G2 and by 40.6% of those shedding strains with bbea-GNT; these percentages were significantly higher (χ² test, P<.02) than those for children infected by strains with bbea-G1 (15.4%), cbea-G1 (9.7%), or cbea-GNT (11.5%).

Different electropherotype–G type associations were not related to significant differences in the number of bowel movements per day; to the order in which diarrhea, fever, and vomiting started; to the occurrence of respiratory symptoms; or to the appearance of mucus or blood in the stools.

The age of the children who shed HRV strains with bbea-G2 was a median of 12 months higher than that of the children infected by strains with bbea-G1 (P<.002), bbea-G4 (P<.04), cbea-GNT (P<.006), bbea-G4 (P<.0004), bbea-GNT (P<.0001), or cbea-G1 (P<.02) (figure 3). The age and the severity scores were not correlated (Spearman’s rank order correlation, r<.02; P=.2).

Figure 2. Score of enteritis caused by the 9 most frequent human rotavirus electropherotype–G type association patterns, plotted by the whiskers method. Lower and upper horizontal bars correspond to the 25th and 75th percentiles, respectively; squares represent the median values. The number of subjects is indicated at the bottom of each column. *Significant (Mann-Whitney U test) compared with bbea-G1 (P<.01), bbea-G4 (P<.05), cbea-G1 (P<.0004), bbea-G1 (P<.0004), bbea-G1 (P<.00001), cbea-G1 (P<.004), and cbea-G4 (P<.0003). §Significant compared with cbea-G1 (P<.03) and cbea-G4 (P<.05). †Significant compared with cbea-G4 (P<.04).
Figure 3. Age of children shedding the 9 most frequent human rotavirus electropherotype–G type association patterns, plotted by the whiskers method. Lower and upper horizontal bars correspond to the 25th and 75th percentiles, respectively; squares represent the median values. The number of subjects is indicated at the bottom of each column. *Significant (Mann-Whitney U test) compared with bbea-G1 (P < .002), bbea-G4 (P < .04), cbea–G not typeable (GNT; P < .006), bcea-G4 (P < .0004), bbbf-GNT (P < .0001), and ceea-G1 (P = .02).

With regard to the temporal distribution, strains with G4 type were prevalent from January 1993 through March 1994, strains with G1 type were prevalent from March 1994 through May 1996, and those with G2 type were prevalent from November 1996 through May 1997 (figure 4).

DISCUSSION

In this study, we attempted to correlate electropherotype, subgroup, and G type of HRV strains with the epidemiological and clinical findings for a large cohort of children with rotavirus gastroenteritis. Of the few previous studies [4–12] in which the problem of the association between the severity of enteritis and the various criteria of classification of HRV (electropherotype, subgroup, and G type) was considered, only in those of Bern et al. [9] and Yolken et al. [10] were HRV strains with G2 type associated with more-severe dehydration than were strains with other G types, and the differences were not statistically significant.

Our observations are consistent with an association of more-severe disease among children shedding HRV strains with short electropherotypes (fundamentally represented by bbbf) than among those shedding strains with long electropherotypes; interestingly, among subjects who shed viruses with long electropherotypes, more-severe disease seemed to be associated with particular strains (bbea-G1 and bbea-G4) that had the same P[8] type.

On the basis of studies suggesting that VP4 plays a role in rotavirus virulence [19], we hypothesized that the severity of clinical symptoms among children with rotavirus gastroenteritis may reflect either differences in virulence among strains or the introduction of a new G and P type in the community. Epidemiological survey work regarding the circulation of HRV in the city of Palermo showed that during the 10 years before the study began, the G2 type had rarely been found [15, 20].

A new introduction of strains with G2 type into Palermo might have encountered a population immunologically naive for this serotype. Similarly, surveys on HRV G types associated with diarrhea in children in several countries have shown periods in which HRV with G2 type was seldom detected in a community [8, 21, 22]. These periods were interrupted by widespread but brief epidemics associated with HRV with G2 type. Similar periodicity could be seen in HRV strains with G3 and G4 types. The constant and widespread presence of G1 in any community [23] may protect the population from strains of serotypes G1, G3, and G4, which usually share P[8] type (Wa-like) and SG II specificities; in fact, an infection with 1 of these 3 G types apparently induces a booster response against the other 2 [24].

Interestingly, the association patterns bbfb-G2P[4] and bbfb-GNTP[4] were the most common identified in the winter season (November through May) 1996–1997 and caused an outbreak of more-severe gastroenteritis. Regarding the strains with bbfb-GNT, the detection of their P[4] specificity raises the possibility that they could be G2 type. Genetic variation in the VP7 gene of HRV G2 type has been described [25]. Monoclonal antibodies directed to different epitopes of the same serotype could be necessary to type these strains [26].
The children infected with bbfb-G2 strains were ∼12 months older than children who shed other strains. We can hypothesize that this epidemic affected children who had experienced an infection with an HRV with another G type some months before; this hypothesis is corroborated by recent data in the literature that implicated G2 type strains in symptomatic HRV reinfections in older children and adults [24, 27, 28].

However, the differences that we observed in this study in the degree of severity of the enteritis caused by HRV strains with some of the long-pattern electropherotypes remain unexplained. According to recent findings that identified NSP4 as a virulence factor acting as an enterotoxin [29], we can speculate that an unknown virulence factor is associated with strains with bbea, but further studies are needed to demonstrate this hypothesis. The 7 strains with anomalous associations, which were probably of animal origin or the result of genetic recombination, were not associated with more-severe enteritis and likely were not able to spread in the human population. Iizuka et al. [30] speculated that gene segment 5 might be involved in the adaptation of naturally occurring reassortants to humans.

In conclusion, our research has succeeded in showing clinical differences among infections caused by different strains of HRV. We suppose that such differences are due to the reintroduction in a geographic area of a strain that had not circulated in that area for a long time, but we cannot exclude the possibility that such differences could be due to intrinsic factors related to HRV strains that strongly express some virulence factor. Further studies of the role of virulence factors in HRV infections are needed to elucidate such a hypothesis.

Acknowledgments

We thank L. Titone and all the members of the medical staff of the Infectious Diseases department of the G. Di Cristina Children’s Hospital in Palermo, Italy, for their collaboration and support. We also thank S. Antinori for critical advice and S. McIntyre for the revision of the English version of this article.

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