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# Rotavirus Diarrhea Severity Is Related to the VP4 Type in Mexican Children

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**This report is of a community-based case control study to assess whether the severity of acute diarrhea by rotavirus (RV) in young children is associated with a particular VP7 (G) or VP4 (P) RV serotype. Five hundred twenty children younger than 2 years of age with diarrhea lasting less than 3 days were age and gender matched with 520 children with no diarrhea. The G and P serotypes were determined with specific monoclonal antibodies, and the VP4 serotype specificity in a subgroup was confirmed by genotyping. Infection with a G3 serotype led to a higher risk of diarrhea than infection with a G1 serotype. Infection with a G3–nontypeable-P serotype was associated with more severe gastroenteritis than infection with a G3 (or G1) P1A[8] serotype. A child with diarrhea-associated dehydration was almost five times more likely to be infected with a G3–nontypeable-P serotype than a child without dehydration ($P < 0.001$). Moreover, the two predominant monotypes within serotype P1A[8] had significantly different clinical manifestations. In this study, the severity of RV-associated diarrhea was related to different P serotypes rather than to G serotypes. The relationship between serotype and clinical outcomes seems to be complex and to vary among different geographic areas.**

Group A rotaviruses (RVs) are the leading cause of acute diarrhea with severe dehydration, which endangers the lives of children under 2 years of age (14). An effective vaccine could prevent the death of 800,000 children per year (7, 20).

Antibody specificity to neutralize different RV strains has been used to classify them into serotypes, and both of the viral surface proteins, VP4 and VP7, induce neutralizing antibodies; hence, the RV serotype is dual and has been named G and P for VP7 and VP4, respectively (15, 18, 19). Based on VP7, 15 different RV serotypes have been classified in group A (19). Ten of these serotypes (G1 to G6, G8 to G10, and G12) infect humans, although five of them (G1 to G4 and, more recently, G9) seem to be responsible for most infections (19, 24, 29, 35), while serotype G5 is emerging as epidemiologically important in Brazil (16, 22). At least 21 different types of VP4, P [1] to P [21], have been defined by genomic analysis (hybridization and sequencing) (19). Ten of these P types have been found among human RV strains and correspond to specific serotypes or subtypes of VP4, as was determined by neutralization assays with monospecific hyperimmune sera directed against this protein (19). Two of these serotypes (subtypes A and B of serotype P1 and subtype A of serotype P2) are the most frequent among human RV strains (11, 26). Recently, two research groups reported the isolation of the first specific monoclonal antibodies (MAbs) that recognize different reference strains of serotypes P1A, P1B, and P2A (5, 25). Coulson (4) introduced the term "monotype" to define the intraserotypic RV variants, which differ in their reactivities with a given MAb. Thus, a previous study identified five monotypes of serotype P1A[8] according to their reactivities with specific MAbs, namely, F45, 1A10, F45-1A10, F45-RV5, and F45-1A10-RV5, while monotypes RV5, 1A10, and RV5-1A10 were identified for serotype P1B[4] and only monotype HS6 was identified for serotype P2A[6] (26).

Enteric infections with RV have a wide spectrum of clinical manifestations ranging from symptom-free infections to mild, severe, and fatal cases of diarrheal disease. Hence, it is of great interest to search for the determinants of these diverse clinical outcomes, including the possible role of the different RV serotypes. Few studies have previously addressed this issue (2, 6, 21, 31, 33), and they have been generally inconclusive due to the small number of RV diarrheal episodes studied and to the lack of the full range of severity of dehydration. As exceptions to this lack of conclusive results, two studies found that serotype G2 in Italy (3) or serotypes G2 and G3 in Bangladesh (2) were associated with more-severe gastroenteritis than serotype G1 or G4. More studies like these, especially studies that include large number of samples and P serotyping with newly developed methods based on MAbs (4, 5, 25, 26), are needed from other regions of the world.

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TABLE 1. Relationship between G and P serotypes in RVs identified in 198 children with diarrhea

| G serotype | No. of RVs of serotype: | | | Total |
|---|---|---|---|---|
| | P1A[8] | P1B[4] | Nontypeable P[a] | |
| G1 | 34 | 0 | 5 | 39 |
| G2 | 0 | 10 | 1 | 11 |
| G3 | 95 | 3 | 30 | 128 |
| Nontypeable G[b] | 1 | 0 | 12 | 13 |
| Mixed | 3 | 0 | 2 | 5 |
| Common | 0 | 0 | 2 | 2 |
| Total | 133 | 13 | 52 | 198 |

[a] P serotypes that were not P1A[8], P1B[4], or P2A[6].
[b] G serotypes that were not G1, G2, G3, or G4.

The aim of this study was to determine whether the infecting RV serotypes (based on both VP4 and VP7) were associated with different levels of severity of gastroenteritis. This study may add useful information for the development of a more effective and safer vaccine after the withdrawal of the human-rhesus reassortant tetravalent RV oral vaccine due to the report of postvaccine intestinal intussusception cases (1).

## MATERIALS AND METHODS

An age- and gender-matched case control study of children under 2 years of age was carried out. The subjects included 520 children with acute diarrhea that had started within 72 h. The controls included 520 children with no diarrhea. All children were enrolled from seven primary health care centers (one in the north, two in the south, and four in the center of Mexico) from October 1994 to March 1995. Control asymptomatic children were included from the outpatient clinics when they had requested medical attention for diseases other than diarrhea within 5 days prior to our enrolling them in the study. Written consent to include each child in the study was asked from the children's parents or guardians.

The clinical information of the 520 symptomatic children was registered in a special form that included a description of the usual pattern of stools 2 days before the onset of diarrhea and during the diarrheic episode, with watery, loose, or solid stools, with or without mucus or blood, and other characteristics, such as fever and vomiting, being noted. The data were collected by the mother and the consulting physician on the day of the visit. The therapeutic plan was given on the basis of the patient's hydration level according to the World Health Organization recommendations (36). Body weight was determined at onset, following rehydration when necessary, after 14 days of evolution and after the final outcome (life or death).

A child was considered asymptomatic and infected by RV if diarrhea was not present for 5 days before an RV-positive fecal sample. Diarrhea was defined as three or more watery or loose stools in a period of 24 h, not corresponding to the usual stool pattern. The clinical characteristics of children with diarrhea caused by different RV serotypes were compared. A numerical score for severity was taken from the Ruuska and Vesikari scale (30) with two modifications. (i) Instead of using percentages to evaluate dehydration intensity, three groups with similar scores were considered: nondehydrated children, dehydrated children, and children with hypovolemic shock, as recommended by the World Health Organization (36). (ii) The parameter related to treatment was omitted since all cases were treated as outpatients. Thus, the highest possible score was 18, instead of the 20 of the Ruuska and Vesikari scale (30).

The primary RV diagnosis was done locally in each of the participating centers by a commercial immunoenzymatic test (Immuno Card; Meridian Diagnostics Inc.) or by genomic viral RNA electrophoresis followed by silver nitrate staining (17). One hundred forty-five and 140 RV-positive samples were detected by the first and second methods, respectively. RV-positive stool samples were frozen at −20°C until they were sent in refrigerated polyfoam boxes to the laboratory at the Biotechnology Institute. The presence of RV was confirmed by a second immunoenzymatic assay (DAKO Corporation) considered, in this laboratory, the reference standard in RV diagnosis (8). Eleven and 2.85% of the samples positive by the Immuno Card test and by genomic viral RNA electrophoresis followed by silver nitrate staining, respectively, were not confirmed to be positive by this reference standard. Serologic and genomic characterization of strains was further performed in the Biotechnology Institute laboratory.

Once the presence of RV was detected, it was serotyped by specific anti-VP7 and anti-VP4 MAbs used as capture antibodies in an immunoenzymatic test, as previously described (24, 25). The MAbs used (and their serotype specificities) are KU4 and 5E8 (G1); 1C10 (G2); 159 (G3); ST-2G7 (G4); 129 (cross-reactive anti-VP7), F45:4 (hereinafter referred to as F45), and 1A10 (P1A); RV5:2 (hereinafter referred to as RV5) (P1B); and HS6 (P2A), as formerly described (26). RV strains reacting with the cross-reactive MAb 129 but not the G1- to G4-specific MAbs are identified as of the common G serotype. In a subgroup of the analyzed samples, the genes for VP4 were characterized by PCR, using specific oligonucleotides for the genomic types P [3], P [5], P [7], and P [8], as previously described (12). A full description of the relationship between the G serotypes with the P serotypes as determined by their patterns of reactivity with anti-VP4 MAbs or by genotyping by reverse transcription-PCR was described previously (26). Personnel in the laboratory who performed the typing of stool specimens were blind to the clinical information. The G and P RV serotypes were then correlated with clinical manifestations and complications, as well as with the child's age, nutritional status, and gender.

The statistical analysis was done with the Statistical Package for Social Sciences program for Windows. Results are presented as percentages, medians, and ranges. Medians were compared by the nonparametric Mann-Whitney U test for independent samples and, in the case of more than two groups, by the nonparametric Kruskal-Wallis test. Proportions were compared by $\chi^2$ test. Odds ratios (OR) and their 95% confidence intervals (CI) were also estimated (10).

## RESULTS

RV was detected in the feces of 264 out of the 520 children with diarrhea (51%), in contrast with only 79 (15%) of the asymptomatic children. A child with diarrhea was six times more likely to be infected with RV than an asymptomatic child (OR = 5.8; 95% CI = 4.2 to 7.8; $P = <0.0001$). All cases had a favorable clinical course, and no deaths were registered. The mean severity scores were 8.3 points for RV-negative patients and 11.1 points for RV-positive patients ($P < 0.01$).

Of the 264 RV-positive specimens (75%) from symptomatic children, 198 had enough sample material left for G and P serotyping, and in 145 of these 198 specimens (73%), both the G and P serotypes could be identified. Table 1 shows the number of RVs identified in these 198 children with diarrhea according to the G-P binomial serotype classification. The predominant serotypes were G3-P1A[8] (50%), G1-P1A[8] (18%), and G3–nontypeable-P (16%). Only 5% of the characterized RVs belonged to the G2-P1B[4] serotype. In no case was the G4 or the P2A serotype found. The most frequently identified P monotypes within serotype P1A[8] were F45-1A10 in 92 specimens (46.5%), F45 in 25 specimens (12.6%), and

TABLE 2. Relationship between G and P serotypes in RVs identified in 75 asymptomatic children

| G serotype | No. of RVs of serotype: | | | Total |
|---|---|---|---|---|
| | P1A[8] | P1B[4] | Nontypeable P[a] | |
| G1 | 17 | 0 | 4 | 21 |
| G2 | 0 | 0 | 0 | 0 |
| G3 | 23 | 0 | 6 | 29 |
| G4 | 1 | 0 | 1 | 2 |
| Nontypeable G[b] | 1 | 0 | 12 | 13 |
| Mixed | 6 | 0 | 0 | 6 |
| Common | 2 | 0 | 2 | 4 |
| Total | 50 | 0 | 25 | 75 |

[a] P serotypes that were not P1A[8], P1B[4], or P2A[6].
[b] G serotypes that were not G1, G2, G3, or G4.

TABLE 3. Illness severity in 159 children with diarrhea caused by RVs relative to the infecting G-P serotype[a]

| G serotype (no. of children) | Median severity score (minimum, maximum) | No. (%) of children dehydrated | No. (%) of children with shock |
|---|---|---|---|
| G3-nontypeable P[b] (30) | 12 (6, 15)[c,d] | 21 (79)[f,g] | 3 (10)[i] |
| G3-P1A[8] (95) | 10 (4, 15)[c,e] | 39 (41)[f,h] | 1 (1)[i] |
| G1-P1A[8] (34) | 10 (4, 15)[d,e] | 4 (12)[g,h] | 0 (0)[i] |

[a] *P* values from single comparisons of the three groups were 0.006 for the severity scores, <0.0001 for the numbers of dehydrated children, and 0.01 for the numbers of children with shock.
[b] Nontypeable-P serotypes were not P1A[8], P1B[4], or P2A[6].
[c] $P = 0.005$.
[d] $P = 0.003$.
[e] $P = 0.47$.
[f] $P = 0.010$.
[g] $P < 0.0001$.
[h] $P = 0.004$.
[i] Values obtained for the G3–nontypeable-P serotype group were significantly different from values obtained for both the G3-P1A[8] and G1-P1A[8] groups ($P = 0.024$).

F45-1A10-RV5 in 14 specimens (7%) among the samples for which the RVs were characterized.

Seventy-five out of the 79 RV-positive specimens (95%) from asymptomatic children (controls) underwent G and P serotyping, and in 49 of them (65%) both the G and P serotypes could be identified. Table 2 shows the numbers of serotypes in these 75 children without diarrhea according to the G-P binomial serotype classification. The predominant serotypes were G3-P1A[8] (31%), G1-P1A[8] (23%), and nontypeable G-nontypeable P (16%). In no case was the P1B or the P2A[6] serotype found. The relative frequencies of occurrence of the most commonly identified P monotypes within serotype P1A[8] were similar to those in children with diarrhea: F45-1A10 was found in 28 specimens (37%), F45 was found in 10 specimens (13%), and F45-1A10-RV5 was found in 5 specimens (7%) for which the RVs were characterized.

We found a higher risk for diarrhea in children infected with a G3 (either P1A[8] or nontypeable-P) serotype than in those infected with the G1-P1A[8] serotype (OR = 2.15; 95% CI = 0.99 to 4.64; $P = 0.05$).

Clinical manifestations were compared only among specimens of the predominant serotypes; due to the small sample size, the samples infected with the G2-P1B[4] type were not analyzed. Infection by a G3–nontypeable-P serotype led to a significantly more severe gastroenteritis (median severity score, 12 points) than infection by a G3-P1A[8] (median severity score, 10 points) or a G1-P1A[8] (median severity score, 10 points) RV type (Table 3). A child with diarrhea-associated dehydration was almost five times more likely to be infected by a G3–nontypeable-P serotype than a child without dehydration (OR = 4.67; 95% CI = 1.83 to 12.13; $P < 0.001$). Furthermore, a child with hypovolemic shock was 14 times more likely to be infected by a G3–nontypeable-P serotype than a child without this severe clinical outcome (OR = 14.22; 95% CI = 1.24 to 369.66; $P = 0.02$). Children infected by the same G serotype (G3) but different P serotypes had different clinical outcomes: infection with a G3–nontypeable-P serotype was associated with a significantly higher maximum number of stools per day and more vomiting than infections with a G3-P1A[8] serotype (Table 4).

The correlation between P genotypes and phenotypes showed an association of monotypes F45, 1A10, F45-1A10, F45-RV5, and F45-1A10-RV5 with the genomic type P[8], which corresponds to serotype P1A, and of monotypes RV5, 1A10, and 1A10-RV5 with the genomic type P[4], which corresponds to serotype P1B. Table 5 compares the clinical features of children infected by the two predominant P monotypes: F45-1A10 and F45. The median of the severity scores was significantly higher (10.5 points) for children infected with the F45-1A10 monotype than for those infected with the F45 monotype (10 points). A child with diarrhea-associated dehydration was three times more likely to be infected by the F45-1A10 monotype (as opposed to the F45 monotype) than a child without dehydration (OR = 3.38; 95% CI = 0.96 to 6.22; $P = 0.05$). A child with vomiting was three times more likely to be infected by an F45-1A10 monotype (as opposed to the F45 monotype) than a child without vomiting (OR = 3.42; 95% CI = 1.12 to 10.48; $P = 0.03$). The five patients who presented with blood in their stools had monotype F45-1A10. Of the four patients with hypovolemic shock, one was infected with monotype F45-1A10-RV5 and the others were infected with strains that could not be serotyped for VP4.

No statistically significant differences were found among children with diarrhea infected by the diverse G-P serotypes (and monotypes) with regard to age, nutritional status, and gender.

## DISCUSSION

This community-based study of young children with diarrhea from different regions of Mexico examined the possible association between the infecting (G-P) RV serotypes and their

TABLE 4. Clinical manifestations of 125 children with diarrhea caused by RVs relative to the infecting G-P serotype

| G serotype (no. of children) | Value for indicated clinical sign[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Diarrhea | | | Vomiting | | | | Median of maximum temp (°C) (range) |
| | Median of avg no. of stools/day (range) | Median of maximum no. of stools/day (range) | Median no. of days evident (range) | No. (%) of children | Median of avg no. of episodes/day (range) | Median of maximum no. of episodes/day (range) | Median no. of days evident (range) | |
| G3-nontypeable P[b] (30) | 7 (2–19) | 12 (4–30) | 4 (2–12) | 27 (90) | 3.9 (0–17.5) | 6 (0–30) | 3 (0–5) | 38 (36–39) |
| G3-P1A[8] (95) | 6 (1–18) | 10 (1–30) | 4 (1–11) | 82 (86) | 3 (0–7.7) | 4 (0–15) | 3 (0–6) | 38 (37–39) |

[a] Ranges are minimum to maximum values. Differences in values between the two groups of children were not significant except for the medians of the maximum numbers of stools per day ($P = 0.03$), the medians of the average numbers of vomiting episodes per day ($P = 0.01$), and the medians of the maximum numbers of vomiting episodes per day ($P = 0.02$).
[b] Nontypeable-P serotypes were not P1A[8], P1B[4], or P2A[6].

TABLE 5. Illness severity in 117 children with diarrhea caused by RVs of serotype P1A[8] relative to the P monotype[a]

| Monotype (no. of children) | Median severity score (minimum, maximum) | No. (%) of children with vomiting | No. (%) of dehydrated children |
|---|---|---|---|
| F45-1A10 (92) | 10.5 (4, 15) | 79 (86) | 36 (39) |
| F45 (25) | 10 (4, 15) | 16 (64) | 4 (16) |

[a] *P* values were as follows: 0.03 for the median severity scores and numbers of children with vomiting and 0.05 for the numbers of dehydrated children.

clinical manifestations. Our results reveal that in our country, at least during the study period, enteric infection with an RV having a G3 serotype increased the risk for symptomatic infection relative to the risk associated with a G1 serotype RV infection, regardless of the P serotype. Furthermore, the illness severity and the risk for dehydration and hypovolemic shock were related to the P serotype; when the G3-P binomial comprised a nontypeable-P serotype, the gastroenteritis was more intense and severe than when the infecting RV had a G3-P1A[8] serotype and no difference was found between the clinical manifestations of G1 and the G3 serotypes when these serotypes were associated with the same P serotype (P1A[8]).

These nontypeable-P RV strains may have the serotypes for which we screened (P1A, P1B, and P2A) but were not identified perhaps because of insufficient antigen being detected by our assays; alternatively, VP4 may be antigenically different due to antigenic drift of the screened serotypes, with a consequent lower affinity to the MAbs we used. Yet this explanation is not satisfactory, as it does not account for the marked differences in clinical outcomes among children infected by these nontypeable-P RVs. Thus, we hypothesize that at least some of these strains may belong to P serotypes other than the ones for which we screened, such as P3, P4, and P6 (28). This hypothesis needs to be confirmed by further characterization of these nontypeable-P strains, and we must define how different they are both antigenically and genomically from the known P serotypes.

Moreover, it seems that even within strains having the same VP4 (P1A[8]) type there may be different clinical presentations of the RV infection among different monotypes (F45-1A10 versus F45).

This is the first study that shows different clinical outcomes related to different monotypes within a given P serotype, adding complexity to the problem of analyzing the basis for differences in virulence among RV strains. The specific monotypes involved differ in their reactivities with a single neutralizing MAb, which may therefore have arisen as a result of antigenic drift. More studies are needed to determine the genomic differences associated with these antigenic variants.

Previous studies have also searched for clinical features associated with specific G types. Their results have been inconsistent, and some of these studies have reported no differences in clinical severity (or in ratios of symptomatic to asymptomatic patients), while others have found only minor clinical differences in certain signs (9, 13, 27, 32, 34, 37). However, the great majority of these studies have had a small sample size that may have precluded the detection of significant differences in clinical outcomes. In a large study conducted from 1987 to 1989 with children younger than 2 years of age from rural Bangladesh, Bern et al. (2) identified G serotypes in 63% of 764 RV-associated diarrheal episodes and used a composite score for severity of dehydration based on the sums of results for five clinical signs to classify these children as mildly, moderately, or severely dehydrated. That study found a slightly (and statistically significantly) higher proportion (55%) of children with moderate to severe dehydration when they were infected with a G2 or G3 RV serotype than when they were infected with a G1 or G4 serotype (43%). In that survey the authors were not able to demonstrate a difference in severity of dehydration associated with the P serotype. However, P serotyping was performed for only 40 specimens and no comparison between P1 subtypes was carried out, limiting the ability to detect possible differences in clinical outcomes.

In a recently published study (3), Cascio et al. reported their results for children with diarrhea seen in a hospital in Palermo, Italy, from 1993 to 1997. Unlike in Mexico, where the G2-P1B[4] serotype was rarely identified (10 cases), in that Mediterranean country this serotype was the second most prevalent (42 cases) and was associated (together with the nontypeable-G strains) with more severe disease than the infections by the predominant (G1-P1A[8]) and other serotypes. Interestingly, those authors hypothesized that such clinical differences among infections could be explained by the recent new introduction of the G2 type within the population of Palermo.

Clearly, results are different among these three epidemiological surveys (in Bangladesh, Italy, and Mexico) aimed at identifying the relationship between RV serotype and disease severity. Thus, studies carried out on different continents and during diverse time periods have in common that they identify certain serotypes related to more severe gastroenteritis, but they differ in the identities of the more virulent serotypes. This fact gives support to the hypothesis that the clinical outcomes during an RV infection are predominantly determined by the introduction of a new G or P serotype in a certain community with a population immunologically naïve for this serotype. Immunological naïveté may account for the differences in the relationships of serotype with illness severity observed among diverse geographic areas. Conversely, these data are less compatible with the hypothesis that such differences may be due to the existence of universal intrinsically more virulent RV strains.

The immune status of a subset of the children included in this study was previously characterized (23). Of 71 children analyzed (mean age, 10.3 months), 70 had serologically defined primary RV infections. Thus, as probably most of the RV infections studied in our survey were primary infections and the mean ages were similar among children infected with different RV types, potential selection bias is unlikely.

Given that not all severe (or mild) cases were associated with a particular RV serotype, this possible causal relationship is not necessary or sufficient. Therefore, factors determining disease manifestations, other than viral properties, need to be considered.

This study is one of the largest that has examined the relationship between G and P serotypes and clinical features. Our data lead to the concept of a significant variation in the serotypes associated with more severe diarrhea among different populations. This variation has to be considered in the design of future effective RV vaccines.

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## REFERENCES

1. **Abramson, J. S., C. J. Baker, M. C. Fisher, M. A. Gerber, H. C. Meissner, D. L. Murray, G. D. Overturf, C. G. Prober, M. B. Rennels, T. N. Saari, L. B. Weiner, and R. J. Whitley.** 1999. Possible association of intussusception with rotavirus vaccination. Pediatrics **104:**575.
2. **Bern, C., L. Unicomb, J. R. Gentsch, N. Banul, M. Yunus, B. Sack, and R. I. Glass.** 1992. Rotavirus diarrhea in Bangladeshi children: correlation of disease severity with serotypes. J. Clin. Microbiol. **30:**3234–3238.
3. **Cascio, C., E. Vizzi, C. Alaimo, and S. Arista.** 2001. Rotavirus gastroenteritis in Italian children: can severity be related to the infecting virus? Clin. Infect. Dis. **32:**1126–1132.
4. **Coulson, B. S.** 1987. Variation in neutralization epitopes of human rotaviruses in relation to genomic RNA polymorphism. Virology **159:**209–216.
5. **Coulson, B. S.** 1993. Typing of human rotavirus VP4 by an enzyme immunoassay using monoclonal antibodies. J. Clin. Microbiol. **31:**1–8.
6. **Dagan, R., Y. Bar-David, B. Sarov, M. Katz, I. Kassis, D. Greenberg, R. I. Glass, Z. M. Carmi, and I. Sarov.** 1990. Rotavirus diarrhea in Jewish and Bedouin children in the Negev region of Israel: epidemiology, clinical aspects and possible role of malnutrition in severity of illness. Pediatr. Infect. Dis. J. **9:**314–321.
7. **DeZoysa, I., and R. G. Feachem.** 1985. Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. Bull. W. H. O. **63:**569–583.
8. **Flewett, T. H., C. F. Arias, L. F. Avendaño, A. Ghafoor, M. M. Mathan, L. Mendis, K. Moe, and R. F. Bishop.** 1989. Comparative evaluation of the W. H. O. and DAKOPATTS enzyme-linked immunoassay kits for rotavirus detection. Bull. W. H. O. **67:**369–374.
9. **Flores, J., K. Taniguchi, K. Green, I. Perez-Schael, D. Garcia, J. Sears, S. Urasawa, and A. Z. Kapikian.** 1988. Relative frequencies of rotavirus serotypes 1, 2, 3, and 4 in Venezuelan infants with gastroenteritis. J. Clin. Microbiol. **26:**2092–2095.
10. **Gehlbach, S. H.** 1993. Interpreting the medical literature, 3rd ed., p. 83–98. McGraw-Hill, New York, N.Y.
11. **Gentsch, J. R., P. A. Woods, M. Ramachandran, B. K. Das, J. P. Leite, A. Alfieri, R. Kumar, M. K. Bhan, and R. I. Glass.** 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. **174:**S30–S34.
12. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B. M. Das, and M. K. Bhan.** 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1365–1373.
13. **Georges-Courbet, M. C., A. M. Beraud, G. M. Beards, A. D. Campbell, J. P. Gonzalez, A. J. Georges, and T. H. Flewett.** 1988. Subgroups, serotypes, and electropherotypes of rotavirus isolated from children in Bangui, Central African Republic. J. Clin. Microbiol. **26:**668–671.
14. **Glass, R. I., P. E. Kilgore, R. C. Holman, S. Jin, J. C. Smith, P. Woods, M. J. Clarke, M. Shang, and J. Gentsch.** 1996. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. J. Infect. Dis. **174**(Suppl. 1)**:**S5–S11.
15. **Gorziglia, M., G. Larralde, A. Z. Kapikian, and R. M. Chanock.** 1990. Antigenic relationship among human rotaviruses as determined by outer capsid protein VP4. Proc. Natl. Acad. Sci. USA **87:**7155–7159.
16. **Gouvea, V., L. De Castro, M. Timenetsky, H. Greenberg, and N. Santos.** 1994. Rotavirus serotype G5 associated with diarrhea in Brazilian children. J. Clin. Microbiol. **32:**1408–1409.
17. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrilamide gels. J. Clin. Microbiol. **16:**473–477.
18. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque reduction neutralization. J. Infect. Dis. **149:**694–702.
19. **Kapikian, A. Z., Y. Hoshino, and R. M. Chanock.** 2001. Rotaviruses, p. 1787–1833. *In* D. M. Knipe, P. M. Howley, D. E. Griffin, M. A. Martin, R. A. Lamb, B. Roizman, and S. E. Strauss (ed.), Fields virology. Lippincott Williams & Wilkins, Philadelphia, Pa.
20. **Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock.** 1986. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. J. Infect. Dis. **153:**815–822.
21. **Kilgore, P. E., E. U. Leanne, J. R. Gentsch, J. Albert, A. Mcelroy, and R. I. Glass.** 1996. Neonatal rotavirus infection in Bangladesh: strain characterization and risk factors for nosocomial infection. Pediatr. Infect. Dis. J. **15:**672–677.
22. **Leite, J. P., A. A. Alfieri, P. A. Woods, R. I. Glass, and J. R. Gentsch.** 1996. Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization and sequence analysis. Arch. Virol. **141:**2365–2374.
23. **Menchaca, G., L. Padilla-Noriega, M. Méndez-Toss, J. F. Contreras, F. I. Puerto, H. Guiscafré, F. Mota, I. Herrera, R. Cedillo, O. Muñoz, R. Ward, Y. Hoshino, S. López, and C. F. Arias.** 1998. Serotype specificity of the neutralizing antibody response induced by the individual surface proteins of rotavirus in natural infections of young children. Clin. Diagn. Lab. Immunol. **5:**328–334.
24. **Padilla-Noriega, L., C. F. Arias, S. López, F. Puerto, D. R. Snodgrass, K. Taniguchi, and H. B. Greenberg.** 1990. Diversity of rotavirus serotypes in Mexican infants with gastroenteritis. J. Clin. Microbiol. **28:**1114–1119.
25. **Padilla-Noriega, L., E. R. Werner, E. R. Mackow, M. Gorziglia, G. Larralde, K. Taniguchi, and H. B. Greenberg.** 1993. Serological analysis of human rotavirus serotypes P1A and P2 by using monoclonal antibodies. J. Clin. Microbiol. **31:**622–628.
26. **Padilla-Noriega, L., M. Méndez-Toss, G. Menchaca, J. F. Contreras, P. Romero-Guido, F. I. Puerto, H. Guiscafré, F. Mota, I. Herrera, R. Cedillo, O. Muñoz, J. Calva, M. L. Guerrero, B. S. Coulson, H. B. Greenberg, S. López, and C. F. Arias.** 1998. Antigenic and genomic diversity of human rotavirus VP4 in two consecutive epidemic seasons in Mexico. J. Clin. Microbiol. **36:**1688–1692.
27. **Pitson, G. A., K. Grimwood, B. S. Coulson, F. Oberklaid, A. S. Hewstone, I. Jack, R. F. Bishop, and G. L. Barnes.** 1986. Comparison between children treated at home and those requiring hospital admission for rotavirus and other enteric pathogens associated with acute diarrhea in Melbourne, Australia. J. Clin. Microbiol. **24:**395–399.
28. **Ramachandran, M., J. R. Gentsch, U. D. Parashar, S. Jin, P. A. Woods, J. L. Holmes, C. D. Kirkwood, R. F. Bishop, H. B. Greenberg, S. Urasawa, G. Gerna, B. S. Coulson, K. Taniguchi, J. S. Bresee, and R. I. Glass.** 1998. Detection and characterization of novel rotavirus strains in the United States. J. Clin. Microbiol. **36:**3223–3229.
29. **Ramachandran, M., B. K. Das, A. Vij, R. Kumar, S. S. Bhambal, N. Kesari, H. Rawat, L. Bahl, S. Thakur, P. A. Woods, and R. I. Glass.** 1996. Unusual diversity of human rotavirus G and P genotypes in India. J. Clin. Microbiol. **34:**436–439.
30. **Ruuska, T., and T. Vesikari.** 1990. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand. J. Infect. Dis. **22:**259–267.
31. **Ruuska, T., and T. Vesikari.** 1991. A prospective study of acute diarrhoea in Finnish children from birth to 2 1/2 years of age. Acta Paediatr. Scand. **80:**500–507.
32. **Steele, A. D., P. Bos, and J. J. Alexander.** 1988. Clinical features of acute infantile gastroenteritis associated with human rotavirus subgroups I and II. J. Clin. Microbiol. **26:**2637–2649.
33. **Sufian, M., E. Assouli, Z. Banjar, Mohammed, and F. T. Zamakhchari.** 1992. Rotavirus infection in children in Saudi Arabia. Am. J. Trop. Med. Hyg. **46:**272–277.
34. **Uhnoo, I., and L. Svensson.** 1986. Clinical and epidemiological features of acute infantile gastroenteritis associated with human rotavirus subgroups 1 and 2. J. Clin. Microbiol. **23:**551–555.
35. **Velázquez, F. R., J. J. Calva, M. L. Guerrero, D. Mass, R. I. Glass, L. K. Pickering, and P. G. M. Ruiz.** 1993. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. Pediatr. Infect. Dis. J. **12:**54–61.
36. **World Health Organization.** 1990. A manual for the treatment of diarrhoea for use by physicians and other senior health workers. W. H. O./CDD/SER80.2. Rev 2. World Health Organization, Geneva, Switzerland.
37. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brand, W. J. Rodriguez, H. W. Kim, R. H. Parrott, J. J. Urrutia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299:**1156–1161.