Severity of rotavirus infection in relation to serotype, monotype and electropherotype

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Abstract The aim of this study was to determine whether the severity of symptoms associated with rotavirus infection was related to the serotype of the infecting virus. Severity of clinical symptoms in 108 children admitted to hospital for treatment of rotavirus diarrhoea was retrospectively assessed using a scoring system for frequency and duration of vomiting and diarrhea, degree of fever, acidosis and dehydration, and presence of electrolyte imbalance. Children were 6–30 months old and were fully weaned at onset of symptoms prior to admission to hospital. No other enteric pathogens were detected during the course of the illness. Serotypes and monotypes were identified using a panel of monoclonal antibodies. Gel electrophoresis of rotavirus RNA was performed to determine electropherotypes. Children surveyed were infected with serotype 1 (47), serotype 2 (15) or serotype 4 (46) rotaviruses. Comparisons of severity of clinical symptoms according to infecting serotype revealed no statistically significant differences between serotype 1, 2 or 4 infections. In addition, no differences were detected between different rotavirus strains within each serotype (as judged by electropherotype) including monotypes 1a or 1c. This study failed to reveal differences in virulence between rotavirus strains of different VP7 serotypes infecting young children.

Key words: gastroenteritis; rotavirus; serotype; severity; symptoms.

Rotaviruses are accepted as a major cause of severe, dehydrating, acute infectious diarrhoea in young children in all parts of the world. The genetic diversity of rotavirus strains infecting humans was recognized soon after their detection in 1973. It is now apparent that human rotaviruses are also antigenically diverse. Whether different strains cause different degrees of illness is unclear.

Rotaviruses contain double stranded RNA that can be separated by gel electrophoresis into 11 discrete bands, each corresponding to a single gene. Variations in the mobilities of these 11 genes in different strains result in characteristic gel patterns or 'electropherotypes'. Each gene codes for a single protein. Proteins coded by several genes define serological differences between strains. Rotaviruses are initially classified into Groups A–F by serological tests that detect antigenic determinants on the gene 6 products (VP6). Group A rotaviruses are common worldwide in humans and are further classified into subgroups and serotypes on the basis of identification of non-neutralizing antigenic determinants on VP6 (subgroups I, II) or of neutralizing determinants on the major outer capsid VP7 protein (human serotypes 1, 2, 3, 4, 8, 9). A binary system of classification after identification of both outer capsid neutralizing antigens (VP7 and VP4) may eventually be established.

The VP7 serotype of an infecting strain is at present identified directly for rotavirus excreted in stools using an enzyme immunoassay incorporating validated VP7 specific neutralizing monoclonal antibodies. Serotype 1 rotaviruses can be further classified into monotypes (subtypes) using a panel of VP7 neutralizing monoclonal antibodies.

There is little information about whether human strains of differing subgroup, serotype, monotype or electropherotype vary in their capacity to produce clinical symptoms in young children. Studies of severity of clinical symptoms according to subgroup have given conflicting results. Uhnoo et al. found that a temperature above 39°C was significantly more common in subgroup I infections, whereas subgroup II produced more diarrhoea and vomiting. Conversely Steele et al. reported more fever with subgroup II infections and increased vomiting associated with subgroup I. One of three dominant electropherotypic variants of subgroup II was found to be associated with a significant increase in associated respiratory symptoms. There has been no analysis of clinical severity in relation to serotype. The conflicting results observed in these two studies may be partly explained by differences in the age range of the children studied. In particular, symptoms observed in children more than 3 years of age may be modified by pre-existing immunity due to a previous rotavirus infection. Serum antibody studies indicate that most children experience rotavirus infection during their first 3 years of life.

During the course of an epidemiological study of the serotype of faecal rotavirus strains causing acute diarrhoea in children admitted to hospital, we identified numerous strains corresponding to serotypes 1, 2 or 4 and to monotypes 1a or 1c. The electropherotype of many strains was classified by co-electrophoresis of extracted genomic RNA. We report...
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here the clinical severity of rotavirus illness in children admitted to hospital in relation to the serotype, monotype and electropherotype of the infecting rotavirus strains.

METHODS

A long-term epidemiological study to identify serotype and electropherotype of rotavirus strains infecting hospitalized Melbourne children has been underway since 1974.1-8,10,11 Serotype 1 strains have been further identified as monotypes 1a, 1b, 1c or 1d using a panel of monoclonal antibodies.9 Strains of the same serotype identified during successive winter epidemics were further classified into electropherotypes using co-electrophoresis to establish identity.1,5,10,11

Comprehensive virological data and adequate clinical information were available in 108 cases of children aged 6-30 months. Infants under 6 months of age were excluded to minimize the effect of maternal antibody. We believe that virtually all children under 30 months of age admitted to this hospital with acute gastroenteritis are suffering from a primary infection.12 Another seven cases could have been included had clinical information been adequately recorded.

There were 47 cases of serotype 1, 15 of serotype 2 and 46 of serotype 4. Serotype 3 infections were rare and are excluded from analysis. Within each serotype, patients were subdivided into groups infected with strains shown to have identical serological reactions and a homogeneous electropherotype (Table 1). All rotavirus infections were community acquired, with symptoms evident on admission to hospital, and diagnosis confirmed on faecal specimens obtained within 48 h of admission. The sex and age ranges of patients are listed in Table 1.

Clinical assessment

Hospital records were reviewed by G. L. B., while blinded to the serotype results. The records were compiled by resident medical officers and nursing staff in the Emergency Department and in the wards. The history of symptom onset, frequency of vomiting and diarrhoea (watery stools) was obtained from the parent or guardian. Dehydration was assessed by the resident medical officer according to clinical signs. Serum electrolytes and pH were measured when the admitting doctor believed this to be clinically indicated. The system of Riepenhoff-Talty et al. was used to assign a severity score to the signs and symptoms in each patient (Table 2).13 The maximum number of vomits or stools per day, either reported prior to admission by parents, or recorded in hospital records during inpatient stay was noted. Where the maximum number of stools or vomits per day was not documented clearly, a score of 1 was assigned for 'infrequent' or 'occasional', a score of 2 for 'frequent', and a score of 3 for 'profuse' or 'persistent'. These judgements were made in eight patients. Absence of information about one or more symptoms accounted for the seven exclusions. Duration of diarrhoea and vomiting was counted from symptom onset until the time of the last vomit or watery stool passed during recovery. The maximum temperature recorded prior to or during admission was noted.

Scores were related to the infecting strains in two ways. First, the mean total scores, median and standard deviations for each strain were compared. Second, the children who scored 2 or more for each individual sign or symptom (i.e. moderate or severe) were compared between serotypes, monotypes and electropherotypes. Statistical comparisons were made using the x^2 test, with Yate's correction or using Fisher's exact test where appropriate.

RESULTS

Serotype 1 rotavirus strains identified in 1983 and 1986, serotype 2 strains from 1977 and 1989, and serotype 4 strains from 1974, 1982, 1984, 1988 and 1989, are considered for analysis. Table 1 indicates the monotypes and electropherotypes within serotype 1 which are considered separately. Comparison of overall severity scores and individual symptoms associated with the three serotypes is shown in Table 3. Mean and median scores showed no statistically significant differences between the serotypes. All comparisons of individual signs and symptoms between any two of the three serotypes showed no differences. Specific monoclonal antibodies subdivided serotype 1 strains into 18 monotype 1a strains, 6 monotype 1b and 23 monotype 1c strains. Comparisons between the larger two subgroups (1a and 1c) showed no differences in total, mean or median scores, nor in individual symptom scores (Table 4). The monotype 1c strains could be further subdivided into two electropherotypes, 83A and 83E. No differences emerged when scores or symptoms were compared (Table 5).

Table 1  Characteristics of patients and rotavirus strains

| Virus excreted serotype/ electropherotype | Year | Total no. (male) | Age range (months) | Median |
|------------------------------------------|------|-----------------|-------------------|--------|
| 1a/A1                                    | 1986 | 9 (8)           | 7-24              | 13     |
| 1a/B1                                    | 1986 | 9 (6)           | 7-26              | 14     |
| 1b/A                                     | 1983 | 6 (5)           | 6-27              |        |
| 1c/83A                                   | 1983 | 11 (6)          | 6-19              | 10     |
| 1c/83E                                   | 1983 | 12 (7)          | 6-21              | 12     |
| 2/M                                      | 1977 | 6 (5)           | 7-18              | 13     |
| 2/A                                      | 1989 | 9 (6)           | 10-19             | 14     |
| 4/A                                      | 1974 | 9 (5)           | 6-18              | 13     |
| 4                                        | 1982 | 6 (4)           | 7-20              | 18     |
| 4/C                                      | 1984 | 9 (5)           | 6-24              | 16     |
| 4                                        | 1988 | 10 (5)          | 10-22             | 14     |
| 4                                        | 1989 | 12 (5)          | 8-24              | 14     |

Table 2  Scoring for severity of acute diarrhoeal disease (after Riepenhoff-Talty et al.13)

| Symptom                      | Score |
|------------------------------|-------|
| Diarrhoea                    |       |
| Maximum no. stools per day    | <5    | 5-8 | >8   |
| Duration in days             | <5    | 5-7 | >7   |
| Vomiting                     |       |
| Maximum no. per day          | <4    | 4-6 | >6   |
| Duration in days             | <3    | 3-5 | >5   |
| % bodyweight loss            | <5    | 5-9 | >9   |
| Fever °C                     | <38.5 | 38.5-38.9 | >38.9 |
| Acidosis                     | —     | 7.34-7.30 | >7.30 |
| Electrolyte imbalance        | —     | Present |

Another seven cases could have been included had clinical information been adequately recorded.
Serotype 4 rotaviruses reappeared in 1988 after a 4 year absence. This could have been due to a change in the virus. Comparison of the effect of serotype 4 strains from the two epidemics several years apart showed no differences (Table 6). Infants infected in 1988 clearly had had no prior experience of the serotype 4 prevalent in 1984. Any change in the virus attacking an equally susceptible population could be expected to show changes in symptoms but again there were none observed.

| Table 3 | Rotavirus infection: severity vs serotype |
|---------|-----------------------------------------|
| Serotype | 1 | 2 | 4 |
| n        | 47 | 15 | 46 |
| Score    |   |   |   |
| Mean     | 10.2 | 10.0 | 10.5 |
| s.d.     | 3.1 | 1.7 | 3.5 |
| Median   | 10 | 10 | 10 |
| Symptoms (%) |   |   |   |
| ≥5 stools/day | 85 | 67 | 70 |
| ≥5 days of diarrhea | 40 | 20 | 28 |
| ≥4 vomiting/day | 68 | 80 | 72 |
| ≥3 days of vomiting | 34 | 47 | 41 |
| ≥5% dehydration | 43 | 60 | 46 |
| ≥38.5°C | 40 | 53 | 39 |

P > 0.05 for all comparisons between serotypes.

| Table 4 | Rotavirus infection: severity vs monotype |
|---------|-----------------------------------------|
| Monotype | 1a | 1c |
| n        | 18 | 23 |
| Score    |   |   |
| Mean     | 9.8 | 10.8 |
| s.d.     | 2.2 | 3.7 |
| Median   | 11 | 10 |
| Symptoms (%) |   |   |
| ≥5 stools/day | 83 | 91 |
| ≥5 days of diarrhea | 39 | 48 |
| ≥4 vomiting/day | 83 | 61 |
| ≥3 days of vomiting | 44 | 26 |
| ≥5% dehydration | 33 | 57 |
| ≥38.5°C | 33 | 43 |

P > 0.05 for all comparisons.

| Table 5 | Rotavirus infection: severity vs electropherotype within serotype 1c |
|---------|-----------------------------------------|
| Electropherotype | 83A | 83E |
| n        | 11 | 12 |
| Score    |   |   |
| Mean     | 11.7 | 10.0 |
| s.d.     | 3.9 | 3.6 |
| Median   | 11 | 9 |
| Symptoms (%) |   |   |
| ≥5 stools/day | 100* | 93* |
| ≥5 days of diarrhea | 55 | 42 |
| ≥4 vomiting/day | 64 | 58 |
| ≥3 days of vomiting | 27 | 25 |
| ≥5% dehydration | 64 | 50 |
| ≥38.5°C | 55 | 33 |

P > 0.05 for all comparisons.

*P = 0.26 Fishers exact test.

DISCUSSION

The most reliable way to determine differences in illness caused by different serotypes of rotavirus is to conduct a prospective community-based study. A study in Houston identified a similar spectrum of serotypes in hospitalized and non-hospitalized children but did not identify symptom or severity differences between serotypes. The logistics of combining accurate clinical information about outpatients with comprehensive laboratory studies are daunting. In addition, limitations are imposed by the occurrence of various serotypes in different years. Such a study has not been possible in Melbourne and may only be practical in a location where very large numbers of children with acute diarrhoea are seen. Even then, changes in serotypes are unpredictable. We have therefore sought such information as is available from our epidemiological studies extending over 15 years. This has meant that we have had to rely upon data from inpatients only. It is not surprising therefore that the total scores for clinical severity of rotavirus disease were similar for all strains studied. Admission to hospital would preselect children with more severe disease as judged by Emergency Department medical staff and would fail to account for patients with mild to moderate symptoms not requiring hospital treatment.

Differences in virulence of strains, if they exist, would be more likely to be identified by changes in numbers of children requiring admission to hospital coincident with changes in serotype or electropherotype. However, two or more strains of the same or different VP7 serotypes were dominant during most of the years studied, so it is not possible to dissect differences between strains using that approach.

Differences in virulence of rotavirus strains have been observed in animal models including rabbits, calves and gnotobiotic piglets. The differences have generally not been observed in regard to clinical symptoms, but have been detected by measuring the amount of virus replication in gut tissues, intestinal lumen and faeces, and by comparing histologic lesions in the intestine. None of these measures are possible in children with community acquired infection.

This study is more likely to reveal differences in the severity of individual symptoms (i.e. incidence of fever, vomiting or diarrhoea) between different strains than differences in overall severity of disease. However no statistically significant differences emerged. The small numbers in some of the groups could...
result in masking of a true difference (type II error). This possibility could only be overcome by increasing the size of the groups studied. But group size was dictated by the number of children with each serotype who happened to be admitted over a period of several years, from whom appropriate volume stool samples were obtained, and whose medical notes were adequately recorded. Hence the data are not optimal, but they are the best we can achieve given the practical problems inherent in such a study.

Asymptomatic rotavirus infection of newborn babies in hospital nurseries (where rotavirus infection has become endemic) has been attributed to reduced virulence of these strains, rather than to control of symptoms by passive maternal antibody. Studies with reassortants have shown that the VP6 gene segment (of simian rotavirus SA11 and of neonatal calf diarrhoea virus NCDV) is related to virulence in an experimental animal model although other genes may also influence virulence. Rotavirus strains endemic in newborn nurseries have been shown to possess highly related gene 4 segments that are distinct from those of virulent rotaviruses of the same VP7, serotype.

The serotyping scheme used in this study was based on differences in antigenic epitopes on VP7. Our failure to detect differences in virulence of the strains studied may reflect the fact that all possessed similar VP7 gene segments. Comparison of virulence of strains with differing VP7 classification may (once this is achievable) reveal differences in clinical symptoms between strains.

The observation that symptoms of equal severity were caused by at least three of the four main human serotypes supports the logic that rotavirus vaccine development should include the aim of stimulating protective immunity against all commonly occurring human serotypes. This study gives no support to the concept that stimulation of immunity against only one or two serotypes will be adequate for a rotavirus vaccine.

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