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SEQUENTIAL VARIATION IN GENOMIC RNA PATTERNS OF HUMAN ROTAVIRUSES ISOLATED FROM INFANTILE GASTROENTERITIS

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SUMMARY

The incidence and RNA electrophoretypes of rotaviral isolates obtained from infants and young children with acute gastroenteritis were studied from October, 1985 through April, 1986. Analysis of the viral RNA was carried out by polyacrylamide gel electrophoresis followed by silver staining. Fourteen electrophoretypes were identified. A single dominant electrophoretype was found during the first months of the rotavirus seasonal outbreak. In contrast, a large variety of RNA patterns were observed during the latter portion of the study period. Subgrouping of rotavirus isolates by a double-sandwich enzyme-linked immunosorbent assay using monoclonal-detecting antibodies showed that all strains belonged to subgroup II. Mixed rotavirus electrophoretypes appeared in 4 cases (8.16%).

KEY-WORDS: Rotavirus, RNA, Gastroenteritis; Molecular epidemiology, Genomic RNA patterns, Sequential variation, Electrophoretypes, Children.

INTRODUCTION

Rotaviruses have been recognized as being one of the main causes of childhood diarrhoea [7]. The viral genome consists of 11 segments of linear double-stranded RNA (dsRNA), with molecular weights ranging from $2.5 \times 10^6$ to $0.4 \times 10^6$ daltons [14]. Polyacrylamide gel electrophoresis (PAGE) of rotavirus dsRNA, in conjunction with a sensitive silver staining technique

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[6], has proven to be a very useful method for the identification and distinction of rotavirus isolates. Human rotaviruses show a large diversity in their segmented genomic patterns, and electrophoretic analysis has been established as a valuable means of studying the epidemiology of rotavirus infections [10, 12, 15, 17].

In the present report, we analysed faecal samples collected from infants and young children with gastroenteritis between October, 1985 and April, 1986, a period which included an outbreak of rotavirus infection, in order to conduct an epidemiological survey of the propagation of the virus. We describe the sequential variation in genomic RNA patterns detected.

MATERIALS AND METHODS

Collection of specimens.

Two-hundred and sixty faecal specimens were obtained from October, 1985 through April, 1986 from infants and young children under 5 years of age who had been admitted to the Paediatric Department of the Hospital Clinico Universitario of Valencia, Spain, with symptoms of gastroenteritis or diarrhoea with dehydration.

Preparation of specimens.

Ten to twenty percent suspensions of faeces in phosphate-buffered saline (PBS), pH 7.4, were made up, homogenized and clarified by centrifugation at 1500 g for 10 min at 4°C. Liquid faecal specimens were diluted to 1/2 with PBS and centrifuged. The supernatants were used to perform electron microscopic examination of samples, nucleic acid analysis and enzyme-linked immunosorbent assay (ELISA) for subgrouping of rotavirus strains.

Electron microscopy.

Previously prepared 10-20 % faecal suspensions were centrifuged at 50,000 rpm for 90 min (L8-70M Ultracentrifuge, Beckman Instruments), absorbed onto carbon-Formvar-coated 400-mesh grids and negatively stained with 3 % phosphotungstic acid, pH 7.0. The preparations were examined under an electron microscope (Zeiss EM 10C/CR, voltage 80 KV) at a magnification of 31,500X. Samples were considered negative when no virus particles were found during 15 min of observation.

Nucleic acid analysis.

The PAGE technique for rotavirus RNA detection has been previously described [3, 4, 8, 10]. Electrophoresis was performed on 10 % polyacrylamide gel slabs without SDS by using the discontinuous buffer system of Laemmli [9] and conducted for double-stranded RNA.

PAGE = polyacrylamide gel electrophoresis.

ELISA = enzyme-linked immunosorbent assay.

OD = optical density.

PBS = phosphate-buffered saline.

SDS = sodium dodecyl sulphate.
16 h at room temperature at 15 mA constant current. The gels were stained with silver nitrate as described by Herring et al. [6]. Comparisons of different rotavirus strains were made by mixing and then co-electrophoresing them.

**ELISA for subgrouping of rotaviruses.**

Double-sandwich ELISA subgroup assays were performed as previously described by Beards et al. [2]. Capture antibody was a hyperimmune rabbit anti-rotavirus serum raised against complete and incomplete rotavirus particles of subgroups I and II. As «detecting» antibody, three monoclonal antibodies were used: (1) a rotavirus-group-specific monoclonal antibody, (2) a rotavirus subgroup-I-specific monoclonal antibody and (3) a rotavirus subgroup-II-specific monoclonal antibody.

**RESULTS**

During this survey, rotavirus particles were detected in 65 (25 %) of the 260 faecal specimens examined by electron microscopy from October, 1985 to April, 1986. A peak of occurrence was found in December, 1985 and January/February, 1986, when rotaviruses were detected in 52/135 patients (38.51 %).

Of the 65 rotavirus-positive specimens obtained, 49 (75.38 %) contained a sufficient number of rotaviral particles for RNA analysis. The numbers of rotavirus-positive patients, specimens with demonstrable RNA patterns of the virus and different electrophore types detected each month are summarized in table I. As shown in this table, rotaviruses were found throughout the study period.

Adenovirus (3.46 %), astrovirus (1.53 %), coronavirus-like particles (1.15 %) and calicivirus (0.76 %) have also been detected among the 260 faecal samples studied; on 5 occasions, some of these viruses were associated with rotavirus.

| Month/year | Rotavirus-positive specimens | RNA-analysis-positive | Nb of electrophoretypes |
|------------|------------------------------|-----------------------|-------------------------|
| 10/1985    | 3                            | 2                     | 1                       |
| 11/1985    | 3                            | 3                     | 1                       |
| 12/1985    | 17                           | 12                    | 6                       |
| 1/1986     | 16                           | 12                    | 5                       |
| 2/1986     | 19                           | 14                    | 8                       |
| 3/1986     | 4                            | 4                     | 4                       |
| 4/1986     | 3                            | 2                     | 2                       |

**TABLE I. — Monthly number of rotavirus-positive specimens by electron microscopy, RNA analysis-positive and different electrophore types detected.**
Fourteen different electrophoretypes were found. Figure 1 shows typical RNA migration patterns displayed by rotavirus isolates. Whenever appropriate, differences between isolates with similar RNA patterns were confirmed by co-electrophoresis (results not shown).

All rotavirus isolates analysed during this period had the so-called «long» electrophoresis pattern, with fast-moving segments 10 and 11, but contained RNA segments with differences in their electrophoretic mobility.

Mixed rotavirus electrophoretypes showing extra RNA fragments with respect to the 11 regular genome segments appeared in four cases (8.16%).

The classification scheme proposed by Lourenco et al. [10] to characterize and compare rotavirus electrophoretypes was used in this study as follows: the 11 RNA bands were divided into 4 groups including, respectively, bands 1,

![Figure 1](image-url)

**FIG. 1. — Representative RNA electrophoretypes displayed by rotavirus strains isolated in the October, 1985 through April, 1986 rotavirus season.**

The gel is a 10 % polyacrylamide gel which has been silver-stained. Genome segments are numbered on the left in decreasing order of size. Black circles indicate the presence of extra RNA bands in lanes A and J, as compared with the 11 regular bands which make up the rotaviral genome. Lane A shows a clear mixture of patterns Ia,IIa,IIIb,IVa and Ib,IIb,IIIb,IVa; lanes B, C, G, H and I, pattern Ib,IIb,IIIb,IVa; lanes D, F and J, pattern Ib,IIb,IIIb,IVa; and lane E, pattern Ib,IIa,IIIb,IVa.
2, 3 and 4 (group I); bands 5 and 6 (group II); bands 7, 8 and 9 (group III); and bands 10 and 11 (group IV). Differences in the relative migration of RNA bands within a group are indicated by a small letter, with each pattern referred to as a, b, c, etc.

The monthly incidence and distribution of the different rotaviral electrophoretic types detected are summarized in Table II. One of the electrophoretic migration patterns, Ib,IIb,IIIb,IVa, accounted for the largest proportion (36.7%) of all rotaviruses identified. Five isolates obtained during the first two months of the study period had this electrophoretic pattern. In contrast, strains collected during the following months had a large variety of RNA patterns, although the electrophoretype that had appeared first occurred more frequently than the others. During the final months, it was difficult to find any predominant electrophoretype.

| Electrophoretypes | 10/85 | 11/85 | 12/85 | 1/86 | 2/86 | 3/86 | 4/86 |
|-------------------|------|------|------|------|------|------|------|
| Ib,IIb,IIIb,IVa   | 2    | 3    | 4    | 6    | 3    | ...  | ...  |
| Ic,IIb,IIIB,IVa   | ...  | ...  | 2    | 3    | 2    | ...  | ...  |
| Ib,IIa,IIb,IVa    | ...  | ...  | ...  | 1    | ...  | ...  | ...  |
| Ic,IIa,IIb,IVa    | ...  | ...  | 2    | 1    | ...  | ...  | ...  |
| Ic,IIa,III,IVa    | ...  | ...  | 1    | ...  | ...  | ...  | ...  |
| Ib,IIa,IIId,IVa   | ...  | ...  | 2    | ...  | 1    | ...  | ...  |
| Ib,IIb,IIc,IVa    | ...  | ...  | ...  | 1    | 1    | ...  | ...  |
| Ib,IIb,IIId,IVa   | ...  | ...  | ...  | 2    | ...  | ...  | ...  |
| Ic,IIa,IIIa,IVa   | ...  | ...  | ...  | ...  | 2    | ...  | ...  |
| Ib,IIa,IIIc,IVa   | ...  | ...  | ...  | 2    | ...  | ...  | ...  |
| Ib,Ic,IIIf,IVa    | ...  | ...  | ...  | ...  | ...  | ...  | ...  |
| Ib,IIb,IIIf,IVa   | ...  | ...  | ...  | ...  | ...  | ...  | ...  |
| Ic,IIa,IIId,IVa   | ...  | ...  | ...  | ...  | 1    | ...  | ...  |
| Ib,IIb,IIId,IVa   | ...  | ...  | ...  | ...  | 1    | ...  | ...  |
| Ib,IIa,IIIIf,IVa  | ...  | ...  | ...  | ...  | ...  | ...  | ...  |

In an attempt to more precisely characterize the rotavirus isolates, we determined their serological subgroups using two subgrouping monoclonal antibodies in an ELISA test. The specificity of both monoclonal antibodies was assessed by titering them against dilutions of two known subgroup I and subgroup II strains. The reactivity appeared to be highly specific. As expected, all rotavirus isolates studied were identified as subgroup II, with OD values higher than 1.200 when a monoclonal antibody specific for this subgroup was used as the detecting antibody. With the rotavirus subgroup-I-specific monoclonal antibody, OD values between 0.165 and 0.380 were obtained (fig. 2).
FIG. 2. — Scattergram of the double-sandwich ELISA results using different monoclonal detecting antibodies.

Capture antibody was a hyperimmune rabbit anti-rotavirus serum. As detecting antibodies, three monoclonal antibodies were used: (1) a rotavirus group-specific monoclonal antibody, (2) a rotavirus subgroup-I-specific monoclonal antibody, and (3) a rotavirus subgroup-II-specific monoclonal antibody. Each point (●) represents the average absorbance of replicate wells. The 49 rotavirus isolates tested were identified as subgroup II, with OD values higher than 1,200.

**DISCUSSION**

Our results have further demonstrated the extensive genomic heterogeneity of rotaviruses and the occurrence of different RNA patterns within an outbreak, as published by others [3, 10, 12]. However, some remarkable findings of our survey should be noted: no «short» electrophoresis patterns were
detected, in contrast to many other reports. The predominance of the «long» electrophoretype has also been universally observed in recent years [17]. This fact could have immunological consequences, as sequential illnesses associated with different rotavirus serotypes have been described [13].

The observation of a predominant electrophoretype in a given rotavirus season is in agreement with results of other studies reported from many parts of the world [4, 11, 12].

We have detected 8.16 % of RNA patterns with more than 11 bands, suggesting the possibility of simultaneous or sequential infection by more than one rotavirus strain. The frequency of such a finding is approximately 10^-7% according to different reports [10, 16]. Mixed rotavirus infection could be the first step in genetic reassortment in vivo, with an intermediate step being necessary in the establishment of stable reassorted virus [5].

Electrophoresis of rotaviral RNA unfortunately cannot be used to determine antigenic serotypes of field isolates [1]. When type-specific monoclonal antibodies become available, serotyping of rotavirus strains will be transferable to many laboratories.

**RÉSUMÉ**

**VARIATION SÉQUENTIELLE DES ÉLECTROPHORÉGRAMMES DES ROTAVIRUS HUMAINS ISOLÉS DES GASTRO-ENTERITES AIGUÉS INFANTILES**

On a étudié l'incidence et les profils électrophorétiques de l'ARN génomique des souches de rotavirus obtenues d'octobre 1985 à avril 1986, de gastro-entérites aigües de l'enfant. L'analyse de l'ARN par électrophorèse sur gel de polyacrylamide a permis l'identification de 14 électrophorétypes différents. Pendant les premiers mois de la période d'étude, on a détecté un électrophorétype dominant ; au contraire, dans les mois suivants, on a observé une grande variété de profils électrophorétiques.

La détermination des sous-groupes des rotavirus par analyse immunoenzymatique avec des anticorps monoclonaux détecteurs, a permis de constater que toutes les souches sont du sous-groupe II. Dans 4 échantillons (8,16 %) on a détecté des électrophorétypes mixtes.

**MOTS-CLEFS**: Rotavirus, Gastroentérite, ARN; Enfant, Epidémiologie moléculaire, Electrophorétypes, Profils de l'ARN génomique, Variation séquentielle.

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