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ELECTROPHEROTYPES OF ds-RNA OF ROTAVIRUS IN INFANTS AND YOUNG CHILDREN WITH GASTROENTERITIS IN BANGLADESH

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Abstract

One hundred suspected rotavirus stools, collected at the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh, from January 1982 to March 1984, were examined by the polyacrylamide gel electrophoresis (PAGE) technique, to detect electropherotyping of double-stranded ribonucleic acid (ds-RNA) of rotavirus strains present in Bangladesh. Of the samples, 39% were positive for rotavirus by both enzyme-linked immunosorbent assay (ELISA) and PAGE, whereas only 1% were positive by PAGE alone. Electropherotyping of the ds-RNA of rotavirus has shown that 6 different strains of rotavirus, 3 each of short (S) and long (L) ds-RNA banding pattern were infecting the study population. The predominant electropherotype during 1982-83 was the L_1 type, which was replaced by the L_2 type in 1984. The short type was present in 1982.

Key words: Rotaviruses; Gastroenteritis; Diarrhoea; RNA, Viral; Electrophoresis, Polyacrylamide Gel; Immunoenzyme Technics.

Introduction

Rotavirus has been associated with diarrhoeal disease in man and some animals in many parts of the world (1,2). Electropherotyping of the double-stranded ribonucleic acid (ds-RNA) genome of rotavirus is an important method for differentiation among rotavirus strains (3).

It is now generally agreed that the rotavirus genome consists of 11 segments of ds-RNA (3,4). Although minor differences are evident in the banding pattern, the following always is true: there is a grouping of four large segments, then two of medium size, followed by a closely-running triplet which cannot always be resolved, and finally, two small segments. This pattern makes the rotavirus genome readily distinguishable from the patterns produced by other genera of the reoviridae.

Differences in the migration patterns of any of the 11 ds-RNA segments are sufficient to distinguish among isolates (2,3,5); this factor is invaluable for studies on the evolution and spread of newly appearing strains (6). Thus, genome electropherotyping is now a commonly used method for both taxonomic and epidemiologic studies (5,7).

In this preliminary study, we looked at the occurrence of human rotavirus electropherotypes among patients at the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The results provide new information concerning the number of different electropherotypes of human rotavirus in this community.

Materials and methods

One hundred suspected rotavirus stools (0.1 ml) were collected directly in sterile vials containing 1 ml PBS, pH 7.2, and were stored at –20°C. All samples were taken from infants and young children with mild-to-severe secretory-type diarrhoea, attending the ICDDR,B hospital at Dhaka, Bangla-

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desh, from January 1982 to March 1984. All stool samples were examined for rotavirus, by the ELISA (8).

Nucleic acid extraction

Genome RNA of rotavirus from feces was extracted by the method described by Rodger and Holmes (3). Virus preparations were deproteinized with phenol and sodium dodecyl sulfate (SDS). Electrophoresis of deproteinized RNA was carried out in 10% polyacrylamide slab gels with a 3% stacking gel, using the discontinuous buffer system without SDS as described by Laemmli (9). After electrophoresis, the gels were stained by the silver staining technique described by Herring *et al.* (10).

Results

Electropherotypes of viral RNA obtained from 40 of 100 stool specimens are shown in the Table. A positive result in both ELISA and polyacrylamide gel electrophoresis was seen for 39 samples, while one sample was positive only by PAGE. Two major electropherotypes of ds-RNA of rotavirus found during this period were "long" (L) and "short" (S). Segment 11 of strains with "short" electrophoretic pattern lines up approximately with segment 10 of strains with "long" patterns (11). The most predominant types in 1982, 1983 and 1984 were, respectively, L_1, L_1 and L_2 (Table). S types were found only in 1982.

The banding patterns of the ds-RNA of rotavirus are shown in Figs. 1, 2 and 3. The S patterns are shown in lanes 2, 3 and 6 and the L RNA patterns in rows, 1,4,5,7,8,10 and 11. There are some differences in the banding patterns of segments in both S and L RNA banding..

L and S electropherotypes were subtyped according to their difference in segment positions. Subtype L_1 is shown in rows 1,4,5 and 11, while subtype L_2 (differing in segments 3 or 4-9) is shown in row 7; and subtype L_3 (differing in segments 3 or 4 & 7-9) is shown in rows 8 and 10. S_1 and S_2 are shown in rows 2 and 6 (differing in segments 7, 8 & 9) respectively.

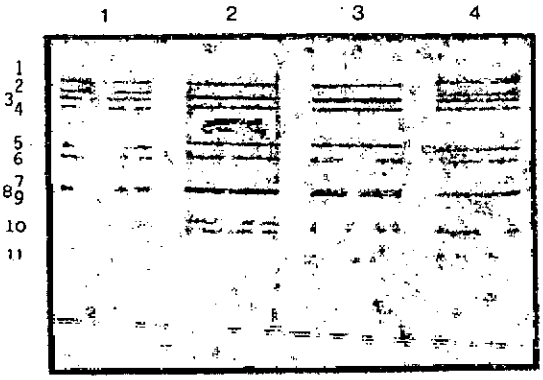

Fig. 1 – Electrophoretic banding pattern of ds-RNA of human rotavirus isolated in Bangladesh. The gel shows a number of fairly clear differences in the electropherotypes. Rows 1 and 4 show the long (L_1) banding pattern and row 2 shows short banding (S_1) pattern. Row 3 shows ds-RNA of rotavirus with 13 segments, designated as S_3 type (two extra bands between segments 5-6 and 10-11).

TABLE – ROTAVIRUS ELECTROPHEROTYPES RECOGNIZED IN BANGLADESH

Year	No. of samples positive for rotavirus (n=40) by		Electropherotypes recognized
	both PAGE & ELISA	PAGE only	
1982	8	1	S_1(1)*, S_2(1), S_3(1), L_1(5), L_3(1)
1983	22	–	L_1(9), L_2(7), L_3(6)
1984	9	–	L_1(2), L_2(7)

* Numbers in parentheses indicate the number of samples showing the same electropherotypes.

The strain which gave a negative result in the ELISA but positive in PAGE showed 13 bands (row 3, Fig. 1). This banding pattern shows two extra bands between segments 5-6 and 10-11. compared with S type (row 2, Fig. 1). This strain is labeled as S_3, due to its close similarity with the banding pattern of S types.

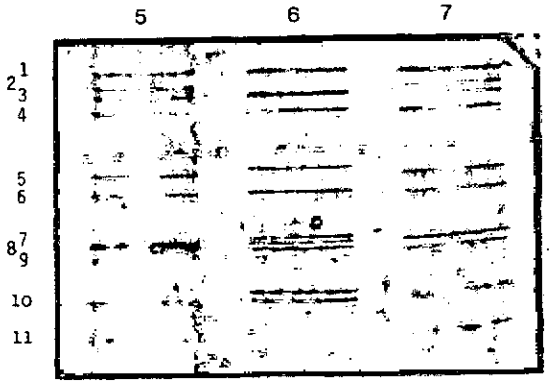

Fig. 2 – Showing different subtypes of long and short electrophoretic banding patterns. Row 5 shows L_1 type, row 7 shows L_2 type (differing in segments 3 or 4 and 9) and row 6 shows S_2 type (differing in segments 7, 8 and 9).

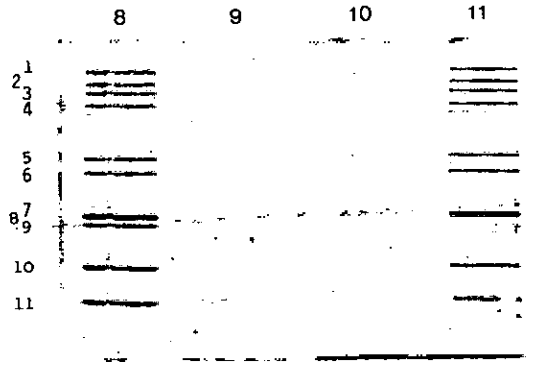

Fig. 3 – Electrophoretic banding pattern of ds-RNA of rotavirus showing subtype L_3 (differing in segments 3 or 4 and 7-9) in rows 8 and 10.

Discussion

Rotavirus isolates obtained from a sample of 10 patients showed six different electropherotypes of their ds-RNA genome. Changes in the prevalent rotavirus genotype usually exhibit a sequential pattern, with a limited number persisting for a year (12, 13) or even up to two years (7), and several less common types co-existing with a predominant type (7). In agreement with these observations, we found that in 1982 the S types also were present with L types, but not in the later years.

The subtype L_1, which predominated in 1982-1983, was replaced in 1984 by subtype L_2. The changing pattern of rotavirus electropherotypes in a community (Table) may indicate that new strains could emerge by reassortment during possible multiple infections. This is supported by reports of reassortment of rotavirus RNA *in vitro* under certain conditions (14, 15). Another plausible explanation is that the virus is in a state of continuous antigenic drift, due to pressure imposed on it by the host immune system and changes in the environment. An analogous situation is seen with the influenza virus, which too has a segmented genome (16).

The strain which gave a negative result in the ELISA test and was shown by the PAGE to have 13 bands with an S type needs further identification. So far, all human rotaviruses with short patterns have been found to belong to sub-group 1 and serotype 2 (11).

This preliminary investigation found 6 different electropherotypes of rotavirus, 3 each of S and L banding patterns, which could be of the same or different serotypes. Further studies are required to determine the prevalence of the predominant subtype L_2 or to document the appearance of a new one.

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