

Original Article

Electrophoretic RNA Genomic Profiles of Rotavirus Strains Prevailing Among Hospitalized Children with Acute Gastroenteritis in Tehran, Iran

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Background: Human rotavirus has now been established as the most important cause of childhood gastroenteritis worldwide. The RNA genomic electrophoretic diversity of group A rotavirus strains in Tehran, Iran, during April 2002 through March 2005, was investigated in 1250 stool specimens.

Methods: Stool samples were obtained from young children with acute gastroenteritis. RNA electrophoretic profile of group A rotavirus strains was determined using RNA polyacrylamide gel electrophoresis in those samples found to be positive for rotavirus by enzyme immunoassay.

Results: The predominant RNA profile detected was the long electrophoretic profile (90%) followed by the short profile (8.8%). Four patients (1.1 %) had patterns of mixed infection. Fourteen different electrophoretic RNA patterns, eight of long and six of short, were detected in the study area. A long RNA electrophoretic profile persisted during the years of the study with peak incidence in cool seasons. The long pattern occurred throughout the study period and in patients aged one to 60 months, but the short profile identified only in infants at varying intervals.

Conclusion: Our data demonstrate epidemiologic differences between the incidence of long and short electrophoretic profiles. The long profiles appear to circulate continuously in the area, whereas the short patterns appear in an episodic fashion.

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Keywords: Cell culture • enzyme immunoassay • polyacrylamide gel electrophoresis • rotavirus

Introduction

Human rotavirus (HRV) is a major cause of acute gastroenteritis in young children worldwide. It is an important cause of death in children in the developing countries.^{1,2} Rotavirus, a member of the Reoviridae family, is a nonenveloped virus with a double-stranded RNA (ds RNA) genome of 11 segments

enclosed in a triple-layered protein.³ Rotaviruses constitute a heterogeneous group of viruses showing different antigenic specificities and a high extent of genetic diversity.³ Polyacrylamide gel electrophoresis (PAGE) has high sensitivity and specificity for determination of the prevalent rotavirus strains in the community.³⁻⁵

Electrophoresis of the rotavirus RNA genome allows detection and classification of the viruses into two major groups; the long (L) and the short (S) electrophoretic profiles based on the migration patterns of gene segments 10 and 11 on polyacrylamide gel.³ The variations in the electrophoretic mobility of one or more RNA segments allow different rotavirus strains to be assigned to distinct electrophoretic profiles.³ Electrophoresis of the rotavirus RNA genome has

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often been used as a useful indicator of the genomic diversity of rotavirus isolates in a population over a certain period.⁶⁻⁸ This study reports the occurrence of rotavirus infection among hospitalized young children with acute gastroenteritis and RNA electrophoretic characterization of isolated circulating rotavirus strains during a three-year period in Tehran. It is a contribution to the molecular epidemiology knowledge of rotavirus infection in Iran.

Materials and Methods

Patients' specimens

This study was conducted in five pediatric hospitals in Tehran, Iran during April 2002 through March 2005. Fecal samples were collected from 1250 children under five years old who were admitted with acute gastroenteritis to the infectious diseases units at the hospitals. The labeled fecal samples were transported on ice to the Laboratory of the Virology Department at Iran's Pasteur Institute where they were stored at -20°C until processed.

Rotavirus detection

All fecal samples were screened for rotavirus group A-specific antigen by enzyme immunoassay (EIA) (Rotavirus-IDEA, Dako Denmark). These were performed according to instructions supplied by the manufacturers with the kit.

Polyacrylamide gel electrophoresis (PAGE) of rotavirus ds-RNA

The rotavirus ds RNA was extracted from all rotavirus-positive specimens according to previously described methods.^{9,10} Briefly, a 10% emulsion was made in extraction buffer containing sodium dodecyl-sulfate (SDS). The suspension was then mixed with an equal volume of phenol/chloroform, vortexed, and centrifuged at 10,000 rpm for five minutes, and then the top aqueous phase containing RNA was removed. The RNA was precipitated with two volumes of ethanol at -20° C for two hours, collected by centrifugation, and resuspended in diethylpyrocarbonate water. For analysis, 30 uL of RNA was electrophoresed in a 10% polyacrylamide gel (0.5 mm thick) overnight at 70 volts at room temperature. The gels were fixed in ethanol/acetic acid, stained with silver nitrate, washed, and then they were developed with NaOH/formalin and finally preserved in 0.05 M

sodium carbonate solution and photographed.

Cell culture

The RNA also extracted from cell culture (MA-104) infected with rotavirus strain (SA-11), was used as marker in PAGE.¹¹

Results

A total of 1250 stool samples were collected from children under five years old for the study. Rotavirus was detected in 404 (32.3 %) of the 1250 stool specimens tested for the presence of group A rotavirus antigen by EIA. Infection occurred throughout the years and peaked during the cool seasons of the years. Figure 1 shows the electrophoretotypes detected from the study.

Of the 404 rotavirus-positive specimens tested by PAGE, 341 yielded rotavirus RNA electrophoretic patterns. Three hundred seven (90%) of the isolated strains were of the long electrophoretotype, and 30 (8.8%) of the strains were of the short electrophoretotype. The remaining four (1.1%) strains showed profiles of mixed infections (Table 1). In total, 14 different electrophoretic profiles [eight of the long electrophoretotype (lanes LA-LH) and six of the short electrophoretotype (SA-SF)], were detected in the study, which are shown in Figure 1.

The most commonly detected long and short electrophoretotypes were LA (25%) and SD (3.2%), respectively (Table1). Each year a number of different rotavirus strains, as defined by PAGE RNA electrophoretotypes cocirculated in the area, with a predominant long RNA type, which persisted during the period of this survey (Figure 2).

Over time there was a certain change in the numbers and types of RNA pattern, so that new strains emerged within the studied community (e.g., LB in 2003 and LC in 2004), whereas some strains ceased to exist (e.g., LH in 2004). Some strains existed for short period of time, such as LG in 2004, which emerged and disappeared within a few months. The prevalent long types LD and LE also cocirculated during the study period. Each year rotavirus short RNA profiles identified for short period of time (e.g., SA and SB), however, strain SD persisted in the area for about three years (Figure 2).

The monthly distribution of the long electrophore types appears to be random and no apparent seasonal variations could be detected.

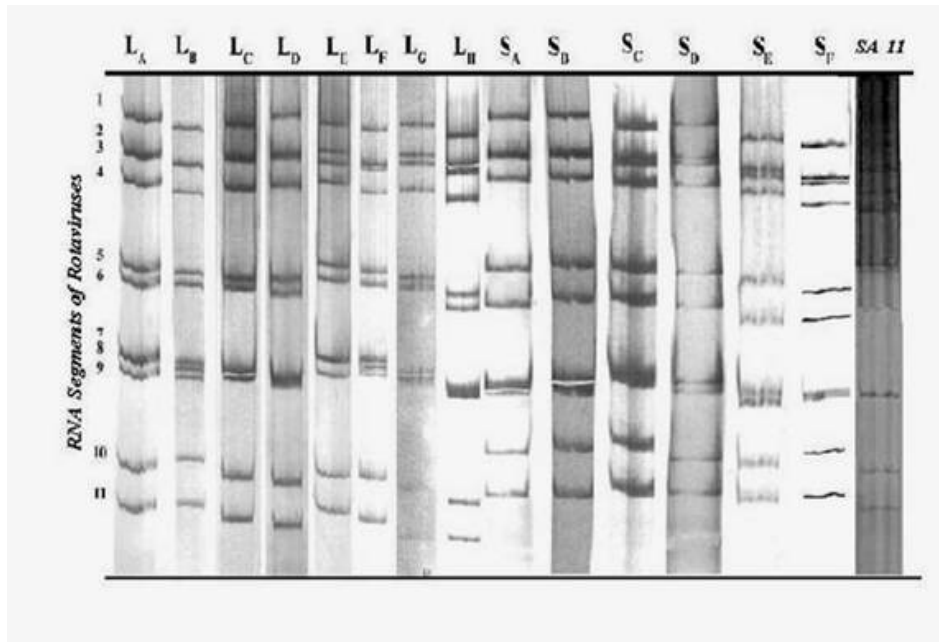


Figure 1. Genomic RNA electrophoresis of representative strains from rotavirus electrophoretotypes identified in Tehran, during April 2002 through March 2005. RNA migration was from the top to the bottom. Lanes LA-LH represents long electrophoretotypes and SA-SF the short electrophoretotypes detected. SA-11 is as the marker.

However, pattern LA was predominant in October and December, and prevalent strains LD and LE peaked in November. The short profiles were more prevalent during autumn-winter and were not observed in warm months of the study period (Figure 2). The distribution of long RNA electrophoretotypes did not appear to vary with age and it occurred in all age ranges (one month to five years old), while the short RNA profiles identified among children aged up to two

years old (Table 2). The prevalent long and short strains were detected from hospitalized children in all of the geographic areas studied in Tehran. Some strains (LB and LD) were concentrated in the eastern and northern parts of the city, respectively, as shown in Figure 3.

Discussion

Rotavirus strains from diarrheic stools in our

Table 1. Frequency of occurrence of 14 different RNA electrophoretotypes of rotavirus in hospitalized children with acute gastroenteritis in Tehran.

Pattern of mobility of RNA segments 10,11 No. of patients (%)	Electrophoretotypes	No. of patients (%) with RNA electrophoretotype during the study period			Total (%)
		Apr. 2002 to Dec. 2002	Jan. 2003 to Dec. 2003	Jan. 2004 to Mar. 2005	
Long patterns 307(90%)	LA	47	25	15	87(25.8)
	LB	0	10	18	28 (8.3)
	LC	0	0	6	6 (1.8)
	LD	19	17	7	43 (12.7)
	LE	46	13	19	78 (23.2)
	LF	3	2	6	11 (3.2)
	LG	0	0	4	4 (1.2)
	LH	32	18	0	50 (15)
Short patterns 30 (8.8 %)	SA	1	1	0	2 (0.6)
	SB	0	0	5	5 (1.5)
	SC	1	3	0	4 (1.2)
	SD	3	5	3	11 (3.2)
	SE	0	0	3	3 (0.9)
	SF	3	1	1	5 (1.5)
Mixed infection 4 (1.1 %)	Mixed patterns	1	1	2	4 (1.1)

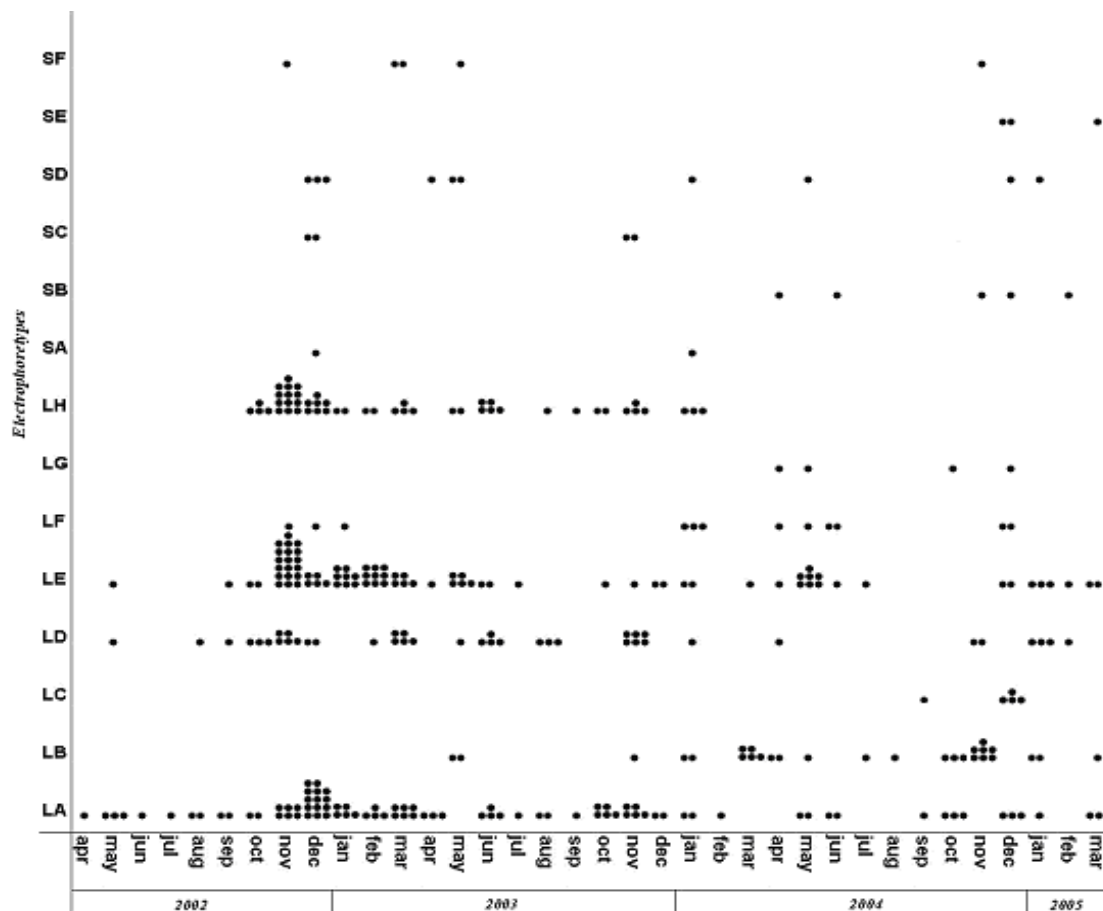


Figure 2. Temporal distribution of rotavirus electrophoretotypes in children with acute gastroenteritis during April 2002 through March 2005 in Tehran.

study were the group A antigen and the strains were analyzed by their ds RNA genomic profiles as determined by PAGE. Rotaviruses were associated with 32.2% of the diarrheal episodes in children less than five years old with peak incidence in infants. This result is similar to other studies in Iran.^{12,13} Rotavirus infection occurred throughout the year and peaked in the cool months of the years.

Rotavirus RNA electrophoretotypes were found to be similar to what have been demonstrated elsewhere in the world. Two major RNA patterns,

designated short and long profiles, constituting a majority (90%) of the electropherotyped specimens. The predominant electrophoretic pattern detected in children from Ahwaz, Iran was also long electrophoretotype.¹³ This incidence appears to be the norm globally.¹⁴⁻¹⁶ The finding of 14 long and short RNA profiles are similar to other studies, which showed a great heterogeneity of rotavirus electropheretotypes. In Australia, Unites States of America (Philadelphia), China, India, and Africa 90, 48, 12, 10, and 16 different electropheretotypes were encountered,

Table 2. Distribution of rotavirus RNA electropheretotypes among different age groups of hospitalized children with acute gastroenteritis in Tehran.

Age groups	Electropheretotypes													
	Long patterns							Short patterns						
	LA	LB	LC	LD	LE	LF	LG	LH	SA	SB	SC	SD	SE	SF
1 – 6 months	10	3	—	6	11	—	1	8	—	1	—	2	1	1
7 – 11 months	17	5	—	9	16	2	1	13	1	—	2	2	—	3
1 – 2 years	35	12	3	12	29	5	—	14	1	4	2	7	2	1
3 – 5 years	10	2	1	7	12	—	—	9	—	—	—	—	—	—
Total	72	22	4	34	68	7	2	44	2	5	4	11	3	5

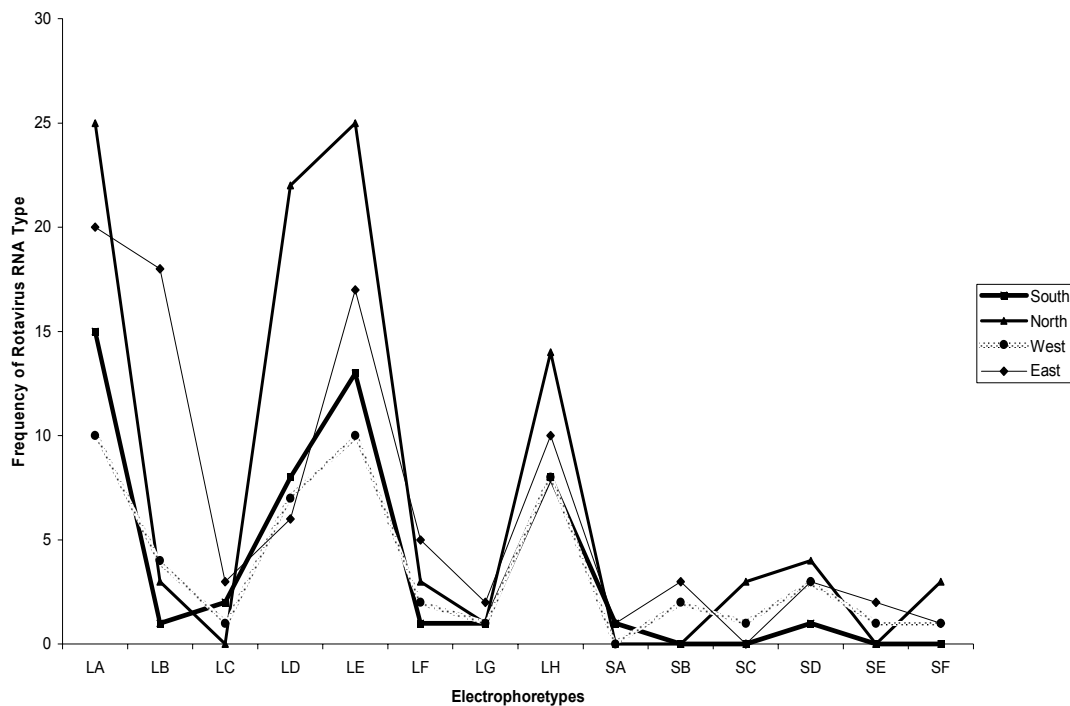


Figure 3. Distribution of rotavirus RNA patterns among hospitalized children with diarrhea from all of the geographic areas studied in Tehran, during April 2002 through March 2005.

respectively.¹⁷⁻²¹

During each seasonal peak of rotavirus activity, several rotavirus RNA electropherotypes existed, with a predominant strain accompanied by less common types. The rotavirus strains occurred in a manner in which some strains disappeared as new strain emerged. It was also shown that, whereas some strains existed for short periods, other persisted during the three-year period of the study. These results confirm the findings of other studies.^{22,23}

The most prevalent strain in years studied was pattern LA thus supporting other reports that a single predominant rotavirus electropherotype may persist for several years.^{19,24} In our study, RNA profile of strain LB was present in the area during May 2003 through March 2005 that had not been detected in 2002. And LC type occurred exclusively in the September and December 2004. However, it is apparent that new strains may suddenly emerge, as strain LG identified during the spring and autumn of 2004, with no previous occurrence having been observed. The most prevalent short profile (SD type) was first appeared in December 2002 and persisted during the study period, whereas a few of strain SB observed only in 2004.

Rotavirus strain variation is a consequence of

either antigenic drift, or antigenic shift and gene reassortment amongst circulating rotaviruses.²⁵ This may be a common mechanism of generation of viral variants in this region, because of the large pool of different viruses circulating in the community and the mixed infection level seen. The long pattern was present in all seasons and peaked in cool months. Short profile persisted for various periods with peak occurrence in November and December, and disappeared in summer. Within each electropherotype, most of the children infected were aged seven to 24 months. This data shows similar epidemiologic pattern of rotavirus infection associated with long and short RNA profiles to what have been found in the developing countries.^{24,26} The results from this study, and other developing countries,²⁷⁻²⁹ have demonstrated the high genomic diversity of circulating rotavirus strains. This underscores the need to continue the global surveillance of rotavirus, especially in developing countries where any future rotavirus vaccine will be needed most.

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