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ANALYSIS OF ROTAVIRAL GASTROENTERITIS IN TBILISI

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Summary. - Electrophoretypes of 107 rotaviral isolates collected in Tbilisi for 18 months revealed seven patterns from which 4 were "long" and 3 "short". The "long" electrophoretypes represented 74.8% of total number of the isolates analysed. One of the "long" electrophoretypes dominated for the whole investigation period. Differences in the seasonal distribution of the isolates with various electrophoretypes were demonstrated and appearance of rotaviral isolates was registered with a "short" electrophoretype which had never been detected before.

Key words: rotaviruses; gastroenteritis; genomic RNA; electrophoretype

Introduction

Rotaviruses are one of the major cause of human gastroenteritis. Due to their complicated antigenic structure and because of cultivation difficulties with the natural isolates alternative approaches such as electrophoretic analysis of rotaviral RNA segments in polyacrylamide gel (PAG) are of great importance. A well-reproducible mobility pattern of the 11 genomic RNA segments (electrophoretypes) inherent to the particular strain allows to identify the rotaviral isolates and is widely used in following the spread of rotavirus infections (reviewed by Estes *et al.*, 1984; Sanders, 1985). The aetiological role of rotaviruses in acute intestinal diseases of children in Georgia was demonstrated in our previous study (Sakvarelidze and Zangaladze, 1986). Present work was aimed at characterization of the electrophoretypes of rotaviral isolates coming from children with gastroenteritis in Tbilisi. The seasonal distribution of various electrophoretypes was followed for up to 18 months among children of different age groups.

Materials and Methods

Faecal samples were collected from October 1984 to March 1986 from 845 children aged 1 month to 3.5 years which had been admitted to Tbilisi Paediatric Infections Hospital with the diagnosis of acute intestinal infection. Aliquots of centrifuged (3000 x g for 15 min) 10 - 20% faecal suspensions were used for electron microscopy (EM), enzyme immunoassay (EIA) and electrophoresis of the genomic RNA of viral isolates.

EM was performed by negative contrast using 1% of aqueous solution of uranyl acetate.

EIA (indirect) was made for detection of rotaviral antigen; specific (for group A viruses) antibodies were derived from rabbit blood sera and chick egg yolk; animals were immunized with SA 11 rotavirus particles purified in CsCl gradient (Ginevskaya *et al., 1988).*

Isolation of viral RNA. Aliquots (0.2 - 0.5 ml) of 10 - 20% faecal suspensions containing rotaviruses confirmed by EM and EIA, were treated with Freon-113 (Serva) and subsequently diluted in phosphate buffered saline (0.01 mol/l phosphate buffer pH 7.4; 0.1 mol/l NaCl). The virus was sedimented by centrifugation (100 000 x g for 2 hr). To the resuspended pellet Na-dodecylsulphate (1%), Na-ethylendiaminetetraacetate (0.001 mol/l), and pronase (0.2 mg/ml) were added, the samples were incubated at room temperature for 15 min and after exposure to Na-acetate the RNA was deproteinized with a mixture of phenol, chlorophorm and isoamyl-alcohol (25:24:1). For repeated deproteinization, the mixture of chlorophorm and isoamyl-alcohol (24:1) was used. The RNA was sedimented using 2.5 volumes of ethanol (at - 20 ^{0}C overnight or at -70 ^{0}C for 2 hr).

Electrophoresis. RNA was sedimented by centrifugation (20 000 x g for 15 min), dried, dissolved in 10 μl of sterile water, 5 μl of the buffer was added (6% Na dodecylsulphate, 15% mercaptoethanol, 0.15 mol/l Tris-HCl pH 6.8; 30% glycerol; 0.01% bromphenol blue) and heated at 100 ^{0}C for 1 min. Electrophoresis was performed according to Laemmli (1970) in 10% separating and 4% concentration gel at 10 mA for 17 hr. The RNA was stained with silver nitrate as described previously (Herring *et al.*, 1982).

Results and Discussion

With EM and EIA rotaviruses were detected in 239 (28.3%) out of 845 children with the diagnosis of acute intestinal infection. Of these, 107 (44.8%) positive samples contained a sufficient amount of material for RNA analysis (RNA-positive samples). Fig. 1 depicts the electrophoregram showing all 7 electrophoretypes seen by us. All isolates had the typical group A rotavirus pattern of RNA segment distribution. The comparison of electrophoretic mobility of segments 10 and 11 with respective segments of WA human rotavirus showed both "long" and "short" electrophoretypes. Based on less manifested differences of the electrophoretic mobility of individual segments, 4 "long" (A-D) and 3 "short" (E-G) variants were defined. "Short" isolates differed mainly in the relative mobility of segments 7.8 and 9; the variability of "long" variants was recorded for this group of segments as well as for segments 2, 3, 5 and 6.

Rotaviruses in the faeces of sick children were detected during the whole follow-up, with increased incidence of rotaviral gastroenteritis in the autumn and winter (Table 1). Isolates with "long" electrophoretypes comprised 74.8% of all RNA-positive samples, their frequency being higher than that of the "short" ones in both the autumn-winter as well as spring-summer seasons. The

Table 1. Seasonal incidence of intestinal infection, rotavirus-positive isolates and their electrophoretypes in Tbilisi from October 1984 to March 1986

	Incidence of cases				
	10.1984-02.1985	03.1985-09.1985	10.1985-02.1986	03.1986	Total
Patiens with acute intestinal infection	204	317	292	32	845
Rotavirus-positive*	77	41	115	6	239
RNA-positive	31	21	54	1	107
A	11	10	13	1	35
B	3	4	8	0	15
C	4	5	4	0	13
D	6	1	10	0	17
E	1	0	10	0	11
F	6	1	4	0	11
G	0	0	5	0	5
Total number of "long"	24	20	35	1	80
Total number of "short"	7	1	19	0	27

* Samples in which rotaviral antigen (by EIA) and rotaviral particles (by EM) were detected

isolates with electrophoretype A occurred most frequently comprising 32.7% of all RNA-positive isolates. Isolates corresponding to all 4 "long" electrophoretype patterns were detected throughout the seasons. The percentage of isolates with "short" electrophoretypes was significantly higher in the autumn-winter than in the spring-summer period (22.6, 35.2, and 4.8%, respectively, of the RNA-positive isolates for each season), and it increased during the second autumn-winter season. The isolates with the "F" electrophoretype proved to be the most frequent "short" ones in the autumn-winter period of 1984 - 1985 which represented 85.7% out of all "short" isolates found during a season. During the next autumn-winter season the relative frequency of electrophoretype "F" isolates decreased by 21.1%, the most common of "short" electrophoretype isolates became of type E (52.6%) but also electrophoretype G rotavirus isolates occurred which had never been noted before.

Distribution of isolates with different electrophoretypes among children of

Table 2. Distribution of isolates with "long" and "short" electrophoretypes among children of different age groups

Season	Number of isolates from children									
	1 to 6 month-old			7 to 12 month-old			Older than 12 months			
	Electrophoretype		Total No. of RNA-positive	Electrophoretype		Total No. of RNA-positive	Electrophoretype		Total No. of RNA-positive	Ale the RNA-positive
	"long"	"short"		"long"	"short"		"long"	"short"		
October 1984 February 1985	7	1	8	10	3	13	7	3	10	31
March-September 1985	9	0	9	7	1	8	4	0	4	21
October 1985 February 1986	12	8	20	12	5	17	11	6	17	54
Total	28	9	37	29	9	38	22	9	31	106

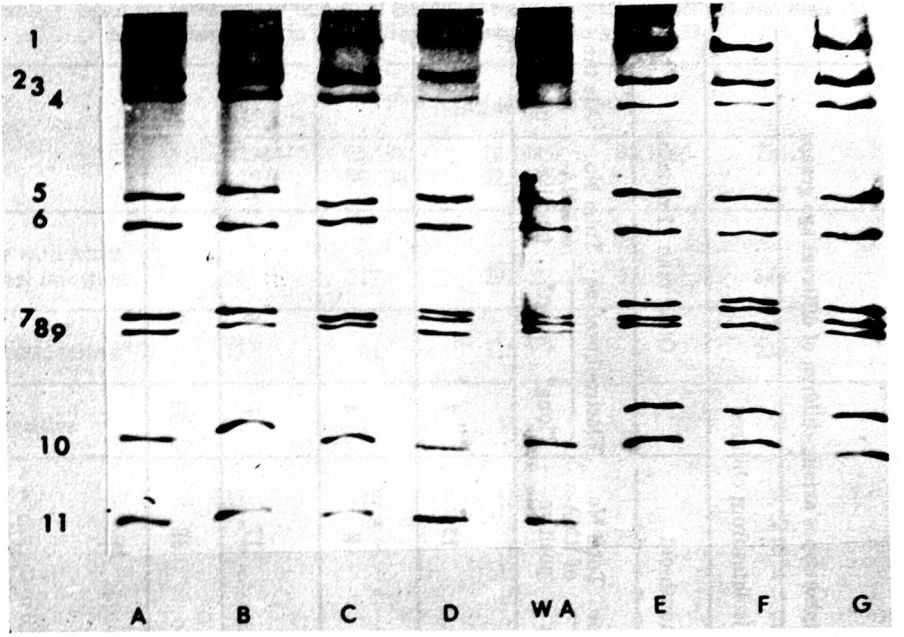

Fig. 1
Electrophoretypes of rotaviruses isolated in Tbilisi
Typical migration profiles of genomic RNA segments of isolates are compared with those of the reference human Wa rotavirus strain. Lanes A-D show "long". Lones E-G "short" electrophoretypes. Genomic segments are numbered in the left.

the three age groups (Table 2) showed no significant differences: The ratio of "long" and "short" isolates remained unchanged and "long" isolates prevailed (75.7, 76.3, and 71.0%, respectively) in each group. Electrophoretype A isolates were the most representative in all age groups; isolates with "short" electrophoretype G were detected only in children older than 6 months.

Our communication for the first time characterizes the rotaviruses circulating in Georgia. Electrophoretyping-detected genomic variability of rotaviruses has been described by many investigators (Espejo *et al.*, 1980; Rodger *et al.*, 1981; Lourenco *et al.*, 1981; Spencer *et al.*, 1983; Dimitrov, *et al.*, 1984; Tam *et al.*, 1986; Szücs *et al.*, 1987; Novikova and Antsupova, 1987; Shindarov *et al.*, 1988) and is more or less typical of various epidemiological situations. During the whole period of our follow-up the same variant of the "long" type proved to be the most representative, simultaneously with other "long" and "short" circulating variants. Prolonged persistence of the dominating electrophoretype indicating a certain stability of rotaviral population was also described in Australia (Rodger *et al.*, 1981), United States (Dimitrov *et al.*, 1984), Bulgaria

(Shindarov *et al.*, 1988), and in many other countries. One can assume that the strains with dominating electrophoretypes are genetically more stable and/or have a higher epidemic potential as compared to other strains. At the same time we detected some variations in the rotavirus population, namely the appearance of a new previously non-identified variant of "short" electrophoretype with changes in segments 7.8 and 9, i. e. including the gene coding for the major neutralization antigen.

At present it is not clear in what extent the genomic variability of rotaviruses correlates with the appearance of virulent strains. Studies of rotaviral isolates using molecular, biological, and serological methods would contribute to the solution of this problem.

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