CHARACTERIZATION OF HUMAN ROTAVIRUSES ISOLATED FROM SYMPTOMATIC AND ASYMPTOMATIC INFECTIONS

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Summary. – The occurence and circulation of different human rotavirus electropherotypes during 1984–1992 in diarrhoeic and healthy subjects were studied using examination of electropherotypes of viral RNA by polyacrylamide gel electrophoresis (PAGE). In 638 of 934 faecal specimens 11 different electropherotypes were distinguished. One long electropherotype pattern was far more frequent (58.6%) than any of the other 10 rotavirus electropherotypes detected throughout the whole study. The broadest spectrum of electropherotypes was detected in the group of children hospitalized with gastroenteritis as compared to the other groups followed. In the group of asymptomatic newborns only single electropherotype was detected, which did not occur in any group of sick children or adult contancts. Hospital-acquired infection was proved in 7.9% of followed hospitalized gastroenteritis cases. All electropherotypes detected in hospitalized children were identical to the strains occurring among community-acquired rotavirus infections. According to these findings we do not suppose that a hospital is a reservoir of rotaviruses responsible for clinically apparent gastroenteritis cases.

Key words: rotaviruses; RNA electropherotypes; viral gastroenteritis; asymptomatic infection

Introduction

Rotaviruses are well-known human and animal pathogens causing diarrhoea in many mammalian and avian species. Human rotaviruses constitute a heterogenous group of viruses showing different antigenic specificity and a high extent of genetic diversity. The virus genome consists of 11 segments of double-stranded RNA separable by PAGE. The PAGE pattern (electropherotype) exhibits extensive diversity. Simultaneous coexistence of rotaviruses with a number of different electropherotypes, a sequential pattern of appearance of the given electropherotypes in the community, and a predominance of one electropherotype each year (or several following years) are features associated with rotavirus molecular epidemiology (Estes *et al.*, 1984). Because of the difficulties encountered in growing human rotaviruses in cell culture (Albert and Bishop, 1984; Tietzová and Petrovičová, 1994), electron miscroscopy still represents the method of choice for their diagnosis. The identification of human group A rotavirus strains by immunological methods which distinguish two subgroups (I and II) and seven antigenic specificities (serotypes 1, 2, 3, 4, 8, 9 and 12) is hampered by difficulties in growing the strains *in vitro* or by their close antigenic relatedness (Kapikian *et al.*, 1981; Gerna *et al.*, 1985).

The examination of electropherotypes of viral RNA by PAGE is an alternative approach for the differentiation of field strains of human rotaviruses.

The aim of the present study was to investigate the occurence and circulation of different human rotavirus electropherotypes during 1982–1992 in diarrhoeic and healthy subjects. This was the first study of the molecular epidemiology of human rotaviruses in Slovak and Czech Republics.

Abbreviations: ELISA = enzyme-linked immunosorbent assay; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate buffered saline; SDS = sodium dodecyl sulphate

Materials and Methods

Specimens. Out of 3,842 faecal samples obtained from diarrhoeic and healthy subjects 934 were positive in ELISA for rotavirus-specific antigen. Positive specimens were divided into 4 groups (I - IV) according to sample sources (see Table 1). Paired faecal samples were taken from 100 children hospitalized for gastroenteritis at the day of admission and during the course of hospitalization.

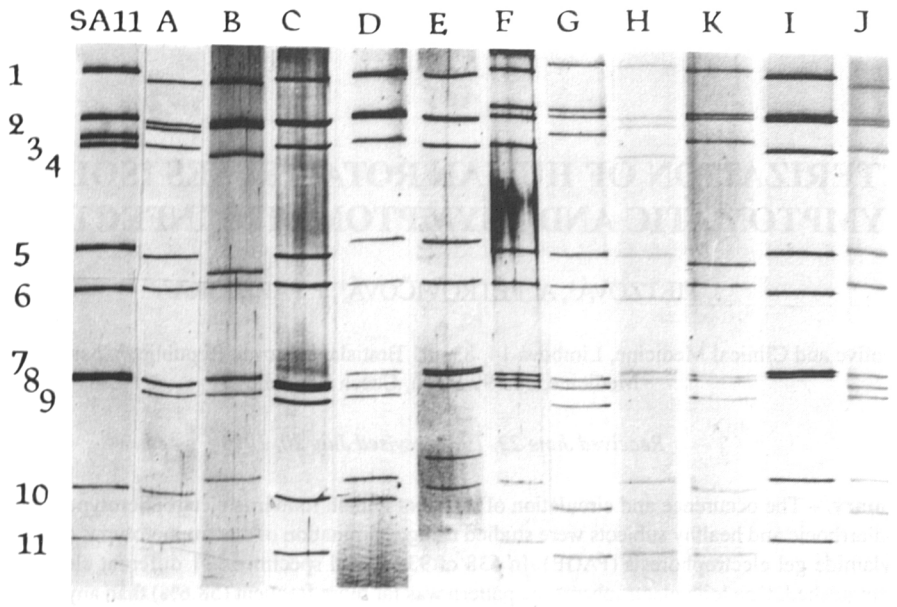

Fig. 1
Rotavirus RNA electropherotypes detected in 934 faecal samples in 1984-1992
Electropherotypes A-J demonstrated on human rotavirus isolates. SA11 – reference monkey rotavirus strain.

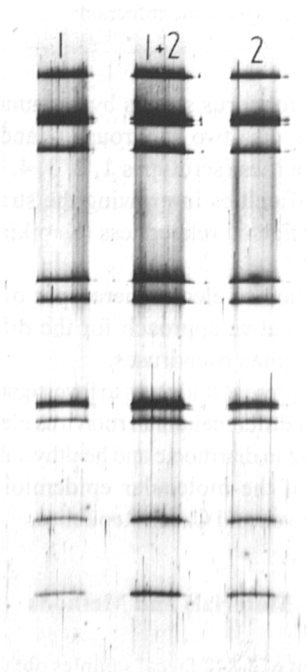

Fig. 2
Co-electrophoresis of RNA of rotavirus, profile B, isolated from neonates with asymptomatic rotavirus infections
Samples of two different rotavirus isolates of the same electropherotype (B) electrophoresed separately (lanes 1, 2) or mixed together (lane 1 + 2).

Rotavirus RNA isolation. 20% rotavirus-positive stool suspensions made in phosphate buffered saline pH 7.4 (PBS)) were extracted with Freon-113 (Serva). The virus was pelleted by ultracentrifugation at 100,000 x g for 2 hrs at 4 °C and resuspended in 200 µl STE buffer (10 mmol/l Tris, 1 mmol/l EDTA, 100 mol/l NaCl, pH 7.4). It was treated with sodium dodecyl sulphate (SDS) and proteinase K (Serva), and extracted with phenol-chloroform (3:2) and chloroform-isoamylalcohol (24:1). Finally, RNA was ethanol-precipitated and kept at -20 °C.

PAGE. The precipitated RNA was pelleted by centrifugation at 10,000 x g for 15 mins and dissolved in 30 µl of distilled water. Then 20 µl of a sample buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.005% bromophenol blue and 0.062% mol/l Tris) was added. PAGE was performed according to Laemmli (1970) (7.5% separating gel and 3% concentrating gel) at 40 mA for 8 hrs at 15 °C. RNA was stained with silver nitrate as described by Oakley *et al.* (1980).

Evaluation of electropherotypes was done using the scheme of Lourenco *et al.* (1981). Individual profiles were designated as follows: A (b-b-e-a), B (b-c-e-a), C (c-b-e-a), D (c-a-d-b), E (c-a-e-b), F (b-b-d-a), G (b-b-b-a), H (b-a-g-a), K (b-d-e-a), I (b-a-h-a), J (b-b-a-b).

ELISA of the rotavirus-specific antigen was done using the ELISA kit of Bioveta.

Results

In 638 of 934 (68.3%) ELISA-positive specimens, bands characteristic of the migration pattern of the rotavirus segmented genome were detected. Among them, 11 different electropherotypes were distinguished: A, B, C, F, G, H, K, I

Table 1. Rotavirus RNA electropherotypes in 4 groups of faecal samples

Group	Total No. of samples	No. of samples with characteristic electrophe-rotype	No. of samples corresponding to certain electrophoretype											No. of samples with incom-plete profile
			Long							Short			XY[a]	
			A	B	C	F	G	H	K	I	D	E	J	
I	126	77	41	–	26	–	1	–	–	–	9	–	–	–	5
II	734	506	322	–	56	5	85	3	8	6	4	9	3	5	39
III	29	18	11	–	3	–	3	1	–	–	–	–	–	–	3
IV	45	37	–	37	–	–	–	–	–	–	–	–	–	–	–
Total	934	638	374	37	85	5	89	4	8	6	13	9	3	5	47
(%)		68.3	58.6	5.8	13.3	0.8	14.0	0.6	1.3	0.9	2.0	1.4	0.5	0.8	5.0

Groups: I – children requiring medical checking; II – children hospitalized with gastroenteritis; III – healthy adult subjects (nursing staff); IV – asymptomatic newborns.
[a] Mixed infection.

Table 2. Incidence of rotavirus electropherotypes in 1984–1992

Group/year	No. of tested samples	Incidence of electopherotypes (%)											
		Long								Short			XY[a]
		A	B	C	F	G	H	K	I	D	E	J	
I + II/													
1984	3	66.7	–	–	–	–	–	–	–	33.3	–	–	–
1985	6	83.3	–	16.7	–	–	–	–	–	–	–	–	–
1986	5	80.0	–	–	–	–	–	–	–	20.0	–	–	–
1987	39	56.4	–	38.4	–	2.6	–	–	–	2.6	–	–	–
1988	93	60.2	–	15.1	5.4	19.3	–	–	–	–	–	–	–
1989	192	60.9	–	18.6	–	8.4	0.6	–	–	5.2	3.7	–	2.6
1990	136	62.5	–	3.6	–	25.0	1.5	5.9	–	–	1.5	–	–
1991	96	62.5	–	10.4	–	17.7	–	–	6.3	–	–	3.1	–
1992	13	92.3	–	7.7	–	–	–	–	–	–	–	–	–
III/													
1990	13	61.5	–	15.4	–	–	23.1	–	–	–	–	–	–
1991	5	60.0	–	20.0	–	20.0	–	–	–	–	–	–	–
IV/													
1984	22	–	100.0	–	–	–	–	–	–	–	–	–	–
1986	15	–	100.0	–	–	–	–	–	–	–	–	–	–

Groups I – IV are characterized in Table 1.
[a] Mixed infection.

(long pattern), and D, E, J (short pattern) (Tables 1 and 2). The electropherotype A (long pattern) was far more common (58.6) than any of the other 10 rotavirus electropherotypes detected (Fig. 1). The broadest spectrum of electropherotypes was detected in the group of children hospitalized with gastroenteritis (group II), where 10 different migration patterns were detected. In 5 cases (0.8%), more than 11 segments of rotaviral RNA were observed, indicating the occurence of infection with different rotavirus strains (mixed infection). On the other hand, in the group of asymptomatic newborns (group IV), only single electropherotype (B) was detected, which did not occur in any group of sick

children or adult contacts. The identity of this migration pattern was confirmed in co-electrophoresis (Fig. 2).

In 100 paired faecal samples taken from hospitalized children the bands characteristic for the migration pattern of the rotavirus segmented genome were detected in 63 pairs (63%). Identical electropherotypes were detected in 58 pairs (96.7%), in 2 pairs different patterns were detected in the second sample (as compared to the pattern detected in the first sample), and in 3 pairs PAGE showed mixed infection in the second sample.

Discussion

The variety of rotavirus RNA electropherotypes constitutes a powerful tool for epidemiological studies. The present study for the first time characterizes the rotaviruses circulating in Slovak and Czech Republics. The circulation of a single predominant RNA electropherotype with minor co-circulating variants demonstrated in present study is typical for rotavirus epidemiology (Estes *et al.*, 1984; Steele *et al.*, 1993). Throughout whole our study only single electropherotype (A - bbea) predominated in contrary to some of the published data indicating year-to-year variation (Begue *et al.*, 1992), summer-winter seasons' variation (Tazi-Lakhasi *et al.*, 1988), or shifts in the predominant electropherotypes in every epidemic (Pipittaja *et al.*, 1991) or in 2 – 3 year intervals (Rodger *et al.*, 1981).

Asymptomatic excretion of rotavirus was detected in 37 of 45 (82.2%) healthy newborns, this value being slightly higher than that in previously published studies of Steele *et al.* (1992). No rotavirus-positive stool samples from newborns taken immediately after the birth were detected and the proportion of rotavirus-shedding infants raised with the time of hospitalization, indicating nosocomially acquired infection. Unlike infection of older children, infection of healthy neonates in obstetric hospital nurseries was asymptomatic. This fact can be explained as a consequence of passive protection via maternal antibody in breast milk or serum, or as natural attenuation of rotavirus strains endemic in obstetric hospital nurseries (Palombo and Bishop, 1994).

Hospital-acquired (nosocomial) infection has been proved in 7.9% of the followed hospitalized gastroenteritis cases and all detected electropherotypes were identical to those prevailing among community-acquired rotaviral infections. According to these findings, in agreement with Steele *et al.* (1993), we suppose that a hospital *per se* is not the reservoir for rotaviral gastroenteritis, and that the source of nosocomially transmitted infection is represented by newly admitted patients and by hospital staff. Our results showed a lower incidence of nosocomial infections than that reported by Gaggero *et al.* (1992).

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