ELECTROPHEROTYPES OF ROTAVIRUS STRAINS CAUSING GASTROENTERITIS IN INFANTS AND YOUNG CHILDREN IN TIRANA, ALBANIA, FROM 1988 TO 1991

F. SUPERTI[1], E. DIAMANTI[2], S. GIOVANNANGELI[1], V. DOBI[2], L. XHELILI[3], G. DONELLI[1]*

[1]Department of Ultrastructures, Istituto Superiore di Sanitá, Viale Regina Elena, 299-00161 Rome, Italy;
[2]Department of Epidemiology, Research Institute of Hygiene, Epidemiology and Immunology Products, Tirana, Albania;
[3]University Paediatric Hospital, Tirana, Albania

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Summary. – During 1988–1991, an epidemiological survey was conducted in Tirana (Albania) on group A rotavirus strains which cause gastroenteritis in infants and young children. Rotaviruses were detected in 312 of 1,241 (25.1%) examined specimens from children with acute diarrhoea. Viruses were detected throughout the study period. Among the 72 rotavirus strains tested for double-stranded RNA (dsRNA) electrophoretic migration pattern, 9 different electropherotypes were recognized, 1 of those being more frequent than the others. At the beginning and at the end of the examined period (1988 and 1990–1991) two different long electropherotypes were predominant, whereas in 1989 (middle period) short electropherotypes were common indicating an involvement of virus strains with short electropherotypes in hospitalization-requiring diarrhoeas occuring in the area surveyed in that year.

Key words: group A rotaviruses; RNA electropherotypes, diarrhoea

Introduction

Rotaviruses are the single most common cause of acute gastroenteritis among infants and young children in the developing and developed regions of the world (Kapikian and Chanock, 1990). The understanding of the epidemiology of rotavirus diarrhoea would advance by describing the circulation of rotavirus strains in a variety of geographic locations over a time.

Examination of electrophoretic patterns (electropherotypes) of segmented viral dsRNA by polyacrylamide gel electrophoresis (PAGE) represents a useful tool in the study of epidemiology of rotavirus infection (Estes *et al.,* 1984). Variations in the electrophoretic mobility of one or more RNA segments allow different rotavirus strains to be assigned to distinct electropherotypes. Molecular epidemiologic studies based on the identification of RNA electropherotypes of rotavirus strains circulating in a community have been reported by many investigators (Chiba *et al.,* 1984; Donelli *et al.,* 1993; Follett *et al.,* 1984; Gomez *et al.,* 1986; Rodger *et al.,* 1981; Ruggeri *et al.,* 1989; Steele and Alexander, 1987; Ahmed *et al.,* 1991; Noel *et al.,* 1991; Tabassum *et al.,* 1994; Krishnan *et al.,* 1994). These studies have provided epidemiologic evidence demonstrating the coexistence of different strains during diarrhoeal outbreaks, the appearance of new strains and the disappearance of old ones from a community, and shifts in the prevalence of strains. Furthermore, these studies have suggested that rotavirus strains may evolve rapidly and show great genetic diversity at the end of an outbreak (Konno *et al.,* 1984). The significance of this genetic variation is not completely understood, but it is likely that certain nucleic acid sequence variations may lead to antigenic differences.

In this paper, we report the results of a diagnostic and epidemiological study on the occurence of rotavirus infections among 1,241 Albanian children with acute diarrhoeal disease. Stool samples, collected from January 1988 to March 1991, were mainly from children admitted to the University Paediatric Hospital of Tirana. A few stool samples were also provided by the Paediatric Hospital of Elbasan. Viral identification was performed by rotavirus enzyme-linked immunosorbent assay (ELISA) and the na-

*Corresponding author

Abbreviations: dsRNA = double-stranded RNA; ELISA = enzyme-linked immunosorbent assay; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate buffered saline

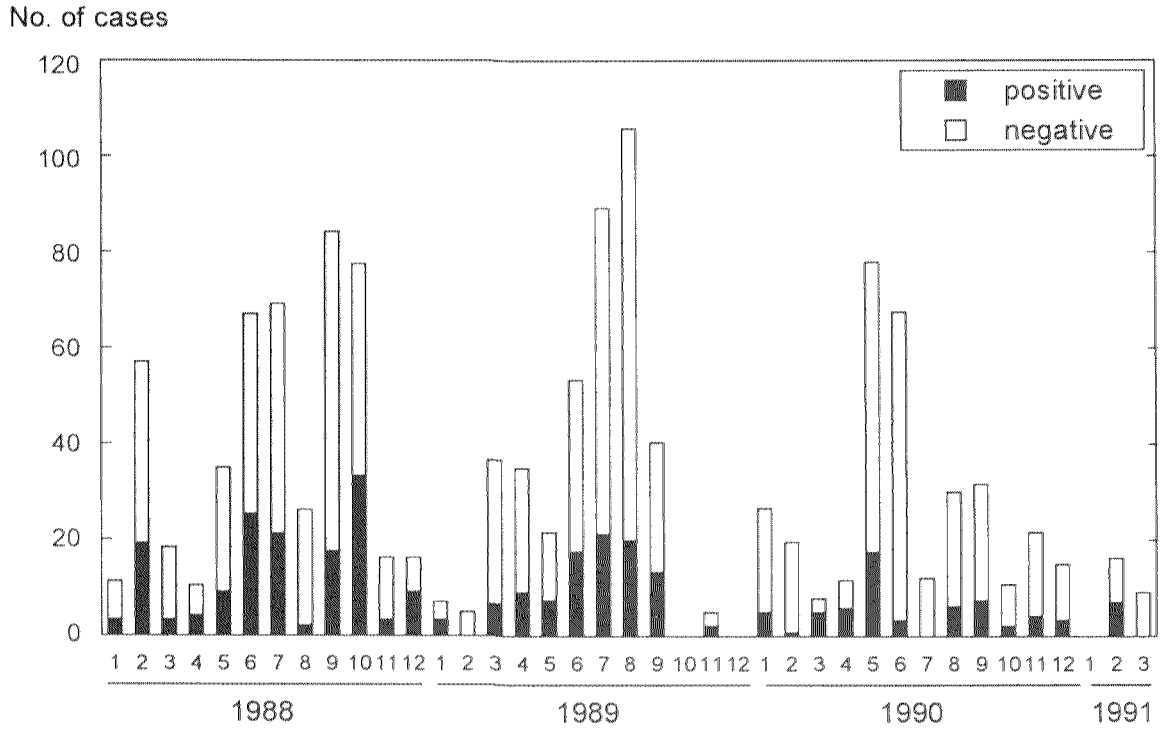

Fig. 1
Rotavirus infections assayed by ELISA in children with diarrhoea in Albania between January 1988 and March 1991

ture of different rotaviral strains was investigated by PAGE of viral genomic RNA.

Materials and Methods

Patients. Stool specimens were obtained from 1,241 patients: 1,209 specimens (97.5%) from 0–3 year-old children and 32 from children above 3 years of age. Specimens were collected from January 1988 to March 1991: 1,224 were from patients admitted with acute diarrhoea to the University Paediatric Hospital of Tirana, and 17 were from Paediatric Hospital of Elbasan. From each patient a single stool was collected during the first five days after admission. No single set of criteria was used for the screening except the fact that each child had diarrhoea when admitted to the hospital. Hence, the examined stool samples represent conveniently procured ones for surveillance purposes rather than proper random ones.

Stool specimens were frozen and stored at –80 °C until processing. Twenty percent suspensions of stools in phosphate buffered saline (PBS, pH 7.2) were clarified by centrifugation at 3,000 x g for 20 mins. The supernatants were collected and utilized for ELISA and rotaviral RNA electrophoresis (PAGE).

ELISA. Stool extracts, prepared as described above, were tested for rotavirus group A-specific antigen by a commercial ELISA [Enzygnost Rotavirus (Ag), Behring Institute, AG, Marburg, Germany] according to the manufacturer's instructions.

RNA extraction. Rotavirus-positive (ELISA) stool samples were diluted two-fold with RNA extraction buffer (0.01 mol/l Tris, 0.1 mol/l NaCl, 0.001 mol/l ethylenediamine tetraacetate, 1% sodium dodecyl sulphate, pH 7.5) and subsequently deproteinized with a mixture of phenol, chloroform and isoamyl alcohol. RNA was precipitated from the aqueous phase by adding 2 volumes of cold ethanol in the presence of 0.3 mol/l sodium acetate overnight at –20 °C. After centrifugation at 8,000 x g for 30 mins, the pelleted RNA was dissolved in sample buffer (Laemmli, 1970) containing 0.003% bromophenol blue and 2.5% Ficoll 400.

PAGE was carried out by the method of Laemmli (1970) in slab gels of 10% acrylamide and 0.35% bis-acrylamide, with a 3.5% acrylamide stacking gel, for 18 hrs at a constant voltage of 140 V. Gels were silver-stained by the method of Herring *et al.* (1982).

Results and Discussion

Rotaviruses were detected in the faeces of 312 of 1,214 patients (25.1%) by ELISA. The number of patients and the results of ELISA by month of admission to the hospital are shown in Fig. 1: infection rates ranged from 17.7% in 1990 to 30.4% in 1988, being 24.7% in 1989. In a few samples screened in 1991, the percentage of rotavirus infection reached 28.0%. The typical epidemiological pattern of in-

Table 1. Distribution of rotavirus infections by patient age in Albanian children with acute diarrhoeal disease (January 1988 – March 1991)

Year	Age (months)				
Number of	0-5	6-11	12-23	24-35	≥36
1988					
Tested	135	216	115	11	9
Positive (%)	(29.6)	(33.3)	(27.8)	(18.2)	(22.2)
1989					
Tested	94	172	102	16	13
Positive (%)	(22.3)	(28.5)	(24.5)	(12.5)	(7.7)
1990					
Tested	82	125	91	25	10
Positive (%)	(15.8)	(20.0)	(18.7)	(8.0)	(20.0)
1991					
Tested	6	14	3	2	0
Positive (%)	(33.3)	(35.7)	(0)	(0)	(0)
Total					
Tested	317	527	311	54	32
Positive (%)	(24.0)	(28.6)	(23.8)	(11.1)	(15.6)

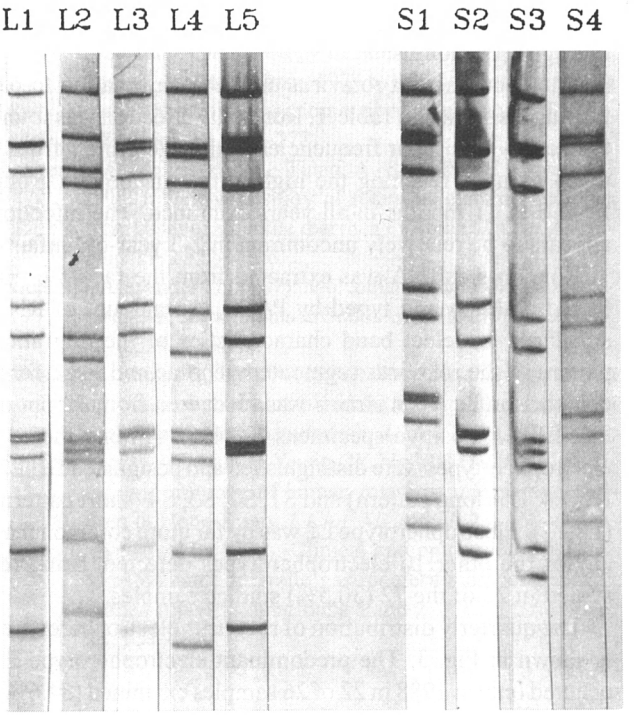

Fig. 2
RNA electropherotypes of rotaviruses identified in children with diarrhoea in Albania between January 1988 and March 1991

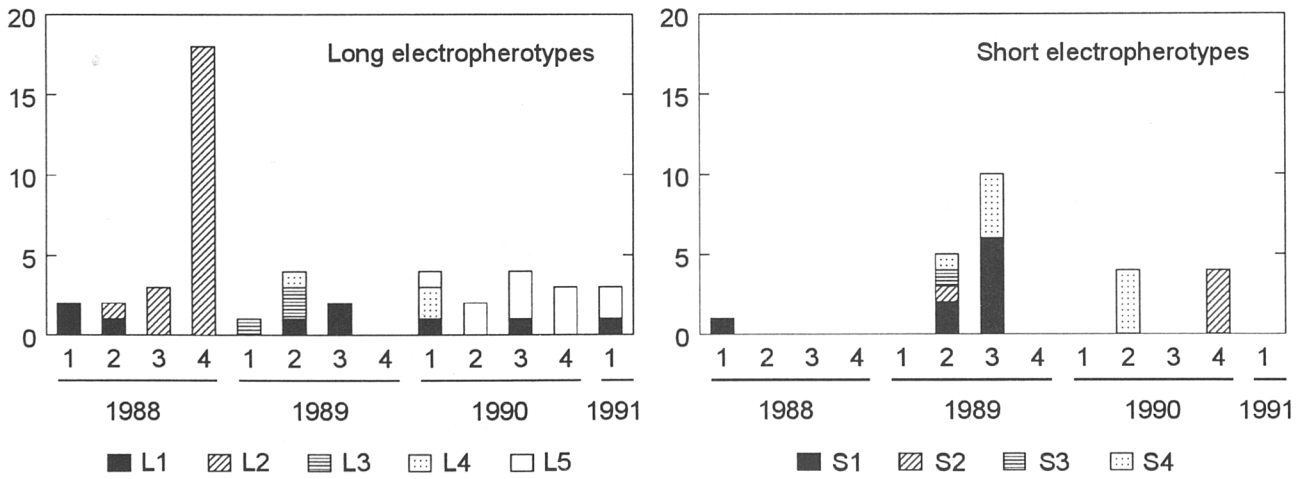

Fig. 3
Quartely distribution of rotavirus RNA electropherotypes in Albania between 1988 and 1991

fection described for countries with temperate climate (Brandt *et al.*, 1983; Konno *et al.*, 1978) was not observed by us; our surveillance was characterized by the presence of cases almost in all months, suggesting an endemic persistence of the virus in the population. These results, comparable to those obtained in two similar studies carried out on children with acute diarrhoea admitted to the main paediatric hospital of Rome, Italy, in the years 1983, 1984, and 1987–1989 (Donelli *et al.*, 1988, 1993), underline the complexity of rotavirus epidemiology. The high number of rotavirus-positive samples observed in hot months is most likely to be related also to the more frequent hospitalization

of persons for bacterial and/or parasitic diarrhoeal disease during these months.

The occurence of rotavirus diarrhoea in relation to patient age is shown in Table 1. Rotavirus infection was found to occur with similar frequencies in the age groups from 0 to 23 months, reaching the highest rate in the age group from 6 to 11 months in all years examined. The infection appears to be relatively uncommon in 2-3 year-old infants.

Rotavirus dsRNA was extracted from the faeces of infected children and typed by PAGE. An amount of RNA sufficient to detect band characteristics of the migration pattern of the rotavirus segmented genome and to perform comparison between strains was recovered from 72 out of 312 ELISA-positive specimens (23.1%). Among these, 9 electropherotypes were distinguished and designated L1, L2, L3, L4, L5 (long pattern) and S1, S2, S3, S4 (short pattern) (Fig. 2). Electropherotype L2 was by far more common than any of the other 10 electropherotypes detected, being revealed in 22 of the 72 (30.5%) studied samples.

The quarterly distribution of rotavirus electropherotypes is shown in Fig. 3. The predominant electropherotype L2 occured only in 1988 in 22 of 26 samples examined (84.6%). In the same year, only one sample exibiting the short pattern of migration (S1) was found.

Seven different electropherotypes (L1, L3, L4, S1, S2, S3, S4) were observed in 1989. During this year, short electropherotypes were predominant, being revealed in 15 of 22 (68.2%) samples tested. S1 and S4 were the more common electropherotypes detected, altogether accounting for 13 of the 22 (59.1%) specimens studied.

In 1990, five electropherotypes (L1, L4, L5, S2, S4) were revealed, one of which was newly introduced. The new long electropherotype (L5) was detected in 7 of 21 (33.3%) samples examined. This electropherotype was also observed in the first quarter of 1991.

The results of the genomic characterization of viral strains allow some considerations to be made. During the period of the study, we detected nine different rotavirus electropherotypes, among which electropherotypes L2 and L5 were by far more frequent than the others. Interestingly, the two predominant viral electropherotypes detected in this study were present only during a limited period of the surveillance, and once a particular electropherotype had disappeared from the population, it never recurred suggesting the occurence of a continuous antigenic drift. In fact, electropherotype L2 was recovered only in 1988, and electropherotype L5 appeared in 1990 and lasted all through the examined period. The presence of a dominant rotavirus type in a given period in a geografical area has been previously reported by several authors (Follett *et al.*, 1984; Dimitrov *et al.*, 1984; Tam *et al.*, 1986; Steele and Alexander, 1987; Ruggeri *et al.*, 1989; Donelli *et al.*, 1993) and proposed to reflect a specific immune status of the population. In this respect we observed in 1989 a predominance of short electropherotypes, altogether accounting for 68.2% of samples tested. These data are in agreement with previous observations of other authors (Uhnoo and Svensson, 1986; Noel *et al.*, 1991; Tabassum *et al.*, 1994; Krishnan *et al.*, 1994), which described the succession of long and short electropherotypes, suggesting that this may reflect a change in the immunity of the population at risk.

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