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Rotavirus serotypes and electropherotypes in Finland from 1986 to 1990

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Summary. Four epidemic seasons of rotaviruses were studied in Helsinki during 1986–1990. This is the first Scandinavian study, where both electropherotypes and serotypes are determined. Out of 5316 fecal specimens 769 (14.5%) rotavirus positive samples were detected by electron microscopy. Of these, 645 isolates (83.9%) gave a clear RNA pattern in gel electrophoresis and they clustered into 87 electropherotypes. An illustrative number of isolates representing each electropherotype (= E-type) was serotyped using VP7 protein-specific monoclonal antibodies for serotypes G1–G4 and without exceptions, within one E-type only a single serotype specificity was found. After establishment of the serotype of each E-type, the distribution of serotypes was scored as 61.2%, 2.0%, 0.5% and 29.8% for G1–G4, respectively; 6.5% remained untypable. Two seasons had one predominant E-type (Season 1, 1986–87, and Season 3, 1988–89, 84.2% and 80.6% of rotavirus positive samples, respectively). Both were followed by a season with no predominant E-type, but several minor E-types. Altogether, 5 short E-types (13/645 samples) with serotype G2 specificity were found, most of them occurring in Season 2. Only 2 E-types (3 samples) belonged to serotype G3. Group C rotavirus was found in 8 specimens. In this study a shift in serotypes, from G1 to G4, was observed in Finland in 1988/89; a similar shift was reported in many European countries at that time.

Introduction

Rotaviruses, genus of the family *Reoviridae*, cause diarrhoea in children in every part of the world [27]. In humans three antigenically distinct groups of rotaviruses have been detected, group A, B and C [39], of which the most common is group A. Group C rotaviruses are found all over the world but rarely, and group B rotavirus infections have only found to occur in China [23]. The genome of rotaviruses consists of 11 double-stranded RNA segments and in polyacrylamide gel electrophoresis (PAGE) the genomic pattern (electropherotype) of each group is easily recognized.

The genome profile of group A rotavirus consists of four size groups of segments (segments 1–4, 5–6, 7–9 and 10–11) [25]. Within these four groups minor segment variation occurs commonly (e.g. [42]). The most prominent variation is between long and short (and super short) RNA patterns: the segment coding NSP5 protein of the short electropherotype (segment 11 of the long electropherotype) is partially duplicated making this segment move slower [37]. In most cases, the reason for minor variations in migration of segments with the same number of nucleotides is not known. Recently, it has been reported that even a single nucleotide substitution may cause a detectable change in the electropherotype [9]. These mutations may cause differences in the secondary structure of the segment and alter its migration in PAGE [27].

In addition to the genetic differences also much antigenic variation occurs in group A rotaviruses. Subgroups I and II are based on antigenic properties of VP6 protein [21]. The major neutralization antigen, VP7 protein, determines the G serotype. The most common serotypes in humans are G1, G2, G3 and G4 [55, 56], but also serotypes G6 [15], G8 [30], G9 [35], G10 [2] and G12 [45] have been found. To date, at least 14 G serotypes have been detected in humans and animals [20]. Some serotypes can be divided into subtypes, (G1a, b and c and G4A and B [8, 14]). Serotyping also can be based on VP4 specificity (P serotypes) [17] and at least 17 P serotypes have been found so far [46].

Experiments have shown that reassortment of gene segments does not occur randomly [19]. Already long ago it was noticed that isolates representing serotype G2 specificity usually have a short RNA pattern, while a long genome profile is common among isolates with serotype G1, G3 or G4 specificity [24]. Also, rotaviruses infecting humans can be divided into three groups by RNA-RNA hybridization [10, 34] indicating that some segment combinations are preferred. Exceptions to the above mentioned segment combinations are found [32, 44], but rarely.

Determination of RNA profiles and/or antigenic characterization of rotavirus isolates for successive epidemic seasons has been used to clarify the local and global epidemiology of rotavirus strains, e.g. [1, 7, 38, 41]. Limited numbers of Finnish samples have been serotyped before [13, 51, 52], but no electropherotyping has been reported. In earlier rotavirus studies samples with serotype G8 specificity [16] and group C rotaviruses [31, 53] have been found in Finland. In this report, we present a comprehensive study of epidemiology of rotavirus electropherotypes and serotypes G1–G4 during four epidemic seasons, 1986–1990, in the capital city area of Finland.

Materials and methods

Clinical samples

5316 stool samples from patients with acute gastroenteritis (mostly from children; 67.1% and 90.0% of patients were $\leqslant 1.5$ and $\leqslant 4.0$ years old, respectively) were sent to the Department of Virology, University of Helsinki, for the study of enteric viruses by electron microscopy (EM) from September 1986 to July 1990. Rotaviruses were seen in 769 samples (14.7%). Most

samples were sent from three metropolitan hospitals: 44.4% from the municipal Aurora Hospital of Helsinki, 37.6% from Children's Hospital, University of Helsinki and 7.7% from Jorvi Hospital situated about 20 km from Helsinki. Other hospitals and health care centers accounted for the remaining 10.3% of samples including single samples sent from more distant parts of Finland.

Electron microscopy

10% suspensions from fecal samples were prepared in 0.05 M Tris-0.1 M NaCl, pH 7.4 with 1 mM $CaCl_2$. Rotaviruses were identified on grids negatively stained with 2% potassium phosphotungstate (pH 5.5–6.0) by electron microscopy (EM) (Jeol, JEM-100CX II, Tokyo, Japan). Rotavirus positive suspensions were stored at −20 °C for further studies.

RNA extraction and gel electrophoresis

Subsequently, a 600 µl sample of 10% stool suspension was subjected to a generally used procedure of nucleic acid extraction: treatment twice with equal volumes of phenol and a mixture of chloroform-isoamylalcohol (= CHIS, 24:1) and once with CHIS alone, followed by ethanolacetate precipitation. Samples were suspended in 50 µl of Laemmli sample buffer without sodium dodecyl sulfate (SDS), and nucleic acids resolved by size in a 7.5% polyacrylamide gel [29] without SDS. All samples were first run in 1.5 mm-thick gels (length 12 cm; 90 V, 17 h, room temperature) and then further coelectrophoresis studies were carried out with 0.75 mm small gels (Mighty Small, Hoefer Scientific Instruments, San Franscisco, CA) with short (7 cm) or longer (9 cm) gels (250 V, 1.5 h and 3 h, respectively, cooling with tap water). The electropherotype of the sample was slightly different in small and big gels: our classification was based on big gels. By using both electrophoresis methods a high resolution was achieved.

Silver staining

Silver staining was performed as modified from Herring et al. [22]. It included a 15–30 min fixation in 10% EtOH, 0.1% acetic acid followed by a 20–30 min staining in a 0.18% $AgNO_3$. After a brief rinse (30 s and 7 min for 0.75 mm and 1.5 mm gels, respectively) in distilled water the gel was developed in 3% NaOH with 8 ml/l formaldehyde until bands were well resolved. Postfixation with 5% acetic acid was made. Simian rotavirus (SA11) RNA extracted from infected MA-104 cells was used as a marker.

Serotyping by ELISA

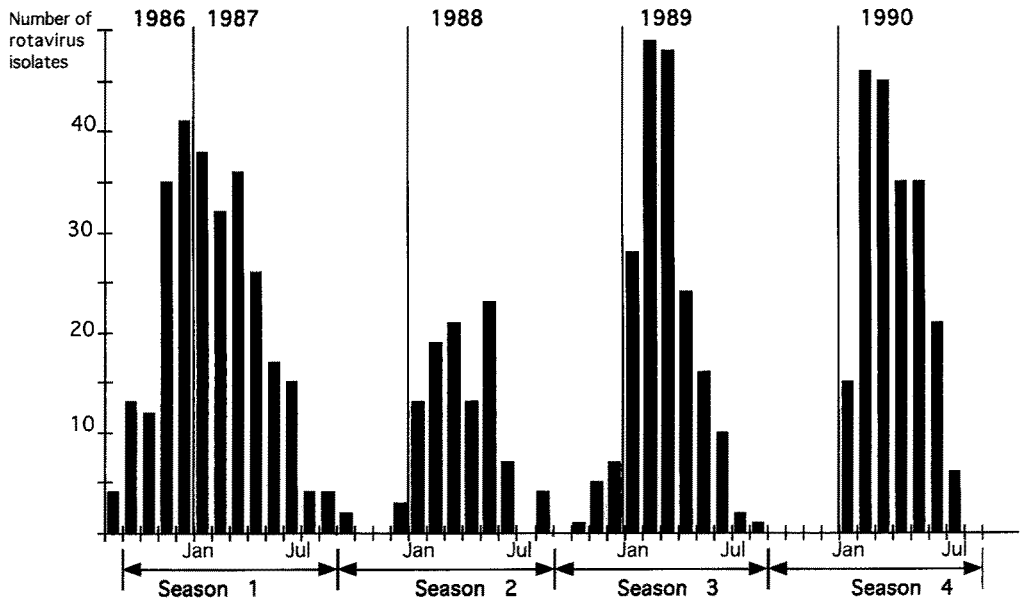
Commercial Serotec ROTA-MA momoclonal antibodies (Mabs) for human rotavirus serotypes G1-G4 (Maps were prepared against KU, S2, YO and ST3, respectively) were used (Serotec Co, Sapporo, Japan [50]). The EIA procedure with the Mabs was followed with some modifications: Microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with monoclonal antibodies diluted in PBS (100 µl/well) with incubation overnight (o/n) at 4 °C. The wells were washed three times with PBS and the washing was repeated between all following steps before addition of substrate. Wells were blocked with PBS, 0.05% Tween 20 (= PBST), 1% BSA (250 µl) o/n at 4 °C. 50 µl of 10% stool suspension diluted in 1:4 in PBST, 10% skimmed milk was added into each well and incubated o/n at 4 °C. The same amount of anti-SA11 hyperimmune rabbit serum (1:1000 in PBST, 2.5% skimmed milk) was added into wells with incubation for 1 h at 37 °C. Peroxidase-conjugated anti-rabbit immunoglobulin (Dakopatts, Copenhagen, Denmark, 50 µl/well) diluted in 1:2000 with PBST was incubated

for 30 min at 37 °C. As a substrate, 1,2-phenylenediamine, dihydrochloride (Dakopatts) in 0.1 M citrate-phosphate buffer, pH 5.0, with H_2O_2 was used, the reaction was stopped with 20% H_2SO_4 and the absorbance values were measured at the wavelength of 492 nm with Multiskan (Labsystems, Helsinki, Finland). An isolate was assigned to a specific serotype if the absorbance value was > 0.2 and if it was higher than twice the value of any other serotype. As controls, cell cultured rotaviruses of each serotype (human rotavirus strains Wa, DS-1, simian rotavirus RRV and porcine Gottfried for G1 to G4, respectively, kindly supplied by L. Svensson, Swedish Institute for Infectious Disease Control, Stockholm, Sweden) were included.

Results

The determination of serotypes and electropherotypes

The temporal distribution of 769 rotavirus positive isolates detected by EM during four successive seasons 1986–90 is shown in Fig. 1. Each season consists of 12 months beginning in September and ending in August of the following year. The actual epidemics lasted from 7 months (Season 4) to 12 months (Season 1) with peaks in winter or spring. Epidemics were separated by a period of 1 to 4 months, in the summer-fall, with no positive isolates except before the beginning of Season 1, when four isolates/month appeared. The greatest number of rotavirus positive samples were detected in Season 1 (273 samples) and it was followed by Season 2 with only 103 positive samples. The last two seasons consisted of about 200 samples each.



	Season 1	Season 2	Season 3	Season 4	Total
Rotavirus positive/season	273	103	191	202	769
All samples	1737	1082	1283	1214	5316

Fig. 1. Monthly distribution of rotavirus positive isolates sent to Department of Virology, University of Helsinki, Finland, from August 1986 to October 1990

Table 1. Seasonal frequency of serotypes and electropherotypes of human group A rotaviruses in Finland during 1986–1990

Season	Serotype*	No. of E-types	(no. of isolates/group)
Season 1 1986/87	G1	11	(<u>203</u>, 14, 7, 2, 2, 1, 1, 1, 1, 1, 1)
	G4	1	(2)
	NT	4	(2[a], 1, 1, 1)
Season 2 1987/88	G1	10	(14, 13, 8, 8, 4[b], 4, 4, 1, 1, 1)
	G2	3	(5, 4, 1)
	G3	1	(1)
	NT	7	(2[a], 2, 2, 2, 1, 1, 1)
Season 3 1988/89	G1	6	(11, 4, 2, 2, 1, 1)
	G2	1	(1)
	G3	1	(2)
	G4	4	(<u>133</u>[c], 1, 1, 1)
	NT	4	(2[d], 1, 1, 1)
Season 4	G1	13	(14, 14, 12[b], 12, 9[d], 8, 6, 2, 1, 1, 1, 1, 1)
	G2	1	(2)
	G4	7	(<u>26</u>[c], 12, 6, 3, 3, 2, 2)
	NT	13	(6, 3, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1)

The predominant electropherotypes are underlined. The pairs of groups with indistinguishable E-types in different seasons are marked with *a–d*

NT Not typable

*The serotype of the E-type was established after at least a half of the samples of the E-type was determined unexceptionally to be of the same serotype

In gel electrophoresis 645 (83.9%) out of 769 isolates showed an RNA pattern of rotavirus A clear enough to allow classification into electropherotypes. Eight samples with group C rotavirus specificity including one family outbreak were scattered throughout the period. The rest, 92/769 isolates were too weak or blurred to determine in PAGE, 12 isolates (1.6%) had more than 11 segments, indicating double infections, and from 12 isolates no material was left after EM detection. During the whole period 87 different electropherotypes of group A rotaviruses could be defined (Table 1). From 16 to 34 E-types were detected during one season. The scheme for determination of the E-types had three steps: Firstly, isolates of one season were compared to each other and they were

Table 2. Distribution of human group A rotavirus serotypes and group C rotaviruses in Finland during four epidemic seasons, 1986–1990

Serotype	1986/87 (n = 241)	1987/88 (n = 80)	1988/89 (n = 165)	1989/90 (n = 159)	Total (n = 645)
G1	234(**97.1**)[a]	58(**72.5**)	21(12.7)	82(**51.6**)	395(61.2)
G2	0(0)	10(12.5)	1(0.6)	2(1.2)	13(2.0)
G3	0(0)	1(1.2)	2(1.2)	0(0)	3(0.5)
G4	2(0.8)	0(0)	136(**82.4**)	54(34.0)	192(29.8)
Untypable	5(2.1)	11(13.8)	5(3.0)	21(13.2)	42(6.5)
Group C	2	1	0	5	8

[a] Number (%) of all samples of the E-types. The serotype of the E-type was established after at least a half of the samples of the E-type was determined unexceptionally to be of the same serotype

clustered into E-types as presented in Table 1. In the second step, the isolates of successive seasons were compared to each other. Altogether, three E-types were represented in consecutive seasons (labelled a–c in Table 1). One of them was the predominant electropherotype of Season 3 (serotype G4) that had identical appearance with an E-type of Season 4 consisting of 26 isolates (serotype G4). The last step was the cross comparison of seasons (1 and 3, 1 and 4 and 2 and 4) with only E-types consisting of more than one isolate (due to the lack of material). Only two E-types looked similar in coelectrophoresis (labelled with d in Table 1): one E-type in Season 2 (two samples, serotype G1) and one in Season 4 (12 samples, serotype G1), all other E-types were unique. Most of the E-types (40/87) represented serotype G1. Beside the two predominant groups, which consisted of 100–200 samples, all other groups had less than 30 samples.

At least half of the isolates of each E-type was studied using serotype EIA and the serotype of 211/413 isolates (51.1%) was identified. Within one E-type, without exception, only a single serotype specificity was found. Thus all samples in an E-type were scored as representing the same serotype (Table 2). Serotype G1 accounted for 61.2% of all samples being the most common serotype in three of the four seasons. The second largest serotype was G4 (29.8%) predominating in one season. Serotypes G2 and G3 were found rarely (2.0% and 0.5%, respectively).

Temporal distribution of electropherotypes and serotypes

Figure 2 presents the temporal distribution of eletropherotypes and serotypes during the 4-year period. In Season 1 (1986/87) one single predominant electropherotype could be found through the whole season from September 1986 to August 1987, but not in the following seasons. It represented serotype G1 specificity and accounted for 203 (84.2%) out of 241 rotavirus positive samples. Also, 10 minor E-types showed serotype G1 specificity (colored in black in

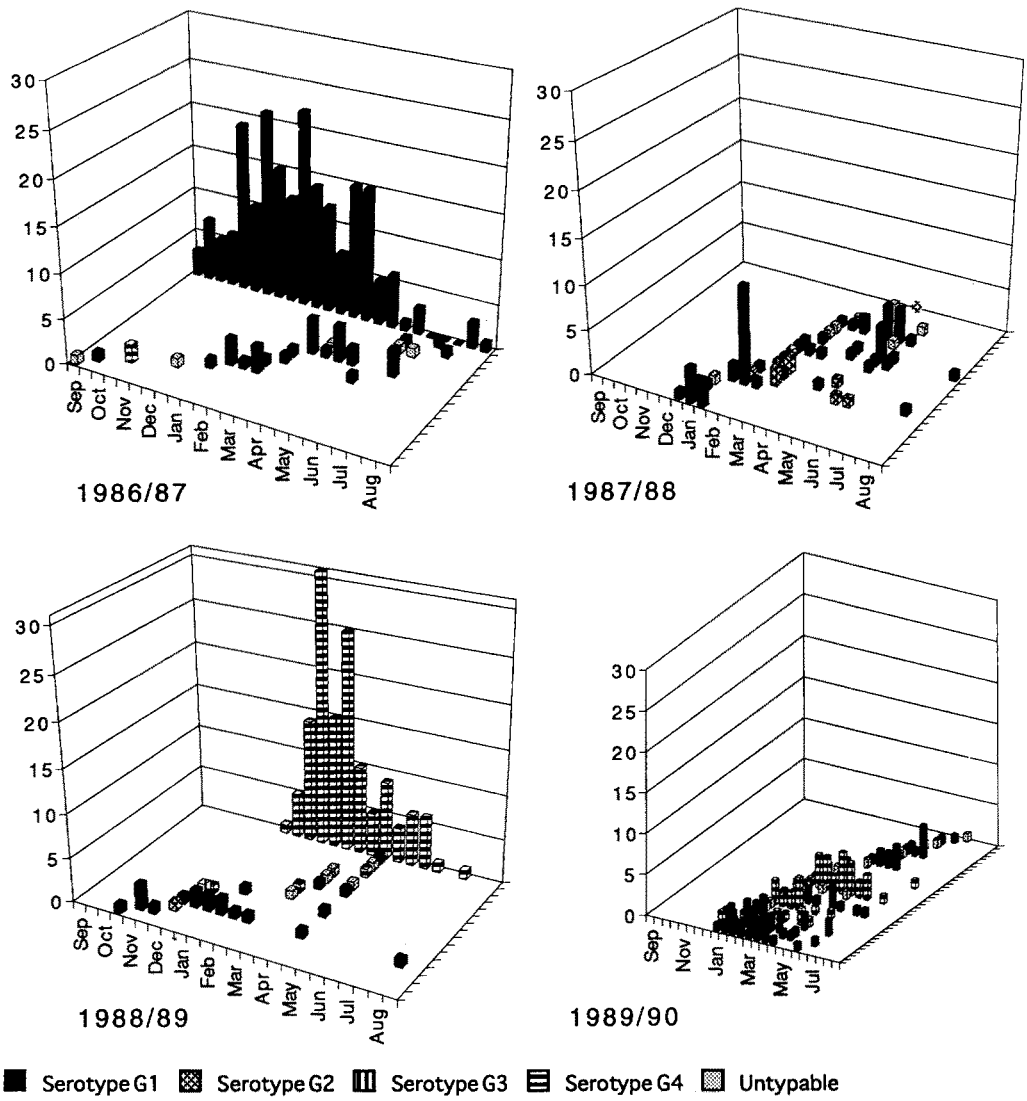


Fig. 2. Three-dimensional graphic presentation of the temporal distribution of electropherotypes during 1986–1990. The E-types are represented in the X-axis in the order of appearance, except the predominant E-types in Season 1 and 3 that are placed to the background. The Z-axis determines the number of specimens per half a month

Fig. 2). The rotavirus epidemic began extraordinarily early this season. As seen in Fig. 2, during the first four months the predominant electropherotype occurred almost exclusively and only then more E-type appeared. No representatives of serotypes G2 and G3 were detected and only one electropherotype showed G4 specificity. Four small groups (total 5 samples) remained untypable.

The Season 2 (1987/88) contrasted to the Season 1 in that no predominant electropherotype appeared. The epidemic began in December and during a short time span most of the 21 E-types of this season appeared. About half of them (10

minor E-types) belonged to serotype G1, which was the most common serotype in this season (72.5%). Three E-types (10 isolates) with short RNA pattern and serotype G2 specificity were found sporadically. One sample with serotype G3 was detected, but none of serotype G4.

In Season 3 (1988/89) again a predominant electropherotype, but now of serotype G4 specificity, appeared accounting for 133 (80.6%) out of 165 isolates. The epidemic began in October with a few minor E-types, mostly of serotype G1. The predominant E-type was detected from the end of December 1988 to July 1989 with the main peak occurring in February 1989. Interestingly, 7 months later at the end of February 1990, in Season 4, a similar electropherotype was detected again for five months (26 samples). In addition to the predominant E-type, three minor groups showed serotype G4 specificity in Season 3. Six E-types belonged to serotype G1 accounting only for 12.7% of all samples. One sample with short RNA pattern and G2 specificity and one E-type with G3 specificity were found during this season.

In Season 4 (1989/90) the rotavirus epidemic lasted only 7 months beginning in January. None of the 34 electropherotypes identified was predominating. Both serotype G1 and G4 occurred commonly (13 and 7 E-types, 51.6% and 34.0% of samples, respectively). As mentioned above, one E-type with 26 samples (of serotype G4) represented the predominant E-type of Season 3. Two samples showed a short RNA pattern and as many as 13 minor E- types (13.2%) remained untypable.

RNA patterns in gel electrophoresis

The characteristics in RNA pattern, especially the migration of the triplet segments including VP7 coding gene, that would indicate the possible serotype, were looked for. Electropherotypes of selected samples representing different serotypes are shown in Fig. 3. Electropherotypes of serotype G2 (Fig. 3C) were easily identified by their short RNA patterns. Electropherotypes of serotype G3 (Fig. 3D), including SA-11, were also recognizable, because their segments 7–9 were closer to each other than in RNA patterns of samples representing other serotypes. The comparison of the electropherotypes of serotype G1 with those of G4 revealed no characteristics which would allow a definite serotype identification (Figs. 3A and B).

Discussion

This report presents the distribution of electropherotypes and serotypes of rotavirus isolates in hospitalized children during four epidemic seasons, 1986–1990, in greater Helsinki area in Finland. This is so far the most extensive study of rotavirus epidemiology reported in Scandinavian countries. Since essentially every hospitalized child with diarrhoea was studied, the findings represented well the epidemiological status in this area. Also, most electropherotypes were found in samples from different hospitals, so E-types did not consist of samples from nosocomial infections (data not shown).

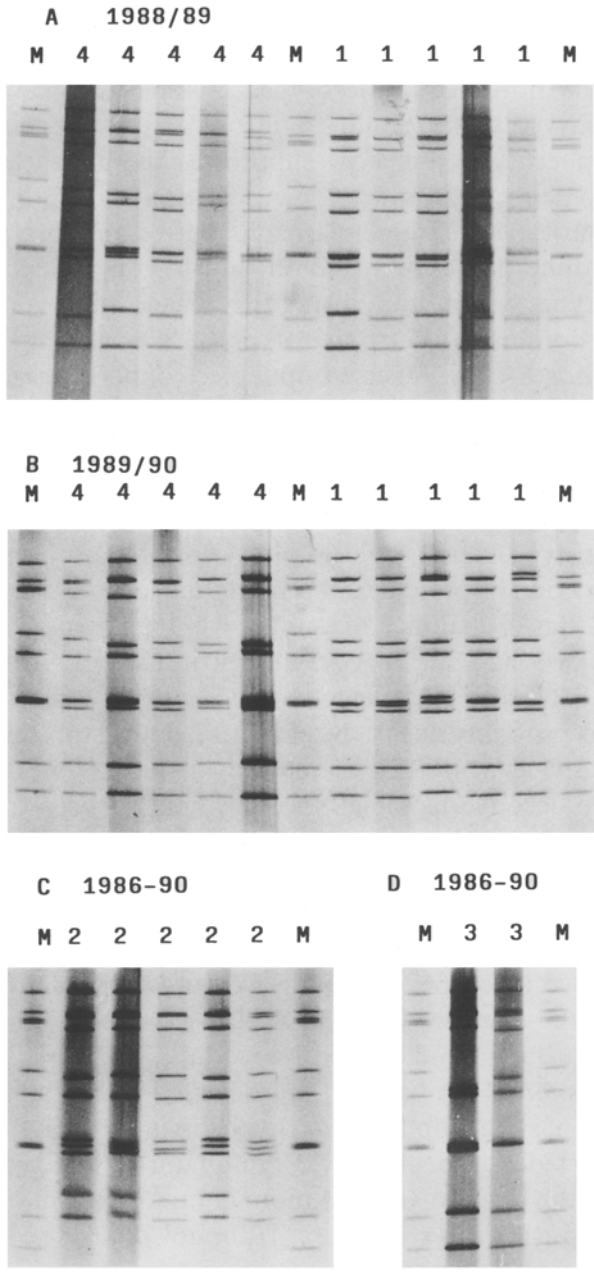


Fig. 3. RNA patterns of selected isolates in PAGE. **A** Electropherotypes representing serotypes G4 and G1 in Season 3 (except the single E-type of G4 from Season 1 in *2*, beside *M*). The predominant strain of Season 3 is in *3*. **B** E-types of G4 and G1 in Season 4. **C** All E-types representing serotype G2. **D** All E-types of serotype G3. SA-11 is used as marker (*M*)

As many as 87 different electropherotypes were detected during the study period. During two seasons one distinct electropherotype predominated. Interestingly, after each of these seasons the following epidemic could arise without any electropherotype predominating over the others. There occurred 16–19

electropherotypes during the seasons with one major E- type, approximately the same number that is commonly reported to appear during one epidemic season (e.g. 8). Instead, even more electropherotypes, 21 and 34, appeared in years when no predominant E-type was around. So, it looks like the occurrence of a predominant electropherotype somehow suppresses the appearance of other E-types during an epidemic season.

The electropherotypes of different seasons were carefully compared to each other. Most electropherotypes occurred only during one epidemic season. Only four electropherotypes were found during two seasons. The most interesting of them was the predominant electropherotype of Season 3 that reappeared in Season 4 as a minor E-type. Overseasonal electropherotypes are reported to occur rather commonly in long- scale surveys [7, 33, 36, 40].

The stool samples were serotyped by EIA using monoclonal antibodies for G1–G4. When a sufficient number of isolates was serotyped, it became clear that each E-type consisted of isolates with similar serotype specificity. So it was unnecessary to serotype every stool sample. Also other authors [7, 8, 40] have come to the same conclusion, when the place and time scale of the study are limited. However, the probability of similar RNA patterns with serotype differences increases in long-scale surveys and/or in samples from different geographical areas [3, 11, 49]. The detection level of our serotype EIA was low (51.1%), which was probably due to the condition of the stool suspensions. They were stored for many years at $-20\,^{\circ}C$ and thawn several times, so the rotavirus particles may have become damaged. In every epidemic season some E-types, most of which had only 1 or 2 samples, remained untypable. We cannot conclude whether this was due to the low sensitivity of our test or whether these possibly represented other serotypes. In addition to the four most common serotypes, serotype G8 has been detected in Finland earlier [13].

In our study, two serotypes, G1 and G4, were common (61.2 and 29.8%, respectively). Also, these two serotypes have been reported to occur most often in surveys in Australia, Japan and United Kingdom [7, 33, 36]. The serotype G2 (showing always a short RNA pattern) was detected rarely, mainly in Season 2. The disappearance of subgroup I and serotype G2 in Sweden [43, 47] and Italy [11] has been reported after being very common during 1977–83. We also have preliminary data showing that G2 was more common in Finland in the beginning of 1980's than during this study period (unpubl. data). The serotype G3 was detected even less often than group C rotaviruses.

Konno et al. [28] described in 1984 an epidemic that started with a single predominant electropherotype and later various RNA patterns appeared during the same epidemic season. Also, in our study, the predominant electropherotype in Season 1 appeared nearly alone and only four months later other electropherotypes of the same serotype were found. The appearance of the major electropherotype in Season 3 was somewhat different: the E-type showing G4 serotype specificity was accompanied by very few other representatives of G4 in that period, but it reappeared in Season 4 and then also other E-types of serotype G4 were found. One explanation for these observations would be that the strain

predominating initially varies as the season progresses or in the next season. To test this hypothesis we will employ nucleotide sequencing of the different electropherotypes. The genetic variation of rotaviruses is caused either by point mutations due to errors made by RNA polymerase or by reassortment of segments in mixed infections [26]. Nucleotide sequencing studied will reveal which of these mechanisms is more important in epidemics. Also, the variation at the protein and antigenic level can be then deduced.

In all studies so far G1 appears to be the most common serotype among humans [54]. This serotype predominated also in Finland during 1986–88 [our data, 4, 52], but then a shift from serotype G1 to G4 happened in 1988–90. This change in serotypes is very interesting, because it seems to have occured in many counteries in Europe at the same time. Noel et al. [36] report a shift from serotype G1 to G4 in London and Birmingham in 1989/1990. In Freiburg, Germany, a nosocomial outbreak of serotype G4B appeared among neonates in 1988 [12]. In other parts of the world, as in the USA, where serotype G3 was common [5, 18], or in Asian countries, where very rapid changes in serotypes were common [6, 48], the shift from serotype G1 to G4 was not seen. In Australia serotype G4 is reported to have a high peak approximately every 3 years [7].

Even though worldwide homological/analogical serotype variation is apparently not occurring [54], similar epidemiological patterns in many European countries can be detected: 1) the rather constant presence of serotype G1, 2) the diminishing of the prevalence of G2 during the past ten years and 3) the shift from G1 to G4 at the same time period. This suggests that larger geographical areas than one country may have the same kind of epidemiology of rotavirus serotypes.

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