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Group A rotavirus G type prevalence in two regions of Hungary

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Summary. Rotaviruses are a major cause of gastroenteritis in children worldwide. Rotaviruses are antigenically complex, with multiple serotypes (G types). The first longitudinal study of group A rotavirus serotype (G type) distribution in Hungary is reported. Neutralizing monoclonal antibodies specific for G1, G2, G3, and G4 were used in an enzyme immunoassay to determine the antigenic variation of group A rotaviruses in two collections of stool specimens assembled from 1984–1992 in Baranya County, southwest Hungary, and from 1988–1992 at the Central Hospital for Infectious Diseases in Budapest. Ninety-two percent of the 1215 virus-positive samples were typed as follows: G1 (81%), G2 (4%), G3 (1%), G4 (5%), or mixed type (1%). G1 was the predominant type during the entire study period with the exception of the 1988/1989 rotavirus season in Baranya County when G4 predominated. Among G1 strains, different electropherotypes were detected with a shift of the predominant G1 electropherotype(s) each 2 to 3 years. G typing from two longitudinal collections established regional differences within Hungary in the prevalence of rotavirus antigenic types among children with rotavirus-associated diarrhea. These are the first longitudinal rotavirus typing results for Hungary and Central Europe.

Introduction

About nine to ten million cases of serious dehydrating diarrhea occur each year worldwide [7, 10, 25, 26]. Group A rotaviruses are the most common cause of severe, acute gastroenteritis among infants and young children worldwide [15, 16, 27, 28]. During the last decade the antigenic complexity of circulating group A rotaviruses has become apparent. The virus outer capsid is composed of

two proteins, the protease-sensitive hemagglutinin (P) and a glycoprotein (G). These proteins induce neutralizing antibodies and are key targets of the host's protective immune response. Group A rotaviruses include 14 serotypes (G types), differentiated by changes of VP7, the major outer capsid glycoprotein [9]. At least ten G types occur among viruses isolated from humans and four of these (G1, G2, G3, and G4) are distributed widely throughout the world [1, 3, 6, 9, 11, 20, 47]. Antigenic types (P types) of human rotavirus VP4 are not as well defined; but six genotypes, which also may represent distinct P types, occur among human rotaviruses [9, 22, 23, 30].

Several studies characterizing the geographic and temporal distribution of rotavirus G types have been published [2, 5, 17–19, 21, 29, 32, 34, 40, 42, 44, 45] including a few reports from Europe [2, 17, 19, 34, 44, 45]. None of these reports are from Central or Eastern Europe. We report a study of the antigenic variability of rotaviruses in Hungary, using a G-specific MAb-based enzyme immunoassay (EIA) and samples collected over eight and four years from children with nonspecific gastroenteritis in two separate regions of the country.

Materials and methods

Definitions

A case of acute nonspecific gastroenteritis was an illness described by a nonspecific gastroenteritis diagnosis (International Classification of Diseases, version 9, Clinical Modification, codes 008.6, 008.8, 009.0–009.3, and 558.9) [31] and collection of a diagnostic stool specimen within the first seven days of illness, to exclude nosocomial cases. A case of acute rotavirus diarrhea was defined by the detection of rotavirus excreted in the stool of a child with acute nonspecific gastroenteritis. The rotavirus epidemic year was defined as July 1 through June 30. Rotavirus types G1 to G4 refer to MAb-based G type assignments on the basis of G antigenic type. Electropherotypes are the patterns of migration of rotavirus RNA gene segments in polyacrylamide gels.

Stool specimens from Baranya County

Baranya County is one of 19 counties in Hungary and is situated west of the Danube on the Hungarian-Croatian border. The population of Baranya County in 1992 was 477,731. All specimens submitted to the regional Laboratory of Virology, Pécs, from children with acute nonspecific gastroenteritis during the period June 1987 through July 1992, were screened for rotavirus. Samples from non-hospitalized children were submitted by pediatricians in Pécs and its outskirts, as well as from five district physicians in the county. Samples from hospitalized children were received from hospitals with pediatric wards in the towns of Komló, Mohács, Siklós, and Szigetvár, and from the County Children's Hospital, Pécs, and from the gastroenterology unit of the Department of Pediatrics, Medical University of Pécs. Previously electropherotyped, but not G-typed, samples collected during the 1984/85 to 1986/87 rotavirus seasons [38] also were included in this report. All stool samples were stored at –20 °C before testing.

Stool specimens from Szent László CHID, Budapest

Szent László Central Hospital for Infectious Diseases (CHID) is the only infectious diseases hospital in Hungary and is in Budapest, about 150 km north of Baranya County.

Rotavirus-positive stool samples submitted from the Department of Pediatrics and detected by Rotalex (Orion Diagnostica, Espoo, Finland) in the hospital virus laboratory were stored at −20 °C and transferred to Pécs every two to three months. The stool samples in this collection were obtained between July 1, 1988 and June 30, 1992, from children five years or younger hospitalized because of acute gastroenteritis. Patients admitted to CHID resided in the metropolitan area of Budapest, which has a population of about two million persons, one-fifth the total Hungarian population.

Detection of rotavirus in stool specimens

All stool samples were tested for rotavirus in a commercial group A EIA (Dakopatts, Glostrub, Denmark) or by electropherotyping [39]. After 1988, electropherotypes were analyzed by silver staining, instead of ethidium bromide staining and UV-light detection.

Demographic and hospitalization data

Demographic and clinical data accompanying samples from Baranya County included the patient's age, date of sample collection, and place of residence. For each sample from the CHID, the patient's medical record number, date of hospital discharge, length of hospital stay, discharge status, and discharge diagnoses were obtained retrospectively.

Monoclonal antibody G-typing EIA

Ten percent stool suspensions were extracted with fluorocarbon and tested in an EIA using monoclonal antibodies (MAb) [28]. Typing MAbs included in the EIA were KU-4 (G1), S2-2G10 (G2), YO-1E2 (G3), and ST-2G7 (G4) [24, 37, 40, 41]. MAbs 631-7-54 and 60-F2D4, against group-specific determinants on the VP6 and VP7 proteins, respectively, also were used. Cut-off points for scoring positive EIA results were determined as the mean OD values plus three standard deviations for each MAb pair using a group of 20 rotavirus-negative specimens. A sample was assigned a single G type only if the reactivity with one G typing MAb was at least twice that of the others tested. Samples that failed to react with VP6-specific MAbs were scored as "VP6-negative." Samples were scored as G1, G2, G3, G4, or "VP7-negative," depending upon their reactivities to the G type-specific or common VP7 MAbs. Samples were scored as "mixed" if they showed reactivity with more than one G type-specific MAb. Samples were called "non-typeable" if reactivity with G type common MAb was positive, but no reactivity was detected in wells containing the G type-specific MAbs.

Statistical analysis

Univariate analysis using the Epi Info statistical software package was performed for adjusted and unadjusted groups [13].

Results

Distribution of samples by study region, year, and patient age

During the 8-year period, 5506 stool specimens from the two regions in Hungary were collected. A total of 3267 (59%) samples were from Baranya County; most of these (61%) were collected during the last four years of the study period. Rotaviruses were detected in 915 (28%) of the Baranya County stool specimens and 367 (40%) of the positive samples were analyzed in the typing assay. The

Table 1. Age distribution of children with acute nonspecific gastroenteritis represented by the two collections of stool samples

Age group	Baranya County			Budapest Metropolitan Area		
	Samples submitted[a]	Rotavirus identified[b]	Number tested[c]	Samples submitted[d]	Rotavirus identified[b]	Number tested[c]
1–5 months	722	130 (18)	48 (37)	432	376 (87)	161 (43)
6–11 months	590	183 (31)	70 (38)	506	445 (88)	204 (46)
1 year	756	272 (36)	107 (39)	591	532 (90)	247 (46)
2 years	508	168 (33)	62 (37)	257	226 (88)	106 (47)
3 years	414	112 (27)	43 (38)	98	89 (91)	40 (45)
4 years	74	14 (19)	11 (79)	42	36 (86)	16 (44)
≥5 years	107	13 (12)	9 (69)	115	100 (87)	48 (48)
Not recorded	96	23 (24)	17 (74)	198	158 (80)	96 (61)
Total	3267 (100)	915 (28)	367 (40)	2239 (100)	1962 (88)	918 (47)

[a]Stool specimens from patients with acute nonspecific gastroenteritis, collected within 7 days of illness

[b]Rotavirus identified by EIA and/or electrophoresis of extracted viral RNA. Numbers in parentheses are percentages

[c]Number tested in the MAb-based G typing assay. Numbers in parentheses are percentages

[d]Samples screened by Rotalex in Budapest and submitted to the Laboratory of Virology, Pécs

number of samples collected in each year differs in part because of differences in the amount of effort spent soliciting their submission. A total of 2239 samples screened as rotavirus-positive were sent from CHID. Testing in Pécs confirmed the presence of rotavirus in 1962 (88%) of the CHID samples and 918 (47%) of them were included in the G typing assay. Testing was stopped when the results indicated that further testing would not change the overall patterns noted (see below).

Rotavirus epidemic cases and the peak-incidence of illness were observed at least one or two months earlier at CHID than in Baranya County in each season. The age distribution of the children in the two groups was similar (Table 1) and an average of 45% of samples from each age group were G typed. More samples from CHID (78%) were from urban residents than among the samples from Baranya County (60%). Stool samples were equally selected for racial composition in both regions; 18% of stool samples were collected from gypsies.

Characterization of rotaviruses by G typing

Of the 1285 samples from both regions tested in the G typing assay, 1215 (95%) reacted with the VP6 common MAb (Table 2). G1 was detected in 81%, G2 in 4%, G3 in 1%, and G4 in 5% of these 1215 specimens. A total of 102 (8%) non-typeable specimens potentially represented types other than G1–G4. Ten

Table 2. Year-to-year distribution of rotavirus G types in two regions of Hungary

Sample origin and season[a]	G type							No. positive/ no. tested
	VP6[b] negative	1	2	3	4	Non-typeable[c]	Mixed[d]	
Baranya County								
1984/1985	6	**5 (100)**	0	0	0	0	0	5/11
1985/1986	3	**17 (100)**	0	0	0	0	0	17/20
1986/1987	0	**19 (95)**	0	0	0	0	1 (5)	20/20
1987/1988	1	**42 (67)**	1 (2)	2 (3)	14 (22)	3 (5)	1 (2)	63/64
1988/1989	0	21 (41)	3 (6)	1 (2)	**22 (43)**	2 (4)	2 (4)	51/51
1989/1990	10	**94 (80)**	0	0	5 (4)	18 (15)	0	117/127
1990/1991	0	**21 (70)**	2 (7)	0	0	7 (23)	0	30/30
1991/1992	0	**43 (98)**	0	0	1 (2)	0	0	44/44
Total	20	262 (76)	6 (2)	3 (1)	42 (12)	30 (9)	4 (1)	347/367 (95)
Budapest metropolitan area								
1988/1989	3	**57 (78)**	7 (10)	4 (5)	2 (3)	2 (3)	1 (1)	73/76
1989/1990	26	**258 (80)**	3 (1)	4 (1)	2 (1)	51 (16)	4 (1)	322/348
1990/1991	19	**255 (84)**	23 (8)	0	7 (2)	19 (6)	0	304/323
1991/1992	2	**157 (93)**	7 (4)	0	4 (2)	0	1 (1)	169/171
Total	50	727 (84)	40 (5)	8 (1)	15 (2)	72 (8)	6 (1)	868/918 (95)
All total	70	989 (81)	46 (4)	11 (1)	57 (5)	102 (8)	10 (1)	1215/1285 (95)

Numbers in parentheses are percentages

[a]From July 1st of each year to June 30th of the subsequent year

[b]Sample failed to react with the common VP6-specific MAb

[c]Sample with reactivity to common VP7-specific MAb but failed to react with G 1–4 type-specific MAbs

[d]Sample with reactivity to more than one G typing MAb

samples had more than one G type reactivity; all contained G1 with G2 or G1 with G4. Samples stored for a longer period of time before testing were more likely to lack reactivity to the VP6 common or VP7 common MAbs. Types were distributed comparably over all age groups studied.

Geographic distribution of G types

G1, G2, G3, and G4 were detected in both regions with comparable overall prevalence (Table 2). During the comparable last four rotavirus seasons only G4 was more frequently observed among the specimens from Baranya County (12%) than among the samples from CHID (2%; $X^2 = 47$, $P < .001$). Differences in the type distribution by referring hospital or rural rather than urban place of residence were not observed within individual seasons.

Year-to-year distribution of G types

G1 was the predominant type during all seasons except during the 1988/89 season in Baranya County when G4 exceeded G1 (Table 2). G1, G2, G3, and G4 co-circulated only in two seasons, in 1987/1988 and 1988/1989. G1 was detected each year, and the proportion of cases infected with this type varied between 41% and 100%. G2 and G3 rotaviruses were detected in a minor proportion of cases. G4 was detected in 22% of the samples during the 1987/88 season, the season prior to its peak season. Among specimens from CHID, G1 was the predominant type each year. Other types co-circulated at low levels without apparent trends.

The non-typeable strains were first detected in Baranya County during the 1987/88 season and were detected more frequently during the 1989/90 (15%) and 1990/91 (23%) seasons ($P < .001$), the two seasons following that in which G4 was predominant. Non-typeable strains also were more common among the CHID samples during the 1989/90 (16%) and 1990/91 (6%) seasons.

We did not observe more severe clinical symptoms associated with a particular G type nor a difference in prevalence of serotypes between hospitalized and non-hospitalized children.

RNA electropherotyping

Samples also were analysed by electropherotyping. Seven seasons of electropherotyping results were available from Baranya County and four seasons from CHID. In both regions a two- to three-year shift was detected among the three predominant electropherotypes, but the G type remained G1. The electropherotypes associated with G1 included multiple and many similar electropherotypes. The predominant electropherotypes were different between the regions in the four comparable seasons. The G4 types which were predominant during the 1987/1988 season in Baranya County had electropherotypes distinct from those associated with G1 types circulating during the same season. Two slightly different electropherotypes were associated with G4 types during the three seasons (1987/88 to 1989/90) in Baranya County when G4 was more active and

this number of electropherotypes was less than the number of electropherotypes associated with G1 in any season. Two electropherotypes were observed in six of the samples scored as mixed. Most (89%) of the samples with non-typeable rotaviruses were successfully analyzed for RNA and exhibited 'long' electropherotypes with at least three distinct patterns. Furthermore, two group C rotaviruses were detected by RNA analysis.

Discussion

During the last decade, information has accumulated about the distribution of rotavirus G types on five continents [2, 47]. The 1019 samples characterized from the 6-year collection in northeast London-West Midlands is the largest European study published [34]. Other G typing results from Europe include a total of 649 stool samples from four collections in England, Finland, Italy, and Sweden, collected during the last decade [2, 17, 19, 44, 45]. We report the distribution of rotavirus G types using 1285 samples collected from 1984–1992 and 1988–1992 in two regions of Hungary. The typing assay permitted the assignment of a G type in 92% of the virus-positive samples.

G1 was the most common type circulating in Hungary. This finding agrees with global and European G typing results [2, 34, 47]. The portion of G1 samples (81%) is close to the percentage observed in Finland (87%) and Italy (72%) [19, 44, 45], but higher than the rate observed in West Midlands (55%) [34], Northeast London (60%) [34], and Sweden (50%) [2]. These regional differences may reflect cycles in the prevalence of G1 samples from year to year because in our study G1 prevalence ranged from 100% to 41% in our two study regions. Similar results were observed in Italy, where G1 occurred at a rate of 43% during the 1981/1982 season and increased to 90% during the 1984/1985 season [19], and in northeast London, where a rate of 98% was detected during the 1987/1988 season and decreased to about 30% during the 1989/1990 season [34]. A longer period of monitoring will be required to assess this fluctuation in the circulation of G1 more accurately.

The overall occurrence of G2 (4%), G3 (1%), and G4 (5%) were similar to that reported from Finland and Italy [19, 44, 45]. In some parts of the world, G3 has been the predominant type [47], including Houston, Texas, where G3 was predominant during 3 of 11 successive seasons [32]. G3 was the second most frequently detected G type in England and Sweden [2] a decade prior to our study and was detected at a low rate (8%) among samples from Finland [2] and in Northeast London (3%) [34]. G3 strains were not detected in Italy [19]. While G4 was absent from all stool specimens tested from Africa [47], Canada [2], and Italy [19], we found type G4 strains in both Hungarian regions. G4 strains were predominant during one season in Baranya County and one season in northeast London [34].

The analysis of our samples by RNA gel electrophoresis showed that several different electropherotypes of the same G type may co-circulate, as is known from previous studies [17, 19, 32, 34]. Among G1 rotaviruses, two to three

electropherotypes were predominant each season among the 6 to 12 co-circulating electropherotyes. The electropherotyping results also suggest that the prevalence of G1 subtypes has a two- to three-year cycle in the study regions, within the limits of the seven and four years of study reported here. Circulating G1 strains may be more antigenically heterogeneous than previously recognized and than viruses of other types, especially in surface-structures of neutralizing epitopes [5, 8, 27, 47]. Molecular characterization of VP7 and VP4 proteins of G1 viruses with distinct electropherotypes may identify antigenically distinct strains. Detailed characterization of circulating G3 and G4 strains may explain why these G types, when predominant, include only a few co-circulating electropherotypes. In this study, electropherotyping complemented G typing and identified differences within a G type suggesting future epidemiologic and laboratory studies.

The combined, published G typing results indicate that circulating rotavirus were antigenically diverse in most regions studied [2, 4, 5, 18, 21, 32, 34, 42, 47]. Although year-to-year cycles of antigenic change are community-wide events, no clear patterns of change have been discovered permitting predictions of predominant types. Further, cycles of changing G1 subtypes appear to be occurring even when G1 is not a predominant type. If the prevalence of G1 subtypes changes from year to year in the natural setting when many G1 types are co-circulating, it will be of interest to monitor changes of G1 subtypes that occur in response to universal immunization of children with a single G1 subtype, as is the plan with current vaccine strategies. It is possible that G1 subtypes change from year-to-year in response to the same immunologic pressures that drive changes in G type prevalence. The recent description of new antigenic rotavirus types [3, 11, 20, 46] is yet another facet of the "puzzling diversity" [12] of rotavirus.

The diversity of circulating rotaviruses may not prevent an effective vaccine program. The published G typing results suggest that rotavirus types are more endemic than influenza viruses. In addition, recent studies [35, 36, 45] suggest that two rotavirus infections are sufficient to stimulate heterotypic immunity protective against serious illness. These two infections can be asymptomatic because asymptomatic infections stimulate immunity to levels comparable to those achieved after symptomatic infections [33, 45]. If a vaccine can induce an immune response equivalent to two natural infections, the immunity thus acquired may be protective against sub-typic variants.

The results of our study in Hungary supplement data available for the distribution of rotavirus G types in Europe. Furthermore, it gives information on the circulation of rotavirus antigenic types for a longer period of time and in a larger children population than is available from other European regions. We believe our observations of subtype and G type variations when taken in the context of other studies, justify continuing monitoring of changes in predominating circulating G types and improved methods for P typing in a target population before and during introduction of a vaccine in any geographic area.

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