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Serotypes and Subgroups of Rotavirus Isolated from Children in Central Brazil

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ABSTRACT

Group A rotavirus, obtained from children of Goiânia, Brazil, during 1987-1994, were analyzed for subgroup and G serotype by enzyme-linked immunosorbent assay with monoclonal antibodies. The index of serotyping obtained was 61.4% with the following proportions: G1–19.7%, G2–28.0%, G3–9.8%, G4–1.5%, and G5–2.3%. It was observed that G1 occurred from 1987 to 1989 and from 1993 to 1994, and G2 from 1990 to 1993. About 94% of the samples (85/90) could be subgrouped with the following results: 55.5% for SG II, 7.8% SG I, and 31.1% for SG non-I-non-II. Unusual relationship patterns were also detected among serotypes, subgroups, and profiles of electropherotypes in 57.0% of the samples: 20 of them were G2/SG II/"long" profile. The results suggest that variation in temporal and regional characteristics should be considered in the development of rotavirus vaccine.

***Key words*:** Rotavirus; Serotyping; Diarrhoea, Infantile

INTRODUCTION

Group A rotavirus is a major aetiological agent of diarrhoea in humans. In developing countries, the virus is significantly responsible for mortality of children (1). Outer-capsid proteins, VP7 and VP4, are responsible for neutralizing the antibody response in host. The presence of different serotypes results from extensive genetic variations in the dsRNA segments encoding these outer-capsid proteins (2,3). Fourteen G serotypes (VP7) (4,5) and 12 P serotypes (VP4) have so far been recognized (6). The virus also presents an inner-capsid, constituted of VP6 protein, that also shows genetic variations, resulting in 4 different subgroups (I, II, I and II, non-I-non-II) (7). Although the genetic mechanism is still not understood, two major patterns of migration of the 11th genomic segment—one slower and another faster—were detected using gel electrophoresis, and are called "short" and "long" pattern respectively. The "short" pattern, in general, is related to subgroup I, whereas the "long" pattern is usually found in subgroup II samples. Since immunity for rotavirus is homotypic, it is necessary to have knowledge about the circulation of serotypes in different places and at different times. The determination of G and P serotypes is equally important, but due to difficult access to routine procedures for typing VP4, studies have been developed mainly in relation to VP7, the major viral neutralization antigen (8).

In this communication, we report the results of subgrouping and G serotyping of group A rotavirus samples obtained from faecal specimens of children of Goiânia, Brazil, during 1987-1994.

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METHODS AND MATERIALS

A total of 2,158 faecal samples were collected from children, aged up to 10 years, with or without diarrhoea, who were admitted to hospitals, ambulatories, or day-care centres in Goiânia city, Brazil, between March 1986 and June 1995. Group A rotavirus was detected by a

combined enzyme immunoassay for rotavirus and adenovirus (EIARA) (9), RNA polyacriamide gel electrophoresis (PAGE) (10) using classification of electropherotypes as described previously (11), and immune electron microscopy (IEM) (12). G serotyping was done using enzyme-linked immunosorbent assay with monoclonal antibodies (EIA-MAb), for G1, G2, G3, and G4 serotypes (Silenus, Australia and Serotec/ Rota MA, Japan) and for G1, G2, G3, G5 and G6 (kindly supplied by Dr. Harry Greenberg, USA). The assays were done using polyclonal or monoclonal antibodies as capture according to Coulson *et al*. (13) and Pereira *et al*. (9) respectively. All samples were also tested against a specific monoclonal antibody for group A rotavirus antigen. A sample was assigned to one serotype when the value of optical density (OD) of the reaction for that serotype was at least two-fold the value corresponding to any other serotype. The subgrouping was done by enzyme-linked immunosorbent assay using monoclonal antibodies as capture to subgroup I and II of rotavirus (kindly supplied by Dr. Harry Greenberg, USA), with reagents from Pereira *et al*. (9). All the samples were also tested with monoclonal antibody for group A rotavirus antigen (kindly supplied by Dr. T.H. Flewett, UK). A sample was considered to belong to one subgroup when the OD value for that subgroup was at least two-fold the OD value for the other subgroup. Samples not reacting with subgroup I or II, but reacting with group A antigen, were considered to be subgroup non-I-non-II, and those that were not reactive with these monoclonal antibodies were considered not subgrouped.

Results were analyzed using the confidence interval of 95% and chi-square test with probability of <0,05 regarded as significant (14).

RESULTS

Two hundred fifty-four (11.8%) of the 2,158 faecal samples obtained between March 1987 and June 1995 were positive for rotavirus, and 132 were tested for G serotypes. These samples were considered representative of all the positive samples, since there was no significant statistical difference (p>0,05) between the samples submitted and the samples not submitted for serotyping in relation to age, sex, and period of collection (data not shown).

The G serotype was obtained in 81 (61.3%) of the 132 samples, and showed the following results: 26 (19.7%) were G1, 37 (28.0%) G2, 13 (9.8%) G3, 2 (1.5%) G4, and 3 (2.3%) G5 (Table 1). Forty-five of 51 non-typeable samples were reactive with monoclonal antibody for group A antigen. Results of analysis of circulation of the serotypes in relation to the years of sample collection are shown in the figure. It was observed that G1 circulated from 1987 to 1989 and from 1993 to 1994. In 1990, it was replaced by G2, predominating up to 1993. G3 serotype occurred in 1988 and 1989, with predominance in the first year. G4 had a circulation in 1988 and 1990, and G5 in 1987 and 1990. Non-typeable samples occurred proportionally in all the years. In relation to subgroups, 94.4% of the samples could be subgrouped: 50 (55.5%) of them were subgroup II, 7 (7.8%) subgroup I, and 28 (31.1%) subgroup non-I-non-II, and 5 (5.6%) did not react with monoclonal antibody for group A antigen.

Table 2 shows the characteristics of 82 samples of rotavirus submitted for serotyping and subgrouping in relation to electropherotype patterns. It was observed

Table 1. Percentile of rotavirus G serotypes from 132 faecal samples obtained from children of Goiânia, Brazil (1987-1994)

Serotype	No.	%	(CI-95%)*
G1	26	19.7	(12.7-27.7)
G2	37	28.0	(20.7-36.1)
G3	13	9.8	(4.8-16.8)
G4	2	1.5	(0.1-5.5)
G5	3	2.3	(0.3-7.2)
Not serotyped	51	38.6	(30.7-47.3)

*CI=Confidence interval

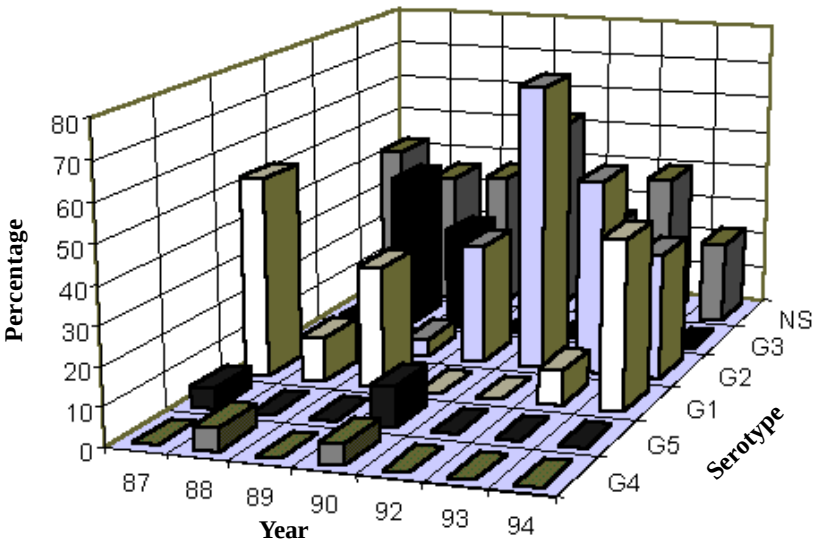

Fig. Percentile of rotavirus G serotypes from 132 faecal samples obtained from children of Goiânia, Brazil, in relation to the year of sample collection (1987-1994)

Table 2. Serotype, subgroup, and electrophoretic patterns of 82 group A rotavirus samples obtained from faecal samples from children from Goiânia, Brazil

	Serotype*/pattern							
	G1		G2		G3		NS†	
SG	Long	Short	Long	Short	Long	Short	Long	Short
I	0	0	4	3	0	0	0	0
II	9	0	20	0	3	0	16	0
NI-NII‡	1	0	1	1	0	0	21	3
Total	10	0	25	4	3	0	37	3

*Sample G4 and G5 were not submitted to reaction for subgroup
†NS= Non-typeable; ‡NI-NII= Non-I-non-II

that 9 of 10 G1 samples were SGII/"long" pattern, and the remaining one was SG non-I-non-II/"long" pattern. Of the 29 G2 samples, 20 were SG II/"long" pattern, and 7 were SG I with 4 presenting "long" pattern. Two G2 samples presented the usual pattern: SG I/"short" pattern. All G3 samples were SG II/"long" pattern. Of 40 non-typeable samples, 16 were SG II/"long" pattern, and 24 were SG non-I-non-II, of which 21 had "long" pattern and 3 "short" pattern.

DISCUSSION

The occurrence of different serotypes of group A rotavirus at different times and in different locations should be considered in vaccine development (15). In this study, 61.4% of the positive rotavirus samples were G serotyped by EIA-MAb. This technique permits the determination of serotypes in high proportion as has been confirmed by various researchers with variable rates from 59% to 92.0% (16-22). Negative results in serotyping of rotavirus samples can be attributed to the occurrence of new serotypes or strains not recognized by monoclonal antibodies used due to variation of antigenic epitopes in VP7 (monotypes) or to incomplete viruses (without VP7) in some specimens tested due to prolonged storage in freezer temperature, as well as repeated freeze-thawing cycles (15,20,23). In our study, 45 of the 51 non-typeable samples were reactive to group A antigen, indicating the presence of VP6 antigen.

We have observed that G1 and G2 occurred at similar rates in the population, and G3, G4 and G5 viruses were also found. According to some authors, G1 serotype predominates in human population in several countries (16-19,23-25). Our results show that G1 had occurred in an important way, but G2 had an equal circulation. A similar result has also been reported from Bangkok (20), but data seem to be uncommon. Although G5 rotavirus is mainly found in swine, it was detected in 3 of our samples. This serotype has been found in high proportion in different geographic regions of Brazil (4,26). This suggests the occurrence of interspecies transmission (27) or a genetic reassortment between samples from different species (4,28).

The concurrent circulation of different serotypes has been reported (29), specially with G1 occurring each year (17,30). We observed that G1 occurred from 1987 to 1989, reappearing in 1993, and that G3 circulated only in 1988 and 1989. During this period, G2 appeared and predominated until 1993. This suggests that, at least for G1 and G2, the circulation of serotypes in our region seems to be mutually exclusive, as has been described by other authors (25). These data reinforce the concept that the success for rotavirus-vaccination depends on the knowledge of circulation pattern of rotavirus serotypes (31,32).

Subgroup characterization of 63.3% of the samples tested was done with monoclonal antibody for SG I and SG II. This percentage is in agreement with some authors (7). We observed that 31.1% of the samples were subgroup non-I-non-II. This high prevalence of SG non-I-non-II was not reported before, and suggests that our samples are very peculiar. Correlation analysis of serotypes, subgroups, and patterns of electropherotypes showed 57.0% of the serotyped samples with unusual relationship patterns. All G1 samples, except one, three G2 and all G3 samples presented the expected serotype/subgroup/electropherotype relationship pattern. Regarding G2 samples, 20 were SG II/"long" pattern, 4 were SG I/"long" pattern, and 2 were SG non-I-non-II. The detection of these unusual samples was reported previously, and some authors considered the unusual samples as consequence of reassortment process occurring between samples from different genogroups arising the intergenogroups (33,34).

SG I/"long" pattern samples have been described in different regions of the world (16,27,35,36), but G2/SG II/"long" pattern samples have rarely been described. In Brazil, Linhares *et al*. (37) reported four similar samples and Bingnan *et al*. (38) described three samples with this pattern obtained from children of Bangladesh. Further molecular studies will be necessary for a better characterization of these uncommon samples. Results of serotypes, subgroups, and electropherotypes suggest the presence of several samples with uncommon characteristics and a shift of G1/G2 samples occurring

in Goiânia, Brazil. These results suggest the need of characterization of rotavirus samples in different locations and at different times before establishing the vaccination process.

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