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G and P genotypes of rotavirus circulating among children with diarrhea in the Colombian northern coast

Summary. A study on the prevalence of rotavirus G and P genotypes was carried out based on 253 stool specimens obtained from children living in the Colombia northern coast region who were less than 3-years-old and who suffered from acute diarrhea. A previous study had detected the presence of rotavirus A in 90 (36.5%) of the 246 samples tested by enzyme immunoassay (EIA), and these strains were investigated in the present study. Of these, 50 strains yielded an RNA electropherotype, most of which (80.0%) had long profiles and 20.0% of which had short profiles. Genotyping of 84 positive samples indicated that 67.9% of the strains could be typed. G1 (57.9%), was the most predominant VP7 genotype, followed by G3 (21.1%), G9 (15.8%) and G2 (5.3%). Among the VP4 genotypes, P[4] (49.1%) was the most prevalent, followed by P[6] 36.4% and P[8] (14.5%). Neither G4 nor G8 nor P[9] types were detected. The most common G-P combinations were G3 P[4] (8.8%) and G9 P[6] (7.0%), followed by G1 P[4] and G1 P[8] (5.3% each). All G1 P[8] strains showed long RNA profiles, whereas G3 P[4] and G9 P[6] displayed both long and short patterns. Mixed infections involved 21.0% of strains. There was a marked diversity among strains collected, and novel strains, including G9, as well as other atypical combinations of G and P genotypes, such as G9 P[6] and G3 P[4], were found. [**Int Microbiol** 2004; 7(2):113–120]

Key words: rotavirus · diarrhea · genotypes · electropherotypes

Introduction

Diarrhea is one of the most common diseases in infants and young children, both in developed and developing countries. The incidence of diarrhea in African, Latin American and Asian countries has been estimated to be over one billion, with approximately 3.3 million deaths per year, in children under 5 years of age [6,22,24,35,37,57,59].

The rotavirus described by Bishop et al. (1973) is the main cause of severe gastroenteritis in infants, children and young animals. The rotavirus genome consists of eleven segments of double-stranded RNA that code for the triple capsid and non-structural proteins. Based on the VP6 protein of the internal capsid, seven distinct groups are distinguished. (A to G). Rotavirus groups A, B and C can infect humans and animals, with group A having been widely recognized to be the cause of severe diarrhea in infants [22,37]. In neutralization assays, different serotypes of the rotavirus group A were determined by using antibodies of high neutralizing activity against the proteins VP7 and VP4. Based on the genetic and antigenic diversity of these external capsid proteins, the rotaviruses have been classified into types G and P, respectively [22,37].

The development of culture techniques for human rotaviruses and the use of standard assays for serotyping have enabled the identification of at least 14 types of G rotavirus, ten of which have been described for humans. So far, 21

type-P rotavirus have been described by using genotyping methods, including RT PCR and sequence analysis.

Epidemiological studies have demonstrated that rotavirus types G1, G2, G3 and G4 are prevalent worldwide [13,26,39, 40,43,44,54,55,56]. However, other unusual strains, including G5, G8, G9 and G10, have been reported in Brazil [2,29,50, 51]. Serotype G9 has been described also in Argentina [8], India [36,47,58], Malawi [15], Nigeria [1] and Ghana [4]. In addition, serotypes G6 and G8 have been detected in Australia [7], and G12 in Thailand [46] and India [20]. In a previous study, we reported, for the cities of Cartagena (10º 25′ N, 75º 33′ W) and Sincelejo (9º 18′ N, 75º 24′ W), frequencies of rotavirus infections of 44% and 28% respectively [59]. To our knowledge, the prevalence of human rotavirus G and P genotypes has not been determined in Colombia so far.

Nonetheless, the molecular characterization of the circulating native strains of rotavirus in Colombia is of great importance for prospective rotavirus vaccine studies. In addition, the current oral tetravalent vaccine, licensed in 1998, which was withdrawn from use in the United States because of a risk of intussusceptions [14,45], covers the four epidemiologically most important G types rotaviruses (G1 to G4) [32]. The aim of the present study was to determine the prevalence of rotavirus G and P genotypes present in fecal samples obtained from children living in the Colombia northern coast who were less than 3 years of age and who had acute diarrhea.

Materials and methods

Samples. The strains used for the present study were those in which rotavirus A had been detected in a previous study on the etiology of acute diarrhea in 253 infants and young children in Cartagena de Indias and Sincelejo, Colombia [59]. Of the 246 stool samples tested by enzyme immunoassay (EIA), 90 (36.5%) revealed the presence of rotavirus A. Sixty-two of the samples were from Cartagena de Indias and 28 were from Sincelejo. These rotavirus-positive samples were electropherotyped and subsequently subjected to RT-PCR.

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analyses. The viral double-stranded RNA from the stool samples was extracted with Trizol (Life Technologies Grand Island, New York) using the method originally described by Chomozcynnky and Sachi, N [16]. SDS-PAGE analysis of the extracted rotaviral RNA was carried out according to methods previously described by Iturriza-Gomara et al. [34].

RT-PCR. Viral RNA was extracted from the stools with GuSCN (Life Technologies Grand Island, New York) following the method described by Boom et al. [11]. The specimens were subjected to RT-PCR assays with the Access RT-PCR kit (Promega, Madison, WI). The primers used for the specific primers were previously described by Gouvea et al. [30] for VP7, and Gentsch et al. [27] for VP4**.** In six of the 90 rotavirus-positive extracts, there was not enough material to carry out genotyping.

Agarose gel electrophoresis. The RT-PCR products (12 µl) were mixed with bromophenol blue and loaded onto a 2% agarose gel (Biorad, Richmond, VA) in TAE buffer, pH 8.5, containing 0.5 µg ethidium bromide ml^{-1}. Statistical analysis was performed with Epi-Info 6.04 A. Relationships between proportions were determined by chi-square tests and 95% confidence level. A value $p \leq 0.05$ was assumed to be a statistically significance difference.

Results

Detection of rotavirus genome by SDS-PAGE. Fifty of 90 of rotavirus-positive specimens (55.5%) yielded an RNA electropherotype, with a profile characteristic (4-2-3-2) of rotavirus group A. Of these, 40 displayed a long pattern and 10 had a short pattern. In addition, several samples differed markedly with respect to the electrophoretic mobility of RNA segments 1 to 4. Figure 1 shows three representative rotavirus strains with long and short profiles.

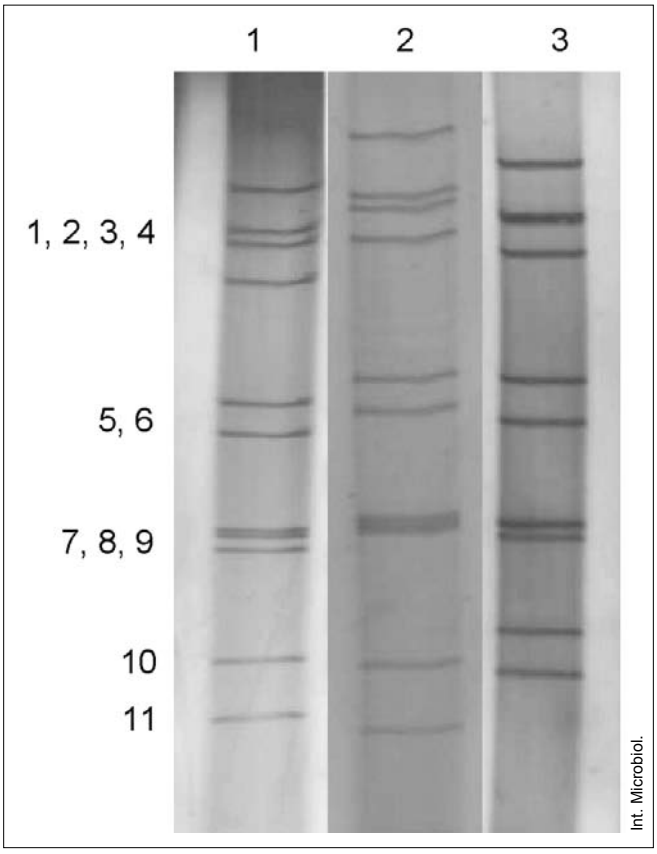

Fig. 1. SDS-PAGE analysis of the double-stranded (ds) RNA genome extracted from three distinct stool samples obtained from infants with acute diarrhea. Numbers at left side indicate the four variable regions of the rotavirus genome. Lanes 1 and 2, long-profile strains; lane 3, short-profile strain. Differences in the migration of the first four gene segments of the dsRNA genome are noted in several samples. Gene segments 2 and 3 in the first variable region (lane 3) are not distinguishable.

Determination of G and P genotypes. Of 84 rotavirus-positive samples that were subjected to RT-PCR, 57 (67.9%) could be genotyped. (Table 1).Of these, the genotype G (VP7 associated) was assigned to 38 strains and the genotype P (VP4 associated) to 55 strains.

Figures 2 and 3 show the agarose gel of the representative amplified products of G and P genotypes.

Table 1. G and P genotypes of rotavirus detected in stool samples from symptomatic infants and young children on the Colombian northern coast

Genotypes	Percent of strains classified as:								
	P[4]	P[6]	P[8]	P[9]	P[4] P[6]	P[6] P[8]	P[4] P[8]	NTP	Total
G1	3 (5.3)	0	3 (5.3)	0	0	1 (1.8)[a]	1 (1.8)[a]	11 (19.3)	19 (22.6)
G2	1 (1.8)	1 (1.8)	0	0	0	0	0	0	2 (2.4)
G3	5 (8.8)	0	0	0	0	0	0	0	5 (6.0)
G4	0	0	0	0	0	0	0	0	0
G8	0	0	0	0	0	0	0	0	0
G9	1 (1.8)	4 (7.0)	0	0	0	0	0	1 (1.8)	6 (7.1)
G1-G3	0	2 (3.5)[a]	0	0	0	0	1 (1.8)[a]	0	3 (3.6)
NTG	9 (15.8)	5 (8.8)	1 (1.8)	0	6 (10.5)[a]	1 (1.8)[a]	0	27[b]	49 (58.3)
Total	19 (22.6)	12 (14.3)	4 (4.8)	0	6 (7.1)	2 (2.4)	2 (2.4)	39 (46.4)	84[c]

[a]Mixed infections; [b]Total that could not be typed for their respective P or G genotypes; [c]Total number of samples subjected to RT-PCR; NTG: Nontypeable for G types; NTP: Nontypeable for P types

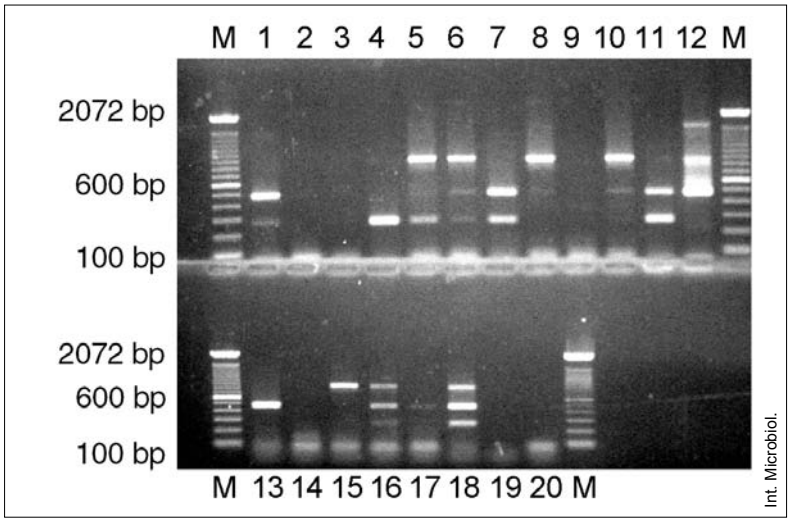

Fig. 2. RT-PCR typing of rotavirus strains. VP4 genotypes detected directly from twenty infants (age range 2–30 months) with acute diarrhea. Lanes: M, DNA marker ladder; 1, 6, 7, 11, 16 and 18, dual infection with P[6] P[4] types (483 bp, 267 bp); 4 and 5, P[6] type (267 bp); 10, 12, 13 and 17, P[4] type (483 bp).

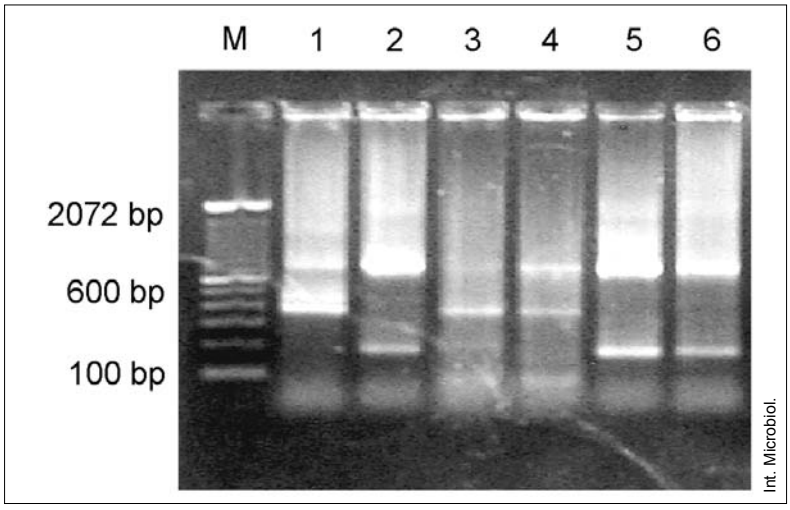

Fig. 3. RT-PCR typing of group A rotavirus strains. VP7 genotypes detected from stool samples obtained from infants with acute diarrhea. Lanes: M, DNA marker ladder; 2, 5, and 6, G1 type (749 bp); 3, G3 type (374 bp); 1 and 4, dual infection with G1 and G3 types.

Table 2. G and P genotypes of rotavirus detected in fecal samples from symptomatic infants and young children in two cities on the Colombian northern coast

Genotypes	Percent of strains classified as:											
	G1	G2	G3	G4	G8	G9	Total	P[4]	P[6]	P[8]	P[9]	Total
Catagena	14 (51.9)	2 (7.4)	5 (18.5)	0	0	6 (22.2)	27	24 (60.0)	15 (37.5)	1 (2.5)	0	40
Sincelejo	8 (72.7)	0	3 (27.3)	0	0	0	11	3 (20)	5 (33.3)	7 (46.7)	0	15

Incidence of genotypes G and P by city. Table 2 shows the distribution of strains in each city. In Cartagena, G1 (51.9%) was prevalent, whereas among the P strains, the P[4] genotype (60.0%) was predominant. In Sincelejo, where only two (G1 and G3) of the four major human G genotypes were detected, the difference in the detection of G1 (72.7%) and G3 (27.3%) genotypes was statistically significant ($p < 0.05$). In contrast, three P genotypes commonly described in human infections were found, with P[8] (46.7%) being prevalent.

Correlation of G and P genotypes. Both G and P genotypes were assigned to 18 of 57 (31.6%) fecal specimens. Tables 1 and 3 show the combinations of both genotypes detected.

Genotypes and electropherotypes. Of 57 samples with single and mixed strains, 36 (63.1%) were positive as determined by PAGE (Table 3). All G1 P[8] strains exhibited long electropherotypes. G3 P[4], G2 P[4] and G9 P[4] strains had identical short electropherotypes. The strains G9 P[6] and G1 P[4] shared long and short patterns. Mixed strains associated with long electropherotypes included G1 P[4] P[8], G1 P[6] P[8], P[6] P[8], and P[4] P[6]; none of these had short electropherotype. Mostly G1 genotypes, in single or mixed infections, were most commonly associated with long electropherotypes. G9 strains showed identical long and short profiles. P[4] and P[6], the second most common single genotypes, had predominately long patterns (fourth, each). In addition, the P[6] genotype was more significantly associated with long electropherotypes than with short ones ($p < 0.05$).

Mixed infections. Twelve samples (21.1%) were involved in mixed infections. Table 1 shows the distribution of mixed infections.

RT-PCR vs. SDS-PAGE. Interestingly, 21 of 57 (36.8%) samples characterized by RT-PCR were negative by SDS-PAGE, and 12 of 50 (24.0%) specimens electropherotyped (displaying long and short patterns) could not be typed by RT-PCR.

Discussion

The present work is a continuation of our prospective study on infants and young children with diarrhea, conducted in two cities in the northern coast of Colombia, between 1998 and 2000 [59]. This study reports for the first time the prevalence of G and P genotypes of group A rotavirus infecting infants and young children with acute diarrhea in Colombia. Furthermore, this is the first report describing the electrophoretic patterns of rotavirus associated with childhood diarrhea in our region. The strategies used in this work, including a set of the most common specific primers for the typing of rotavirus genotypes, allowed characterization of 67.9% of rotavirus strains present in the stool specimens.

Table 3. Relationship among genotypes and electropherotypes

Genotype	Number of strains	Electropherotype		
		Long	Short	Negative
G1 P[8]	3	3	0	0
G3 P[4]	5	0	1	4
G9 P[6]	4	1	1	2
G1 P[4]	3	1	1	1
G2 P[4]	1	0	1	0
G2 P[6]	1	0	0	1
G9 P[4]	1	0	1	0
G1 P[4] P[8]	1	1	0	0
G1 P[6] P[8]	1	1	0	0
G1 G3 P[4] P[8]	1	0	0	1
G1 G3 P[6]	2	0	0	2
P[6] P[8]	1	1	0	0
P[4] P[6]	6	2	0	4
P[8]	1	1	0	0
P[4]	9	4	2	3
P[6]	5	4	1	0
G1	11	8	0	3
G9	1	1	0	0
Total	57	28	8	21

Although the finding that the strains in 27 samples (32.1%) could not be typed (either as G or as P) was rather unexpected; the percentage was lower than that reported in an earlier study carried out in Brazil (55.4%) [55]. However, the percentage of G genotypes that could not be typed in 49 (58.3%) samples was higher than those reported for Ireland [43], Bangladesh [58] and Africa [13]**.** Even so, we assume that these nontypeable G strains may belong to other genotypes for which specific primers were not included in the panel, or it might be due to RT-PCR failure because of the low amount of RNA, rather than to the presence of strong inhibitors.

The prevalence of rotavirus G and P types has been monitored worldwide, and types G1 to G4, [P8] and [P6] are the most frequent [3,10,13,22,23,25,28,36,40,42,43,44,54,55]. In our study, the most common G genotypes were detected, except G4, which was not identified during the course of this study. Instead, unpredictably, G9 (either alone or in a mixed infection), which appears to be an emergent in developed [10,31,48] and developing countries [4,8,23,51,58], was detected in 15.8% of the strains. Consistent with the findings of recently published studies, G9 was third in prevalence, with percentages similar to those found in Brazil, (15.9%) [51], Argentina (18.0%) [9], and India (17.0%) [36].

Epidemiological studies have shown that P[8] strains are the most commonly identified worldwide [3,10,19,23,33,35, 43,52,54]. In this study, other P genotypes, including P[4] (49.1%) and P[6] (36.4%), were found to be predominant, whereas P[8] was less frequent. Our findings are in concordance with the results of studies in Ghana [5], where P typing showed P[4] to be the prevalent strain followed by P[6] and P[8].

Studies in many countries have shown that G1 P[8], G2 P[4], G3 P[8] and G2 P[6] are the G-P combinations most commonly found worldwide [3,7,10,19,25,31,33,43,48,52,54]. In our study, however, the percentages of unconventional G3 P[4] (8.8%) and G9 P[6] (7.0%) strains were slightly higher than those of G1 P[8], G2 P[4] and G2 P[6] (5.3% each). These results confirm that G9, as well as its combinations G9 P[6] and G3 P[4], have been the most frequently described both in American and in Asian developing countries [2,4,9,36,47,51,53].

Mixed infections with different G and P genotypes produce a particular group of strains circulating in the community. In this study, mixed infections involved 21.1% of the strains, which is comparable to the percentages found in India (21.0, 23.0, and 24.0%) [19,36,58], Ireland (18.8%) [42], and even Brazil (16.0%) [50], whereas the number of strains involved in mixed infections in other Latin American countries, such as Argentina (10.0%) [3] and Mexico (12.0%) [49], is moderately low. Also of interest was the fact that, in our study, the percentage of strains involved in mixed infections was almost half (44.0%) that reported for a city of Brazil, where an uncommon rotavirus epidemic strain was reported a few years ago [53].

A notable finding was the marked contrast in the distribution of strains between the two Colombian cities. The incidences of G1 (51.9% and 72.7%) and G3 (18.8% and 27.5%), detected in Cartagena and Sincelejo, respectively, did not differ significantly ($p > 0.05$). However, G9, the second most frequent genotype detected in Cartagena, was absent in Sincelejo. Regarding P genotypes, more P[8] strains were detected in Sincelejo (46.7%) than in Cartagena (2.5%) ($p < 0.05$), while the frequency of P[6] strains were closely similar in the two cities ($p > 0.05$).

In Cartagena, a large diversity of rotavirus strains was observed with regard to G and P genotypes as well as to RNA electrophoretic profile. By contrast, in Sincelejo, only limited circulating G genotypes (only G1 and G3) were present, and there was a total absence of rotaviruses with short electropherotypes, demonstrating a relative homogeneity strains present in this city. It may be that factors such as geographic location and population density contribute to this notable difference. Cartagena de Indias, which is located on the Caribbean coast, has many promising industries and is the first tourist destination in Colombia. Over the past ten years, for different reasons, e.g. due to displacement, the dynamics of the city, including movement of the population, have increased. In contrast, in Sincelejo, which is located approximately 240 kilometers southwest of Cartagena, the current economy is based on agriculture and livestock, which offers less possibilities for dynamic population movement. Our findings agree remarkably well with the results of a study carried out in six Australian cities, where the marked diversity of serotype-G rotavirus detected was closely related to the population base in each city [40].

Previous published reports have shown that long electrophoretic profiles are prevalent overall. Consistent with similar results in Bogotá, Colombia [12], and those reported in other countries [3,17,21,33,48,53,56], a significant percentage (80.0%) of strains bearing a long profile (all of them detected in Cartagena) were found in this study. In addition, visible differences were observed regarding the mobility of segments 1 to 4 of the RNA genome during SDS-PAGE. Those differences suggest that there should be at least eight different migration patterns, possibly due to mixed infections, based on the capability of this segmented virus to form reassortants. Maunula et al. [41] suggested that the wide genetic diversity could be explained by fraction analysis of the sequences of all eleven RNA segments.

Our findings confirm that an absolute correlation between genotype and specific electrophoretic profile remains to be shown. Many studies report differences also among strains of the same genotype with different electrophoretic patterns. In this study, all G1 P[8] strains (in total three) had long profiles, as described previously [4,19,33,42]. Consistent with findings in other published studies [4,36,48,58], G9 P[6] strains shared long and short electropherotypes. A single G3 P[4] strain that was positive on SDS-PAGE-exhibited a short profile, which is consistent with a previous report in Ghana [5], although it differs with the results of studies in Minas Gerais [53]. All G2 P[4] strains had a short pattern, which agrees with the findings of previous published studies [36, 42]. Several reports showed that G1 P[4] strains had predominantly short patterns [19,42]. By contrast, G1 P[4] strains detected in this study had identical long and short electropherotypes.

The association of genotype P[6] in neonatal or asymptomatic infections has been documented [18,38]. We detected a high proportion (90%) of this genotype in symptomatic infants between 3 and 12 months of age. Our findings are consistent with the results of studies done in Brazil [2], Malawi [15], India [47], and Tunisia [56], which have shown that P[6] is a common cause of severe diarrhea in infants overall.

Considering the prevalence of the rotavirus infection and the remarkable genetic variability among rotaviruses, the present work provides basic information about the significant genetic diversity of circulating rotavirus strains, as well as the current formulations of rotavirus vaccines, which are based on the four most common VP7 types.

In summary, this study, in addition to describing the rotavirus G and P genotypes present in human infections in Colombia, shows the displacement of the most common G rotavirus genotypes and the emergence of an uncommon strain (G9), previously not described in Colombia. We think that demographic conditions and geographic localization are favorable for engendering mixed infections with different G and P genotypes, which are capable of forming reassortants and thus of producing unusual strains that then circulate in the community. These findings are of great relevance regarding candidate rotavirus vaccines to be tested in developing countries in Asia, Africa and Latin America [14]. A surveillance study of rotaviral diarrhea is essential for monitoring both new genotypes and changes in the genotypes circulating in Colombia.

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Genotipos G y P de rotavirus encontrados en niños aquejados de diarrea en la costa norte de Colombia

Resumen. El artículo describe un estudio de la prevalencia de los genotipos G y P de rotavirus en 253 muestras fecales de niños menores de 3 años afectados de diarrea aguda que vivían en la costa norte de Colombia. Un estudio anterior reveló la presencia de rotavirus A en 90 (36.5%) de las 246 muestras sometidas a inmunoensayo y dichas muestras fueron las usadas en este estudio. En 50 de las 90 muestras usadas se obtuvo un electroferotipo, un 80.0% de perfil de RNA largo y un 20.0% de perfil de RNA corto. De 84 muestras sometidas a genotipificación, se logró tipificar 67.9%. El genotipo G1(57.9%) fue predominante entre los asociados a VP7, seguido de G3 (21.1%), G9 (15.8%) y G2 (5.3%). Entre los genotipos VP4, el P[4] (49.1%) fue el de mayor prevalencia, seguido de P[6] 36.4% y de P[8] (14.5%). No se detectaron los genotipos G4, G8 o P[9], y las combinaciones G-P más frecuentes fueron G3 P[4] (8.8%) y G9 P[6] (7.0%), seguidas de G1 P[4] y G1 P[8], con 5.3% cada una. Todas las cepas G1 P[8] tenían perfil de RNA largo, mientras que G3 P[4] y G9 P[6] los tenían tanto largos como cortos. Se detectaron infecciones mixtas en el 21.0% de las cepas. Se observó una marcada diversidad en las cepas recolectadas y se identificaron cepas nuevas, como G9, y también combinaciones atípicas, como G9 P[6] y G3 P[4]. [**Int Microbiol** 2004; **7**(2):113–120]

Palabras clave: rotavirus · diarrea · genotipos · electroferotipos

Genótipos G e P de rotavirus encontrados em crianças acometidas de diarréia na costa norte da Colômbia

Resumo. O artigo descreve um estudo de prevalência dos genótipos G e P de rotavirus em 253 amostras fecais de crianças menores de 3 anos, acometidas de diarréia aguda e que viviam na costa norte da Colômbia. Um estudo anterior tinha revelado a presença de rotavirus A em 90 (36.5%) das 246 amostras submetidas ao imunoensaio e as mesmas amostras foram usadas no estudo. Foram obtidos electroferótipos de RNA para 50 das 90 amostras, sendo a maioria (80.0%) de perfil de RNA longo e cêrca de 20.0% de perfil curto. De 84 amostras submetidas a genotipificação, consegui-se tipificar 67.9%. O genótipo G1(57.9%) foi predominante entre os associados à VP7, seguido de G3 (21.1%), G9 (15.8%) e G2 (5.3%). Entre os genótipos VP4, o P[4] (49.1%) apresentou a prevalência mais alta, seguido de P[6] 36.4% e P[8] (14.5%). Os genótipos G4, G8 ou P[9] não foram detectados. As combinações G-P mais frequentes foram G3 P[4] (8.8%) e G9 P[6] (7.0%), seguidas de G1 P[4] e G1 P[8], com 5.3% cada uma. Todas as linhagens G1 P[8] tinham perfil longo, enquanto que as linhagens G3 P[4] e G9 P[6] apresentaram perfis longos e curtos. Foram detectadas infecções mixtas em 21.0% das linhagens. Foi observada uma diversidade marcante nas linhagens coletadas e foram identificadas linhagens novas, como G9, e também combinações atípicas como G9 P[6] e G3 P[4]. [**Int Microbiol** 2004; 7(2):113–120]

Palavras chave: rotavirus · diarréia · genótipos · electroferótipos