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Rotavirus infection in the Paraguayan population from 2004 to 2005: High incidence of rotavirus strains with short electropherotype in children and adults

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

Rotavirus is considered the main viral cause of acute gastroenteritis in children in both developed and developing countries. The aim of the present study was to continue the surveillance of rotavirus in the Paraguayan population in anticipation of a rotavirus vaccination in children.

Material/Methods:

Fecal samples from infants (<5 years of age) and adults with diarrhea (912 and 801 samples, respectively) were collected in Paraguay during 2004–2005. Rotavirus incidence was screened by PAGE and genotyping was performed by reverse transcription (RT)-PCR.

Results:

Rotavirus incidence was 23.8% and 19.4% for children and adults, respectively. The rotavirus incidence was higher in the coolest and driest months of the year. Five different group A rotavirus electropherotypes were detected. Rotaviruses with a long electropherotype were the most frequently detected in children in 2004 and 2005. However, in 2005 (after six years of absence in Paraguay) rotaviruses with a short electropherotype were detected at high frequency in both children and adults. Of these, 14 samples were genotyped (11 from children and 3 from adults) and all of them showed the G2P[4] type.

Conclusions:

This study reinforces the importance of continuous survey of rotavirus infection, extended to all age groups, in order to increase our knowledge about the complexity of rotavirus epidemiology.

Key words:

diarrhea • group A rotavirus • molecular epidemiology

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BACKGROUND

Rotavirus is considered the greatest worldwide viral cause of acute gastroenteritis in children. Its incidence is similar in developed and developing countries, which suggests that its control cannot be carried out by improving only the hygiene and water supply in the population [1]. Therefore, a vaccine would be the best strategy to prevent the severity of this infection.

Rotavirus, a member of the *Reoviridae* family, has an icosahedral, nonenveloped, triple-layered capsid and a genome of 11 double-stranded RNA (dsRNA) segments. The electrophoresis of the genome in a polyacrylamide gel produces a characteristic migration pattern called an "electropherotype". Based on antigenic differences in protein VP6, seven groups of rotavirus have been identified (A–G). Within group A, at least 26 P types and 15 G types have been detected based on differences within the *VP4* and *VP7* genes, respectively [2–4]. Based on the electrophoresis migration pattern, the genomic segments of group A rotaviruses are arranged in four clusters according to their sizes: segments 1 to 4 are included in cluster I, segments 5 and 6 in cluster II, segments 7, 8, and 9 in cluster III, and segments 10 and 11 in cluster IV [3]. Depending on the mobility of segment 11, two electropherotypes, i.e. long or short, can appear [5]. In humans, strains with a short electropherotype usually correspond to subgroup I and G2P[4] strains and those with a long electropherotype to subgroup II and G1P[8], G3P[8], or G4P[8] strains [2].

Epidemiological studies carried out in different regions of the world have shown that rotaviruses with a long electropherotype predominate in children during long periods of time, while epidemics caused by rotaviruses with a short electropherotype appear sporadically. For this reason, it could take several years of study to identify rotaviruses with a short electropherotype [6–8]. Since in Paraguay acute infantile diarrhea is considered the fourth cause of mortality in children under five years old [9], many rotavirus epidemiological studies have been carried out in this country. We have recently shown that in Paraguayan children: 1) rotavirus infections display an epidemic seasonal pattern (with the highest incidence during the coolest months of the year) [10,11], 2) the predominant G types (i.e. G1, G4, and G9), or their molecular variants, change from season to season and, 3) there is evidence of a cross-border migration of rotavirus strains between Paraguay and neighboring countries [12].

In this paper we report the epidemiological pattern of the rotaviruses detected in Asunción, Paraguay, from January 2004 to December 2005, evidence of minor differences between the incidence of rotavirus infection in children and adults, and a high incidence of rotavirus strains with a short electropherotype in children and adults.

MATERIAL AND METHODS

Sample collection

A total of 912 fecal samples from infants (up to 5 years of age) with acute diarrhea and 801 fecal samples from patients over 18 years of age (range: 18–95 years, mean: 40.8 years)

Table 1. Distribution by age group of rotavirus infection in Paraguayan adults with acute diarrhea between 2004 and 2005.

Age (years)	No. of rotavirus-positive samples *	No. of samples
18 to 27	37 (16.9%)	219
28 to 37	40 (19.0%)	211
38 to 47	27 (24.6%)	110
48 to 57	25 (27.5%)	91
>57	26 (15.3%)	170
Total	155	801

* No statistical differences were seen between the age groups ($p=0.078$).

with diarrhea were collected from the Clinical Laboratory of San Roque Private Hospital from January 2004 to December 2005. This study was approved by the Ethics Committee of the Research Institute of Health Sciences, National University of Asunción. The statistical analyses were carried out with the EpiInfo Program.

Molecular analysis

The screening for rotavirus-positive samples was performed by RNA extraction and polyacrylamide gel electrophoresis (PAGE), as described previously [10]. Differences in the mobility of RNA segments from different isolates were resolved by co-electrophoresis. To obtain the G types, rotavirus dsRNA was extracted directly by TRIZOL® (Invitrogen, USA) followed by a nested multiplex reverse transcription (RT)-PCR. *VP7* gene was amplified using a pair of generic primers (Beg9 and End9) [13]. Then a pool of internal primers: 9T1-1, 9T1-2, 9T1-3P, 9T1-4, 9T-9B, and 9Con1, was used [14]. The P types were obtained by a similar RT-PCR strategy using the primers Con2, Con3, 1T-1, 2T-1, 3T-1, 4T-1, and 5T-1 [15]. Amplicons were analyzed by electrophoresis in a 3% agarose gel, stained with ethidium bromide, and visualized under UV light.

RESULTS

Incidence, age group, and seasonality

The group A rotavirus incidence was 23.8% (217/912) and 19.4% (155/801) for children and adults, respectively. In adults, none of the age groups appeared to be more affected than the others (Table 1), whereas in children, taking into account the overall data, the most affected age group was that between 6 and 23 months of age ($p=0.001$) (data not shown). When examining the age groups of children by year, we found that in 2004 the most affected age group was that between 6 and 11 months of age ($p=0.005$) and in 2005 none of the age groups appeared to be more significantly affected than the others (Table 2). It is noteworthy that during 2005, most of the children affected by rotaviruses with a long electropherotype were between 6 to 23 months of age ($p=0.004$), whereas in the case of children infected with rotaviruses with a short electropherotype, none

Table 2. Distribution by age group of rotavirus infection in Paraguayan children with acute diarrhea between 2004 and 2005.

Age (months)	2004		2005		
	No. of rotavirus-positive samples (%)*	No. of samples	No. of rotavirus-positive samples (%)		No. of samples
			Long	Short	
0 to 5	3 (10.0%)	30	7 (11.7%)	5 (8.3%)	60
6 to 11	22 (32.4%)**	68	22 (25.0%)	9 (10.2%)	88
12 to 23	22 (21.6%)	102	29 (18.8%)	18 (11.7%)	154
24 to 35	12 (21.1%)	57	9 (9.9%)	8 (8.8%)	91
36 to 47	5 (12.2%)	41	4 (6.9%)	4 (6.9%)	58
48 to 60	9 (15.3%)	59	17 (16.3%)	12 (11.5%)	104
Total	73	357	88	56	555

* All the rotavirus-positive samples from 2004 display a long electropherotype;

** The age groups with greater incidence appear in bold ($p < 0.005$).

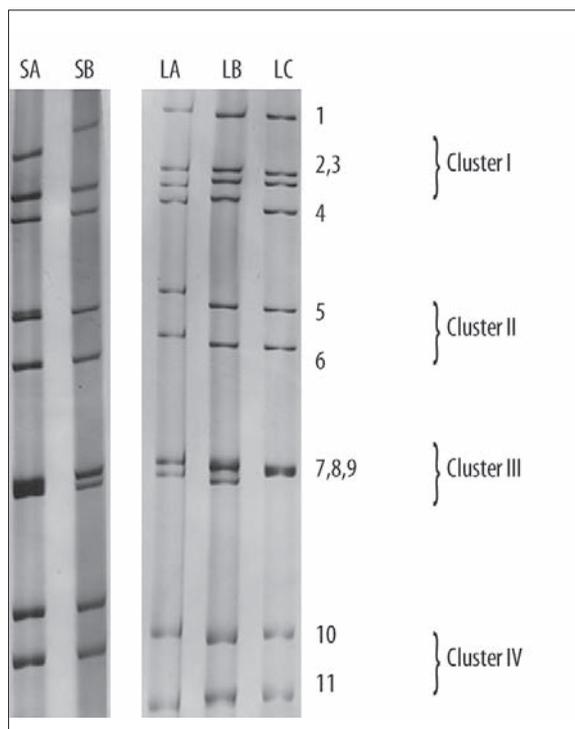


Figure 1. Gel electrophoresis showing the characteristic genomic double-stranded RNA migration pattern of group A rotavirus circulating in Paraguay from 2004 to 2005. Different short electropherotypes are denominated SA and SB and long electropherotypes LA, LB, and LC. The segments and clusters are indicated on the right.

of the age groups appeared to be more significantly affected than the others (Table 2).

The incidence of rotaviruses in Paraguayan children from 2004 to 2005 was seasonal, with the highest incidence during the coolest and driest months of the year. In 2004, the rotavirus epidemic peak started in June, with the highest incidence detected in August, and finished at the end of

October. However, in 2005 we found that the epidemic peak was shorter and delayed in terms of the appearance of cases of rotavirus infection in the infantile population, beginning suddenly in July and decreasing at the end of September.

Molecular characterization

Of the 372 group A rotavirus-positive samples, at least five different electropherotypes were detected; three of them, designated LA, LB and LC, presented a long electropherotype and the other two, designated SA and SB, a short electropherotype. Electropherotypes designated LA, LB, and LC showed differences in all clusters, whereas electropherotypes SA and SB presented differences only in segment 1 (cluster I) (Figure 1).

Electropherotype LC was the most frequently detected in children (71.4%, 155/217) during 2004 and 2005. In adults, the most frequently detected electropherotypes were LC (76.2%) in 2004 and SA (53.7%) in 2005 (Table 3). Although LC was predominant in children during 2005, SA was also detected at high frequency in this group (38.9%).

G and P typing of fourteen strains with a short electropherotype was carried out by RT-PCR. All the 14 samples with a short electropherotype tested (11 from children and 3 from adults) belonged to G2P[4]. The overall results of the G and P genotyping will be published elsewhere.

DISCUSSION

The high incidence of rotavirus in both children and adults agrees with previous studies published by our group [10,16]. Even though our previous work, which was carried out in Paraguayan adults, was performed with a latex agglutination kit for rotavirus detection with low sensitivity and specificity, the present study confirms the incidences reported previously [16]. The wide spectrum of ages in rotavirus-infected adults reported in Paraguay (Table 1) [16] suggests that multiple infections could occur over a person's lifetime. Therefore, since the real prevalence and importance of rotavirus infection in adults remain to be determined [17],

Table 3. Different electropherotypes of rotavirus detected in fecal samples in children and adults between 2004 and 2005.

Electropherotypes*	Children		Adults	
	2004	2005	2004	2005
LA	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
LB	5 (6.8%)	0 (0.0%)	2 (9.5%)	0 (0.0%)
LC	67 (91.8%)**	88 (61.1%)	16 (76.2%)	42 (31.3%)
SA	0 (0.0%)	56 (38.9%)	2 (9.5%)	72 (53.7%)
SB	0 (0.0%)	0 (0.0%)	1 (4.8%)	0 (0.0%)
ND***	0 (0.0%)	0 (0.0%)	0 (0.0%)	20 (14.9%)
Total rotavirus-positive samples	73	144	21	134

* Were assigned as LA, LB, and LC to the long migration patterns and as SA and SB to the short;

** The electropherotypes with greater incidence by year appear in bold;

*** Rotavirus-positive samples of undetermined electropherotype.

the results presented here reinforce the notion that rotavirus infections should be taken into account in the differential diagnosis of diarrheal diseases in adults.

The analysis performed on the rotavirus RNA migration pattern showed that in children infected with rotaviruses with a long electropherotype, the most infected age group was that between 6 to 23 months of age, whereas in children infected with rotaviruses with a short electropherotype, none of the age groups appeared to be more significantly affected than the others (Table 2). These results are in agreement with a study carried out in Italian children from 1993 to 1997 [18].

It has been hypothesized that acquired passive immunity against rotavirus infections might protect infants under 5 months of age, while children older than 23 months of age might have acquired immunity protection by previous infections [19]. Moreover, longitudinal and vaccine trial studies suggest a low heterotypic protection between rotavirus strains with a short electropherotype (G2P[4]) and a long electropherotype (G1, G3, G4 or G9 and P [8]) [20,21]. Therefore, the broad range of ages in Paraguayan children infected with rotaviruses with a short electropherotype could be explained by the exposure of an immunologically naive population to this new type of rotavirus.

Cascio et al. [18] showed that children infected with strains with a short electropherotype, G2P[4] type, and subgroup I presented more severe gastroenteritis than those infected with strains with a long electropherotype, G1P[8] or G4P[8] type, and subgroup II. Since we do not have enough clinical data to score the severity of patients' symptoms, a limitation of our study is the lack of correlation among the clinical symptoms, the infecting strain, and the age group affected.

It has been shown that the seasonality of the infection is not well defined in tropical zones, whereas in temperate zones (with well-defined seasons), rotavirus infections have been reported mainly during the coolest and driest months of the year [22,23]. For instance, in Venezuela and the north of Brazil the seasonality of the infection is not well defined, while in Argentina as well as in the central and southern

states of Brazil, seasonal patterns of rotavirus infections have been described [24–26].

As in previous years [10,11], a seasonal incidence of rotavirus infection was detected in Paraguay (which is situated in a tropical/subtropical zone) during 2004 and 2005. In 2004, the epidemic peak occurred from June to October, while in 2005 it was shorter, beginning in July and ending in September. In order to know whether climatic factors were the cause of this "shortening", we plotted the monthly distributions of mean humidity, mean temperature, the total number of collected samples, and the rotavirus-positive samples (data not shown). In fact, in May, June, and October of 2005 the mean temperature and/or humidity were higher than in previous years [27], and in July 2005 there was a decrease in mean temperature as well as in humidity, in accordance with the sudden increase in the number of rotavirus-positive samples detected. This finding reinforces the idea that the incidence of rotaviruses is influenced by meteorological conditions [22,23,28–30].

Another important finding of this study was the reappearance, at high numbers, of rotavirus strains with a short electropherotype in children. In our country, the short electropherotype had not been previously detected in adults, mainly due to the lack of studies carried out before, but in children, only three rotavirus strains with a short electropherotype were detected in 1999 [10,11,31].

The reappearance of rotaviruses with a short electropherotype in Paraguayan children could be linked to the high prevalence of G2P[4] rotavirus strains, usually associated to a short electropherotype, detected in two large Argentinean cities during 2004 (J. Stupka, personal communication). This hypothesis is supported by data from RT-PCR analysis of Paraguayan strains, which showed that the G2P[4] type was associated with a short electropherotype; consequently, the SA strains detected in our infantile population could have come from Argentina. It is interesting to note that G2P[4] strains were not detected in Rio de Janeiro, Brazil, during 2000–2004 [32,33], and only three (1.1%) G2P[4] strains were detected in São Paulo, Brazil, during the same period [34].

Since one of the rotavirus vaccines currently available (Rotarix®; G1P[8]) shares neither the G nor P type with the rotavirus G2P[4], usually associated with a short electropherotype [21], and epidemics caused by rotavirus with a short electropherotype appear sporadically [6–8], it would be important to monitor the prevalence of this strain after the introduction of the rotavirus vaccine in Paraguay.

CONCLUSIONS

Our study reinforces the importance of continuous epidemiologic surveys of rotavirus infection, extended to all age groups, and the monitoring of cross-border migration of rotavirus strains among neighboring countries in order to increase our knowledge about the complexity of rotavirus epidemiology in developing countries [2].

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