ACUTE DIARRHEA IN PARAGUAYAN CHILDREN POPULATION: DETECTION OF ROTAVIRUS ELECTROPHEROTYPES

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Summary. – Group A rotavirus infections were detected in 93 of 410 fecal samples from children with acute diarrhea, admitted in three main hospitals of Asunción, Paraguay, from August 1998 to August 2000. Most of the rotavirus-infected patients were admitted during the winter season in the three epidemic years. The rotavirus infection rate was highest in infants from 6 to 23 months of age. In the 93 samples examined, 10 different rotavirus electropherotypes were recognized, but two of them largely predominated. Only one sample showed a short electropherotype pattern, thus indicating a minor involvement of the rotavirus subgroup I in rotaviral acute diarrhea in the area and the time during which the survey was carried out.

Key words: group A rotavirus; electropherotypes; infantile diarrhea

Introduction

Rotavirus is the major etiologic agent of acute diarrheal disease in infants and young children (Kapikian and Chanock, 1996). The rotavirus genome contains 11 segments of double-stranded RNA (dsRNA). The migration patterns of the RNA segments in polyacrylamide gel electrophoresis (PAGE), called the electropherotypes, have been useful for diagnosis and molecular epidemiological studies of rotavirus infection (Estes *et al*., 1984). On the basis of the antigenic properties of the VP6 and the electropherotypes, rotaviruses have been classified into groups A to G (Parashar *et al*., 1998). Group A rotaviruses are the most pathogenic and prevalent in humans and in various animal species. This group may exhibit two migration patterns on the basis of the relative mobility of the RNA segments 10 and 11, namely long and short. In human rotaviruses, the short pattern has been shown to correspond to a subgroup called I, and the long pattern to the subgroup II. Both long and short electropherotypes may also exhibit different mobility patterns if one regards the other segments (Estes, 2001; Kalica *et al*., 1981). The identification of RNA electropherotypes of rotavirus strains may provide evidence of the coexistence of different strains, the appearance of new strains or the disappearance of old ones in a community over a period of time (Rodger *et al*., 1981; Ruggeri *et al*., 1989). In Paraguay, acute diarrheal disease is the third cause of mortality in children between 1 and 4 years of age (Dirección General de Planeamiento y Evaluación, 1999). Epidemiological studies carried out in children with acute diarrhea in Asunción, Paraguay, have shown the presence of rotavirus in 23.7–33 % of the cases (Chamorro, 1984; Achucarro *et al.,* 1989). Recently, Coluchi *et al*. (2002) have found that 70 of 220 samples (31.8%) taken from children with diarrhea during the epidemic year 1999 in Asunción were positive for rotavirus. All of the samples examined by Coluchi's team showed the typical pattern of rotavirus group A. In the present work we report the occurrence of a rotavirus infection and the study of their electropherotypes in 410

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Abbreviations: IPS = Instituto de Previsión Social; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate children with acute diarrheal disease admitted to three main hospitals in Asunción, Paraguay, during three consecutive epidemic years (1998–2000).

Patients and Methods

Patients. Fecal samples from 393 infants (up to 4 years of age) with acute diarrheal disease were routinely collected between August 1998 and December 1999. No samples were collected in April 1999, although patients with acute diarrheal disease were admitted to the hospitals during that month. Out of the 393, 286 were collected at the Hospital de Clinicas, 80 at the Main Hospital of the IPS (both public hospitals), and 27 at the San Roque Hospital (private hospital). In the year 2000, a group of 17 fecal samples previously diagnosed as rotavirus-positive by the latex agglutination assay, were collected at the San Roque Hospital for electropherotype analysis. All fecal samples were frozen and stored at -20°C until processing.

RNA extraction. A 10–20% fecal suspension in 400 ml of phosphate-buffered saline pH 7.0 (PBS) was treated with 1% sodium dodecyl sulfate (final concentration); and subsequently deproteinized with an equal volume of phenol-chloroform (1:1) by vortexing and centrifugation at 12,000 x g for 10 mins. RNA was ethanol-precipitated overnight at -20°C. After centrifugation at 12,000 x g for 10 mins, the pelleted RNA was dissolved in the sample buffer (0.0625 mol/l Tris-HCl pH 6.8, 5 mol/l urea, 5% 2-mercaptoethanol, 3% ASDS and 0.01% Bromophenol Blue) for 10 mins at 56°C.

PAGE. The electrophoretic separation of dsRNAs was performed on a 7.5% polyacrylamide gel with a 4.5% stacking gel as described by Laemmli (1970). After electrophoresis for 2 hrs the gels were silver-stained (Sanguinetti et al., 1994). All samples in which RNA extraction and PAGE were rotavirus-positive were run simultaneously on another gel to compare their electropherotypes with those of other rotavirus strains.

Results and Discussion

Rotavirus was detected by PAGE in 76 out of 393 fecal samples collected from August 1998 to December 1999, covering all months during this period, except for April 1999.

The number of samples positive for rotavirus, distributed per month, is shown in Fig. 1. Rotavirus infection showed a seasonal pattern, with the highest frequency during the coolest months of the year; i.e., between July and September, a typical feature of subtropical regions of the South hemisphere (da Silva Domingues *et al*., 2000; Rodger *et al*., 1981). The distribution of rotavirus infection for different age groups is shown in Table 1. The age group ranging from 6 to 23 months showed the highest infection rate, while the lowest infection was observed in infants from 0 to 5 months and 2 to 4 years of age. Passively acquired immunity against rotavirus infection may account for the apparent relative resistance in the group of infants under 5 months, while the group of 2 to 4 years of age might have acquired immunity by previous infections (Cubitt *et al*., 2000; Espinoza *et al*., 1997; Parashar *et al*., 1998). In accordance with several previous reports (Ardern-Holmes *et al*., 1999; Ryan *et al*., 1996); the infection rate for males (59.1%) was higher than that for females (38.7%).

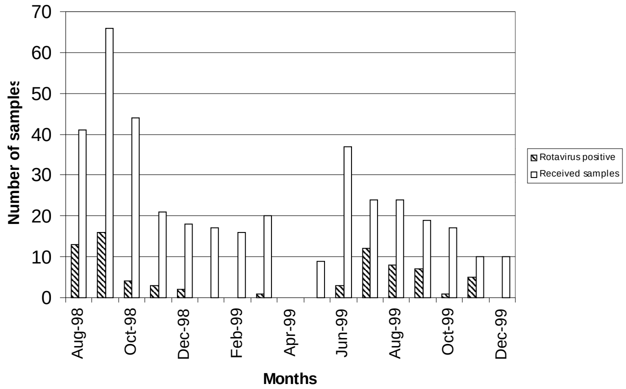

Fig. 1

Monthly variation of rotavirus-positive samples collected from August 1998 to December 1999

Only 62 out of the 93 positive samples (66.7%) were electropherotyped due to the small amount of the rest of the samples. The rotavirus strains detected showed a typical 4-2-3-2 gene segment pattern, which is characteristic for group A rotaviruses. Among the 62 samples 10 electropherotypes were distinguished, as shown in Fig. 2, where only predominant electropherotypes are shown. All of them belonged to the long pattern except for only one sample, as shown in Fig. 3. The electropherotypes designated LA and LE were the most frequent. Apparently, rotaviruses of the electropherotypes LA and LE coexisted in the population of Asunción during 1998. However, in 1999, the electropherotype LA was not detected and the electropherotype LE predominated at the beginning of the epidemic period. At the end, however, five less frequent electropherotypes were present. Interestingly, in 2000, the electropherotype LA reappeared and was found in 10 of the 17 rotavirus samples collected during the coolest months. These samples were taken at the private San Roque Hospital. Based on the electropherotypes alone we cannot conclude that strains that disappeared in 1999 reappeared in 2000. However, it is clear that the electropherotype LA predominated during the epidemic in 2000. On the other hand, the presence of a single predominant RNA electropherotype with minor variants, the appearance of new strains and the disappearance of the formers are known to be typical features of rotavirus epidemiology (De Sierra *et al*., 2002; Konno *et al*., 1984; Rodger *et al*., 1981; Ruggeri

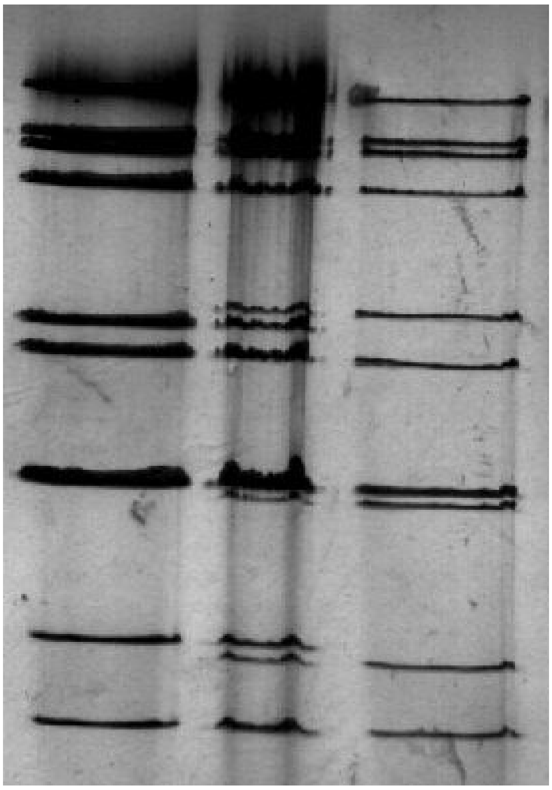

Fig. 2

Infections with rotaviruses with long electropherotypes and with rotaviruses with mixed electropherotypes

LA, LE = long electropherotypes. M = mixed electropherotype.

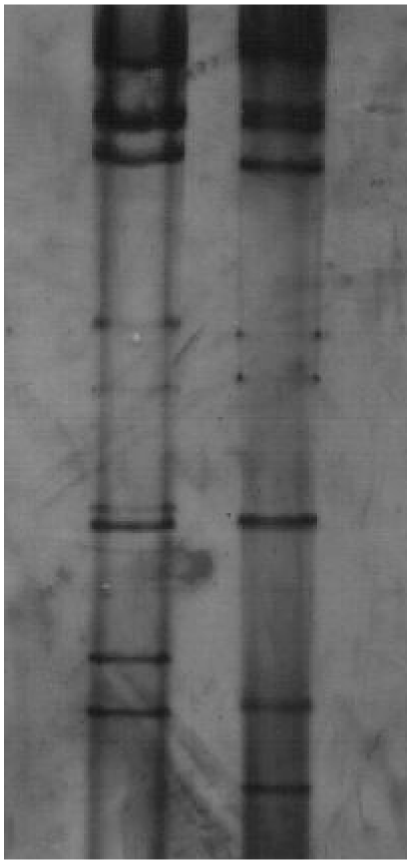

Fig. 3

Infections with rotaviruses with long and short electropherotypes

SF = short electropherotype. LE = long electropherotype.

et al., 1989; Superti *et al.*, 1995). Some published studies performed in short periods of time allowed detection of only long electropherotypes (Hortal *et al.* 1986; Ruggeri *et al.*, 1989). Further studies may allow us to find variation between long and short electropherotypes, since we have found only one short electropherotype among the 93 positive samples. De Sierra *et al.* (2002) have found predominance of the long electropherotype for many epidemic years, alternating with the short electropherotype. One out of the 93 positive samples (nearly 1%) showed more than 11 segments of rotaviral RNA (Fig. 2), which suggested a co-infection (mixed infection) with different rotavirus strains. In many cases where a large number of samples were examined, the presence of mixed rotavirus electropherotypes have been reported (Rasool *et al.*, 1989; Spencer *et al.*, 1983; Tietzová *et al.*, 1995). This fact indicates the probability of genetic reassortment in an individual infected with two or more viral strains. Our study reinforces the notion that RNA electropherotyping represents a powerful tool for diagnosis, differentiation of subgroups and molecular epidemiological studies of rotaviruses (Watanabe *et al.*, 2001).

Table 1. Distribution of rotavirus infection by age in children with acute diarrhea from August 1998 to December 1999

Age group (months)	No. of positive samples/No. of tested samples (%)
0 to 5	10/81 (12.3)
6 to 11	22/101 (21.8)
12 to 23	30/120 (25)
24 to 35	6/44 (13.6)
> 36	4/43 (9.3)
ND[a]	4/4 (100)

[a]ND = no data were available.

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