

## An Outbreak of Diarrhoea Associated with Rotavirus Serotype 1 in a Day Care Nursery in Rio de Janeiro, Brazil

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*Faeces from 17 children less than 1.6 years old and 15 adults more than 22 years old were collected during an outbreak of gastroenteritis in a day care nursery and screened for the presence of adenovirus and rotavirus by enzyme immunoassay (EIARA) and other viruses by electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE). Ten samples (58.8%) from children and one (6.7%) from adults were positive for rotavirus and all samples were negative for bacteria and parasites. No other viruses were observed in EM. An enzyme immunoassay test using monoclonal antibodies (Mab-EIA) to determine the subgroup(s) and the serotype(s) of rotavirus was performed and the results showed that all positive samples belong to serotype 1, subgroup II of group A rotaviruses. In PAGE test all samples had the same profile and the 10 and 11 dsRNA segments corresponded to the "long" profile of group A of rotaviruses. These results corroborated the Mab-EIA results and indicate a sole source of infection. The major symptoms observed were: vomiting (60%), fever (70%) and diarrhoea (100%). In previous years (1989 to 1991) we observed only rotavirus serotype 2 in this same day care nursery, but no outbreak was reported.*

Key words: diarrhoea – outbreak – rotavirus – serotype 1

Rotaviruses are now well established as the most frequent viral pathogen detected in cases of acute gastroenteritis in children under five years of age in both developed and developing countries. In developed countries, rotaviruses are responsible for 40 to 60% of cases of severe dehydrating diarrhoea, although deaths are rare. In the developing countries, rotavirus is an equally important pathogen, and deaths from dehydration are common (Snyder & Merson 1982, Ho et al. 1988, Gangarosa et al. 1992). Groups A, B and C rotaviruses have been found in stools of both humans and animals (Penarand et al. 1989, Ushigima et al. 1992), groups D, E, and F rotaviruses have been associated with yearly epidemic of diarrhoea in adults and children in China (Fang et al. 1989). Twelve human serotypes of group A rotavirus have been described. In Japan, major human rotaviruses are group A, and few outbreaks or sporadic infections due group C have been reported (Bridger et al. 1986, Beards et al. 1992). While the detection of rotaviruses by enzyme immunoassay (EIA) and polyacrylamide gel electrophoresis (PAGE) has

become a simple routine procedure, the serotyping of individual strains can provide additional insights into the epidemiological features of rotavirus diarrhoea. These epidemiological studies are very important because a number of candidate vaccines have been developed and tested, but the degree of protection afforded by each vaccine has varied greatly. One of the factors affecting the efficacy of candidate vaccines is the serotype or subtypes of rotaviruses circulating naturally in the communities. Although several reports on the epidemiology of rotavirus serotypes and subtypes in different settings have appeared in recent years, little is known about differences in the distribution between countries during the same year and within the same country from year to year (Matsui et al. 1989, Nakagomi et al. 1989).

Studies on the etiology of acute gastroenteritis in Rio de Janeiro and other States of Brazil, carried out during the last ten years, demonstrated the presence of rotaviruses, enteric adenoviruses and astroviruses. Rotaviruses are the most common viruses found in the diarrhoeic faecal specimens (Pereira et al. 1983 a, b, 1993, Leite et al. 1985, 1991, Linhares et al. 1989, 1992).

In the present report we describe an outbreak of diarrhoea in the day care nursery "Berta Lutz" at the Oswaldo Cruz Foundation, Rio de Janeiro, caused by rotavirus serotype 1, subgroup II.

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### MATERIALS AND METHODS

For virological analysis, faeces from 17 children under 1.6 years of age and 15 adults more than 22 years old were obtained between May 19-25/1992. Ten percent of faecal suspensions were prepared in 10 mM Tris, 15 mM CaCl<sub>2</sub>, pH 7.4, clarified by centrifugation at 2,000 g/10 min/4 °C and tested by EIARA (Pereira et al. 1985). Simultaneously, polyacrylamide gel electrophoresis and electron microscopy (EM) were performed as described elsewhere (Leite et al. 1985, Pereira et al. 1983).

Rotavirus-positive samples were tested by enzyme immunoassay using monoclonal antibodies (MAb-EIA) to determine the subgroup(s) and serotype(s). Both tests were performed in polyvinyl chloride microplate (Dynatech Laboratories Inc., Alexandria, USA). Standard dilution (1/10,000) of sheep anti-rotavirus capture serum (EIARA test) was used and, as top sera: (i) for subgroup test – MAb 255/60 (subgroup I) and MAb 631/9 (subgroup II), diluted in conditions previously described (Greenberg et al. 1983) and kindly supplied by Dr Vera Gouvea (FDA/NHI, USA); (ii) for serotyping test, we used the MAb to rotaviruses serotypes 1 to 4, purchased from Silenus Laboratories PTY LTD (Australia), and used at dilution of 1/1,000. Rabbit anti-mouse IgG conjugated with Horseradish peroxidase was obtained from Sigma Laboratories Inc. (USA) and used at a dilution of 1/1,000. Samples giving OD readings higher than at least twice as high as conjugate controls were taken as positive.

For bacteriological analysis, swabs and faecal samples were cultured for identification of *Salmonella* sp., *Shigella* sp. and enteropathogenic *Escherichia coli* (EPEC, ETEC) by the use of

enrichment media (Tetrathionate Kauffmann and Silliker medium) followed by the use of the selective media Holt Harris Teague and Hektoen Enteric Agar. About five and ten colonies were transferred to the Costa and Vernin Medium (Costa & Hofer 1972). The biochemical and antigenic characterization was in accordance with the methods described by Edwards and Ewing (1986).

For parasitological analysis, the stool samples were collected in polypropylene bags containing merthiolate-formalin preservative. Concentration of parasites was done by Willis brine flotation and by Hoffman, Pons & Janer's gravity sedimentation method (Beaver et al. 1984).

### RESULTS

The presence of rotavirus was demonstrated by EIARA, PAGE and/or EM in 10 (58.8%) of 17 faecal tested samples from 17 children and one (6.7%) of 15 faecal samples from 15 adults (Table I). PAGE revealed the same profile in all samples and the 10 and 11 dsRNA segments corresponded to the "long" profile, typical of group A, subgroup II (Fig.). The subgroup MAb-EIA test corroborated the PAGE observations and the serotyping MAb-EIE test showed that all positive samples belonged to the serotype 1 (Table II). No viruses other than rotavirus were detected by EM, EIARA or PAGE techniques. All samples were negative for both parasites and pathogenic bacteria. Therefore, rotavirus was the unique pathogen observed in the positive faecal samples. Major clinical symptoms observed among rotavirus positive children were: fever (70%), vomiting (60%) and diarrhoea (100%) (Table I). Neither respiratory nor other clinical symptoms were observed.

TABLE I

Rotavirus positive samples and major clinical symptoms

			Major clinical symptoms		
			Diarrhoea	Vomiting	Fever
Children	1.6 years 17 <sup>a</sup>	Pos. 10(58.8%) Neg. 7(41.2%)	10(100%) 3(42.8%)	6(60%) wt	7(70%) wt
Adults	22 years 15	Pos. 1(6.7%) Neg. 14(93.3%)	1(100%) 1(7.1%)	wt wt	wt wt

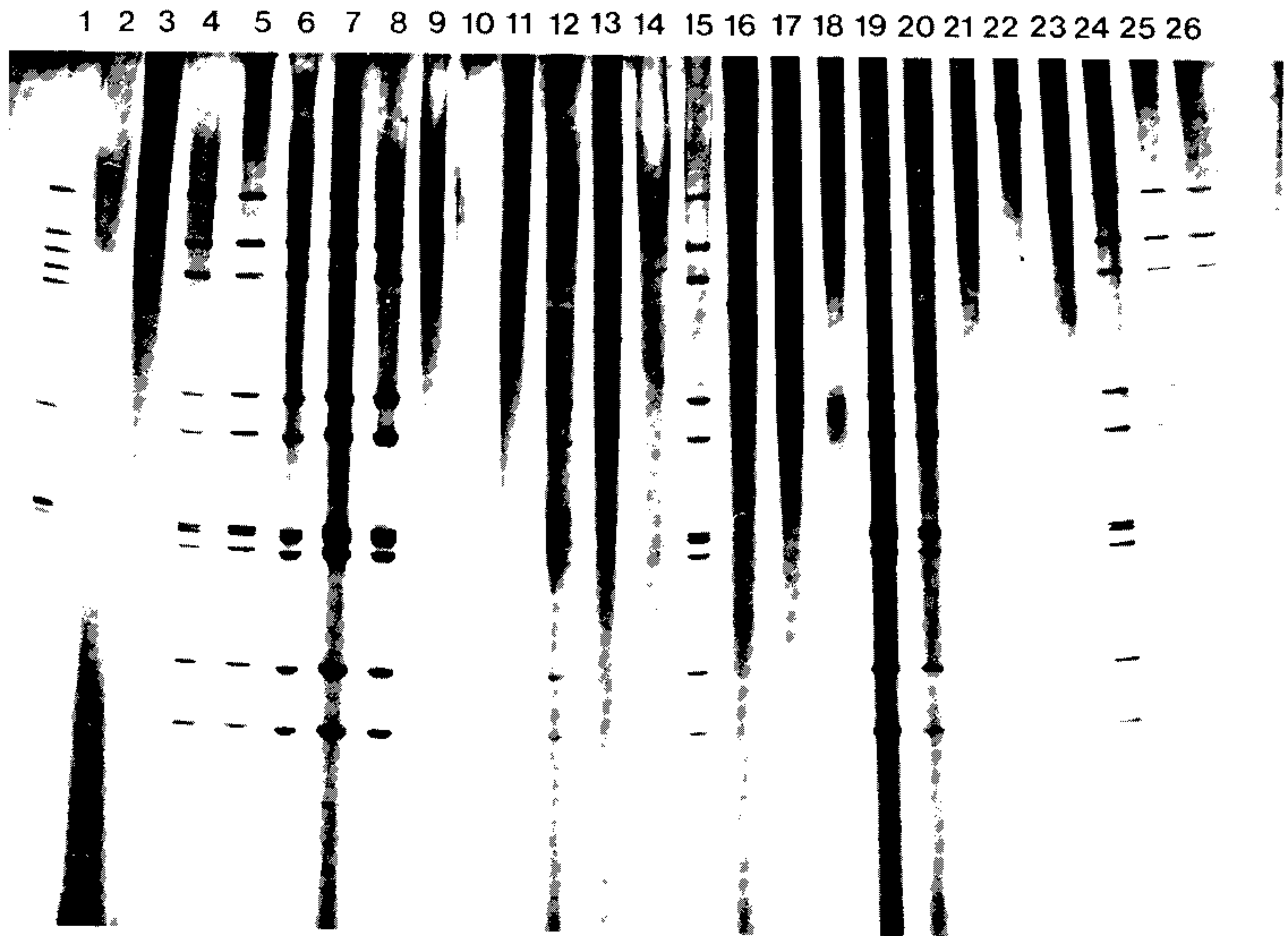
a: four faecal samples were obtained from one child during four different days. All positive samples contained only rotavirus.

wt: without.

TABLE II  
Prevalence of rotavirus serotypes in a day care nursery in Rio de Janeiro

No. Samples/Date	Positives	Negatives	Serotypes	Subgroup
22/1989 <sup>a</sup>	2(9.0%)	20	2	II
05/1990 <sup>a</sup>	4(40.0%)	3	2	II
15/1991 <sup>a</sup>	5(33.3%)	10	2	II
17/1992 <sup>b</sup>	10(58.8%)	7	1	II
15/1992 <sup>c</sup>	1(6.7%)	14	1	II

*a*: 1989 to 1991 all faecal samples were obtained from children.  
*b*: faecal samples were obtained from children.  
*c*: faecal samples were obtained from adults.



PAGE of nucleic acids extracted from faecal samples. 1: rotavirus SA-11 (mutant with abnormal segments 4 and 5). 2-3, 9-11, 13, 16-18, 21-23: negative samples. 4-8, 12, 14-15, 19-20, 24-26: positive samples.

DISCUSSION

Studies in Rio de Janeiro, carried out during the past ten years, have demonstrated the presence of rotaviruses, adenoviruses and astroviruses, associated with acute gastroenteritis in children under five years old (Pereira et al. 1983 et al. 1983 a, b, Leite et al. 1985, 1991). In our studies, we have shown that, as reported for other states of Brazil and other countries, human rotaviruses are the most common aetiologic agent

of acute diarrhoea in infants and young children (Brown et al. 1988, Beards et al. 1989, Linhares et al. 1989, 1992, Gomez et al. 1990, Bingnan et al. 1991, Chakravarti et al. 1992, Pereira et al. 1993).

In the present report we describe an outbreak of diarrhoea in a day care nursery where rotavirus serotype I was the unique entero-pathogen observed. Ten (58.8%) out of 17 faecal samples from 17 children and one (6.7%) out of 15 faecal samples from 15 adults were positive for

rotavirus by EIARA, PAGE and/or EM tests (Table I). All samples had the same profile in PAGE technique (Fig.), suggesting a unique source of infection. In this day care nursery, all children under 1.6 years old stay together in the same area with a private toilet and bathroom. They have no contacts with other groups including older children. Only the nurse and the principal have access the all groups. The principal is a 37 years old woman and the only rotavirus positive adult who suffered severe diarrhoea starting four days after her four years old son, had a similar disease at a separate school. The other adults are in charge of preparing food and cleaning of all children. From 1989 to 1991 we observed the endemic presence of rotavirus mainly of serotype 2 in this day care nursery (Table II). In our previous studies we demonstrated that rotavirus seasonality is reflected by a peak through May-July (Pereira et al. 1993).

In Rio de Janeiro and other cities of Brazil we observed the prevalence of rotaviruses of subgroup II, serotypes 1 and 2 (De Castro et al. manuscript in preparation, Linhares et al. 1988, Pereira et al. 1993). These results are in accordance with observations in several other countries (Brown et al. 1988, Beards et al. 1989, Gomez et al. 1990, Bingnan et al. 1991, Chakravarti et al. 1992, Woods et al. 1992).

This is the second outbreak of rotavirus gastroenteritis reported in Rio de Janeiro during the last ten years. The first (Sutmoller et al. 1982) occurred in a private school and was associated with both rotavirus subgroup I and *Shigella sonnei*.

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