

## ROTAVIRUS ASSOCIATED DIARRHOEA DURING INFANCY IN THE CITY OF S. LUÍS (MA), BRAZIL: A TWO-YEAR LONGITUDINAL STUDY

Klaus Eberhard STEWIEN(1), Luís C.F. da CUNHA(2), Aymoré de C. ALVIM(2), Stelito A. dos REIS FILHO(2), Maria A.B. ALVIM(2), Alice A.P. BRANDÃO(2) & Maria de N.R. NEIVA(2).

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### SUMMARY

A total of 479 diarrhoeic children and 337 children without diarrhoea (controls) less than 5 years old were investigated in a two-year study in the city of S. Luís (MA), with the purpose to determine the incidence, the age distribution and the seasonality of rotaviruses, as well as to establish the severity of the disease in this region between the North and the Northeast of Brazil. rotavirus incidence was highest in children of the 1st. year of life, showing an average of 25% per year among the diarrhoeic patients attending the two main hospitals and three health units at the periphery of the city. It was shown that rotaviruses are significant enteropathogens in children less than 18 months old. Frequency of rotaviruses dropped in diarrhoeic patients 18 to 23 months old to only 4%, the same percentage observed in children of the control group. A typical seasonal distribution of rotaviruses was not seen during the two years of study. There was a peak in the incidence of rotaviruses in 1986, during the rainy season, and two peaks in 1987, one in the rainy season and one in the dry season. It was also shown that severity of diarrhoea in rotavirus positive cases was higher than in the negative cases. Rotavirus diarrhoeic patients had more loose stools per day, and higher frequencies of vomiting and fever, resulting more often (> 2 times) in moderate or severe dehydration. Finally, it is concluded that the introduction of immunoprophylaxis may reduce significantly the high mortality rates in early childhood observed in S. Luís.

**KEY WORDS:** Rotavirus; Diarrhoea; Seasonal distribution.

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### INTRODUCTION

In developing countries acute diarrhoea is the major cause of death and one determinant of malnutrition of children under 5 years of age<sup>9, 10, 11, 12, 16</sup>. Surviving children due to repeated intestinal infections, frequently do not attain their normal growth and development or present chronic malnutrition, a condition that eventually ends up in death<sup>10, 17</sup>. In Brazil infant mortality is highest in the Northeastern Region, where more than 14% or the children die during the first 5 years of life. More than 50% of all such recorded deaths are due to acute diarrhoea as the main or an associated cause of death.

Longitudinal studies carried out in several parts of the world have shown that rotavirus usu-

ally is the main etiologic agent of acute diarrhoea in children up to two years of age, when infant mortality is highest<sup>1, 3, 4, 7, 14</sup>. The development of a rotavirus vaccine should decrease the high diarrhoeal mortality rates observed in developing countries<sup>2, 15</sup>. To direct such vaccine development, information on the importance of rotavirus in different parts of the world is needed.

This paper presents the findings of a 2 year longitudinal study in S. Luís, in the State of Maranhão, with the purpose to establish the incidence, age and seasonal distribution, as well as the severity of acute diarrhoea caused by rotavirus in the region which represents the transition between Brazil North and Northeast.

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(1) Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 1374. 05508 São Paulo, SP, Brazil.

(2) Center of Health Sciences, University of Maranhão, São Luis, MA, Brazil.

## MATERIAL AND METHODS

### Study area

S. Luis city, capital of the State of Maranhão (MA), is located in an island by the same name near the continent, measuring 988 square km. The topography is usually flat with several beaches in the north, quite a number of swamps and two rivers, Anil and Bacanga, the latter partially dammed. It has tropical climate (hot and humid), with temperatures varying from 25.2 degrees C to 30.3. Air humidity is high all year round, usually above 80%. Rainy season comes from January to June. The so-called Winter ends up in June. The socio-economic level of the population (around 500,000 inhabitants as 1985) is mainly low.

### Study population

Stools of 479 children with acute diarrhoea and 337 without diarrhoea or respiratory symptoms (controls) from 0 to 5 years of age were obtained when they were seen at the out-patient clinics of the two main Children's Hospitals located at the central zone of the city and at 3 peripheral health units, representing a random sample of a population easily reached. The hospitals (Hospital Infantil do Estado do Maranhão and Hospital Materno-Infantil do INAMPS) receive the majority of diarrhoea cases in city of S. Luis (MA). Stool samples were collected daily (4 to 5 times a week) from the 1st child seen at the out-patient clinic of one of the hospitals or at one of the peripheral health units. The following information was then obtained, such as name, date of birth (age), sex, address as well as signals and symptoms of disease (WHO criteria), degree of dehydration, aspect of stools and type of treatment (oral or intravenous). All children belonged to the low socio-economic class.

### Stool samples

Stool samples were collected daily from each child with or without a rectum bougie, and removed in dischargeable/sterile flasks (NUNC, Denmark) to the Health Sciences Center Virology Laboratory under refrigeration. All samples were stored at -20 degrees C up to 3 months during which time the tests were carried out.

### Laboratory tests

All specimens were tested for the presence of

rotavirus by a latex agglutination assay (Slidex Rota-Kit, Biolab-Merieux France) and by the direct double antibody sandwich enzyme-linked immuno-assay - ELISA (Dakopatts, Dako Corp., Denmark), described by GRAUBALLE et al., 1981<sup>5</sup> and by GRAUBALLE & JARZABEK, 1984<sup>6</sup>. Both tests were run according to the manufacturer's instructions. In the ELISA, alternate wells of polystyrene 96-well plates were coated overnight at 4 degrees C with a 1:50 dilution of hyperimmune rabbit anti-human rotavirus antibody and normal rabbit immunoglobulin. Plates were washed five times with a hand-hold wash apparatus (Immunowash, Nunc, Denmark) and faecal specimens, prepared as 10% suspensions were added to duplicate wells coated with anti-rotavirus and control sera.

After incubation for 2 h at 37 degrees C, the specimens were aspirated and the plates were washed as described above. Next steps were performed as described in the test procedures of the kit. Absorbance in each well was measured at a wavelength of 490 nm on a microplate reader (Dynatech Microplate Reader, MR 600, USA). A specimen was considered positive if the absorbance of the test wells minus the absorbance of the control wells was greater than 0.1 and the absorbance of the test wells divided by the absorbance of the control wells was greater than 6.

Data presented in Tables and Figures are based on the results achieved by the ELISA, because this assay is more sensitive than electron microscopy and is comparable to the indirect enzyme immuno-assay used as the World Health Organization reference standard<sup>5,6</sup>.

## RESULTS

### Rotavirus detection rates

Human rotavirus was detected by the immunoenzymatic assay (ELISA) in 19% (90/479) of the 479 children under 5 years of age suffering from acute diarrhoea, as shown in Table 1.

In the same period children without diarrhoea (control group) presented only 6% (21/337) of rotavirus positive results by ELISA. Rotavirus frequency was 24% (68/280) among patients attended at hospitals (out-patient clinic), against 11% (22/

TABLE 1

Rates of detection of Rotavirus among children under 5 years of age, by latex agglutination and by ELISA. S. Luís (MA), Brazil, May 1986 - April 1988.

Children examined	Total N°studied	Latex agglutination		ELISA	
		N° positive	%	N° positive	%
<b>With diarrhoea</b>					
- 1st. year	248	45	18	46	19
- 2nd. year	231	42	18	44	19
- Total	479	87	18	90	19
<b>Without diarrhoea</b>					
- 1st. year	171	10	6	11	6
- 2nd. year	166	8	5	10	6
- Total	377	18	5	21	6

TABLE 2

Rotavirus incidence among children with and without acute diarrhoea, according to age group. S. Luís (MA), Brazil, 1986 to 1988.

Age (months)	Children with diarrhoea		Children without diarrhoea	
	N° *	%	N° *	%
0 - 5	30 (124)	24	4 (51)	8
6 - 11	39 (148)	26	9 (99)	9
12 - 17	14 (95)	15	2 (59)	3
18 - 23	1 (23)	4	1 (25)	4
24 - 59	6 (88)	7	5 (103)	5
<b>TOTAL</b>	<b>90 (479)</b>	<b>19</b>	<b>21 (337)</b>	<b>6</b>

\* N° - Number of positives (total number of examined children).

199) among the patients treated at the peripheric Health Units (data not shown).

### Age distribution

Table 2 shows that rotavirus incidence is highest in age groups of 0 - 5 months and 6 - 11 months when diarrhoea patients presented 24% and 26%, respectively, with an average of 25% (69/272) in patients of the first year of life. Children without diarrhoea (< 1 year) had an incidence of 9%, only. One can also see that the incidence of rotavirus decreases among children aged 12 - 17 months, reaching 15% and 3%, respectively for patients and control groups. In the age group of 18 - 23 months the incidence of rotavirus was the same for patients and control groups (4%). Among children 2 years old or above, patients presented 7% and control groups 5% of positivity for rotavirus.

### Seasonal distribution

Figure 1 shows the bimonthly distribution of rotavirus detected by patients with diarrhoea. One can observe the presence of rotavirus throughout the study period. Prevalence peaks occurred in 3 different times: May/June 1986, March to June 1987 and November/December 1987.

### Clinical symptoms and treatment

Table 3 compares positive and negative cases according to its main symptoms and treatment re-

ceived. Complete records could be obtained from a total of 415 patients with acute diarrhoea. One can observe among the rotavirus positive cases higher frequency of daily evacuations, vomiting, fever and moderate or severe dehydration. More than 85% of both groups of patients were treated by oral rehydration (ORT).

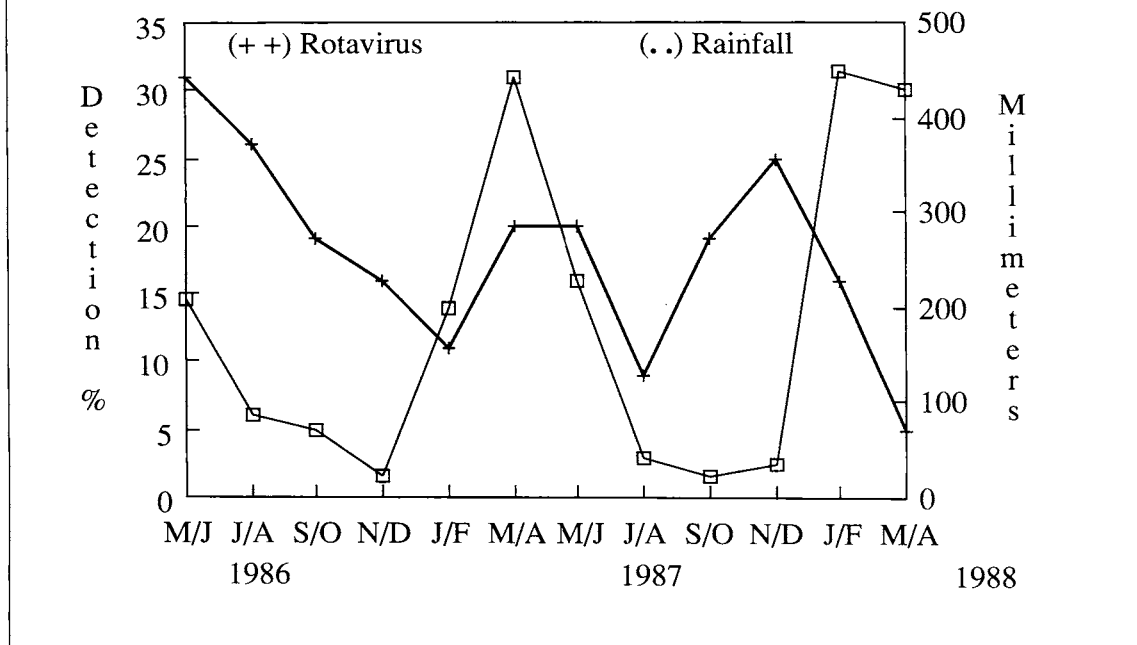
TABLE 3

Clinical symptoms and treatment of 415 children with acute diarrhoea. S. Luís (MA), Brazil, 1986 and 1988.

	Patients With Rotavirus (n = 78)		Without Rotavirus (n = 337)	
	N°	%	N°	%
<b>Symptoms</b>				
- Number of evacuations				
2 - 3/daily	15	19.3	151	46.3
4 and plus/daily	63	80.7	181	53.7
- Vomiting	44	56.4	127	37.7
- Fever	48	61.5	125	37.1
<b>Dehydration</b>				
- absent or mild	40	51.3	262	77.7
- moderate or severe	38	48.7	75	22.3
<b>Treatment</b>				
- ORT	67	85.9	303	89.9
- Intravenous	11	14.1	34	10.1

N° - Number of positives.  
n - Total number of examined children.

**Figure 1**  
**Seasonal fluctuation in the incidence of rotavirus detected in children with diarrhoea and rainfall given in millimeters from May 1986 to April 1988**



### DISCUSSION

Results of this 2 year longitudinal study carried out in the city of S. Luís (MA) show that rotavirus have an importante role in the etiology of acute diarrhoea disease during childhood. Rotavirus was identified in 25% of patients (< 1 year of age) treated in the two main children hospitals and in health units of the city periphery (Table 1). Rotavirus positive cases were more frequent at the hospitals that receive more severe cases, than at Health Units in the peripheric area of the city (24% against 11%) for children < 5 years of age.

As shown in Table 2, incidence of rotavirus was very similar among children of age groups 0 - 5 months and 6 - 11 months, 24% and 26% among patients against 8% and 9% among controls, respectively. Rotavirus incidence dropped drastically among patients aged 12 - 17 months (15%); it was reduced to only 4% for the age group 18 - 23 months, as well as for the control groups. It should be emphasized that the number of children aged 18 - 23 months was low in hospitals and health units

during all the period of study. These results show that the high circulation of rotavirus ceases in the community when infants living in that region became 18 months old.

Other studies have shown less drastic decrease in the age distribution of rotavirus in childhood<sup>1, 3, 7, 14</sup>, except that one referred by GEORGE et al., 1984<sup>4</sup>, in Bangui, Central African Republic.

The progressive decrease in the proportion of diarrhoea cases of children under 5 years of age could be ascribed to the acquisition of immunity against the disease. Thus, one could hope that immunoprophylaxis for the control of acute diarrhoea caused by rotavirus will be a success.

Figure 1 shows that the bimonthly distribution of rotavirus did not present a typical seasonal profile during the study period, presenting differences from 1986 to 1987. A peak of rotavirus incidence was observed during 1986 rainy season and two peaks in 1987, one in the rainy season and another

at the end of the dry season. This phenomenon was also observed by STINTZING et al. (1981), in Addis Ababa<sup>13</sup>. These findings show that the rotavirus incidence can vary from one year to the next in regions of tropical climate and is not necessarily related to rain precipitation.

Among the clinical symptoms (Table 3), it is worth emphasizing that the frequency of daily evacuations, vomiting and fever is higher among cases of diarrhoea caused by rotavirus than among clinical cases from other causes. It is also worth commenting that the frequency of cases followed by moderate or severe dehydration was more than double among rotavirus positive children, reaching 48.7%. These findings corroborate previous observations of LINHARES et al.<sup>8</sup> 1985, in Belém (PA). The high number of dehydration present in cases of acute diarrhoea caused by rotavirus in this region are certainly due to its high temperatures and high rates of poverty and malnutrition. Studies carried out by BLACK et al.<sup>2</sup> 1981, in Bangladesh found out that acute diarrhoea caused by rotavirus or enterotoxigenic *E. coli* is responsible for 77% of the cases of severe dehydration in children under 5 years of age. One conclusion could be drawn from these findings: vaccine prophylaxis programmes carried out in regions where these agents present high incidence as observed in S. Luís would permit a drastic reduction of childhood mortality rates.

## RESUMO

### Diarréia aguda associada a rotavírus durante a infância na cidade de São Luís (MA). Estudo longitudinal de 2 anos.

Um total de 479 casos de diarréia aguda e de 337 crianças sem diarréia (controles) foi investigado em um estudo longitudinal de 2 anos na cidade de São Luís (MA) em crianças menores de 5 anos de idade, a fim de estabelecer a incidência e a distribuição etária e sazonal de rotavírus, bem como determinar a severidade da doença nesta região do Brasil. A incidência de Rotavírus apresentou os maiores índices durante o primeiro ano de vida das crianças, com média anual de 25% entre os pacientes atendidos nos principais hospitais pediátricos da cidade e em Unidades de Saúde da periferia. O estudo mostrou que a relevância dos rotavírus como agentes etiológicos da diarréia aguda na infância já acaba aos 18 meses de idade das crianças desta região, quando os valores de positividade

caem a apenas 4%, como no grupo controle. A distribuição bimensal de rotavírus não apresentou perfil tipicamente sazonal, ocorrendo 1 pico de incidência no ano de 1986, durante a estação chuvosa, e 2 picos no ano de 1987, dos quais um na estação chuvosa e outro, durante a época seca do ano. O estudo também mostrou que a doença diarreica é mais severa entre os casos positivos para rotavírus do que entre negativos, apresentando maior frequência de evacuações diárias, de vômitos e febre, levando ao índice acima de 2 vezes maior de casos de desidratação moderada ou severa. Finalmente, é feita uma análise em relação ao efeito que a imunoprofilaxia poderá ter sobre os elevados índices de mortalidade infantil registrados nesta região do país.

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