**Abstract**

**Objective:** to determine the prevalence of rotavirus in the etiology of acute diarrhea in children from Natal city, RN, Brazil and investigate the existence or not of a seasonal distribution of this pathogen in our environment.

**Methods:** fecal samples from 1,903 children (boys and girls) with ages ranging from 1 month to 10 years, living in Natal-RN, who presented acute diarrhea episodes in a period from January 1996 to December 1998, were analyzed. We searched viral particles directly in the feces by a passive agglutination reaction using anti-rotavirus specific-group monoclonal antibodies coated latex particles.

**Results:** 151 children (7.9%) of the studied population presented a positive reaction, revealing the presence of rotavirus particles in feces. Considering, however, only the children (ages from 1 to 24 months) who are more susceptible to rotavirus infection, we verified that from 1,065 examined children, 136 of them (12.8%) presented positive reaction for rotavirus, and the great majority of all children with positive reaction (96.3%) had ages ranging from 6 to 24 months. Analysis of the distribution of the cases of rotavirus infection in the three year revealed that the incidence of infection was higher in July, August, and September.

**Conclusions:** the results indicated rotavirus infections have an important role in the etiology of acute diarrhea cases. The majority of the acute diarrhea cases positive for rotavirus occurred during the first two years of life, reaching mainly children from 6 to 24 months, with the highest incidence of infection during the months of July, August, and September.


**Introduction**

Rotaviruses are globally distributed and have a broad spectrum of hosts. They affect several vertebrate species, including humans, mainly in their first 2 years of life. These viruses belong to the Reoviridae family, *Rotavirus* genus. They exhibit a double-stranded RNA genome with 11 segments of different sizes involved by three concentric protein layers, which form a capsid of icosahedral symmetry.⁴,¹⁰,¹¹,¹⁴

Human rotaviruses were simultaneously discovered in Australia, England and Canada in the 70s. Today, they are considered one of the main causes of infectious diarrhea in humans, and contribute significantly to the high levels of morbidity and mortality among infants in the whole world, especially in developing countries, where malnutrition levels are very high.⁷,⁹,¹⁰,¹³,¹⁷
As rotaviruses are more resistant than most viruses - to high temperatures, acidic pH, lipid solvents, and non-ionic detergents in general -, they are extremely stable in environmental conditions, and remain in nature for an undetermined length of time. Moreover, conventional treatment of water by flocculation seems to be less efficient in inactivating rotaviruses than other enteric viruses, which significantly contributes to its dissemination in communities.3,4,9,12 These characteristics place rotavirus in the list of the main causes of diarrhea all over the world. It is responsible for a large number of hospitalizations of children aged 1 to 24 months. This pathogen is transmitted both in the community and in hospitals, and is responsible for high levels of nosocomial infection.8,10,13,16,17

The mechanisms of pathogenic action of the rotavirus are not well defined. It is known that its replication occurs mainly in the jejunal mucosa, in the mature epithelial cells that recover villi, where absorption takes place. Studies with gnotobiotic animals infected by human rotavirus reported the presence of significant microscopic changes in the intestinal mucosa where flattened, and rounded cells were observed. These changes, which affect mainly the lateral and apical cells lining the villi, are responsible for the mechanisms that determine diarrhea. The destruction of disaccharidase (lactase, maltase, sucrase and isomaltase) enzyme-producing cells causes a reduction in the concentration of these enzymes in the intestinal lumen. The digestion of the corresponding disaccharides is affected, and results in a high osmotic load in the colon. This may determine an increase in the secretion of water and electrolytes into the intestinal lumen, causing diarrhea with hydroelectrolytic imbalance. This condition may lead to death, especially when affecting undernourished children.5 Lactose-intolerance may be the basic mechanism of this process. However, some authors believe that changes in sodium transport, associated with glucose and with poor absorption caused by the extensive epithelial lesions, may play an important role in the onset of diarrhea.

We analyzed fecal samples of children with episodes of acute diarrhea in Natal, capital of the state of Rio Grande do Norte, Brazil. The objective of this study was to determine the prevalence of rotavirus infection in the samples, to investigate the role these agents play in the etiology of acute diarrhea, and to establish the existence of seasonal distribution of infection caused by this pathogen in our environment.

Materials and Methods

**Sample collection and processing** – We collected fecal samples of 1,903 children, both boys and girls, who were treated in outpatient services or hospitalized in public or private institutions from January 1996 to December 1998. The children presented with episodes of acute diarrhea of presumable infectious nature. Stool cultures did not reveal enteropathogenic bacteria in their feces. Subjects’ ages ranged from 1 month to 10 years; 56.0% of them were from 1 to 24 months old. We used a representative sample of 1 to 2 ml or 1 to 2 g of feces from each child, dissolved in 2 ml of diluent, vigorously agitated in a Vortex mixer, and left to rest for 10 minutes at room temperature. Preparations were then centrifuged at 800 g for 10 minutes to obtain a limpid supernatant, in which the presence of rotavirus particles was investigated.

**Sample analysis** – Two aliquots of 50 ml of supernatant from each fecal sample were analyzed for the detection of rotavirus particles by passive agglutination reaction. We used a commercial kit (Slidex Rota-kit 2 bioMérieux), which consists of a suspension of anti-rotavirus specific-group monoclonal antibodies coated latex particles which detects the corresponding antigen in the fecal sample supernatant, and of a suspension of non-antibodies coated latex particles. We placed 50 ml of the suspension of antibodies coated latex particles (reaction) on one end of a plate, and, on the other end, 50 ml of the suspension of non-antibodies coated latex particles (negative control). After that, 50 ml of the supernatant was added to each end of the plate, and the components were mixed to obtain a homogeneous preparation. The presence of agglutination was investigated by optical microscopy. The sample was positive when agglutination was visible within 2 minutes on the end of the plate corresponding to reaction, and a homogenous suspension was found on the negative control end; and it was negative when homogenous suspensions were observed on both ends of the plate, which revealed absence of agglutination. Statistical significance analysis was performed with the chi-square test using the Web Chi Square Calculator software provided by the Georgetown University, Washington DC, http://www.georgetown.edu/chall/webtools/web-chi.html.

Results

We analyzed fecal samples of 1,903 children aged 1 month to 10 years, 1,055 (55.4%) boys and 848 (44.6%) girls. These children presented with episodes of acute diarrhea of presumable infectious nature. Of this total, 151 (7.9%) fecal samples were positive for the presence of rotavirus particles in the feces. These particles were detected by the passive agglutination test of anti-rotavirus specific-group monoclonal antibodies coated latex particles. Out of the 1,903 children, 1,752 (92.1%) were negative for these pathogens. Of the children who tested positive, 92 (4.8%) were boys and 59 (3.1%) were girls (Table 1).

Most of the fecal samples that tested positive were from 1 to 24-month old children. The distribution of the percentage of children that presented positive reaction for rotavirus per age group revealed the existence of differences in the infection prevalence indices for this pathogen. Values ranged from zero, in 6 to 10-year old children, to 12.8%, in 1 to 24-
month old children. These values are statistically significant (R=0.001) in comparison with the other age groups studied.

The 2nd and 3rd semesters of life are the periods when children are at a higher risk of acquiring rotavirus infection in any part of the world. A total of 1,065 children 1 to 24-month old were studied, and 136 (12.8%) were infected by this virus, as demonstrated by detection of viral particles in the feces, while 929 (87.2%) tested negative for this pathogen. Of the 136 children with acute diarrhea and presence of rotavirus particles in the feces, 131 were in the 6 to 24-month old group.

When distributed according to age groups, the percentage of children with acute diarrhea and positive reaction for rotavirus was higher in the 6-12 and 13-18-month old groups (17.2 and 16.6%, respectively). This revealed a statistically significant difference (R=0.001) when compared with the other age groups.

Cases of infection by this pathogen were recorded in all the months of the year. However, the analysis of the percentage of children infected by rotavirus and with diagnosis confirmed by detection of viral particles in the feces confirmed the seasonal distribution of the symptomatic cases of the infection. The cases we studied were diagnosed along 3 years, and most of them occurred in the months of July, August, and September, with the peak of incidence in September (Figure 1).

Table 1 - Distribution of rotavirus infection cases diagnosed in 1-month to 10-year old children with acute diarrhea episodes, according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive Reaction</th>
<th>%</th>
<th>Negative Reaction</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>92</td>
<td>4.8</td>
<td>963</td>
<td>50.6</td>
<td>1.055</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
<td>3.1</td>
<td>789</td>
<td>41.5</td>
<td>848</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>7.9</td>
<td>1.752</td>
<td>92.1</td>
<td>1.903</td>
</tr>
</tbody>
</table>

P = 0.184 by the chi-square test

Discussion

This study analyzed fecal samples of children with acute episodes of diarrhea in Natal, Rio Grande do Norte, Brazil. The results revealed that 56.0% of these children were up to 24 months old. This finding concurs with literature as to the age of greater incidence of acute diarrhea, which, as a rule, decreases with age. We also investigated the prevalence of rotavirus in the etiology of these cases of acute diarrhea, and the seasonal distribution of the cases diagnosed. The results revealed that most cases diagnosed affected 6 to 24-month old children, and that the smallest number of cases was observed in the groups of children younger than 6 months and older than 24 months. The epidemiological profile of rotavirus infection observed in the studied children is significantly similar to what is found in children of the same age group in any other part of the world.10,11,13

The low incidence of symptomatic infections by this pathogen in the first 5 months of life is probably due to the presence of antibodies IgA in the intestinal mucosa. These antibodies are passively transported from mother to child by the milk, as breast feeding lasts 3 to 4 months on average. The presence of these antibodies in the intestinal mucosa at this age seems to provide a certain protection, although partial, against rotavirus infection. These children, thus, do not get infected by this pathogen, or have an asymptomatic form of the infection.2,10,18 After weaning, children gradually lose the protection these antibodies provide, and become susceptible to rotavirus infection. Therefore, the largest number of acute diarrhea episodes caused by this virus occur from 6 to 24 months of age.8,11,13 This study showed that after the first 2 years of life, the number of cases of rotavirus infection decreases, maybe because most children at this age have already somehow been in contact with this pathogen. Although there is a large variety of these viruses in nature, these children probably develop antibodies against group-specific antigens common to all rotaviruses and which provide cross-protection. These antibodies do not prevent infection, but seem to often make it asymptomatic or of little clinical importance.13,15,16

The distribution of percentages of rotavirus-infected children along the months of the year in the 3 years studied shows that infection by this pathogen occurs in all months of the year. We also observed, however, a higher prevalence in the months of July, August, and September, pointing to
Table 3 - Distribution of rotavirus infection cases diagnosed in 1 to 24-month old children with acute diarrhea episodes

<table>
<thead>
<tr>
<th>Age group</th>
<th>Positiv reaction</th>
<th>%</th>
<th>Negativ reaction</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5 months</td>
<td>5</td>
<td>4.5</td>
<td>105</td>
<td>95.5</td>
<td>110</td>
</tr>
<tr>
<td>6-12 months</td>
<td>56</td>
<td>17.2</td>
<td>269</td>
<td>82.8</td>
<td>325</td>
</tr>
<tr>
<td>13-18 months</td>
<td>53</td>
<td>16.6</td>
<td>267</td>
<td>83.4</td>
<td>320</td>
</tr>
<tr>
<td>19-24 months</td>
<td>22</td>
<td>7.1</td>
<td>288</td>
<td>92.9</td>
<td>310</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>12.8</td>
<td>929</td>
<td>87.2</td>
<td>1,065</td>
</tr>
</tbody>
</table>

P < 0.001 by the chi-square test.

In Europe, rotavirus infection is also seasonally distributed in Italy, France, England, the Netherlands, and Finland, where gastroenteritis caused by this virus has higher incidence in February and March.7

This study found a prevalence of 7.9% rotavirus infections in all the children with acute diarrhea episodes (age: 1 month to 10 years). When only the 1 to 24-month old children were considered, prevalence was 12.8%. There was no statistically significant gender difference (P=0.184) in the incidence of rotavirus infection (Table 1). Statistically significant difference in the prevalence of the infection in different age groups (R=0.001) was observed, as 90.0% of the diagnosed cases of rotavirus infection were found in the 1 to 24-month age group. Moreover, no cases were diagnosed in children older than 6 years (Table 2). However, if we consider only the children up to 24 months of age, who are exposed to a higher risk of rotavirus infection,8,10,11,13 prevalence reached 17.2 and 16.8% in the 2nd and 3rd semesters of life, against 4.5 and 7.1% in the 1st and 4th semesters, respectively. This statistically significant difference (R=0.001) was probably a result of the presence of maternal antibodies in the 1st semester of life and of the partial protection acquired from the 4th semester on as a consequence of exposure to this virus (Table 3).

The rotavirus infection prevalence index for the children up to 24 months of age in this study is similar to the one reported by Pereira et al.11 in a study of children younger than 5 years of age from 14 Brazilian states, where the incidence of infection by this pathogen ranged from 13% in the state of Pernambuco to 20% in the state of Santa Catarina. However, the prevalence index found in this study for the 6 to 24-month age group is significantly below the 30.0% found in Poland and the 20-40% in different studies carried out in Italy, both studies with children in this same age group who were hospitalized because of acute diarrhea. It is also lower than the 25.0% index found in children younger than 5 years of age in a Spanish study.8,13,16

The results of this study show a higher incidence of acute diarrhea in children younger than 24 months, which decreases as they get older. They also show that rotavirus plays an important role in the etiology of this clinical entity in Brazil, and that infection by this pathogen is more prevalent in the first 2 years of life, affecting mainly children from 6 to 24 months of age, with higher prevalence in the months of July, August, and September. No case of rotavirus infection was detected in children older than 6 years of age.

References

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