Prevalence of rotavirus in feces of children with acute diarrhea: clinical signs and associated symptoms

Prevalência de rotavírus em fezes de crianças com diarreia aguda: sinais e sintomas clínicos associados

Altacílio Aparecido Nunes[1], Luane Marques de Mello[2], Renata Neiva Parrode[3], João Paulo Maneira Bittar[4]

RESUMO

Introdução: O rotavírus é agente etiológico muito comum em diarreias agudas em crianças, em todo o mundo, afetando principalmente crianças menores de cinco anos de idade. A mortalidade anual global associada a infecções por rotavírus foi estimada em 454.000 a 705.000 mortes por ano, predominantemente nos países em desenvolvimento. Os objetivos deste estudo foi descrever os sorotipos circulantes e a prevalência de rotavírus em uma região do Brasil, bem como, apresentar a caracterização clínica da doença diarréica em crianças. **Métodos:** Estudo transversal que selecionou 124 amostras de um mesmo número de crianças de até cinco anos de idade, com gastrenterite aguda, atendidas em uma Unidade Básica de Saúde. **Resultados:** Trinta e uma amostras foram positivas para rotavírus A com maior ocorrência de setembro a março ($p < 0,05$). Com relação ao genótipo G, 16 (80%) amostras foram classificadas como G1. O genótipo P mostrou que todas as cepas eram P[8]. A desidratação foi encontrada 2,45 vezes mais entre crianças infectadas com rotavírus. **Conclusão:** Espera-se que a determinação da prevalência da infecção e doença provocada pelo rotavírus e a caracterização do perfil de cepas virais circulantes possa contribuir para informação sobre a biologia molecular e a epidemiologia da infecção pelo referido agente.

UNITERMOS: Rotavirus, Diarreia, Gastroenterite, Crianças, Vacina.

ABSTRACT

Introduction: *Rotavirus is a very common etiologic agent in acute diarrheas in children worldwide, affecting mainly children under five years of age. The overall annual mortality associated with rotavirus infection was estimated at 454000-705000 deaths annually, predominantly in the developing countries. The aim of this study was to describe the prevalence and serotypes of rotavirus in a region of Brazil, as well as to present a clinical evaluation of this diarrheal disorder in children.* ***Methods:*** *Cross-sectional study which selected 124 samples from the same number of children under five years of age with acute gastroenteritis, attended to in a Basic Health Unit.* ***Results:*** *Thirty-one samples were positive for rotavirus A, with higher occurrence from September to March ($p < 0.05$). With respect to genotype G, 16 (80%) samples were rated as G1. P genotype showed that all strains were P[8]. Dehydration was found 2.45 times more often among children infected with rotavirus.* ***Conclusion:*** *It is hoped that determining the prevalence of infection and disease caused by rotavirus and the characterization of the profile of circulating viral strains may contribute to information on the molecular biology and epidemiology of infection by that agent.*

KEYWORDS: *Rotavirus, Diarrhea, Gastroenteritis, Children, Vaccine, Symptoms.*

INTRODUCTION

Rotavirus is a very common cause of acute diarrhea in children worldwide, chiefly affecting children younger than five years and the yearly global mortality associated with rotavirus infections has been estimated to be 454,000 to 705,000 deaths annually, predominantly in developing countries. The fact that the incidence of rotavirus is similar in developed and developing countries suggests that the disease is not adequately controlled with improvements in sanitation alone. On this basis, in view of the tremendous morbidity and mortality and economic burden of rotavirus worldwide, and because of the structure of the virus and the lack of effective antiviral therapy, the future of rotavirus management is thought to be prevention through vaccination (1-3).

The World Health Organization (WHO) has encouraged the introduction of a rotavirus vaccine into national immunization programs, particularly in poorer countries.

[1] Professor Doutor. Departamento de Medicina Social da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo.
[2] Doutora em Patologia. Professora Colaboradora do Centro de Atenção Primária da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo.
[3] Graduada em Medicina. Residente de Clínica Médica da Universidade Federal do Triângulo Mineiro.
[4] Graduado em Medicina. Especializando em Acunpuntura da Universidade Federal de São Paulo.

06-502_prevalence_of_rotavirus.pmd 147 28/06/2010, 19:03

Therefore, epidemiological studies about the distribution and prevalence of rotavirus serotypes in all geographic regions are important for an adequate choice of the antigen components that are candidates for a vaccine, for the evaluation of the results, and for guidance about the adoption of new measures for the control, prevention and treatment of the disease (3-6).In Brazil, morbidity and mortality associated with diarrhea have decreased in the last 2030 years. This has been attributed to improvements in drinking water supply and sanitation, the promotion of breastfeeding and the increased use of oral rehydration therapy nationwide. With the decrease in bacterial diarrhea, rotavirus infection has gained importance as a cause of diarrhea in children < 5 years of age (7).

Rotavirus diarrhea occurs from early infancy, when it is highly prevalent, to adult age, with most infections being associated with mild gastrointestinal signs and symptoms (5). However, in a smaller proportion of cases the disease may manifest as severe diarrhea, causing patient dehydration, convulsions, coma and, occasionally, death. Immunity to the severe form of the disease can be achieved by the occurrence of previous infections, often asymptomatic and naturally acquired (3, 5, 6) therefore, implementing a vaccine program whereby very young infants receive the vaccine for protection against more severe subsequent infections, represents the ideal means of preventing moderate to severe rotavirus infections. Thus, the main objective of artificial immunization against rotavirus is not the prevention of infection with this agent, but rather the prevention of the occurrence of severe cases of the disease since it is known that cross-immunity between different rotavirus serotypes in inadequate (5, 6).

In Brazil, the number of studies on the prevalence of viral diarrhea and the characterization of rotavirus strains in towns distant from the large capital cities are still infrequent. Thus, the objective of the present study was to describe the epidemiology of acute infant diarrhea, especially those provoked by rotavirus, and to characterize the circulating strains and their association with signs and symptoms.

METHODS

This was a cross-sectional study conducted on 124 fecal samples collected from an equal number of children up to five years of age seen at a Basic Health Unit in the state of Minas Gerais, Brazil.

Inclusion criteria and eligibility

We selected children aged 0 to 5 years with signs and symptoms of acute gastroenteritis (lasting up 14 days), assisted in a pediatric clinic of a basic health unit during the period from 2005 to 2007, where parents or guardians consent for the participation of their children.

Exclusion criteria

We excluded children over 5 years old and whose parents or guardians refused to allow their participation. We also excluded children with a confirmed diagnosis of bacterial diarrhea.

Viral identification/characterization

The feces were collected in sterile plastic and packed in polystyrene boxes containing dry ice and transported to the laboratory where they were kept at temperatures appropriate to the analysis. Rotavirus was detected and characterized by two methods, i.e., polyacrylamide gel electrophoresis (PAGE) with electrophenotype classification and ELISA, respectively. G and P genotypes were identified by RT-Nested-PC, after viral dsRNA extraction by techniques described in the literature.[8]

Statistical analysis

Data were stored and analyzed statistically with the Epi Info software version 6.04 using the chi-square test and/or Fisher exact test, and Z test for proportions, with the level of significance set at 5%. For the analysis of association between variables the Prevalence Ratio (PR) estimator was used, with its 95% confidence interval (95% CI). The sample size was calculated taking into account a prevalence of 10-38% of rotavirus diarrhea, one size population of 500 children aged chosen and a margin of error of 3%.

Ethical aspects

The research protocol was approved by the Research Ethics Committee of the University of Uberaba, the parents or persons responsible gave written informed consent to have the sample collected from the children, and an epidemiological chart was filled out for each patient.

RESULTS

Seventy-four of the selected children (59%) were boys and 50 (41%) were girls ($p < 0.05$). Of the 124 samples studied, 31 (25%) were positive for rotavirus, 18 of them (59%) from boys and 13 (41%) from girls ($p < 0.05$).

The determination of prevalence regarding age range and seasonality revealed a higher percentage of positive reactions among infants aged seven to 12 months ($p < 0.05$),

06-502_prevalence_of_rotavirus.pmd 148 28/06/2010, 19:03

with a higher peak of occurrence from September to March [($p < 0.05$) (Table 1)]. The April-August period in the Southeast region of Brazil corresponds to the dry season, with a mean temperature of 23.2 ºC, a rainfall index of 140 mm and moderate relative humidity of atmospheric air, close to the mean for the rainy season. September-March corresponds to the rainy season, with higher mean temperatures, a rainfall index above 1750 mm (year) and a relative humidity of atmospheric air above 70%.

TABLE 1 – Socio-demographic variables and percent distribution of rotavirus A strains according to age range and seasonality – 2005/2007 period

Variables	N	%	P
Sex			
Male	74	59	< 0.05*
Female	50	41	
ELISA for *Rotavirus A*			
Positive	31	25	< 0.05 #
Negative	93	75	
Age range (months)			
0 to 6	5	15.0	< 0.05**
7 to 12	15	50.0	
13 to 24	6	20.0	
25 to 60	5	15.0	
Climatic season			
Dry	6	20.0	< 0.05*
Rainy	25	80.0	

* Fisher exact test and/or and **X^2 for trend ; # Z test.

Regarding the G genotyping of the 20 strains analyzed, 16 (80%) were classified as G1, two (10%) as G4, one as G3 (5%), and one as G9 (5%) [$p = 0.0001$]. P genotyping showed that all strains were P[8]. The most common G and P association was G1P[8] – 80%, followed by two G4P[8] strains and two G9P[8] strains. Genotype analysis in relation to the period of characterization demonstrated that G1 occurred in both years, while genotypes G3 and G9 mainly occurred in 2006. The occurrence of signs and symptoms in the various age ranges considering the etiology of diarrhea is presented in Table 2.

It can be seen that fever was present in all patients, including those with a negative ELISA for rotavirus, accompanied in order of frequency by abdominal pain and vomiting. Comparison of the proportions of symptoms between age ranges revealed a significant difference only regarding abdominal pain among patients with a virus-positive ELISA ($p = 0.0005$). However, when the association between signs/symptoms and the presence of rotavirus as the etiological agent of diarrhea was determined according to positive ELISA results considering all age ranges, the prevalence of dehydration was found to be 2.45-fold higher among children with rotavirus-induced diarrhea (Table 3).

TABLE 3 – Association between clinical signs and symptoms and rotavirus-induced diarrhea according to positive ELISA result.

Signs/symptoms	Prevalence ratio (PR)	95% Confidence interval (95% CI)	p
Fever	1.0	–	–
Vomiting	1.27	0.79 to 2.05	0.45
Nausea	1.50	0.94 to 2.39	0.16
Abdominal pain	1.18	0.83 to 1.67	0.45
Dehydration	2.45	1.12 to 5.36	< 0.05*

* Statistically significant.

DISCUSSION

Estimates obtained in other investigations conducted at the outpatient clinic level in various countries have revealed rotavirus positivity rates ranging from 12 to 50% (9-11). In the present study there was a 25% prevalence of rotavirus while in previous studies mainly conducted in the others regions of Brazil prevalence rates ranged from 11 to 37-38% (7-16). Thus, the results obtained in the present study agree with most Brazilian studies and also with those conducted in other parts of the world. When the prevalence of *rotavirus A* is considered in relation to sex, the results obtained here agree with those reported in previous studies which also observed a significant difference between genders, with higher positivity rates among males (8-17).

TABLE 2 – Distribution of signs and symptoms of children with rotavirus A-induced diarrhea (detected by ELISA) and of children with diarrhea and with negative ELISA for Rotavirus according to age range – 2005/2007 period

	Rotavirus-positive ELISA					*Rotavirus-negative ELISA*				
Age range (months)	0-6 N (%)	7-12 N (%)	13-24 N (%)	25-60 N (%)	*P* *	0-6 N (%)	7-12 N (%)	13-24 N (%)	25-60 N (%)	*P* *
Signs/symptoms										
Fever	5 (100)	15 (100)	6 (100)	5 (100)	*0.99*	14 (100)	45 (100)	19 (100)	15 (100)	*0.99*
Vomiting	2 (40)	8 (53)	3 (50)	1(20)	*0.40*	6 (44)	18 (40)	6 (33)	3 (20)	*0.74*
Nausea	2 (40)	9 (60)	3 (50)	1 (20)	*0.78*	6 (44)	18 (40)	3 (16)	3 (20)	*0.37*
Abdominal pain	3 (60)	4 (60)	6 (100)	4 (80)	*0.0005*	8 (55)	36 (80)	10 (52.6)	6 (40)	*0.10*
Dehydration	2 (40)	5 (33)	2 (33)	0	*0.53*	3 (22)	4 (8.9)	2 (9.9)	2 (13.3)	*0.75*

** Fisher exact test.

06-502_prevalence_of_rotavirus.pmd 149 28/06/2010, 19:03

In the present study, *rotavirus A* infection occurred in all age ranges, including infants younger than six months, although the frequency was higher among 7 to 18-month-old children. Other studies have shown an important occurrence of symptomatic infection in this age range, possibly reflecting the socioeconomic-sanitary conditions of the population (7-9, 12-14, 17). Rotavirus infection in the 0 to 6 month age range frequently occurs in an asymptomatic manner, in contrast to what was observed in the present study, in which practically all patients of all age ranges presented some symptoms. Particularly important was the significant association between diarrhea with a rotavirus-positive ELISA and dehydration, suggesting that the children studied here had a moderate or severe form of the disease, or that most of the patients might not have had previous contact or infections with rotavirus since it has been scientifically proved that infection by this agent confers some natural immunity to subsequent infections. Specifically, infection causes serum and intestinal antibody responses that protect against severe diarrhea upon reinfection with rotavirus (19). One important study evaluated the protection conferred by each rotavirus infection by monitoring 200 infants from birth through 2 years of age (2) and found that children who had 1, 2, or 3 previous rotavirus infections had progressively lower risks of additional rotavirus infections (adjusted relative risk, 0.62, 0.40, and 0.34, respectively) and diarrhea (adjusted relative risk, 0.23, 0.17, and 0.08, respectively). Furthermore, subsequent infections were significantly less severe than initial infections (P=.024) and more likely to be caused by rotavirus of a different G type (P=.054). The researchers concluded that protection against future infections increased with each rotavirus infection, although natural infection best protects against more severe disease and is less protective against less severe or asymptomatic disease.

On the other hand, a lower proportion of infection was observed in children older than two years, supporting the notion that, after this age range, the occurrence of rotavirus infection tends to decrease, perhaps owing to the fact that large part of children older than this age have already had contact with the virus, with the consequent acquisition of an immune response (20, 21).

In Brazil, seasonality is variable, with an increased incidence of rotaviruses during the cold months or during the dry season between April-August in the Center-West and Southeast regions. On the other hand, the states of the North and Northeast the occurrence of the virus is recorded throughout the year. A considerable variability of electrophenotypes was also observed here, suggesting the occurrence of seasonal patterns. However, there was a greater concentration of cases during the warmer months with higher rainfall, possibly suggesting that the different dsRNA segments of rotaviruses have peculiar and variable characteristics of geographic and meteorologic determination. Some studies have clearly demonstrated a seasonal trend for viral diarrheas, especially those of rotavirus etiology (22-24). This finding was confirmed in the present study which showed a greater occurrence in warmer seasons, in contrast to what occurs in cold climate countries, where the highest incidence is observed during periods of low temperatures (1, 3, 5, 25).

In the present study we observed a predominance of the G1 genotype suggesting the possibility of changes in the prevalence of different genotypes in different geographic regions. Regarding the time of occurrence, several studies have systematically indicated that a given genotype predominates during a period of up to two years, with a new hegemonic antigenic variant arising thereafter, indicating the need for new vaccinal strategies (3, 4, 6, 8, 13). In addition, considering P genotyping, all genotypable strains were P[8], in agreement with studies conducted in different parts of the world (19, 20) and suggesting that this component may be important for the development of vaccines of universal use in a large number of countries. The predominant G serotype varies by region and year. G1 tends to be the predominant pathogenic serotype in the United States and is also the predominant serotype worldwide, although to a lesser extent, occurring about 53% of the times. Other predominant serotypes worldwide are G2 (11%), G3 (14%), G4 (5%), and other genotypes (20, 22, 24).

The prevalence of *rotavirus A* observed in the current study supports data obtained in previous studies in Brazil and in the world (7, 17, 19, 25-27). Thus, the present findings can make a contribution by supporting the information related to the socioeconomic impact of viral diarrhea, such as the loss of working days on the part of the parents, the increased demand for health services and hospitalization, which exposes children to other risks, such as contracting other diseases, with additional costs and morbidity and increased mortality due to preventable diseases. The present results suggest the need for constant monitoring of rotavirus infections according to rigorous methodological and analytical criteria in view of the possible occurrence of biases of various sources and types. The findings thus obtained are directly implicated in the development of vaccines for the combat of the microorganism in question, considering the regional differences.

The determination of the prevalence of rotavirus infection and disease and the characterization of a profile of the viral strains circulating in the municipality are expected to contribute to the information we have about the molecular biology an epidemiology of rotavirus infection in Brazil. These findings can definitely be used in the analysis of vaccine candidates against this important etiologic agent of childhood diarrhea in our country, thus reducing the impact of this disease.

Because it was a cross-sectional study, the findings described here may contain restrictions, such as the presence of selection and measurement bias. To minimize and overcome these limitations, there is need for further prospecti-

06-502_prevalence_of_rotavirus.pmd 150 28/06/2010, 19:03

ve studies on the subject are conducted with larger samples and better selected.

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✉ Endereço para correspondência:
Altacílio Aparecido Nunes
Av. Bandeirantes, 3900 – Monte Alegre
14025-520 – Ribeirão Preto, SP – Brasil
☎ (16) 3602-2569
altacilio@fmrp.usp.br

Recebido: 9/9/2009 – Aprovado: 8/10/2009

06-502_prevalence_of_rotavirus.pmd 151 28/06/2010, 19:03