Study of the dynamic of rotavirus circulation by identification of their electropherotypes

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Abstract

An electrophoretical study of rotavirus strains was carried out to determine the RNA migration patterns and to follow the dynamics of virus circulation. Fifty five positive fecal samples for rotavirus, obtained from 0-5 year old children during 2005 and 2006, were subjected to the Polyacrylamide Gel Electrophoresis. It was possible to define the RNA migration pattern of 37 (62%) rotavirus strains, displaying eight distinct profiles, all of them compatible with group A rotavirus. All rotavirus were detected from May to September showing an outstanding seasonality. In 2005 were detected only rotavirus strains presenting long migration patterns (100%=15/15), named L1, L2, L3 or L4. The L2 profile was predominant. However, in 2006, a few rotavirus strains displaying long migration patterns (L1 and L2) were detected (14%=3/22) from May to June, although rotavirus strains presenting short migration patterns (S1, S2, S3 or S4) were detected between July and August. These viruses of short RNA migration patterns were the most predominant strains (86%=19/22). The variability of RNA migration patterns detected showed a great genomic heterogeneity of rotavirus strains. By monitoring viral nucleic acid electrophoretic characteristics was possible to follow the dynamics of rotavirus circulation between 2005 and 2006.

Keywords: Diarrhea. Rotavirus. Electrophoresis.

1 Introduction

Acute diarrhea is one of the most frequent childhood infectious diseases throughout the world. The disease might be associated with different pathogens, such as: viruses, bacteria, protozoa and helminths (ESTES; KAPIKIAN, 2007). Among the viral agents, the rotaviruses stand out (CARMO, 2006; HOSHINO; KAPIKIAN, 2000). They are widely spread and may cause infection in children of different social classes, mainly from six months to two years old, being associated with nosocomial outbreaks of acute diarrhea in day care centers and pre-schools (LINHARES, 2000). Acute diarrheal disease is associated with high mortality rates especially in developing countries. It is estimated that by the age of five, all children will be attacked by at least one episode of gastroenteritis caused by rotavirus: one in five will seek ambulatory service; one in every 65 will be hospitalized; and one in 293 will die (PARASHAR et al., 2003). In Brazil, according to the Ministry of Health, childhood diarrhea from 20.6 % to 37.6 % of the hospitalized patients and 40 % of the deaths were due to the rotavirus (O´RYAN et al., 2001).

The transmission occurs through the oral-fecal route, through interpersonal contact, ingestion of water and foods, and contact with contaminated objects (ESTES; KAPIKIAN, 2007).

The rotavirus belongs to the *Reoviridae* family, whose particles consist of two concentric capsids. The structural proteins VP6, VP4 and VP7 are involved in the classification of the rotavirus into groups, serotypes and genotypes. Thus, the protein VP6 classifies the rotavirus in groups A to G. The rotaviruses of group A are those of greatest epidemiological importance because of their involvement in more than 90 % of the cases of rotavirus infection. The proteins VP7 and VP4 are responsible for the induction of neutralizing antibodies and classify the

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rotavirus into serotypes and genotypes (ESTES; KAPIKIAN, 2007).

The viral genome consists of eleven double strand RNA segments, which can be separated by polyacrylamide gel electrophoresis (PAGE) (DESSELBERGER, 1996; ESTES; KAPIKIAN, 2007; HERRING et al., 1982; RAMIG, 2004). The electrophoretic pattern of the group A rotavirus genome is composed of four groups of RNA segments of high molecular weight (segments from 1 to 4 - Group I), segments of medium weight (segments 5 to 6 - Group II and segments 7 to 9 - Group III) and two smaller segments (segments 10 and 11 - Group IV). Differences in the migration pattern of the tenth and eleventh genomic segments are useful to classify the group A rotavirus as of long, short and super short profiles (HOSHINO; KAPIKIAN, 2000).

Despite the analysis of the electrophoretic profiles has been widely used to characterize samples of rotavirus, revealing and monitoring the circulation and co-circulation of different profiles during an epidemic, and several studies on detection and classification of rotavirus have been carried out in Brazil, in different geographical regions, there are few reports in the literature on surveys carried out in Minas Gerais (ROSA E SILVA; CARVALHO; GOUVEA, 2002; ROSA E SILVA; NAVECA; CARVALHO, 2001). The objective of this study is therefore to characterize the electropherotypes of rotavirus strains and through them to follow the dynamics of rotavirus circulation in 2005-2006, in Juiz de Fora, MG.

2 Materials and methods

This study was carried out with 55 fecal samples, positive for rotavirus of the group A, previously subjected to immunoenzymatic tests for detection of rotavirus and adenovirus antigens by using the kit EIERA according to the directions of the manufacturer (Bio-Manguinhos, Oswaldo Cruz Foundation, Rio de Janeiro). These samples belong to the collection of fecal samples from the laboratory of virology, UFJF. The fecal samples were collected, from naught to five years old children , who were presenting clinical evidence of gastroenteritis and were treated in the healthcare network in Juiz de Fora, MG, during 2005 (18) and 2006 (42). The feces, in natura, were collected after spontaneous evacuation and a signed consent from was obtained from the parents or other responsible adult. The study was approved by the Committee of Ethics in Research from UFJF (CEEA-029/2005).

2.1 Extraction and purification of the viral RNA

The fecal specimens were processed according to Boom and others (1990). Fecal suspensions to 10%, in buffer Tris-HCl-Ca_2 0.01M pH 7.2, clarified (13,000 rpm - 5min - 4ºC), were treated by buffer containing isotiocianate of guanidine and silica. After incubation for 30 minutes at room temperature in constant agitation, the material was centrifuged (1 min - 13,000 rpm) and the supernatant discarded. The silica precipitate containing the viral RNA absorbed was washed by solution of ethanol 70% and acetone, respectively. After centrifugation, the supernatant was discarded and the precipitate submitted to a temperature of 56°C for 15 minutes for evaporation of the acetone residues. After drying, the precipitate containing the viral RNA was resuspended in H_2O Milli-Q and maintained for 5 minutes at 56°C. After centrifugation, the supernatant was transferred to sterile microtube and stored at -20°C or maintained at 4°C, if used immediately.

2.2 Polyacrylamide gel electrophoresis (PAGE)

RNA extracted of rotavirus positive strains were submitted to PAGE, according to Laemmli (1970), to determine the viral RNA migration patterns (electropherotypes). All reactions were carried out, using a rotavirus strain of known electrophoretic profile (positive control). After electrophoresis (100V-3h), gels were stained with silver nitrate by methods described by Herring and others (1982).

3 Results and discussion

Of 55 positive samples tested by PAGE, the electrophoretic profile of RNA was obtained for 37 samples (67.3 %), 15 samples of 2005 and 22 of 2006.

An outstanding characteristic of the rotavirus infections is seasonality. The samples used in this study were detected between May and September (Table 1), including the autumn and winter seasons. In this period, are generally registered the lowest rates of pluviometric precipitation and the lowest temperatures.

These results corroborate previous studies carried out in the same area (GOUVEA et al., 2009; ROSA E SILVA; NAVECA; CARVALHO, 2001) and in other Brazilian cities (PEREIRA et al., 1993, GUSMÃO et al., 1999). According to Moe and Shirley (1982), low temperatures have a direct influence on the survival of the rotavirus in the wild. Though this study was carried out in countries with a temperate climate, in which the seasonality is quite outstanding, it has been confirmed in Brazil, in the Central-Western and South-eastern regions (CAMPOS et al., 2003; CARDOSO et al., 2003; ROSA E SILVA; CARVALHO; GOUVEA, 2002).

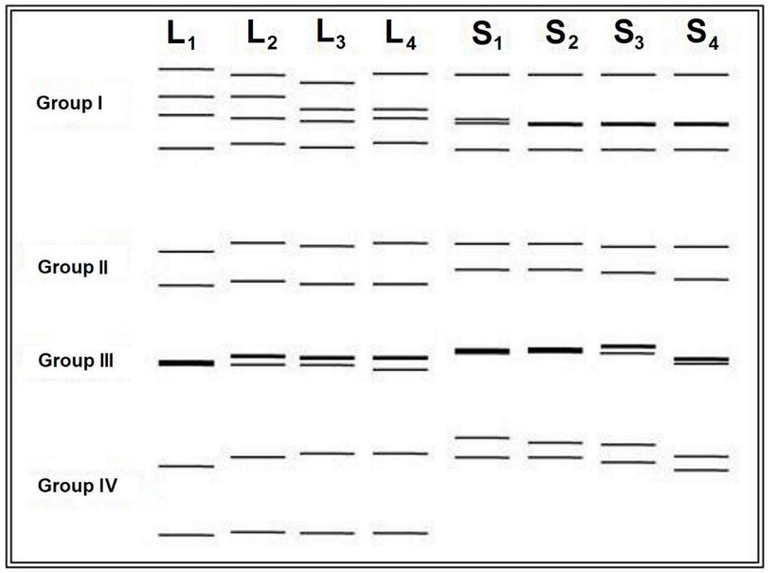
Table 1

Temporal distribution of electrophoretypes of rotavirus strains detected in 2005-2006.

Electropherotypes	2005					2006				Total
	May	Jun	Jul	Aug	Sep	May	Jun	Jul	Aug	
S1	___	___	___	___	___	___	___	5	3	8
S2	___	___	___	___	___	___	___	5	3	8
S3	___	___	___	___	___	___	___	___	2	2
S4	___	___	___	___	___	___	___	___	1	1
L1	___	___	___	___	3	___	1	___	1	5
L2	1	6	1	1	___	1	___	___	___	10
L3	___	1	___	___	___	___	___	___	___	1
L4	___	___	___	___	2	___	___	___	___	2
Total	1	7	1	1	5	1	1	10	10	37

Source: The authors (2010).

All the tested samples showed characteristic profiles of rotavirus from group A. In 2005, all (15/15) presented long profiles, whereas in 2006 86% (19/22) presented short profiles and 14% (3/22) presented long profiles. Analysis of the viral genome through PAGE allowed identification of eight different electrophoretypes. The differences found between the long and short profiles were identified by numbers (Electrophoregram1).



Electrophoregram1: Electrophoregram of rotavirus strains detected in Juiz de Fora, MG, in 2005-2006. Long profiles (L); short profiles (S)

Source: The authors (2010).

The electrophoretic diversity of the samples studied expresses great genetic variability common to rotavirus and other viruses of segmented RNA genomes. Such variability is reached through constant changes resulting from the lack of corrective activity of RNA polymerase, involved in replication of these viruses, as well as from events of genetic reassortment that can take place in cases of co-infection by two different viral samples (DESSELBERGER, 1996).

Rotavirus strains displaying L2 electrophoretic profile were responsible for 50% of the cases in 2005, reappearing in 2006 as an isolated event in May. In May, June, July and August 2005 and May 2006, they were responsible for all rotavirus cases except one in June 2005, associated with a rotavirus strain displaying L3 electrophoretic profile in its single occurrence (Table 1).

In accordance with most of the investigations, there is a predominance of rotavirus strain displaying long RNA migration pattern. In 2006, all cases of rotavirus diarrheal occurred from May to August. In July and August occurred the most diarrheal disease caused by rotavirus. In May and June only rotavirus strains presenting L2 and L1 profiles were detected. In July, rotavirus strains presenting short profiles emerged and those that had S1 and S2 profiles were responsible for all cases. In August was observed co-circulation of five different electrophoretypes, mainly of short profile (90%=9/10). Short profile was the most predominant in this year. The emergence and predominance of rotavirus strains presenting short profile was observed in other Brazilian study (DOMINGUES et al., 2008), thus confirming the importance of them as the main strain responsible for diarrhea caused by rotavirus in 2006.

5 Conclusions

During this study the determination of the electrophoretical patterns allowed to observe the circulation of at least eight different strains of group A rotavirus. The rotavirus infections occurred from May to September, period corresponding to autumn-winter station, and emerge in Southeast region of Brazil. The variety of rotavirus strains detected by PAGE showed the great potential of genetic variability commonly observed in rotavirus infections occurring during a year. It was also possible to follow the dynamic of rotavirus strains circulation that included the occurrence, disappearance, co-circulation and predominance of rotavirus strains in 2005-2006. Monitoring the electrophoretypes pointed to important changes in 2006, when long profiles were switched by rotavirus strains displaying short profile, then showing the emergence of a completely different strain of group A rotavirus, which was the predominant type circulating in 2006.

Estudo da dinâmica de circulação de rotavírus através da identificação de seus eletroferótipos eletroferótipos

Resumo

Foi realizado um estudo eletroforético de amostras de rotavírus, visando determinar os perfis de migração do RNA viral, bem como acompanhar a dinâmica da circulação dos mesmos. Para tanto, 55 amostras fecais diarréicas, positivas para rotavírus, obtidas de crianças de 0-5 anos, durante os anos de 2005 e 2006, foram submetidas à técnica de eletroforese em gel de poliacrilamida. Foi possível definir o perfil eletroforético de 37 (62%) delas, tendo sido observados oito perfis distintos, todos compatíveis com rotavírus do grupo A. Todas as amostras de rotavírus foram detectadas de maio a setembro, indicando uma sazonalidade na ocorrência destas infecções em Juiz de Fora. Em 2005 todas as amostras apresentaram perfis longos (100%=15/15), denominados de L1 a L4, com predominância de amostras de perfil L2. Em 2006, poucas amostras de perfil longo (14%=3/22) foram detectadas nos meses de maio e junho, tendo sido substituídas pelas amostras de perfil curto (C1-C4), que emergiram em julho, predominando neste ano (86%=19/22). A variabilidade dos perfis eletroforéticos detectados mostrou uma grande heterogeneidade genômica e através do monitoramento foi possível acompanhar a dinâmica de circulação das amostras de rotavírus entre 2005 e 2006.

Palavras-chave: Diarréia. Rotavírus. Eletroforese.

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