Rotavirus genotyping in gastroenteritis cases of an infantile population from Western Brazilian Amazonia

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ABSTRACT

Methods: During the period from 2000 to 2002, 79 rotavirus-positive stool samples were collected from children presenting diarrhea in the Western Brazilian Amazon. Molecular characterization of the G and P genotypes was performed using RT-PCR and electrophoretotyping analysis by polyacrylamide gel electrophoresis. A total of 59 samples were confirmed as group A rotavirus. Molecular characterization of the VP7 (glycoprotein) and the VP4 (protease-sensitive), are important for the genetic diversity of rotaviruses. Results: The VP7 or VP4 antigens are used to classify G and P genotypes, respectively. In the host, both proteins induce neutralizing antibodies, which are essential for protection against rotavirus infections². On the basis of antigenic and genetic diversities, 23 G and 30 P rotavirus genotypes have been identified among strains of both human and animal origin, the serotypes G1 to G4 being the most common cause of disease worldwide³. Ten G types and 11 P types (mostly, G1-G6, G8-G10, and G12, and P[4], P[6], P[8], and P[9], respectively) have been recovered in association with human infections. The combinations G1P[8], G2P[4], G3P[8], and G9P[8] are the most frequently found⁴.

In this study, the first genotyping of rotavirus in the Western Brazilian Amazon was confirmed by a combined Enzyme Immunoassay for rotavirus and Adenovirus (EIARA Biomanguinhos/Oswaldo Cruz Foundation, United Kingdom), which uses latex particle agglutination. Samples were then confirmed by a combined Enzyme Immunoassay for rotavirus infections².

Conclusions: The proportion of the rotavirus genotypes observed was not different from that in other areas of Brazil. This study is the first genotyping of rotavirus in the Western Brazilian Amazon.

Keywords: Group A rotavirus. Genotyping. Brazilian Amazonia.

RESUMO


Palavras-chaves: Rotavírus grupo A. Genotipagem. Amazônia brasileira.

Rotavirus is the main cause of acute gastroenteritis in children under five years old worldwide. It is estimated that 130 million children are infected with rotavirus gastroenteritis, causing 2 to 4 million hospital admissions and 600,000 deaths every year¹.

Rotavirus belongs to the Reoviridae family, and the capsid is made of a triple protein layer. The genome is composed of eleven segments of double-stranded RNA, and each segment encodes for a specific protein. Two proteins that make up the outer capsid of the virion, the VP7 (glycoprotein) and the VP4 (protease-sensitive), are

important for the genetic diversity of rotaviruses. The VP7 or VP4 antigens are used to classify G and P genotypes, respectively. In the host, both proteins induce neutralizing antibodies, which are essential for protection against rotavirus infections².

On the basis of antigenic and genetic diversities, 23 G and 30 P rotavirus genotypes have been identified among strains of both human and animal origin, the serotypes G1 to G4 being the most common cause of disease worldwide³. Ten G types and 11 P types (mostly, G1-G6, G8-G10, and G12, and P[4], P[6], P[8], and P[9], respectively) have been recovered in association with human infections. The combinations G1P[8], G2P[4], G3P[8], and G9P[8] are the most frequently found⁴.

Recently, a study on the etiology of gastroenteritis in the infantile population showed group A rotavirus to be the most frequent enteropathogen, occurring in 23.6% of the total diarrhea cases; the second group consisted entirely of pathogenic categories of diarrheogenic Escherichia coli differentiated by PCR technique, occurring in 18.2% of cases, followed by Salmonella sp. (9.3%), Adenovirus (6.3%), and Shigella sp. (5.1%)⁵.

This study aimed to characterize G and P genotypes infecting an infantile population admitted with viral gastroenteritis and to extend our knowledge of rotavirus infection in individuals from Western Brazilian Amazonia.

From March 2000 to March 2002, 330 stool samples were collected from diarrheic children presenting acute gastroenteritis at the Cosme Damião Infantile Hospital, located in downtown Porto Velho, the capital of the State of Rondonia, Brazil. The hospital admits children from the periphery of the city. The stool samples were collected from children after consent was given by their parents or other legal guardians, through written signature. This study received approval from the Committee on Ethics (Center of Tropical Medicine Ethical Committee - Porto Velho, number 463). The rotavirus was diagnosed through two tests. First, the samples were analyzed using the commercial kit Virotec Rota (Omega Diagnostics, Scotland, United Kingdom), which uses latex particle agglutination. Samples were then confirmed by a combined Enzyme Immunoassay for Rotavirus and adenovirus (EIARA Biomanguinhos/Oswaldo Cruz Foundation, Healthy Ministerial of Brazil).

The rotavirus-positive stool samples were dissolved in 0.01M Tris-HCl-CaCl₂ (pH 7.2) and clarified by centrifugation at 800g, and the supernatants were frozen at -20°C. The viral RNA was extracted as described by Boom et al., followed by separation by polyacrylamide gel electrophoresis (PAGE)⁶. The segments of double-stranded RNA (dsRNA) were visualized after silver nitrate staining⁷.

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The reverse transcriptase reaction and PCR were performed as described by Das et al. and Gentsch et al., respectively. For G genotypes, the first PCR was performed using the consensus primer pair 9con1/9con2. In this reaction, one fragment of 904bp was amplified, and the product was submitted to the nested reaction, the combination between primers 9con1 and a pool of G-specific primers, 9T-1, 9T-2, 9T-3P, 9T-4, and 9T-9B genotypes for variable regions of the VP7 gene, specifically to G1, G2, G3, G4, and G9. For P genotypes, the first PCR was performed using the consensus primer pair con2/ con3; one fragment of 876bp was amplified. In the nested reaction, the combinations were between con3 primer and the pool of P-specific primers for variable regions of the VP4 gene, 1T-1, 3T-1, and 4T-1, specifically to the P[8], P[4], P[6], and P[9] genotypes. The products were visualized under UV in 1% agarose gels stained with ethidium bromide.

Of 79 rotavirus-positive stool samples collected, 75% gave PAGE results positive for group A rotavirus. The most frequent profile was the long PAGE profile, columns 2 and 3 the short profile. The stool of children with different PAGE results. Column 1 shows the stool samples from children in Porto Velho revealed both long and short migration profiles. Luz et al., in a study comprising most rotavirus strains of serotypes G1, G3, G4, and G9, showed a predominance of rotavirus strains with long electropherotype. In fact, this electropherotype profile was observed in 74% of our samples. Rotavirus strains displaying short electropherotype represented 25.5% of total and are usually associated with G2 serotypes, which was confirmed in this study.

On the basis of the current binary system for rotavirus characterization, the majority of isolates from diarrheic children fall into four groups: G1P[8], G2P[4], G3P[8], and G4P[8] (Table 1), the G1P[8] genotype being the most common, found in 61% of samples; this was followed by G2P[4] in 20%, G3P[8] in 6.7%, and G4P[8] in 3.3%. Some samples with G1 and G2 genotypes did not have had their P genotype identified; these represented 3.3% and 5% of the samples, respectively. All short profiles were G2P[4] genotypes. We did not find an occurrence of mixed genotypes in our samples.

Studies carried out by our group with populations of ambulatory or hospitalized children in Porto Velho, State of Rondonia, showed rotavirus as the main cause of gastroenteritis, occurring in 23.6% of the total diarrhea cases.

In the current study, the PAGE profiles of stool samples from children in Porto Velho revealed both long and short migration profiles. Luz et al., in a study comprising most rotavirus strains of serotypes G1, G3, G4, and G9, showed a predominance of rotavirus strains with long electropherotype. In fact, this electropherotype profile was observed in 74% of our samples. Rotavirus strains displaying short electropherotype represented 25.5% of total and are usually associated with G2 serotypes, which was confirmed in this study.

On the other hand, according to Leite et al., a significant predominance of G2P[4] and G2P[NT] strains following the introduction of the Rotarix vaccine in Brazil has been observed. As efficacy in the protection achieved against the fully heterotypic G2P[4] immunization was low, there is growing concern for regarding the lower protection against G2P[4] group A rotavirus of vaccinated children. Therefore, only continuous monitoring of rotavirus disease burden and genotype surveillance will provide this information.

We did not find an occurrence of mixed genotypes in our samples as shown by other studies. Additionally, the newly emergent G9 genotype was still not present in Brazil in 2002, when the collection was performed. It may be noticed that from 1997 to 1999, the G9, the 5th most epidemiologically important serotype, was not yet identified in São Luis, Brazil, as it has only recently emerged worldwide.
Extensive and continuous monitoring of rotavirus disease burden and surveillance studies are necessary to assess the question that emerges regarding the early post-vaccination era, that is, whether the monovalent rotavirus A vaccine will contribute to additional changes in genotype distribution in the Western Brazilian Amazon and nationwide.

In conclusion, our study provides relevant data about the first genotyping of rotavirus from children in the Western Brazilian Amazon and about epidemiological surveillance of rotaviruses. The results of our study should help future investigations into the genotypes that circulate after the introduction of the vaccine against rotavirus in Brazil.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES