

BRIEF COMMUNICATION

SHIFT IN HUMAN ROTAVIRUS DISTRIBUTION IN BELO HORIZONTE, BRAZIL DETECTED BY RIBONUCLEIC ACID ELECTROPHORESIS

Millan Scarabeli Alves Coelho da SILVA(1), Francisco José PENNA(2), Ricardo Jenner DUARTE(1), Pedro Augusto Rocha PEREIRA(3),
Andreia Elisa CURSINO(1), Luciano Amedée PÉRET-FILHO(2), Edilberto Nogueira MENDES(3) & Paula Prazeres MAGALHÃES(1)

SUMMARY

Rotavirus has been considered the main agent of infectious diarrhea especially among younger children. We addressed the prevalence of rotavirus-associated diarrhea and the diversity of circulating electropherotypes by immunochromatography and RNA electrophoresis. Stool samples were taken from 391 children (267 with diarrhea) from the lower socioeconomic stratum who sought treatment in the Hospital Infantil João Paulo II/Belo Horizonte, during 2005 and 2006. Rotavirus was detected in 79/20.2% of subjects, 64/24.0% with diarrhea and 15/12.1% with no diarrhea. The virus was strongly associated with diarrhea ($p = 0.003$). A total of 76/19.4% and 69/17.6% rotavirus-positive children were identified by immunochromatography and electrophoresis, respectively. Rotavirus-associated diarrhea was more frequently detected in dry months ($p < 0.001$) and almost exclusively in children aged up to three years. Long profile strains prevailed (54/78.3%) but a shift toward short electropherotype was identified. Despite the decrease seen in 2006, rotavirus infection is still very common in our area. Although viral RNA electrophoresis is useful as a typing method, it should not be used exclusively in the diagnosis of rotavirus infection. We confirmed a shift from long to short profile strains, as already described for other South American countries.

KEYWORDS: Rotavirus; Infectious diarrhea; Diagnosis; Electropherotyping; Immunochromatographic assay.

Diarrhea is recognized as one of the most important public health problems in low-income countries. Annually, it accounts for more than 1.3 million deaths among children aged less than five years, representing around 15% of all deaths in this age group². Diarrheal disease can be associated with numerous and diversified agents, including bacteria, parasites, and viruses, the most prevalent cause of endemic diarrhea in children¹⁷. Among these, rotavirus has been considered the dominant agent of infectious diarrhea worldwide. It is estimated that severe acute diarrhea caused by this virus leads to approximately 40% of all hospital admissions related to diarrhea globally and causes about 450,000 deaths worldwide each year among children younger than 5 years^{15,21,22}.

Rotavirus, which forms a genus of the Reoviridae family, is classified into seven groups (A-G), group A being recognized as the single most important cause of severe acute enteritis in infants and young children in both developed and developing countries. *Rotavirus* B does not appear to be of epidemiological importance apart from in China, and group C has been associated with rare and sporadic cases and small outbreaks¹⁹.

Diagnosis of the infection when indicated is usually based on the

antigenic properties of the virus. Among others, immunochromatographic assays which are considered highly sensitive and specific have been widely used.

Rotavirus genome consists of 11 double-stranded RNA segments that could be separated by polyacrylamide gel electrophoresis (PAGE) to form peculiar RNA profiles named electropherotypes¹. Based on the migration pattern of gene segments 10 and 11 rotavirus strains can be distinguished as having either a long or short electrophoretic profile. This kind of assay constitutes a useful tool for investigating several aspects of the epidemiology of rotavirus-associated disease. Furthermore, a correlation between specific electropherotypes and genotypes has been described. G1P[8], G3P[8], and G4P[8] strains usually exhibit a long profile, and G2P[4] strains present a short electrophoretic profile⁸.

Since there is limited information regarding rotavirus infection in Belo Horizonte, Minas Gerais, Brazil we aimed to investigate the prevalence of diarrhea associated with rotavirus and to monitor the diversity of circulating electropherotypes of rotavirus strains. Data generated may be useful to better understand clinical and epidemiological

(1) Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

(2) Departamento de Pediatria, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

(3) Departamento de Propedêutica Complementar, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Correspondence to: Paula Prazeres Magalhães. Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Microbiologia. Av. Antônio Carlos 6627, 31270-901 Belo Horizonte, Minas Gerais, Brasil. Phone: 55 31 34092759. Fax: 55 31 34092733. E-mail: ppmagalhaes@ufmg.br

aspects of the disease and could ultimately provide clues for designing strategies to prevent rotavirus-associated diarrhea.

This study is part of a prospective investigation into acute infectious diarrhea. It was approved by the Ethics Committee of the Universidade Federal de Minas Gerais. An informed consent was signed by parents/guardians of the children recruited for the study. All fecal samples were obtained from non-hospitalized children aged from 0 to 5 years who sought assistance at Hospital Infantil João Paulo II, a pediatric reference center that attends to patients from Belo Horizonte and the surrounding area. In order to investigate the seasonality of rotavirus infection, we divided the year into dry (from April to September) and rainy (from October to March) seasons based on local climate data.

We analyzed stool samples obtained from 391 patients between January 2005 and December 2006. Among them 267 presented with acute diarrhea and 124 had experienced no diarrhea in the previous 15 days. Acute diarrhea was characterized by the occurrence of three or more loose or watery stools or at least one bloody loose stool in a 24-hour period, for no more than seven days. All individuals were from the lower socioeconomic stratum (mean annual income ~ US\$3,000) and had no history of hospitalization or antimicrobial therapy in the last 15 days.

Fecal samples were sent to the laboratory and stored at -70 °C until being processed for rotavirus detection and typing by a commercial immunochromatographic assay (VIKIA® Rota-Adeno, bioMérieux, Marcy l'Etoile, France) and RNA electrophoresis. The immunochromatographic assay was performed according to the manufacturer's instructions.

For electropherotyping, viral RNA was extracted using a phenol-chloroform-isoamyl alcohol 25:24:1 (v/v) mixture and submitted to electrophoresis by using a discontinuous-pH system: a stacking gel (3.75% acrylamide + bis-acrylamide, pH 6.8) and a resolving gel (7.0% acrylamide + bis-acrylamide, pH 8.8). Electrophoresis was performed in Tris-Glycine buffer (25 mM Tris-HCl, 0.2M glycine, pH 8.6) at 80 V for around 18 h. Gels were then fixed for 10 min in 10% ethanol and 0.5% acetic acid and stained with 0.2% silver nitrate. After rinsing in distilled water, the gels were immersed in a solution containing 3.0% sodium hydroxide and a 1:200 dilution of 37.0% formaldehyde until the bands were resolved. Rotavirus strain Hoshi (positive control, long profile) and Bluetongue virus (negative control) were used as standards in all runs. Electrophoretic patterns were grouped as either short or long profiles.

A patient was considered to be rotavirus-positive when either test was positive. Data were analyzed by employing an χ^2 test with Yates' correction or Fisher's exact test. Differences were taken to be significant when $p < 0.05$.

Rotavirus was detected in fecal samples obtained from 79 (20.2%) subjects, 76 (19.4%) by the immunochromatographic test and 69 (17.6%) by electrophoresis of the viral RNA. When results generated by each assay were compared to data obtained by both techniques, sensitivity, specificity, and positive and negative predictive values for immunochromatography and electrophoresis were 96.2%, 100%, 100%, and 99.0% and 86.1%, 99.7%, 94.2%, and 96.6%, respectively. The correlation between the two tests as determined by the kappa correlation index was 0.48, which is considered a moderate agreement level.

Infection by rotavirus was significantly associated with diarrhea ($p = 0.003$). A total of 64 out of 267 (24.0%) patients with acute diarrhea and 15 of the 124 (12.1%) subjects with no diarrhea were shown to be infected by the virus. Although no statistical difference was observed, the virus was more frequently found in 2005 (49 of 215, 22.8%) than in 2006 (30 of 176, 17.0%). Also, the virus was more common in children from six to 24 months of age (55/210, 26.2%). In fact, infection by the virus was only detected in children aged less than three years with the exception of one aged between 55 and 60 months. Rotavirus was similarly distributed among girls (34 of 159, 21.4%) and boys (45 of 232, 19.4%). In total, the prevalence of rotavirus infection was significantly higher in dry months ($p < 0.001$; 73 of 229, 31.8% versus six of 162, 3.7% in the rainy season).

Patients infected with the virus presented with watery diarrhea ($p = 0.033$), vomiting ($p < 0.001$), and dehydration ($p = 0.010$) more commonly than rotavirus-negative children. The presence of blood in the stool was not observed among rotavirus-infected children ($p < 0.001$).

Among the 69 rotavirus-positive children identified by electrophoresis, 14 (20.3%) were infected by short pattern strains, 54 (78.3%) by long profile, and one (1.4%) by a strain exhibiting an undetermined electropherotype. A temporal variation in the distribution of electropherotypes was observed: short rotavirus strains were only detected in 2006 and long strains of the virus were more commonly found in 2005 (43 of 215, 20.0%) than in 2006 (11 of 176, 6.3%) ($p < 0.001$). The undefined rotavirus strain was identified in the feces of a child who was attended to in 2005. Long and short strains of the virus were evenly distributed in boys and girls. Although no statistical significance was reached, rotavirus exhibiting both short (14 of 14, 100%) and long (49 of 54, 90.7%) migration patterns predominated in dry months. The distribution of rotavirus electropherotypes was associated with age ($p = 0.012$). A higher prevalence of short profile strains was observed in children older than two years. Conversely, no statistical association between clinical data and the electropherotype of the strain was detected. None of the rotavirus-infected children presented with bloody stools.

Rotavirus vaccination was not reported for any child infected with the virus.

In this 2-year study on infectious diarrhea in children, nearly 20% of the subjects were infected by rotavirus, as determined by employing two different detection methods in order to improve data accuracy. More than 80% of rotavirus-positive children presented with diarrhea. These results are similar to those reported for several industrialized and non-industrialized countries, including Brazil^{3,13,14,18}.

Despite the fact that a similar virus distribution was observed throughout the study period, our data suggest a decrease in rotavirus infection rates. We have no ready explanation for these findings but it is plausible to assume that the lower infection rate detected in 2006 could have resulted from the impact of the rotavirus vaccine which was included in the Brazilian immunization schedule in 2006. In fact, a post-vaccination reduction in the rates of morbidity, severity, and mortality associated with gastroenteritis, especially rotavirus infection has been demonstrated^{4,5}.

With regard to the sex distribution of the infection, conflicting data

have been reported^{11,6,10,13}. Higher rotavirus detection rates during drier cooler months are consistent with findings mainly reported for non-tropical regions. With regard to tropical countries, a universal pattern has yet to be established¹⁶. Rotavirus infection was not statistically associated with age, although it appears to be more prevalent in children younger than two years, similarly to previously reported data^{7,23}.

We found that the association between the rotavirus-positive status and frequent watery stools, vomiting, and dehydration among diarrhea patients has been universally recognized. On the other hand the occurrence of bloody stools is not usually observed²³.

A shift was detected from long profile strains observed mostly in 2005 towards short profile electropherotypes seen only in 2006. In fact, similar findings were reported for other places in Brazil^{7,11} and in other South American countries^{12,20}. It is possible that this shift represents a trend in the distribution of rotavirus strains circulating in South America, probably driven by the periodic but unpredictable fluctuation in the prevalence of different rotavirus strains all over the world as a result of genetic drift and/or genetic shift⁹. This switch from long to short profile could also be the result of a raised immunity caused by vaccination, since the vaccinal strain used in Brazil exhibits a long profile electropherotype. This explanation may apply to the association between rotavirus profile and age.

In conclusion, despite the decrease observed in the second year of the study, we demonstrated that rotavirus infection is still very common in our area. Our data also showed that although viral RNA electrophoresis is useful as a typing method, it should not be used exclusively in the diagnosis of rotavirus infection. Finally, we confirmed a shift from long to short profile rotavirus strains which had already been described for South American countries.

RESUMO

Alteração da distribuição de rotavírus detectada por eletroforese de RNA viral

Rotavírus é considerado o principal agente de diarreia infecciosa especialmente entre crianças mais jovens. O objetivo deste estudo foi avaliar a prevalência de diarreia associada a rotavírus e a diversidade de eletroferotipos circulantes empregando-se imunocromatografia e eletroforese de RNA. Espécimes fecais de 391 crianças (267 com diarreia aguda) de nível socioeconômico baixo, atendidas no Hospital Infantil João Paulo II/Belo Horizonte, durante 2005 e 2006, foram incluídos na investigação. Rotavírus foi detectado em 79/20,2% das crianças, 64/24,0% com e 15/12,1% sem diarreia. Associação entre infecção pelo vírus e diarreia foi observada ($p = 0,003$). Um total de 76/19,4% e 69/17,6% crianças rotavírus-positivas foram detectadas por imunocromatografia e eletroforese, respectivamente. Rotavírose foi mais frequentemente detectada em meses secos ($p < 0,001$) e quase exclusivamente em crianças com até três anos de idade. Embora predomínio de amostras com perfil longo (54/78,3%) tenha sido observado, identificou-se aumento da frequência de amostras com perfil curto. Apesar da queda da frequência de rotavírose em 2006, a doença ainda é muito comum na nossa região. Os dados demonstram que eletroforese de RNA viral é um método adequado para tipagem de rotavírus, mas não deve ser empregado como único método para diagnóstico da infecção pelo vírus. A alteração do padrão de distribuição de amostras, com aumento de frequência de perfil

eletroforético curto em detrimento da queda de ocorrência de amostras com perfil longo, confirma dados previamente descritos para outros países da América do Sul.

ACKNOWLEDGMENTS

Standard rotavirus strains were kindly supplied by Dr. Edel Figueiredo Barbosa Stancioli from the Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Pró-reitoria de Pesquisa da UFMG.

REFERENCES

1. Andreasi MAS, Batista SMF, Tozetti IA, Ozaki CO, Nogueira MM, Fiaccadori FS, *et al*. Rotavirus A em crianças de até 3 anos de idade, hospitalizadas com gastroenterite aguda em Campo Grande, Estado do Mato Grosso do Sul. *Rev Soc Bras Med Trop*. 2007;40:411-4.
2. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, *et al*. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969-87.
3. Carmo GMI, Yen C, Cortes J, Siqueira AA, Oliveira WK, Cortez-Escalante JJ, *et al*. Decline in diarrhea mortality and admissions after routine childhood rotavirus immunization in Brazil: a time-series analysis. *PLoS Medicine*. 2011;8:e1001024.
4. Desai R, Oliveira LH, Parashar UD, Lopman B, Tate JE, Patel MM. Reduction in morbidity and mortality from childhood diarrhoeal disease after species A rotavirus vaccine introduction in Latin America: a review. *Mem Inst Oswaldo Cruz*. 2011;106:907-11.
5. Dulgheroff ACB, Figueiredo EF, Moreira LP, Moreira KC, Moura LMS, Gouvêa VS, *et al*. Distribution of rotavirus genotypes after vaccine introduction in the Triângulo Mineiro region of Brazil: 4-year follow-up study. *J Clin Virol*. 2012;55:67-71.
6. Gouvea VS, Dias GS, Aguiar EA, Pedro AR, Fichman ER, Chinem ES, *et al*. Acute gastroenteritis in a pediatric hospital in Rio de Janeiro in pre- and post-rotavirus vaccination settings. *Open Virol J*. 2009;3:26-30.
7. Gouvêa VS, Domingues ALS, Naveca FG, Pedro AR, Bevilacqua CC. Changing epidemiology of rotavirus-related hospitalizations in Rio de Janeiro, Brazil, from 2002 to 2006. *Open Virol J*. 2007;1:47-50.
8. Iturriza-Gómara M, Isherwood B, Desselberger U, Gray J. Reassortment *in vivo*: driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *J Virol*. 2001;75:3696-705.
9. Koopmans M, Brown D. Seasonality and diversity of group A rotaviruses in Europe. *Acta Paediatr Suppl*. 1999;88(426):14-9.
10. Latipov R, Utegenova E, Kuatbayeva A, Kasymbekova K, Abdykarimov S, Juraev R, *et al*. Epidemiology and burden of rotavirus disease in Central Asia. *Int J Infect Dis*. 2011;15:e464-9.
11. Leite JPG, Carvalho-Costa FA, Linhares AC. Group A rotavirus genotypes and the ongoing Brazilian experience: a review. *Mem Inst Oswaldo Cruz*. 2008;103:745-53.
12. Martínez M, Amarilla AA, Galeano ME, Aquino VH, Fariña N, Russomando G, *et al*. Predominance of rotavirus G2P[4] and emergence of G12P[9] strains in Asunción, Paraguay, 2006-2007. *Arch Virol*. 2010;155:525-33.
13. Nitiema LW, Nordgren J, Ouermi D, Dianou D, Traore A, Svensson L, *et al*. Burden of rotavirus and other enteropathogens among children with diarrhea in Burkina Faso. *Int J Infect Dis*. 2011;15:e646-52.

14. Orlandi PP, Silva T, Magalhães GF, Alves F, Cunha RPA, Durlacher R, *et al.* Enteropathogens associated with diarrheal disease in infants of poor urban areas of Porto Velho, Rondônia: a preliminary study. *Mem Inst Oswaldo Cruz*. 2001;96:621-5.
15. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, *et al.* Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis*. 2009;200(Suppl 1):S9-15.
16. Pitzer VE, Viboud C, Lopman BA, Patel MM, Parashar UD, Grenfell BT. Influence of birth rates and transmission rates on the global seasonality of rotavirus incidence. *J R Soc Interface*. 2011;8:1584-93.
17. Procop, GW. Gastrointestinal infections. *Infect Dis Clin North Am*. 2001;15:1073-108.
18. Rosa e Silva ML, Naveca FG, Carvalho IP. Epidemiological aspects of rotavirus infections in Minas Gerais, Brazil. *Braz J Infet Dis*. 2001;5:215-22.
19. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol*. 2005;15:29-56.
20. Stupka JA, Carvalho P, Amarilla AA, Massana M, Parra GI, Argentinean National Surveillance Network for Diarrheas. National Rotavirus Surveillance in Argentina: high incidence of G9P[8] strains and detection of G4P[6] strains with porcine characteristics. *Infect Genet Evol*. 2009;9:1225-31.
21. Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, *et al.* 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis*. 2012;12:136-41.
22. Widdowson MA, Steele D, Vojdani J, Wecker J, Parashar U. Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. *J Infect Dis*. 2009;200(Suppl 1):S1-8.
23. Wierzbza TF, Abdel-Messih IA, Abu-Elyazeed R, Putnam SD, Kamal KA, Rozmajzl P, *et al.* Clinic-based surveillance for bacterial- and rotavirus-associated diarrhea in Egyptian children. *Am J Trop Med Hyg*. 2006;74:148-53.

Received: 5 June 2012

Accepted: 27 September 2012