Genotyping of gastroenteric viruses in hospitalised children: first report of norovirus GII.21 in Brazil

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This retrospective study (April-September 2003) was designed to investigate the roles of the main viruses responsible for cases of acute infantile gastroenteritis in hospitalised children up to two years of age. The viruses were identified in 64.7% (88/136) of the cases and the detection rates of rotavirus A (RVA), norovirus (NoV) and astrovirus were 41.9% (57/136), 30.3% (24/79) and 12.7% (7/55), respectively. RVA and NoV were detected in 20 of the 24 reported nosocomial infection cases. This study identified the first circulation of the genotype NoV GII.21 in Brazil and highlights the need to establish differential diagnoses through active laboratorial surveillance.

Key words: gastroenteric viruses - genotyping - nosocomial infections

Rotavirus A (RVA), norovirus (NoV) and human astrovirus (HAstV) have been described as the most important agents responsible for sporadic cases and outbreaks of acute gastroenteritis (AGE) worldwide (Patel et al. 2008). Previous reports have demonstrated that RVA and NoV are common causes of nosocomial diarrhoea in paediatric populations admitted to hospitals (Tran et al. 2010). These viruses survive for extended periods under adverse environmental conditions and the morbidity of the viruses is associated with AGE infections (Bruijning-Verhagen et al. 2012).

The diagnosis of RVA using polyacrylamide gel electrophoresis (PAGE) and enzyme immunoassays (EIAs) has facilitated the identification of the aetiological agent in AGE infections and EIAs have been used for rapid diagnosis in public health laboratories (Pereira et al. 1983). The establishment of molecular detection methods in the 1990s and, in particular, the development of polymerase chain reaction (PCR) protocols, facilitated the demonstration of the association of NoV and HAstV with infantile AGE. However, because the tests are not routinely performed in developing countries, the occurrence of these viruses as aetiological agents may be underestimated (Whilhelmi et al. 2003, Moreno-Espinosa et al. 2004).

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This study was designed to investigate the prevalence of RVA, NoV and HAstV in cases of infantile gastroenteritis, i.e., cases that required hospitalisation and cases that developed AGE following hospitalisation (nosocomial infection). Importantly, this study was performed in 2003, three years before the rotavirus vaccine (Rotarix®) was introduced into the vaccination schedule of the Brazilian Immunisation Program. In addition, the viruses were also characterised molecularly to provide genotype information for the molecular epidemiological study of circulating viruses during the pre-vaccination period.

Children (112) aged less than two years and suffering from AGE, who were admitted to the Municipal Hospital Getúlio Vargas Filho, Niterói, state of Rio de Janeiro (RJ), Brazil, between April-September 2003 and children (24) who developed gastroenteritis three days after hospitalisation were included in this study (136 total cases). The Ethical Committee on Research at the Fluminense Federal University and at the Hospital (64/03) approved this study. RVA screening was performed using the EIA IDEA Rotavirus (Oxoid Ltd, England) and/or RIDASCREEN Rotavirus (R Biopharm Group, Germany) kits and PAGE. The semi-nested PCR for the RVA G and P genotyping were performed as previously described (Gentsch et al. 1992, Das et al. 1994, Leite et al. 1996). NoV PCR was used to screen for NoV and was based on the degenerate primers Mon 431-434, which are specific to region B and are located within the 3'-end of open reading frame (ORF)1 (RNA polymerase) (Beuret et al. 2002). The primers targeting the 3'-end of the major capsid gene (region D) were to molecularly characterise the virus (Vinjé et al. 2004). To detect the HAstV genome, a single PCR was performed using a set of primers (Mon 269-270) targeting the ORF2 region (Noel et al. 1995). The PCR products obtained from region D of NoV

and HAstV were purified (QIAquick® PCR Purification Kit, Qiagen®) and sequenced (ABI Prism 3100 Genetic Analyzer and Big Dye Terminator Cycle Sequencing Kit v.3.1; Applied Biosystems, CA, USA). A phylogenetic tree was constructed using the MEGA 4 software program with the neighbour-joining method and the genetic distance was calculated using the Kimura 2-parameters model with 2,000 pseudo-replicas for genotypic strain classification. The statistical analysis was performed using the Statistical Package for the Social Science version 17.0 software program.

Table I shows the results of the detection of viruses according to the age distribution. The samples were screened for RVA using EIAs and PAGE and both methodologies produced similar results. The 57 RVA-positive samples demonstrated a long electrophoretic pattern. The only RVA genotypes detected in this study were G1 (33), G9 (24) and P8 (47) and they exhibited a distribution of G1P8 [n = 29 (50.9%)], G9P8 [n = 18 (31.6%)], G1P? [n = 4 (7%)] and G9P? [n = 6 (10.5%)]. The results are consistent with a previous study performed in samples from hospitalised children in RJ (Carvalho-Costa et al. 2006, 2011, Leite et al. 2008). Previously, the G1 genotype was the most common genotype found worldwide and this genotype is the component of the attenuated monovalent RVA vaccine (Rotarix®) that was licensed for routine infant immunisation in Brazil in 2006 (Leite et al. 2008). The RVA-negative samples were tested for NoV. The RVA and NoV-negative samples were then analysed for HAstV. However, the determination of co-infection was not performed, which limits the assessment of the true burden of the viruses in this population.

NoV was detected in 24 of the 79 RVA-negative samples (30.3%) and the genotypes were characterised as GII.4 (n = 17), which included variants 2001, 2002 and 2003, GII.6 (n = 1) and GII.21 (n = 1) (GenBank accessions JF816241-JF816255). An increased prevalence of NoVGII strains, primarily GII.4, has been reported in outbreaks and sporadic AGE cases (Siebenga et al. 2009, Ferreira et al. 2010, Zheng et al. 2010). The detection of the GII.4 variants reflects the rapid movement of these viruses throughout the world; the frequency of reports of these variants increased during the first decade of the XXI century (Siebenga et al. 2009, Zheng et al. 2010). This study identified the first circulation of genotype GII.21 in Brazil (Figure). This genotype was originally described as a possible recombination between GII.b and GII.18 (Chhabra et al. 2010). However, the Norovirus Genotyping Tool version 1.0 (rivm.nl/mpf/norovirus/typingtool) has identified the genotype as GII.21, similar to samples from Iraq (AY675554) and India (EU019230).

HAstV was detected in 12.7% (7/55) of the cases. Using nucleotide sequencing, the virus was characterised as HAstV type 1; this genotype is the most prevalent worldwide and is associated with a large number of severe gastroenteritis cases requiring hospitalisation (Gabbay et al. 2005, Victoria et al. 2007).

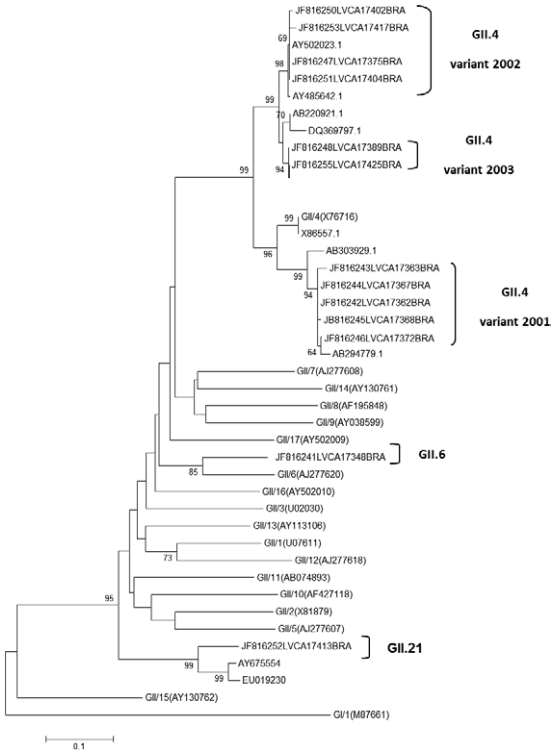
Nosocomial infections were identified in 24 of the cases and were associated with RVA (n = 13) and NoV (n = 7) (Table II).

TABLE I

Distribution of the virus studied according to age group

Age group (months)	Number of positive/number of studied (%)			
	Rotavirus	Norovirus[a]	Astrovirus	Total
0-6	28/70 (40)	10/42 (23.8)	3/33 (9)	41/70 (58.5)
7-11	10/29 (34.4)	4/19 (21)	2/14 (14.2)	16/29 (55.1)
12-18	14/23 (60.8)	6/9 (66.6)	1/3 (33.3)	21/23 (91.3)
19-24	5/14 (35.7)	4/9 (44.4)	1/5 (20)	10/14 (71.4)
Total	57/136 (41.9)	24/79 (30.3)	7/55 (12)	88/136 (64.7)

a: p = 0.0187.



Phylogenetic tree based on 56 deduced aminoacid within the capsid region (region D) of genogroup (G) II norovirus (NoV) strains collected in the state of Rio de Janeiro, Brazil, in 2003 and GII NoV reference strains. Outgroup: GI/1Norwalk reference strain. Prototypes strains denomination: genogroup/genotype following by GenBank accessions between brackets. Strains denomination: GenBank accessions, initials of the lab, registration number of the sample in the laboratory and country of origin of the samples.

Previous reports have demonstrated that RVA and NoV are common causes of nosocomial infections; these reports cite evidence such as aerosol transmission (especially for NoV infection) and the contamination of environmental surfaces and fomites (Wilhelmi et al. 2003, Tran et al. 2010).

TABLE II
Viruses and clinical characteristics of nosocomial infections

Viruses	Total of cases +/- (%)	Months of infection	Sex F/M (n)	Genotype (n)	Median age (months)	Clinical characteristics[a] (n)
Rotavirus	13/24 (54)	Jul, Aug, Sep	7/6	G1P8 (8), G9P8 (5)	8.7 (02-24)	Pneumonia (13)
Norovirus	7/11 (63)	May, Jun, Jul	0/7	GII.4 (7)	7.7 (01-16)	Pneumonia (7)
Negative	4/24	May, Jun, Jul, Sep	1/3	-	4.5 (0-10)	Cardiopatic (1), pneumonia (3)

a: causes of hospitalization before acute gastroenteritis; F: female; M: male; +: positive; -: negative.

Due to the short evaluation period of this study, the seasonality of the viruses could not be inferred; however, the timing of the RVA and NoV infections (p = 0.0149) confirmed previous findings demonstrating increased positivity during the driest months (June-August) of the year in Southwest Brazil (Bittencourt et al. 2000, Araújo et al. 2002, Carvalho-Costa et al. 2006).

More than 50% of the AGE cases investigated in this study were in children aged up to six months, reflecting the well-established trend that this viral infection is more severe in very young children (Barnes et al. 1998, Carvalho-Costa et al. 2006). In addition, NoV was detected in 55.5% (10/18) of the children aged more than one year and in 22.9% (14/61) of the children under one year of age and this difference was statistically significant (p = 0.0187). Previous studies have shown that NoV infections occur more frequently in children aged less than 24 months (Victoria et al. 2007, Patel et al. 2008). No significant difference was observed in the age distribution of the children with RVA and HAstV gastroenteritis.

The clinical manifestations reported in our study (Supplementary data) are consistent with other reports showing that vomiting, fever and anorexia may not be indicative of RVA infection (Vaz et al. 1999, Carvalho-Costa et al. 2006). These data are contrary to data from a number of studies proposing that RVA diarrhoea is more likely to be associated with fever, vomiting and dehydration than diarrhoea caused by other pathogens. The symptoms may occur alone or in combination, resulting in the hospitalisation of the children (Araújo et al. 2002, Nguyen et al. 2004). A correlation between the symptoms and viral infection was not observed, with the exception of anorexia in the RVA cases (p = 0.0138) and the association between anorexia and abdominal pain (p = 0.0076).

In conclusion, this study demonstrates that RVA, NoV and HAstV are important aetiological agents in the more severe cases of AGE and are responsible for nosocomial infections in young children. In this context, we emphasise the importance of performing differential diagnoses in AGE cases, highlighting the relevance of laboratorial surveillance. In addition, this study describes for the first time the circulation of the NoV GII.21 genotype in Brazil, which is a valuable contribution to the databases that enable molecular epidemiology and viral evolutionary studies.

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