



Noroviruses associated with outbreaks of acute gastroenteritis in the State of Rio Grande do Sul, Brazil, 2004–2011



Juliana da Silva Ribeiro de Andrade^{a,*}, Monica Simões Rocha^a,
Felipe Aníbal Carvalho-Costa^a, Julia Monassa Fioretti^a,
Maria da Penha Trindade Pinheiro Xavier^a, Zenaida Maria Alves Nunes^b,
Jeanice Cardoso^c, Alexandre Madi Fialho^a, José Paulo Gagliardi Leite^a,
Marize Pereira Miagostovich^a

^a Laboratory of Comparative and Environmental Virology – Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Ministry of Health, Rio de Janeiro, RJ, Brazil

^b Central Laboratory of State of Rio Grande do Sul, State Foundation of Production and Health Research, Porto Alegre, RS, Brazil

^c Department of Epidemiologic Surveillance, State Department of Health, Porto Alegre, RS, Brazil

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ABSTRACT

Background: Acute gastroenteritis norovirus (NoV) in a country of continental dimensions like Brazil has resulted in under-reporting of the number of outbreaks, as well as the genotypes associated.

Objectives: To demonstrate the role of NoV in outbreaks occurring in the State of Rio Grande do Sul, Southern Brazil, we determined its prevalence, as well as the genotypes associated, and evaluated clinical and epidemiological aspects.

Study design: NoV investigation was carried out in rotavirus group A negative stool samples from 2265 patients from 741 outbreaks that occurred in the State of Rio Grande do Sul, Brazil, during a period of eight years (2004–2011). NoV detection and nucleotide sequencing for genotype characterization was carried by using sets of primers targeting a conservative Rd-Rp polymerase genome region and the viral capsid gene, respectively.

Results: NoVs were detected in 817 stool samples (36.1%) and associated with 327 outbreaks (44.1%). NoV GII.2, GII.3, GII.4, GII.6, GII.12, GII.13, GII.14, GII.15, GII.17, GII.21; and GI.1 and GI.3 were characterized. GII.4 was the most frequently detected (72.3%), with five variants identified (Asia.2003, Hunter.2004, Yerseke.2006a, Den Haag.2006b, New Orleans.2009). This study describes the first detection of GI.1 and GII.13 and GII.15 in Brazil and demonstrates NoV winter-spring seasonality in this region of the country.

Conclusions: NoVs were responsible for almost 50% of outbreaks, with about 70% of them resulting from genotype GII.4 and its variants. The seasonality observed could help health authorities to establish a system of active surveillance in order to reduce NoV impact especially in congregate settings.

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1. Background

Noroviruses (NoVs) (genus *Norovirus*) belong to the family *Caliciviridae* and possess a single-stranded positive-sense RNA genome that encodes three open-reading frames (ORF 1–3) [1]. Genetically

diverse, NoVs are divided into five genogroups (GI–GV), of which GI, GII, and GIV have been shown to infect humans [2]. These genogroups are divided into at least 35 genotypes and the genetic classification is based on phylogenetic analysis of the capsid region (ORF-2) [2].

The major impact of NoV infections in public health has been demonstrated by their global distribution, which are primarily responsible for water- and food-borne outbreaks of acute gastroenteritis (AG) worldwide [3–5]. NoVs are transmitted by the fecal–oral route and most AG outbreaks are described in confined places and large gatherings, such as cruises, resorts and nursing homes [6–8].

The emergence of new variants of NoV GII.4 is associated with epidemics of large public health impact. The rapid evolution and spread of these viruses require frequent studies to determine

Abbreviations: AG, acute gastroenteritis; NoVs, noroviruses; ORF, open-reading frame; RVA, rotavirus A; RS, Rio Grande do Sul; EIA, enzyme immunoassay; PAGE, polyacrylamide gel electrophoresis; nt, nucleotide.

* Corresponding author at: Avenida Brasil, 4365, Manguinhos, CEP 21040-360 Rio de Janeiro, RJ, Brazil. Tel.: +55 21 2562 1875; fax: +55 21 2562 1851.

E-mail addresses: juliana@ioc.fiocruz.br, juliana.andra36@hotmail.com (J.d.S.R. de Andrade).

prevalence, molecular epidemiology, and geographical distribution, in order to establish measures to prevent AG outbreaks and provide data that could contribute to the development of an effective vaccine for the control of AG [9–11].

In Brazil, despite studies conducted in different regions demonstrating the high prevalence and diversity of NoVs circulating in the country, there is still no active laboratory surveillance network for the diagnosis of these viruses [11–14]. In Southern Brazil, only one study has reported NoV GII in Porto Alegre city [15], despite the several AG outbreaks with no etiologic defined whose initial investigation has focused on group A rotavirus (RVA).

2. Objectives

This study aimed to associate NoV infection to AG outbreaks that occurred in the state of Rio Grande do Sul (RS), Southern Brazil, over eight years (2004–2011), providing epidemiological and molecular characterization of genotypes and variants responsible for those outbreaks. Clinical characteristics of the AG cases were also assessed.

3. Study design

3.1. Study area

The State of Rio Grande do Sul is the coldest Brazilian state, located between latitudes N – 27°04' and S – 33°45'. The climate is subtropical and the four seasons are well marked. The mean temperature varies from 14 to 17 °C (winter) and 23 to 25 °C (summer) and the rainy season includes the months of September to November. It has about 11 million inhabitants and covers a land area of 268,781,896 km [2], comprising 496 municipalities grouped into seven mesoregions [16].

Table 1

Primers used in the polymerase chain reaction for amplification of the genome of human norovirus.

Primers	Sequence (5'-3') ^a	Orientation	Localization	Region	Position
Primers to detection NoV ^b					
Mon431 ^f	TGG ACI AGR GGI CCY AAY CA	+	RNA Pol ^c	B	5093–5112 ⁱ
Mon432 ^e	TGG ACI CGY GGI CCY AAY CA	+	RNA Pol	B	5093–5112 ⁱ
Mon433 ^f	GAA YCT CAT CCA YCT GAA CAT	–	RNA Pol	B	5285–5305 ⁱ
Mon434 ^e	GAA SCG CAT CCA RCG GAA CAT	–	RNA Pol	B	5285–5305 ⁱ
Primers to Classification of Genogroups ^d					
Cap A ^e	GGC WGT TCC CAC AGG CTT	–	VP1	D	6897–6914 ⁱ
Cap B2 ^e	TAT GTI GAY CCW GAC AC	+	VP1	D	6738–6754 ⁱ
Cap B1 ^e	TAT GTT GAC CCT GAT AC	+	VP1	D	6738–6754 ⁱ
Cap C ^f	CCT TYC CAK WTC CCA YGG	–	VP1	D	6667–6684 ⁱ
Cap D3 ^f	TGY CTY ITI CCH CAR GAA TGG	+	VP1	D	6432–6452 ⁱ
Cap D1 ^f	TGT CTR STC CCC CAG GAA TG	+	VP1	D	6342–6451 ⁱ
Primers to Classification of Genogroups ^g					
G1SKF ^e	CTGCCGAATTGTAATGA	+	VP1	C	5671–5689 ⁱ
G1SKR ^e	CCAACCCARCATRTTACA	–	VP1	C	5058–5076 ⁱ
G2SKF ^f	CNTGGGAGGGCGATCGCAA	+	VP1	C	5401–5423 ⁱ
G2SKR ^f	CCRCNCGCATRHCRTRTACAT	–	VP1	C	5401–5423 ⁱ
Primers to classification of NoV GII ^h					
EVP2F ^f	GTR CCR CCH ACA GTT GAR TCA	+	VP1	P2	6381–6403 ^j
EVP2R ^f	CCG GGC ATA GTR GAY CTR AAG AA	–	VP1	P2	6381–6403 ^j

^a IUPAC code to indicate degenerate positions: I, iosine; R, purine (A/G); Y, pyrimidine (C/T); S, C/G; I, A/T/C/G; W, A/T; K, G/T; H, A/T/C.

^b Reference: [19].

^c Pol, Polymerase.

^d Reference: [20].

^e Genogroup I.

^f Genogroup II.

^g Reference: [21].

^h Reference: [22].

ⁱ Primer positions based on Norwalk (M87661) for NoV GI.

^j Primer positions based on Lordsdale (X86557) for NoV GII.

3.2. Clinical specimens

Stool samples were obtained in the context of AG outbreak monitoring under the Unified Health System (SUS-Brazil) comprising a hierarchical network in which samples are provided by spontaneous demand from outpatients assisted in different Municipal Health Centers. These samples, through the Central Laboratory of the State (LACEN-RS), are forwarded to the Regional Rotavirus Reference Laboratory, Laboratory of Comparative and Environmental Virology (RRRC-LVCA) for investigating RVA presence by polyacrylamide gel electrophoresis (PAGE) [17] and enzyme immune-assay (EIA) (Premier Rotaclone, Meridian Bioscience, Inc.; Ridascreen, R-Biopharm).

3.3. Case definition and inclusion criteria

For this study, one stool sample of 2265 patients with a negative diagnosis for RVA from 741 AG outbreaks that occurred in the period 2004–2011, in 97 out of 496 municipalities of the state, were selected for NoV investigation. AG outbreaks were characterized by the Surveillance Epidemiologic Service as an increase in the number of cases above the expected range for the population involved in that specific period following the definition of the Brazilian Ministry of Health [18].

To characterize NoV genotypes, 112 samples were selected on a geographic and temporal distribution, in which 50% of the municipalities of each mesoregion of State of Rio Grande do Sul were selected by random drawing in Excel 2010. One sample of each outbreak per year was selected afterwards.

3.4. Fecal suspension, RNA extraction, and reverse-transcription

Fecal suspensions were prepared in 10% Tris/HCl/Ca²⁺ (0.01 M, pH 7.2). The extraction of viral RNA was performed using the methodology of the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions.

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