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Norovirus in feces and nasopharyngeal swab of children with and without acute gastroenteritis symptoms: First report of GI.5 in Brazil and GI.3 in nasopharyngeal swab

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ABSTRACT

Background: Noroviruses (NoVs) are an important cause of acute gastroenteritis (AGE), worldwide.
Objectives: To evaluate the frequency, viral load and molecular profile of NoV in fecal and nasopharyngeal swab samples from hospitalized children, and to determine children's secretor status.
Study design: From May 2014 to May 2015, 219 children were included in the study, 96 with gastroenteric symptoms and 123 without gastroenteric symptoms. All fecal and nasopharyngeal swab samples were screened by TaqMan RT-qPCR duplex (GI/GII NoV) and quality samples were characterized by genomic sequencing.
Results: Norovirus positivity rate in feces was 15.4% in asymptomatic and 18.8% in the symptomatic group. The median viral loads in feces were 2.69×10^8 GC/g and 4.32×10^7 GC/g from children with or without AGE symptoms, respectively. In nasopharyngeal swab samples, the NoV positivity was 11.4% in symptomatic children, with a median viral load of 2.20×10^7 GC/mL and 6.5% in asymptomatic children, with an average viral load of 1.73×10^6 GC/mL. In only two cases NoV was detected in both samples. A considerable genomic variability was observed in feces, with six genotypes being detected, as follows: GII.4, GII.6, GI.3 and GII.3, GI.2 and GI.5. Two GI.3 was detected in nasopharyngeal swab.
Conclusions: Our data reveal considerable NoV frequencies in both nasopharyngeal and fecal samples from symptomatic and asymptomatic children. Higher viral loads were detected in samples from AGE symptomatic children, when compared to asymptomatic children. High genomic variability was observed, with this being the first report of GI.5 NoV in Brazil and of GI.3 in nasopharyngeal swab samples.

1. Background

Noroviruses (NoVs) are an important cause of acute gastroenteritis (AGE). In a systematic review it was estimated that the NoV account for 18% of all AGE cases in the world [1]. The *Norovirus* genus belongs to the *Caliciviridae* family, and is further subdivided into six genogroups and approximately 40 genotypes [2], based on the complete genomic sequence of the gene encoding the VP1 capsid protein [3]. Even though the viral receptor remains unknown, it is admitted that human histoblood group antigens (HBGAs) are putative receptors or co-receptors, at least for some NoV strains. Therefore, individuals who express such antigens in their mucosa (positive secretor status – Se +) would be more susceptible to NoV infection when compared to those that do not express those antigens in their mucosa (negative secretor status – Se –) [5].

NoVs are transmitted by the fecal-oral route, through direct person-to-person contact or contact with fomites, by ingestion of contaminated food or water, and ingestion of aerosolized particles from vomit of infected individuals [6–8]. One study reported NoV positivity in 0.5% (3/562) of nasopharyngeal swabs from children with respiratory symptoms, suggesting that NoV may be transmitted by the respiratory route [9].

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2. Objectives

The main objectives of this study were to evaluate the frequency, viral load and molecular profile of NoV in fecal and nasopharyngeal swab samples from children under six years of age, presenting or not AGE symptoms, hospitalized in a public child care referral hospital, and also to determine the secretor status of the children participating in the study.

3. Study design

3.1. Design and study population

This is an observational, cross-sectional study, conducted in samples obtained from children up to six years of age. Samples were collected from children hospitalized between May 2014 to May 2015 at the Materno Infantil Hospital, Goiânia, Goiás, Brazil. The study population was divided into two groups, the first group comprised children with AGE symptoms (diarrhea with or without vomiting and/or fever) at the time of sample collection and the second group included children who did not present any of those symptoms. The AGE asymptomatic population consisted of children that were attended for other reasons, such as surgery, congenital conditions, respiratory tract infections, and others.

The study population comprised 219 children between 0 and 70 months, with a mean of 15 months old. Considering the symptoms, 44% (96/219) of the children were included in the AGE symptomatic group. One fecal sample and one nasopharyngeal swab were obtained from each child participating in the study and all children's clinical data were obtained from medical records.

Samples were only collected after a consent form was signed by the parents or legal guardians of the child. The study was approved by the Ethics Committee on Research of the Clinical Hospital/Federal University of Goiás (protocol: 37305314.7.0000.5078).

3.2. Sample processing

All samples were stored at 4 °C until they were processed, stool samples and nasopharyngeal swabs were transported, processed, and tested separately. After collection, each nasopharyngeal swab was diluted in a sterile tube containing 2 mL of minimum essential media (MEM), resulting in an approximated dilution of 1:10, and transported immediately to the laboratory. The tubes were homogenized by vortexing, the swabs were removed from the tube that were centrifuged at 1300 × *g* at 10° C for 10 min. The supernatants were collected and used for viral RNA extraction. The cell pellets were stored for secretor status evaluation by genotyping and phenotyping. Stool suspensions were prepared (20% in PBS, pH 7.4), and all clinical samples were stored at −80 °C until further testing.

3.3. GI/GII NoV duplex RT-PCR TaqMan

Samples (20% fecal suspensions and swab supernatants) were extracted using a commercial kit (QIAamp Viral RNA Mini kit-Qiagen, Freigburg, Germany), following the manufacturer's instructions. Viral loads were determined by quantitative GI/GII NoV Duplex RT-PCR TaqMan (RT-qPCR) assay targeting the polymerase/capsid junction region (Table 1), using the AgPath-ID™ One Step RT-PCR kit (Life Technologies, Grand Island, NY), as it has done by Schultz et al. [13], adapted for a duplex reaction. For the standardization of the duplex RT-qPCR previously sequenced samples were used in previous studies conducted in our laboratory [14,15]. A negative control (MilliQ water) and a positive control were included in each run. To verify the presence of inhibitors TaqMan® Exogenous Internal Positive Control Kit (Life Technologies, Grand Island, NY) was used. Viral load was determined by a standard curve built with serial dilutions (10^7–10^1) of recombinant plasmids containing GI and GII NoV inserts (genotypes: GI.3 and GII.4). Curve validation was obtained by the correlation coefficient ($R > 0.99$) and efficiency of reaction. Samples with Cycle threshold (Ct) <40 cycles were considered positive.

3.4. Molecular characterization of NoV positive samples

To determine the NoV genotypes, samples with the highest loads were submitted to conventional RT-PCR assays using the same primer pair used in the RT-qPCR assay. Products that had sufficient DNA concentration were purified by using 65% isopropanol and 70% ethanol, and then submitted to sequencing reaction (Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA)), in duplicates, in an automatic sequencer (DNA ABI PRISM 3130, Applied Biosystems). Sequences quality analysis was determined by the interface phred/prhap [16]. Consensus genomic sequences and prototype sequences of each NoV GI and GII genotypes, obtained from GenBank, were subjected to alignment using Clustal X program [17]. Phylogenetic tree was constructed using the program MEGA version 7.0 [18]. Analysis were based on the neighbor-joining method, considering the nucleotide substitution model Kimura two parameters and 1000 replicates with boostraps values above 80%.

3.5. Determination of secretor status

Secretor status of the children were determined using nasopharyngeal swab cells by an enzyme immunoassay (EIE), using Lectin-UEA (Ulex europaeus agglutinin) specific for Fucα1-2Gal-R, which is secreted in saliva and mucous, only in secretors individuals, following the protocol described by Nordgren et al. [19], with modifications, instead of saliva epithelial cells from the respiratory tract were used as clinical material. Briefly, plates were sensitized with 100 μL of cells from nasopharyngeal swab pellet diluted 1:10 in carbonate-bicarbonate buffer (pH 9.6), and incubated at 37 °C for 2 h, followed by overnight incubation at 4 °C. Plates were washed with PBS (pH 7.4) and blocked at room temperature for one hour with PBS solution with 3% BSA. The conjugate (Lectin-UEA-HRP) diluted 1:1500 in PBS solution with 0.3% BSA was added to each well, and plate was incubated for 1.5 hs at 37 °C. The substrate was then added (H2O2 + TMB), followed by reading (absorbance determination at 450/620 nm). The cut-off value was determined by the mean of the absorbance of five negative controls plus the value of two standard deviations. An error margin of 10% above and below the cut-off value was considered. Two positive and negative controls were run on each plate. All samples were run in duplicate. Results from samples characterized as negative secretor phenotype were further confirmed by genotyping of the partial region of gene FUT2 as described by Lindesmith et al. [20], using cells from nasopharyngeal swab.

3.6. Data analysis

Data from the children's medical records were analyzed together with the obtained results. Statistical analysis were performed using IBM SPSS software, version 20, and the chi-square test ($\chi 2$) and Fisher's exact test were applied, when appropriate. Statistical significance was accessed, considering 95% confidence intervals, and p values < 0.05.

4. Results

The NoV positivity rate in feces was 15.4% (19/123) among children in the asymptomatic group and 18.8% (18/96) in the

Table 1
Oligonucleotide primers and probes used in the study.

Genogroup	Oligonucleotides	Sequence (5′ – 3′)	Orientation	Position[a]	Reference
GI	JJVMF	CCATGTTCCGTTGGATGC	+	5283–5300	10
	G1SKR	CCAACCCARCCATTRTACA	–	5358–5377	12
	RING1(c)	FAM[b]-AGATYGCGITCICCTGTCCA – BHQ[d]	–	5321–5340	10
GII	QNIF2	ATGTTCAGRTGGATGAGRTTCTCWGA	+	5012–5037	11
	G2SKR	CCRCCNGCATRHCCRTTRTACAT	–	5367–5389	12
	QNIFS	VIC[c]-AGCACGTGGGAGGGCGATCGMG – BHQ[d]	+	5042–5061	11

Sequences describes by Hill et al. [10], Loisy et al. [11] and Kojima et al. [12].
[a] Genomic location for isolates Norwalk virus, GI – NoV (ID: M87661), Lordsdale virus (GII – NoV) (ID: X86557).
[b] probe labeled with the fluorophore FAM.
[c] probe labeled with the fluorophore VIC.
[d] BHQ – Black Hole Quencher.

Table 2
Characteristics of patients considering positivity for NoV in fecal or swab samples.

	Fecal samples			Nasopharyngeal samples		
	Positivity/n	(%)	*p*	Positivity/n	(%)	*p*
Sex						
Female	18/91	19.8		10/91	11	
Male	19/128	14.8	0.3	9/128	7	0.3
Age (months)						
0–24	25/170	14.7		16/170	9.4	
25–48	9/33	27.3	0.6	2/33	6.1	0.8
49–72	3/16	18.8	0.5	1/16	6.3	0.7
Presence of symptoms (AGE)						
Yes	18/96	18.8		11/96	11.5	
No	19/123	15.5	0.5	8/123	6.5	0.2
Symtoms						
Diarrhea and fever	6/30	20		4/30	13.3	
Diarrhea and vomiting	1/2	50	0.4	1/2	50	0.2
Diarrhea, fever and vomiting	11/45	24.5	0.7	5/45	11.1	0.16
Diarrhea, fever, vomiting and abdominal pain	0/19			1/19	5.3	0.46
Secretor status						
Se+	35/202	17.3		18/202	8.9	
Se –	2/17	11.8	0.6	1/17	6	0.67
Viral load (median)						
Symptomatic children	2.69×10^8 GC/g[a]			2.20×10^7 CG/mL[b]		
Asymptomatic children	4.32×10^7 GC/g[a]			1.73×10^6 CG/mL[b]		

n –number of samples.
[a] GC/g, genomic copies per gram.
[b] GC/g, genomic copies per mL.

symptomatic group. Viral load in feces varied from 5.58×10^6 to 2.38×10^{11} GC/g in samples of children with symptoms (median 2.69×10^8 GC/g) and from 2.79×10^4 to 2.38×10^{10} GC/g (median 4.32×10^7 GC/g) in samples of children in the asymptomatic group (Tables 2 and 3).

In nasopharyngeal swab samples the NoV positivity was 11.4% (11/96) in symptomatic, with viral loads that ranged from 2.33×10^6 to 1.25×10^9 GC/ml (median of 2.20×10^7 GC/mL) and 6.5% (8/123) in asymptomatic, with viral loads varying from 4.22×10^4 to 1.77×10^8 GC/mL (median of 1.73×10^6 GC/mL). NoV was detected in both feces and nasopharyngeal swab of the same child, in only two cases. One patient had AGE symptoms, whereas the other was asymptomatic. The viral load was 4.33×10^7 GC/g in feces and 1.77×10^8 GC/ml in nasopharyngeal swab of one child, and 2.44×10^9 GC/g in feces and 7.91×10^6 GC/mL in nasopharyngeal swab from the other child (Tables 2 and 3). In addition, among the 11 children with AGE symptoms that were positive for NoV in nasopharyngeal swab samples, 63.3% (7/11) also had respiratory symptoms such as coughing and/or wheezing, accompanied by fever, at the time of sample collection. Among the eight children without AGE symptoms, 50% (4/8) also had respiratory symptoms. The only two children who were positive for NoV in both samples (fecal and nasopharyngeal swab) also presented respiratory symptoms.

Considering fecal sample positivity, a higher frequency of GII NoV (70%) was detected, when compared to GI NoV (30%); however, this was not observed in nasopharyngeal swab samples, of which 52% (10/19) were positive for GII NoV and 48% (9/19) for GI. There was no statistical significance in NoV positivity, when considering characteristics of children such as sex, age, presence of symptoms and secretor status (Table 2).

A considerable genomic variability was observed among the 16 NoV-positive samples that could be sequenced, with six genotypes being detected in feces (14 samples total), as follows: GII.4 (35.7%, n = 5), GII.6 (21.4%, n = 3), GI.3 and GII.3 (14.4%, each, n = 2), and GI.2 GI.5 (7.1% each, n = 1). Four GII.4 samples (44.5%), three GII.6 samples (33.3%), one GII.3 and GI.3 samples (11.1% each) were all from children with AGE symptoms, whereas two GI.3 (33.3%),

Table 3
Characteristics of all patients positive for norovirus (in fecal and/or nasopharyngeal swab).

Patient ID	Sex	Age (months)	Month colettion	AGE symptoms	Secretor status	NoV Genogroup/Genotype	Viral load (GC/g) in feces	Viral load (GC/mL) in nasopharyngeal swab
1	F	11	May/2014	–	+	GII	9.62×10^{6}	–
2	F	2	June/2014	–	+	GII	–	4.34×10^{5}
3	F	62	June/2014	–	+	GI	9.11×10^{7}	–
4	M	8	July/2014	–	+	GI	2.79×10^{8}	–
5	M	17	July/2014	Diarrhea and vomiting	+	GII	–	4.80×10^{6}
6	F	1	July/2014	–	+	GII	–	4.62×10^{6}
7	F	2	July/2014	–	+	GI	–	8.33×10^{5}
8	M	2	July/2014	Diarrhea, fever and vomiting	–	GI	2.14×10^{7}	–
9	M	1	Aug/2014	–	+	GI.3	4.33×10^{7}	1.77×10^{8}
10	M	9	Aug/2014	Diarrhea, fever and vomiting	+	GI	–	5.99×10^{7}
11	M	44	Aug/2014	–	+	GII	1.79×10^{8}	–
12	M	1	Aug/2014	–	+	GI	–	1.89×10^{6}
13	M	35	Aug/2014	Diarrhea and fever	+	GII.3	1.71×107	–
14	M	11	Aug/2014	Diarrhea, fever and vomiting	+	GII.4	1.79×10^{8}	–
15	F	8	Aug/2014	Diarrhea and fever	+	GII	3.59×10^{8}	–
16	F	48	Set/2014	Diarrhea, fever and vomiting	+	GII	3.97×10^{6}	–
17	M	1	Set/2014	–	+	GI.3	–	6.20×10^{6}
18	F	1	Set/2014	–	+	GI	4.32×10^{7}	–
19	F	27	Set/2014	Diarrhea and fever	+	GI	–	1.02×10^{8}
20	F	2	Set/2014	–	+	GII.4	8.13×10^{7}	–
21	F	8	Set/2014	–	+	GII	1.49×10^{7}	–
22	F	7	Set/2014	Diarrhea and fever	+	GI	–	1.25×10^{9}
23	F	48	Set/2014	–	+	GII.3	3.55×10^{7}	–
24	M	41	Set/2014	Diarrhea and fever	+	GII.6	8.59×10^{8}	–
25	M	12	Oct/2014	Diarrhea, fever and vomiting	+	GII	2.44×10^{9}	7.91×10^{6}
26	M	27	Oct/2014	Diarrhea and fever	+	GII.4	6.95×10^{8}	–
27	F	19	Oct/2014	Diarrhea, fever and vomiting	+	GII.4	2.14×10^{9}	–
28	F	13	Oct/2014	–	+	GII	7.53×10^{4}	–
29	M	70	Oct/2014	Diarrhea, fever and vomiting	+	GI.3	5.58×10^{6}	–
30	F	1	Oct/2014	–	+	GII	–	4.22×10^{4}
31	M	1	Oct/2014	Diarrhea, fever and vomiting	+	GI	2.38×10^{11}	–
32	M	24	Oct/2014	Diarrhea, fever and vomiting	+	GII.4	1.19×10^{8}	–
33	F	1	Oct/2014	–	+	GI	2.79×10^{4}	–
34	F	23	Oct/2014	Diarrhea, fever and vomiting	+	GI	–	5.84×10^{8}
35	M	16	Oct/2014	Diarrhea and fever	+	GII	–	5.39×10^{6}
36	F	24	Nov/2014	Diarrhea, fever and vomiting	+	GI	–	2.58×10^{8}
37	M	30	Nov/2014	Diarrhea, fever, vomiting and abdominal pain	+	GII	–	2.20×10^{7}
38	F	7	Nov/2014	Febre, diarreia e vômito	+	GII	–	1.46×10^{7}
39	M	9	Nov/2014	Diarrhea and vomiting	+	GII.4	1.93×10^{11}	–
40	M	5	Dec/2014	–	+	GII	4.82×10^{7}	–
41	F	16	Dec/2014	Diarrhea and fever	+	GI	1.72×10^{8}	–
42	F	5	Dec/2014	–	+	GII	2.80×10^{6}	–
43	F	1	Dec/2014	–	+	GII	1.83×10^{5}	–
44	F	16	Jan/2015	–	+	GI.5	1.46×10^{9}	–
45	M	40	Jan/2015	–	+	GI.2	4.43×10^{6}	–
46	F	12	Jan/2015	–	–	GII	–	1.57×10^{6}
47	M	3	Feb/2015	Diarrhea, fever and vomiting	+	GII.6	2.79×10^{10}	–
48	M	1	Feb/2015	Diarrhea, fever and vomiting	+	GII	5.69×10^{7}	–
49	F	27	Feb/2015	Diarrhea, fever and vomiting	+	GII	2.82×10^{7}	–
50	M	4	Mar/2015	Diarrhea and fever	+	GII	–	2.33×10^{6}
51	F	16	May/2015	–	+	GII	2.38×10^{10}	–
52	M	22	May/2015	Diarrhea and fever	+	GII	1.27×10^{9}	–
53	F	49	May/2015	–	+	GII	1.55×10^{10}	–
54	M	4	May/2015	–	–	GII	2.88×10^{6}	–

ID: Identification; F: female; M: male; AGE: acute gastroenteritis; –: negative.

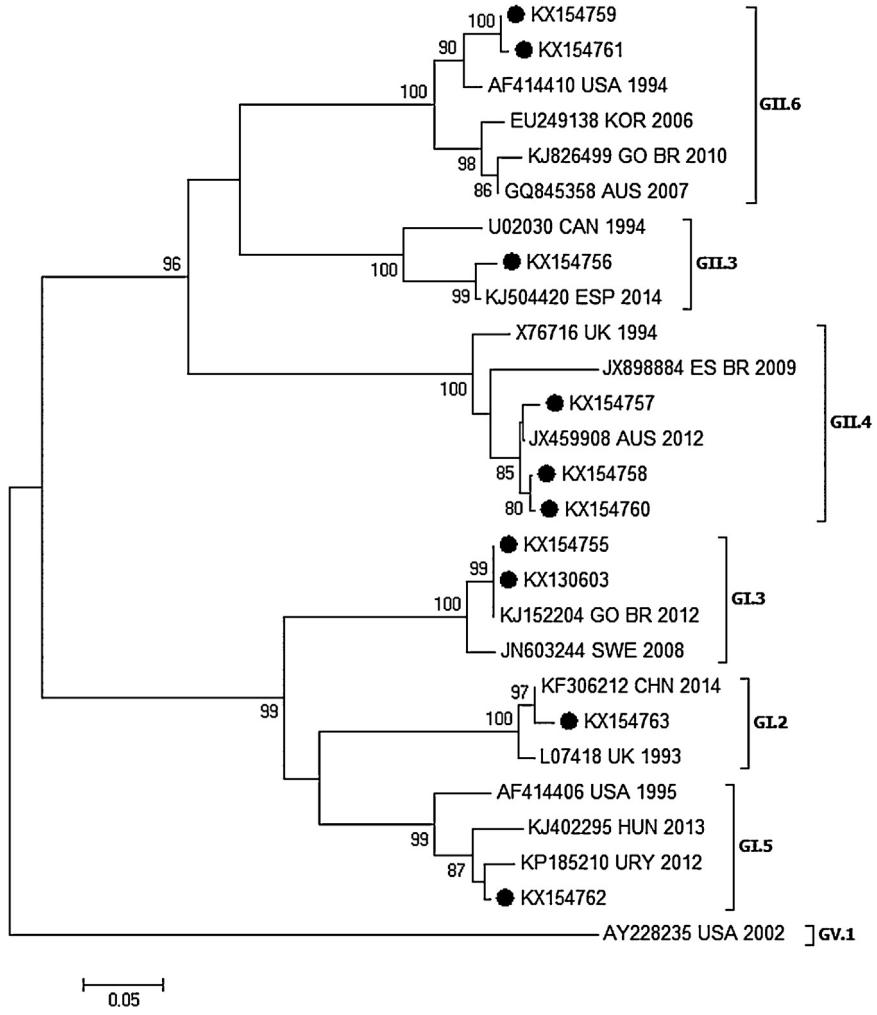

Fig. 1. Phylogenetic analysis of partial sequence of the RNA-dependent RNA polymerase/capsid gene (C region) (389/378 bp) detected in fecal and nasopharyngeal swab samples from HMI patients. Samples characterized in this study are marked by a full circle. The evolutionary history was inferred using the Neighbor-Joining method. Bootstrap values were obtained in 1000 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood method. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2015).

one of each GI.2, GI.5, GII.3 and GII.4 samples (16.7% each) were obtained from asymptomatic children. Only GI.3 NoV was detected in nasopharyngeal swab samples (two samples), both obtained from asymptomatic patients, one patient that was positive for NoV in both samples (feces and nasopharyngeal swab) and the other from a patient who was positive only in nasopharyngeal swab sample.

Three GII.4, two GII.3, two GII.6 and two GI.3 in feces and two GI.3 in nasopharyngeal swab showed 100% nucleotide identity, therefore each of these groups of samples were designated by single access number (GI.3/nasopharyngeal swab – KX130603, GI.3/fecal sample – KX154755, GII.3 – KX154756, GII.4 – KX154757, GII.4 – KX154758, GII.6 – KX154759, GII.4 – KX154760, GII.6 – KX154761, GI.5 – KX154762 and GI.2 – KX154763) (Fig. 1).

5. Discussion

In this study, considerable NoV positivity indexes were found in both AGE symptomatic (18.8%) and asymptomatic (15.4%) groups of children. It is estimated that NoV detection rate in hospitalized children with AGE varies from 3 to 35.4% and among asymptomatic children from 0 to 16% [21–24], including in Brazil.

This is the first investigation of NoV in both nasopharyngeal swab and fecal samples of the same children, being observed a considerable positivity rate in symptomatic and asymptomatic children. A previous study [9], has investigated nasopharyngeal swab samples for NoV presence and the detection rate was only 0.5% (3/562), but it is important to note that the population of the study consisted of children with respiratory symptoms previously tested and negative for respiratory viruses. Surprisingly, in our study, 19 children had NoV positivity in swab samples, and high positive index for NoV was observed in children that were no positive for the virus in feces (89.5%); furthermore, 42% of children did not have AGE symptoms at the time of sample collection.

Only two children had NoV positive in both, fecal and swab sample, and only one had AGE symptoms, including vomiting. This was surprising, since one of the hypothesis for the presence of virus in the respiratory tract could be reflux, because of vomiting, of particles to the respiratory tract [9]; however, only one child had vomiting in our study. Furthermore, a high positivity index for NoV nasopharyngeal swab sample was observed without the presence of the virus in feces, suggesting that NoV particles would not be coming from the gastroenteric tract.

Regarding the presence of respiratory symptoms in patients who were positive for NoV in nasopharyngeal swab sample, we have not investigated the presence of other respiratory viruses; therefore, these symptoms cannot be attributed to NoV. In addition, this was cross-sectional study, and we did not continue to monitor

these children so it may be possible that they could have presented AGE symptoms after the nasopharyngeal swab was collected.

Although, the RT-qPCR methodology is being increasingly used for NoV screening in samples from children with AGE symptoms [25–27], viral loads are not always reported. Our data on viral loads corroborate results from Barreira et al. [28] that also observed higher viral loads, one $\log_{10}$ higher, in samples from symptomatic, when compared to asymptomatic children. Interestingly, higher loads (one $\log_{10}$ higher) were also observed in nasopharyngeal swab samples of children with AGE, when compared to asymptomatic children.

The higher frequency of GII NoV (70%) in feces, when compared to GI NoV (30%), is in accordance with frequencies reported by many studies, including those conducted in Brazil [22,25,29,30], reinforcing the predominance of GII circulation in many parts of the world [31]. However, it should be noted that the frequencies of GII (52%) and GI NoV (48%) in nasopharyngeal swab samples were similar.

Although, a higher percentage of NoV-positive stool samples were also detected in samples from secretor positive children (94.6%), we observed that 92% of the total children participating in our study were characterized as positive secretor status, so it is not possible to say that there was an association between the secretor status and NoV positivity.

A considerable NoV variability was detected in this study, with GII.4 genotype being the most frequent variant in fecal samples. This GII.4 predominance has been reported worldwide, mainly in samples from symptomatic cases [23,32–34]. The GII.6 genotype was the second most detected and mainly associated with symptomatic cases, corroborating data by Currier et al. [32]. However, Vicentini et al. [34] reported GII.6 NoV only in association with asymptomatic cases.

In Brazil, the predominance of GII.4 followed by GII.6 in symptomatic cases has been reported [35,36]; however, it should be noted that these studies were conducted only in samples from symptomatic patients. A considerable NoV variability was also detected in a day care center in Goiânia, Brazil, and the most frequent genotype was GII.6 followed by GII.4 [15]. This is the first time that GI.5 NoV is detected in fecal samples from Brazil. This genotype has been detected in other Latin American countries, such as Mexico [33,37–39].

Unfortunately, only two samples of nasopharyngeal swab could be sequenced, and were both characterized as GI.3, with this being the first report of GI.3 in this type of sample.

Considering the phylogenetic analysis, GII.6 samples had higher nucleotide identity with the Miami292_1994 sample [40] than with samples identified more recently in other countries [41,42], including samples previously detected in the same city in Brazil [15]. The GII.4 samples had higher nucleotide identity with the pandemic GII.4 Sydney [43] strain that is still predominantly circulating [31]. In addition, GI.3 samples showed high nucleotide identity (99%) with a sample detected in a previous study also conducted in bone marrow transplanted patients in the city of Goiânia [14].

We have found considerable NoV frequencies and loads in feces and nasopharyngeal swab samples from both AGE symptomatic and asymptomatic children, and also high genomic variability in feces, reporting for the first time, GI.5 NoV in Brazil and GI.3 in nasopharyngeal swab samples. We hope to have contributed for a better understanding of NoV molecular epidemiology, given that this information will be crucial for the development of an effective vaccine against NoV. Furthermore, data on NoV detection and viral load in nasopharyngeal swab samples in children with and without AGE symptoms are novel, and should be investigated by other groups.

Competing interests

None.

Ethical approval

Ethical approval was obtained from the Research Ethics Committees of Clinical Hospital – Federal University of Goiás/protocol: 37305314.7.0000.5078

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