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Role of *Noroviruses* in Sporadic Acute Gastroenteritis Cases from Children Attended in a Large Referral Children's Hospital in Buenos Aires City, Argentina

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Abstract

Background: *Noroviruses* (NV) are recognized as the leading cause of sporadic and epidemic acute gastroenteritis worldwide, in all age groups. While there is increasing knowledge that NV is responsible for many acute gastroenteritis outbreaks in Argentina, studies to estimate prevalence in sporadic cases are scarce.

Methods: Descriptive, observational, cross-sectional study was conducted with children less than 5 years with acute gastroenteritis attending the outpatient department at the "Ricardo Gutiérrez" Children's Hospital (RGCH) in Buenos Aires city between June 2017 and June 2021. Sociodemographic, clinical and epidemiological data were recorded. Stool samples were tested and genotyped for norovirus. Association between *Norovirus* detection and demographic and clinical variables was assessed.

Results: A total of 350 children with acute diarrhea were included, of which stool samples were collected for 332 (94.9%). *Norovirus* was detected in 81 cases (24.4%). Vomiting and moderate/severe diarrhea were more frequent in *Norovirus* positive than *Norovirus* negative children. However, presence of watery diarrhea and history of *Rotavirus* vaccination were significantly associated with *Norovirus* etiology. GII and GII.4 were the most frequently detected genogroup and genotype, respectively.

Conclusion: *Noroviruses* were detected with high frequency, mostly in children between 6 months and 2 years old, reinforcing the hypothesis of a new updated scenario of *Norovirus* predominance over *Rotavirus*. Watery diarrhea, complete vaccination against *Rotavirus* and vomiting are three key parameters that should raise suspicion of possible *Norovirus* gastroenteritis. Continuous and active *Norovirus* surveillance in this age group is important since children represent a priority group for *Norovirus* vaccine design and development.

Keywords: Diarrhea; *Norovirus*; Argentina; Children

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Introduction

Acute diarrhea is still a major public health concern worldwide since it is associated with high morbidity and mortality in children under 5 years of age, particularly in developing countries [1]. Diarrheal diseases cause not only infections and death, but also malnutrition leading to stunting and cognitive impairment [2,3].

Etiological studies have shown the disease is caused mainly by viruses, especially in young children [4-7]. Rotaviruses and *Noroviruses* are widely described as the most common enteropathogens in children under 58. Moreover, in countries where universal *Rotavirus* vaccination has reached significant coverage levels, disease burden attributable to *Norovirus* (NV) is greater than that resulting from *Rotavirus* in the first years of life [8-13].

Noroviruses are recognized as the leading cause of sporadic and epidemic acute gastroenteritis worldwide, in all age groups, with high disease burden in young children, immunocompromised individuals, and the elderly [14]. This pathogen is responsible for over 200,000 deaths in children under 5 [15]. Recent studies in developing countries have described the presence of NV accounted for 15 to 30% of sporadic acute diarrheal episodes in

both outpatient and hospital settings [16-18].

Noroviruses can be classified into genogroups based on complete VP1 amino acid sequences, of which GI and GII predominate in humans. Each genogroup can be further divided into genotypes based on nucleotide sequences of the capsid and polymerase regions [19]. GII.4 is the most frequently detected strain worldwide, associated with nearly 70% of the *Norovirus* infections. As GII.4 has shown epochal evolutionary dynamics, distinct variants are expected to emerge periodically replacing previous dominant strains [20].

In Argentina, around 1-1.25 million cases of acute gastroenteritis are reported each year in the public health sector, half of which occur in children under 5, resulting in a rate of 1,450 to 1,680 per 10,000 and *Rotavirus* has been identified as the most common etiological agent of diarrheal cases in that age group [21,22]. However, results from sentinel surveillance for gastroenteritis showed 40-60% of acute episodes in children remained undiagnosed [23]. After routine *Rotavirus* vaccination was incorporated to the national immunization program in 2015 (2 doses of monovalent vaccine at 2 and 4 months of age), rapid and significant decline in *Rotavirus* infection numbers was observed, suggesting NV could now be the leading cause of acute diarrhea in children, as has occurred in other countries after the introduction of *Rotavirus* vaccines [24].

While there is increasing knowledge that NV are responsible for many acute gastroenteritis outbreaks, few studies have been conducted to estimate prevalence in sporadic acute diarrhea cases in children, and the information on this in the last two decades is scarce [25-29].

The study aim was to assess the role of NV in sporadic acute diarrhea cases in children under 5 years of age attending the outpatient department at a large tertiary children´s hospital in Buenos Aires city, in order to contribute knowledge on the burden of *Norovirus* associated acute diarrhea and improve policies for disease control as well as accelerate vaccine development.

Materials and Methods

Study population

A descriptive, observational, cross-sectional study was conducted. Children under 5 years of age with symptomatic acute gastroenteritis attending the outpatient department at the "Ricardo Gutiérrez" Children's Hospital (RGCH), one of the three largest pediatric referral centers in Buenos Aires city, between June 2017 and June 2021, were included.

Study enrollment was carried out through non-random convenience sampling to reduce potential selection bias. Data were recorded on case report forms detailing: demographics (age, gender), medical history and epidemiology (breastfeeding, premature birth, malnutrition or weight below 10th percentile, household members with diarrhea, daycare attendance), rotavirus vaccination (number of doses), diarrheal episode (date of symptom onset and of consultation, presence of vomiting, stool characteristics: watery/mucous/mucohemorrhagic/hemorrhagic, absent/mild/moderate/severe dehydration, prior antimicrobial treatment, and disease severity according to the vesikari score).

Stool samples were submitted to the argentine reference laboratory for viral gastroenteritis (INEI-ANLIS "Dr. Carlos G. Malbrán") for *Norovirus* testing and genotyping, as previously described. Briefly, after nucleic acid isolation (QIAampTM Viral RNA Mini Kit, Qiagen), samples were screened for NV GI and GII by a real-time quantitative reverse transcription polymerase chain reaction targeting the ORF1/ORF2 junction region (AgPath-IDTM One-Step RT-PCR, Applied Biosystems). *Norovirus* positive samples were further genotyped through amplification and sequencing of partial regions encompassing the 3'-end of the polymerase (RdRp) gene and the 5'-end of the capsid (VP1) genes (QiagenTM One-Step RT-PCR Kit, Qiagen). Genotype assignment of RdRp and VP1 partial sequences were obtained using the online software human calicivirus typing tool. *Norovirus* positive samples were additionally tested for other viral and bacterial enteropathogens using ELISA (Group A *Rotavirus*), RT-qPCR (*sapovirus* and astrovirus), qPCR (Group F adenovirus), and conventional culture with biochemical identification (*Salmonella* sp, *Shigella* sp, *Campylobacter* sp, diarrheagenic *E. coli, Aeromonas* sp, and *Yersinia sp*.) to determine presence of mixed infections.

Statistical analysis

For quantitative variables, calculation of median and Interquartile Range (IQR), or mean and standard deviation (and 95% confidence interval) was performed. Also, proportions for categorical variables were described. Wilcoxon rank sum or t-test (depending on the distribution of data), and *Chi-square* were used for continuous data and proportions, respectively.

Association between NV detection and demographic and clinical variables was assessed. The odds ratio and 95% Confidence Interval (95% CI) were used to measure potential association and p-value <0.05 considered statistically significant.

Results

From June 2017 to June 2021 a total of 350 children with acute diarrhea were identified and included in the study. Median age was 21.8 months (IQR:11-30 months) and 58.6% were males. Most of the children were eutrophic (98.3%) and 42.7% were breastfed. Seventy five (21.4%) children had at least one household member with diarrhea and 73.6% had been vaccinated for rotavirus (85.8% had received both doses). The most accompanying symptoms were fever (62.6%) and vomiting (54.3%). Fifty-six percent of patients presented watery diarrhea, followed by muco-hemorrhagic (42.6%), and hemorrhagic diarrhea (1.4%); 94.6% were well hydrated and no child suffered severe dehydration or shock. With respect to disease severity 41.4% were classified as mild, 49.4% as moderate, and as 9.2% severe according to the Vesikari Scoring System.

Oral rehydration solution and fluid therapy were prescribed in almost all patients (97.7%). Only eight patients were admitted for intravenous rehydration therapy.

Even though acute diarrhea cases were uniformly distributed throughout the year, two seasonal were observed in consultations, January to March and June to August. In Argentina, the government implemented SARS-CoV-2 pandemic lockdown

and distancing policies from March to November 2020, with 'homeschooling' as part of the adopted measures, producing a sharp decline in clinic visits related to diarrheal disease. Thus, as foreseeable, no outpatient came to hospital between March and October 2020 after the SARS-CoV-2 pandemic lockdown was implemented (Figure 1).

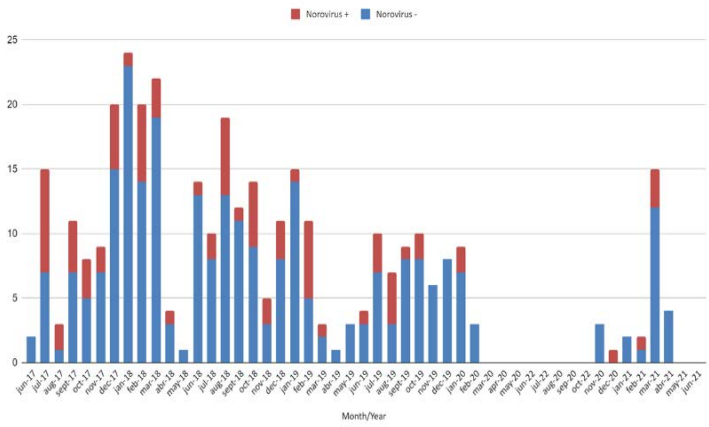

Figure 1: Monthly distribution of acute diarrhea and norovirus cases from outpatient children under 5 years. RGCH, 2017-2021. *Norovirus* positive and *Norovirus* negative cases are color-coded.

Stool samples were collected for 332 (94.9%) patients and NV was detected in 81 acute diarrhea cases (24.4%). Positive NV cases were evenly distributed throughout the year, although higher frequencies were observed in the months of July/August (2017-2019) and February (2018-2019). Sixty-five percent were male and the median age was 18.0 months (SD: 12.8 months); most cases (61.7%) occurred in children under 2 years, especially between 6 and 18 months of age (64.0%). With respect to epidemiological features, only 23.5% of positive cases referred a symptomatic household member, and 13.0% attended daycare; 84.8% had been vaccinated against *Rotavirus*, of which 90.7% had received two doses. Fever and vomiting were present in 55.6% and 61.7% of cases, respectively. Most *Norovirus* positive cases presented watery diarrhea (64.2%), followed by mucohemorrhagic (34.6%) and hemorrhagic (1.23%) stools. Although moderate/severe diarrhea was present in 59.2%, almost all patients (95.1%) showed no significant loss of fluids and were sent home with information about monitoring for emergency signs, recommended home fluids or oral rehydration therapy.

Vomiting and moderate/severe diarrhea were more frequent in *Norovirus* positive than *Norovirus* negative children, but the difference was not statistically significant. Conversely, presence of watery diarrhea and history of *Rotavirus* vaccination were significantly associated with NV etiology (Table 1).

Table 1: Comparison of clinical and epidemiological characteristics between *Norovirus* positive and negative cases.

	***Norovirus* (+) (n=81)**	***Norovirus* (-) (n=251)**	**OR (CI 95%a)**	**P**
Sex (Male)	65.40%	55.80%	1.5 (0.9-2.5)	0.06
Age (Months) (Median; IQR)	18	19		0.59
	(IQR= 11-27)	(IQR= 12-30)		
Rotavirus vaccination (Yes)	84.80%	69.60%	2.4 (1.2-4.8)	0.003
2 doses	90.70%	85.60%	1.6 (0.6-4.6)	0.17
Symptomatic household members (Yes)	23.50%	21.10%	1.1 (0.6-2.1)	0,33
Fever (Yes)	55.60%	64.10%	0.7 (0.4-1.2)	0.08
Vomiting (Yes)	61.70%	52.60%	1,4 (0.9-2.4)	0.07
Watery diarrhea (Yes)	64.20%	46.20%	1.5 (0.9-2.6)	0.05
Moderate and severe diarrhea (Vesikari scale) (Yes)	59.30%	41.00%	1.0 (0.6-1.7)	0.48
Normally hydrated (Yes)	95.10%	94.00%	1.2 (0.4-3.8)	0.38

Of the samples that tested positive for NV, 27 (33.3%) showed bacterial coinfection. The most frequently isolated microorganisms were Campylobacter spp (13, 48.1%) followed by Shigella spp (9, 33.3%), Aeromonas spp (3, 11.1%), and diarrheagenic *E. coli* and Salmonella spp (1 case each, 3.7%). Yersinia spp coinfection was not detected among the *Norovirus* cases. Viral co-infections were detected in 5 cases with Rotavirus (6.2%), 5 with Astrovirus (6.2%), 4 with Group F Adenovirus (4.9%), and 3 with *Sapovirus* (3.7%). No *Norovirus* cases exhibited coinfection with two or more enteropathogens.

Genotyping showed 63 NV (77.8%) were GII, 17 (21.0%) GI, and 1 (1.2%) was a mixed GI/GII infection. Six different genotypes were identified for GII (GII.2, GII.4, GII.5, GII.6, GII.12, and GII.14) and 3 for GI (GI.3, GI.5, and GI.6). The most frequent genotypes were GII.4 Sydney [P16] (28.4%), GII.2 [P16] (4.9%), and GII.14 [P7] (4.9%) for GII strains, and GI.5 [P5] (6.2%) and GI.3 [P3] (4.9%) for GI strains. Of note, in 29 (35.8%) strains the genotype could not be identified (Figure 2).

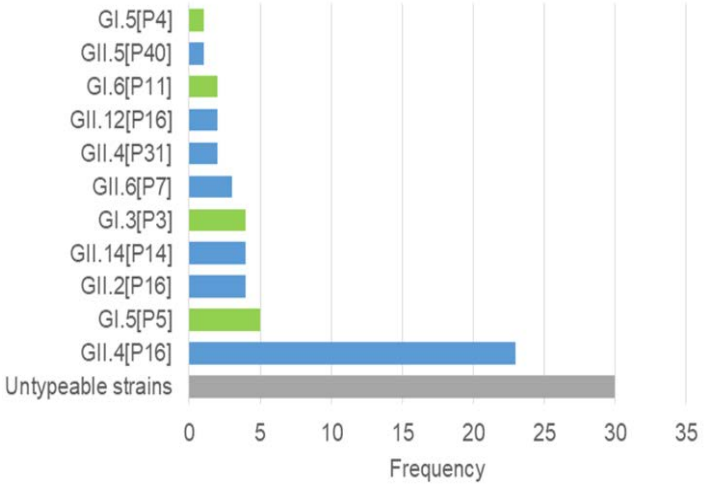

Figure 2: *Norovirus* genotypes frequency of detection. RGCH, 2017-2021 (n=50). GI and GII genogroup strains are color-coded in green and blue, respectively.

Discussion

In this study we assessed the role of norovirus as the cause of acute symptomatic gastroenteritis in children less than 5 years of age, treated as outpatients at a large tertiary referral children´s hospital in Buenos Aires city. Overall, NV was detected in 24.4% of stool samples analyzed. These results are in agreement with those of recent studies conducted in Argentina and Latin America through both active and passive surveillance programs [30]. We detected NV cases throughout the year, with higher rates during warmer and colder months. This seasonal pattern differs from other studies describing a single winter peak [31]. Highest prevalence occurred in children between 6 months and 2 years old, the same age group classically affected by *Rotavirus*, reinforcing the hypothesis of a new updated scenario of *Norovirus* predominance over *Rotavirus*, especially in countries with optimal *Rotavirus* vaccination coverage [32].

Our results describe the clinical and epidemiological profile of NV infections in a sample population of children under 5 years of age. However, they only represent a very small fraction of the total number of cases, as acute diarrhea is generally underreported [33]. With respect to NV in particular, lack of accessible diagnostic methods at most healthcare centers further undermines estimates on the role of this pathogen as a common etiological agent. In this study, patients who developed watery diarrhea and had complete vaccination against *Rotavirus* were likely to have *Norovirus* infection. Although not statiscally significant, many of the cases also presented vomiting. These three parameters, when present, should raise suspicion of possible NV gastroenteritis.

Also noteworthy, was the high proportion of both bacterial and viral coinfections among *Norovirus* cases. While coinfections have been extensively described in developing countries, the contribution of multiple enteropathogens to disease severity and prognosis remains under debate [34-36]. In our study, *Norovirus* cases with bacterial and viral coinfections were more associated with moderate and severe than to mild cases (P-value= 0.028).

Analysis of viral diversity showed GII was the most predominant geno group and GII.4 was found in one third of the positive cases. As in previous studies, genotypes found in sporadic cases were more diverse than those associated with outbreaks [37,38]. Moreover, polymerase genotype GII.P16 was detected in association with GII.2, GII.4 and GII.12 capsid genotypes. Spread of this polymerase occurred rapidly after its association with GII.4 Sydney during the 2015-2016 seasons. It is thought to have significant epidemic potential due to its ability to generate recombinant strains with several genotypes [39]. Twenty nine *Norovirus* strains could not be genotyped as it was not possible to amplify or sequence the specific target for dual genotyping, mainly because viral loads in stool samples were low, and correlated with higher RT-qPCR Ct values (>30) compared to genotyped strains (data not shown). Interactions between viral load and host-related factors, pathogens, transmission mode and timing of specimen collection remain unclear. Since our population comprised immunocompetent, ambulatory patients, predominance of mild episodes could help explain the high Ct values.

Conclusion

Continuous and active NV surveillance in children is important for several reasons. First, as *Rotavirus* vaccines have been increasingly incorporated into National Immunization Programs, *Norovirus* is becoming more prevalent as the leading cause of acute gastroenteritis. Second, it has been proposed that children are reservoirs of genetic diversity; therefore early detection could allow identification of emerging strains with pandemic potential circulating before detection during outbreaks. Also, as with other high-risk populations, children represent a priority group for norovirus vaccine design and development, to prevent and control the increasing burden of disease.

During the course of the COVID-19 pandemic in 2020, acute gastroenteritis episodes declined. It was assumed that *Norovirus* cases had decreased as well. Conversely, in the first semester of 2021, *Norovirus* infections increased, probably because social activities and schools reopened, as restrictions implemented during the COVID-19 lockdown were loosened. For this reason, active monitoring of acute diarrhea and *Norovirus* infections is important in the post pandemic scenario, during which improved personal hygiene measures such as hand washing were put into practice, but rates of routine rotavirus vaccination fell.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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