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Short Communication

Norovirus passive surveillance as an alternative strategy for genetic diversity assessment in developing countries

Juan I. Degiuseppe[a,*], Karina L. Roitman[a], Karina A. Rivero[b], Juan A. Stupka[a], Argentine Surveillance Network for Viral Gastroenteritis[a]

[a] Laboratory of Viral Gastroenteritis, INEI-ANLIS "Dr. Carlos G. Malbrán", Av. Vélez Sársfield 563, Buenos Aires 1281, Argentina
[b] Microbiology Internship Program, INEI-ANLIS "Dr. Carlos G. Malbrán", Av. Vélez Sársfield 563, Buenos Aires 1281, Argentina

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ABSTRACT

In developing countries, the acute gastroenteritis outbreaks submitted for viral testing are limited due to deficient surveillance programs. The aim of this study was to analyze a passive surveillance strategy for monitoring the molecular epidemiology of norovirus (NV) and counterbalance the genetic diversity data gap.

Laboratory-confirmed rotavirus negative sporadic stool samples (N = 523) collected between 2010 and 2017 from children were selected from our archival collection and were tested for NV and sequencing was performed on the positive samples. Passive surveillance information was compared with the genetic diversity data that was available from local norovirus-confirmed gastroenteritis outbreaks.

Each year, norovirus detection in the sporadic samples ranged from 12 to 29%. GI and GII norovirus were detected in 7 (1.3%) and 101 (19.3%) of the specimens, respectively. Four GI and six GII capsid genotypes were identified. Six out of 9 strains detected in the NV outbreaks panel were also identified in the set of sporadic samples either coincidently in the same year, the previous or the later year. Also, this set of samples depicted even better the circulating epidemic strain. Thus, implementing norovirus testing and genotyping in stool samples collected with other purposes represent a suitable strategy for providing genetic diversity information.

Introduction

Noroviruses are the leading cause of symptomatic acute gastroenteritis in people of all-age groups worldwide [1] and they have been widely described as the responsible for the majority of gastroenteritis outbreaks in semi-closed settings [2,3].

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 the polymerase regions [4]. Either way, GII.4 is the most frequently detected strain worldwide as is associated with near 70% of the norovirus infections. Unlike other genotypes, GII.4 has shown epochal evolutionary dynamics, with periodical emergence of genetically distinct variants replacing previous dominant strains [5].

Monitoring the norovirus genetic diversity is important for two main reasons: (i) genotypes may suggest the possible routes of transmission in outbreak settings [6]; and (ii) understanding the genetic diversity and evolution is vital for norovirus vaccine development. Norovirus outbreaks are the most common scenarios to study its molecular epidemiology. However, in many developing countries the amount of reported and tested acute gastroenteritis outbreaks is disturbingly limited even though they often represent a mandatory notification health event. Under notification occurs mainly because most of the cases go unreported if people do not seek medical care, either due to lack of healthcare access or due to the mildness and self-limited nature of the symptoms [7]. Also, molecular diagnostic assays are not usually available in clinical settings because they are cost-prohibitive and viral testing in cases of diarrhea in the general population is not often a priority, so they remain undiagnosed.

* Corresponding author.
E-mail addresses: jdegiuseppe@anlis.gob.ar (J.I. Degiuseppe), karina.roitman2@gmail.com (K.L. Roitman), krivero@anlis.gob.ar (K.A. Rivero), jstupka@anlis.gob.ar (J.A. Stupka).

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Table 1
Norovirus (NV) frequency of detection in passive surveillance set of samples in Argentina, 2010–2017.

Year	Number of sporadic samples selected	NV GI positive samples	NV GII positive samples	NV global frequency of detection (%)
2010	49	0	14	28.6
2011	51	2	4	11.8
2012	39	0	10	25.6
2013	35	0	9	25.7
2014	112	2	30	28.6
2015	73	1	8	12.3
2016	114	1	18	16.7
2017	50	1	8	18.0
Total	523	7	101	20.7

Thus, our study aimed to investigate the potential benefits of a passive surveillance strategy to counterbalance the norovirus genetic diversity data gaps.

Materials and methods

In Argentina, the Rotavirus Surveillance Network includes sentinel hospitals distributed nationwide that monitor rotavirus in sporadic acute gastroenteritis symptomatic children through immunochromatography or ELISA. Sentinel hospitals are encouraged to submit all the rotavirus-positive for genotyping and a fraction (~10%) of rotavirus-negative samples for archival purposes.

For logistical and cost considerations, a set of anonymized rotavirus-negative samples from archival collection from January 2010 until December 2017 were randomly selected on the basis of the total number of samples received per year. After nucleic acid isolation, samples were screened for norovirus GI and GII by real-time quantitative reverse transcription polymerase chain reaction and norovirus-positive samples were further genotyped by amplification and sequencing of ~575 bp of a partial region of the 3'-end of ORF1 and 5'-end of ORF2 of the genome as previously described [8]. Genotype assignment was retrieved using the online software Human Calicivirus Typing Tool (https://norovirus.ng.philab.cdc.gov). Norovirus sequences from each representative strain were submitted to GenBank with the accession numbers MW649127-649130 (GI strains) and MW649143-649152 (GII strains).

For each year, we examined the differences between genotypes detected through this passive surveillance strategy and those detected in the norovirus-confirmed gastroenteritis outbreaks [9].

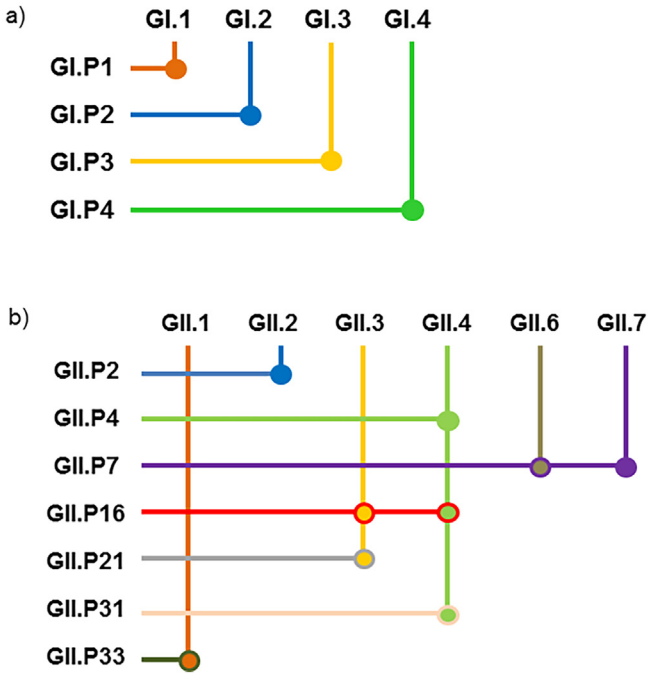

Fig. 1. Summarized polymerase and capsid norovirus genotypes detected from sporadic stool samples panel in Argentina, 2010–2017. Association of the combination of polymerase types (in rows) and capsid types (in columns) detected in norovirus sporadic samples from passive surveillance strategy. Each genotype, GI (a) and GII (b) is represented with a specific color. Double-colored circles indicate recombinant strains.

Results

A total of 523 stool samples were tested for norovirus. GI and GII norovirus were detected in 7 and 101 of the specimens, respectively. This represented a norovirus detection rate in the passive surveillance samples panel that ranged from 12 to 29% for each year (Table 1). Dual typing was successfully determined from 65 samples out of 108 positive samples (~60%). Four GI (GI.1, GI.2, GI.3, and GI.4) and six GII capsid genotypes (GII.1, GII.2, GII.3, GII.4, GII.6, and GII.7) were found (Fig. 1). GII.4 was the most frequent genotype and Den Haag/2006, New Orleans/2009 and Sydney/2012 variants were retrospectively detected. Regarding polymerase genotypes, four GI (GI.P1, GI.P2, GI.P3, and GI.P4) and seven GII (GII.P2, GII.P4, GII.P7, GII.P16, GII.P21, GII.P31, and GII.P33) were identified. Six out of the 13 strains identified in the passive surveillance panel were recombinants (Fig. 1). Moreover, the polymerase genotypes that were found associated with GII.4 strains were GII.P4, GII.P31 and GII.P16, accordingly to the contemporaneous circulation patterns described worldwide. In comparison with the genetic diversity observed in norovirus acute gastroenteritis outbreak of the same period, of the nine strains detected in the outbreak panel, six were also identified in the set of sporadic samples either coincidently the same year, the previous or later years (Fig. 2). Between both passive surveillance set of samples and outbreaks, GII genogroup was the most prevalent with GII.4 being the dominant genotype.

Discussion

Developing countries experience difficulties with the plan and continuous support of health events surveillance programs. Thus, we intended to explore a passive approach as an alternative strategy to overcome the challenge of assessing norovirus genetic diversity.

As reported elsewhere [10,11], the genotypes found in the sporadic panel were more diverse than that the associated with outbreaks. Noteworthy, this set of samples not only contemporaneously agreed with the non-GII.4 norovirus genotypes related to acute gastroenteritis outbreaks but also depicted even better the circulating epidemic strain, as we found it in associations with several polymerase genotypes, and more GII.4 variants. This represent an advantage for early detection of emerging strains with pandemic potential that circulate before being detected in an outbreak setting [10].

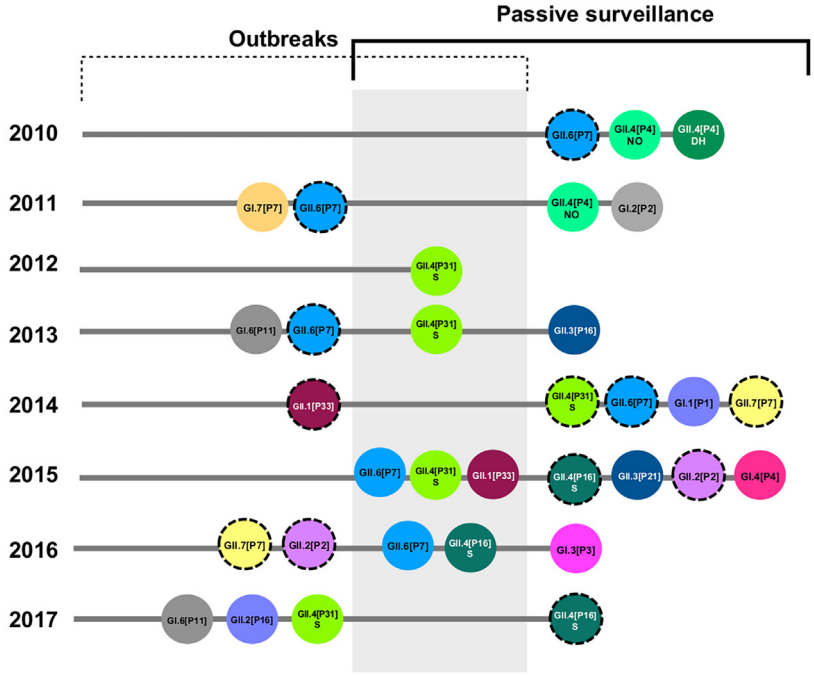

Fig. 2. Comparison between norovirus outbreaks and passive surveillance genotypes detected in Argentina, 2010–2017. For each year, norovirus partial polymerase and capsid genotypes are listed for outbreaks (dotted bracket) and sporadic set of samples (continuous bracket). The gray-shaded area represents the strains detected coincidently the same year in both groups and the dotted genotypes indicate those that were detected the previous or later years.

These results attempt to encourage researchers from public health and reference laboratories that have limited access to test acute gastroenteritis outbreaks to be able to get at least a limited set of rotavirus-negative stool samples from sporadic symptomatic cases in children under 5 years of age during the cold season to increase the yield of norovirus detection (i.e., 100 samples might potentially lead to 10–30 norovirus positive samples).

While the selection of the panel is biased, this study highlights somehow the importance of norovirus in those symptomatic children who remained undiagnosed. Following the decline of rotavirus gastroenteritis after the introduction of vaccines, it is important that countries aspire to understand the epidemiology and the evolutionary dynamics of norovirus as a framework for control and prevention policies, such as vaccines in high-risk populations [1,12]. Until improvements on health information systems are achieved, norovirus genetic diversity should be conducted through alternative strategies. Therefore, we consider that passive surveillance represents a suitable tool for providing norovirus circulating genotypes information for those countries where data gaps from outbreaks exist.

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Competing interests

None declared.

Ethical approval

Not required.

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