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Norovirus compared to other relevant etiologies of acute gastroenteritis among families from a semirural county in Chile☆

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ABSTRACT

Objective: To determine the dynamics of norovirus disease, a major cause of acute gastroenteritis (AGE), compared to other relevant etiologies, among families living in a lower middle income area.
Study design: Families with three or more members and with one or more healthy children <24 months of age were followed for 1–2 years to detect any AGE. Stool samples were tested for viral and bacterial pathogens and a questionnaire was completed for those with norovirus or rotavirus AGE.
Results: Between April and June 2016, 110 families were enrolled, with 103 of them completing ≥12 months of follow-up. A total of 159 family AGE episodes were detected, mostly affecting one individual (92%). At least one pathogen was detected in 56% (94/169) of samples, of which 75/94 (80%) were sole infections. Norovirus was most common ($n = 26$), followed closely by enteropathogenic *Escherichia coli* (EPEC) ($n = 25$), rotavirus ($n = 24$), and astrovirus ($n = 23$). The annual incidence of family AGE was 0.77, and 0.12 for norovirus. Most norovirus AGE occurred in children <4 years old (96%). Only 13/159 (8%) index AGE cases resulted in a secondary case, of which four were associated with norovirus. The majority of norovirus strains were GII (85%), with a mild predominance of GII.4 (9/26; 35%); most norovirus isolates (69%) were recombinants.
Conclusions: The family incidence of AGE in this lower middle income community was nearly one episode per year, mostly caused by viruses, specifically norovirus closely followed by rotavirus and astrovirus. Norovirus infections primarily affected children <4 years old and secondary cases were uncommon.

Summary

Based on prospective, family-based surveillance over 2 years, annual incidence of family AGE was 0.77 with low (8%) occurrence of secondary cases. Norovirus was most common (26/169); predominantly occurred as sole infection, in children <4 years, with 15% secondary cases.
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Introduction

Acute gastroenteritis (AGE) continues to be a major public health problem worldwide, with estimated incidence rates in low- to middle-income countries of 2.3 to 4.1 episodes per year in children younger than 5 years of age (Fischer Walker et al., 2012). In middle- to high-income countries, incidence ranges from 0.1 to 3 episodes per year in this age group (Roy et al., 2006). Worldwide, norovirus has been associated with 18% of these episodes (Ahmed

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et al., 2014). In Latin America, norovirus accounts for 14–16% of AGE, based on reports in children less than 5 years of age (Riera-Montes et al., 2018) and in medical settings (hospitals, emergency rooms, and/or outpatient clinics). Few prospective cohort studies following healthy children have been performed in order to determine the natural history of norovirus disease, providing invaluable information on the dynamics of infection over the first years of life (Saito et al., 2014; Lopman et al., 2015; ORyan et al., 2009).

Norovirus can be acquired through several routes, including person-to-person transmission, in which close contact among family members may play a role in intrafamilial spread (Phillips et al., 2011; Götz et al., 2001); family-based studies are nevertheless scarce. One study originally designed to investigate rotavirus epidemiology (Gastañaduy et al., 2015), evaluated stool samples from family contacts of children with norovirus diarrhea obtained 5–9 days after the 'primary' AGE, including controls and relatives. The infection attack rate (iAR) was highest among household contacts of children with norovirus AGE (33%), compared to those of other etiology diarrhea cases and healthy controls (8% and 18%, respectively). Recently, an active family-based study from the Netherlands compared the burden and transmission of norovirus and rotavirus AGE, reporting a secondary attack rate of 15% for norovirus and 28% for rotavirus (Quee et al., 2020). These studies provide a basis for intra-domiciliary transmission of norovirus.

Family-based studies in a different socio-demographic context are required in order to expand these findings, allowing for better determination as to the impact of infection within this nucleus of transmission.

The aim of this study was to estimate the burden of norovirus disease among families with at least one child <24 months of age living in a semirural, lower middle income area, and to compare it with that of other relevant etiologies. Additionally, the importance of different household members (age, relationship) in introducing AGE into the household, the severity of norovirus and rotavirus episodes, and the genotypes of norovirus isolates were examined.

Methods

Study design and population

This was a prospective, active, family-based surveillance performed in Colina, a suburban county near the capital city of Santiago, Chile, with approximately 121 000 inhabitants, of whom 32% live under the poverty line (Ministry of Social Development and Family, 2017). Families were contacted on a 'first come, first served' basis after visiting one of two public community health care centers in the county as part of their routine well-baby medical visits. Families meeting the inclusion criteria of at least three members, including one healthy child <24 months of age, and at least one responsible adult accessible by phone, were invited to participate. Informed consent was requested from one responsible adult, and from other adults in the household (and assent from children aged 8–18 years) if they suffered an episode of AGE during the study period. Consecutive enrollment continued until 110 families were included in the study, in order to reach 100 families followed for a minimum of 12 months (estimating a maximum dropout rate of 10%). This protocol was approved by the local institutional review board.

Surveillance method

An initial baseline questionnaire was applied to the responsible adult, who was instructed to call the study nurse if a case of AGE occurred. In addition, study personnel contacted the families by phone on a weekly basis to enquire whether any member had developed AGE symptoms.

Upon detection of an AGE case, the study team visited the home, identified the affected individuals, and applied a questionnaire on symptoms. Stool samples were to be obtained from the affected individuals within 48 h of the identification of a case and transported as described below. If AGE occurred in another household member in concordance with the index episode, stool samples were requested and tested following the same timeframe.

Sample collection

Stool samples were collected from diapers or in a plastic pot and stored temporarily in the home refrigerator until they could be transported in coolers to the Enteric Virus Laboratory at the Faculty of Medicine, Universidad de Chile. Stool was aliquoted for RNA, which was immediately extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Germany) and stored at −20 °C until testing. Another aliquot was stored in RNA-later at −70 °C for subsequent genotyping. A third aliquot in Cary Blair medium was maintained at room temperature for bacterial pathogen detection. Viral and bacterial pathogen testing was performed within 24 h of receiving the sample.

Sample testing

Norovirus and sapovirus were detected by reverse transcription polymerase chain reaction (RT-PCR) assays targeting conserved sequences in the polymerase region, using a pool of degenerate primers, 289hi for RT and 290hijk for PCR (Jiang et al., 1999; O'Ryan et al., 2000). Rotavirus was detected by Rotaclone ELISA (Meridian, Cincinnati, OH, USA), enteric adenoviruses by commercial rapid diagnostic test GastroVir-Strip (Coris Bioconcept, Gembloux, Belgium), and astrovirus by RT-PCR, as described previously (Belliot et al., 1997; Finkbeiner et al., 2009).

Salmonella spp, *Shigella* spp, *Campylobacter* spp, and *Yersinia* spp were cultured in selective media. A multiplex PCR was used to determine *Escherichia coli* pathotypes (Vidal et al., 2004).

Norovirus was confirmed and genogrouped by amplification and sequence analysis of a 544-bp segment comprising the ORF1/2 junction region using the following primer pairs: Mon432 (5′-TGGACICGYGGICCYAAYCA-3′)–G1SKR (5′-CCAACCCARCCATTR-TACA-3′) for genogroup I and Mon431 (5′-TGGACIAGRGGIC-CYAAYCA-3′)–G2SKR (5′-CCRCCNGCATRHCCRTTRTACAT-3′) for genogroup II, as described previously (Kojima et al., 2002; Anderson et al., 2001). Amplicons were purified using the EZNA Gel Extraction Kit (OMEGA Bio-tek Inc., Doraville, GA, USA) and cloned into plasmid vector pCR2.1-TOPO with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) by transformation of *E. coli* Top10 cells. Vectors were purified with Wizard Plus SV Minipreps DNA Purification System (Promega, USA) and sent to Macrogen (Korea) for sequencing. Sequences were assembled by Vector NTI Advance 11.0. A 213-bp region of the 3′ end of the RNA-dependent RNA polymerase gene (ORF1) and a 344-bp region of the 5′ end of the capsid gene (ORF2) were identified manually and analyzed separately. Norovirus genotypes were assigned using MEGA 7.0.21 software to generate a genetic distance tree using the maximum-likelihood method, including reference sequences for each genotype (Kumar et al., 2016), and using an online genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool) (Kroneman et al., 2011). For sequences with recombination in the ORF1–ORF2 overlapping region according to this analysis, a recombinant of special interest according to our literature review (see Results) was selected and analyzed further with SimPlot version 3.5.1 to identify a putative recombination point. The SimPlot analysis was performed by setting the window width and the step size to 200 bp

and 20 bp, respectively. A consensus sequence obtained from three to four GenBank sequences of each genotype of the recombinant was used as reference, and a third dataset from a non-recombinant genotype was used as negative control.

Sample size

Based on Fischer Walker et al. (Fischer Walker et al., 2012) and Roy et al. (Roy et al., 2006), an initial estimate of one to three AGE family episodes during a 12-month period was made, for a total of 100–300 episodes, of which 18% would be due to norovirus, for a total of 18–54 family norovirus AGE. It was estimated that for 100 children followed for 1 year, with an AGE rate of approximately 1.5 per year (Fischer Walker et al., 2012), there would be a total of 150 episodes, of which 15–20% would be norovirus-positive, leading to approximately 23–30 cases. For families, it was estimated that there would be a median of three to four members with one acute diarrhea episode per member per year for a total of 300–400 additional AGE cases, of which 10–15% would be due to norovirus, for an additional 30–60 cases. These numbers were considered sufficient to describe the epidemiology of AGE and norovirus AGE in families, responding appropriately to the primary objectives. Since findings from the first year were lower than these estimates, the family surveillance was continued for a second year.

Data analysis

The number and incidence of overall family episodes, norovirus and rotavirus AGE, number of secondary transmissions, and number of individuals affected during a family episode were described. The number and incidence of overall AGE and norovirus AGE occurring in the different age groups were determined, as well as the proportions of norovirus sole infections and co-infections with other viral and/or bacterial pathogens. Finally, the severities of norovirus and rotavirus AGE in children <4 years of age were compared.

Statistical differences were established using Pearson's Chi-square test or Fisher's exact test for categorical variables, the independent samples *t*-test for normal continuous variables, and the Mann–Whitney test for non-normal continuous variables. Epi Info 7 and R version 3.5.1 were the statistical packages used for the analysis.

Definitions

An AGE episode was defined as an increased frequency of unformed stools, abnormal for the individual and sufficient to modify their daily routine, with or without vomiting, fever, or other symptoms.

A family episode of norovirus AGE was defined as one or more members with a norovirus-positive AGE. The primary case was the first identified case; secondary or subsequent norovirus AGE cases were those occurring within 2 weeks of the primary case.

AGE severity in children <4 years of age was measured using the Vesikari score (Ruuska and Vesikari, 1990): a score of <7 was considered mild, 7–10 moderate, and 11–20 severe. In adapting dehydration to the score, a score of 1 was given if the child had received rehydration salts and 0 if not.

Results

A total of 110 families were enrolled between April and June of 2016. Seventeen families (15.4%) were lost to follow-up or dropped out before completing 24 months of surveillance: nine moved out of the study area, one was excluded due to poor adherence to the protocol, four were excluded due to loss of contact, and three declined to continue participation. Of those families that dropped out, surveillance ranged from 0 to 19 months. Only families with >12 months of surveillance were included in the final analysis ($n = 103$); for these families, surveillance ranged from 12 to 27 months (median 25, interquartile range 24–25 months). The data represent a total of 2483 months of family surveillance.

All families consisted of at least a mother and an index child; 83/103 (81%) included a father, 28/103 (27%) included grandparent(s), and 21/103 (20%) included an additional family member in the household. The responsible family member was the mother in 93% of enrolled households; in five cases it was the grandmother and in two cases it was the father. All families routinely prepared food at home and had a refrigerator. All index children were up to date on their well-baby visits and vaccinations included in the National Immunization Program; none of the index children had received the rotavirus vaccine. Further demographic information is shown in Table 1. There was no relationship between attending daycare at enrollment, mother's education level, whether a mother worked outside the home, number of people in the household, people per bed, or people per room and the risk of overall or norovirus AGE.

The numbers of participants and months of follow-up by age category were as follows: (1) <2 years of age, $n = 105$ (two siblings were born after initial family enrollment), months = 1433; (2) 2–4 years old, $n = 28$, months = 1441 (including index children who changed age category during the study); (3) 5–17 years old, $n = 80$, months = 2079; (4) 18–64 years old, $n = 245$, months = 6157; (5) ≥65 years old, $n = 3$, months = 82. This resulted in a total of 461 individuals under surveillance for 11 204 person-months.

Acute gastroenteritis surveillance

A total of 159 family AGE episodes were detected, with a total of 174 individuals affected. The majority (146 episodes) affected only one individual (92%), while 11 episodes involved two individuals (7%) and two episodes involved three individuals (1%). A total of 169/174 AGE samples were obtained (Figure 1).

Table 1
Demographic information for 103 families, index children, and mothers under surveillance for AGE.

Variables	
Families	
House (vs. apartment), *n* (%)	85 (83)
Homeowner, *n* (%)	65 (63)
Public water system, with their own water meter, *n* (%)	100 (97)
Public sewage system[a], *n* (%)	97 (94)
Number of people in household, median (IQR)	4 (3–9)
People per bedroom, median (IQR)	2 (0.8–5)
People per bed, median (IQR)	1.5 (0.8–4)
Index children	
Age at enrollment (months), median (range)	8 (0–21)
Male, *n* (%)	50 (49)
Attends daycare, *n* (%)	37/88 (42)[b]
Age at admission (months), mean (range)	20 (13–28)
Mothers	
Age at enrollment (years), median (IQR)	27 (18–45)
Education level, *n* (%)	
Elementary school	29 (28)
High school	61 (59)
Technical degree	10 (10)
Some university	2 (2)
Completed university	1 (1)
Works outside the home, *n* (%)	22 (22)
Type of employment: employee, *n* (%)	18/22 (82)
Type of contract: permanent, *n* (%)	20/22 (91)
Hours per week, median (IQR)	44 (20–60)

AGE, acute gastroenteritis; IQR, interquartile range.
[a] Versus septic (one family had no bathroom).
[b] This question was added after the start of the study. Due to loss to follow-up, data were available for 88/103 index children.

One or more pathogens were detected in 94/169 (56%) AGE samples, with a total of 115 pathogens. A sole infection was detected in 75/94 (80%) samples. The distribution of pathogens identified is shown in Figure 1 and Table 2. A virus was identified in 64/94 (68%) samples, bacteria in 16 (17%), and a mixed bacterial/viral infection in 14 (15%). Norovirus was the most common pathogen identified, followed closely by enteropathogenic *Escherichia coli* (EPEC), rotavirus, and astrovirus. All mixed infections involved either norovirus/sapovirus, rotavirus, or astrovirus. Mixed infections were as follows: five norovirus/EPEC, one norovirus/sapovirus/EPEC, one norovirus/*Salmonella* spp, one norovirus/astrovirus, one norovirus/astrovirus/EPEC, four rotavirus/EPEC, one rotavirus/astrovirus, one sapovirus/EPEC, two sapovirus/astrovirus, one astrovirus/adenovirus, and one astrovirus/EPEC.

The annual incidences at the family level and by age group for overall AGE, norovirus, and rotavirus are shown in Table 3. One index child had three episodes of norovirus and three index children had two episodes. Children <2 years old were the age group most infected by norovirus (17/26; 65%), and nearly all infections occurred in children <4 years of age (25/26; 96%).

Secondary symptomatic infections were far less common than expected. Only 13/159 index AGE cases (8%) resulted in a secondary infection. The majority began in an index child <2 years of age (8/13, 62%); nine (69%) began in a child <4 years old and 11 (85%) in a child <9 years old. However, there was a significant difference in the occurrence of secondary infection when the primary infection occurred in a child ≤4 years of age (9/149; 6%) versus in an individual >4 years of age (4/10; 36%; $p = 0.004$). A secondary infection, suggesting household transmission, was identified in 5/26 (19%) norovirus family episodes. In 4/13 (31%) cases with a secondary infection and in 1/2 cases with a tertiary episode, the agent detected in the index case was norovirus (Figure 2).

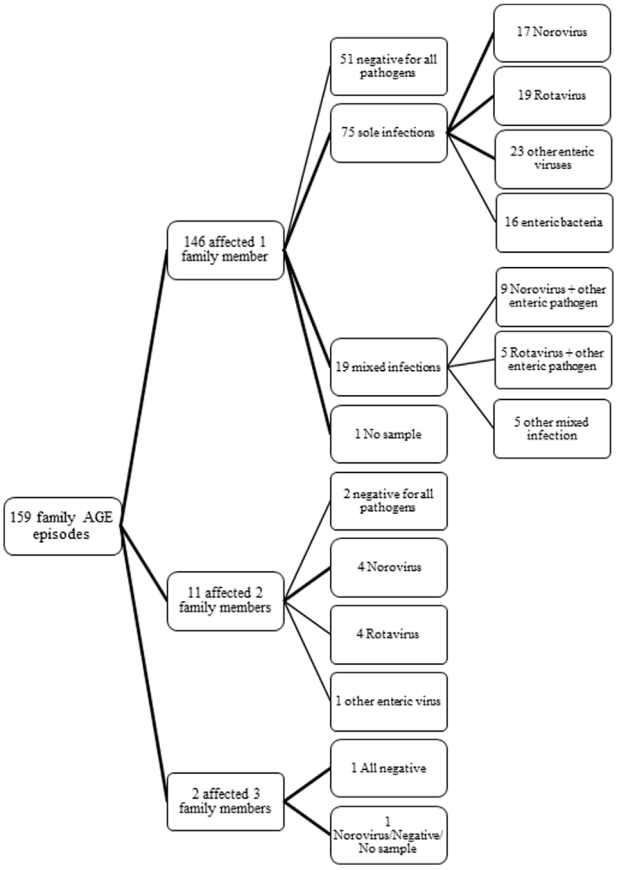

Figure 1. Etiology of 159 family acute gastroenteritis (AGE) episodes.

Norovirus genotyping

Most of the norovirus strains were GII (22/26; 85%), primarily GII.4 (9/26; 35%), according to the partial ORF2 sequence. Figure 3 shows the genetic distance tree of the isolates determined in this study compared to reference sequences, according to both ORF1 and ORF2 partial sequences; Table 4 shows the genotype distribution.

Isolates from children with recurrent infections had different genotypes, suggesting re-infections. Two of three patients with two infections were infected with different genogroups by ORF2 (GI.3/GII.1 and GII.1/GI.3, respectively) and the third was infected by different genotypes within the same genogroup (GII.7/GII.6). ORF2 genotypes from the patient with three infections were GII.2, GI.2, and GII.6. For family episodes in which more than one member was affected, we could only genotype the isolates in both samples from one family and both had the same genotype (GII.7). Analysis of both partial ORF1 and ORF2 regions showed that 18/26 (69%) norovirus isolates were putative recombinants (Table 4), with GII.P16-GII.4 and GII.P7-GII.6 being the most frequent. All recombinant strains except GII.P16-GII.1 have been reported previously in Latin America (Fajardo et al., 2014a; Barreira et al., 2017; Hernandez et al., 2016). Therefore, this recombinant was further analyzed using SimPlot (Figure 4), which confirmed the presence of recombination and located the recombination breakpoint to position 294 (nucleotide 5107–5113 according to the complete genome sequences used as reference), in agreement with previous studies that estimated the recombination point to be located between nucleotides 4981 and 5117 in other strains (Bull et al., 2005; Fumian et al., 2012; Fajardo et al., 2014b).

Severity of norovirus compared to rotavirus AGE

Table 5 summarizes the severity of norovirus compared to rotavirus AGE episodes in children ≤4 years of age. Norovirus episodes were less severe than rotavirus based on the Vesikari score and days of fever. In the case of both pathogens, severity did not differ between those cases that resulted in a secondary infection and those that did not for either pathogen (data not shown). Two children were hospitalized, one with rotavirus and one with norovirus.

Discussion

Over nearly 2 years of active, prospective surveillance of 103 families from a semirural, lower middle income population, almost 0.8 AGE episodes occurred annually per family, a majority of which occurred in children <2 years of age. In this age group, the number of AGE episodes was nearly one per year; this number decreased to approximately 0.4 episodes per year in the 2–4 years age group, with very few cases detected in older individuals. Only a few individuals over 65 years lived in the households, so no conclusions can be made regarding this age group. Lower detection rates in adults may be attributed to a true lower incidence or to under-reporting in those individuals.

For children, the rates encountered in this population were significantly lower than the roughly 2–4 episodes per year reported in a meta-analysis of lower middle income countries between 1990 and 2010 (Fischer Walker et al., 2012) and are similar to those reported in developed countries (Roy et al., 2006). In China, a more recent family survey-based study estimated a monthly incidence of 12.6% in children 0–4 years of age; this implies nearly 1.5 episodes per year (Chen et al., 2013), which is

Table 2
Pathogen distribution in samples collected from AGE cases over the 24 months of family surveillance

	Samples positive for indicated pathogen, *n* (%) (*n* = 169)[a]	% of all pathogens detected (*n* = 94)	Samples positive only for the indicated pathogen, *n* (%) (*n* = 169)	% of specific pathogen that were sole infections
Norovirus	26 (15)	23%	17 (10)	65%
Rotavirus	24 (14)	21%	19 (11)	79%
Astrovirus	23 (14)	20%	16 (9)	70%
Sapovirus	8 (5)	7%	4 (2)	50%
Enteric adenovirus	4 (2)	3%	3 (2)	75%
Diarrheagenic *Escherichia coli*[b]	28 (17)	24%	15 (9)	54%
Campylobacter	1 (1)	1%	1 (1)	100%
Salmonella	1 (1)	1%	0	0
Shigella	0	0	0	0
Yersinia	0	0	0	0
Negative for all pathogens	75 (44)	-	-	-

AGE, acute gastroenteritis.
[a] The sum of all positive detections exceeds the number of positive samples due to some samples testing positive for more than one pathogen.
[b] Twenty-five were enteropathogenic, two were Shiga toxin-producing, and one was enterotoxigenic.

Table 3
Number, percentage, and annual individual-level incidence of overall, norovirus, and rotavirus AGE occurring by age group.

	Families		Age groups (years) (*n* = 174)							
	n (%)	AI	<2 *n* (%)	AI	2–4 *n* (%)	AI	5–17 *n* (%)	AI	18–64 *n* (%)	AI
Norovirus	24 (19)	0.12	17 (65)	0.14	8 (31)	0.07	0 (0)	0	1 (4)	0.00
Rotavirus	21 (13)	0.10	17 (71)	0.14	3 (12)	0.02	1 (4)	0.01	3 (12)	0.01
All AGE	159 (100)	0.77	109 (63)	0.91	45 (26)	0.37	4 (2)	0.02	16 (9)	0.03

AGE, acute gastroenteritis; AI, annual incidence. Note: There were no diarrhea episodes in adults ≥65 years of age.

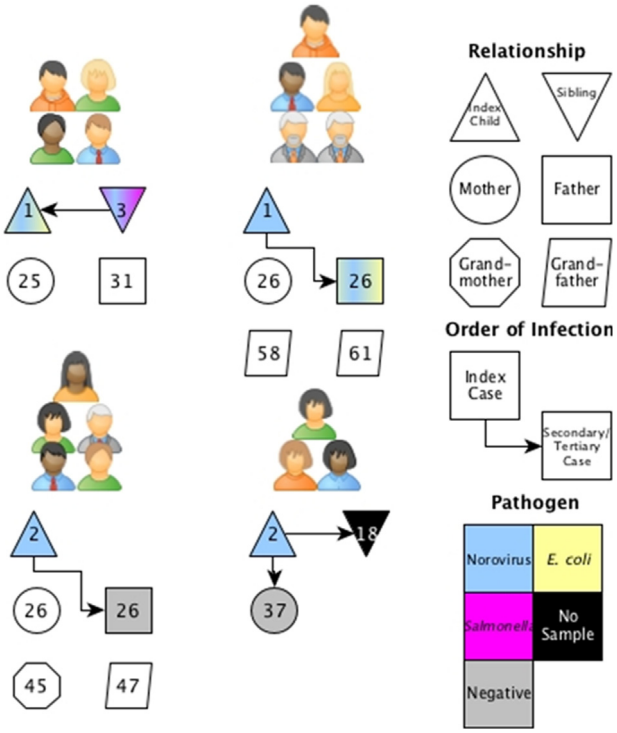

Figure 2. Cases of norovirus infection that spread to other family members. The number within each shape indicates the age of each family member (in years).

more similar to the present study findings. The previous study reported a significant drop in AGE in older individuals, although not to the low levels observed in our population, where only one in every 30–50 individuals reported an AGE episode annually. In a study on predominantly Hispanic families living in the USA, Perry et al. described a significantly higher risk of primary and secondary AGE episodes in children <2 years of age, with an up to 8-fold higher risk than older AGE (Perry et al., 2005). Quee et al. recently published the results of an active family-based study in the Netherlands and reported 3.1 AGE per 100 persons per week, predominantly in children <2 years of age, with a significant decline in individuals >18 years old (Quee et al., 2020). The present study results complement these studies by including a lower middle income population, which can most likely be extrapolated to similar regions.

A potential causal microorganism was detected in 56% of AGE cases, of which norovirus was the most common, followed closely by EPEC, rotavirus, and astrovirus. Sapovirus was detected in only 5% of cases, while other pathogens such as enteric adenovirus and enteric bacteria other than EPEC were rarely detected in these Chilean families. Importantly, most norovirus and rotavirus cases were detected as sole infections, although the detection of more than one pathogen was not uncommon (26% and 21% for norovirus and rotavirus, respectively, mostly with diarrheagenic *E. coli*). These results may be somewhat surprising in a country where rotavirus vaccines are not routinely used, as in such situations rotavirus has been reported to be two to three times more common than norovirus (Shen et al., 2019; Bányai et al., 2018). In countries where rotavirus vaccines are being used, a significant drop in rotavirus prevalence has occurred to a relative frequency of 1:1 (or even less) compared to norovirus (Bucardo et al., 2014; Challappa et al., 2016); however, these findings have mostly been reported from hospital or emergency room settings, where rotavirus is known to be more common. In outpatient-based settings, where less severe AGE cases are seen, rotavirus and norovirus frequencies are similar even in the absence of rotavirus vaccination (Riera-Montes et al., 2018; Quee et al., 2020). In the Colina families, incidence rates of norovirus and rotavirus were similar in children <2 years of age, at nearly 0.15 episodes per child per year; the

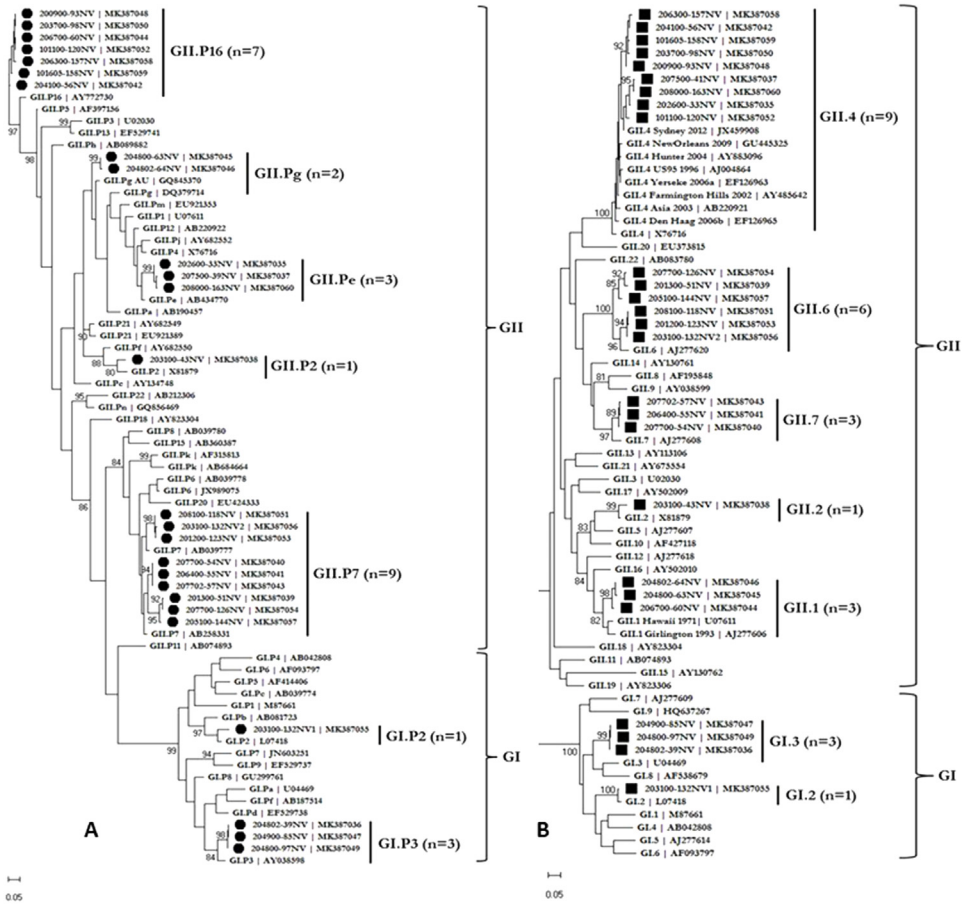

Figure 3. Genetic distance tree of 26 norovirus isolates detected in individuals with acute gastroenteritis from a cohort of families from a lower middle income area in Chile. The sequence of a segment of the ORF1/2 junction region of isolates identified in this study was compared to reference sequences of different genotypes. The results obtained using the partial ORF1 region (from 213 bp of the 3′ end of the RNA-dependent RNA polymerase (RdRp) gene) and partial ORF2 region (from 344 bp of the 5′ end of the capsid gene (Vp1)), are shown in images (A) and (B), respectively. Strains isolated in Chile are indicated by circles (RdRp) and squares (Vp1), with their respective accession numbers.

Table 4
Norovirus genotypes according to ORF1–ORF2 and putative recombinants.*= Recombinants.

	Number	%
GII.P16-GII.4*	6	23%
GII.P7-GII.6*	6	23%
GII.P7-GII.7	3	11.5%
GII.Pe-GII.4*	3	11.5%
GI.P3-GI.3	3	11.5%
GII.Pg-GII.1*	2	7.7%
GI.P2-G1.2	1	3.8%
GII.P16-GII.1*	1	3.8%
GII.P2-GII.2	1	3.8%

frequency of rotavirus, but not norovirus, decreased significantly in the 2–4 years age group. Both norovirus and rotavirus were detected in only a few AGE cases after 5 years of age.

Overall, secondary attack AGE was uncommon in this population and less than expected (Mughini-Gras et al., 2016; Heusinkveld et al., 2016), with one out of every 10 family episodes associated with possible intrafamilial transmission. In those AGE cases positive for norovirus, one out of six (4/26) resulted in an additional AGE case within the family, suggesting the possibility of intrafamilial spread. In Ecuador, 33% of norovirus-positive cases

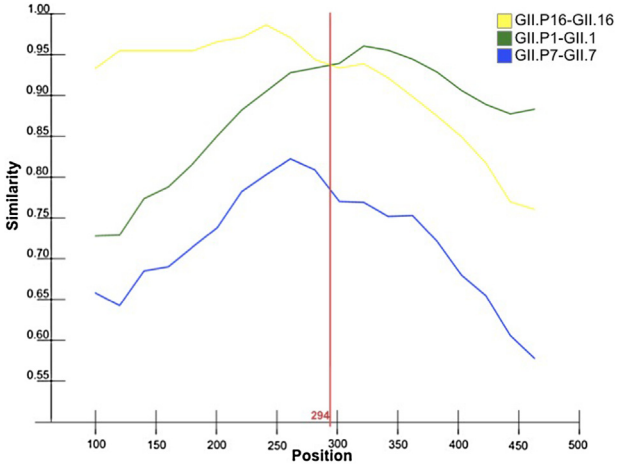

Figure 4. Simplot analysis of recombinant GII.P16-GII.1. The *x*-axis represents the base position and the *y*-axis represents the similarity between the sample sequence (putative-recombinant norovirus strain of GII.P16-GII.1) and the reference sequences (GII.P16-GII.16, GII.P1-GII.1, GII.P7-GII.7). GenBank accession numbers of sequences used as reference: AY772730, AY682551, GU292831, KF920739 for GII.P16-GII.16; U07611, JX289822, AF414421 for GII.P1-GII.1; and AB258331, MN038126, MH279830 for GII.P7-GII.7.

Table 5
Severity of AGE episodes caused by norovirus or rotavirus in children ≤4 years of age

Variables	Norovirus	Rotavirus	Mixed norovirus infections	*p*-Value[a]
Number	25	20	13	
Vesikari score, mean (range)	7.2 (2–12)	9.6 (3–17)	7 (2–11)	0.017
Severity, *n* (%)				
Mild	11 (44)	4 (20)	6 (46)	0.070
Moderate	11 (44)	8 (40)	5 (38)	
Severe	3 (12)	8 (40)	2 (15)	
Days of diarrhea, median (IQR)	6 (4–7)	6 (3.75–6.5)	5 (2–7)	NS
Maximum episodes of diarrhea per day, median (IQR)	4 (4–5)	6 (4–7.25)	4 (4–5)	0.073
Days of vomiting, median (IQR)	1 (0–2)	2 (1–2.25)	1 (0–1)	NS
Maximum episodes of vomiting per day, median (IQR)	2 (0–3)	2 (1.75–3)	1 (0–2)	0.097
Days of fever, median (IQR)	0 (0–0)	0 (0–1)	0 (0–1)	0.050
Maximum temperature[b], median (IQR)	39.4 (38.95–39.45)	38.5 (38–38.63)	38.95 (38.25–39.43)	NS
Used rehydration salts, *n* (%)	7 (28)	10 (50)	3 (23)	NS
Hospitalized, *n* (%)	1 (4)	1 (5)	0 (0)	NS

AGE, acute gastroenteritis; IQR, interquartile range; NS, not significant.
[a] Comparison between norovirus and rotavirus.
[b] Temperature ≥38 °C was considered fever; any temperature <38 °C was not recorded.

resulted in more than one secondary case with a stool sample positive for norovirus, irrespective of symptom status (samples were obtained from individuals both with and without diarrhea) (Gastañaduy et al., 2015). It is likely that if stool samples from asymptomatic individuals had been tested in our study, we would have found higher intrafamilial transmission; however, the goal was to identify significant, symptomatic secondary cases. For rotavirus, secondary infections were also uncommon (4/23, 17%). In the Netherlands, a household-based study found a secondary attack rate in symptomatic individuals of 15%, similar to the present study findings, and 51% for asymptomatic norovirus, compared to 28% and 22% for rotavirus, respectively (Quee et al., 2020).

Norovirus AGE was in general less severe than rotavirus AGE, replicating findings from other studies conducted in hospital or emergency room settings (ORyan et al., 2010; Pringle et al., 2015). Nevertheless, six out of 10 norovirus episodes were moderate to severe, and one child was hospitalized. A possible hospitalization rate of 3% is similar to the rate observed for rotavirus, which in the early 2000s was estimated to be 1:65 (Parashar et al., 2003). However, in order to obtain robust confidence intervals on the rate of norovirus-associated hospitalization, a significantly higher number of families would have to be surveyed. The evaluation of AGE severity is challenging, as it relies on family records, and families are not trained to evaluate some of the variables included in severity scores, specifically dehydration. Thus, these scores should be considered an approximation and not an exact metric.

Two out of three norovirus isolates were putative recombinants, stressing the genomic plasticity of norovirus, a finding that should be considered in the development of future vaccines. All but one recombinant strain had been reported previously in Latin America (Fajardo et al., 2014a; Barreira et al., 2017; Hernandez et al., 2016). The recombination breakpoint for the new recombinant isolate, GII.P16-GII.1, was identified by sequence analysis.

This study has limitations inherent to complex field studies that rely on the continued and relatively intense participation of families. The detection of AGE occurred only if the responsible family member notified the event directly or during the weekly phone calls. Mild episodes and episodes occurring during vacation periods may have been missed. Nevertheless, we had a high yield of weekly family contact by phone (approximately 95%), and we are confident that we captured most of the clinically significant AGE. The low rate of AGE among family members older than 5 years of age was an unexpected finding (Sacri et al., 2014). After the first year of surveillance, the responsible adult was reminded during the weekly phone calls to notify adult cases; nevertheless, the number of cases in older individuals did not increase. The pathogen yield was 56%, and most infections were caused by a sole pathogen. Multiplex gene detection-based platforms can increase pathogen detection, especially for bacteria, but the interpretation of such detection can be difficult (Calderaro et al., 2018). We have high confidence in the norovirus and rotavirus detection results, as all were successfully genotyped.

For vaccine purposes, this family-based study indicates that if a successful vaccine program were to be implemented in infants within a similar lower middle income population, vaccination of approximately 100 infants (within 100 families, most with one and at most three other children) could prevent at best 26 norovirus AGE cases (assuming that norovirus is also the key driver of the AGE with mixed infections), 18 of which would be moderate to severe, one hospitalization, and two to five secondary cases due to intrafamilial transmission. A vaccine including both norovirus and rotavirus could potentially prevent 50 AGE cases, of which 35 would be moderate to severe, including two hospitalizations.

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Ethical approval

This protocol was approved by the IRB of the Faculty of Medicine of the University of Chile. Informed consent was requested from one responsible adult, and from other adults in the household (and assent from children aged 8–18 years) if they suffered an AGE episode during the study period.

Conflict of interest

The funding agency had no role in the study design, the collection, management, analysis, and interpretation of the data, or in the preparation, review, and approval of the manuscript. MOR received financial support from an Investigator-Initiated Sponsored Research Grant. The other authors declare no conflict of interest.

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