

Symptomatic and Asymptomatic Rotavirus and Norovirus Infections During Infancy in a Chilean Birth Cohort

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Background: Rotavirus and more recently norovirus have been recognized as 2 of the most common causes of acute diarrhea in children. Comparative analysis of these infections in a birth cohort has not been performed and can provide relevant insight on clinical and viral behaviors.

Methods: Mother-infant pairs from middle-low socioeconomic background living in the Metropolitan Region of Chile are being followed for 18 months in 2 outpatient clinics. Infants are evaluated monthly for asymptomatic excretion of rotavirus and norovirus and during acute diarrhea episodes (ADE) for rotavirus, norovirus, and bacterial enteropathogens. Severity of ADE is evaluated using the Vesikari score.

Results: Between July 1, 2006 and September 1, 2008 a total of 198 children were followed for a mean of 15.7 months. Asymptomatic rotavirus and norovirus infections were detected in 1.3% and 8% of 2278 stool samples compromising 14% and 57% of infants, respectively. Incidence of ADE was approximately 0.8 for the first year of life and approximately 0.6 for the 13 to 18 month age group. Rotavirus and norovirus were detected in 15% and 18% of 145 ADE evaluated. Mean Vesikari score was 10.4 and 7.4 for rotavirus and norovirus respectively ($P = 0.01$) and severity was not associated with age of patients for either virus. Reinfections were more common for norovirus asymptomatic episodes: 44% versus 19% ($P = 0.01$) and borderline for symptomatic episodes: 40% versus 11% ($P = 0.08$). Rotavirus genotype G9P8 and norovirus genogroup II (GII) predominated although most asymptomatic episodes for both viruses were nontypable. None of 19 symptomatic GII norovirus infections had a previous documented GII infection compared with 10 of 31 asymptomatic GII infections (OR = 0.95% CL = 0, 0.59; $P = 0.008$).

Conclusions: Children had suffered a mean of approximately 1.4 ADE by 18 months of age of which 15% and 18% were caused by rotavirus and norovirus, respectively. In general rotavirus infections were more severe than norovirus infections and for both viruses severity was not related to age. Norovirus reinfections were significantly more common than rotavirus reinfections but for GII norovirus a primary infection seems to confer protection against clinically significant reinfections.

Key Words: rotavirus, norovirus, calicivirus, diarrhea, cohort

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An estimated 1.4 billion children younger than 5 years of age develop acute diarrhea every year in developing countries of whom 123.6 million will require outpatient medical care and 9 million hospitalization.¹ Acute gastroenteritis is responsible for approximately 1.8 million deaths, primarily in the most impoverished areas of the world.² These estimates are somewhat lower than the approximately 3 million annual deaths from diarrhea that was reported 10 years earlier,^{3,4} indicating progress in prevention and treatment of acute diarrhea. Diarrhea associated deaths are uncommon in industrialized countries but hospitalizations and medical visits continue to be substantial worldwide irrespective of development status.^{1,5} Chile is a rapidly developing country with a current population of 16.5 million. In the 1990s, children living in a low socioeconomic level community in Santiago, Chile, had an overall incidence of 2.1 diarrhea episodes in each infant per year.⁶ Improved socioeconomic conditions within the country suggest that this impact may have changed during the past decade. The most recent estimates indicate that acute diarrhea is associated with approximately 150,000 outpatient visits every year in Chile.⁷

Four enteric viruses, rotavirus, human caliciviruses (HuCVs), astrovirus, and enteric adenoviruses, cause greater than 50% of acute nonbloody gastroenteritis in children worldwide.⁸ Rotavirus is the most common cause of moderate-to-severe gastroenteritis in children younger than 2 years of age causing from 30% to 50% of diarrhea-associated hospitalizations in this age group.^{9–11} This virus is detected in 10% to 30% of less severe episodes, requiring outpatient visits. Norovirus is a HuCV that causes food and waterborne gastroenteritis outbreaks that affect adults and children and more recently has been identified as a significant cause of mild to moderate and even severe gastroenteritis in children in as much as 20% of episodes during the first 2 years of life.^{12–16}

The natural history of rotavirus infection has been well described in a Mexican cohort study followed by a limited number of more recent cohort studies that include testing periodic stool samples for rotavirus. These studies have provided valuable information on the incidence rates of both asymptomatic^{17,18} and symptomatic^{17–19} infections during the first 2 years of life, including the association between age, number of episodes, and symptoms; serotype data were included in 2 of these studies.^{17,19} Prospective evaluation of symptomatic norovirus infections in children have been studied in Finland within a rotavirus vaccine study¹⁵ and in 1 large study, including both children and adults monitored for 6 months.²⁰ Both studies underscore the high proportion of diarrhea cases associated with norovirus in young children. Asymptomatic infections are common as demonstrated in another Mexican cohort in which 50% of children below 2 years of

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age had at least 1 asymptomatic norovirus infections.²¹ The high frequency of symptomatic and asymptomatic infections is supported by a seroprevalence study that reports over 80% seropositivity by 2 years of age.²²

To our knowledge, there are no published studies that compare rotavirus and norovirus symptomatic and asymptomatic episodes in the same cohort. Simultaneous evaluation of both viruses can provide relevant insight on clinical and viral behavior. Our primary aim was to compare the natural history of rotavirus and norovirus infections, studied simultaneously in this cohort. In addition, we aimed to update the incidence rate of acute diarrhea in a middle-low socioeconomic birth cohort within the Metropolitan Region of Santiago, Chile. This study is part of a larger initiative which has as mid-long term goal to identify virological and host-related factors and/or interactions associated with susceptibility to rotavirus and norovirus infection and severity of disease.

METHODS

Overall Study Design

Mother-infant pairs living in 2 areas (Colina and Peñalolén) within the Metropolitan Region of Santiago were contacted within 15 days after labor and/or birth and are being followed monthly in 2 outpatient clinics up to 18 months of age. Enrollment began in July 2006 and inclusion criteria included ≥ 35 weeks gestational age, birth weight > 2500 g, absence of neonatal diseases requiring hospitalization, a literate mother willing to make phone contact with study personnel when needed. For this first report we established a cutoff date of September 1, 2008.

After signing informed consent, mothers were scheduled for their monthly well-baby visits at the outpatient clinics with personnel fully dedicated to the study and were instructed to bring approximately 2 g of recently emitted stool for rotavirus and norovirus detection. Mothers were also instructed to contact study personnel (by phone or personal contact) if the child has an acute diarrheal episodes (ADE) to receive information on oral rehydration, a recommendation to visit the emergency room if the episode was deemed moderate to severe, and to schedule a visit to the outpatient clinic within 48 hours.

During the well-baby clinic visit, study personnel obtained information on potentially missed diarrhea episodes occurring during the month. For each ADE, during the visit to the outpatient clinic personnel obtained information from the mother about fever (recorded and not recorded), number of vomiting episodes per day, number of stool evacuations per day, need for oral and/or intravenous rehydration and/or hospitalization, and duration of the episode. Study personnel estimated the most severe dehydration status, occurring during the ADE based on well-established clinical parameters²³ and the Vesikari score was calculated after the episode had ended.²⁴ Mothers were instructed to bring a fresh stool sample obtained within 72 hours of diarrhea onset. ADE stools were tested for rotavirus and norovirus and for bacterial enteropathogens. In addition, a 3 mL blood sample and a saliva sample were obtained from infants after a first rotavirus and/or norovirus asymptomatic or symptomatic episode for host-related studies (to be described in a future manuscript). The protocol was approved by both Ethical Committees of the Faculty of Medicine and of the Eastern Metropolitan Health Service. Detailed implementation of the birth cohort can be found in the electronic version (www.pidj.com).²⁵

Stool Sample Collection and Processing

For well-baby visits approximately 1 to 2 g of stool were collected by the mother from a clean diaper, deposited into a provided clean plastic cup and kept in the house refrigerator for 0 up to 48 hours before the clinic visit. For ADE, the mother

provided a similar sample in a clean plastic vial, part of which was swabbed and placed in Cary-Blair transport media. Stool collection through a thin rubber tube was performed by study personnel if the mother failed to bring a stool sample. Plastic cups were stored at 4°C for a maximum of 96 hours in the microbiology laboratory before testing for rotavirus and norovirus. The remaining stool was stored at -20°C for further analysis. Samples in transport media were maintained at environmental temperature until processing for bacterial pathogens within 72 hours.

Rotavirus was detected by a commercial ELISA kit (IDEIA, DakoCytomation) and norovirus by a noncommercial 9-valent ELISA²⁶ and/or by reverse transcription-polymerase chain reaction (RT-PCR) targeting conserved sequences in the polymerase region of HuCVs.²⁷ Primers used for RT-PCR were a pool of degenerate primers 289hi for RT and 290hjk for PCR, which amplify both norovirus and sapovirus. Swabs from transport media were cultured for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* according to standard techniques using selective media. Enteropathogenic *Escherichia coli*, enterotoxigenic *E. coli*, and enterohemorrhagic *E. coli* were studied by multiplex polymerase chain reaction.²⁸ Rotavirus G and P types were determined by RT-PCR as previously described including primers for G1-G4, G8, and G9 (for rotavirus G), and P4, P6, and P8 (for rotavirus P).²⁹ Norovirus genogroups GI and GII were determined by capsid RT-PCR using primer R2 for RT, F1, and G1 for GI PCR and Mon381 and Mon383 for GII PCR.³⁰ In addition, we tested 45 randomly selected norovirus positive samples nontypable with the above primers with the technique described by Kojima and colleagues.³¹

Definitions

ADE was defined as an increase in frequency of stool emissions and/or decrease in stool consistency for the individual child, according to the mother’s perception. A symptomatic or asymptomatic episode was considered rotavirus positive when the ELISA OD was > 0.250 . For norovirus, accepted criteria for positivity by ELISA was an OD reading > 0.5 and at least 3-fold higher than the negative control and by RT-PCR a band at the expected size level (320–330 bp). A time period of ≥ 15 days was required to separate 2 episodes caused by the same virus. Mother-infant pairs were excluded from the study if any chronic or medically significant disease interfered with the possibility of follow-up or whether they failed to comply with a minimum of 20% of the scheduled well-baby visits, including a stool sample.

Statistical Analysis

Categorical and continuous variables were analyzed with a 2 tailed χ^2 test with Yates correction or the Fisher exact test and an unpaired *t* test, respectively. Odds ratio (OR) and 95% confidence limits (CL) are shown if appropriate. Calculation of incidence rates for illness was based on the total duration of follow-up for each child because all episodes were captured after retrospective collection. For asymptomatic infections, incidence calculation was based on the total number of stools tested per child. Centers for Disease Control and Prevention Program Epi Info version 3.3.2, February 9, 2005, was used for all statistical analysis. A *P* value < 0.05 was considered statistically significant.

RESULTS

Enrollment and Compliance With Well-Baby Scheduled Visits

Between July 1, 2006 and September 1, 2008, a total of 246 infant-mother pairs were enrolled of whom 48 were excluded (18.4% and 20% from Colina and Peñalolén, respectively) because

TABLE 1. Enrollment, Follow-up, and Acute Diarrhea Episodes (ADE) in the Birth Cohort

Characteristic	
Infants enrolled/completing follow-up (%) [*]	246/198 (80%)
Duration of follow-up	
7–10 mo	19 children
11–14 mo	48 children
15–18 mo	131 children
Total child-months of follow-up	3106
Including a stool sample (% total)	2278 (73%)
ADE: total/studied (%) [†]	234/145 (62%)
By age groups: N (% total)/studied [‡]	
0–6 mo	52 (22%)/36
7–12 mo	107 (46%)/67
13–18 mo	75 (32%)/42
No. ADE per child: N (% total)/studied [‡]	
Children >1 episode	123/87
1 episode only	62 (50%)/35
2 episodes	30 (24%)/24 [*]
>3 episodes	31 (25%)/28 [§]
Median age of first ADE	9 mo

^{*}Mother-infant pairs complying with >20% scheduled well-baby visits, including a stool sample.

[†]Episodes studied are those that had clinical information required for Vesikari score and a stool sample obtained.

[‡]In 12 both episodes were studied and in 12 only 1 episode was studied.

[§]In 13 of 28 children all episodes were studied, in the 15 remaining children episodes were studied as follows: 1 child 5 of 6 episodes; 1 child 2 of 6 episodes; 1 child 1 of 6 episodes; 1 child 2 of 5 episodes; 2 children 3 of 4 episodes; 1 child 2 of 4 episodes; 5 children 2 of 3 episodes; 2 children 1 of 3 episodes; 1 child 1 of 2 episodes.

they failed to complete a minimum of 20% visits with a stool sample. Of the 198 children in active follow-up, 106 and 92 were from Colina and Peñalolén, respectively. All children were followed for at least 7 months at the cutoff date, most (66%) were followed for more than 15 months (mean follow-up of 15.7 months; median 16 months) for a total follow-up of 3106 child-months (1772 for Colina and 1334 for Peñalolén) (Table 1). Compliance of scheduled visits with a stool sample was 73% (87% for Colina and 54% for Peñalolén) and a total of 2278 asymptomatic stool samples (1552 from Colina and 726 from Peñalolén) were studied for norovirus and rotavirus. None of the infants received a rotavirus vaccine.

Acute Diarrhea Episodes

A total of 234 ADE episodes were identified during the 3106 months of follow-up in the 198 children. The proportion of ADE episodes adjusted to the months of follow-up were very similar between sites, 132 of 1772 (7.5%) and 102 of 1334 (7.6%) for Colina and Peñalolén respectively. The first ADE occurred at a median age of 9 months. The percentage of ADE cases peaked at 46% between 7 and 12 months of age and 32% of cases occurred between 13 and 18 months of age (Table 1). The incidence rates of ADE by semester of life adjusted by the number of months of follow-up were for 0 to 6 months: 52 of 1188 (for a mean of 0.26 ADE per child for the first semester), 7 to 12 months 107 of 1119 (mean of 0.57 ADE), 13 to 18 months 75 of 799 (mean of 0.56 ADE). Overall, 123 of 198 infants (62%) had at least 1 ADE episodes, slightly more in Colina than Peñalolén 73 of 106 (63%) versus 50 of 92 (54%) ($P = 0.05$). Roughly one-half of the children suffering an ADE had only 1 episode, one-fourth had 2 episodes, and another one-fourth had 3 or more episodes. A total of 145 (62%) ADE could be fully evaluated clinically and for etiology, and the proportion of evaluable cases by age groups was well distributed. Most children (52/61, 85%) with multiple episodes had at least 1 and up to 5 episodes fully characterized.

Rotavirus and Norovirus Asymptomatic and Symptomatic Infections

Overall, rotavirus and norovirus were detected in 56 and 219 stool samples respectively. All rotavirus samples were positive by ELISA. For norovirus, 124 samples were positive only by RT-PCR, 47 by ELISA, and 48 positive by both ELISA and RT-PCR. Asymptomatic rotavirus infections were detected in 31 of 2278 (1.3%) monthly stool samples of 27 of 198 (14%) infants; 4 children had 2 rotavirus episodes. Asymptomatic norovirus was detected in 187 stools (8% monthly samples) corresponding to 113 (57%) infants. Multiple asymptomatic norovirus infections ranged from 2 to 7 episodes in 35 infants. Asymptomatic coinfections with both rotavirus and norovirus were detected in only 4 of 2278 (0.2%) stool samples.

For ADE, rotavirus was detected in 21 children, representing 15% of all studied episodes. No child had 2 symptomatic rotavirus infections and coinfection with a bacteria was detected in 3 ADE. Norovirus was detected in 26 of 145 ADE (18%). Only 1 child had 2 documented symptomatic norovirus infections and 1 child had a coinfection with a bacteria (*Campylobacter spp*) (Table, Supplemental Digital Content 1, <http://links.lww.com/A1354>). Most of these symptomatic episodes were also detected by RT-PCR (60%) followed by dual ELISA/RT-PCR positivity (27%).

Comparison of Age-Specific Incidence and Primary or Reinfection Status With Severity of Rotavirus and Norovirus Infections

Incidence of asymptomatic rotavirus infections per semester of life was similar: 0 to 6 months 10 of 925 samples tested (for a mean of 0.065 episodes per child for the first semester), 7 to 12 months 9 of 837 (0.065 episodes per child), 13 to 18 months 12 of 516 (0.14 episodes per child; $P = 0.1$ compared with other age groups). For norovirus asymptomatic infections incidence rates were 0.34, 0.50, and 0.24 for the first, second, and third semester of life ($P = 0.04$ and 0.03 for the higher incidence at 7 to 12 months of age compared with <7 months and ≥ 13 months of age, respectively).

Rotavirus and norovirus positivity of ADE was similar for the 3 semesters of life: 11%, 16%, and 14% for rotavirus and 17%, 18%, and 19% for norovirus. Median age for symptomatic rotavirus and norovirus infections were 9.5, and 11 months, respectively. ADE caused by rotavirus were in general more severe than episodes caused by norovirus as observed both by the proportion of mild episodes (1/18 vs. 13/25, $P = 0.004$) and the mean Vesikari score (10.4 vs. 7.4, $P = 0.01$) (Table 2). Nevertheless, norovirus caused 3 compared with 5 rotavirus severe ADE. Severity of rotavirus or norovirus ADE was not associated with age of infection except possibly for a mild trend toward increasing severity with increasing age for norovirus ADE. Two children with rotavirus infection required hospitalization and 1 an emergency room visit; 1 child with a norovirus infection required an emergency room visit. Asymptomatic reinfections were more common for norovirus compared with rotavirus infection: 83 of 187 (44%) versus 6 of 31 (19%), $P = 0.01$; a similar trend was observed for a symptomatic reinfection 10 of 25 (40%) versus 2 of 18 (11%), $P = 0.08$. Both symptomatic rotavirus reinfections occurred after a primary asymptomatic infection and were of moderate severity. No severe norovirus reinfection was identified and the mean Vesikari score did not differ significantly between primary infections or reinfections.

Rotavirus and Norovirus Genotypes and/or Genogroups by Infection Status

Rotavirus genotype G9P8 and norovirus genogroup GII predominated in both asymptomatic and symptomatic episodes.

TABLE 2. Association Between Age and Primary Versus Reinfection Status With Severity of Rotavirus and Norovirus Infections

Infection	No. Episodes Asymptomatic	No. ADE With Indicated Severity*				Mean Severity Score
		Mild	Moderate	Severe	Total	
Rotavirus	31	1	12	5	18	10.4 [†]
Age episode						
0–6 mo	10	0	3	1	4	10.5
7–12 mo	9	0	7	2	9	10.2
13–18 mo	12	1	2	2	5	10.8
Primary infection [‡]	25	1	10	5	16	10.2
Reinfections	6	0	2	0	2	9.0
Norovirus	187	13	9	3	25	7.4 [†]
Age episode						
0–6 mo	68	4	2	0	6	5.8
7–12 mo	85	6	4	1	11	7.2
13–18 mo	34	3	3	2	8	8.7
Primary infection [‡]	104	8	4	3	15	7.6
Reinfections	83	5	5	0	10	6.7

*Vesikari score: 0 to 6 mild, 7 to 11 moderate, and 12 to 20 severe.

[†] $P = 0.01$ for difference in mean Vesikari score between rotavirus and norovirus ADE.

[‡]Defined as primary infection when the asymptomatic or symptomatic episode corresponded to the first positive detection of the indicated virus in stools for a given child.

TABLE 3. Episodes of GII and Nontypable Norovirus Infections in Relation With a Prior Episode Associated With the Same or Different Genogroup

Prior NV Infection	GII Infections		Nontypable Infections	
	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic
No NV	10	14	4	45
GII	0*	10* [†]	0	13 [‡]
GI	2	0	0	2
Nontypable	7	7	2	27
Total	19	31	7 [§]	89 [§]

* $P = 0.008$ for difference in previous GII infections.

[†]The 10 episodes occurred in 5 children (3 with 1 reinfection each, 1 with 3, and 1 with 4 asymptomatic reinfections).

[‡]Three children also had a previous nontypable infection.

[§]In 1 symptomatic episode and 2 asymptomatic episodes none of the previous norovirus positive sample(s) was(were) available for genotyping.

Five of 15 asymptomatic rotavirus tested were G9P8 and 10 were nontypable. For 17 symptomatic rotavirus episodes tested, 16 were G9P8 and 1 was G2P4. Of 120 asymptomatic norovirus positive samples tested, 90 (75%) did not amplify for either group, 33 (28%) were GII and only 5 samples belonged to GI (Table 3). For 26 symptomatic norovirus episodes tested, 19 were GII and 7 were nontypable; symptomatic GI infections were not detected. Only 10% of 45 samples nongrouped by the first set of primers were grouped after applying the Katayama protocol.

A significant finding was that 0 of 19 confirmed symptomatic GII norovirus infections had a previous documented GII infection compared with 10 of 31 documented asymptomatic GII infections (Odds ratio: 0, exact lower and upper 95% confidence limits: 0 and 0.59; $P = 0.008$) (Table 3). Overall, 0 of 26 symptomatic norovirus infections had a previous GII infection compared with 23 of 119 asymptomatic infections (Odds ratio: 0, exact lower and upper 95% confidence limits: 0, and 0.70; $P = 0.01$).

DISCUSSION

Chilean infants living in low-middle income environments suffered a mean of approximately 1.4 ADE during the first 18

months of life, which is lower than the 2 episodes infant/year reported in the 1990s.⁶ Near 40% of children will not have an ADE during the first 18 months of life whereas nearly 30% will have 2 or more episodes. The relatively late occurrence of the first ADE in this population, median at 9 months of age, together with previous reports of a significant proportion of moderate-to-severe ADE occurring during the second year of life in Chile⁹ suggest that similar or possibly higher incidence rates can be expected for the second and third year of life.

Norovirus and rotavirus accounted for 33% of ADE evaluated with a slightly higher detection rate for norovirus over rotavirus. Overall virus-virus and bacteria-virus coinfections occurred in less than 10% of fully evaluated episodes. Rotavirus symptomatic infections were in general more severe than norovirus symptomatic infections as documented by the Vesikari score, although almost half of norovirus infections caused moderate-to-severe diarrhea. The age of infection did not have a significant impact on disease severity for either virus. These findings are relevant for several reasons. First, noroviruses clearly establish themselves as a cause of ADE among infants with a similar if not higher incidence than rotavirus. It also becomes clear that overall norovirus infections are less severe than rotavirus infections but this does not preclude the possibility of having moderate-to-severe norovirus infections. These results are compatible with hospital-based studies that have detected norovirus in severely ill children³²; hospitals would tend to concentrate children with severe norovirus infections. The virology or biology behind the overall difference in severity of norovirus and rotavirus will be further evaluated in future studies.

Asymptomatic detection of norovirus in stools was common and more than half the children had at least 1 RT-PCR and/or ELISA positive sample during follow-up, many with repeated positivity. We have interpreted these detections as “asymptomatic infections” but this may be an overstatement. Prolonged shedding is a possibility in some of the cases despite our episode definition that required at least 2 weeks between positive samples. Norovirus shedding for 3 or more weeks has been reported.³³ Nevertheless, these results are comparable with an earlier report from Mexico.²¹ False positivity of our RT-PCR is possible although consistency of negative controls routinely used during the procedure and the

specificity of the band size amplified reduce this possibility. The fact that most asymptomatic norovirus positive samples did not amplify with RT-PCR directed to capsid sequences even after applying a second set of primers is intriguing. It is possible that some of these correspond to sapoviruses because the RT-PCR used detects sapoviruses. It is also possible that a lower viral load of asymptomatic stools, associated with prolonged storage, hampered or possibility of capsid amplification. In addition, some of these noroviruses may not amplify with the capsid primers selected. We will explore these possibilities in the near future to clarify the true significance of these asymptomatic positive samples.

Symptomatic rotavirus reinfections were uncommon and in line with numerous studies on natural infection and protection.^{17,34,35} Norovirus reinfections in contrast, both symptomatic and asymptomatic were common with as many as 7 episodes in a given child before 18 months of age. Repeated symptomatic episodes were less common (10 reinfections vs. 15 primary infections) with a mild trend toward less severity. One of the most relevant findings of this study was that none of 19 symptomatic GII infections compared with 10 of 31 asymptomatic GII infections had a documented prior GII infection. This finding strongly raises the possibility of GII genogroup-specific induced protection in this population against symptomatic reinfection, and supports further studies focusing on the broadness of protection potentially conferred by epitopes in GII noroviruses. GII noroviruses are consistently reported as the most common genogroup causing disease in children.^{36,37} Within 1 year we will have completed 18 months of follow-up for the entire cohort that will allow including more data on recurrent infections, including subtype analysis, which may or may not further support this protective trend.

Several limitations of our study need to be addressed. Two sites were included that although sharing the same protocol, had differences in parental adherence and performance. The similarity of the results between the 2 sites after adjustment of the follow-up periods and stool sample collections make them comparable. We were able to study 62% of ADE episodes because mothers did not always report the episode in a timely manner. The few cases requiring hospitalization and emergency room visits were studied. We obtained monthly stool samples in contrast to weekly stool samples obtained in a few of the most comprehensive cohort studies previously performed. Asymptomatic infection rates could thus for be higher than the rates detected in our study. The difficulty of such frequent sampling although is differentiating new episodes from an episode with prolonged shedding, especially if RT-PCR is used. For norovirus positivity we used 2 criteria based on ELISA and/or RT-PCR. Previous studies from our group and others have shown that these techniques are complimentary because some stools react to one but not the other and choosing the correct primer for RT-PCR may be difficult.³⁸

In summary, this study provides new important insights on the dynamics of norovirus as compared with rotavirus infections in young Chilean infants and supports the significant role of both viruses in symptomatic and asymptomatic intestinal infections.

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