First Episodes of Norovirus and Sapovirus Gastroenteritis Protect Against Subsequent Episodes in a Nicaraguan Birth Cohort

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Background: Norovirus and sapovirus cause a large burden of acute gastroenteritis (AGE) in young children. We assessed protection conferred by norovirus and sapovirus AGE episodes against future episodes.

Methods: Between June 2017 and July 2018, we recruited 444 newborns in León, Nicaragua. Weekly household surveys identified AGE episodes over 36 months, and AGE stools were tested by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) for norovirus genogroup (G)I/GII and sapovirus. We used recurrent-event Cox models and negative control methods to estimate protection conferred by first episodes, controlling for observed and unobserved risk factors, respectively.

Results: Sapovirus episodes conferred a 69% reduced hazard of subsequent episodes using the negative control method. Norovirus GI (hazard ratio [HR] = 0.67; 95% confidence interval [CI] = 0.31, 1.3) and GII (HR = 0.20; 95% CI = 0.04, 0.44) episodes also appeared highly protective. Protection against norovirus GII was enhanced following two episodes.

Conclusions: Evidence of natural immunity in early childhood provides optimism for the future success of pediatric norovirus and sapovirus vaccines.

Keywords: Children; Diarrhea; Gastroenteritis; Nicaragua; Norovirus; Sapovirus

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Following the global deployment of rotavirus vaccines, human caliciviruses are among the most commonly detected pathogens in causing acute gastroenteritis (AGE).[1,2] In low- and middle-income countries, where the majority of deaths due to gastroenteritis occur, two meta-analyses estimate that norovirus was detected in 15% to 17% of tested AGE stools,[3,4] and sapovirus had the second highest attributable incidence for AGE in children under 2 years of age.[5] Despite this high burden of disease, there are no licensed vaccines against norovirus or sapovirus. For rotavirus, a seminal birth cohort study showed that children with one, two, and three previous rotavirus infections had progressively lower risks of subsequent infections and episodes than children without previous infections; this information on natural immunity informed rotavirus vaccine development.[6] Understanding comparable patterns of protection following natural norovirus and sapovirus infections may inform the overall feasibility of future vaccines, provide insights on the vaccine schedules and doses, and serve as a benchmark for comparison with vaccine-induced immunity.

While natural calicivirus infection is expected to confer protection against future infections, protective associations can be confounded by unobserved factors that cannot be readily controlled for using standard covariate adjustment. Negative control methods, in which pathogen-specific risks are compared among children with a history of infection with the same or a different pathogen, have previously been used to correct for unmeasured bias in studies of natural immunity by intrinsically controlling for susceptibility to enteric infections.[7] We determined the protection conferred by first and

second episodes on future infections of norovirus and sapovirus using robust statistical methods to account for confounding risks and additionally considered host genetic factors that modify susceptibility to norovirus infections.

METHODS

Study Design

We obtained data from a birth cohort study of 444 children in León, Nicaragua.[8] Between June 2017 and July 2018, mothers of singleton infants living in 14 contiguous health sectors of the Perla Health District were offered participation.[8] Participating children received weekly household visits until 36 months of age to assess for AGE episodes, defined as increase in stool frequency to >3 stools in a 24-hour period or a substantial change in stool consistency (bloody, watery, very loose) and/or vomiting. We collected reflex stools during AGE episodes and tested them for norovirus and sapovirus. Household characteristics were assessed at enrollment, and updated covariates related to AGE risk behaviors and nutrition monthly. The child's mother provided informed consent, and the Institutional Review Boards of the National Autonomous University of Nicaragua, León and the University of North Carolina at Chapel Hill provided approval for the study.

Specimen Collection and Laboratory Analysis

We have described stool sample collection, transport, and preliminary processing including viral RNA purification elsewhere.[8,9] The screening of norovirus genogroup (G)I and GII and sapovirus was performed by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR).[8,9] Due to increasing evidence of the importance of host histo-blood group antigen phenotype in modifying risk of norovirus gastroenteritis,[9] we also measured the child's secretor status as described elsewhere.[10]

Statistical Analysis

We used two different statistical approaches to estimate hazard ratios (HRs) for subsequent episodes following one or two prior episodes: (1) a discrete-time interval Andersen–Gill model, which is an extension of the Cox proportional hazards model for recurrent events and (2) a negative control method comparing the event rates of norovirus and sapovirus episodes to the rates of other AGE episodes with similar risk factors.[11] To address potential confounding, the Andersen–Gill model adjusted for covariates that have been associated with AGE risk in prior studies: vaginal versus cesarean delivery, presence of a toilet in the home, breastfeeding in the last week, and consumption of high-risk foods in the past month (i.e., seafood, raw produce, and food consumed outside the home). To control for potentially synergistic protective effects associated with concurrent calicivirus infections, we also adjusted for the presence of coinfection during the first norovirus GI, norovirus GII, or sapovirus infection. For the norovirus GI and GII outcomes, we further adjusted for the child's secretor phenotype.

The negative control approach was recently proposed by Northrup et al[11] to control for unmeasured confounders in natural immunity studies. We considered any AGE episode for which we did not detect the virus of interest in a stool sample to be a negative control event. For individuals with no prior infection, we define A and B as the time to first occurrence of the outcome of interest and a negative control outcome, respectively. After one (or two) infections of the virus of interest have occurred, we define C and D as the time to next occurrence of the outcome of interest and a negative control outcome, respectively. Then we estimate the hazard ratio using a Mantel–Haenszel type estimator:

$$HR = \left\{\sum I(B < A) I(C \leq D)\right\} / \left\{\sum I(A \leq B) I(D < C)\right\}$$

where $I(X < Y)$ is an indicator function equal to 1 if $X < Y$ and 0 otherwise. Confidence intervals were constructed using a bootstrap approach.

RESULTS

The 444 children enrolled in the birth cohort were 51% male and 55% born by vaginal delivery, and 73% lived in a household with a toilet. The children experienced 1,702 total AGE episodes over 36 months. Eighty children experienced a symptomatic, RT-qPCR-confirmed norovirus GI, 120 norovirus GII, and 109 children a sapovirus AGE episodes. Of these, 13 children (16.3%) experienced subsequent norovirus GI, 23 (19.2%) norovirus GII, and 16 (14.7%) sapovirus AGE episodes. Five GI episodes were coinfected with GII; nine GI episodes were coinfected with sapovirus; and five GII episodes were coinfected with sapovirus.

The adjusted Andersen–Gill model found that a first episode of sapovirus reduced the hazard of a subsequent episode by 83% (adjusted HR = 0.17; 95% confidence interval [CI] = 0.08, 0.37), compared with 49% in the unadjusted model (HR = 0.51; 95% CI = 0.32, 0.84). The negative control model confirmed this protective association (HR = 0.31; 95% CI = 0.06, 0.83) (Table). Only one child experienced a third sapovirus episode; thus, we did not estimate the protective efficacy of two prior sapovirus episodes. A first episode of norovirus GI (adjusted HR = 0.38; 95% CI = 0.18, 0.80) and GII (adjusted HR = 0.30; 95% CI = 0.17, 0.52) were both associated with a decreased hazard of subsequent episodes; effect sizes were smaller in the unadjusted models (GI HR = 0.86; 95% CI = 0.50, 1.47 and GII HR = 0.55; 95% CI = 0.36, 0.85). The negative control method similarly adjusted for confounding for norovirus GI (HR = 0.67; 95% CI = 0.31, 1.3) and norovirus GII (HR = 0.20; 95% CI = 0.04, 0.44), although to a smaller degree than the covariate-adjusted method. A second episode of norovirus GI was not associated with a decreased hazard of a subsequent episode. However, in a post hoc analysis, we removed one outlier child who had experienced four norovirus GI episodes within 1 year and found a protective association of two

TABLE. Hazard Ratios for Future Norovirus and Sapovirus Episodes Among Children With Prior Episodes in a Nicaraguan Birth Cohort in the First 36 Months of Life, 2017–2021

Episode history	Unadjusted Andersen–Gill Model	Adjusted Andersen–Gill Model[a]	Negative Control Method
Sapovirus			
One prior episode (n = 109)	0.51 (0.32, 0.84)	0.17 (0.08, 0.37)	0.31 (0.06, 0.83)
Norovirus GI			
One prior episode (n = 80)	0.86 (0.50, 1.5)	0.38 (0.18, 0.80)	0.67 (0.31, 1.3)
Two prior episodes [b](n = 13)	1.0 (0.33, 3.3)	1.0 (0.32, 3.3)	1.7 (0.33, ∞)
Norovirus GII			
One prior episode (n = 120)	0.55 (0.36, 0.85)	0.30 (0.17, 0.52)	0.20 (0.04, 0.44)
Two prior episodes (n = 23)	0.32 (0.08, 1.3)	0.18 (0.04, 0.73)	0.14 (0.00, 0.67)

[a]Adjusted for delivery route, presence of a toilet in the home, breastfeeding in the last week, consumption of high-risk foods in the last month, and coinfection with another calicivirus (sapovirus, norovirus GI, norovirus GII). Norovirus models were additionally adjusted for child's secretor status.

[b]On exclusion of one outlier child who experienced four norovirus GI episode within 12 months, the *associations* in the Andersen-Gill models are protective.

prior norovirus GI episodes against future episodes (adjusted HR = 0.38; 95% CI = 0.05, 2.7).

DISCUSSION

In a population-based birth cohort in León, Nicaragua, we identified protective associations between first and subsequent AGE episodes associated with human norovirus and sapovirus. We implemented two methods to control for confounding by risk factors that predispose young children to enteric infections. The latter approach used time to negative control events (i.e., AGE episodes not associated with caliciviruses) to reduce confounding associated with risk factors common to AGE episodes of various etiologies.

While the two approaches showed different magnitudes of association for sapovirus and norovirus GI following one prior episode, both approaches suggest that the crude relationship between prior and subsequent episodes is substantially confounded. The direct adjustment approach controlled for measured confounders directly, which may lead to higher precision than the negative control method, which controls for confounding in an indirect way. Furthermore, the negative control method does not utilize the full sample, possibly leading to similar precision issues. It is also possible that unmeasured confounders were adjusted for by the negative control method, but not by the direct adjustment approach, leading to slightly different estimates. Additional research is needed to identify these factors, with more granular collection of nutritional and behavioral risk factor data.

A birth cohort study of norovirus in Peru suggested a similar level of protection against symptomatic norovirus GII.4, GII.6, and GII.17 after a prior symptomatic or asymptomatic homologous infection.[12] Our study did not include asymptomatic infections or identify these specific genotypes, but our findings also suggest that prior infections confer protection within genogroups. In addition, an analysis of data from the Malnutrition and the Consequences for Child Health and Development (MAL-ED) study found modest decreases in subsequent norovirus GII and sapovirus episodes following a first diarrhea episode (HR = 0.82 and 0.73, respectively), using similar negative control methods that also appeared to correct for additional confounding compared with covariate adjustment.[7] It is unclear why we observed a greater level of protection in our study than this prior study; however, our adjustment for the child's secretor status in the norovirus analyses resulted in a stronger protective association. In this cohort, 89% of children were secretor positives, thus at high risk for norovirus infections. Without adjusting for secretor phenotype, the few nonsecretor children might skew the results in favor of a less protective association. Concerning sapovirus, it is possible that the differences in protective associations between sites could be explained by differences in the diversity of sapovirus genotypes by location; a location with a lower diversity of circulating genotypes may exhibit higher protection against future episodes.

Immunity induced by a natural norovirus or sapovirus infection is not likely to extend beyond the infecting genogroup. Early norovirus challenge studies in adults have shown protection within a norovirus genogroup but little cross-protection across genogroups.[13] For sapovirus, studies using hyperimmune sera found strong reactivity against homologous sapoviruses, but little cross reactivity between genogroups, providing evidence that sapovirus genogroups are antigenically distinct.[14] Therefore, protection should be examined not only for each virus genus as a whole but also on the genogroup or genotype level. Relatedly, we studied two closely related caliciviruses that have the potential to confer cross-reactive immunity. We controlled for calicivirus coinfections in the Andersen–Gill model to account for more potent immunity observed following a coinfection than might have been observed following a singular infection. We did not test samples for a wide array of enteric pathogens and thus could not rule out other pathogens from causing disease symptoms. However, the detection of norovirus and sapovirus in diarrheal stools suggests that some degree of immunity would be gained, regardless of the true etiology of the episode.

Limitations of this work include the small number of repeat norovirus and sapovirus episodes, leading to imprecise estimates. However, the protective associations were mostly consistent across viruses. One child in the cohort experienced four norovirus GI within 1 year, suggesting unique norovirus risk factors that were not sufficiently controlled. In contrast to the study on norovirus from Peru,[12] we only tested stools from symptomatic episodes; thus, we do not contribute to understanding the level of protection conferred by asymptomatic infections nor the cumulative protective effects of multiple symptomatic and asymptomatic infections. It is possible that symptomatic infections induce a more robust immune response than asymptomatic episodes, and thus we observed strong associations that might have been attenuated by including asymptomatic infections. Indeed, Rogawski McQuade et al[7] observed greater magnitudes of protection for diarrhea episodes compared with asymptomatic infections, giving support to this hypothesis. Finally, due to small numbers of genotype-specific reinfections, we could not assess the level of genotype-specific protection within genogroups.

These findings support the hypothesis that norovirus and sapovirus AGE episodes in early childhood confer protection against future episodes in the first 3 years of life, providing optimism for the future success of pediatric calicivirus vaccines. Norovirus vaccines are currently under development, and these data on the development of natural immunity to norovirus early in life are needed. Sapovirus vaccine development efforts have not yet begun, but the lessons learned from the norovirus experience could be adapted to this related virus.

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