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Symptomatic and asymptomatic norovirus infections in early life; The PREVAIL Cohort, 2017-2020

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Introduction: Norovirus is the leading cause of medically attended acute gastroenteritis in the United States. Efforts to reduce the disease burden are constrained by uncertainty around fundamental aspects of norovirus epidemiology. This study describes characteristics of norovirus infections and explores potential risk factors for symptomatic infections in early life.

Methods: The Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal birth cohort study followed 245 children from birth to 2 years of age with weekly stool sample collection and symptom surveys. Stool samples were tested by reverse transcriptase-realtime polymerase chain reaction to detect norovirus genogroup (G)I and GII; positive samples were genotyped. Infections accompanied by diarrhea and/or vomiting were considered symptomatic. Children were categorized as adherent if they participated for ≥18 months and submitted ≥70% of samples.

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Results: A total of 72 GI and 330 GII norovirus infections (among 156 children) were identified. One-fifth (20.8%) of adherent children experienced ≥1 norovirus infection by 6 months of age, increasing to 84.2% children by 2 years of age. About one-third of infections were symptomatic, including half of infections with cycle threshold values <25. Infection with norovirus genotype GII.4 Sydney was the strongest predictor of symptomatic infection in adjusted analyses, as was older age and higher viral load. Childcare attendance, breastfeeding, mother's secretor status, and prior infections were not predictive of symptom status.

Conclusion: This study highlights fundamental characteristics of norovirus epidemiology in early life with implications for understanding the full natural history of the disease, disease transmission and prevention approaches.

Keywords: Norovirus; Genotype; Child; Birth cohort; Gastroenteritis; United States

INTRODUCTION

Norovirus is the leading cause of medically-attended acute gastroenteritis (AGE) in the United States [1]. Considerable uncertainty around fundamental aspects of norovirus epidemiology, including the frequency of exposure early in life, prevalence of asymptomatic infections, and immune response [2], challenge efforts towards developing an effective vaccine [3].

Asymptomatic norovirus infections and their potential contribution to transmission are not well quantified. Limited data suggest a large portion of norovirus infections, up to one-third, may be asymptomatic [4–8]. There is also indication that viral load may imperfectly correlate with symptom status [7,8] raising the potential that asymptomatic infections with high viral loads may contribute to norovirus transmission [5]. How these characteristics vary by virus and host characteristics is unclear.

Most epidemiologic reports on norovirus include outbreak surveillance and studies focused on medically-attended AGE [9]. From these studies, young children aged 6-24 months have the highest incidence of norovirus infections in the outpatient and emergency department settings [1] and are considered primary contributors to norovirus transmission [10]. Further, early childhood exposures shape an individual's immunologic protection against subsequent homotypic and heterotypic infections [11].Birth cohort studies provide a more complete view of norovirus epidemiology and can contribute to the development of effective preventive strategies [2,12,13].

The Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal (PREVAIL) birth cohort provides an opportunity to examine norovirus infections among an immunologically-naïve population over the first two years of life. In this study, we describe the characteristics of norovirus infections and assess potential risk factors for symptomatic and

asymptomatic infections including viral and host characteristics, behaviors, and environmental exposures.

METHODS

Study design

From April 17, 2017-July 16, 2018, PREVAIL enrolled mothers who resided in the Cincinnati, Ohio region during their third trimester of pregnancy. Maternal demographic information and saliva samples were collected at enrollment. Children were followed until their second birthday with weekly symptom surveys, weekly stool sample collection, and additional data collection during episodes of AGE. Information on childcare was collected at 6 weeks and 4, 6, 12, 18, and 24 months of age, along with duration of breastfeeding. Additional methodology details have been previously described [14]. The study protocol was reviewed and approved by the Centers for Disease Control and Prevention, Cincinnati Children's Hospital Medical Center and the participating hospitals' IRBs.

Enteropathogen infections and AGE symptoms

All stool samples underwent reverse transcriptase-realtime quantitative polymerase chain reaction (RT-qPCR) testing to detect GI and GII noroviruses. RT-qPCR positive samples were genotyped after sequencing of conventional reverse transcription-polymerase chain reaction products (Supplemental Material). Cycle-threshold (Ct) values from RT-qPCR were used as a proxy for viral load (lower Ct values represent higher viral load). To identify potential co-infections, 16 additional enteropathogens were tested by RT-qPCR and/or the Luminex xTAG® Gastrointestinal Pathogens Panel (Supplemental Material).

Norovirus infections were defined based on timing and genotype of norovirus detected in stool. The initial positive sample was considered the start of an infection. Subsequent detections of the same genotype were classified as the same infection if within 30 days of the prior detection, regardless of any intervening negative samples. Similar to other birth cohort studies [13,15], any detection of a new genotype or detection >30 days from the prior detection was considered a new infection. The duration of viral shedding was defined as the number of days from the first to last detection of norovirus within an infection.

Symptom data from weekly surveys, illness surveys, and medical records were combined to identify episodes of AGE, defined as ≥3 looser than normal stools and/or ≥1 episode of vomiting in 24-hours. An infection was considered symptomatic if it started ≤4 days prior to or ≤14 days after the start of AGE symptoms; infections that started outside this range were considered asymptomatic. Severity of symptoms were classified using a 20-point modified Vesikari score (MVS) [16] (Supplemental Material).

Incidence rate was calculated by dividing the total number of norovirus infections by the total weeks at risk in the cohort. Weeks at risk was the sum of weeks in which a stool sample was submitted by participants. Norovirus-positive samples were not subtracted from weeks at risk since infections were based on genotype detected and children remained at risk of infection from other genotypes throughout the follow-up period.

Fucosyltransferase 2 (*FUT2*) genotype (secretor status), a genetic mutation which impacts binding of norovirus to its target cells, of mothers and children was determined from saliva samples to evaluate potential genetic susceptibility to infection [17] (Supplemental Material).

Statistical analysis

Characteristics of mothers and children and a description of norovirus infections are initially provided for all participants and infections. Subsequent analyses were restricted to participants who submitted ≥70% of weekly samples and who were followed for ≥18 months (adherent participants). Co-infections were excluded from symptom analyses. The chi-square, Mann-Whitney U and Kruskal-Wallis tests were used for comparing data in descriptive analyses.

Generalized estimating equation (GEE) models utilizing a binomial family function and exchangeable correlation structure were fit to estimate predictors of infection symptom status among adherent children. Four GEE models were fit including an unadjusted model using all infections and three models adjusted for predictors selected *a priori* using 1) all infections, 2) infections during the first year of life and 3) infections in the second year of life. An adjusted generalized linear model (GLM) was similarly fit among first infection only. Predictors included health insurance type, childcare at the time of infection, breastfeeding status, mother and child secretor status, norovirus genogroup/genotype, whether the child had previous norovirus infections, child's age, and minimum Ct value detected during the infection (Supplemental Material). Results are presented as odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs). All analyses were conducted in R version 4.4.0 [18].

RESULTS

A total of 245 mother-child pairs were enrolled. The median duration of active study participation (submitted symptom survey and/or sample) was 103 weeks [IQR: 102-104] and 13,944 stool samples were analyzed (Table 1). Approximately three-quarters of mothers (73.1%) and children (69.0%) were genotypically secretors. The median duration of breastfeeding was 28.9 weeks and half of children were cared for solely at home at 12 months (49.8%) and 24 months (44.9%) of age, while the remaining children attended childcare at least once per week (Table 1).

101 mother-child pairs were considered adherent (Table 1). Non-adherent children participated in the study for a significantly shorter duration (median weeks=103 [IQR: 59-104]) compared to adherent children (median =103 [IQR: 103-104], p<0.001). Non-adherent and adherent mother-

child pairs submitted a median of 18 [IQR: 6-40] and 86 [IQR: 77-94] stool samples, respectively. Non-adherent children were more likely to have mothers with a high school education or less, public health insurance, and self-classify as Black (Table 1).

Overview of norovirus infections among all children

In the entire cohort, 72 GI and 330 GII norovirus infections were identified among 156 children (Table 1). One in five (21.6%, n=53) children experienced ≥1 norovirus infection by 6 months of age, increasing to over half (51.0%, n=125) within the first year of life and nearly two-thirds (63.7%, n=156) by age 2. The median age at infection among all children was 11.9 months [IQR: 7.6-17.3] (Table 1). Eighty-nine children (36.3%) did not have any detected norovirus infection during follow-up; 34.8% (n=23/66) of these children were FUT2 non-secretors (status unknown=23). Overall, 13.6% (n=53/391) infections were among FUT2 non-secretors (status unknown=11). The incidence of norovirus infection was 1.7 (95% CI: 1.5-1.9) per child-year, peaking during winter months (Figure 1).

Among GI infections, GI.3 was the most frequently detected genotype (67.6%, n=46/68) followed by GI.6 (25.0%, n=17/68). GII genotypes were more widely distributed with GII.4 Sydney (27.9%, n=86/308) and GII.6 (23.7%, n=73/308) detected in approximately one in five of all genotyped infections (Supplemental Figure 1).

Few co-infections were identified (n=34/402, 8.5%) [19]. Excluding coinfections, 65.2% (n=214/328) of norovirus infections with known symptom status were asymptomatic.

Norovirus infections among adherent children

We observed 253 (45 GI and 208 GII) norovirus infections among 101 adherent children (Table 2). Genotypic distributions were similar as those described for the full cohort (Supplemental Figure 1). Eighteen co-infections were identified. Among norovirus-only infections with known symptom status, two-thirds (65.3%, n=143/219) were asymptomatic, a proportion nearly identical to the full cohort (Table 2).

The median age at first norovirus infection for adherent children was 9.4 months [IQR: 6.1-12.3]. By 2 months of age, 4.0% of children had experienced a norovirus infection, and that proportion increased rapidly in early life to 61.4% (n=62) and 84.2% (n=85) at 1 and 2 years of age (Table 1, Figure 2). 71.1% (n=64/90) of infections that occurred during the first year of life were asymptomatic compared to 61.2% (n=79/129) in the second year. No children experienced a symptomatic infection before 4 months of age (Figure 2) and only 3.0% had by by 6 months of age. The proportion increased to 20.8% by 1 year of age and further rose to 51.5% by age 2 (Figure 2). Asymptomatic infections generally occurred at a higher level than symptomatic infections (Figure 3). This relationship was most pronounced with GI and GII.other genotypes, but less consistent for GII.4 Sydney (Figure 3).

Norovirus viral shedding and cycle thresholds by symptom status among adherent children

The median duration of viral shedding was 13.0 days [IQR: 1.0-23.0] with longer shedding seen for GII infections (GII.4 Sydney: 12.0 [IQR: 1.0-23.0]; other GII: 15.0 [IQR: 1.0-25.5]) compared to GI infections (8.0 [IQR: 1.0-21.0]) (Table 2). When all genogroups were combined, children with symptomatic norovirus infections shed virus longer than those with asymptomatic infections (median=18 days [IQR: 7.5-28.0] vs. 10 days [IQR: 1.0-22.0], p-value=0.004).

Minimum Ct values were comparable by genogroup/genotype with median values ranging from 24.5-26.0 (p=0.358). The median Ct value was significantly lower in symptomatic infections (23.0 [IQR: 21.0-27.0]) compared to asymptomatic infections (27.0 [IQR: 23.0-31.0], p-value<0.001). Yet, over half of infections with Ct values less than 25, representing infections with the highest viral loads, were asymptomatic (Ct <20: 52.6%; Ct 20-24: 51.3%) (Figure 4). Among the infections with Ct values <25, the proportion of children considered secretors was comparable for both asymptomatic (84.3% secretors) and symptomatic (87.8% secretors) infections.

Symptom status and healthcare utilization among adherent children

The median duration of symptoms was 3 days [IQR: 2-5] and similar across genogroups/genotypes (p-value=0.200) (Table 2). Vomiting was the most common symptom reported, present in 84.2% (n=64/76) of symptomatic infections, followed by diarrhea (77.6%, n=59/76) and fever (31.5%, n=23/73). The median duration of vomiting and diarrhea were 1.0 day [IQR: 1.0-2.0] and 3.0 days [IQR: 2.0-4.3], respectively. Children experienced vomiting without diarrhea in 22.4% (n=17/76) of symptomatic infections. Children typically experienced a maximum of 3.0 [IQR: 2.0-5.0] vomiting and 4.0 [IQR: 3.0-5.0] diarrhea episodes in a 24-hour period (median values). Vomiting was more frequently reported in GII.4 Sydney (85.7%, n=18/21) and other GII (87.5%, n=42/48) infections compared to GI (57.1%, n=4/7) infections but not significantly (p=0.117). Nearly all children with GII.4 Sydney infections experienced diarrhea (95.2%, n=20/21); diarrhea was also common in both GI (71.4%, n=5/7) and other GII (70.8%, n=34/48) infections. Fever was reported frequently in GI infections (50.0%, n=3/6), but this was not significantly higher than in GII.4 Sydney (23.8%, n=5/21) or other GII (32.6%, n=15/46) infections (p=0.460) (Table 2). Symptom characteristics were similar when comparing infections occurring in the first and second years of life (Supplemental Table 1).

Most symptomatic infections (88.2%, n=67/76) were considered mild according to their MVS scores (Table 2). Severity by genogroup/genotype did not differ significantly, though the highest proportion of moderate and severe infections occurred among GII.4 Sydney infections (moderate=14.3%, n=3/21; severe=9.5%, n=2/21). Severity did not differ by child's year of life (Supplemental Table 1).

Overall, medical care was sought for one-third (32.9%, n=25/76) of symptomatic infections, resulting in 15 outpatient visits and 10 emergency department or hospital admissions among 23 children (Table 2). Differences in care seeking were observed by genogroup/genotype, though not

significantly (p=0.167). The majority (85.7%, n=6/7) of symptomatic GI infections were treated at home. One in five (21.7%, n=15/69) children with a symptomatic GII infection (GII.4 Sydney or other GII) sought care in the outpatient setting while an additional 13.0% (n=9/69) presented to the ED or were hospitalized. Out of 51 GII.4 Sydney infections with known symptom status among adherent children, 41.2% (n=21) were symptomatic and 11.8% (n=6) resulted in an ED visit or hospital admission (Table 2).

Predictors of symptom status

Unadjusted models using all infections among adherent children suggest the strongest predictor of experiencing symptoms was infection with GII.4 Sydney (OR=2.95, 95% CI: 1.11-7.82) (Supplemental Table 2). When adjusting for all predictors (Figure 5), infection with GII.4 Sydney (OR=3.45, 95% CI: 1.18-10.12) remained a significant predictor of experiencing symptoms. Increased age at infection (OR=1.10, 95% CI: 1.01-1.19) had a significant but modest impact on symptomatic status. Lower viral load (higher Ct value) was associated with reduced odds of symptomatic infection (OR=0.89 for a one unit increase in Ct value, 95% CI: 0.83-0.95). The high OR (3.68, 95% CI: 0.90-15.07) observed for children considered FUT2 secretors suggests secretors may have increased odds of symptomatic infection, however, the CI was wide and crossed the null. Childcare setting, breastfeeding, mother's secretor status, and whether the child had prior infections were not predictive of symptom status.

Model convergence was not achieved when including all predictors in models stratified by child's year of life (Supplemental Table 2). In the first year of life, children who attended large childcare settings were at greatest risk of symptomatic infection (OR=4.97, 95% CI: 1.42-17.40) compared with those who were cared for solely at home or in another's persons home; an inverse relationship was found in the second year of life (OR=0.31, 95% CI: 0.14-0.73). Breastfeeding increased the odds of symptomatic infection (OR=5.92, 95% CI: 1.64-21.33) during the second year of life only. Higher Ct values were associated with lower odds of symptomatic infection during the second year of life (OR=0.85, 95% CI: 0.77-0.95), as well as for first infections (OR=0.87, 95% CI: 0.74-0.99) (Supplemental Table 2).

DISCUSSION

This U.S. birth cohort highlights fundamental characteristics of norovirus infection and disease in early life. Five out of six of the cohort's adherent children experienced at least one norovirus infection by 2 years of age. The majority of infections were asymptomatic, particularly among the youngest children. Half of infections with high viral loads were asymptomatic, raising questions about transmission dynamics in early life. In adjusted analyses, the strongest predictor of experiencing symptoms was being infected with norovirus GII.4 Sydney.

The frequency of severe norovirus infections underscores the importance of preventive measures, including an effective norovirus vaccine. GII.4 viruses are suspected of causing more severe disease than other genotypes in older adults [20,21], however, potential biases in estimating severity have prevented more definitive conclusions [9]. Our adjusted models found GII.4 Sydney to be a strong predictor of symptomatic infection and we further found over one-quarter of symptomatic GII.4 Sydney infections resulted in an ED visit or hospitalization. The propensity of GII.4 to cause severe disease, combined with heterotypic protection it may promote [11], emphasizes its importance as a component of future norovirus vaccines.

Our data highlight the under-appreciated enormity of asymptomatic norovirus infections in early life, which are critical to understanding the full natural history of the disease. In this cohort, two-thirds of infections were asymptomatic, a finding nearly identical to that of ORChID, a similarly structured Australian-based birth cohort study [13]. Notably, infections among children aged 0-5 months were most likely to be asymptomatic, similar to findings in Peru and Australia [13,15], bolstering evidence of protection provided by maternally derived antibodies [22]. The adjusted associations observed for breastfeeding stratified by year of life suggests breastfeeding may postpone rather than prevent infection, as has been suggested for rotavirus [23].

We demonstrate the strong transmission potential from asymptomatic infections among young children. Despite lower viral load being associated with reduced risk of symptoms in this study and in others [24], we found half of infections among children with the highest viral loads were asymptomatic. We were unable to assess the direct impact of symptom status on transmission; however, prior studies suggest high viral load may be independently associated with transmissibility, regardless of symptom presence or absence [5].

We found childcare may expose children to norovirus early, thereby reducing the risk of symptomatic infection in the second year of life. Recognizing the frequency of early exposures may better inform the timing of norovirus vaccination before the opportunity for natural infection. This timing must be balanced, in some circumstances, by delaying vaccination until maternal antibodies wane [22]. One of the most advanced candidates in the norovirus vaccine field to date vaccinated children beginning at age 5 months [25] and was discontinued after failing to meet its efficacy endpoints [26]. A comprehensive understanding of the prevalence of pre-existing immunity among children at the time of vaccination, interference by maternal antibodies, and other factors may contribute to effective vaccine development.

A majority of our analyses were conducted among adherent mother-child pairs to most accurately quantify infection statistics. Excluding non-adherent children may limit the generalizability of these results to broader populations with potentially different exposure and infection patterns. GEE models were constrained by sample size which prevented modeling of children 0-6 months of age, among whom predictors may differ when compared to older children, and assessment of important factors such as interaction between secretor status and genogroup/genotype [27]. Lastly,

FUT2 genotype assessment was limited to a mutation found in many populations (G428A) but does not capture different polymorphisms more common in others (e.g. Asian), possibly resulting in misclassification and limiting the generalizability of findings [28].

This longitudinal study of children from birth to 2 years of age provides insight into early norovirus exposures and characterizes the range of childhood norovirus infections from asymptomatic to those that require hospital care. Understanding the clinical and epidemiologic patterns in norovirus infections among young children and the risk factors that contribute to the development of symptoms can aid in identifying appropriate vaccine approaches and strategies. Further assessment of these data to evaluate immune response to infection and subsequent protection against future infections will lead to even greater insight into vaccine development.

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Contributions: JMB, CPM, SAM, and DCP conceptualized this study. JMB, JLC, CPM, RMB, SCC, and ARB assisted with data curation. HB, KN, EB and JV performed laboratory analyses and results interpretation. JMB performed formal analysis. MAS acquired financial support for the project. ALM and MAS were responsible for conducting the research and investigation process. DCP, ALM and MAS were responsible for development and design of methodology. MAS, ALM, UDP, JV, SAM, and DCP provided oversight and supervision. JMB conducted data visualization and drafted the manuscript. JLC, CPM, SAM and DCP critically reviewed and edited the manuscript. All authors reviewed the manuscript and had the final responsibility for the decision to submit for publication.

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Table 1. Child characteristics and stool sample collection among all 245 children in the PREVAIL cohort (n=245)

	All children N=245	Adherent[1] N=101	Non-adherent N=144	p-value[2]
Demographics				
Maternal education				
High school or less	115 (46.9%)	18 (17.8%)	97 (67.4%)	**<0.001**
More than high school	130 (53.1%)	83 (82.2%)	47 (32.6%)	
Insurance type				
Private	106 (43.3%)	78 (77.2%)	28 (19.4%)	**<0.001**
Public	139 (56.7%)	23 (22.8%)	116 (80.6%)	
Maternal race				
Asian or multiracial	12 (4.9%)	6 (5.9%)	6 (4.2%)	**<0.001**
Black	107 (43.7%)	17 (16.8%)	90 (62.5%)	
White	126 (51.4%)	78 (77.2%)	48 (33.3%)	
Maternal secretor status				
Non-secretor	51 (20.8%)	24 (23.8%)	27 (18.8%)	0.671
Secretor	179 (73.1%)	76 (75.2%)	103 (71.5%)	
Unknown	15 (6.1%)	1 (1.0%)	14 (9.7%)	
Child secretor status				
Non-secretor	48 (19.6%)	21 (20.8%)	27 (18.8%)	0.783
Secretor	169 (69.0%)	80 (79.2%)	89 (61.8%)	

Unknown	28 (11.4%)	0 (0%)	28 (19.4%)	
Duration of breastfeeding (weeks, median [IQR])	28.9 [4.4-55.9]	47.7 [16.4-63.0]	7.1 [1.0-19.9]	**<0.001**
Childcare location[3]				
Solely at home at 12 months	101 (49.8%)	48 (47.5%)	53 (52.0%)	0.623
Solely at home at 24 months	79 (44.9%)	48 (49.0%)	31 (39.7%)	0.284
Weeks active in study (median [IQR])	103 [102-104]	104 [103-104]	103 [58.8-104]	**<0.001**
Stool samples submitted (median [IQR])	52 [14- 82]	86 [77-94]	18 [6-40]	**<0.001**
Experienced ≥1 norovirus infection by age				
6 mo	53 (21.6%)	21 (20.8%)	32 (22.2%)	0.912
12 mo	125 (51.0%)	62 (61.4%)	63 (43.8%)	**0.010**
24 mo	156 (63.7%)	85 (84.2%)	71 (49.3%)	**<0.001**
Median age at first infection (mo)	7.6 [4.9-11.5]	9.4 [6.1-12.3]	6.3 [4.0-9.9]	**0.002**

[1] Adherent children were those who participated in the study for ≥18 months and submitted ≥70% of weekly samples.

[2] Comparing adherent and non-adherent mothers/children

[3] Excludes children with missing information (n=35 at 12 months; n=62 at 24 months)

Table 2. Characteristics of norovirus infections by genogroup/genotype among 101 adherent children in the PREVAIL cohort.

Characteristic	All infections N=253	GI N=45	GII.4 Sydney N=57	Other GII (non-GII.4) N=151	p-value[1]
Year of age					
First year of life	106 (41.9%)	12 (26.7%)	29 (50.9%)	65 (43.0%)	**0.044**
Second year of life	147 (58.1%)	33 (73.3%)	28 (49.1%)	86 (57.0%)	
Symptom status[2]					
Asymptomatic	143 (65.3%)	30 (81.1%)	30 (58.8%)	83 (63.4%)	0.073
Symptomatic	76 (34.7%)	7 (18.9%)	21 (41.2%)	48 (36.6%)	
Duration of shedding (days, median [IQR]	13.0 [1.0-23.0]	8.0 [1.0-21.0]	12.0 [1.0-23.0]	15.0 [1.0-25.5]	0.440
Minimum Ct value (median [IQR])	26.0 [22.0-30.0]	26.0 [23.0-31.0]	24.5 [22.0-30.0]	25.5 [22.0-30.0]	0.358
Among symptomatic infections (excluding co-infections)[3]					
Symptom duration (days, median [IQR])	3 [2, 5]	2 [2, 5]	4 [2, 5]	2 [2, 4]	0.200
Symptom type					
Diarrhea	59 (77.6%)	5 (71.4%)	20 (95.2%)	34 (70.8%)	0.075
Vomiting	64 (84.2%)	4 (57.1%)	18 (85.7%)	42 (87.5%)	0.117
Fever[4]	23 (31.5%)	3 (50.0%)	5 (23.8%)	15 (32.6%)	0.460
Diarrhea					
Maximum episodes in 24 hours (median [IQR])	4.0 [3.0-5.0]	3.0 [3.0-4.0]	4.5 [1.3-6.0]	4.0 [3.0-5.0]	0.946
Duration (days, median [IQR])	3.0 [2.0-4.3]	1.5 [1.0-2.0]	3.5 [2.0-5,3]	3.0 [2.0-4.3]	0.097
Vomiting					

Maximum episodes in 24 hours (median [IQR])	3.0 [2.0-5.0]	3.0 [2.5-4.5]	3.0 [2.0-5.0]	3.0 [2.0-4.8]	0.910
Duration (days, median [IQR])	1.0 [1.0-2.0]	1.0 [1.0-1.5]	2.0 [1.0-3.0]	1.0 [1.0-2.0]	**0.048**
Illness severity (MVS)[5]					
Mild (score 0-8)	67 (88.2%)	6 (85.7%)	16 (76.2%)	45 (93.8%)	0.221
Moderate (score 9-10)	5 (6.6%)	1 (14.3%)	3 (14.3%)	1 (2.1%)	
Severe (score ≥11)	4 (5.3%)	0 (0%)	2 (9.5%)	2 (4.2%)	
Care seeking					
Home care	51 (67.1%)	6 (85.7%)	12 (57.1%)	33 (68.8%)	0.167
Outpatient	15 (19.7%)	0 (0.0%)	3 (14.3%)	12 (25.0%)	
ED/Hospital	10 (13.2%)	1 (14.3%)	6 (28.6%)	3 (6.3%)	

Abbreviations: IQR, interquartile range; ED, emergency department; MVS, modified Vesikari score

[1] Comparing distributions across genogroups

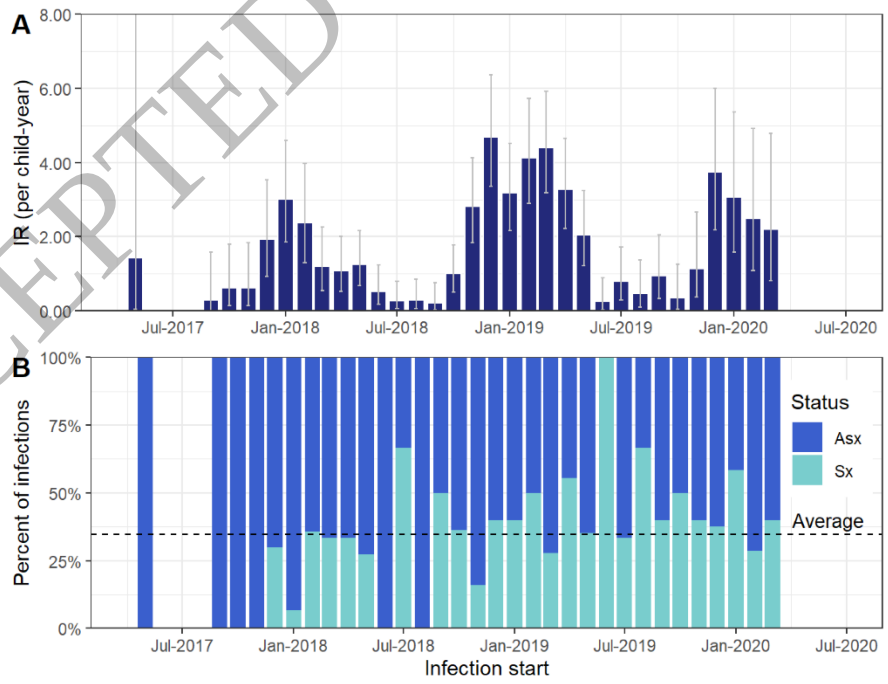
[2] Excluding coinfections (n=18) and the 16 infections with unknown symptom status (5 GI, 4 GII.4 Sydney, and 7 other GII infections). Symptom status was unknown if data were not available for the weeks leading up to or following the start of the infection or if symptoms that occurred within the 14-day window were attributed to another pathogen.

[3] Statistics in subsequent rows are calculated among symptomatic infections only and exclude co-infections.

[4] Data on fever was missing for 3 infections.

[5] Calculated using a modified Vesikari score incorporating diarrhea duration and number of episodes, vomiting duration and number of episodes, fever, behavioral signs, and treatment.

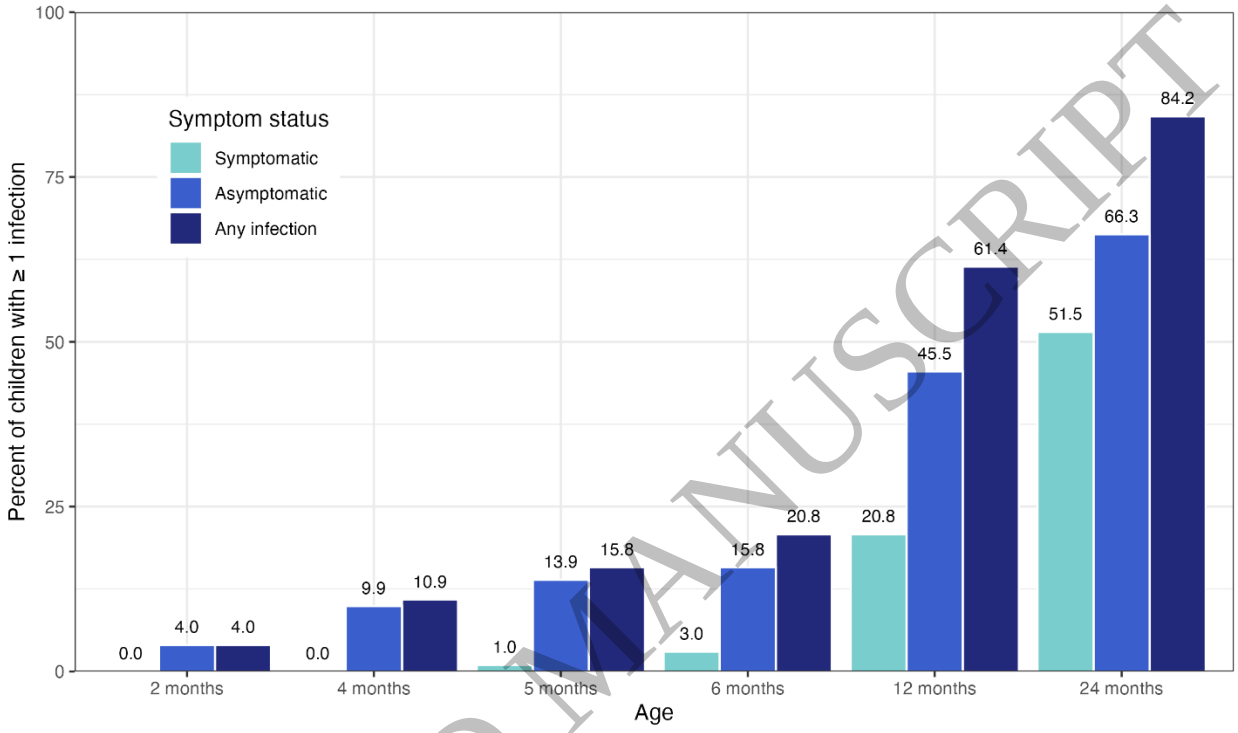
Figure 1. Norovirus incidence rate and 95% confidence interval (error bars) among all 245 children enrolled in the PREVAIL cohort (A) and infection symptomatic status[1] (B) by month.



Abbreviations: IR, incidence rate; Asx, asymptomatic; Sx, symptomatic

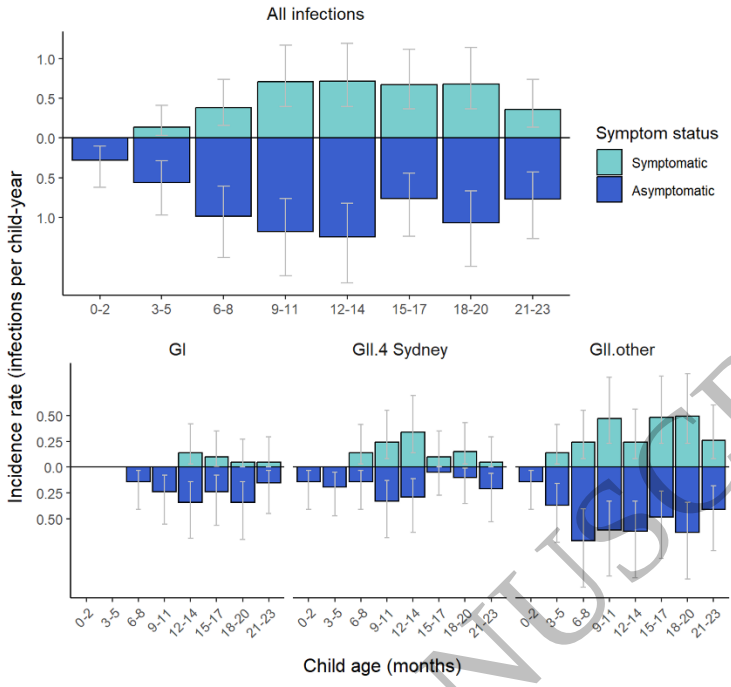
[1] Excludes infections with unknown symptom status and mixed infections

Figure 2. Percentage[1] of 101 adherent children experiencing one or more norovirus infections by age and symptomatic status in the PREVAIL cohort.



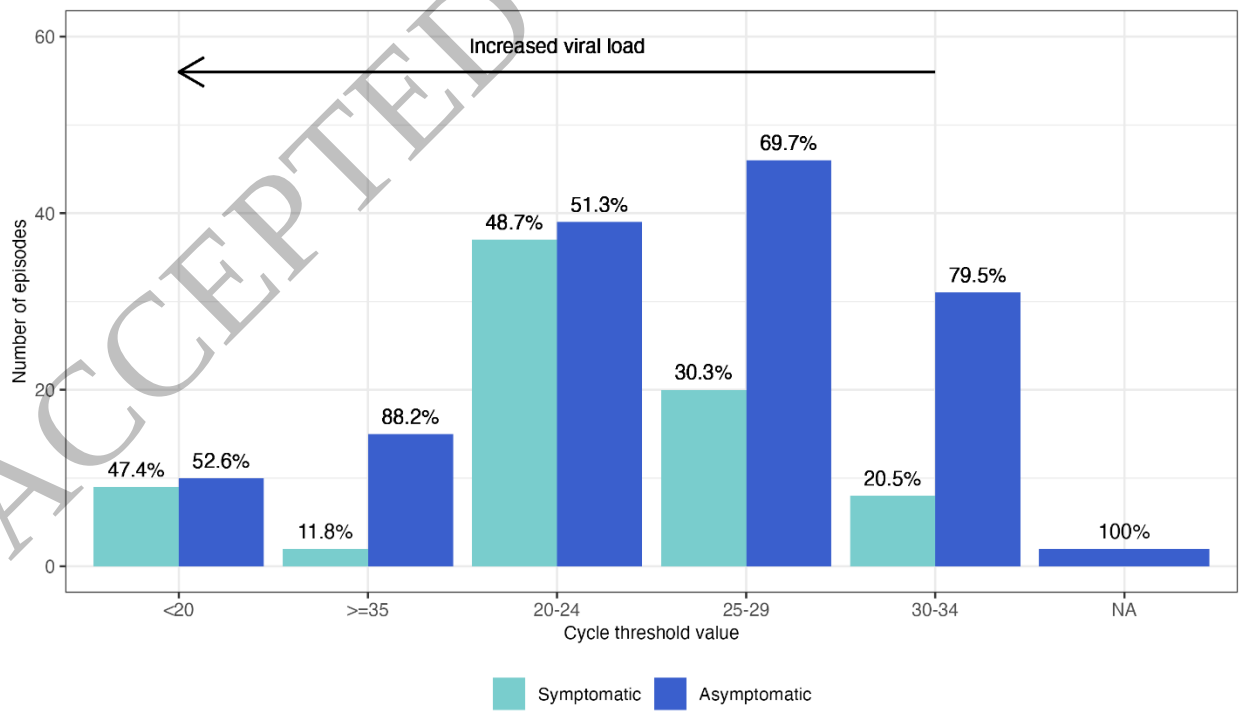
[1] The sum of symptomatic and asymptomatic percentages may not equal the any infection percentage. Symptomatic and asymptomatic infection percentages exclude co-infections and infections with unknown symptom status. All infection percentages include co-infections and those with unknown symptom status. Additionally, children may have had both a symptomatic and asymptomatic infection by a given age and would be included in both categories.

Figure 3. Norovirus infection incidence rate[1] by symptomatic status per child-year and 95% confidence intervals (error bars) among 101 adherent children in the PREVAIL cohort



[1]Excludes co-infections and norovirus infections with unknown symptom status.

Figure 4. Minimum cycle-threshold value per infection stratified by symptomatic status[1] among 101 adherent children in the PREVAIL cohort.



[1]Excludes co-infections and norovirus infections with unknown symptom status.

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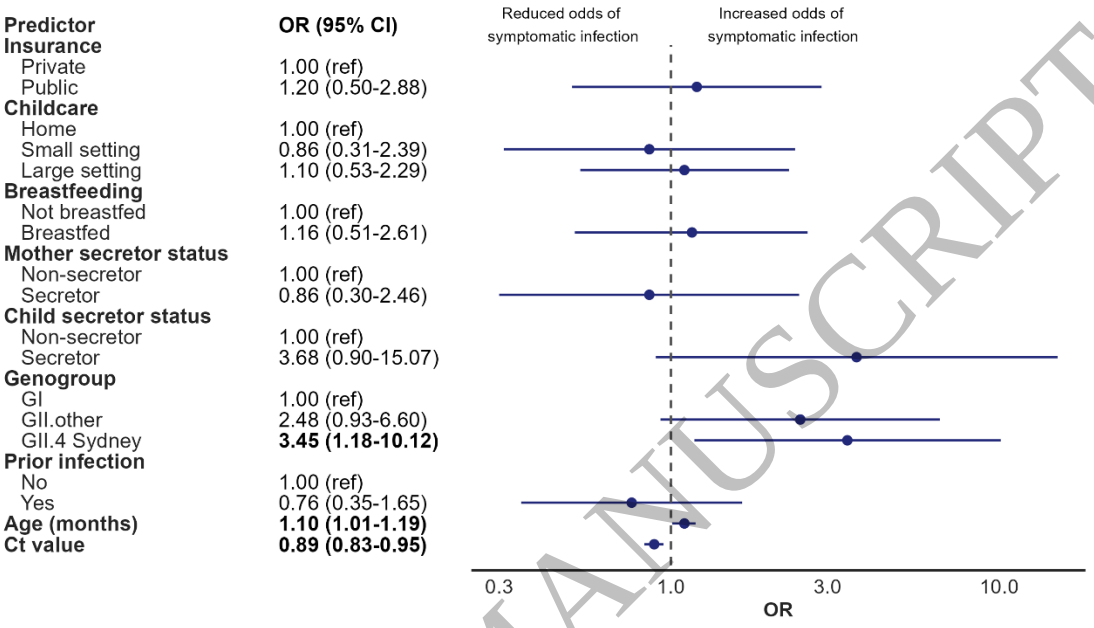
Figure 5. Adjusted generalized estimating equation model results describing predictors of norovirus infection symptomatic status[1] (n=212) among adherent children in the PREVAIL cohort.



Abbreviations: OR, odds ratio; CI, confidence interval; sx, symptoms

[1] Excludes co-infections and norovirus infections with unknown symptom status.

[2] Definitions: Childcare: Childcare was considered at home if the child was in their own home or another's home with no other children; small childcare centers are those with 5 or fewer other children; large childcare centers are those with more than 5 other children. Breastfeeding: Describes whether the child was breastfed at the time of infection. Mother/child secretor status: Refers to *FUT2* status of the mother/child. *FUT2* genotype was categorized based on detection of a 428 G > A point mutation. Individuals with the point mutation in both *FUT2* alleles (AA) were classified as non-secretors. Individuals with GG or GA genotypes were classified as secretors.

Genogroup: Genogroup was stratified into GI, non-GII.4 strains of GII, and GII.4.

Prior infection: Whether the child had at least one norovirus infection prior to the current infection.

Ct value: Minimum cycle threshold value identified among stool samples collected during the norovirus episode. OR represents the relationship between symptom status and a one unit increase in Ct value.