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MAJOR ARTICLE

The Epidemiology of Sapovirus in the Etiology, Risk Factors, and Interactions of Enteric Infection and Malnutrition and the Consequences for Child Health and Development Study: Evidence of Protection Following Natural Infection

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Background. Sapovirus is one of the principal agents of acute viral enteritis in children. Because it has not been routinely included in diagnostic evaluations, the epidemiology and natural history remain poorly described.

Methods. A birth cohort of 1715 children from 8 countries contributed surveillance samples (n = 35 620) and diarrheal specimens (n = 6868) from 0 to 24 months of age. Sapovirus was detected by quantitative polymerase chain reaction concurrently to other enteropathogens using multiarray cards. Logistic regression was used to identify risk factors, and longitudinal models were employed to estimate incidence rates and evaluate evidence of protective immunity.

Results. Sapovirus was detected in 24.7% (n = 1665) of diarrheal stools and 12.8% (n = 4429) of monthly surveillance samples. More than 90% of children were infected and 60% experienced sapovirus diarrhea in the first 2 years of life. Breastfeeding and higher socioeconomic status were associated with reduced incidence of infection and illness. Specimens with sapovirus detected had an increased odds of coinfection with rotavirus (odds ratio [OR], 1.6 [95% confidence interval {CI}, 1.3–2.0]), astrovirus (OR, 1.5 [95% CI, 1.3–1.7]), adenovirus (OR, 1.3 [95% CI, 1.1–1.5]), and *Shigella* (OR, 1.4 [95% CI, 1.3–1.6]). Prior infection with sapovirus conferred a risk reduction of 22% for subsequent infection (hazard ratio [HR], 0.78 [95% CI, .74–.85]) and 24% for subsequent diarrhea (95% CI, 11.0%–35.0%; HR, 0.76).

Conclusions. Sapovirus is a common cause of early childhood diarrhea. Further research on coinfections is warranted. Evidence of acquired immunity was observed even in the absence of genotype-specific analysis for this pathogen of known genetic diversity.

Keywords. sapovirus; diarrhea; coinfections; immunity.

Diarrhea remains a leading cause of death and long-term disability around the world [1]. Since the development and global deployment of rotavirus vaccines, a growing proportion of epidemic and endemic gastroenteritis burden has been attributed to the viruses of the Caliciviridae family, including noroviruses and sapoviruses [2–5]. While noroviruses have received comparably greater attention, advances in diagnostic assays have facilitated increased understanding of sapovirus epidemiology and genetic variation in recent years [6, 7]. Sapoviruses are single-stranded RNA viruses first identified in humans in 1976 by electron microscopy [8] and soon after classified as an etiologic cause of acute gastroenteritis internationally [9, 10].

The Etiology, Risk Factors, and Interactions of Enteric Infection and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, an 8-site multiyear birth cohort study, demonstrated that 10 pathogens are

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responsible for 97.5% of diarrhea in children living in low- and middle-income countries (LMICs) [11, 12]. Sapovirus was second to *Shigella* only in incident cases of acute diarrhea in this study [13], with an attributed incidence of 22.8 (95% confidence interval [CI], 18.9–27.5) cases per 100-child years. This high rate of illness is surprising as relatively little work has been done on the epidemiology and clinical severity of sapovirus in the community setting.

These findings highlight sapoviruses as enteropathogens of greater public health significance than previously recognized. Longitudinal, community-based studies are needed to describe the natural history and potential protective immunity of sapovirus infection and clinical disease, particularly in early life where the majority of enteric mortality and morbidity occurs. We examine the epidemiology of sapovirus, the burden of infection, and clinical characteristics of sapovirus diarrhea among children 0–24 months of age. The longitudinal design and large scope of the study also permit the evaluation of the protective effect of prior infection on subsequent risk of infection, a notable gap in research on sapovirus to date.

MATERIALS AND METHODS

Study Population

MAL-ED was concurrently conducted in 8 countries (Bangladesh, Brazil, Pakistan, Peru, South Africa, Tanzania, Nepal, and India) under a common protocol [14]. Singleton infants weighing >1500 g were enrolled within 17 days of birth and visited twice weekly over the first 24 months of life to create a complete daily surveillance history for symptomatic illness. All subjects were enrolled with informed parental permission under national and international institutional review board approvals. Enrollment and samples for this analysis were collected between November 2009 and February 2012. Inclusion in this analysis was limited to children completing the 24 months of surveillance.

Stool Collection and Processing

Surveillance and diagnostic techniques have been extensively described [13, 15, 16]. Stool was collected monthly during the first year of the study and quarterly during the second year. Additional samples were collected when diarrhea was detected by twice-weekly active surveillance. Stool samples were available for 95.6% of episodes detected by surveillance. Stool was aliquoted and frozen at −70°C pending analysis. Nucleic acids were extracted from 200 mg of stool using the QIAamp Fast stool kit (Qiagen), with a 2-minute bead beating step. As extrinsic controls, 10^6 copies of phocine virus herpesvirus (gift from Martin Shutten, Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands) and 10^7 MS2 bacteriophage per sample were spiked to the buffers to monitor the efficiency of extraction and the presence of inhibitors of amplification. Equal volumes (20 µL) of DNA and RNA extracts were added to 50 µL of buffer and 4 µL enzyme mix from the Ag-Path-ID One Step reverse-transcription polymerase chain reaction (PCR) kit and 6 µL of water for a reaction volume of 100 µL. The complete list of primer sets employed for the detection of 40 enteropathogens can be found elsewhere [17–19]. Primers selected for sapovirus-targeted RNA-dependent RNA polymerase, precise primer sequences are previously published for this and other pathogens [11].

Specimens were mixed and added to array card and amplified in a ViiA7 instrument (Life Technologies). Cycling conditions were as follows: 45°C for 20 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The cycle for a positive assay was 35 in runs in which intrinsic controls of amplification and template-free negative controls yielded valid results.

Data Descriptions and Definitions

Birthdate, sex, weight, anthropometrics, and demographic data were collected at birth. Anthropometric data were acquired monthly within 48 hours of the date of birth of the participant and normalized using 2016 World Health Organization normative references [20]. Children were categorized as exclusively breastfed from birth until intake of any liquid other than breastmilk, medicine, or vitamins; as receiving a mixed diet from when exclusivity terminated until the breastfeeding was discontinued; and as weaned thereafter. Diarrhea severity was evaluated using the Community Diarrheal Assessment (CODA) score [21, 22], assigning scores of 1–15 based on maternal report of the presence and duration of fever, vomiting, anorexia, liquid stools, and maximum stool output in a 24-hour period during the defined episode. A wealth index comprised of access to improved water and sanitation, assets, maternal education, and income (WAMI) was used to measure socioeconomic status, as described elsewhere [23].

Statistical Analysis

Kaplan-Meier survival analysis was conducted to estimate time to first infection and sapovirus incidence per 100 child-months. Incidence rates of sapovirus infection and clinical disease were calculated allowing for multiple failures (infections or clinical episodes, respectively) per child. New episodes of infection or disease were separated from prior episodes by either a sapovirus-negative stool sample or a period of ≥14 days from prior detection.

A Cox proportional hazards model was used to generate hazard ratios (HRs) comparing risk of incident sapovirus infection among children with vs without prior exposure. To provide conservative estimates, we excluded infections occurring within 30 days of each other as possible episodes of persistent shedding, resulting in the removal of 1175 stools from the immunity analysis. Children contributed person-time to the "unexposed"

group from birth until their first infection, and to the "exposed" group thereafter. HRs were conducted using a Breslow method for ties and robust variance to account for within-child clustering of infections. The model accounted for within-child clustering (nonindependence) of infections using a robust variance estimator [24, 25].

Odds of sapovirus detection in diarrheal samples, compared to surveillance stools, was calculated using logistic regression to assess whether sapovirus was associated with diarrhea in each age group and country. Associations between demographic, socioeconomic, and anthropometric indicators, and the population-averaged odds of sapovirus infection and diarrhea, were explored using a generalized estimating equations approach to adjust for within-child correlations.

We used a Pearson χ^2 test to examine whether levels of coinfection with 9 of the other most prevalent pathogens (*Shigella*, rotavirus, adenovirus 40/41, enterotoxigenic *Escherichia coli* [ETEC], norovirus, astrovirus, *Campylobacter*, enteroaggregative *E. coli* [EAEC], and *Giardia*) were higher in sapovirus than in all samples in the cohort and to determine which species were associated with sapovirus at greater than expected rates if coinfections sorted randomly. To evaluate interaction between pathogens, we employed logistic regression to estimate the odds of detection of prevalent pathogens in diarrheal samples with and without concurrent sapovirus infection in a subsample of stools with at least 2 pathogens present.

RESULTS

Sapovirus Epidemiology in the First 2 Years of Life

A total of 1715 children contributed 42 488 stools (35 620 monthly surveillance stools and 6868 diarrheal samples) to the analysis. Valid determinations of sapovirus were available from 97.5% of available specimens (n = 41 408). The distribution of samples by age and site is detailed in Table 1. Across sites, sapovirus was detected in 12.8% (n = 4429) of monthly surveillance stools and 24.7% (n = 1665) of diarrheal samples from birth to 24 months.

Median time to first detection of sapovirus was 8 months of age. By 12 months of age, 75.0% of children had experienced infection, and by 24 months cumulative detection of sapovirus by PCR was nearly universal (93.6%) in surveillance stools. Incidence of infection showed a rapid increase between 3 and 6 months (Figure 1A and 1B; Table 1), with rates stabilizing at 16.0–18.4 episodes per 100 child-months between 6 and 18 months of age and then decreasing slightly to 14.0 (95% CI, 13.3–14.7) episodes per 100 child-months between 18 and 24 months of age across sites (Table 1).

Table 1. Incidence of Sapovirus Infection and Symptomatic Illness in 8 Low- and Middle-Income Countries

Characteristic	No. of Children	Child-Months at Risk	Total No. of Surveillance Specimens	No. of Sapovirus Infections	Incidence Rate (95% CI) of Sapovirus Infection	Total No. of Diarrheal Specimens	No. of Sapovirus Diarrheal Episodes	Incidence Rate (95% CI) of Sapovirus Diarrhea
Total	1715	40 968.4	34 662	5677	13.9 (13.5–14.2)	6746	1600	3.9 (3.7–4.1)
Age group, mo								
0–5	1712	9365.7	7696	619	6.6 (6.1–7.2)	1642	173	1.8 (1.6–2.1)
6–11	1714	10 107.1	8402	1695	16.8 (16.0–17.6)	2079	551	5.5 (5.0–5.9)
12–17	1714	10 052.3	8672	1765	17.6 (16.8–18.4)	1680	512	5.1 (4.7–5.6)
18–24	1711	11 443.3	9892	1598	14.0 (13.3–14.7)	1345	364	3.2 (2.9–3.5)
Country								
Asia								
Bangladesh	210	5029.2	4317	1064	21.2 (19.9–22.5)	1384	398	7.9 (7.2–8.7)
India	227	5422.5	4774	795	14.7 (13.7–15.7)	640	171	3.2 (2.7–3.7)
Nepal	227	5445.4	5047	666	12.2 (11.3–13.2)	911	178	3.3 (2.8–3.8)
Pakistan	246	5881.2	4638	1071	18.2 (17.2–19.3)	1841	378	6.4 (5.8–7.1)
Africa								
South Africa	237	5637.2	4585	517	9.2 (8.4–10.0)	119	19	0.3 (.2–.5)
Tanzania	209	4996.2	4243	482	9.6 (8.8–10.5)	159	33	0.7 (.5–.9)
South America								
Brazil	165	3893.3	2844	132	3.4 (2.9–4.0)	91	11	0.3 (.2–.5)
Peru	194	4663.5	4214	950	20.4 (19.1–21.7)	1601	412	8.8 (8.0–9.7)

Individuals, samples, person-time, and incidence rate of sapovirus diarrhea and infection are expressed as number of detections per 100 child-months, shown by age group and site. All detections, whether asymptomatic or diarrheal, were considered episodes for calculation of incidence rates for infection. Only episodes of diarrhea with sapovirus detected were included as events for calculation of incidence rates for diarrhea. New episodes of infection were defined as separated from prior episodes by either sapovirus-negative stool samples or a period of ≥14 days from prior sapovirus-positive stools.

Abbreviation: CI, confidence interval.

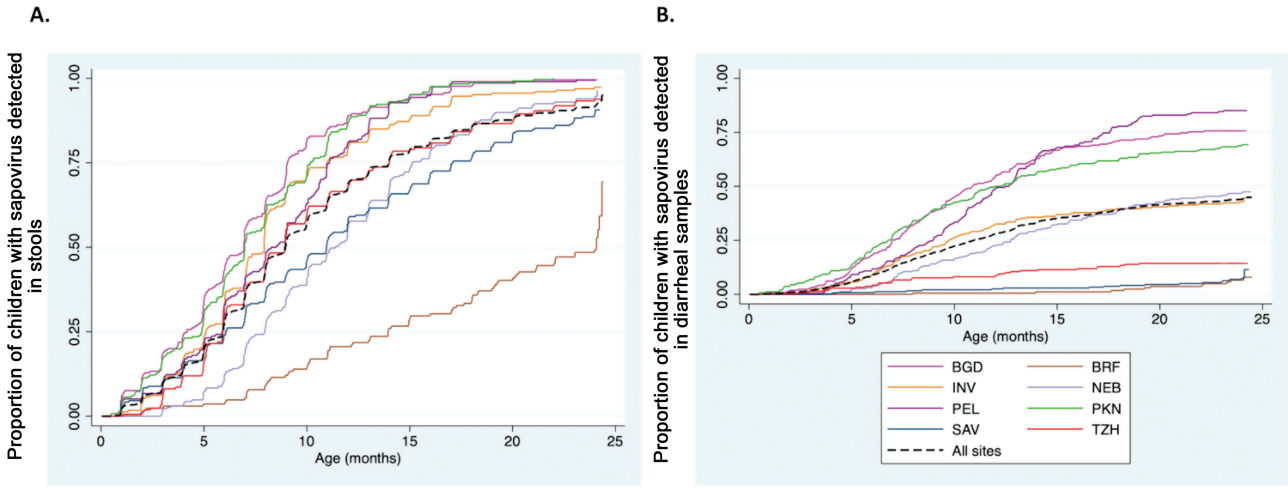

Figure 1. Time to first sapovirus infection and symptomatic episode in 8 low- and middle-income countries. Detection of sapovirus in stools (*A*) and diarrhea samples (*B*) increases rapidly between 3 and 6 months of age. Median time to first infection was 8 months, and by 24 months 94% of children had been infected. Sapovirus diarrhea was experienced by half of the cohort with approximately equal proportions of disease in the first and second years of life. Abbreviations: BDG, Bangladesh—Dhaka; BRF, Brazil—Fortaleza; INV, India—Vellore; NEB, Nepal—Bhaktapur; PEL, Peru—Loreto; PKN, Pakistan—Naushero Feroze; SAV, South Africa—Venda; TZH, Tanzania—Haydom.

By 12 months, 35.6% of children had experienced at least 1 episode of clinical diarrhea with sapovirus detected; this rose to 49.9% by 24 months of age. Age-specific incidence rates of sapovirus diarrhea demonstrated a similar distribution, increasing from 1.8 (95% CI, 1.6–2.1) episodes per 100 child-months between 0 and 5 months of age to a table peak of 4.7–5.9 episodes between 6 and 17 months of age, followed by a decline to 3.2 (95% CI, 2.9–3.5) episodes of sapovirus diarrhea per 100 child-months in children 18–24 months of age. Detection of sapovirus was more frequent in diarrheal samples than surveillance stools; association of sapovirus and clinical diarrhea was statistically significant across all age groups except for 0–2 months (Figure 2A), and across all study sites with the exception of South Africa (Figure 2B). Neither the mean duration (4.6 days) nor severity (CODA score 3.0) of sapovirus diarrhea significantly differed from all-cause diarrhea (4.9 days; CODA score 2.9).

Incidence of sapovirus diarrhea was heterogenous across sites, with children in Peru and Bangladesh experiencing 8 or more episodes per 100 child-months, compared to <0.5 episodes in the same time period in Brazil or South Africa. These trends were proportional to the frequency of all-cause diarrhea across sites (Figure 3). Notably, in sites with the lowest levels of sapovirus detection, there were disparate trends in

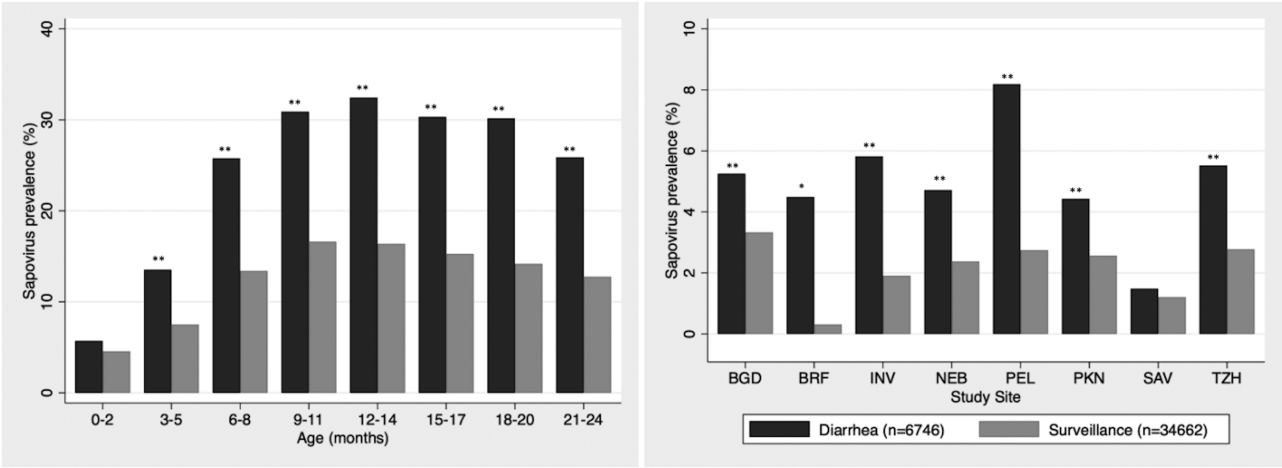

Figure 2. Sapovirus detection in surveillance (asymptomatic) and diarrheal stool samples among children 0–24 months of age in 8 low- and middle-income countries. Prevalence of sapovirus in stools from children with diarrhea or from surveillance stools by age (*A*) and study site (*B*) in 8 low- and middle-income countries worldwide. Sapovirus is consistently associated with diarrhea throughout early childhood. *$P < .01$; **$P < .001$. Abbreviations: BDG, Bangladesh—Dhaka; BRF, Brazil—Fortaleza; INV, India—Vellore; NEB, Nepal—Bhaktapur; PEL, Peru—Loreto; PKN, Pakistan—Naushero Feroze; SAV, South Africa—Venda; TZH, Tanzania—Haydom.

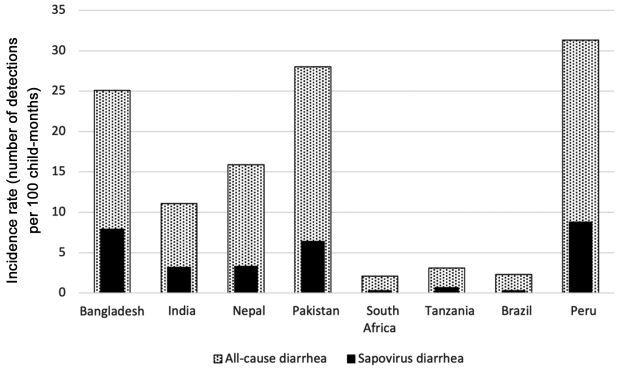

Figure 3. Incidence of sapovirus diarrhea and all-cause diarrhea among children 0–24 months of age in Etiology, Risk Factors, and Interactions of Enteric Infection and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study sites in 8 low- and middle-income countries.

the relationship between infection and symptomatic illness: in Brazil, detection occurred nearly exclusively in children with diarrhea, whereas in South Africa, frequency of detection was more evenly distributed between diarrheal and surveillance samples (Figure 2B).

The mean duration of exclusive breastfeeding and weaning was 53.8 and 511.1 days, respectively. Children in the cohort underwent progressive linear growth shortfalls: mean enrollment length-for-age *z*-score was −1.1, which fell to −1.4 at 12 months and −1.7 at 24 months. The average weight-for-age *z*-score at enrollment was −1.2, which increased to −0.9 at 12 months before dipping again to −1.1 at 24 months. The majority of children (85.5%) had access to improved drinking water at baseline, though only 21.2% had access to improved sanitation. Just under half (44.8%) had caregivers who had completed a primary education. Mean WAMI score was 0.55, ranging from 0.21 in Tanzania to 0.83 in Brazil. After adjustment for age and site, odds of sapovirus infection and diarrhea was significantly higher among children who received mixed feeding (infection OR, 1.7, $P < .001$; diarrhea OR, 2.9, $P < .001$) or were fully weaned (infection OR, 1.6, $P < .001$; diarrhea OR, 2.6, $P = .001$) before their second birthday, and significantly reduced per unit increase in WAMI score (infection OR, 0.27, $P = .001$; diarrheal OR, 0.61, $P = .023$) (Table 2).

Association With Other Enteric Infections

Detection of multiple enteropathogens in a single stool was noted in 38.2% of stools. To examine coinfection with sapovirus in the cohort, we compared the prevalence of 9 common pathogens (EAEC, *Giardia*, *Campylobacter*, norovirus, adenovirus, astrovirus, *Shigella*, ETEC, and rotavirus) in sapovirus-positive stools and all stools, and observed a higher than expected prevalence of every pathogen examined except EAEC in stools with a concurrent sapovirus infection (Table 3). The odds of detecting each pathogen was then compared between the sapovirus-positive vs -negative stools, among a subsample of coinfected

Table 2. Factors Associated With Sapovirus Infection and Symptomatic Illness in 8 Low- and Middle-Income Countries

	Sapovirus Infection			Sapovirus Diarrhea		
Characteristic	OR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value
Age (mo)	1.03	(1.02–1.03)	.000	1.01	(1.00–1.02)	.039
Site						
Bangladesh	Ref			Ref		
India	0.74	(.66–.82)	.000	0.44	(.35–.56)	.000
Nepal	0.56	(.50–.63)	.000	0.46	(.37–.57)	.000
Pakistan	0.83	(.75–.92)	.000	0.83	(.69–.99)	.040
South Africa	0.60	(.53–.68)	.000	0.06	(.04–.11)	.000
Tanzania	0.47	(.41–.54)	.000	0.09	(.06–.15)	.000
Brazil	0.23	(.19–.28)	.000	0.07	(.03–.13)	.000
Peru	0.80	(.72–.89)	.000	0.90	(.74–1.09)	.268
Breastfeeding category						
Exclusive	Ref			Ref		
Mixed	2.80	(2.24–3.51)	.000	5.54	(3.19–9.61)	.000
Weaned	2.45	(1.92–3.13)	.000	4.33	(2.41–7.80)	.000
Anthropometrics						
LAZ	1.00	(.96–1.04)	.876	1.00	(.92–1.08)	.931
WAZ	0.99	(.96–1.03)	.666	0.99	(.92–1.07)	.885
Socioeconomic status						
WAMI score	0.72	(.58–.88)	.001	0.61	(.41–.93)	.023

Adjusted ORs of experiencing sapovirus infection and diarrhea among children 0–24 months of age by age, geographic location, diet, nutritional indicators, and socioeconomic status.

Abbreviations: CI, confidence interval; LAZ, length-for-age *z*-score; OR, odds ratio; WAMI, water and sanitation, assets, maternal education, and income; WAZ, weight-for-age *z*-score.

Table 3. Prevalence of Coinfections With Sapovirus and Other Enteropathogens in 8 Low- and Middle-Income Countries

	Prevalence of Coinfections			Odds of Detecting Each Pathogen in Sapovirus-Positive (n = 2235) vs Sapovirus-Negative (n = 13 601) Stools With ≥2 Pathogens Present		
Pathogen	All Stools (n = 41 408)	Sapovirus-Positive Stools (n = 6094)	χ^2 *P* Value	Adjusted OR	(95% CI)	*P* Value
Adenovirus	5977 (14.5)	1247 (20.8)	<.001	1.3	(1.1–1.5)	<.001
Astrovirus	4988 (12.1)	1201 (20.1)	<.001	1.5	(1.3–1.7)	<.001
Campylobacter	9321 (31.8)	1833 (41.6)	<.001	1.1	(.9–1.3)	.479
Giardia	13 515 (32.9)	2386 (39.9)	<.001	1.2	(1.0–1.3)	.022
Norovirus	6917 (16.8)	1096 (18.3)	.001	0.9	(.8–1.0)	.206
Rotavirus	2223 (5.4)	497 (8.3)	<.001	1.6	(1.3–2.0)	<.001
Shigella	4659 (11.3)	974 (16.2)	<.001	1.4	(1.3–1.6)	<.001
ETEC	2337 (8.0)	422 (9.6)	<.001	1.0	(.8–1.2)	.800
EAEC	8103 (27.7)	1227 (28.0)	.618	1.1	(.9–1.3)	.437

Prevalence of key pathogens was higher than expected in sapovirus-positive samples, relative to all samples. After adjustment for site, age, WAMI (water and sanitation, assets, maternal education, and income) score, and breastfeeding, the odds of detecting adenovirus, astrovirus, *Campylobacter*, *Giardia*, rotavirus, and *Shigella* were greater in coinfected stool samples with sapovirus concurrently present, relative to those without sapovirus present.

Abbreviations: CI, confidence interval; EAEC, enteroaggregative *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; OR, odds ratio.

stools (stools with ≥2 pathogens present, n = 15 836). After adjustment for age, site, WAMI score, and breastfeeding category, the strongest apparent interaction was with rotavirus, which had 1.6 times the odds (OR, 1.6 [95% CI, 1.3–2.0]) of being detected in a coinfection with sapovirus present, relative to a coinfected stool without sapovirus present. Similarly, the excess odds of detecting concurrent astrovirus was 50% (OR, 1.5 [95% CI, 1.3–1.7]), *Shigella* 40% (OR, 1.4 [95% CI, 1.3–1.6]), adenovirus 30% (OR, 1.3 [95% CI, 1.1–1.5]), and *Giardia* 20% (OR, 1.2 [95% CI, 1.0–1.3]).

Protection Following Naturally Acquired Infection

In a model (n = 40 233 stools) adjusted for site, early childhood feeding (breastfeeding category), and WAMI score, we observed evidence of protection following infection. After removal of stools within the window of persistent shedding, 4919 total stools representing any sapovirus detection and 1153 representing sapovirus diarrheal episodes were included in models of protective immunity. Prior sapovirus infection was associated with decreased risk of subsequent sapovirus detection by 22% (95% CI, 16.0%–28.0%) and the risk of subsequent sapovirus diarrhea by 24% (95% CI, 11.0%–35.0%), relative to children with no prior sapovirus detection, after adjustment for site, feeding status, and WAMI score (Table 4). The number of prior detections also exhibited a dose-response association with subsequent infection and clinical disease. Children with 1 prior infection exhibited a 15% (95% CI, 9%–22%) decrease in subsequent infections, whereas those with ≥2 infections had a 31% (95% CI, 25%–37%) decrease, relative to those with no prior evidence of sapovirus detection. Those with 1 prior infection

Table 4. Evidence of Natural Immunity to Sapovirus Infection and Symptomatic Illness in 8 Low- and Middle-Income Countries

Exposure	Prior Detection	HR for Subsequent Infections	(95% CI)	*P* Value	HR for Subsequent Diarrhea	(95% CI)	*P* Value
Prior detection (any)	None	Ref			Ref		
	Any	0.78	(.72–.84)	<.001	0.76	(.65–.89)	.001
No. of prior detections (any)	0	Ref			Ref		
	1	0.85	(.78–.91)	<.001	0.83	(.71–.97)	.020
	≥2	0.69	(.63–.75)	<.001	0.67	(.56–.82)	<.001
Prior sapovirus diarrhea	None	Ref			Ref		
	Any	0.89	(.84–.95)	<.001	1.31	(1.15–1.49)	<.001
No. of prior sapovirus diarrhea episodes	0	Ref			Ref		
	1	0.90	(.84–.96)	.002	1.24	(1.07–1.44)	.004
	≥2	0.86	(.79–.94)	.001	1.35	(1.13–1.60)	.001

In an analysis of 4919 stools representing infections occurring at least 30 days apart, prior infection with sapovirus was noted to decrease the risk of subsequent infection and disease, after adjustment for site, early childhood feeding, and socioeconomic status. Children with prior sapovirus detection had a 22% lower risk of subsequent detection (95% CI, 16%–26%) and 24% lower risk of subsequent sapovirus diarrhea (95% CI, 11%–35%). Children who had ≥2 prior sapovirus detections had a 31% (95% CI, 25%–37%) decrease in their subsequent risk of sapovirus diarrhea.

Children with prior sapovirus diarrhea had an 11% lower risk of subsequent sapovirus detections (95% CI, 5%–16%), but a 31% increased risk of subsequent symptomatic (diarrheal) episodes (95% CI, 15%–49%). Children with ≥2 prior diarrheal episodes similarly had reduced risk of future infection (14% [95% CI, 6%–21%]) but increased risk of future symptomatic episodes (35% [95% CI, 13%–60%]).

Abbreviations: CI, confidence interval; HR, hazard ratio.

had a 17% decrease (95% CI, 3%–29%), and those with ≥2 prior infections had a 33% (95% CI, 8.0%–44%) decrease in episodes of subsequent diarrhea, compared to children with no prior sapovirus detections. Although experiencing prior sapovirus diarrhea (symptomatic episodes) was also associated with a reduction in future incidence of any infection (11% [95% CI, 5%–16%]), a 31% increased risk of subsequent symptomatic (diarrheal) episodes (95% CI, 7%–44%) was observed among these children relative to children without sapovirus diarrhea. A similar dose response was noted, with 1 prior episode associated with a 24% increased risk (95% CI, 7%–44%) and 2 prior diarrheal episodes with a 35% increase (95% CI, 13%–60%) in risk of future diarrheal episodes relative to children who had not experienced sapovirus diarrhea.

No changes to results were noted when a sensitivity analysis was run excluding low-incidence sites (Brazil, Tanzania, South Africa).

DISCUSSION

Reports of sapovirus burden and contribution to disease in LMICs remain limited [26, 27]. Here we report on the incidence of disease in 8 countries in a multisite birth cohort of 1715 children and >40 000 stool samples. The study found that sapovirus was one of the principal enteropathogens identified in samples from children with diarrhea in a follow-up study using TaqMan array cards, which employed detection targets not included in the diagnostic algorithm of the initial microbiologic protocol.

One of the most notable findings that emerged was the heterogeneity in sapovirus detection across 8 countries with a high burden of diarrheal disease and growth faltering in early life. Greater duration of breastfeeding was significantly associated with reduced incidence during a critical period for child growth and survival. However, these risk factors do not explain the 20-fold difference in incidence rates of sapovirus diarrhea observed in Tanzania and South Africa as compared to Peru and Bangladesh when examined under a common protocol. Differences in symptomatic disease were more pronounced than in asymptomatic carriage between the high-sapovirus and low-sapovirus sites, and interestingly, the proportion of infections that were associated with symptoms differed vastly at sites with lowest overall detection. No other pathogens examined in the MAL-ED study exhibited this level of intersite heterogeneity, and environmental and host-level factors that may explain this finding merit further research. Innate resistance to calicivirus infection has been previously demonstrated with select genogroups of rotavirus and norovirus, but in this cohort neither sapovirus infection or diarrhea was associated with secretor or Lewis status of the child or the mother [28]. Another innate factor that may modulate the pathogenicity of sapoviruses in these contexts is differences in the gut microbiota, whose differential composition has been shown to either potentiate or resist colonization by different enteropathogens [29]. Enteropathogen-induced changes on commensal gut microbiota are beginning to be characterized for such diverse pathogens as norovirus [30, 31] and *Campylobacter* [32] and can demonstrate select changes in microbiota that may alter host susceptibility to other infections.

The occurrence of multiple pathogen coinfections is well-documented, but remains poorly understood. In this study we observed coinfections with ≥2 enteropathogens in more than a third of all samples. The potential of microbe–microbe interactions is broadly recognized, but the nature and impact of these interactions are only beginning to be elucidated. We detected evidence of increased prevalence and odds of coinfection with several other enteropathogens in sapovirus-positive stools, a finding that has not been replicated in other analyses of coinfections in the same cohort [32, 33]. Viral coinfections have been hypothesized to be associated with community-acquired *Clostridioides difficile* infection [34]. Interactions between enteric viral infections [35] are less well described, a natural consequence of the less advanced characterization of the human virome. We noted in this study that the odds of sapovirus coinfection were greatest for rotavirus. Given this observation, it is notable that following the introduction of rotavirus vaccination, no changes in sapovirus infections were noted [36]; however, given the heterogeneity in sapovirus incidence observed, extrapolation to other settings based on observations at 1 site should cautioned against.

Incidence estimates of community-based sapovirus infection and diarrhea are comparable, though higher than prior estimates in similar settings [26, 27]. A study conducted in Peru found similar rates of infection and diarrhea in the first and second year of life and recorded few cases of infection with the same genotype among children, prompting authors to suggest that this was early evidence of protective immunity [26]. Here we present findings that demonstrate evidence of protective immunity following natural infection. Prior detection decreased the risk of subsequent detection in stool by 22% and symptomatic diarrhea by 24%. We observed evidence of dose-related protection, as children with 1 prior detection experienced a 15% reduction in risk, whereas those with multiple prior detections experienced a 31% reduction in risk. The relative low level of protection observed may be a result of the large genetic diversity among sapoviruses. The finding that children with sapovirus diarrhea, despite this protection, were more likely to subsequently experience sapovirus diarrhea suggests that there are factors of host vulnerability to illness that are yet to be described, though they are not explained by anthropometric indices of undernutrition, breastfeeding, or histo-blood group antigens, which have been examined in this cohort previously [28]. Nevertheless, demonstration of protection points to the value of genotyping sapovirus infections across epidemiologic settings in order to guide vaccine development.

The global distribution and burden of disease combined with the expanding technologies that allow combinatorial vaccines from different viral pathogens may allow for the inclusion of sapovirus in future vaccines to better control acute gastroenteritis in children [37].

Notes

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References

1. Kotloff KL. The burden and etiology of diarrheal illness in developing countries. Pediatr Clin North Am **2017**; 64:799–814.
2. Becker-Dreps S, Bucardo F, Vilchez S, et al. Etiology of childhood diarrhea after rotavirus vaccine introduction: a prospective, population-based study in Nicaragua. Pediatr Infect Dis J **2014**; 33:1156–63.
3. Hemming M, Räsänen S, Huhti L, Paloniemi M, Salminen M, Vesikari T. Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of RotaTeq vaccine into the national immunization programme in Finland. Eur J Pediatr **2013**; 172:739–46.
4. Pitkänen O, Vesikari T, Hemming-Harlo M. The role of the sapovirus infection increased in gastroenteritis after national immunisation was introduced. Acta Paediatr **2019**; 108:1338–44.
5. Bucardo F, Reyes Y, Svensson L, Nordgren J. Predominance of norovirus and sapovirus in Nicaragua after implementation of universal rotavirus vaccination. PLoS One **2014**; 9:e98201.
6. Oka T, Katayama K, Hansman GS, et al. Detection of human sapovirus by real-time reverse transcription-polymerase chain reaction. J Med Virol **2006**; 78:1347–53.
7. Oka T, Wang Q, Katayama K, Saif LJ. Comprehensive review of human sapoviruses. Clin Microbiol Rev **2015**; 28:32–53.
8. Madeley CR, Cosgrove BP. Letter: caliciviruses in man. Lancet **1976**; 1:199–200.
9. McSwiggan DA, Cubitt D, Moore W. Calicivirus associated with winter vomiting disease. Lancet **1978**; 1:1215.
10. Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home. J Med Virol **1979**; 4:249–54.
11. Liu J, Gratz J, Amour C, et al. Optimization of quantitative PCR methods for enteropathogen detection. PLoS One **2016**; 11:e0158199.
12. Platts-Mills JA, Babji S, Bodhidatta L, et al; MAL-ED Network Investigators. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health **2015**; 3:e564–75.
13. Platts-Mills JA, Liu J, Rogawski ET, et al; MAL-ED Network Investigators. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. Lancet Glob Health **2018**; 6:e1309–18.
14. MAL-ED Network Investigators. The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. Clin Infect Dis **2014**; 59(Suppl 4):S193–206.
15. Houpt E, Gratz J, Kosek M, et al; MAL-ED Network Investigators. Microbiologic methods utilized in the MAL-ED cohort study. Clin Infect Dis **2014**; 59(Suppl 4):S225–32.
16. Richard SA, Barrett LJ, Guerrant RL, Checkley W, Miller MA; MAL-ED Network Investigators. Disease surveillance methods used in the 8-site MAL-ED cohort study. Clin Infect Dis **2014**; 59(Suppl 4):S220–4.
17. Liu J, Gratz J, Amour C, et al. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. J Clin Microbiol **2013**; 51:472–80.
18. Rogawski ET, Liu J, Platts-Mills JA, et al; MAL-ED Network Investigators. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. Lancet Glob Health **2018**; 6:e1319–28.
19. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet **2016**; 388:1291–301.
20. World Health Organization Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl **2006**; 450:76–85.
21. Lee G, Peñataro Yori P, Paredes Olortegui M, et al. An instrument for the assessment of diarrhoeal severity based on a longitudinal community-based study. BMJ Open **2014**; 4:e004816.
22. Lee GO, Richard SA, Kang G, et al; MAL-ED Network Investigators. A comparison of diarrheal severity scores in the MAL-ED multisite community-based cohort study. J Pediatr Gastroenterol Nutr **2016**; 63:466–73.
23. Psaki SR, Seidman JC, Miller M, et al; MAL-ED Network Investigators. Measuring socioeconomic status in multicountry studies: results from the eight-country MAL-ED study. Popul Health Metr **2014**; 12:8.
24. Huber PJ. The behavior of maximum likelihood estimates under nonstandard conditions. Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability. Berkeley: University of California Press, **1967**:221–33.
25. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica **1980**; 48:817–38.
26. Sánchez GJ, Mayta H, Pajuelo MJ, et al; Sapovirus Working Group. Epidemiology of sapovirus infections in a birth cohort in Peru. Clin Infect Dis **2018**; 66:1858–63.
27. Shioda K, Cosmas L, Audi A, et al. Population-based incidence rates of diarrheal disease associated with norovirus, sapovirus, and astrovirus in Kenya. PLoS One **2016**; 11:e0145943.
28. Colston JM, Francois R, Pisanic N, et al. Effects of child and maternal histo-blood group antigen status on symptomatic and asymptomatic enteric infections in early childhood. J Infect Dis **2019**; 220:151–62.
29. Schnupf P, Gaboriau-Routhiau V, Cerf-Bensussan N. Modulation of the gut microbiota to improve innate resistance. Curr Opin Immunol **2018**; 54:137–44.
30. Almand EA, Moore MD, Outlaw J, Jaykus LA. Human norovirus binding to select bacteria representative of the human gut microbiota. PLoS One **2017**; 12:e0173124.
31. Patin NV, Pena Gonzalez A, Hatt JK, et al. The role of the gut microbiome in resisting norovirus infection as revealed by a human challenge study. mBio **2020**; 11:e02634-20.
32. Rouhani S, Griffin NW, Yori PP, et al. Gut microbiota features associated with campylobacter burden and postnatal linear growth deficits in a Peruvian birth cohort. Clin Infect Dis **2020**; 71:1000–7.
33. Olortegui MP, Rouhani S, Yori PP, et al. Astrovirus infection and diarrhea in 8 countries. Pediatrics **2018**; 141:e20171326.
34. Jangi S, Lamont JT. Asymptomatic colonization by *Clostridium difficile* in infants: implications for disease in later life. J Pediatr Gastroenterol Nutr **2010**; 51:2–7.
35. Makimaa H, Ingle H, Baldridge MT. Enteric viral co-infections: pathogenesis and perspective. Viruses **2020**; 12:904.
36. Lambisia AW, Onchaga S, Murunga N, Lewa CS, Nyanjom SG, Agoti CN. Epidemiological trends of five common diarrhea-associated enteric viruses pre- and post-rotavirus vaccine introduction in coastal Kenya. Pathogens **2020**; 9:660.
37. Blazevic V, Lappalainen S, Nurminen K, Huhti L, Vesikari T. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. Vaccine **2011**; 29:8126–33.