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Naturally Acquired Immunity Against Rotavirus Infection and Gastroenteritis in Children: Paired Reanalyses of Birth Cohort Studies

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Background. Observational studies in socioeconomically distinct populations have yielded conflicting conclusions about the strength of naturally acquired immunity against rotavirus gastroenteritis (RVGE), mirroring vaccine underperformance in low-income countries. We revisited birth cohort studies to understand naturally acquired protection against rotavirus infection and RVGE.

Methods. We reanalyzed data from 200 Mexican and 373 Indian children followed from birth to 2 and 3 years of age, respectively. We reassessed protection against RVGE, decomposing the incidence rate into the rate of rotavirus infection and the risk of RVGE given infection, and tested for serum antibody correlates of protection using regression models.

Results. Risk for primary, secondary, and subsequent infections to cause RVGE decreased per log-month of age by 28% (95% confidence interval [CI], 12%–41%), 69% (95% CI, 30%–86%), and 64% (95% CI, –186% to 95%), respectively, in Mexico City, and by 10% (95% CI, –1% to 19%), 51% (95% CI, 41%–59%) and 67% (95% CI, 57%–75%), respectively, in Vellore. Elevated serum immunoglobulin A and immunoglobulin G titers were associated with partial protection against rotavirus infection. Associations between older age and reduced risk for RVGE or moderate-to-severe RVGE given infection persisted after controlling for antibody levels.

Conclusions. Dissimilar estimates of protection against RVGE may be due in part to age-related, antibody-independent risk for rotavirus infections to cause RVGE.

Keywords. rotavirus; gastroenteritis; immunity; natural infection; birth cohort.

Rotavirus gastroenteritis (RVGE) remains a leading cause of pediatric gastrointestinal disease burden globally [1], with approximately 10 million severe cases and 193 000 deaths annually among children <5 years old [2]. Live oral vaccines are being integrated into national immunization plans of developing countries following a decade of use in higher-income settings. Because immunity against rotavirus infection is imperfect, understanding how naturally acquired and vaccine-derived protection influences rotavirus epidemiology is critical for planning interventions and assessing vaccine impact [3–6].

Birth cohort studies have sought to measure naturally acquired immune protection against rotavirus among young children [7–11]. Under comparable designs, studies have yielded near-identical estimates of naturally acquired protection against rotavirus infection. However, protection against RVGE, and against moderate-to-severe RVGE in particular, has appeared to differ considerably. In a cohort of Mexican children, 2 infections seemed to confer complete protection against moderate-to-severe RVGE [9], while some children in Vellore, India, experienced severe disease following ≥3 infections [8]. These apparent differences in protection mirror variation in vaccine performance. Despite delivering >90% protection against moderate-to-severe RVGE in the United States and Europe [12], estimated protection has ranged from 81% in middle-income Latin American countries, including Mexico [13], to 39%–48% in low-income African and Asian settings [14, 15].

Several hypotheses have been proposed to account for differences in the epidemiology of diarrheal pathogens across settings. Nutrition-related immune deficiencies, host-genetic factors, and physiological damage to the gut from previous infections or chronic conditions may contribute to susceptibility and weak immune protection in low-resource, high-burden settings such as Vellore. Recent multicenter cohort studies have been undertaken to determine how these risk factors relate to diarrhea susceptibility

[16–18] and the performance of oral polio and rotavirus vaccines [19]. A question the earlier cohort studies can answer, however, is whether age-dependent host responses to rotavirus infection further confound direct comparison of estimates from studies undertaken in distinct settings. Children exposed to differing levels of rotavirus transmission are expected to acquire primary, secondary, and later infections at distinct ages, when they may experience differential risk for RVGE given infection [20].

We revisited 2 large-scale birth cohort studies undertaken in Mexico City, Mexico and Vellore, India, to understand why protection against RVGE appeared to differ between the settings despite similar levels of protection against rotavirus infection [8, 9]. In side-by-side analyses of the studies, we evaluated serum antibody concentrations and age as determinants of rates at which children acquired rotavirus infection, and as determinants of risk for rotavirus infections to cause RVGE.

METHODS

Birth Cohort Studies

The studies followed similar protocols [8, 9] aiming to identify all rotavirus infections among children during follow-up, and to characterize gastrointestinal symptoms associated with each infection. Children were enrolled at birth in slum communities and followed to ages 24 and 36 months in Mexico City and Vellore, respectively. Surveillance for rotavirus infection included scheduled testing of asymptomatic stool specimens for

rotavirus (weekly in Mexico City and biweekly in Vellore). Sera were drawn every 4 and 6 months in Mexico City and Vellore, respectively, to detect rises in anti-rotavirus immunoglobulin G (IgG) and immunoglobulin A (IgA) titers (Table 1). Both studies defined IgG seroconversion by a 4-fold rise in titers; the definition of IgA seroconversion (4-fold rise in Mexico City vs 3-fold rise in Vellore) accommodated antibody waning over the longer period between tests in India.

Field workers visited homes to collect diarrheal stool when mothers reported changes in children's stool pattern; clinical specimens were collected if children presented for care. Diarrhea episodes were defined as ≥3 watery stools in 24 hours (or ≥2 more than considered "normal" by the mothers), and ended the first day bowel movements returned to the usual daily pattern. Rotavirus infections were considered asymptomatic if no diarrhea occurred within 5 (in Mexico City) or 7 (in Vellore) days before and after rotavirus detection in stool, or during the interval over which a serological response was detected. Physicians assessed diarrheal severity on the 20-point Vesikari scale in clinic or based on symptom data from field workers and mothers. For consistency, we defined moderate-to-severe diarrhea as a Vesikari score of ≥11. In total, 200 children were recruited in Mexico City and retained in follow-up for 77% of the planned child-months of observation. Our analysis of the Vellore cohort included the 373 children (of 452 newborns recruited) who completed all 36 months of follow-up, consistent with primary

Table 1. Study Design, Enrollment, and Follow-Up

Study Attributes	Mexico City Cohort	Vellore Cohort
Duration of follow-up	24 mo	36 mo
Frequency of asymptomatic stool testing	Weekly	Every 2 wk
Frequency of serological testing	Every 4 mo	At least every 6 mo
Definition of rotavirus shedding	ELISA positive	2x ELISA positive or RT-PCR positive
Definition of seroconversion	4-fold rise in IgG or IgA	4-fold rise in IgG or 3-fold rise in IgA
Study population	200 children	373 children[a]
Child-months of observation	3699/4800 (77%)[b]	13 341/13 428 (99%)
Asymptomatic stool samples tested	15 503	26 902
Of all scheduled tests	15 503/20 800 (75%)	26 902/29 094 (92%)
Of all scheduled tests while child was retained in follow-up	15 503/16 029 (97%)	26 902/28 906 (93%)
Diarrheal episodes tested, of all reported diarrheal episodes	963/1133 (85%)	1829/1856 (99%)
Serum samples tested, of all scheduled tests	1037/1080 (96%)	2565/2598 (99%)
Infections detected	315	1103
From diarrheal episodes	89/315 (28%)	282/1103 (26%)
From asymptomatic shedding	88/315 (28%)	237/1103 (21%)
From seroconversion only	139/315 (44%)	584/1103 (53%)
Mean (SD) age, d, asymptomatic infections detected by seroconversion only	445 (167)[c]	626 (308)[d]
Mean age, d, asymptomatic infections detected by shedding	339 (187)[c]	542 (342)[d]
Rotavirus-negative diarrhea samples	874/963 (91%)	1547/1829 (85%)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgA, immunoglobulin A; IgG, immunoglobulin G; RT-PCR, reverse-transcription polymerase chain reaction; SD, standard deviation.

[a]Sample restricted to children who completed 3 years of follow-up (83% of initial cohort of 452 children).

[b]Ninety-eight of 200 enrolled children (49%) in Mexico City completed the scheduled 2 years of follow-up.

[c]In the Mexico City cohort, the mean age of asymptomatic infections detected by shedding is significantly lower than the mean age of asymptomatic infections detected by seroconversion alone (P < .0001 by cluster bootstrap).

[d]In the Vellore cohort, the mean age of asymptomatic infections detected by shedding is significantly lower than the mean age of asymptomatic infections detected by seroconversion alone (P < .001 by cluster bootstrap).

analyses of the studies; no differences in baseline nutritional and demographic covariates were observed between those who completed or dropped out of follow-up in Vellore [8].

Statistical Analyses

Descriptive Analyses

Within each cohort we calculated age-specific incidence rates of rotavirus infection, asymptomatic infection, and RVGE, as well as all-cause and rotavirus-negative diarrhea, by fitting Poisson regression models measuring the incidence rate as a continuous function of age (in months). Polynomial terms for age were added until the Bayesian information criterion indicated no improvement in model fit. We estimated models using generalized estimating equations (GEE) with robust standard errors to account for repeated sampling of children.

Age and Previous Infection as Determinants of Risk for Rotavirus Infection to Cause RVGE

Primary analyses of the datasets provided similar estimates of relative rates at which children were reinfected with rotavirus after 1, 2, or 3 previous infections. However, these studies suggested weaker protection against incidence of RVGE and moderate-to-severe RVGE in Vellore. Based on differences in age at which infections occurred between the 2 settings, we hypothesized that older age may affect risk for RVGE given rotavirus infection. We examined this first by testing for differences in the proportions of primary, secondary, and subsequent rotavirus infections causing RVGE within age-specific strata defined by intervals between serological tests. For consistency with our use of GEE with robust standard errors in regression analyses, we constructed confidence intervals around proportions using a cluster bootstrap of individuals [21]. We used the same approach to compare risk of moderate-to-severe RVGE, in this case computing credible intervals via the Jeffreys prior [22] due to sparse counts within strata.

To characterize the effect of age on risk (conditional probability) of RVGE or moderate-to-severe RVGE given infection, we calculated the relative risk for these symptomatic manifestations during primary, secondary, and later rotavirus infections as a function of age using the modified Poisson regression approach with robust standard errors [23]. We tested different transformations of the exposure variable (age), identifying via the Bayesian information criterion that a natural log transformation of age provided a superior fit over inclusion of first-, second-, or third-order polynomials for age.

Role of Serum Antibody and Age in Risk for Rotavirus Infection and RVGE

We next used data on serum IgG and IgA antibody titers to better understand the independent roles of acquired immunity and age in protection against rotavirus infection, and protection against RVGE or moderate-to-severe RVGE given infection. We used repeated-events Cox proportional hazards models to measure how time to reinfection related to age and serum IgA and IgG antibody levels. We used Poisson regression models to calculate relative risks for RVGE and moderate-to-severe RVGE given infection acquired at differing ages and serum antibody titers. As in the other analyses, model parameters were estimated via GEE with robust variance to account for repeated sampling of children. Because no threshold IgA and IgG concentration is known to confer complete protection against rotavirus infection or RVGE [24–27], we measured antibody titers as continuous variables, calculating hazard ratios and relative risks associated with each 2-fold rise in titers above previously established [9] minimum dilutions (≤1:50 IgA and ≤1:400 IgG). Consistent with the analysis described above, we included age as a log-transformed, continuous variable. To ensure reliable baseline titer measurements, we limited analyses to the first infection occurring in each serological interval if multiple infections were reported.

Association of Previous RVGE With Risk for Rotavirus Infection and RVGE

Observations that RVGE occurred frequently on successive infections in Vellore led us to hypothesize that certain children may be predisposed to repeat infections, or to experiencing diarrhea when infected, for instance, due to frequent exposure or immune factors. We tested for an association between risk of RVGE given infection and susceptibility to future infection and RVGE; differences between the studies prevented us from conducting further side-by-side analyses adjusting for other potential predictors of susceptibility. Specifically, we measured the association between the symptom status of a child's previous infection (ie, whether or not previous infection caused RVGE) and time to reinfection, as well as risk for reinfection to cause RVGE. Consistent with the analyses described above, we used repeated-events Cox proportional hazards models to estimate hazard ratios for rotavirus reinfection, and used Poisson regression models to estimate the relative risk that secondary or later infections would cause RVGE as a function of whether the child's preceding infection was symptomatic. In both cases, we used robust error variance to account for repeated sampling of children, and controlled for age (log-transformed) and serum IgA and IgG titers. To further rule out confounding due to differential immune responses after symptomatic and asymptomatic infections, we also tested whether antibody titer (IgA or IgG) was dependent upon whether the previous infection was symptomatic, controlling for previous titer and age (log-transformed). We did this analysis within the subset of serological measurements separated by 1 infection, estimating regression models via GEE with robust standard errors to account for repeated sampling of children.

RESULTS

Cohort Monitoring

The studies detected 315 and 1103 rotavirus infections in Mexico City and Vellore, respectively, with 89 (28%) and 282

(26%) involving RVGE (Table 1). Of all infections, 139 (44%) and 584 (53%) in Mexico City and Vellore, respectively, were detected solely from serological responses; their timing was imputed by the original investigators as the midpoint of the interval between serological tests.

In Mexico City, rates of acquiring rotavirus infection peaked at 1.33 per year (95% confidence interval [CI], 0.95–1.83) at age 19.8 months, and increased with age, consistent with rates of asymptomatic infection. In Vellore, acquisition rates increased earlier in life and peaked at 2.08 per year (95% CI, 1.36–3.24) at age 5.5 months, again consistent with asymptomatic infection (Figure 1). RVGE incidence rates declined with age, and tended not to differ between the 2 settings.

There were 874 (91%) and 1547 (85%) rotavirus-negative diarrhea episodes in Mexico City and Vellore, respectively. Reported incidence of all-cause and rotavirus-negative diarrhea was equivalent between the cohorts or higher in Mexico City across ages (Figure 1).

Age-Related Reductions in Proportions of Rotavirus Infections Causing RVGE

In both cohorts, the proportion of rotavirus infections causing RVGE and moderate-to-severe RVGE declined with the number of previous infections (Figure 2 and Supplementary Table 1). However, the association between the number of infections a child had experienced and the conditional probability for an infection to cause RVGE or moderate-to-severe RVGE differed between the 2 cohorts. In Mexico City, second, third, and fourth infections had 61% (95% CI, 37%–77%), 50% (95% CI,

9%–77%), and 79% (95% CI, 3%–99%) lower risk for causing RVGE, respectively, compared to first infections. We observed no reduction in risk by the second infection in Vellore, and only a 40% (95% CI, 9%–64%) reduction after 3 previous infections (Supplementary Table 1). No children in Mexico City experienced moderate-to-severe RVGE following ≥2 previous infections, but in Vellore, previous infection was not associated with reduced risk for infections to cause moderate-to-severe RVGE (Supplementary Table 1).

When we compared infections at similar ages, however, reductions in proportions of primary, secondary, and later rotavirus infections causing RVGE or moderate-to-severe RVGE were less apparent (Figure 2). On the contrary, RVGE and moderate-to-severe RVGE occurred in a higher proportion of secondary and later infections compared to primary infections among infants 0–3 and 4–7 months of age in Vellore.

In Poisson regression models measuring relative risk of RVGE given infection, we found that each log-month increase in the age at which primary, secondary, and later infections occurred was associated with 28% (95% CI, 12%–41%), 69% (95% CI, 30%–86%), and 64% (95% CI, –186% to 95%) lower risk for infections to cause RVGE, respectively, in Mexico City, and with 10% (95% CI, –1% to 19%), 51% (95% CI, 41%–59%), and 67% (95% CI, 57%–75%) lower risk in Vellore (Table 2). Similarly, risk for infections to cause moderate-to-severe RVGE declined with each log-month of age by 44% (95% CI, 11%–65%) and 93% (95% CI, 76%–98%) for primary and secondary infections in Mexico City, respectively. In Vellore, we calculated
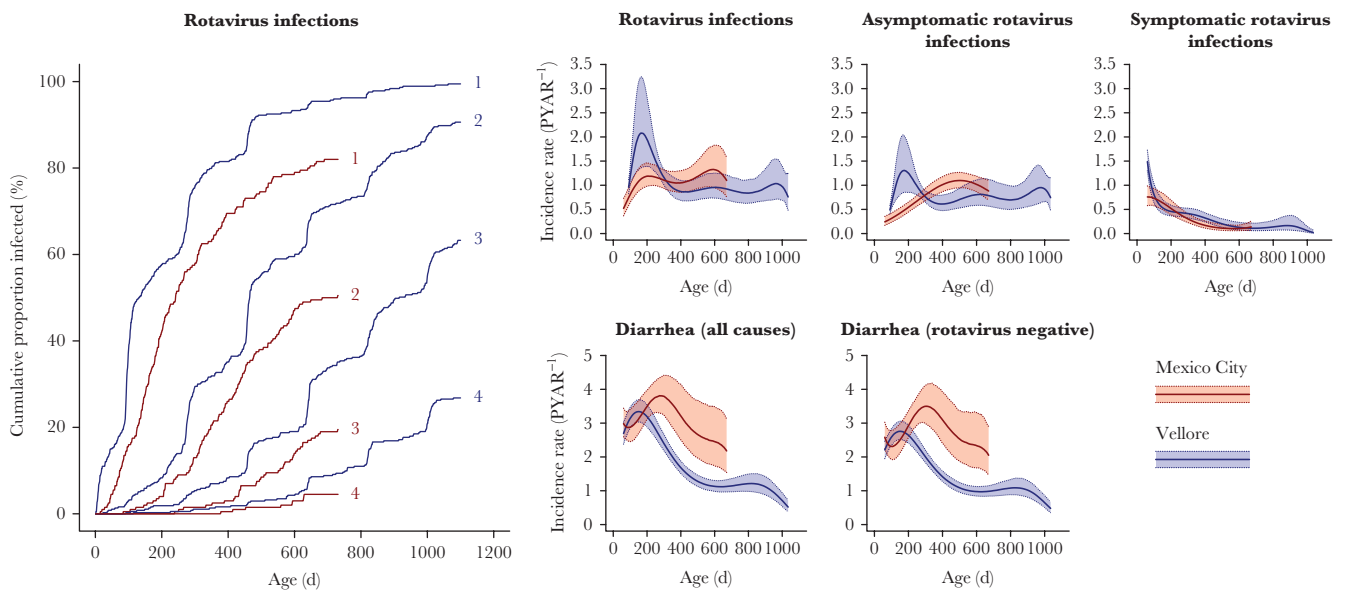

Figure 1. Cumulative and age-specific incidence of rotavirus infection and rotavirus gastroenteritis (RVGE), all-cause diarrhea, and rotavirus-negative diarrhea. We plot cumulative incidence of rotavirus infection (left panel) and age-specific incidence rates of rotavirus infection and gastroenteritis by age (right panels), fitted using polynomial terms for age with robust standard errors to account for repeated observations from children. Shaded areas denote 95% confidence intervals. Abbreviation: PYAR, person-years at risk.
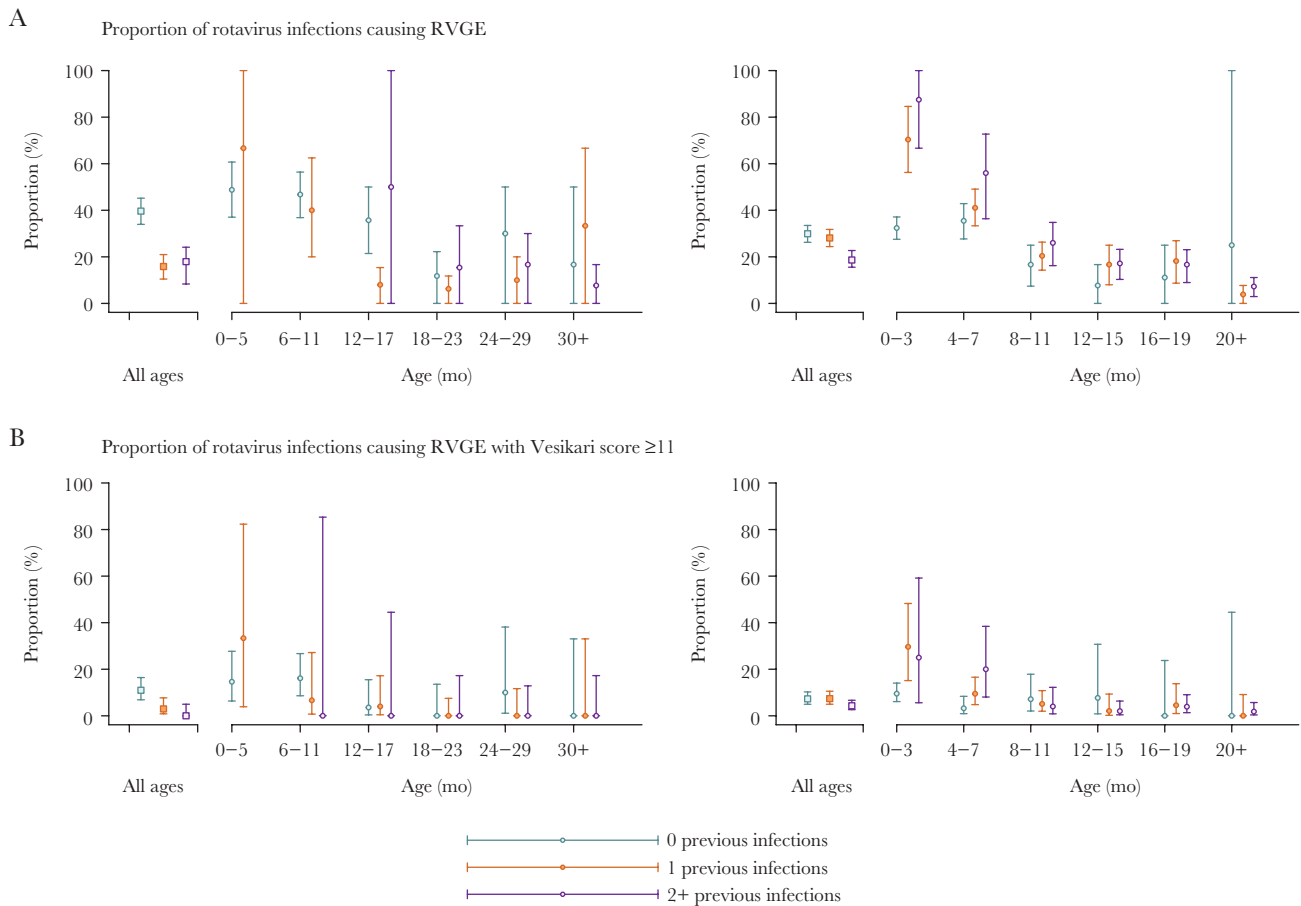
A Proportion of rotavirus infections causing RVGE

B Proportion of rotavirus infections causing RVGE with Vesikari score ≥11

- 0 previous infections
- 1 previous infections
- 2+ previous infections

Figure 2. Aggregate and age-specific proportions of primary, secondary, and subsequent rotavirus infections involving diarrhea. Vellore (left panels) and Mexico City (right panels) cohort data with point estimates and 95% confidence intervals obtained by cluster-bootstrap resampling (*A*) or the Jeffreys prior (*B*). Age strata are defined as the interval between serological measurements in each study. Error bars denote 95% confidence/credible intervals. Asterisks denote where at least 1 significant within-age group risk difference appears across infections: *$P < .05$; ** $P < .01$; ***$P < .001$. Abbreviation: RVGE, rotavirus gastroenteritis.

14% (95% CI, −10% to 33%), 62% (95% CI, 45%–74%), and 74% (95% CI, 51%–86%) lower risk for primary, secondary, and later infections, respectively, to cause moderate-to-severe RVGE with each log-month of age (Table 2). These outcomes confirmed patterns that were visually evident in the proportion of infections causing symptoms within broader age groups (Figure 2). Patterns we identified in the main analyses persisted in sensitivity analyses of the Vellore dataset excluding G10P[11] infections (Supplementary Figure 1), which were not associated with protection in previous analyses [28].

Role of Age and Immunity in Rotavirus Infection and RVGE Given Infection

Serum IgA and IgG concentrations increased with age, but this association disappeared when controlling for the number of previous infections (Supplementary Table 2). We found that each 2-fold increase in serum IgA titer was associated with a 13% (95% CI, 4%–21%) lower rate of acquiring rotavirus in Mexico City, and with an 8% (95% CI, 2%–12%) lower rate of infection in Vellore when controlling for age (Table 3).

Table 2. Effect of Age on the Relative Risk of Rotavirus Gastroenteritis (RVGE) and Moderate-to-Severe RVGE Given Infection

	RR per Log-month (95% CI)[a,b]	
No. of Previous Infections	Mexico City	Vellore
RVGE		
0	**0.72 (.59–.88)**	0.90 (.81–1.01)
1	**0.31 (.14–.70)**	**0.49 (.41–.59)**
≥2	0.36 (.05–2.86)	**0.33 (.25–.43)**
RVGE with Vesikari score ≥11		
0	**0.56 (.35–.89)**	0.86 (.67–1.10)
1	**0.07 (.02–.24)**	**0.38 (.26–.55)**
≥2	…	**0.26 (.14–.49)**

Values in boldfaced text indicate statistically significant effect size estimates (defined as a 95% confidence interval excluding 1).

Abbreviations: CI, confidence interval; RR, relative risk; RVGE, rotavirus gastroenteritis.

[a]Relative risks are computed from Poisson regression models fitted with generalized estimating equations with a robust error variance to account for repeated sampling of individual children; models include an interaction between the number of previous infections and the log-transformed age of children at the time of infection.

[b]Effect sizes for the decline per log-month of age should be interpreted in the context of differences in the age range within the 2 studies (2 years in Mexico City; 3 years in Vellore).

However, we note that effect sizes may not be directly comparable due to the differential frequency of serum collection in the 2 settings, as antibody waning can occur. We also estimated 9% (95% CI, 0%–7%) and 17% (95% CI, 14%–20%) lower rates of rotavirus infection in Mexico City and Vellore, respectively, with each 2-fold increase in serum IgG concentration. In contrast to this evidence for antibody-dependent protection against infection, there were no significant associations between serum antibody titer and risk for rotavirus infections to cause RVGE or moderate-to-severe RVGE after controlling for age. Consistent with analyses described above (Table 2), we estimated lower risk for rotavirus infections acquired at older ages to cause RVGE or moderate-to-severe RVGE, independent of serum antibody levels.

Association Between Rotavirus Susceptibility and Previous Symptomatic Infection

Our observation in Vellore of elevated RVGE risk during secondary and later infections during the first 7 months of life prompted us to consider whether certain children may have especially high risk for acquiring rotavirus, or for experiencing RVGE when infected. We found that children in Vellore who experienced RVGE during a rotavirus infection had 74% (95% CI, 19%–156%) higher risk for their next infection to cause RVGE as well, independent of age and serum IgA and IgG titers (Table 4). This association was not apparent among children in Mexico City; we also observed no independent association between previous symptomatic infection and time to next infection. The association in RVGE risk across rotavirus infections was not likely to be attributable

to differential immune responses after symptomatic and asymptomatic infections; in addition to controlling for age and serum antibody titers in the analysis, we identified no difference in the magnitude of serum IgA or IgG responses following symptomatic vs asymptomatic infections in either cohort (Table 4).

DISCUSSION

Birth cohort studies have been foundational to our understanding of naturally acquired immunity against rotavirus. Evidence of naturally acquired protection against rotavirus infection and RVGE from studies in Mexico City and other settings provided important rationale behind vaccine development [9, 29, 30]. Lower estimates of protection against RVGE from natural infection in Vellore have aided efforts to address the underperformance of live oral vaccines in low-income settings [8, 31, 32]. Whereas naturally acquired protection against reinfection appeared equivalent in Mexico City and Vellore, uncertainty has persisted as to why immunity against RVGE would appear less protective among Indian children. Because rotavirus infections occur at earlier ages and with higher frequency in Vellore, we conducted paired analyses of the original datasets assessing the role of age and previous immunity in risk for rotavirus infection and RVGE.

In both settings, older age appears to be associated with lower risk for rotavirus infections to cause RVGE or moderate-to-severe RVGE, independent of acquired immunity. In contrast, serum IgA and IgG titers are associated with partial protection against infection. These outcomes suggest that acquired

Table 3.   Serum Antibody and Age as Determinants of Rotavirus Infection and Risk for Infection to Cause Rotavirus Gastroenteritis

Predictors	Incidence of Infection of Any Symptom Status		Risk for Infection to Cause RVGE of Any Severity		Risk for Infection to Cause RVGE With Vesikari Score ≥11	
	HR (95% CI)	aHR (95% CI)	RR (95% CI)	aRR (95% CI)	RR (95% CI)	aRR (95% CI)
Mexico City						
Serum IgA titer						
Per 2-fold increase[a]	**0.81 (.76–.87)**	**0.87 (.79–.96)**	**0.78 (.64–.94)**	0.85 (.70–1.02)	0.75 (.44–1.28)	0.82 (.47–1.45)
Serum IgG titer						
Per 2-fold increase[b]	**0.82 (.77–.88)**	**0.91 (.83–1.00)**	0.89 (.78–1.02)	0.99 (.88–1.11)	0.95 (.70–1.29)	1.06 (.81–1.39)
Age						
Per log-month[c]	**0.81 (.69–.95)**	1.00 (.83–1.19)	**0.43 (.29–.63)**	**0.51 (.35–.75)**	**0.27 (.12–.62)**	**0.33 (.11–.99)**
Vellore						
Serum IgA titer						
Per 2-fold increase[a]	**0.80 (.76–.84)**	**0.92 (.88–.98)**	1.01 (.92–1.12)	1.07 (.98–1.17)	0.91 (.71–1.17)	1.01 (.80–1.28)
Serum IgG titer						
Per 2-fold increase[b]	**0.83 (.81–.86)**	**0.83 (.80–.86)**	1.03 (.97–1.09)	0.97 (.90–1.04)	0.97 (.85–1.11)	0.89 (.75–1.06)
Age						
Per log-month[c]	**0.90 (.87–.92)**	**0.88 (.85–.91)**	**0.76 (.70–.82)**	**0.74 (.68–.80)**	**0.70 (.60–.83)**	**0.67 (.55–.81)**

Values in boldfaced text indicate statistically significant effect size estimates (defined as a 95% confidence interval excluding 1).

Abbreviations: aHR, adjusted hazard ratio; aRR, adjusted relative risk; CI, confidence interval; HR, hazard ratio; IgA, immunoglobulin A; IgG, immunoglobulin G; RR, relative risk; RVGE, rotavirus gastroenteritis.

[a]The minimum dilution is <1:50; increases in serum IgA titers are measured on a scale of 2-fold increases over this level.

[b]The minimum dilution is <1:400; increases in serum IgG titers are measured on a scale of 2-fold increases over this level.

[c]Effect sizes for the decline per log-month of age should be interpreted in the context of differences in the age range within the 2 studies (2 years in Mexico City; 3 years in Vellore).

Table 4. Antibody-Independent Association of Previous Symptomatic Infection With the Rate of Subsequent Rotavirus Infection and Risk for Infection to Cause Rotavirus Gastroenteritis

Predictors	Incidence of Next Infection[a]		Risk for Next Infection to Cause RVGE[b]		Fold Increase in Antibody Titer[c]	
	HR (95% CI)	aHR (95% CI)	RR (95% CI)	aRR (95% CI)	Relative Increase in Serum IgA, Adjusted (95% CI)	Relative Increase in Serum IgG, Adjusted (95% CI)
Mexico City						
Symptomatic previous infection						
ref. asymptomatic previous infection	1.44 (.98–2.14)	1.08 (.72–1.61)	0.58 (.21–1.57)	0.69 (.25–1.93)	0.90 (.49–1.71)	1.06 (.52–2.16)
Vellore						
Symptomatic previous infection						
ref. asymptomatic previous infection	**1.22 (1.00–1.49)**	1.07 (.88–1.31)	**1.93 (1.32–2.81)**	**1.74 (1.19–2.56)**	0.87 (.62–1.22)	0.84 (.56–1.24)

Values in boldfaced text indicate statistically significant effect size estimates (defined as a 95% confidence interval excluding 1).

Abbreviations: aHR, adjusted hazard ratio; aRR, adjusted relative risk; CI, confidence interval; HR, hazard ratio; IgA, immunoglobulin A; IgG, immunoglobulin G; RR, relative risk; RVGE, rotavirus gastroenteritis.

[a]We calculated HRs and aHR with repeated-events Cox proportional hazards models of time to next infection using a robust error variance to account for repeated observations from children. As in the other analyses, we adjusted for age at the time of the preceding serum draw (log-transformed) and serum IgA and IgG concentrations.

[b]We calculated RRs and aRR with Poisson regression models using generalized estimating equations with a robust error variance to account for repeated observations from children. As in the other analyses, we adjusted for age at time of infection (log-transformed) and serum IgA and IgG concentrations.

[c]We calculated the association between the increase in antibody concentration resulting from an infection and whether this infection was symptomatic or asymptomatic using regression models of concentration, estimated with a robust error variance to account for repeated observations from children. Unadjusted estimates are not presented as the change in concentration can only be measured when controlling for previous serum IgA or IgG measurement; models further adjust for age (log-transformed), as in the other analyses.

immune protection, by reducing the acquisition rate, defers reinfection to older ages when RVGE or moderate-to-severe RVGE is less likely. Between-cohort differences in the age distribution of primary, secondary, and subsequent infections may have confounded previous comparisons of protection calculated using RVGE incidence endpoints.

Stratifying the proportion of rotavirus infections by age and previous infection reveals a counterintuitive observation among young children in Vellore: At matched ages, secondary and later infections are more likely than primary infections to cause RVGE. Whereas this finding may reflect maternal antibodies preventing diarrhea during the earliest infections, it could also be an example of the Simpson paradox, if children susceptible to acquiring 2 or more infections in the first months of life are also more likely to experience RVGE when infected. Indeed, we find that having experienced RVGE during past infection is associated with future risk for RVGE given infection among children in Vellore, but not in Mexico City, independent of age and serum antibody concentrations.

However, our analysis cannot explain why individual variation in susceptibility to RVGE given infection emerges in Vellore but not Mexico City. As the studies were undertaken in distinct settings, socioeconomic risk factors are not directly comparable between studies; in previous analyses, household hygiene measures and involvement in making bidis (indigenous cigarettes) predicted rotavirus infection in Vellore, but do not have socioeconomic analogues in Mexico City. Although primary analyses did not point to a consistent set of nutritional factors predicting infection or RVGE, associations between these

nutritional measures and susceptibility to rotavirus are difficult to interpret because diarrheal illnesses can cause poor growth, malnourishment, and micronutrient deficiencies. In addition, conflicting evidence about associations between nutrition and rotavirus susceptibility in observational and intervention studies underscores the need to interpret associations cautiously. For instance, vitamin A supplementation exacerbated susceptibility to rotavirus infection and RVGE in Guinea-Bissau [33], despite a protective association between vitamin A intake and diarrhea in observational studies [34]. Similarly, although longer breast-feeding was associated with lower RVGE incidence in Mexico City [9], such an association was not evident in Vellore [8]. Although few host-genetic correlates of rotavirus susceptibility and immune response have been investigated, associations with histo-blood group antigen phenotypes including Lewis positivity and FUT2 secretor status have been reported [35–38]. Recent cohort studies [16–19] investigating relationships among nutritional, genetic, and immunological risk factors and diarrhea endpoints may suggest specific covariates leading to differences such as those observed between the Mexico City and Vellore studies.

Our finding of decreasing RVGE risk with older age on primary, secondary, and subsequent rotavirus infections suggests age is one such contributing factor [4]. Infections tended to occur at younger ages and with higher frequency in Vellore compared to Mexico City, but age-specific RVGE incidence was similar in the 2 cohorts. In populations exposed to high rates of transmission, secondary and later infections are expected to occur in closer succession, and thus at younger ages, than in settings where exposure to rotavirus occurs with lower frequency.

Although factors determining why age is associated with lower risk of RVGE given infection are not known, postnatal intestinal development, immune maturation, and establishment of gut microbial communities contribute to age-dependent risk for infection to cause RVGE in animal models [39–44].

Our analysis has several limitations. Low-replicating rotavirus infections that would otherwise be asymptomatic could be misclassified as RVGE episodes if they co-occur with other diarrhea-causing conditions (chronic or infectious), thus biasing estimates of protection. However, our finding of higher reported rotavirus-negative and all-cause diarrhea incidence in Mexico City, rather than Vellore (Figure 1), suggests misclassification of rotavirus-positive diarrhea as RVGE would not fully explain discrepancies in estimates of protection. Additional rotavirus infections could be misclassified due to imperfect diagnostic specificity. Whereas around half of infections were detected from seroincidence data, only 1 infection, at most, can be ascribed to the time between serological tests. This, too, is unlikely to undermine our inferences because the time between serological tests was well below the average time between infections; seroincidence data were also coupled with routine stool collection to identify asymptomatic rotavirus shedding. We cannot verify that mothers in the cohorts were equally likely to report diarrhea to field workers; however, similar age-specific proportions of rotavirus infections associated with diarrhea and moderate-to-severe diarrhea suggest consistent reporting between studies. Last, our analysis does not distinguish homotypic and heterotypic reinfections according to G-type or P-type, as not all infections were genotyped, and the studies were underpowered to detect significant differences in homotypic and heterotypic protection based on population-level estimates of this effect [45].

Findings from our reanalysis suggest the immunological mechanism of naturally acquired immunity against RVGE should be revisited. Age-associated reductions in the probability for rotavirus infections to cause RVGE may work in tandem with naturally acquired immunity in protecting older children. The cohort studies undertaken in Mexico City and Vellore demonstrate that previous infection provides moderate protection against reinfection. Our findings suggest variation in the efficacy and impact of live oral rotavirus vaccines against RVGE in low- and high-incidence settings may relate to the age at which rotavirus infections occur. Laboratory and epidemiological studies of factors underlying host- and age-related determinants of RVGE risk are important to improve rotavirus vaccine impact.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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