ROTAVIRUS INFECTION IN INFANTS AS PROTECTION AGAINST SUBSEQUENT INFECTIONS

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Abstract

Background Rotavirus is the leading cause of severe diarrhea in infants. To provide a base line for assessing the efficacy of rotavirus vaccines, we evaluated the protection that is conferred by natural rotavirus infection.

Methods We monitored 200 Mexican infants from birth to two years of age by weekly home visits and stool collections. A physician assessed the severity of any episodes of diarrhea and collected additional stool specimens for testing by enzyme immunoassay and typing of strains. Serum collected during the first week of life and every four months thereafter was tested for antirotavirus IgA and IgG.

Results A total of 316 rotavirus infections were detected on the basis of the fecal excretion of virus (56 percent) or a serologic response (77 percent), of which 52 percent were first and 48 percent repeated infections. Children with one, two, or three previous infections had progressively lower risks of both subsequent rotavirus infection (adjusted relative risk, 0.62, 0.40, and 0.34, respectively) and diarrhea (adjusted relative risk, 0.23, 0.17, and 0.08) than children who had no previous infections. No child had moderate-to-severe diarrhea after two infections, whether symptomatic or asymptomatic. Subsequent infections were significantly less severe than first infections ($P = 0.024$), and second infections were more likely to be caused by another G type ($P = 0.054$).

Conclusions In infants, natural rotavirus infection confers protection against subsequent infection. This protection increases with each new infection and reduces the severity of the diarrhea. (N Engl J Med 1996;335:1022-8.)

INFECTIONS with group A rotaviruses are common among infants and young children worldwide.[1-6] The outcome ranges from asymptomatic infection to severe, life-threatening diarrhea.[6-10] Rotavirus is the leading cause of severe diarrhea among children and causes an estimated 870,000 deaths annually in developing countries.[1-3,11,12] The disease burden of rotavirus is so great that development of a vaccine is a global priority for public health.[11,12]

Epidemiologic studies of rotavirus have suggested that natural immunity is acquired after early exposure to the virus and that many children acquire immunity only after several infections.[10,13-18] However, observations from longitudinal studies assessing protection conferred by a natural rotavirus infection appear to be conflicting, showing a range of results from virtually no protection to complete protection.[19-24] These variable results might be explained either by a failure to detect all reinfections or by variations in prevailing neutralization serotypes. Rotavirus strains are serotyped according to the neutralization of one of the two proteins present in the outer capsid: VP7, a glycoprotein, is neutralized in G types, and VP4, a protease-cleaved hemagglutinin, is neutralized in P types.[25,26] G types 1, 2, 3, and 4 are responsible for most infections in children.[27,28]

The strategy for developing an effective oral rotavirus vaccine involves the use of live, attenuated strains that should protect an infant by the same mechanism as natural infection.[29] The failure of a primary natural rotavirus infection to protect completely against subsequent infection may indicate the need for multiple vaccinations or for the administration of a polyvalent vaccine that can elicit full protection against all prevalent serotypes. We have addressed these issues in a longitudinal study of a cohort of Mexican children monitored from birth to two years of age.[15] The aims of this study were to determine the occurrence of primary (initial) and subsequent infections, to quantify the protective efficacy of natural symptomatic and asymptomatic rotavirus infections against subsequent infection and disease, and to compare the distribution of G types between initial and subsequent infections to assess whether protection against reinfection was related to the serotype.

METHODS

Study Design

The study was conducted in San Pedro Mártir, a community on the southwestern outskirts of Mexico City, and approved by the institutional review board of the Instituto Nacional de la Nutrición; written informed consent was obtained from the parents of the children. The enrollment criteria and methods have been published

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previously.[15] A cohort of 200 newborns was recruited at birth, from October 1987 to October 1988, and monitored for two years. Approximately 15 newborns were recruited each month, after which field workers visited each household weekly to interview the mother and collect specimens, regardless of whether the children had symptoms. The interviewer inquired about feeding and eating patterns, the child's stool frequency and consistency the day before the visit, and the occurrence of diarrhea since the previous visit. Changes in the stool pattern were detected through the use of diaries kept for each child since birth. If diarrhea was suspected in a child, a physician was notified through a 24-hour paging system. The physician collected information from the mother, examined the child, and reviewed the diary kept by the field worker to confirm diarrhea and score the severity of the episode.

Stool samples were collected weekly from each child, and additional stool samples were obtained whenever diarrhea occurred. A blood sample was taken during the first week of life and every four months thereafter. Samples were kept on ice and transported within three hours of collection to the laboratory. Stools were processed within 24 hours, and serum was stored at −70°C.

Definitions

An episode of diarrhea was defined as the occurrence of three or more watery stools in a period of 24 hours or loose or watery bowel movements that exceeded by two or more the usual daily number of bowel movements during the previous four weeks. An episode was considered to have ended on the first day bowel movements returned to the usual daily pattern; we have previously validated this definition.[15,30,31] Rotavirus infection was considered to be present if one or both of these conditions were met: rotavirus was detected in stool samples or there was a fourfold increase in the titer of antirotavirus IgA or IgG antibodies (or both) from one serum sample to the next. When infection was identified on the basis of a serologic response alone, the date of infection was defined as the midpoint between the dates of collection of the two serum samples. A rotavirus infection was considered asymptomatic if a child did not have diarrhea during the five days before and five days after rotavirus was detected[32] or during an interval in which a serologic response was identified. A rotavirus infection was defined as symptomatic if diarrhea occurred within five days before or five days after the detection of the virus.[32] Because one or more episodes of diarrhea were observed in the interval between two blood collections and episodes of diarrhea that occurred were associated with several etiologic agents,[15] we defined a rotavirus infection for which the symptom status was unknown as an infection identified only on the basis of a serologic response in an interval between two blood collections in which diarrhea occurred but excretion of rotavirus was not detected.

Assessment of the Severity of Diarrhea

The severity of each episode of diarrhea was evaluated by a physician within 24 hours of notification of the episode and on each subsequent day of illness using a 20-point scoring system described previously.[33,34] A score of 1 to 9 was defined retrospectively as indicating mild disease, and a score of 10 or more, moderate-to-severe disease.[33,34]

Rotavirus Testing

Stool specimens were tested for rotavirus by enzyme immunoassay.[15] The G type of specimens positive for rotavirus was established by a monoclonal-based enzyme immunoassay[27] or by reverse-transcriptase polymerase chain reaction.[35]

Titration of Antirotavirus IgA and IgG Antibodies

Serum samples were analyzed for the presence of antirotavirus IgA and IgG antibodies by enzyme immunoassay with a single lot of semipurified rotavirus strain YO (G3) and mock-infected MA104 cells as positive and negative antigens, respectively.[36] All serum samples from a child were serially diluted (1:100, 1:200, 1:400, and so on) and tested against both antigens on the same plate. The titer was the reciprocal of the last dilution that exceeded an optical density of 0.200, a cutoff point we established by analyzing the distribution of the optical-density readings for the two antigens. Serum-free wells and a serially diluted serum specimen served, respectively, as negative and positive internal controls. The titer of the positive control serum could not differ by more than one dilution from plate to plate. A serologic response was defined as a fourfold increase in the titer of IgA or IgG antibodies.

Statistical Analysis

The number of age-specific child-months at risk was counted for each child. The number of child-months at risk for a first infection was counted from birth until the child became infected, and the number of child-months at risk for a subsequent infection was defined by the interval between infections. The time at risk for a child who dropped out of the study was included for the respective period of observation. The outcomes assessed for first and subsequent infections were any rotavirus infection, rotavirus-associated diarrhea, mild or moderate-to-severe rotavirus-associated diarrhea, and asymptomatic rotavirus infection. The incidence of each outcome was calculated as the number of episodes per 100 child-months at risk.

The Cox proportional-hazards model, generalized to allow for repeated infections per child, was used to obtain adjusted relative risks and their 95 percent confidence intervals to compare the incidence of repeated infections with the incidence of a first infection, with adjustment for potential confounding factors.[37-39] Potential confounding factors were identified from univariate and stratified analyses. For each outcome we fit a generalized proportional-hazards model, which used the length of time from birth to the occurrence of the first infection and the interval between infections and included time-dependent covariates. The number of previous infections (0, 1, 2, or 3) was included in these models as a dummy variable; the reference group was the group with no previous infections. Proportional-hazards modeling was done with S-Plus statistical software.[40]

Adjusted relative risks were also used to calculate the efficacy of infection in protecting against subsequent outcomes, as follows: (1 − the adjusted relative risk) × 100. The age-dependent probability of rotavirus infection was calculated as one minus the Kaplan–Meier estimate of the probability of survival. To assess discordance (negative agreement) of G types between paired stool samples obtained during the first and second infections, Cohen's kappa statistic for two raters and multiple nomial categories was used.[41] StatXact 3 for Windows (Cytel Software, Cambridge, Mass.) was used to compute the exact, one-sided P value of the negative kappa statistic.[42] The chi-square test or t-test was used to compare groups as appropriate.[43]

RESULTS

Cohort Monitoring

The 200 children in the study were monitored for 3699 child-months, which represented 77 percent of the total expected monitoring time. Stool samples (n = 15,503) were collected during 89 percent of the child-weeks of observation and during 85 percent of the episodes of diarrhea; the collection rates were similar for all age groups. All scheduled serum samples (n = 1080) were collected, and 96 percent of them were tested for antirotavirus antibodies.

Identification of First and Subsequent Rotavirus Infections

A total of 316 rotavirus infections occurred among the 200 children; 177 infections were detected by fecal excretion of virus and were reported previous-

ly,[15] 244 were identified on the basis of a serologic response, and 105 were identified by both methods (Table 1). Of the 316 infections, 52 percent were primary infections and the remaining were repeated infections. Fecal excretion of rotavirus was detected more frequently in primary than in subsequent infections (74 percent vs. 36 percent; chi-square = 45.2; $P<0.001$). In contrast, fewer serologic responses were detected in primary infections than in later infections (72 percent vs. 83 percent; chi-square = 4.8; $P=0.03$). The overall incidence of rotavirus infection and rotavirus-associated diarrhea was 1.0 and 0.3 episode per child-year, respectively.

The cumulative probability of rotavirus infections increased with increasing age (Fig. 1). A primary infection was detected in 34 percent of children by six months of age, in 67 percent by one year, and in 96 percent by two years. A second infection was detected in 4 percent of monitored infants by six months of age, nearly 30 percent by one year, and 69 percent by two years. A third infection occurred in 7 percent of infants by one year of age and in 42 percent by two years. By two years of age, a fourth infection was observed in 22 percent of children and a fifth infection in 13 percent.

Morbidity of Rotavirus Infections

Diarrhea was most common in primary rotavirus infections (47 percent); the frequency decreased in second (25 percent), third (32 percent), and fourth or fifth (20 percent) infections (chi-square for trend = 6.9; $P=0.009$). The percentage of symptomatic infections associated with moderate-to-severe diarrhea also decreased from 28 percent in primary infections to 19 percent and 0 percent, respectively, in second and further infections (chi-square for trend = 3.5; $P=0.06$). The severity of 43 subsequent infections for which the symptom status was known was significantly less than that of the infections that occurred immediately before them (mean reduction in score, 2.5; 95 percent confidence interval, 0.8 to 4.3; $P=0.024$) (Fig. 2). Infections for which the symptom status was unknown were not included in this analysis.

TABLE 1. DETECTION OF HUMAN ROTAVIRUS INFECTIONS ON THE BASIS OF FECAL EXCRETION OF VIRUS AND SEROLOGIC RESPONSE.*

METHOD OF DETECTION	1ST INFECTION (N = 164)	2ND INFECTION (N = 102)	3RD INFECTION (N = 40)	4TH AND 5TH INFECTIONS (N = 10)	TOTAL (N = 316)	P VALUE†
	number (percent)					
Excretion	122 (74)	35 (34)	18 (45)	2 (20)	177 (56)	<0.001
Serologic response	118 (72)	88 (86)	29 (72)	9 (90)	244 (77)	0.03
Both	76 (46)	21 (21)	7 (18)	1 (10)	105 (33)	<0.001

*Of the 316 infections detected, 52 percent were first, 32 percent second, 13 percent third, and 3 percent fourth or fifth infections.

†The chi-square test was used to compare values for primary infections with those for subsequent infections.

Potential Confounding Factors for Rotavirus Infection

We compared the groups of children with one, two, or more than two infections with the group of children with no infections. Factors significantly associated with a higher number of infections were older age, male sex, a shorter duration of breastfeeding, and a larger number of persons sleeping in the child's room. These variables were evaluated as potential confounding factors in the modified proportional-hazards model. Older age and crowded sleeping conditions were not found to be significant covariates in this model. Sex and duration of breastfeeding were retained in the final model to calculate the adjusted relative risks and efficacy values.

Protection Conferred by Natural Rotavirus Infection

Rotavirus infections protected against subsequent rotavirus infections and disease (Table 2). The incidence of any rotavirus infection decreased from 11.3 infections per 100 child-months among children with no previous infections to 4.2 per 100 child-months among children with three previous infections. The adjusted efficacy in protecting against infection was 38 percent after one infection and increased to 60 percent after two infections and 66 percent after three infections. Similarly, the incidence of rotavirus-associated diarrhea decreased from 4.4 episodes per 100 child-months among children with no previous infections to 0.5 per 100 child-months among children with three previous infections. The adjusted efficacy in protecting against rotavirus-associated diarrhea was 77 percent after one infection and increased after two (83 percent) and three (92 percent) infections. The degree of protection was greatest against moderate-to-severe illness and reached 100 percent after two infections. Natural rotavirus infection was less efficacious in protecting against mild diarrhea (73 percent and 75 percent after one and two infections, respectively) and least efficacious in protecting against asymptomatic infection (38 percent after one, 62 percent after two, and 74 percent after three infections). To assess the possible bias introduced by excluding infections for which the symptom status was unknown, we reanalyzed the data and included this group. The cumulative efficacy with increasing number of infections persisted.

Protection after Symptomatic and Asymptomatic Rotavirus Infections

The incidence of subsequent rotavirus infection was similar after a first symptomatic or asymptomatic

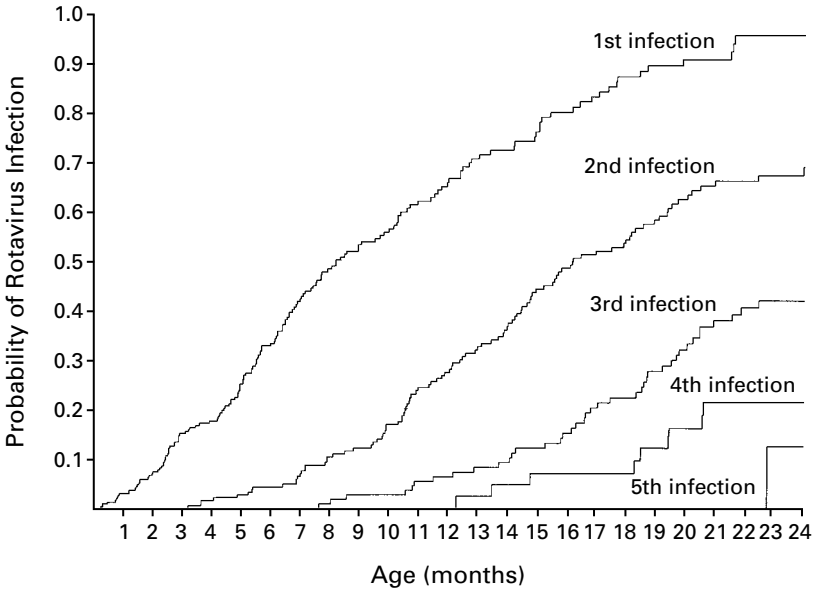

Figure 1. Cumulative Probability of First and Subsequent Natural Rotavirus Infections during the First Two Years of Life.

infection (8.1 vs. 7.1 episodes per 100 child-months; adjusted relative risk, 1.1; 95 percent confidence interval, 0.7 to 1.7), as was the incidence of rotavirus-associated diarrhea (2.0 vs. 1.1 episodes per 100 child-months; adjusted relative risk, 1.7; 95 percent confidence interval, 0.7 to 4.1). No moderate-to-severe illness was detected after two consecutive infections, whether the infections were symptomatic or asymptomatic.

G Types of Rotavirus Identified in Fecal Specimens

The G type was determined for 76 percent of the 177 strains of rotavirus identified in fecal specimens. G3 was the most frequently isolated type (35 percent), followed by G1 (20 percent), G2 (15 percent), and G4 (6 percent). To test the hypothesis that protection against subsequent infection might depend on the serotype, we assessed whether the second infection was less likely to be caused by the G type that caused the first infection. The G types were determined in 22 pairs of strains isolated from first and second infections (Fig. 3). In 2 of these 22 pairs (9 percent), the G type was the same in both the initial and the second infections. This observed proportion of concordant G types was lower than the expected proportion (24 percent) calculated as part of Cohen's kappa statistic ($P = 0.054$ by a one-sided exact t-test). The number of subsequent infections whose G type matched that of the initial infection was too small to permit separate statistical analysis of the relation between the severity of infection and G type.

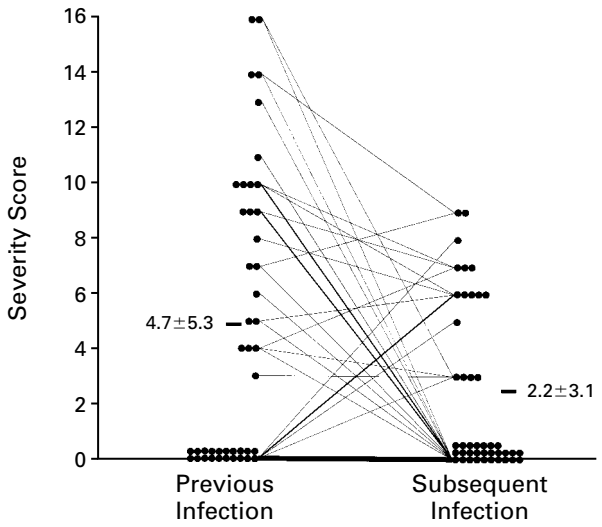

Figure 2. Severity of 43 Rotavirus Infections as Compared with the 43 That Followed Them.

A score of 1 to 9 indicated mild disease, and a score of 10 or more, moderate-to-severe disease. In 15 pairs of infections, both episodes were asymptomatic. In two pairs the severity score decreased from 10 in the earlier episode to 0 in the later one, in two pairs the score decreased from 9 in the earlier episode to 0 in the later one, and in two pairs the score increased from 0 in the earlier episode to 6 in the later one. The short horizontal bars indicate the mean (±SD) for each group.

TABLE 2. EFFICACY OF NATURALLY OCCURRING HUMAN ROTAVIRUS INFECTIONS IN PROTECTING AGAINST SUBSEQUENT ROTAVIRUS-ASSOCIATED OUTCOMES.*

OUTCOME AND NO. OF PREVIOUS INFECTIONS	NO. OF EPISODES	INCIDENCE†	ADJUSTED RELATIVE RISK (95% CI)‡	ADJUSTED EFFICACY (95% CI)§
	episodes/100 child-mo			%
Any infection¶				
0	164	11.3		
1	102	8.3	0.62 (0.50–0.83)	38 (17 to 50)
2	40	5.4	0.40 (0.28–0.59)	60 (41 to 72)
3	9	4.2	0.34 (0.17–0.67)	66 (33 to 83)
Any diarrhea‖				
0	64	4.4		
1	16	1.3	0.23 (0.12–0.40)	77 (60 to 88)
2	8	1.1	0.17 (0.08–0.36)	83 (64 to 92)
3	1	0.5	0.08 (0.01–0.56)	92 (44 to 99)
Moderate-to-severe diarrhea‖				
0	18	1.2		
1	3	0.2	0.13 (0.04–0.45)	87 (55 to 96)
2	0	0.0	Undefined	100
Mild diarrhea‖				
0	46	3.2		
1	13	1.1	0.27 (0.14–0.50)	73 (50 to 86)
2	8	1.1	0.25 (0.11–0.55)	75 (45 to 89)
3	1	0.5	0.01 (0.0–2.0)	99 (−100 to 100)
Asymptomatic infection				
0	71	4.9		
1	47	3.8	0.62 (0.42–0.91)	38 (9 to 58)
2	17	2.3	0.38 (0.21–0.66)	62 (34 to 79)
3	3	1.4	0.26 (0.08–0.83)	74 (17 to 92)

*CI denotes confidence interval.

†The group with no previous infections was monitored for 1450 child-months; the group with one previous infection, 1233 child-months; the group with two previous infections, 747 child-months; and the group with three previous infections, 215 child-months.

‡The risk was adjusted for sex and breast-feeding status.

§Efficacy was calculated as the percent reduction in the risk of an outcome as compared with the risk for children who were not yet infected.

¶This category includes symptomatic infections, asymptomatic infections, and infections for which the symptom status was unknown.

‖The incidence of the 88 infections for which the symptom status was unknown among children with no previous infections (2.0 infections per 100 child-months) was similar to the incidences among children with 1 previous infection (3.2 infections per 100 child-months), 2 previous infections (2.0 infections per 100 child-months), or 3 previous infections (2.3 infections per 100 child-months). Data on these infections are not included in this table.

DISCUSSION

In this study, we quantified the level of protection conferred by natural rotavirus infection against subsequent infection and disease. Natural rotavirus infection was associated with protection against the entire range of outcomes, from asymptomatic infection to moderate-to-severe diarrhea. Protection was greatest against moderate-to-severe disease, less against mild illness, and least against asymptomatic infection. Complete protection against moderate-to-severe diarrhea resulted after two infections, regardless of whether the infections were symptomatic or asymptomatic. Repeated infections with the same G type were less likely to occur, even when the probability of occurrence of the most common G types was considered, suggesting homotypic protection.

The identification of rotavirus infections on the basis of fecal excretion of virus and serologic response was complementary. More infections were detected on the basis of serologic response (77 percent) than on the basis of fecal excretion (56 percent); however, in 41 percent of the infections detected through the excretion of virus, no serologic response could be identified. Possible explanations for this observation include underdetection of fecal excretion of virus as a result of the weekly monitoring regimen, inability of the rotavirus assay to detect low levels of excretion, and relative inability of serum antibody responses to reflect immune responses at the site of infection, especially among children with primary infections. In these children, measurement of local immunity may have increased detec-

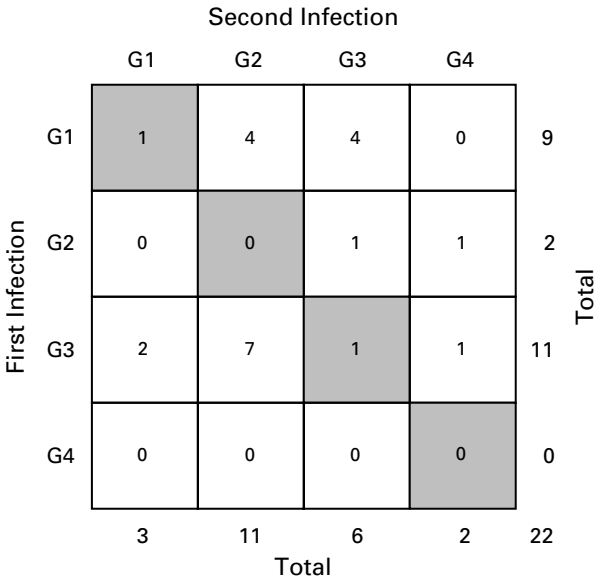

Figure 3. Distribution of Rotavirus G Types Identified in Fecal Specimens Obtained during the First and Second Infections in 22 Children.

The boxes indicating a shared G type are shaded.

tion.[44] Although in 88 infections identified only on the basis of a serologic response the symptom status was undefined, the cumulative efficacy of natural protection with an increasing number of infections remained similar whether or not these infections were included in the analyses.

Six cohort studies have reported on the degree of protection conferred by a natural rotavirus infection against subsequent reinfections.[19-24] These estimates and our own are not directly comparable because the seven studies used different methods and populations. Some studies did not enroll subjects at birth,[22,24] some did not use an operational definition of diarrhea,[19-21] and some relied on the parents' reports rather than on active surveillance for diarrhea.[19,22,24] In addition, some did not evaluate the severity of diarrhea[20,24] or did not identify asymptomatic infections.[21,23] One of these studies had dropout rates as high as 50 percent[20]; one had dropout rates that were distributed unequally between the comparison groups and relied on serologic response alone to identify diarrhea-associated rotavirus infections[19]; and several failed to assess[19] and control adequately[19,20] or at all[21,22,24] for potential confounding factors. Also, a limited diversity of circulating strains or a lower degree of reinfection in other studies may have accounted for the higher estimates of efficacy.[20,22,24] Moreover, previous studies[19-24] evaluated the degree of protection after only one infection and not after several infections, as in our study.

It is encouraging that the degree of protection conferred by asymptomatic infection was similar to that afforded by symptomatic infection. The occurrence of two rotavirus infections, whether symptomatic or asymptomatic, resulted in complete protection against moderate-to-severe illness. This finding implies that an attenuated vaccine that caused asymptomatic infection could induce protective immunity. That two natural infections were required for complete protection against moderate-to-severe illness implies that more doses of an attenuated vaccine will be required to achieve efficacy similar to that from exposure to a wild-type strain adapted to the human intestine.

Several live oral rotavirus vaccines have been evaluated in large field trials. These vaccines have been protective against severe disease, but none have completely protected against rotavirus diarrhea.[1,29] Our results provide a base line against which the efficacy of candidate vaccines can be compared and provide insights into how the epidemiology of disease may change after immunization.[29] They also suggest that each rotavirus infection will decrease a child's subsequent risk of infection as well as of both severe and mild disease.

This study has several implications for vaccine development. At least two doses (or "takes") of a polyvalent vaccine, which includes the most common G types, appear to be required to induce sufficient cumulative immunity to prevent the moderate-to-severe illness most likely to be associated with a first infection. Widespread immunization with such a vaccine is expected to prevent severe illness, reduce the transmission of wild-type virus, and ultimately decrease the disease burden of this most common cause of severe diarrhea in infants and young children.

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