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Neonatal Infection with G10P[11] Rotavirus Did Not Confer Protection against Subsequent Rotavirus Infection in a Community Cohort in Vellore, South India

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Abstract

Background—Various observational studies have suggested that neonatal rotavirus infection confers protection against diarrhea due to subsequent rotavirus infection. We examined the incidence of rotavirus infection and diarrhea during the first 2 years of life among children infected with the G10P[11] rotavirus strain during the neonatal period and those not infected with rotavirus.

Methods—Children were recruited at birth and were followed up at least twice weekly. Stool samples, collected every 2 weeks for surveillance and at each episode of diarrhea, were screened by enzyme-linked immunosorbent assay and were genotyped by polymerase chain reaction.

Results—Among 33 children infected neonatally with G10P[11] and 300 children not infected with rotavirus, there was no significant difference in the rates of rotavirus-positive diarrhea (rate ratio [RR], 1.05 [95% confidence interval {CI}, 0.61–1.79]), moderate or severe rotavirus-positive diarrhea (RR, 1.42 [95% CI, 0.73–2.78]), or asymptomatic rotavirus shedding (RR, 1.25 [95% CI, 0.85–1.83]).

Conclusion—Neonatal G10P[11] infection with a strain resembling a vaccine candidate did not confer protection against subsequent rotavirus infection or diarrhea of any severity in this setting.

Rotavirus is the most significant cause of diarrhea in children, causing ~611,000 deaths annually [1]. Most children in the world are infected with this agent during the first few years of life, although death occurs mainly in developing countries where access to acute medical care is generally poor. A live oral vaccine, Rotashield, introduced in 1998, was subsequently withdrawn because of its association with intussusception [2]. Two live oral

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vaccines made by GlaxoSmithKline and Merck were licensed in 2004 and 2006, respectively, after large-scale clinical trials [3, 4].

Special efforts are being made in developing countries, such as Indonesia and India, to develop additional vaccine candidates, because affordability and availability are strongly linked to the domestic manufacture of vaccines. Globally, oral vaccine candidates that have been developed or that are in development are monovalent or multivalent and are derived from animal-based strains, such as the Chinese lamb rotavirus vaccine; animal-human reassortants, such as Rotashield and RotaTeq; human strains, such as Rotarix; and asymptomatic neonatal strains, such as RV3 in Indonesia and 116E and I321 in India [5].

RV3, a G3P2A rotavirus [6], was first isolated from newborns at Children's Hospital in Melbourne. The development of this strain as a vaccine was based on an observation that neonates infected with this rotavirus strain in hospital nurseries usually were asymptomatic and were later protected against severe disease during early childhood [6]. On the basis of similar clinical findings, 2 strains isolated from newborns in India are also being prepared as candidate vaccines. Strain 116E is a G9P[11] reassortant of a human parent strain and a VP4 gene of bovine origin [7]. A nosocomial outbreak of infection in Bangalore led to the identification among neonates of another outbreak, caused by strain I321, a G10P[11] bovine-human reassortant strain [8]. Unlike strain 116E, strain I321 had a base of 9 bovine gene segments, and only gene segments 5 and 7, which encode nonstructural proteins 1 and 3, were of human origin.

All of the neonatal vaccine candidates have been identified from observational studies in a single type of setting, with relatively small numbers of infants infected neonatally during their stay in a nursery; in these studies, infants were followed for 2 years, and the incidence of infection was compared with that among uninfected children. With regard to strain I321, a cohort study conducted between 1998 and 1999 identified 48 children infected neonatally with G10P[11] and 28 uninfected children; after a 2-year follow-up period, 1 child (2.3%) in the first group had rotavirus-positive diarrhea, compared with 11 children (39.3%) in the second [9]. In Vellore, India, we have demonstrated that G10P[11] strains are responsible for symptomatic diarrhea in neonates and infants and that at least 4 genes of the virus, VP4, VP6, VP7, and NSP4, show >90% sequence homology to I321 [10]. Our initial study was hospital based and did not address the question of whether infection with G10P[11] during the neonatal period conferred protection from rotavirus infection and/or disease during the postneonatal period. We now report results from a community-based birth cohort that compared children infected neonatally and those uninfected with the G10P[11] strain.

SUBJECTS AND METHODS

Study population

We studied a cohort of children from birth, with the primary aim of studying the natural history of rotavirus infection. The study was based in 3 urban slums in Vellore, South India. The slums are overcrowded, consisting of closely clustered houses with many rubbish dumps and open drains, without secure tenancy or ownership of property, and without water and toilets.

The study area was mapped between November 2001 and August 2002, and women of child-bearing age were identified. Pregnant women were identified through repeated household surveys and from local antenatal clinics. The babies of those women intending to remain in the area for 3 years were eligible for enrollment, except if the woman lived in a brick-built house with ≥5 rooms or the baby was born with gross congenital anomalies or a birth weight <1500 g. Recruitment was from March 2002 to August 2003 and was done

consecutively. Children were visited routinely twice a week by a field-worker, who examined the child and inquired about the incidence of diarrhea and other morbidities. Stool samples were collected every 2 weeks. Each time a child had diarrhea, identified either through a routine field-worker visit or reporting by the mother, the field-worker made daily home visits and assessed the severity of the diarrhea. Stool samples were collected every 2 days until the diarrhea ceased. Appropriate treatment was given, and children were referred to the hospital when symptoms were severe.

Diarrhea was defined as the passage of 3 watery stools during a 24-h period or a change in the number or consistency of stools, as reported by the mother. An episode was defined as at least 1 day of diarrhea, preceded and followed by ≥2 days without diarrhea [11]. A rotavirus infection was considered to be symptomatic if rotavirus was detected in at least 1 stool sample collected between 7 days before the onset of diarrhea and 7 days after the diarrhea had ceased and was considered to be asymptomatic if rotavirus was detected in the stool sample of a child without diarrhea. Genotyping data were used to assess whether an asymptomatic infection was continuing or an instance of reinfection, as determined from consecutive samples obtained up to 28 days apart. At scheduled time points, 2-mL blood samples were collected for a study of the natural history of rotavirus infection, the results of which will be reported separately.

The severity of diarrhea was assessed by use of the scoring system of Ruuska and Vesikari [12]. Because accurate temperature measurements were not possible in the field, temperatures were recorded as normal, low-grade fever, or high-grade fever, as reported by the caregivers. An episode of diarrhea was considered to be mild for a score ≤5, moderately severe for a score of 6–10, and severe for scores 110. The field-workers recording the observations regarding illness and severity of illness were unaware of the genotyping results. The study was approved by the Research Committee of the Christian Medical College, Vellore, and the Institutional Ethics Committees of the London School of Hygiene and Tropical Medicine and Baylor College of Medicine, Houston.

Laboratory methods

Mothers were instructed to collect stool samples in the morning, for collection by a field-worker by 10 A.M. The samples were transported within 2 h of collection and were processed immediately for rotavirus screening; 2-g aliquots were stored at −70°C for strain characterization and future testing. All diarrheal samples were screened for bacterial enteropathogens, by culture, and for parasites, by microscopic examination.

Samples were screened for rotavirus by use of a latex agglutination (LA) assay (Meridian Diagnostics) until September 2002 (2% of samples), because a limited number of samples were being tested daily during the early phase of recruitment. The LA assay was used to avoid the wasting of reagents and to reduce study costs. Thereafter, within 24 h of collection, all samples were examined for group A rotavirus by means of ELISA (Rota IDEIA; DakoCytomation).

Viral RNA was extracted from rotavirus-positive fecal suspensions by use of guanidine isothiocyanate–silica, as described by Boom et al. [13]. RNA was eluted and reverse transcribed, and the resulting cDNA was used as the template for both VP4 and VP7-specific typing by polymerase chain reaction (PCR) amplification using oligonucleotide primers described elsewhere [14-17]. Samples that were positive for rotavirus antigen by ELISA but negative by the genotyping PCR were confirmed as rotavirus positive by testing for the VP6 gene by PCR amplification, as described elsewhere [18].

Statistical analysis

For the primary analyses, children infected with the G10P[11] strain during the neonatal period were categorized as exposed. The neonatal period was defined as infection within the first 28 days of life. We restricted the analysis to children who had been followed throughout the first 2 years of life, because most rotavirus-positive diarrhea is likely to occur during this time; thus, our analyses would be comparable to those of previous studies [9]. Follow-up time was calculated from the postneonatal period to the child's second birthday. Periods of ≥1 week during which a child was away from the surveillance area were removed from the person-time calculation.

Baseline continuous data for the exposed and unexposed groups were compared by use of Student's *t* test, when distributed normally, and by the Wilcoxon test, when continuous but not normally distributed; otherwise, categorical data were compared by use of χ^2 tests. Socioeconomic status was defined as class I for households that had none or only 1 of the following: a member who owned the house, a house with >2 rooms, a member with >10 years of schooling, a head of household who was a skilled worker, or a color television, fan, motorized vehicle, steel cupboard, or tape recorder or petroleum gas for cooking fuel. Class II included households that had >1 of the above-listed characteristics.

To account for multiple incidence of illness within a child, frailty survival models (including random-effects terms for a child) were fitted to obtain variance-corrected incidence rate ratios (RRs). All analyses were done with STATA (version 8.0; Stata).

RESULTS

For the present study, 619 infants were eligible. The mothers of 167 infants refused to join the cohort, because they did not want blood drawn from their children, and we excluded from the main analysis 25 infants infected during the neonatal period with a rotavirus strain other than G10P[11] and 28 infants who had stool samples obtained only after the neonatal period. We calculated the rate of rotavirus infection during the first month of life as 16.8 cases of infection/100 child-months (64 cases of infection). Of these cases of infection, 10 (16%) were associated with diarrhea, and 6 of these 10 cases of symptomatic rotavirus infection were due to the G10P[11] strain. During the neonatal period, 38 children were infected with G10P[11], and 361 were not infected with any rotavirus strain; of these, 5 and 61 children, respectively, were lost to follow-up during the subsequent 2 years, owing to migration out of the study area (3 exposed and 36 unexposed children), caregivers' refusal to continue in the study (2 exposed and 23 unexposed children), and death (2 unexposed children; 1 death was due to a congenital cardiopulmonary disorder and the other to diarrhea). Thus, the cohort for these analyses was composed of 33 children infected neonatally with G10[P11] (exposed group) and 300 children not infected neonatally with rotavirus (unexposed group) who were followed up for 2 years, resulting in 636.4 child-years and representing 95.6% of the total expected monitoring time. During the neonatal period, the time of collection of the first stool sample was comparable for the exposed and unexposed groups—namely, a median of 6 days (interquartile range [IQR], 3–10 and 3–12 days, respectively; $P = .89$). The number of surveillance stool samples collected was the same for both groups—that is, a median of 2 samples (IQR, 1–2 samples; $P = .59$). During the early phase of recruitment, LA assays were used for screening, with 2% of the total samples tested by this method. For the exposed group, 20.6% of samples collected during the neonatal period were screened by LA assay, whereas 18.3% of samples from the unexposed group were tested by LA assay ($P = .58$); thus, between the exposed and unexposed groups, there was no statistically significant difference in the proportions of samples tested by each screening method.

Rotavirus was detected in 28 (85%) of the 33 exposed children within the first 2 weeks of life. In addition, for 5 children (15%), rotavirus was detected during diarrheal episodes; the other 28 children (85%) had asymptomatic shedding of rotavirus. Of these 5 episodes, 1 was mild, 1 was moderate, and 3 were severe. The median Ruuska and Vesikari score for these episodes was 11 (IQR, 7–11). The median duration of diarrhea was 10 days (IQR, 8–14 days).

Of the total of 69,264 scheduled routine visits, children were at home on 63,931 occasions (92.3%); the family was away on 393 occasions (0.7%), and the information was collected by proxy on 5015 occasions (7%). There were 19,503 stool samples collected from the 333 children during 15,964 (90.1%) child-fortnights of observation, of which 2852 samples were associated with diarrhea related to 914 (5.3%) child-fortnights of follow-up. Stool samples were collected for 1194 (94.5%) of 1264 diarrheal episodes.

Table 1 shows baseline characteristics for the exposed and unexposed groups. The 2 groups were comparable with respect to mother's age and education, socioeconomic status, sex, birth weight, number of siblings, and duration of breast-feeding. In the exposed and unexposed groups, the number of children who experienced ≥1 episode of rotavirus-confirmed diarrhea was 14 (42%) and 114 (38%), respectively ($P = .62$), and the number who had severe rotavirus-confirmed diarrhea was 3 (9%) and 21 (9%), respectively ($P = .66$). The number of episodes of rotavirus-confirmed diarrhea was 19 (16%) in the exposed group and 165 (14%) in the unexposed group ($P = .87$; table 1). The median number (range) of episodes of diarrhea per child was 4 (3–6) among exposed children and 5 (3–7) among unexposed children ($P = .15$). Table 2 shows the incidence rates and RRs for episodes of the different categories of diarrhea in the 2 groups. No significant difference was found in the rates of (1) all diarrhea, (2) all severe diarrhea, (3) all rotavirus-confirmed diarrhea, (4) moderate or severe rotavirus-confirmed diarrhea, (5) severe rotavirus-confirmed diarrhea, and (6) asymptomatic rotavirus shedding ($P > .3$). There also was no significant difference in the total number of episodes of rotavirus infection (asymptomatic shedding or rotavirus-confirmed diarrhea) between the 2 groups (RR, 1.17 [95% confidence interval {CI}, 0.85–1.60]; $P = .31$). The median time to detection of the first incidence of rotavirus infection after the neonatal period was 0.62 years for the exposed group and 0.72 years for the unexposed group (log-rank test, $P = .54$) (hazard ratio, 1.36 [95% CI, 0.75–2.49]; $P = .31$). The median (range) duration of rotavirus-confirmed diarrhea was 4 days (3–6 days) and 3 days (2–5 days) for the exposed and unexposed groups, respectively ($P = .31$). For each of the 6 diarrheal outcomes mentioned above, we also examined rates of episodes by child age and found no statistically significant differences between the exposed and unexposed groups (data not shown).

Table 3 shows the incidence of type-specific rotavirus-confirmed diarrhea and asymptomatic shedding. Typing was not possible for 7 episodes of rotavirus infection and 22 episodes of asymptomatic shedding in the exposed group and for 45 episodes of rotavirus infection and 147 episodes of asymptomatic shedding in the unexposed group. The proportion of samples that were PCR positive for VP6 but negative for VP4 and VP7 in the genotyping assays may have been due to the low level of virus present in samples. It has been shown previously, by quantitative PCR, that fecal samples from infected subjects without symptoms have significantly less virus than do samples from patients with gastroenteritis [18]. The rates of rotavirus-confirmed diarrhea and asymptomatic shedding were not significantly different between the exposed and unexposed groups, for any type-specific rotavirus ($P > .2$).

We also analyzed rates of rotavirus-confirmed diarrhea and asymptomatic rotavirus shedding among children infected neonatally with non-G10 rotavirus strains. Twenty-two of these 25 children were followed for 2 years. The incidence of rotavirus-confirmed diarrhea

was 0.30 (95% CI, 0.19–0.48), which is not significantly different from that among children infected neonatally with G10 ($P = .79$) or among children uninfected with rotavirus during the follow-up period ($P = .65$). However, the rate of asymptomatic shedding of rotavirus was 0.43 (95% CI, 0.38–0.49) among children infected neonatally with non-G10 rotavirus strains, which is significantly higher than that among children not infected neonatally ($P = .02$) or among children infected neonatally with G10 rotavirus strains ($P = .005$).

In addition, we examined the rates of outcomes among all children recruited into the cohort, regardless of whether children had been followed for 2 years. The duration of follow-up was 65.7 child-years for the exposed group and 596.9 child-years for the unexposed group. In the exposed and unexposed groups, respectively, there were 149 versus 1350 episodes of all types of diarrhea (RR, 1.02 [95% CI, 0.80–1.31]); 6 versus 81 episodes of severe diarrhea (RR, 0.67 [95% CI, 0.26–1.74]); 19 versus 171 episodes of rotavirus-confirmed diarrhea (RR, 1.01 [95% CI, 0.59–1.71]); and 3 versus 23 episodes of severe rotavirus-confirmed diarrhea (RR, 1.19 [95% CI, 0.34–4.17]).

In the exposed group, 1 (5.3%) of 19 rotavirus-positive diarrheal episodes was associated with the identification of an additional pathogen, indicating a mixed infection; in the unexposed group, 4 (2.4%) of the 165 rotavirus-positive diarrheal episodes were caused by mixed infections. This difference was not significant ($P = .42$).

DISCUSSION

In our cohort, neonatal G10P[11] infection did not confer protection against the subsequent development or the severity of rotavirus-positive diarrhea or asymptomatic rotavirus infection. Our study was community based and included careful and frequent follow-up of study participants. Our sample size was comparable or larger than that in previous studies. Although some of our CIs were large, the lower limits of the CIs were reasonable: these were 0.61, 0.73, and 0.34 for all types of rotavirus-confirmed diarrhea, moderate or severe diarrhea, and severe diarrhea, respectively; 0.85 for asymptomatic shedding; and 0.71 for all types of diarrhea. This finding suggests that significant subsequent protection from exposure to neonatal G10P[11] infection is unlikely. Our findings are in contrast to those from a study done in Bangalore between 1997 and 2000, in which neonatal infection with a G10P[11] strain, I321, was shown to protect from subsequent rotavirus-positive diarrhea [9]. However, the study in Bangalore was small, with 44 exposed and 28 unexposed children, and whether the 2 groups were comparable with respect to risk factors for diarrhea and how intensively these risk factors were evaluated is unclear. The I321 strain was being developed as a vaccine candidate but will not continue to phase II studies.

To our knowledge, our study was the first to examine whether the G10P[11] strain conferred any homotypic or heterotypic protection. During the postneonatal period, diarrhea due to G10 rotavirus strains occurred with similar rates among those with neonatal exposure to G10P[11] and those without exposure. Rates of asymptomatic infection with this homotypic genotype also were similar. Between both groups of children, no differences were observed between genotypes causing postneonatal infection, suggesting that G10P[11] infection during the neonatal period did not confer either homotypic or heterotypic protection. However, these subgroup analyses were based on small numbers of children, and the RRs had wide CIs. Thus, we cannot rule out the possibility of smaller but significant levels of protection, which might be detected with a larger sample size.

The rates of disease in our population were as high as those found in other settings where protection against subsequent rotavirus-positive diarrhea after asymptomatic neonatal infection was demonstrated [6, 19]. Rates of disease also were similar to those found in a

study of a large Mexican cohort, in which repeated natural infections with rotavirus were protective, particularly against severe disease; however, the involvement of neonatal infection in protection was not studied specifically [20].

The neonatal strains reported from India—namely, 116E and I321—have the same VP4 gene sequence that is homologous to bovine strain P[11]. These bovine-human reassortant strains may be recent products of the continuous evolution of rotavirus strains. These neonatal strains are thought to cause asymptomatic infection or to have attenuated virulence [21], and colonization of an infant's gut may be due to a lack of maternal anti-VP4 antibodies [22].

However, in a study of 30 adult volunteers and 30 children (some with preexisting antibodies) given a single oral dose of strain I321, strain 116E, or a placebo, the vaccine strains were found to be poorly immunogenic and to replicate poorly [5]. Low immunogenicity may have contributed to the lack of protection associated with the naturally acquired G10 infections in our cohort of children, which raises questions about the suitability of these G10 strains.

In a previous study [10], we reported that a G10P[11] strain circulating in this population was not a completely attenuated strain and that it caused symptomatic diarrhea in 63% of neonates admitted to a neonatal unit. Characterization of the genes encoding VP4, VP6, VP7, and NSP4 in these strains found in the neonatal nursery and in the community study reported here revealed high sequence homology (>90% at the nucleotide level and 94%–98% at the amino acid level) to 4 corresponding genes of the I321 strain obtained from a Bangalore neonatal unit [10]. Genomic regions of the G10P[11] strains obtained in Vellore are more homologous to strain I321 than are strains that have been detected previously in humans throughout the world, as determined by comparison to sequences made available in Genbank between 1993 and 1999 and to circulating bovine strains [10]. The degree of diversity between the G10 strains obtained in Vellore and the I321 strain was consistent with the degree of diversity expected between strains of the same genotype that had been circulating for more than a decade. Whether these differences could account for the lack of protection after G10 infection in Vellore, as opposed to the protection conferred by I321 in previous studies, cannot be determined on the basis of the available data. In a recent safety trial of a I321 candidate vaccine, children 8 weeks old showed no significant immune response to this vaccine strain, compared with a placebo group [23]. This result may have been due to the lower replication of animal or reassortant strains. Infection with a human strain may result in a better immune response, because of higher replication than that seen with animal or reassortant strains.

Our findings suggest that, during the neonatal period, natural rotavirus infection with bovine strains may not offer protection against reinfection and subsequent rotavirus-positive disease. Our data indicate that the efficacy of a rotavirus vaccine may depend on the source of the strain used and may vary between different communities, highlighting the need for efficacy studies in a range of settings.

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Table 1

Baseline characteristics of children infected with G10P[11] rotavirus (exposed group) and those not infected with rotavirus (unexposed group) during the neonatal period.

[a] Birth weight was not available for 1 child in the exposed group and 6 children in the unexposed group.

[b] All children were breast-fed.

[c] Overall, 97 mothers (27%) had gone to primary school, 82 (25%) to middle school, and 44 (13%) to high school; 11 mothers (3%) had completed high school, and 9 mothers (3%) had gone to college.

[d] Class I was defined as households that had none or only 1 of the following: a member who owned the house, a house with >2 rooms, a member with >10 years of schooling, a head of household who was a skilled worker, or a color television, fan, motorized vehicle, steel cupboard, or tape recorder or petroleum gas for cooking fuel. Class II was defined as households that had >1 of the above-listed characteristics.

Characteristic	Exposed group (*n* = 33)	Unexposed group (*n* = 300)	*P*
Age of mothers, median years (range)	24 (22–26)	23 (21–26)	.49
Male sex, no. (%) of children	17 (52)	150 (50)	.87
Birth weight, mean ± SD, kg[a]	2.90 ± 0.44	2.91 ± 0.43	.46
Duration of breast-feeding, median (range), months[b]	19 (15–21)	17 (11–22)	.39
Siblings, no. (%) of children			
0	7 (21)	98 (33)	.18
≥1	26 (79)	202 (67)	
Mother's education, no. (%) of children			
None	6 (18)	85 (28)	.21
Any[c]	27 (82)	215 (72)	
Socioeconomic status, no. (%) of children[d]			
Class I	17 (51)	187 (62)	.23
Class II	16 (49)	113 (38)	

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Table 2

Incidence of diarrhea and rotavirus infection among children infected with G10P[11] rotavirus (exposed group) and those not infected with rotavirus (unexposed group) during the neonatal period.

NOTE. The total duration of follow-up was 63.2 child-years for the exposed group and 573.2 child-years for the unexposed group. CI, confidence interval; RR, rate ratio.

Category	Exposed group (*n* = 33)	Unexposed group (*n* = 300)	RR (95% CI)	*P*
All types of diarrhea				
No. of episodes	118	1146		
Incidence (95% CI), per child-year	1.867 (1.429–2.439)	2.001 (1.834–2.183)	0.93 (0.71–1.24)	.63
Severe diarrhea				
No. of episodes	6	77		
Incidence (95% CI), per child-year	0.095 (0.037–0.243)	0.134 (0.102–0.177)	0.71 (0.27–1.88)	.48
Rotavirus-confirmed diarrhea				
No. of episodes	19	165		
Incidence (95% CI), per child-year	0.301 (0.181–0.500)	0.288 (0.242–0.342)	1.05 (0.61–1.79)	.87
Moderate or severe rotavirus-confirmed diarrhea				
No. of episodes	13	83		
Incidence (95% CI), per child-year	0.206 (0.110–0.385)	0.145 (0.114–0.184)	1.42 (0.73–2.78)	.31
Severe rotavirus-confirmed diarrhea				
No. of episodes	3	23		
Incidence rate (95% CI), per child-year	0.048 (0.015–0.156)	0.040 (0.026–0.061)	1.18 (0.34–4.18)	.80
Asymptomatic rotavirus shedding				
No. of episodes	34	247		
Incidence (95% CI), per child-year	0.538 (0.376–0.770)	0.431 (0.378–0.492)	1.25 (0.85–1.83)	.27

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Table 3

Incidence of type-specific rotavirus-positive diarrhea and of asymptomatic infection among children infected with G10P[11] rotavirus (exposed group) and those not infected with rotavirus (unexposed group) during the neonatal period.

NOTE. CI, confidence interval; RR, rate ratio.

Rotavirus type, category	Exposed group (*n* = 33)	Unexposed group (*n* = 300)	RR (95% CI)	*P*
G1				
Diarrhea				
No. of episodes	5	53		
Incidence (95% CI), per child-year	0.079 (0.033–0.192)	0.092 (0.070–0.122)	0.86 (0.34–2.17)	.74
Asymptomatic shedding				
No. of episodes	8	46		
Incidence (95% CI), per child-year	0.127 (0.056–0.284)	0.080 (0.058–0.111)	1.58 (0.66–3.76)	.32
G2				
Diarrhea				
No. of episodes	2	34		
Incidence (95% CI), per child-year	0.032 (0.008–0.127)	0.059 (0.042–0.083)	0.53 (0.13–2.22)	.34
Asymptomatic shedding				
No. of episodes	1	24		
Incidence (95% CI), per child-year	0.016 (0.002–0.112)	0.042 (0.028–0.062)	0.38 (0.05–2.80)	.27
G9				
Diarrhea				
No. of episodes	2	13		
Incidence (95% CI), per child-year	0.032 (0.008–0.127)	0.022 (0.013–0.040)	1.40 (0.29–6.76)	.69
Asymptomatic shedding				
No. of episodes	0	10		
Incidence (95% CI), per child-year	…	0.018 (0.009–0.032)	…	
G10				
Diarrhea				
No. of episodes	2	12		
Incidence (95% CI), per child-year	0.032 (0.008–0.127)	0.021 (0.012–0.037)	1.51 (0.34–6.75)	.61
Asymptomatic shedding				
No. of episodes	1	8		
Incidence (95% CI), per child-year	0.016 (0.002–0.112)	0.014 (0.007–0.028)	1.13 (0.14–9.06)	.91
Mixed				
Diarrhea				
No. of episodes	1	8		
Incidence (95% CI), per child-year	0.016 (0.002–0.112)	0.014 (0.007–0.028)	1.13 (0.14–9.06)	.91
Asymptomatic shedding				
No. of episodes	1	10		
Incidence (95% CI), per child-year	0.016 (0.002–0.112)	0.017 (0.009–0.032)	0.91 (0.12–7.09)	.93
Unusual (G4 or G8)				

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Rotavirus type, category	Exposed group (*n* = 33)	Unexposed group (*n* = 300)	RR (95% CI)	*P*
Diarrhea				
No. of episodes	0	0		
Incidence (95% CI), per child-year	…	…		
Asymptomatic shedding				
No. of episodes	1	2		
Incidence (95% CI), per child-year	0.016 (0.002–0.112)	0.004 (0.001–0.014)	4.53 (0.41–50.01)	.22
Untypeable				
Diarrhea				
No. of episodes	7	45		
Incidence (95% CI), per child-year	0.111 (0.053–0.232)	0.079 (0.059–0.105)	1.41 (0.64–3.13)	.73
Asymptomatic shedding				
No. of episodes	22	147		
Incidence (95% CI), per child-year	0.348 (0.229–0.529)	0.257 (0.218–0.302)	1.36 (0.87–2.13)	.20