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Neonatal rotavirus-associated necrotizing enterocolitis: Case control study and prospective surveillance during an outbreak

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After the death of a premature infant from rotavirus-associated necrotizing enterocolitis, we instituted prospective surveillance for this disease in our neonatal intensive care unit. During the 4-month study period an additional six cases of necrotizing enterocolitis and eight cases of hemorrhagic gastroenteritis occurred. Rotavirus infection was documented in 11 of these 15 symptomatic infants, in comparison with only eight rotavirus infections in 147 asymptomatic or minimally symptomatic babies ($P < 0.0001$). Stools from 110 nursery personnel tested during the outbreak did not contain rotavirus. However, 12 of 59 staff members had serum IgM antibody against rotavirus, suggesting recent infection. In a case-control study we compared babies with severe gastrointestinal illness with a control group randomly selected from asymptomatic babies in the nursery during the time of the outbreak. Univariate analysis found six categorical variables and nine continuous variables that were significantly associated with disease. Multivariate logistic regression analysis, however, found only birth weight ($P < 0.0001$), rotavirus infection ($P < 0.0001$), and age at time of first nonwater feeding ($P < 0.02$) to be associated with gastrointestinal illness. This study provides further evidence for the role of infection in some cases of neonatal necrotizing enterocolitis and hemorrhagic gastroenteritis. (J PEDIATR 1988;112:87-93)

Necrotizing enterocolitis is a severe, often fatal disease of newborn babies, characterized by bowel ischemia, infarction, or both and the sequelae thereof.[1] The cause of NEC is probably multifactorial, with both infectious and noninfectious risk factors.[2-6] Numerous specific infectious agents have been implicated in outbreaks, as well as in sporadic cases of the disease.[6]

Rotaviruses are the most common agents responsible for gastroenteritis in older infants and children,[7] but their role in causing neonatal disease is controversial. Several investigators have found a high prevalence of asymptomatic

NEC	Necrotizing enterocolitis
GI	Gastrointestinal
NICU	Neonatal intensive care unit
HGE	Hemorrhagic gastroenteritis
EIA	Enzyme-linked immunoassay

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neonatal infection,[8,9] whereas we and others have reported an association between rotavirus infection and severe neonatal gastrointestinal disease, including NEC.[10-14]

In May 1983 an infant with NEC and bowel perforation was transferred to our NICU for surgical bowel resection. Five days later a previously asymptomatic (for GI disease) infant developed fulminant NEC with bowel perforation and died within 24 hours. His stool, surgically resected intestine, and autopsy intestinal tissue all were positive for rotavirus by multiple testing methods. Serologic studies confirmed rotavirus infection in both infants. We immediately initiated a two-part investigation: prospective surveillance for rotavirus prevalence in the NICU, and a case control study of risk factors for severe GI disease. In this report we describe the results of our investigation.

METHODS

Rotavirus prevalence survey. Prospective rotavirus screening of every patient in the NICU was performed for 18 weeks. Rectal swabs were obtained from all babies at least weekly for the first 3 months and at more frequent intervals during peak periods of GI disease incidence. Additional rectal swabs were collected at the onset of symptoms from patients in whom GI disease developed on nondesignated screening days. A total of 162 neonates were studied with 475 rectal swab specimens. During study weeks 2 and 3, a single rectal swab specimen was obtained from each of 110 nursery personnel, and surface swabs were collected from 12 environmental sites in contact with neonates or their diapers. Throughout the study period, sera from all infants were collected from specimens in the chemistry laboratory that remained after ordered tests had been completed. During study week 6, sera were also obtained from 59 members of the nursery staff.

Case control study. A patient with NEC was defined as an infant with GI distress (manifested by one or more of the following: abdominal distention, tenderness, abdominal wall discoloration, visible bowel loops, feeding intolerance, bilious nasogastric drainage, and diarrhea); bloody stools; and characteristic radiologic findings (one or more of the following: pneumatosis intestinalis, biliary air, and peritoneal air). These patients conformed to proposed staging criteria 2 and 3.[15] A baby with hemorrhagic gastroenteritis was defined as a baby with GI distress and bloody stools without the characteristic radiologic changes of NEC, stage 1 disease.[15] Milder GI symptoms (i.e., transient GI distress without bloody stools or radiographic abnormalities) were ubiquitous in our NICU population; for the purposes of this study, infants with GI symptoms milder than NEC or HGE (as well as babies with no GI symptoms) were considered asymptomatic.

Chart review of all 15 symptomatic infants and 45 asymptomatic babies for more than 70 continuous and categorical variables provided the data used in the case-control study and risk factor analysis. The variables studied were in the broad categories of maternal-obstetric, neonatal, and postnatal factors. The 45 asymptomatic control infants were selected by a random number selection table from the 147 asymptomatic study patients in the nursery during the outbreak period.

Potential risk factors for NEC and HGE were screened with univariate analyses of categorical and continuous variables. Certain continuous variables were normalized for days at risk in study patients and control infants and expressed as a decimal fraction (number of events per number of days at risk). Because the latest date of onset of GI disease in a case was 30 days of hospitalization, 30 days was chosen as the limit of total days at risk in the control infants, and clinical histories were not reviewed for risk factors beyond that period. Pearson correlation matrices were calculated to investigate the extent of latent confounding relationships among the variables. Multivariate logistic regression analysis was used to determine the independent significance of certain risk factors by adjusting for other variables.

Statistical analysis was performed on a VAX computer with SPSS-X programs for the corrected chi-square, Student t test, and Pearson correlation procedures. Logistic regression was implemented by means of a FORTRAN program, BLOGIT,[16] which performs a likelihood-ratio test. Odds-ratio plots were drawn with the aid of a Hewlett-Packard plotter.

Laboratory evaluation. All rectal swab specimens were tested for rotavirus with the Rotazyme assay (Abbott Laboratories, North Chicago, Ill.) by following the instructions provided by the manufacturer. Positive swabs, as well as 100 randomly chosen negative swabs, were subjected to confirmatory testing with three additional EIAs.[17] When sufficient specimen was available, electron microscopy and tissue culture for rotavirus were performed by previously described techniques.[18]

Serologic studies for IgM and IgG antibodies to rotavirus in patients and staff were performed by EIA as previously described.[19] A positive serologic test result was defined as the presence of IgM to rotavirus in the absence of detectable rheumatoid factor or a significant rise in IgG antibody in paired sera.[19]

Only patients with confirmed infection were considered to be rotavirus-positive. Patients with Rotazyme-positive specimens that could not be confirmed by additional fecal or serologic testing were included in the rotavirus-negative category. As a result, seven Rotazyme-positive specimens (one from a symptomatic infant, six asymptomatic) were classified as rotavirus-negative.[17]

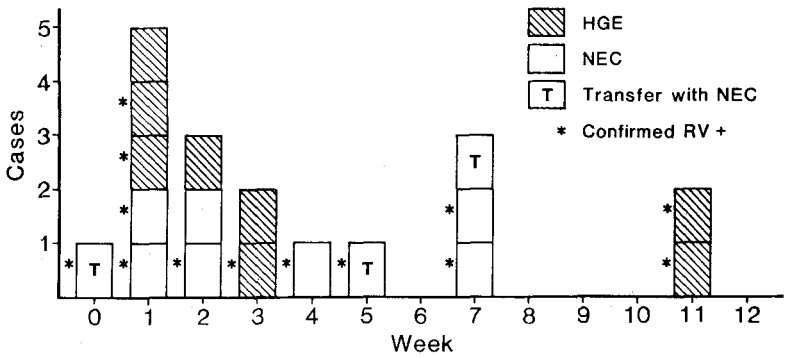

Fig. 1. Distribution of cases of NEC and HGE by study week. *RV*, rotavirus.

Routine bacteriologic and viral cultures were performed on stool specimens from symptomatic infants by standard techniques.

RESULTS

GI disease and prevalence of rotavirus. Fifteen infants in our NICU developed NEC or HGE during the 4 months after the arrival of a baby with severe NEC and bowel perforation. Eleven of these affected babies became ill within the first 4 weeks (Fig. 1). By use of a combination of EIAs (the three EIAs were 100% concordant in their results) and serology tests, 11 of 15 symptomatic patients were found to be infected with rotavirus (Table I). This incidence compared with only 8 of 147 asymptomatic babies ($P < 0.0001$). An adequate quantity of swab sample to permit additional testing was available from four of the babies with NEC and EIA positivity. Characteristic rotavirus particles were seen by electron microscopy in all four fecal samples, and rotavirus was successfully passaged in tissue culture inoculated with feces from two of these symptomatic infants. One infant had rotavirus in his stool on routine screening 24 hours before the development of NEC. Two babies intermittently shed rotavirus: one, with NEC, for 4 weeks; the other, an asymptomatic infant, for 1 week. Both had intervening negative samples.

During the study period, three babies were transferred to our NICU after the development of NEC at referring hospitals (Fig. 1). The first of these patients arrived 5 days before the outbreak began and is presumed to be the index case. Although stool specimens were not available retrospectively on that patient, his admission serum contained IgM antibody to rotavirus. The second transferred patient with NEC arrived during week 5 of the study period. His disease developed at a satellite community nursery that shares numerous staff members with our NICU on a rotating basis. Stool samples were not available from that baby either, but he also had serum IgM antibody to RV. Neither stool nor serum was available from the third transferred patient. No additional cases of NEC or HGE occurred in the NICU between week 12 and the end of the study (week 18).

Table I. Rotavirus in stool of study groups

Patients	n	RV-positive	
		n	%
Symptomatic	15	11	73*
NEC	7	6	86
HGE	8	5	63
Asymptomatic	147	8	5*

RV, rotavirus.
*Incidence of rotavirus in symptomatic group differs from incidence in asymptomatic group by $P < 0.0001$.

None of the 110 nursery personnel had rotavirus detected in their stools during the point prevalence rectal swab survey. Of 59 staff members from whom sera were available, however, 12 (20%) were found to have IgM antibody to rotavirus, indicating recent infection. Rotavirus antigen was not found on any of the environmental surfaces tested.

Routine bacterial and viral cultures of stool for common enteric pathogens were negative in all patients and control infants, and electron microscopic studies did not show any nonrotavirus viral particles. There was no difference in the predominant bacteriologic flora between symptomatic and asymptomatic infants.

Case control study. Comparisons were made between the 15 cases of NEC and HGE and 45 randomly selected control cases. Univariate analysis associated 15 separate factors with the development of severe GI disease ($P < 0.05$). The strongest associations were with rotavirus infection, the presence of a patent ductus arteriosus, birth

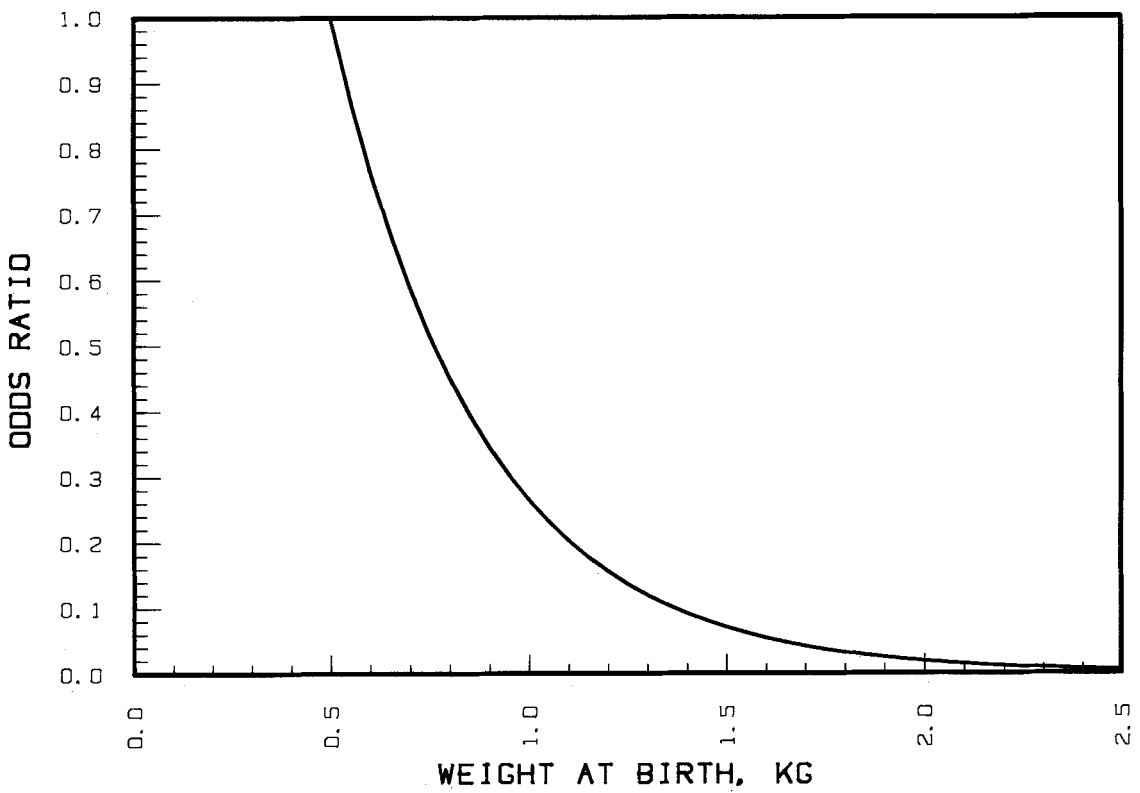

Fig. 2. Relationship between odds ratio and birth weight for 60 neonates in NICU (adjusted for rotavirus status). Odds ratio is set at 1.0 for an infant weighing 500 g.

Table II. Multivariate logistic regression analysis*

Test variable	Controlling variable (P value)	
	Weight	Weight and RV infection
RV infection	0.0001	
PDA	0.16	0.17
Phototherapy	0.09	0.14
Jaundice	0.17	0.21
Bronchopulmonary dysplasia	0.81	0.21
Surgical PDA repair	0.13	0.31
Red cell transfusions (total)	0.38	0.42
Red cell transfusions (wk 1)	0.84	0.59
Phototherapy (wk 1)	1.00	0.84
Phototherapy (total)	0.48	0.47
Age at first nonwater feeding	0.017	0.02

RV, rotavirus; PDA, patent ductus arteriosus.
*BLOGIT: see Methods section in this article.

weight, gestational age, the total number of red blood cell transfusions during the hospitalization, and exposure to phototherapy (each with P <0.0002). Also strongly associated were the following: jaundice, bronchopulmonary dysplasia, and the number of red blood cell transfusions in the first week of life (each with P = 0.001); and surgical repair of patent ductus arteriosus and hours of phototherapy in the first week of life and during the total hospitalization (each with P <0.003). Pearson correlation tables revealed a strong relationship among several of those variables, particularly between birth weight and many of the variables found to be most significant by univariate testing (data available on request), prompting us to perform multivariate logistic regression analysis (Table II) to determine the independence of these seemingly associated factors. When control for birth weight is incorporated into the analysis, only rotavirus infection (P = 0.0001) and age at first nonwater feeding (P < 0.02) are significant. When there is control for both weight and rotavirus infection, age at first nonwater feeding remains the only additionally significant factor. The relative risk of rotavirus infection for the development of NEC or HGE was 38.5; the odds ratios of birth weight and the age at first feeding are displayed in Figs. 2 and 3. Analysis of the number of days between the first day of feeding and the onset of symptoms of NEC or HGE revealed no apparent pattern.

We further analyzed the data on infants infected with rotavirus to identify factors that distinguished infected

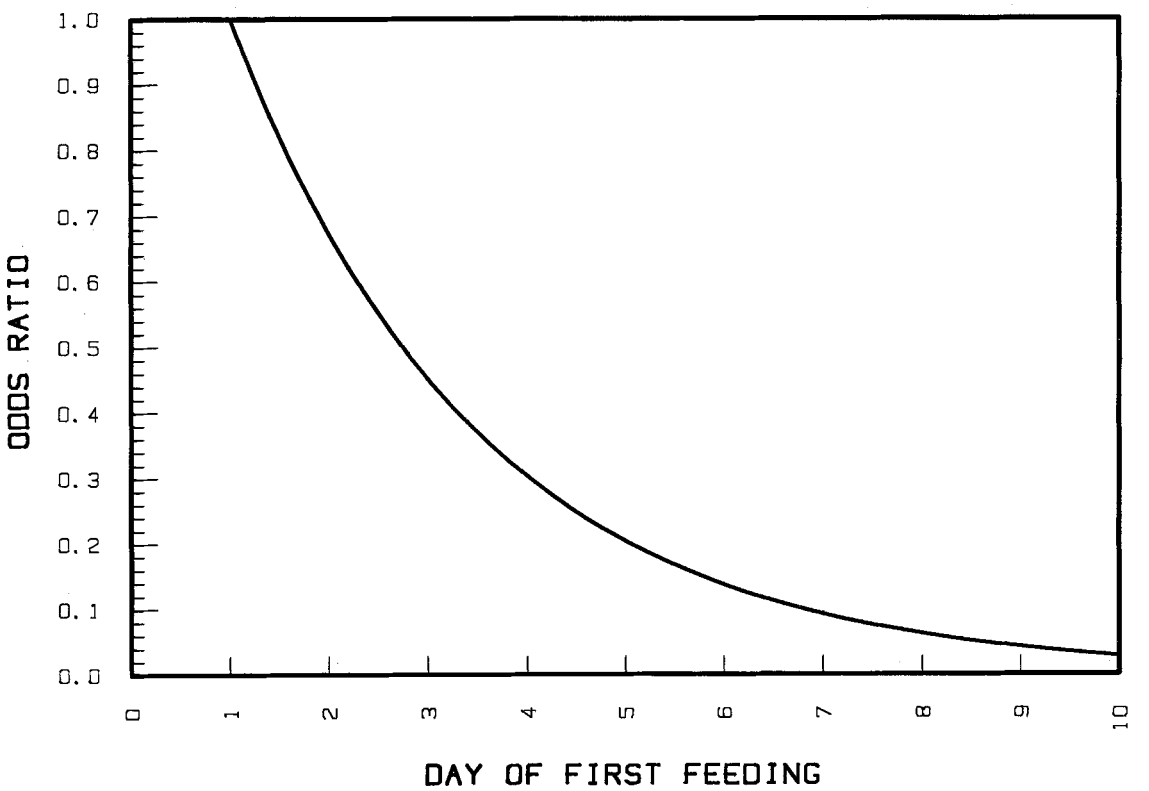

Fig. 3. Relationship between odds ratio and age at first nonwater feeding for 53 neonates in NICU (adjusted for birth weight and rotavirus status). Odds ratio is 1.0 for an infant fed on day 1.

symptomatic babies from those who were infected but remained asymptomatic. Nineteen patients became infected during the study period: 11 symptomatic with NEC or HGE, eight asymptomatic. Significant differences ($P < 0.05$) between those populations included birth weight, age at time of first nonwater feeding, the duration of time spent in phototherapy, and jaundice. The numbers of patients in these two subgroups of rotavirus-infected babies were too small to allow meaningful logistic regression analysis.

DISCUSSION

NEC occurs in both sporadic and epidemic forms,[1,6] the latter suggesting the possible role of infectious agents in pathogenesis.[20] Other circumstantial evidence for an infectious cause includes the efficacy of infection control techniques in halting the spread of disease within nurseries[21] and the increased incidence of illness among nursery staff during outbreaks of NEC.[22] Numerous investigators have identified specific bacterial pathogens apparently associated with individual cases or clusters of NEC.[6] Recently, several outbreaks of NEC have been linked to viral infections, particularly rotavirus[10] and coronavirus.[23,24] Both of these organisms, however, have been reported in the stools of asymptomatic neonates, raising the issue of possible cofactors to explain their virulence in babies in whom NEC or HGE develops. The occurrence of an apparent nosocomial case of rotavirus-associated NEC in our NICU provided the opportunity to survey prospectively for this entity and search for potential cofactors.

Simple univariate analysis of maternal, fetal, and neonatal variables identified "risk factors" for the development of NEC or HGE similar to those reported by others using similar analysis.[2-5] However, logistic regression analysis of these variables eliminated all but three independently associated factors: rotavirus infection, birth weight, and age at first feeding. The eliminated "risk factors" (e.g., jaundice, phototherapy, patent ductus arteriosus, bronchopulmonary dysplasia, transfusions) characterize the small, sick infant susceptible to NEC and HGE less well than does the single category of birth weight. This finding is consistent with the observations of others who dispute the traditionally accepted "risk factors" for NEC.[25-27] The age at first feeding, although not apparently associated strongly with GI disease by univariate statistics ($P = 0.098$), does contribute significant additional information about infants with NEC or HGE, even when birth weight and rotavirus infection are included and controlled for. Recently, howev-

er, a prospective study of delayed feedings failed to show benefit in preventing NEC.[28]

Rotaviruses are the most common cause of diarrheal disease among young children, but their role in neonatal GI disease is disputed. These agents are found in the stools of asymptomatic infants in some nurseries.[8,9] This report may be the strongest evidence to date of an etiologic role for rotavirus in NEC and HGE. Prospective sampling of all infants during the course of an outbreak avoids many of the pitfalls of other methods for determining cause. Asymptomatic shedding of virus, if it occurs, should be readily demonstrable with such a study design. We did not observe such a pattern; rather, the presence of rotavirus was strongly associated with severe GI symptoms. In one instance, our progressive sampling protocol detected rotavirus 24 hours before the onset of symptoms. In two other babies, intermittent viral shedding, previously not well recognized in rotavirus infection, was demonstrated with this prospective approach.

It is possible that a second pathogen is required to produce severe symptomatic disease. In this study, as well as our earlier experience with rotavirus-associated NEC,[10] we found identical bacterial flora (and the absence of traditional bacterial enteric pathogens) in the stools of symptomatic and asymptomatic babies. Hence, we cannot distinguish between an effect of rotavirus alone and disease caused by rotavirus and one or more normally present bacterial species.

The explanation for the different reported outcomes of babies infected with rotavirus may rest with the virus itself, with more virulent strains causing symptomatic disease. Recombination among the rotaviruses has been documented and may be the mechanism for evolution of virulence within a nursery setting. Additionally, recent evidence for genetic conservation of rotavirus from asymptomatic newborn infants has been reported.[29] The fourth gene from rotavirus isolated from asymptomatic neonates appears to differ from the fourth gene of rotavirus from older, symptomatic infants and children.[29] Alternatively, host factors may distinguish which infected babies will merely shed virus and which will develop life-threatening NEC. Our attempt to clarify this latter issue identified distinguishing characteristics that separated the sick infected babies from their infected but asymptomatic nursery mates. An additional host factor, which may be important, is the level of circulating maternal antibody in the at-risk infant. Premature infants with low levels of specific and nonspecific IgG may suffer greater consequences of their infections than do older infants.

We conclude that the small infant who is fed early appears to be at greatest risk for the development of severe GI disease when infected with rotavirus. Infection control measures remain the mainstay in preventing or halting nosocomial outbreaks of NEC.

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