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An outbreak of rotavirus-associated neonatal necrotizing enterocolitis

An outbreak of necrotizing enterocolitis and hemorrhagic gastroenteritis occurred in two nurseries during 25 days in August 1982. Eleven of the 40 patients in these nurseries during that time developed disease (attack rate 27.5%). In seven of the 10 patients with gastrointestinal disease, stool samples tested for human rotavirus were positive by ELISA, whereas in 20 unaffected infants, no stools tested demonstrated HRV (P = 0.0001). Eleven staff members had serologic evidence of recent HRV infection. Comparison of risk factors traditionally associated with the development of NEC between the affected and unaffected infants revealed no significant differences. Rotavirus infection was the only finding that was highly correlated with this epidemic. (J PEDIATR 103:454, 1983.)

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NECROTIZING ENTEROCOLITIS is a severe and potentially fatal disease of newborn infants. Primarily affecting premature neonates, NEC usually manifests as gastrointestinal distress with heme-positive stools and characteristic radiologic, surgical, and pathologic findings.[1,2] Strong, albeit often circumstantial, evidence exists to indicate that infection causes or contributes to some cases of NEC. Clustering of cases is well described,[3] numerous infectious agents have been reported in association with outbreaks of NEC,[4-10] antibiotic prophylaxis has been shown to decrease the incidence of disease,[11,12] human milk may be protective against developing severe NEC,[4] and infection control measures seem to reduce the incidence of disease.[3]

We describe a serious outbreak of neonatal NEC and hemorrhagic gastroenteritis associated with the identification of human rotavirus in stool specimens.

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MATERIALS AND METHODS

Study population. All patients in the medium-risk (level II) and high-risk (NICU) nurseries who were hospitalized during August 1982 were studied. We defined NEC as gastrointestinal distress with heme-positive stools and characteristic radiologic changes. Manifestations of gastrointestinal distress included abdominal distention, tenderness, abdominal wall discoloration, visible bowel loops, feeding intolerance, bilious nasogastric drainage, and diarrhea. Radiologic findings included pneumatosis intestinalis and portal or peritoneal air. Hemorrhagic gastroenteritis was defined as gastrointestinal distress and heme-positive stools, without the radiologic changes of NEC. Control infants were present in the nurseries at the same time but did not have gastrointestinal symptoms.

ELISA	Enzyme-linked immunosorbent assay
HGE	Hemorrhagic gastroenteritis
HRV	Human rotavirus
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit

The medical and nursing staffs of the nurseries were surveyed for symptoms of viral or any other illnesses they may have experienced between July and September 1982.

Laboratory evaluation. Stools and sera were collected in 10 of the patients when they were symptomatic and

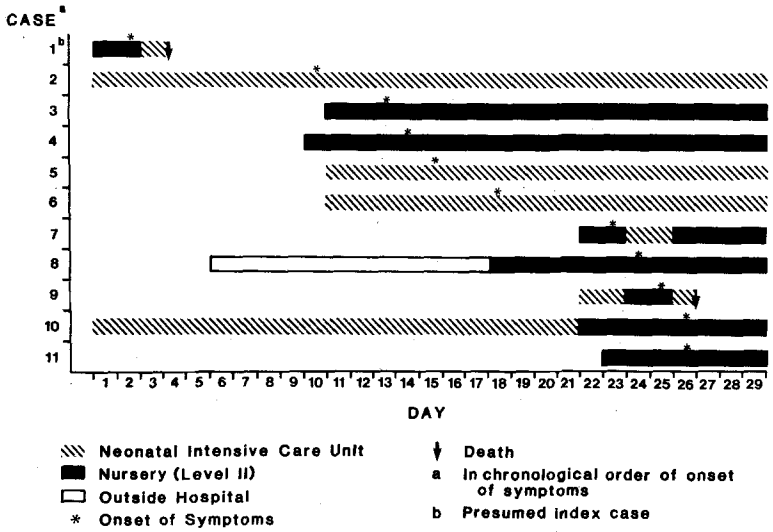

Figure. Location of symptomatic infants during outbreak.

simultaneously in 20 unaffected neonates in the same nurseries. The one remaining patient and nine controls were unavailable either because of death or early discharge from the nursery. Stools were cultured on blood, MacConkey, xylose-lysine-deoxycholate, and Mueller-Hinton agars in the microbiology laboratory by standard techniques. Additionally, dark-field examination of stool specimens for *Campylobacter* was performed. Tissue cultures of human embryonic lung fibroblasts were inoculated and assayed for the presence of *Clostridium difficile* toxin.[13] Viral cultures of the stool, nasopharyngeal washings, and urine of affected babies were performed using human embryonic lung fibroblast, human epithelial cell 2, and Rhesus and cynomolgus monkey kidney cell lines. Stool specimens were prepared by standard technique and studied by electron microscopy.[14] Enzyme-linked immunosorbent assays, as previously described, were performed on the stools of patients and controls for HRV, enteric-type adenovirus, and coxsackie A and B viruses.[15,16] An experimental coronavirus ELISA was performed using antibody directed against the 229E strain of coronavirus. A microtiter ELISA for HRV was performed using goat antibody to HRV on the solid phase and guinea pig antibody to HRV on the liquid phase. Specificity was documented by performing control reactions in wells coated with nonimmune goat serum, as described previously.[15]

Sera from patients, controls, and staff (both symptomatic and asymptomatic) were assayed for IgM antibody to HRV by ELISA techniques previously described.[17]

All comparisons were analyzed for significance by the two-tailed Fisher exact test.

RESULTS

Eight cases of NEC and three of HGE occurred among 40 neonates in two University of Colorado Health Sciences Center nurseries during the study period, for an attack rate of 27.5%. There is frequent movement of babies between the level II nursery and the NICU as care requires. The distribution of the affected babies and the pattern of their internursery transfers are shown in the Figure. The outbreak may have arisen in the level II unit with patient 1, who was subsequently transferred to the NICU. We presume that he introduced the disease to the NICU, where patient 2 became symptomatic eight days after the death of patient 1. Patients 3 and 4 became ill in the level II nursery. Patients 5 and 6 developed disease in the NICU five and eight days, respectively, after the onset of disease in patient 2 in the same unit. The remainder of the cases arose in the level II unit.

The precise pathway of transmission has not been elucidated, but may well have included symptomatic staff members. Two physicians and four nurses from the level II nursery and six nurses from the NICU reported gastrointestinal tract illness in the two weeks before the outbreak, during the outbreak, or in the two weeks following.

The clinical characteristics, signs of disease, and hospital course of the patients are summarized in Table I. Six patients had bacterial sepsis, four had perforations requiring surgery, and two patients died of septic shock.

No significant risk factors distinguished patients from controls, although all patients had some risk factor(s). The groups were nearly identical in birth weight distribution. Patients were neither more asphyxiated at birth nor more

Table I. Clinical characteristics of patients

Patient	Birth weight (gm)	Gestational age (wk)	APGAR score		Major medical problems*	NEC/HGE	Age at onset (days)
			1 min	5 min			
1	2200	35	1	3	TTN, polycythemia, asphyxia	NEC	2
2	900	28	6	7	RDS, PDA lig.	HGE	11
3	2500	35	5	8	TTN, Rh x-Tx	NEC	3
4	2340	35	3	1	Asphyxia	HGE	5
5	1300	29	8	8	RDS, PDA	NEC	5
6	820	29	9	10	RDS, PDA indo.	NEC	8
7	1920	37	8	9	SGA	NEC	2
8	1720	37	6	8	Down syndrome, CHD	NEC	19
9	1940	32	8	9	TTN	NEC	4
10	1050	28	4	6	RDS	HGE	26
11	2150	34	8	7	TTN, GC	NEC	4

*CHD, Congenital heart disease; GC, maternal gonorrhea; indo, treated with indomethacin; lig, ligation; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; Rh, Rh incompatibility; SGA, small for gestational age; TTN, transient tachypnea of newborn; x-Tx, exchange transfusion.
†AB, Apnea and bradycardia; AD, abdominal distention; AT, abdominal tenderness; D, diarrhea; T, thrombocytopenia; V, vomiting/gastric residuals; VL, visible loops of bowel; WD, abdominal wall discoloration.

stressed in the postnatal period by other factors previously associated with the development of NEC. Included among the risk factors analyzed were umbilical catheters, congenital heart disease, patent ductus arteriosus, hypotension, respiratory distress syndrome, bronchopulmonary dysplasia, ventilator use, apnea and bradycardia, anemia, polycythemia, jaundice, exchange transfusions, intraventricular hemorrhage, previous surgery, cardiac catheterization, and chromosomal or other anomalies. Previous antibiotic exposure was nearly identical in the two groups. Although none of the patients had been fed human milk exclusively (compared with six controls), more asymptomatic infants had been exclusively fed formula. None of the feeding patterns were associated with disease. All infants with NEC and HGE had been fed prior to onset of symptoms.

No *Salmonella, Shigella, Campylobacter,* or *Yersinia* species were detected in culture. Only two patients had *Escherichia coli* strains in their stool, and none had *Staphylococcus aureus. Klebsiella pneumoniae* was present in 70% (seven of 10) of stool specimens from patients and in 65% (11 of 17) from controls. *Klebsiella* was also detected in peritoneal cultures in patients 4 and 10. There was no *Clostridium difficile* toxin in any of the patients.

Virus was not cultured from stool filtrate, nasopharyngeal washings, or urine, nor were viral particles seen on electron microscopy of stool. ELISA for coronavirus, adenovirus and coxsackie virus yielded negative results. Rotavirus antigen, however, was detected in the stools of seven of 10 symptomatic infants by ELISA (Table II). Specificity was confirmed by lack of reaction with preimmune antiserum.[15] In none of the asymptomatic controls were stool ELISA HRV positive ($P = 0.0001$).

In 40 nursery personnel tested, 11 (two physicians, nine nurses) had elevated IgM titers to HRV, indicating recent infection (Table III). The attack rate for infection among staff members was 27.5%, identical to the attack rate in the neonates. Ten of these 11 personnel worked in the level II unit, and only four of them had gastrointestinal tract symptoms. Thus, of the 12 staff members who responded affirmatively to the survey for gastrointestinal tract symptoms, serologic studies for HRV were positive in four. Seven asymptomatic personnel also had IgM antibody to HRV. Three babies with NEC had elevated IgM to HRV, and one of the infants with HGE had a serologic rise. One of these serologically positive infants did not have detectable HRV antigen in the stool, but should be counted as an eighth baby among the 10 symptomatic patients tested who had evidence of HRV infection. None of the asymptomatic infants had IgM antibody to HRV.

All infected infants as well as all infants present in the nursery with them ("exposed") were strictly isolated. One of the exposed babies (patient 11) subsequently developed

Signs at disease onset†	Blood culture isolates	Outcome
AT, AD, V, T	Enterococcus, *C. perfringens*	Perforation, shock, death
AD, VL, D, V, AB, T	*S. marcescens*	Recovered
AT, AD, V, AB, T		Recovered
V, AB, T	*K. pneumoniae*	Recovered
AT, AD, WD, VL, D, V, AB, T	*K. pneumoniae*	Perforation, recovered
AD, WD, VL, D, V, AB		Perforation, recovered
AD, WD, VL, D, V, T	*E. coli*	Recovered
AT, D		Recovered
AT, AD, WD, VL, D, V, AB, T	*C. perfringens*	Perforation, shock, death
AD, D, V, AB		Recovered
D, V, AB		Recovered

disease; this was the final case of the cluster. It was necessary to close one of the nurseries to new admissions for seven days to fulfill the criteria of our isolation protocol, and absolute separation of nursing staff into the "exposed" or "clean" areas was maintained. Gowning and gloving were strictly enforced when entering the cohort area, and a change of gown and gloves was required when moving from baby to baby within the group.

Three weeks after the final case of the cluster, another baby in the level II nursery developed NEC. By this time, isolation procedures had been relaxed because none of the original infants remained in the nursery. Strict bedside enteric precautions were imposed, and no additional cases arose. Stool ELISA for HRV yielded negative findings in that infant, but he did have a positive serologic response to HRV, indicating recent infection.

DISCUSSION

Rotavirus was detected in the stools of 70% of the patients with NEC and HGE in this outbreak, whereas no HRV was present in the stools of unaffected infants from the same nurseries ($P = 0.0001$). An eighth infant in the cluster had serologic evidence of HRV infection, as did a ninth baby who developed disease three weeks after the last case of the cluster. In other clinical factors analyzed, including risk factors previously associated with NEC, there was no difference between the infected and control patients. All affected babies, however, had at least some suspected risk factor(s). There was no gastrointestinal tract disease among infants in the well-baby (level I) nursery at the same hospital.

Table II. Incidence of rotavirus antigen in stools

	Patients (n)	*Rotavirus +*	*Rotavirus −*
NEC	7	5*	2
HGE	3	2†	1
Controls	20	0‡	20

*Patients 3, 6, 7, 8, and 9.
†Patients 2 and 4.
‡Incidence of positive stool ELISA in patients with gastrointestinal tract disease (NEC, HGE) differs from that in asymptomatic controls with $P = 0.0001$.

Table III. Serologic (IgM) rotavirus response

	Tested	*Positive*
Nursery personnel	40	11
Neonates		
NEC	7	3*
HGE	3	1†
Asymptomatic	14	0‡

*Patients 3, 5, and 8.
†Patient 4.
‡Incidence of positive serology in patients with gastrointestinal tract disease (NEC and HGE) differs from that of asymptomatic infants with $P = 0.02$.

ELISA has been shown to be a sensitive tool for detecting HRV antigen, at least equal to the sensitivity of electron microscopy.[18] An IgM response to HRV has also been detected more reliably with ELISA techniques.[17] Our specimens for electron microscopy were shipped chilled, not frozen, and subjected to long delays in reaching Canada for analysis. This may account for the failure to find HRV particles in the stool samples by electron microscopy.

Rotavirus is the most common cause of nonbacterial gastroenteritis in childhood.[19,20] In newborn nurseries, HRV infection varies in prevalence from 0% to 49%.[21-26] Most HRV infections in the neonate, however, are asymptomatic or only minimally symptomatic, and serious disease in this group is uncommon. A diarrheal outbreak in a newborn unit was associated with HRV in 56% of symptomatic babies, but HRV was also present in 35% of asymptomatic infants.[27] Again, diarrhea was mild and of brief duration.

NEC may have many causes, and various infectious agents may play a role. Numerous outbreaks have failed to yield any associated pathogens.[3,28,29] It is unlikely that a single infectious agent or other risk factor will be found responsible for all NEC, and multifactorial explanations must be pursued.

In an animal model, rotavirus and enterotoxigenic *E. coli* have been shown to be more pathogenic in combination than alone.[30] A similar association between HRV and enteropathogenic *E. coli* strains has been proposed in neonatal diarrhea.[31] In our nurseries, *Klebsiella* species were ubiquitous in both symptomatic and asymptomatic babies. A potent synergism between HRV and Klebsiella might explain why nurseries with either agent alone have minimal NEC, whereas the combination in our nurseries was so dramatic. Five of our patients had both HRV and *K. pneumoniae* in their stools, and a sixth baby had *K. pneumoniae* in his stool and a serologic response to HRV. *Klebsiella* has been linked with epidemic NEC[32] and with periods of increased incidence of NEC.[33] The possibility of concurrent HRV (or other viral) infection was not investigated in those reports. Further studies of the importance of HRV, acting singly and in combination, in neonatal disease are necessary.

Early and strict implementation of infection control measures can be effective in stopping the spread of NEC through nurseries.[3] In this outbreak, the role of nursery personnel as vectors of disease seems quite plausible. Rotavirus is often an asymptomatic or minimally symptomatic disease in adults,[20] who nevertheless can introduce or transmit the infection to others. We suggest institution of bedside enteric precautions for the first NEC case in a nursery, with strict patient and staff separation into cohorts invoked at the appearance of a second case. Until further data are available on the relationship between HRV and NEC, all patients with NEC ideally should be evaluated for HRV infection. Although the detection of HRV in neonates does not always herald serious illness, the presence of HRV in patients with NEC and HGE should be considered significant for purposes of infection control and warrants the screening of other babies in the nursery.

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REFERENCES

1. Mizrahi A, Barlow O, Berdon W, Blanc WA, Silverman WA: Necrotizing enterocolitis in premature infants. J PEDIATR **66**:697, 1965.
2. Santulli TV, Schullinger JN, Heird WC, Gongaware RD, Wigger J, Barlow B, Blanc WA, Berdon WE: Acute necrotizing enterocolitis in infancy: A review of 64 cases. Pediatrics **55**:376, 1975.
3. Book LS, Overall JC Jr, Herbst JJ, Britt MR, Epstein B, Jung AL: Clustering of necrotizing enterocolitis: Interruption by infection-control measures. N Engl J Med **297**:984, 1977.
4. Kliegman RM: Neonatal Necrotizing enterocolitis: Implications for an infectious disease. Pediatr Clin North Am **26**:327, 1979.
5. Kliegman RM, Fanaroff AA, Izant R, Speck WT: Clostridia as pathogens in neonatal necrotizing enterocolitis. J PEDIATR **95**:287, 1979.
6. Cashore WJ, Peter G, Lauermann M, Stonestreet BS, Oh W: Clostridia colonization and clostridial toxin in neonatal necrotizing enterocolitis. J PEDIATR **98**:308, 1981.
7. Frantz ID III, L'Heureux P, Engel RR, Hunt CE: Necrotizing enterocolitis. J PEDIATR **86**:259, 1975.
8. Powell J, Bureau MA, Paré Claude, Gaildry M-L, Cabana D, Patriquin H: Necrotizing enterocolitis: Epidemic following an outbreak of *Enterobacter cloacae* type 3305573 in a neonatal intensive care unit. Am J Dis Child **134**:1152, 1980.
9. Johnson FE, Crnic DM, Simmons MA, Lilly JR: Association of fatal Coxsackie B_2 viral infection and necrotizing enterocolitis. Arch Dis Child **52**:802, 1977.
10. Chany C, Moscovici O, Lebon P, Rousset S: Association of coronavirus infection with neonatal necrotizing enterocolitis. Pediatrics **69**:209, 1982.
11. Egan EA, Mantilla G, Nelson RM, Eitzman DV: A prospective controlled trial of oral kanamycin in the prevention of neonatal necrotizing enterocolitis. J PEDIATR **89**:467, 1976.
12. Gryfack LJ, Scanlon JW: Oral gentamicin therapy in the prevention of neonatal necrotizing enterocolitis. Am J Dis Child **132**:1192, 1978.
13. Chang TW, Lauermann M, Bartlett JG: Cytotoxicity assay in antibiotic-associated colitis. J Infect Dis **140**:765, 1979.
14. Middleton PJ, Abbott GD, Szymanski MT, Bartolussi R, Hamilton JR: Orbivirus acute gastroenteritis of infancy. Lancet **1**:1241, 1974.
15. Kapikian AZ, Yolken RH, Greenberg HB, Wyatt RG, Kalica AR, Chanock RM, Kim HW: Gastroenteritis virusus. *In* Lennette EH, Schmidt NJ: Diagnostic procedures for viral, rickettsial and chlamydial infections, ed 5. Washington, D.C., 1979, American Public Health Association, pp 927-995.
16. Yolken RH, Lawrence F, Leister F, Takiff HE, Strauss SE: Gastroenteritis associated with enteric type adenovirus in hospitalized infants. J PEDIATR **101**:21, 1982.
17. Yolken RH, Wyatt RG, Kim HW, Kapikian AZ, Chanock RM: Immunological response to infection with human reovirus-like agent: Measurement of anti-human reovirus-like agent immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay. Infect Immun **19**:540, 1978.
18. Yolken RH, Kim HW, Clem T, Wyatt RG, Chanock RM, Kalica AR, Kapikian AZ: Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. Lancet **2**:263, 1977.
19. Steinhoff MC: Rotavirus: The first five years. J PEDIATR **96**:611, 1980.
20. Blacklow NR, Cukor G: Viral gastroenteritis. N Engl J Med **304**:397, 1981.
21. Murphy AM, Albrey MB, Crewe EB: Rotavirus infections of neonates. Lancet **2**:1149, 1977.
22. Chrystie IL, Totterdell BM, Banatvala JE: Asymptomatic endemic rotavirus infections in the newborn. Lancet **1**:1176, 1978.
23. Steinhoff MC, Gerber MA: Rotavirus infections of neonates. Lancet **1**:775, 1978.
24. Totterdell BM, Chrystie IL, Banatvala JE: Rotavirus infections in a maternity unit. Arch Dis Child **51**:924, 1976.

25. Rodriguez WJ, Kim HW, Brandt CD, Fletcher AB, Parrott RH: Rotavirus: A cause of nosocomial infection in the nursery. J PEDIATR **101:**274, 1982.
26. Dean AG, Bowden DK, Easa D, Waxman SH, Courtney P, Poon KA: Rotavirus in newborn nurseries: Negative results from Honolulu and the New Hebrides. Do hospital techniques protect against this gastroenteritis virus? Hawaii Med J **39:**170, 1980.
27. Cameron DJS, Bishop RF, Veenstra AA, Barnes GL: Noncultivable viruses and neonatal diarrhea: Fifteen-month survey in a newborn special care nursery. J Clin Microbiol **8:**93, 1978.
28. Virnig NL, Reynolds JW: Epidemiological aspects of neonatal necrotizing enterocolitis. Am J Dis Child **128:**186, 1974.
29. Ryder RW, Buxton AE, Wachsmuth IK, Mason E, Barrett FF: Heat-stable enterotoxigenic *Escherichia coli* and necrotizing enterocolitis: Lack of an association. J PEDIATR **91:**302, 1977.
30. Tzipori SR, Makin TJ, Smith ML, Krautil FL: Clinical manifestations of diarrhea in calves infected with rotavirus and enterotoxigenic *Escherichia coli.* J Clin Microbiol **13:**1011, 1981.
31. Bishop RF, Hewstone AS, Davidson GP, Townley RRW, Holmes IH, Ruck BJ: An epidemic of diarrhea in human neonates involving a reovirus-like agent and "enteropathogenic" serotypes of *Escherichia coli.* J Clin Pathol **29:**46, 1976.
32. Hill HR, Hunt CE, Matsen JM: Nosocomial colonization with *Klebsiella,* type 26, in a neonatal intensive-care unit associated with an outbreak of sepsis, meningitis, and necrotizing enterocolitis. J PEDIATR **85:**415, 1974.
33. Bell MJ, Feigin RD, Ternberg JL: Changes in the incidence of necrotizing enterocolitis associated with variation of the gastrointestinal microflora in neonates. Am J Surg **138:**629, 1979.