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Outbreak of Necrotizing Enterocolitis Caused by Norovirus in a Neonatal Intensive Care Unit

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Objectives To investigate an outbreak of necrotizing enterocolitis (NEC) in a neonatal intensive care unit (NICU) and to identify the etiology, describe illness risk factors, and develop control measures.

Study design A retrospective case-control study was performed including newborns with NEC and newborns without NEC, examining demographic factors and exposures to medications, staff members, and procedures before illness. Stool samples from affected newborns were collected and tested for bacteria, parasites, and viruses.

Results We confirmed a NEC outbreak in the NICU in January 1998 with 8 cases, including 2 deaths, clustered in time and space. Norovirus-like particles were identified in all available stools from cases; norovirus (NoV) was confirmed with reverse transcriptase polymerase chain reaction in 4 of 6 samples. NEC cases were younger, had lower Apgar scores, and received antibiotics longer than 25 control subjects. Three NICU health care personnel had more contact with cases than control subjects; 1 staff member recalled having gastroenteritis symptoms around the time of the outbreak.

Conclusions This report associates NoV with NEC. NoV appeared to precipitate NEC in predisposed infants. Spatial clustering and epidemiologic links between cases and a health care worker with gastroenteritis suggests that NoV should be investigated among the etiologies of NEC outbreaks and that interventions targeted to interruption of NoV transmission should be considered. (J Pediatr 2008;153:339-44)

Necrotizing enterocolitis (NEC) is a serious life-threatening condition affecting newborns, particularly those with low birth weight and prematurity. Additional risk factors for NEC are reported and include birth asphyxia, polycythemia, umbilical vessel catheterization, congenital heart disease, exchange transfusions, aggressive enteral feeding, hyperosmolar feeding formulas, indomethacin use, hypoalbuminemia, perinatal infection, respiratory distress syndrome, hypoxic ischemic encephalopathy, low Apgar scores, maternal pre-eclampsia, prolonged rupture of membranes, chorioamnionitis, and maternal cocaine use during pregnancy. Illness typically is diagnosed and categorized by criteria proposed by Bell and others in 1978, which take into account historic, systemic, gastrointestinal, and radiologic findings. No simple diagnostic test to determine the etiology of NEC is available, because it appears that NEC is the final common pathway of a variety of insults to the immature gut. However, when cases occur in clusters in neonatal nursery settings, a variety of infectious agents including viruses have been implicated as precipitating agents for this illness.

In January 1998, neonatal intensive care unit (NICU) staff in a large urban teaching hospital reported that NEC was developing in an unexpected number of newborns. Concurrently, staff of the hospital’s emergency department and of the occupational health clinic reported an increase in the number of cases of acute gastroenteritis, characterized by vomiting, among hospital personnel and patients being evaluated in the emergency department. We hypothesized that a transmissible infectious agent was involved in the NEC outbreak and sought to confirm and define the outbreak, identify risk factors for illness and the etiologic agent, and to determine whether cases of NEC occurring during the outbreak differed from those that had occurred sporadically in the preceding months and were not likely due to an infectious agent.
METHODS

The NICU was a 21-bed unit requiring special access for entry and staffed by 35 nurses and 35 physicians. Infants admitted to the NICU were delivered at the hospital, transferred from surrounding community hospitals, or transferred from other university hospitals when census exceeding capacity.

We reviewed records of newborns admitted to the NICU between January 1997 and December 1998. We identified those who met the criteria for definite and advanced NEC, as proposed by Bell3; history of perinatal stressor (prematurity, intrauterine growth retardation, pulmonary disease, abnormalities of labor/delivery, multiple birth, maternal or infant infection, umbilical catheter, or exchange transfusion), systemic signs of illness (temperature instability, lethargy, apnea, bradycardia, deterioration of vital signs, shock, or gastrointestinal hemorrhage), gastrointestinal signs or symptoms (poor feeding, increasing preavage results, emesis, or occult blood in stool, plus persistent blood in stool or marked abdominal distention), and abnormal abdominal radiographic findings (significant bowel distention with ileus, bowel wall edema, peritoneal fluid, pneumatosis intestinals, portal vein gas, or pneumoperitoneum). We calculated monthly attack rates expressed as the number of NEC cases per 100 admissions to the NICU. Monthly attack rates were plotted as a function of time. We compared incidence densities with a Poisson model, as described by Rothman and Greenland.5

From medical records and nursing notes, we logged the bed assignments of affected newborns from admission to illness onset and applied the Griston test for spatial clustering. This statistical test examines the geographic locations of ill and non-ill persons by comparing the observed number of adjacent borders shared by persons in the illness-containing area with an expected number with the assumption (null hypothesis) that ill persons are randomly distributed throughout the area being assessed.6

We conducted a case-control study categorizing as cases the newborns in the NICU with a gestational age at birth <34 weeks in whom NEC developed in January 1998. We categorized as control subjects the newborns in the NICU with a gestational age at birth <34 weeks in whom NEC did not develop. Newborns with a gestational age at birth >34 weeks were excluded. We abstracted demographic, epidemiologic, and clinical data from the medical records of the newborns and their respective mothers by means of a standardized form. Demographic data collected included date of birth, sex, race, weight and gestational age at birth, and birth order, when applicable. Clinical data included Apgar scores at 1 and 5 minutes of life; evidence of severe (<10th percentile of weight for age) intrauterine growth retardation; age at first enteral feeding and type of feeding; administration of antibiotics and duration of antibiotic therapy; administration of total parenteral nutrition, indomethacin, cafféine, surfactant, hista-mine, blocking agents, corticosteroid, granulocyte-colony stimulating factor, and cardiovascular pressor agents; diagnosis of patent ductus arteriosus, and respiratory distress syndrome or hyaline membrane disease and patent ductus arteriosus; place-
Analysis of the temporal distribution of cases by day showed a peak of 3 cases presenting on January 7, 1998, with 5 single cases presenting at irregular intervals between January 2 and January 16, 1998 (data not shown). The relative risk of NEC developing in January 1998 compared with the preceding 12 months was 10.4 (95% CI, 4.0-27.0; \( P < .0001 \)). The January 1998 rate of 23 NEC cases/100 NICU admissions would also exceed the monthly rate for the following 11 months. No additional cases of NEC were identified after January 17—the date control measures were implemented—although new patients continued to be admitted to the unit. Control measures included the cohorting of staff and infants, the mandatory donning of gowns and gloves when providing any care to newborns in the NICU, and the encouraging of hand washing. Hand washing was further facilitated in the NICU by the later replacement of antiquated sinks. Additionally, staff, parents, and visitors were asked to avoid coming to the NICU when they had symptoms of vomiting or diarrhea and to keep out until 72 hours had passed after resolution of symptoms.

The 8 cases of NEC appeared to be clustered in space (Figure 2). Six affected infants were housed in isolettes or incubators located at the end of the unit, which was farthest from the entry. Affected newborns were geographically clustered to a degree greater than expected by chance alone (Grimson test for spatial clustering; \( P = .01 \)). Newborns in adjacent isolettes or incubators tended to be cared for by the same staff member, because individual nursing assignments usually included patients in close physical proximity. To fairly distribute the workload of nursing staff in the unit, newborns requiring more intensive nursing care were distributed evenly throughout the NICU. The bed assignment method was stable with time, except for active cohorting after the diagnosis of NEC.

The case-control study demonstrated significant differences between the 8 cases and 25 control subjects (Table I). Infants in whom NEC developed were a median of 21 days old at illness onset (range, 5-38 days). They were born at significantly younger gestational ages, had lower birth weights, and had lower Apgar scores at 1 and 5 minutes of life compared with control subjects. Results of statistical analyses are summarized in Table I. Newborns in whom NEC developed had received antibiotics longer than control subjects, although no specific antibiotic was associated with the occurrence of NEC. Although there was no statistical difference in the frequency of administration of granulocyte colony stimulating factor, pressor agents, or corticosteroids in the period before illness onset in cases and during their entire NICU stay in control subjects, newborns in whom NEC developed were more likely than control subjects to have received histamine2 blocking agents, indomethacin, surfactant, and caffeine. Cases also were more likely to have been diagnosed with hyaline membrane disease and to have required intubation, although the duration of intubation was similar in cases and control subjects. Diagnosis of patent ductus arteriosus was similar in both groups. Cases were more likely to have had catheterization of the umbilical artery and were older at the time of first enteral feeding. Although no newborn in whom NEC developed was breastfed, a protective effect of breastmilk could not be assessed because few control newborns were fed breastmilk (≈ 20%). No deaths occurred in newborns in whom NEC did not develop.

Evaluation of contact between newborns and medical staff showed that cases had more contact with 3 NICU staff members (identified here as A, B, and C) than control subjects. Caregiver B, who was strongly associated with cases, recalled having vomiting and diarrhea around the time of the outbreak and provided care while ill. Of the 7 newborns in whom NEC developed in whose medical records Caregiver B made notations, 3 were documented within 7 days of onset of illness. Caregiver C made notations in all 6 of the medical records of cases in the 7 days before illness onset. No single medical care provider was found to have contact documented in the medical records with all the cases, although caregiver A was a physician who presumably had contact with all newborns in the NICU on a daily basis. This physician’s notations in the medical records of cases appeared to indicate an increased level of attention devoted to specific infants.

Bacterial, parasitic, and Clostridium difficile toxin assays on stool samples from case newborns yielded no evidence of pathogens. General viral cultures and rotavirus antigen en-
zyme immunoassay results were also negative. Six stool samples from cases were available for further analysis. Round, structured, 710-Angstrom particles morphologically compatible with a calicivirus were observed through electron microscopy of all 6 specimens. The presence of NoV in 4 of the stool specimens was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). Southern hybridization in 3 of the 4 specimens confirmed the presence of NoV; the fourth specimen was of a volume too small for further analysis. The 2 samples that tested negative with RT-PCR were also of very small volume.

We compared the clinical characteristics on presentation of the 8 cases presenting during the outbreak (epidemic cases) and 11 cases that had occurred sporadically in the preceding 7 months (endemic cases; Table II). Epidemic cases differed from endemic cases only in 1 laboratory characteristic: they had significantly lower percent of neutrophil band forms in peripheral leukocyte counts, although the ranges overlapped. Need for surgical intervention and rate of death did not differ in the 2 groups. Results of statistical analyses are summarized in Table II.

**DISCUSSION**

We present an outbreak of NEC associated with NoV. This association is supported by the identification of the virus in stools from cases clustered in time and space, epidemiologic implication of a staff member who recalled symptoms of gastrointestinal illness, and the occurrence of the outbreak during the traditionally-accepted winter peak of NoV illness in the United States.7 Affected newborns previously had described risk factors for NEC.

The presentation of NEC associated with NoV was clinically indistinguishable from that of NEC likely not associated with NoV. This finding highlights the multifactorial nature of NEC in which insults to the gut of various infectious and noninfectious types result in similar clinical illness. It also indicates that a NoV etiology of NEC cannot be determined clinically and must be confirmed virologically.

Evidence implicating viruses in NEC comes from investigations of NEC outbreaks. Stool samples, tissue samples, or both in these studies were found to contain either viral antigens (rotavirus,8,9 adenovirus10), virus-like particles with electron microscopy (coronavirus-like11), or viruses demonstrated by growth in tissue cultures (coxsackie B2,12 rotavirus,9 coronavirus,13 torovirus,14 echovirus 2215). Occasionally, serologic evidence of infection has been reported (rotavirus,9 echovirus 2215). In rotavirus- and coronavirus-associated outbreaks of NEC, medical staff have been weakly implicated in

| Characteristic                        | Cases* (n = 8) | Control subjects† (n = 25) | Odds ratio (95% CI‡) | P-value |
|--------------------------------------|---------------|---------------------------|----------------------|---------|
| Gestational age at birth in weeks (median, range) | 28 (24-33)    | 32 (25-34)               | –                    | .003§   |
| Birthweight in grams (median, range)   | 1073 (763-2106) | 1755 (782-2226)  | –                    | .21§    |
| Apgar score (median, range)           |               |                          |                      |         |
| 1 minute                              | 5 (1-9)       | 8 (2-9)                  | –                    | .03§    |
| 5 minutes                             | 8 (1-9)       | 9 (6-9)                  | –                    | .03§    |
| Days receiving antibiotics (median, range) | 7 (3-15)     | 3 (0-16)                 | –                    | .02§    |
| Medications                           |               |                          |                      |         |
| Indomethacin                          | 3             | 1                        | 12 (1-136)           | .01     |
| Caffeine                              | 6             | 7                        | 8 (1-75)             | .03     |
| Surfactant                            | 5             | 4                        | 9 (1.1-83)           | .02     |
| Histamine, blocker                    | 3             | 1                        | 14 (1-463)           | .04     |
| Hyaline membrane disease              | 5             | 3                        |                      |         |
| Procedures                            |               |                          |                      |         |
| Umbilical cord catheterization        | 5             | 3                        | 12 (1-136)           | .01     |
| Intubation                            | 5             | 4                        | 9 (1-83)             | .02     |
| Age at first enteral feeding, days (median, range) | 5 (2-17)     | 2 (1-9)                  | –                    | .002§   |
| Documented contacts¶                  |               |                          |                      |         |
| Caregiver A                           | 3             | 1                        | 14 (1-463)           | .04     |
| Caregiver B                           | 7             | 9                        | 12 (1-322)           | .02     |
| Caregiver C                           | 6             | 7                        | 8 (1-75)             | .04     |
| Death                                 | 2             | 0                        | 5 (3-11)***          | .01     |

*Cases were neonates ≤34 weeks gestational age housed in the NICU in whom NEC developed in January 1998.
†Control subjects were neonates ≤34 weeks gestational age housed in the NICU in whom NEC did not develop in January 1998.
‡95% CI.
§Wilcoxon rank sum test results are presented.
¶Odds ratios for exposures to caregivers reflect likelihood of illness in newborns exposed to the caregiver compared with newborns not exposed to the caregiver.
**Relative risk is presented. Odds ratio in this case is undefined.

Table I. Summary of significant findings of case-control study of necrotizing enterocolitis in neonates housed in the neonatal intensive care unit during January 1998 in whom necrotizing enterocolitis developed (cases) and in whom it did not (control subjects)
the transmission of infection, with a small proportion of staff members having either serologic findings suggestive of recent infection or viral particles detected in stool samples.8,9,13 Our findings implicate NoV as an additional agent associated with an outbreak of NEC in a NICU.

This investigation was limited by the lack of available samples from control subjects and staff for NoV testing. However, because studies evaluating the prevalence of NoV in newborns suggest that the detection of NoV in infants with symptoms of acute gastroenteritis is an uncommon event,16 we conclude that the detection of the virus in stool specimens from newborns affected during this outbreak suggests that NoV caused, or at a minimum contributed to, the NEC epidemic.

NoV association with NEC has significant implications for the investigation of cases of NEC, particularly when presenting in clusters, and for the development of control measures during outbreaks of NEC. During investigations of clusters of NEC cases, fecal samples should be collected from affected and non-affected newborns and from staff members. Samples should be submitted for general viral diagnostic testing and for testing for NoV. Institution of barrier precautions, cohorting of patients and staff, and enhanced hygiene are appropriate while waiting for results of virologic testing. In the case of NoV, control measures are hampered by this virus' low infectious dose and stability in the environment, but should include appropriate hand washing techniques, disposal of waste and soiled materials, and disinfection.17

### Table II. Summary of significant findings of comparison of necrotizing enterocolitis (NEC) cases among neonates housed in the NICU who developed NEC in January 1998 (epidemic cases) and those who developed NEC between June and December 1997 (endemic cases)

| Characteristic                                      | Epidemic cases* (n = 8) | Endemic cases† (n = 11) | Odds ratio (95% CI‡) | P-value |
|-----------------------------------------------------|-------------------------|-------------------------|----------------------|---------|
| Age at NEC onset in days (median, range)            | 21 (5-38)               | 14 (6-32)               | –                    | .27§    |
| Male sex                                            | 5                       | 5                       | 0.6 (0.1-6)          | .66     |
| Gestational age at birth in weeks (median, range)   | 28 (24-33)              | 27 (23-35)              | –                    | .56§    |
| Birthweight in grams (median, range)                | 1073 (763-2106)         | 1101 (493-2275)         | –                    | .74§    |
| Intrauterine growth retardation at birth¶           | 0                       | 1                       | 0 (0-27)             | 1.0     |
| Presenting signs                                    |                         |                         |                      |         |
| Blood in stool                                      | 4                       | 4                       | 2 (0.2-17)           | .66     |
| Documented apnea                                     | 4                       | 4                       | 2 (0.2-17)           | .66     |
| Abdominal distention                                 | 5                       | 10                      | 0.2 (0-3)            | .26     |
| Abdominal tenderness                                 | 6                       | 7                       | 2 (0.1-21)           | 1.0     |
| Vomiting                                            | 6                       | 7                       | 2 (0.1-21)           | 1.0     |
| Lethargy                                            | 2                       | 5                       | 0.4 (0-4)            | .63     |
| Temperature instability                              | 2                       | 1                       | 3 (0.2-121)          | .55     |
| Hyperglycemia                                       | 2                       | 3                       | 0.9 (0.1-11)         | 1.0     |
| Hypoglycemia                                        | 0                       | 0                       | Undefined            | –       |
| Hypotension                                         | 2                       | 2                       | 1 (0.1-22)           | 1.0     |
| Increased oxygen requirements                       | 4                       | 6                       | 0.8 (0.1-8)          | 1.0     |
| Radiography findings on presentation                |                         |                         |                      |         |
| Pneumatosis intestinalis                            | 7                       | 8                       | 3 (0.2-85)           | .60     |
| Evidence of perforation                              | 0                       | 1                       | 0 (0-27)             | 1.0     |
| Persistent dilated bowel loops                       | 2                       | 0                       | Undefined            | .16     |
| Portal air                                          | 2                       | 7                       | 0.2 (0-2)            | .17     |
| Leukocyte count on presentation (median, range)      | 15.6 (5.4-22.3)          | 12.6 (2.8-34.4)         | –                    | .74     |
| Percent segmented neutrophils                       | 46 (19-64)              | 40 (9-55)               | –                    | .26     |
| Percent band forms                                   | 0.5 (0-16)              | 17 (0-39)               | –                    | .02     |
| Percent lymphocytes                                  | 28 (5-54)               | 31 (9-54)               | –                    | .93     |
| Lowest total leukocyte count (median, range)***      | 8.9 (4.7-14.2)          | 11.6 (2.8-29.6)         | –                    | .30     |
| Highest total leukocyte count (median, range)***     | 19.1 (13.3-31.3)        | 17.2 (5.4-43.7)         | –                    | .97     |
| Lowest platelet count (median, range)***            | 146 (53-271)            | 198 (11-438)            | –                    | .36     |
| Highest platelet count (median, range)***           | 375 (124-454)           | 335 (100-686)           | –                    | 1.0     |
| Death                                               | 2                       | 2                       | 1 (0.1-22)           | 1.0     |

*Epidemic cases were neonates ≥34 weeks gestational age housed in the NICU in whom NEC developed in January 1998, the outbreak period.
†Endemic cases were neonates of any gestational age housed in the NICU in whom NEC developed between June and December 1997.
‡95% CI.
§Wilcoxon rank sum test results presented.
¶Growth retardation: <10th percentile of weight for age.
**Highest and lowest counts per mL during the illness.
We would like to acknowledge Rose Vitagliano, Rebecca L. Frankhauser, and Stephan Monroe for their contributions to this work and Thomas Fekete for his editorial assistance.

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