An Outbreak of Necrotizing Enterocolitis Associated with a Novel *Clostridium* Species in a Neonatal Intensive Care Unit

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An outbreak of necrotizing enterocolitis (NEC) occurred in 6 neonates within a 2-month period. Blood cultures from 3 of these neonates grew the same strain of what appears to be a novel clostridial species for which the name “*Clostridium neonatale*” has been proposed. A point-prevalence survey that used rectal swabs was performed in our intensive-care and intermediate-care nurseries, and it indicated that 20.8% of neonates carried this same “*C. neonatale*” strain despite having no evidence of NEC. In conclusion, we describe an outbreak of NEC associated with the novel species, and we suggest that, in larger neonates, carriage of this type of *Clostridium* species may be a necessary step in the multistage pathogenesis of NEC.

Necrotizing enterocolitis (NEC) is a gastrointestinal disease with high mortality rate (20%–40%) that is most commonly associated with premature neonates [1–8]. Although the pathogenesis of the disease is not completely understood, neonates with NEC present with bloody stools, feeding intolerance, and bowel mucosa damage due to intestinal ischemia [1, 2, 4, 6]. Perforation occurs when the ischemic damage is severe, and aggressive surgical intervention is often required. Microbial overgrowth of the gut is thought to contribute to the pathogenesis of NEC [1, 2, 4, 6, 9]. Whether NEC is caused by a specific infectious agent has not been delineated, but the occurrence of outbreaks suggests that there may be an infectious agent associated with this disease [1, 4, 6, 9, 10]. Furthermore, the hallmark finding of pneumatosis intestinalis (gas in the bowel wall) is thought to be derived from bacterial fermentation, particularly the fermentation of lactose [1]. A wide range of microorganisms have been associated with NEC, including various *Clostridium* species, coagulase-negative *Staphylococcus* species, and various gram-negative rods [1, 6–9, 11]. Recently, suggestions have been made that probiotics or treatment with microorganisms such as *Bifidobacterium* species may competitively replace pathogens or protect from inflammatory mediators that are associated with NEC and may reduce the risk of this disease in neonates [4, 6, 12, 13].

We describe an outbreak of suspected NEC that involved 8 neonates born between 30 weeks and term gestation, in whom a new species of clostridium, for which the name “*Clostridium neonatale*” has been proposed [14], was found in the blood cultures and stools of some. A point-prevalence survey of neonates in the neonatal intensive care unit (NICU) indicated that *C. neonatale* was found in the gut of 20.8% of neonates without symptoms of NEC. Although a causal role could not be conclusively shown, the role that this new species may play in NEC warrants further study.
Figure 1. Time course and age of patients with suspected cases of necrotizing enterocolitis (NEC) in the neonatal intensive care unit. All patients with suspected NEC had bloody stools. Those that were confirmed by radiology to have had NEC are indicated by black bars, and those where radiology was either inconclusive or negative are indicated by white bars.

MATERIALS AND METHODS

Culture for Clostridium species. Stool samples and rectal samples (swabs) were inoculated onto prereduced blood agar supplemented with vitamin K and hemin plates and incubated at 37°C in an anaerobic chamber. Suspected clostridial colonies were isolated for purity and were initially identified by use of the Anident rapid anaerobe identification strips (bioMerieux-Vitek). Subsequent biochemical, cellular fatty acid, and 16SrDNA analysis were performed at the Canadian Science Centre for Human and Animal Health. A detailed analysis of the taxonomic characterization will be published separately. Clinical isolates have been deposited with the American Type Culture Collection (ATCC; type strain BAA-265), and the 16S sequence has been deposited in GenBank (accession number AF275949).

Blood cultures from neonates were performed by injection of up to 5 mL of blood into BacT/Alert Pediatric bottles (Organon Teknika). The blood cultures were incubated in an automated BacT/Alert blood culture machine for a maximum of 5 days. Anaerobic culture for bottles flagged as growth-positive was as described above. Samples of the formula used to feed neonates in the NICU were cultured to determine whether any Clostridium species were present.

Pulsed-field gel electrophoresis (PFGE). The PFGE method used for analysis of these clostridial isolates was the same as reported in Alfa et al. [15], with the addition of thiourea (Sigma Chemical) to both the gel and the running buffer (0.05 mM final concentration). This reduces the “smearing” effect often associated with PFGE analysis of Clostridium species.

RESULTS

The neonatal facility at this 500-bed tertiary-care teaching institution includes a 13-bed NICU and a 20-bed intermediate-care nursery (ICN). Although the first case of NEC was diagnosed at this center, that neonate was subsequently transferred to a separate tertiary-care facility for bowel surgery and subsequently died (the only neonate of the outbreak who died). The neonate who presented as the second NEC case was also transferred to another center for bowel resection and then re-

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gestational age at birth, weeks</th>
<th>Sex</th>
<th>Abdominal radiograph</th>
<th>Blood culture at time of S-NEC</th>
<th>Stool culture at time of S-NEC</th>
<th>Therapy at time of S-NEC</th>
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<tbody>
<tr>
<td>1*</td>
<td>Term</td>
<td>F</td>
<td>NEC</td>
<td>ND</td>
<td>ND</td>
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<td>M</td>
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<td>“C. neonatale”</td>
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<td>8</td>
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<td>“C. neonatale”</td>
<td>“C. neonatale”</td>
<td>Amp, Gent, Metro</td>
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NOTE. Amp, ampicillin; Gent, gentamicin; Metro, metronidazole; ND, not done; NEC, necrotizing enterocolitis; S-NEC, suspected NEC; Vanco, vancomycin.

* This neonate underwent bowel resection surgery, was doing well after surgery, and then suddenly died. No other deaths occurred.

† This neonate underwent bowel resection surgery and, although there was no stool culture performed at the time of NEC, “Clostridium neonatale” was detected in the rectal swab culture at the time of the point-prevalence survey that was done 43 days after the onset of NEC in this neonate.

‡ This patient’s radiograph is shown in figure 2.
turned to this center. All other neonates remained at St. Boniface General Hospital for the entire course of their illness. The time course of the NEC outbreak is given in figure 1. There were 8 suspected cases of NEC over a 2-month period, compared with the historical rate of ~1–2 cases/year. All 8 cases had bloody stool, and table 1 lists other characteristics of these neonates. In our center, stool and blood samples are recommended for culture from neonates suspected of having NEC. The blood cultures from 3 neonates grew clostridial isolates that were all identified as *Clostridium clostridioforme* by the rapid anaerobe-identification panel used in this center (Anident code 6320210). A historical assessment of our blood-culture database indicated that we had never previously isolated *C. clostridioforme* from blood cultures of either neonates or adults. The cluster of 3 positive blood cultures with a unique anaerobe was felt to be significant enough to warrant submission of the isolates to the reference laboratory for species confirmation. The reference laboratory determination that this was a novel species prompted us to consider whether this species of clostridia might represent the etiologic agent for NEC. Of interest, the first case of NEC in this outbreak did grow a *Clostridium* species from a peritoneal swab taken when bowel surgery was performed. However, the isolate was not stocked and therefore was not available for further characterization. The radiograph (figure 2) from case 5 of the outbreak demonstrates the classic presentation of pneumatosis intestinalis in a neonate who also had a positive blood culture for a *Clostridium* species that was ultimately classified by the National Microbiology Laboratory (Canadian Science Centre for Human and Animal Health) as "*C. neonatale*.

A point-prevalence study was performed to determine whether *C. neonatale* was carried by neonates in our NICU and ICN. Although stool samples would have been preferable because they provide a larger sample, we wanted to sample all neonates on a given day; therefore, rectal swabs were obtained from all 24 neonates: 10 in the NICU and 14 in the ICN. Of the 24 neonates screened, 10 had no clostridial isolates and 14 had ≥1 species of clostridia (27 isolates). The species that were detected included *C. neonatale*, 5 isolates (21%); *Clostridium difficile*, 5 isolates (21%); *Clostridium paraputrificum*, 3 isolates (12.5%); *Clostridium butyricum*, 1 isolate (4.2%); and *Clostridium perfringens*, 3 isolates (12.5%). All species identification was performed by the National Microbiology Laboratory (Canadian Science Centre for Human and Animal Health). Using 16SrDNA sequencing of the 5 outbreak isolates from 3 neonates (blood and stool isolates listed in table 1) and rectal swab isolates from 5 neonates who were included in the point-prevalence survey, we established that the same species was detected. The analysis showed 99.9%–100% identity with each other for all strains analyzed. Of the 24 neonates surveyed, 5 carried *C. neonatale*. Charts were reviewed for all neonates who carried any species of clostridium in their bowel. One of these neonates had previously been part of the outbreak (case 2) and, therefore, had received antibiotic therapy for NEC. At the time of the prevalence survey, this neonate was still colonized with *C. neonatale* but no longer had NEC. Furthermore, there were 4 neonates who neither previously nor currently had NEC, despite the presence of *C. neonatale* in their rectal swab sample. One neonate who had only *C. paraputrificum* detected in the rectal swab sample was not part of the original NEC outbreak but had confirmed NEC at the time of the point-prevalence survey and was treated appropriately.

The clonality of the *C. neonatale* isolates from both the outbreak and the prevalence survey was confirmed by PFGE of the blood and stool isolates as well as the point-prevalence rectal swab isolates (figure 3). All isolates showed identical PFGE banding patterns.

The 3 neonates in the outbreak who had positive blood cultures for *C. neonatale* had received formula, but none of the formula samples evaluated grew any microorganisms. Culture supernatant fluid from broth cultures of *C. neonatale* did not

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**Figure 2.** Radiograph of neonate (case 5), showing necrotizing enterocolitis. This neonate had extensive bowel wall involvement (thick arrow) and a positive blood culture for "*Clostridium neonatale*." Air can also be seen in the portal vein distribution of the liver (thin arrow).
C. neo-

interest, the reference lab analysis demonstrated that positive blood cultures with the same strain of a significant potential to invade, given that 3 neonates had neonatale” has been proposed [14]. It appears that this strain has been described an outbreak of NEC that was associated with a novel species of clostridia for which the name “C. neo-

produce any cytopathic effect when tested in human foreskin fibroblast cell culture (data not shown).

DISCUSSION

We have described an outbreak of NEC that was associated with a novel species of clostridia for which the name “C. neonatale” has been proposed [14]. It appears that this strain has a significant potential to invade, given that 3 neonates had positive blood cultures with the same strain of C. neonatale. Of interest, the reference lab analysis demonstrated that C. neonatale is a strong fermenter of lactose, with lactic acid as a major end product and moderate amounts of butyric and acetic acid as end products. Furthermore, the strain was motile and was shown to digest milk, with gas production. These characteristics all fit with suggestions elsewhere [1, 12] that organisms capable of fermenting lactose and producing gas and/or butyric acid may be the critical characteristics of microorganisms capable of participating in what appears to be a complex, multifactorial pathogenesis that ultimately leads to the development of NEC in neonates. Because this clostridium strain was originally identified by Anident panels as C. clostridiiforme, it would be reasonable to expect that C. clostridiiforme might have been reported elsewhere in association with neonates. However, C. clostridiiforme has not been reported from stools of infants during the first year of life [16], whereas all the other species that we isolated from our point-prevalence survey have been detected within the first few weeks after birth [16]. In addition, C. clostridiiforme has not been reported as associated with NEC, although there are several reports of unidentified Clostridium species associated with NEC [7–9].

Although NEC is thought to be more prevalent in preterm neonates, the outbreak in our center involved mostly neonates that were near term (5 of 6 confirmed cases were born at ≥35 weeks gestation), which may explain the low mortality rate (1 of 6 confirmed cases). Indeed, our data support the suggestion by Kosloske et al. [7, 8] that those neonates with clostridia-associated NEC were larger, more mature neonates than those with NEC associated with other organisms. The role of Clostridium species in NEC is further supported by studies that have evaluated inflammatory markers in stool [9] and by probiotic therapy that has used Bifidobacterium species to prevent disease in the quail model of NEC [12]. As suggested by Caplan et al. [13], this may reflect how Bifidobacterium species negate the damage due to inflammatory mediators of the bowel (e.g., endotoxin translocation and/or butyric end products of Clostridium species).

The ability of staff (particularly physicians) to carry various Clostridium species on their fingers if hand washing is not adequately performed has been suggested as a potential means of transmitting this organism, and this may be related to outbreaks of NEC [17]. Staff frequently place their fingers in the mouths of neonates to soothe them or to test the integrity of the hard palate, and this may facilitate transfer of such organisms to the neonate. We did not assess this in the current study.

Although our data do not completely demonstrate a causal role for C. neonatale, because it could also be detected in neonates without symptoms of NEC, its invasive potential is apparent because it was detected in blood cultures of 3 neonates during the outbreak. This represents 100% of the blood cultures taken from neonates with suspected NEC during the outbreak. Indeed, the pathogenesis of clostridia-associated NEC may be analogous to the multifactorial disease due to C. difficile, where the presence of a particular pathogen is needed in combination with other critical factors that ultimately lead to disease manifestation. Further studies are needed to determine whether this particular species is the primary cause of NEC or whether it is one of many potential infectious etiologies that have the characteristics needed to contribute to the ultimate development of NEC. Formal classification as a new species (to be submitted in a separate manuscript), submission of strains to ATCC, and deposition of the 16SrDNA gene sequence to GenBank should facilitate future comparisons.
Acknowledgments

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References