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Probiotic administration can prevent necrotizing enterocolitis in preterm infants: A meta-analysis

Christine S.M. Lau [a,b], Ronald S. Chamberlain [a,b,c,*]

[a] Department of Surgery, Saint Barnabas Medical Center, Livingston, NJ, USA
[b] Saint George's University School of Medicine, Grenada, West Indies
[c] Department of Surgery, New Jersey Medical School, Rutgers University, Newark, NJ, USA

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ABSTRACT

Purpose: Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in preterm infants, affecting ~6–7% of very-low-birth-weight (VLBW) infants. Early intervention and aggressive treatment has improved clinical outcomes, but considerable morbidity continues to accrue to NEC survivors. This meta-analysis examines the impact of probiotics on the incidence of NEC and complications among VLBW infants.

Methods: A comprehensive literature search for all published randomized control trials (RCTs) assessing the use of probiotics to prevent NEC in VLBW infants was conducted using PubMed, Cochrane Central Registry of Controlled Trials, and Google Scholar (1966–2014). The incidences of NEC, sepsis, overall mortality, and time to reach full enteral feeds were analyzed.

Results: 20 RCTs involving 5982 preterm VLBW infants were analyzed. Risk of NEC was reduced by 49.1% (RR = 0.509; 95% CI, 0.385–0.672; $p < 0.001$), and overall mortality by 26.9% among infants receiving probiotics (RR = 0.731; 95% CI, 0.577–0.926; $p = 0.009$). An 8.1% reduction in sepsis was also observed in infants receiving probiotics (RR = 0.919; 95% CI, 0.823–1.027; $p = 0.137$). Time to reach full enteral feeds was reduced by 1.2 days among infants receiving probiotics (MD: −1.217; 95% CI, −2.151 to −0.283; $p = 0.011$).

Conclusion: The use of probiotic supplementation in preterm VLBW infants is associated with a significant reduction in the risk of NEC and overall mortality. Additional studies are required to determine the optimal genus, species, and dose of probiotic.

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in preterm infants [1]. NEC is characterized by ischemic necrosis of the intestinal mucosa, with associated inflammation and invasion of enteric gas forming organisms, and is a leading cause of morbidity and mortality among preterm infants, with the risk of developing NEC inversely proportional to birth-weight [2]. More specifically, Fitzgibbons et al reported on 71,808 very-low-birth-weight (VLBW) infants in the Vermont Oxford Network and found that NEC affected approximately 3% of all infants between 1251 and 1500 g and up to 12% of infants <750 g [3]. Mortality ranged from 15.9% in infants between 1251 and 1500 g to 42.0% in those <750 g [3]. Extremely-low-birth-weight (ELBW) infants, defined as infants born <1000 g, have a three times greater risk of developing NEC, compared to very-low-birth-weight (VLBW) infants, defined as infants <1500 g [3]. Even among those who survive NEC, long-term consequences, including intestinal stricture, short bowel syndrome, and both growth and neurodevelopmental delays are common [4,5].

The diagnosis of NEC is primarily made by clinical suspicion and confirmed with diagnostic imaging. Initial symptoms affected infants exhibit include feeding intolerance, increased gastric residuals, abdominal distension, and bloody stools [2,6]. Abdominal radiography is the current modality of choice to confirm the diagnosis of NEC, and is especially useful in diagnosing NEC in the later stages [6]. The most accepted clinical criteria for diagnosing NEC is the Bell's criterion, which includes both clinical and radiological findings as detailed in Table 1 [7,8].

The etiology of NEC is multifactorial, involving a combination of dysbiosis, intestinal immaturity, and an excessive inflammatory response to luminal microbial stimuli [9]. Many different species of bacteria have been implicated in the development of NEC. Smith et al studied 163 premature infants, and reported that premature infants who develop NEC were generally those who were colonized predominantly by Gram-positive bacteria, while those who did not develop NEC had more diverse colonization with Gram-positive and Gram-negative bacteria. They also reported that colonization by *Staphylococcus* spp. was significantly more prevalent among infants who developed NEC compared to those who did not develop NEC [10].

To date, a number of approaches have been evaluated and adopted for the prevention of NEC. The most common method is to withhold enteral feeds in infants at risk of developing NEC; however, the precise timing to begin initial feeds remains controversial. Some studies suggest

Abbreviations: NEC, necrotizing enterocolitis; VLBW, very-low-birth-weight.

* Corresponding author at: Department of Surgery, Saint Barnabas Medical Center, Professor of Surgery, Rutgers University, New Jersey Medical School (NJMS), 94 Old Short Hills Rd. Livingston, New Jersey, USA 07039. Tel.: +1 973 322 5195; fax: +1 973 322 2471.
E-mail address: rchamberlain@barnabashealth.org (R.S. Chamberlain).

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Table 1
Bell's staging criteria for necrotizing enterocolitis [7,8].

Stage 1 (suspected NEC)	
Systemic manifestations	Lethargy, temperature instability, apnea, bradycardia
Gastrointestinal manifestations	Gastric residuals, mild abdominal distension, occult blood in stool
Abdominal radiographs	Nonspecific, distension with mild ileus
Stage 2 (definite NEC)	
Signs and symptoms	Above signs and symptoms, plus: absent bowel sounds, abdominal tenderness, marked abdominal distension
Abdominal radiographs	Pneumatosis intestinalis or portal venous air
Laboratory changes	Metabolic acidosis, thrombocytopenia
Stage 3 (advanced NEC)	
Signs and symptoms	Above signs and symptoms, plus: unstable vital signs, evidence of septic shock, marked gastrointestinal hemorrhage
Abdominal radiographs	Pneumoperitoneum
Laboratory changes	Metabolic and respiratory acidosis, disseminated intravascular coagulation

that rapid increase in feeding increases the risk of NEC [9,11,12]. Conversely, completely withholding enteral feeds leads to a prolonged use of parenteral nutrition, causing subsequent intestinal atrophy, increased permeability and inflammation, and late-onset sepsis [9,12]. In a meta-analysis by Morgan et al (2011), these authors demonstrated that delaying enteral feeds beyond four days of age was not associated with a reduced risk of NEC and that the delay in enteral feeds was actually associated with a longer time required to establish full enteral feeds [13]. The use of human milk in the prevention of NEC has also been studied, and has been shown to be protective against NEC. Sullivan et al studied 207 infants and reported that infants fed exclusively human-milk had significantly lower rates of NEC compared to infants receiving both human and bovine milk [14]. Several authors have also suggested that avoidance of H2 blockers may be useful, given that gastric acid prevents the cascade of infectious and inflammatory events leading to NEC [15,16].

More recently, probiotics have been proposed in the prevention of NEC. Since abnormal bacterial colonization has been implicated in the pathogenesis of NEC, it is hypothesized that probiotics, which are live microbial food supplements that improve intestinal microbial balance, may be beneficial in prevention [17]. Various species of probiotics have been studied, with the most common being species within the *Lactobacillus* and *Bifidobacterium* genus. More recently, *Saccharomyces boulardii*, a yeast, has also been considered as a probiotic.

Yang et al demonstrated a 66% reduction in the risk of NEC in preterm infants with the use of probiotics and a 42% reduction in the risk of mortality, with no significant decrease in the risk of culture-positive sepsis [18]. The most recent Cochrane Review reported a 59% reduction in the risk of NEC and a 34% reduction in the risk of mortality [19]. A number of randomized controlled trials (RCTs) not included in the study by Yang et al or Cochrane Review has since been published, and the results are conflicting [20–24]. Serce et al (2013) conducted a study involving 208 VLBW infants (104 receiving probiotics and 104 receiving placebo), and reported 7 cases of NEC cases in both the *Saccharomyces* and placebo groups (RR = 1.00, $p = 1.00$). There was also no significant difference in culture-positive sepsis (RR = 0.69, $p = 0.29$) or mortality (RR = 0.89, $p = 0.74$) [20]. Conversely, Jacobs et al studied 1099 VLBW infants (548 receiving probiotics and 551 receiving placebo), and reported a significant reduction in NEC incidence (RR = 0.46; 95% CI, 0.23–0.93; $p = 0.03$) with the use of probiotics, but no significant difference in sepsis (RR = 0.81; 95% CI, 0.61–1.08; $p = 0.16$) or mortality (RR = 0.97; 95% CI, 0.58–1.62; $p = 0.91$) [21].

This current meta-analysis provides an updated perspective on the impact of probiotics on the incidence of NEC, sepsis, overall mortality, and time to reach full enteral feeds, while further clarifying the conflicting findings that have been found in recent RCTs. Given the high morbidity and mortality of NEC, the use of probiotics could be a simple, readily available, low-cost supplement to help with this global problem and may play an important role in the future care of preterm VLBW infants.

1. Materials and methods

1.1. Study selection

A comprehensive search of all published RCTs evaluating probiotics to prevent NEC in VLBW infants was conducted using PubMed, Cochrane Central Registry of Controlled Trials, and Google Scholar (1966–2014). Additional citations were searched, using the references of the articles retrieved from prior publications. The last search was conducted on July 28, 2014 and only articles written in English were considered. Keywords searched included combinations of "probiotics", "*Lactobacillus*", "*Bifidobacterium*", "*Saccharomyces*", "necrotizing enterocolitis", "neonate", "infant", "preterm", and "very-low-birth-weight". The following inclusion criteria were used: randomized controlled trials (RCTs) involving VLBW (<1500 g) preterm (<37 weeks gestational age) infants with enteral administration of probiotics initiated within 10 days of life and continued for a minimum of 7 days. In case of duplicate publications, only the most recent and updated report of the clinical trial was included.

1.2. Data extraction

Articles retrieved from the searches were assessed for eligibility, and data pertaining to patients, intervention, control groups, outcomes, and methodology, were abstracted (Fig. 1). Clinical outcomes of interest included incidences of NEC stage ≥2 (according to the modified Bell staging criteria), incidence of sepsis (confirmed with a positive blood culture), overall mortality (death owing to any cause prior to discharge), and time to reach full enteral feeds (in days) [7].

1.3. Statistical analysis

For each trial, relative risk (RR) with a 95% confidence interval (95% CI) for NEC, sepsis, and mortality was calculated. Meta-analysis of the pooled data was performed using the Comprehensive Meta-Analysis software Version 3 (Biostat, Englewood, NJ). For studies reporting zero events in any group, a continuity correction factor of 0.5 was adopted to calculate the RR and variance. In the event of zero events in both groups, the RR was not calculable and the study was excluded from the meta-analysis. Both the fixed-effects model and random-effects model were considered, depending on the heterogeneity of the included studies. To assess the heterogeneity between studies, both Cochrane's Q statistic and I^2 statistic were used. Heterogeneity was considered statistically significant when $p < 0.05$ or $I^2 > 50$. If heterogeneity was observed, data were analyzed using a random-effects model. Conversely, in the absence of heterogeneity, a fixed-effects model was assumed. The publication bias regarding the RR of NEC in VLBW infants receiving probiotics was first visually evaluated by funnel plot, and further evaluated using Egger's and Begg's tests. A two-tailed p-value of <0.05 was considered statistically significant. Subgroup analysis was performed based on which genus of probiotics was used — *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, and a mixture of probiotics.

2. Results

2.1. Demographic characteristics of the studies

A total of 20 RCTs were identified, involving a total of 5982 preterm VLBW infants (Table 2) [25–39]. 2983 of these infants received enteric probiotic supplementation, and 2999 infants received a placebo. Among infants who received probiotics, 1058 received *Lactobacillus*,