Effectiveness of probiotics in reducing Necrotizing Enterocolitis (NEC) severity in preterm or low birth weight infants: A review of the literature

abstract

BACKGROUND AND OBJECTIVE: Necrotizing enterocolitis (NEC) is a debilitating and often deadly bowel disease affecting premature and low birth weight neonates. Probiotics may be useful in decreasing incidence and severity of NEC. Our objective was to review the most recent literature on the use of probiotics to reduce NEC severity in preterm and/or low birth weight neonates.

METHODS: A PubMed search using keyword “NEC, necrotizing enterocolitis, probiotic*” was conducted. Studies were considered if at least one probiotic was used in an attempt to reduce NEC incidence or severity in preterm or low birth weight infants. Original studies from 2012-2014 were considered. Meta-analyses and summary articles were excluded.

RESULTS: Ten studies met inclusion criteria. Four found statistically significant results in the reduction of incidence of NEC in neonates, one found a protective effect against the need for NEC surgery, and five studies found no benefit in preventing NEC.

CONCLUSIONS: Of the six randomized controlled trials included in this study, only two found a statistically significant reduction in NEC incidence. Although the efficacy is unclear, all ten studies did support the safety of probiotics administration. Further research utilizing specific promising probiotic strains, including *Bifidobacterium infantis* and *Lactobacillus rhamnosus*, should be carried out to continue determining efficacy of these strains.
Necrotizing enterocolitis (NEC) is one of the most devastating complications endured by hospitalized infants. NEC is an ischemic bowel disease, often manifesting initially as abdominal distention and bloody stools, and progressing as far as bowel resection or even death. The exact etiology of NEC is unknown. The incidence and prevalence of NEC varies based on location worldwide, race, birth weight, and gestational age. Low birth weight and premature infants are more likely to be affected by NEC. Estimates of NEC incidence in the United States fall around 0.1% of all births, with risk of NEC varying from 3-12% of very low birth weight (<1500 grams) infants, with highest risk in the lowest birth weight category of 501-750 grams. Much research has been done in the last quarter-century in an attempt to gain knowledge surrounding this deadly illness. Animal models have given insight into potential mechanisms of the ischemia, while human studies have provided breakthroughs on clinical prevalence and management. Because of the devastating outcomes of developing NEC, research into prevention strategies is a high priority. Some prophylactic measures, such as exclusive human milk feedings, are well accepted in practice; while others, such as the use of probiotics to assist in preventing NEC, are more contested.

Although the exact etiology of NEC is unknown, it is generally accepted that the pathophysiology of the disease is a complex mix of various influencing factors. It is not as well known how factors such as the type, amount, and diversity of microorganisms in the gut of infants contributes to the development of NEC. However, prematurity, mucosal injury, inflammatory response, bacterial translocation, bacterial overgrowth, low gut bacteria species diversity, and the presence of potential pathogenic organisms are thought to potentially contribute.

Because the exact etiology is still unknown, treatment of NEC is general, supportive care. Typically, patients who are exhibiting clinical symptoms of NEC are made NPO (nil per os, nothing by mouth) for bowel rest and undergo a ~14 day course of broad spectrum antibiotics to mediate the bowel ischemia and infection. This bowel rest can further lengthen the amount of time it takes to reach full enteral feedings, lengthening hospital stays and increasing costs of care.

Because of the devastating burden NEC brings to individuals, families, and health care systems, there is great interest in discussing strategies to prevent its occurrence. One such strategy is the administration of probiotics. Probiotics are live microorganisms, such as bacteria, that are hypothesized to be beneficial in assisting with the prevention of NEC in neonates. An infant with a gut microbiome that is void of the 'beneficial' bacteria or low in bacterial diversity may be at an increased risk for NEC. By adding these ‘beneficial’ bacteria, they may outcompete the ‘pathogenic’ bacteria, promote a stronger gut wall structure to prevent mucosal injury, and assist in promoting a positive immune response for the infant. Probiotics are relatively accessible and inexpensive.

Not all probiotics are created equal. Some strains are more widely tested in the use of NEC prevention. Each probiotic strain has different properties and may behave in differing ways in the gut. Gram-positive microorganisms such as Lactobacilli and Bifidobacteria...
are thought to be beneficial in balancing out the coliforms typically found in a preterm newborn’s gut.\textsuperscript{11}

There have been a number of studies reported on the use of probiotics to prevent NEC in the past decade. This analysis focuses on the most recent studies, published from January 2012 to August 2014, to provide a brief summary of the most current work in the field of probiotic use to prevent NEC.

**METHODS**

A review of the literature was performed using PubMed database by searching “NEC, probiotic*, necrotizing enterocolitis”. Studies published from 2012-2014 with risk of NEC as at least one outcome of study were included. Only original studies were utilized. Review articles and meta-analyses were not included.

**RESULTS**

Of the search results, 10 studies were found to meet inclusion criteria. Four of these were classified as cohort studies, while the remaining six were randomized trials. The majority of the studies (n=7) were conducted outside of the United States. A variety of different protocols were used throughout, including varying probiotic strains, dosages, and initiation and duration timelines. Sample sizes ranged from a prospective randomized controlled trial of 101 infants to 5351 infants in one observational cohort study.

A double-blind, randomized controlled trial was conducted in 13 NICUs from Italy and New Zealand.\textsuperscript{12} A total of 743 very low birth weight infants were randomized to receive bovine lactoferrin (BLF) + *Lactobacillus rhamnosus GG* (LGG) (6 x 10\textsuperscript{9} colony forming units/day) or bovine lactoferrin alone (100 mg/day) or placebo. Animal studies had shown a beneficial effect of lactoferrin on reducing NEC, and LGG is thought to increase the efficacy of lactoferrin, although this had not previously been studied in a human model. The findings showcased a significantly lower incidence of NEC in the BLF and BLF+LGG groups vs. control (BLF + LGG vs. control: RR 0.00 (p<0.001)). The incidence of death and/or NEC was also significantly reduced in both the BLF and BLF+LGG groups vs. control (BLF + LGG vs. control: RR = 0.37 (0.18-0.77) p=0.006).

Although the incidence of NEC was significantly lower in the intervention groups, no direct comparisons were made between the two treatment groups. Therefore, it remains unclear whether the BLF or LGG was contributing to decreased NEC rates, although since both BLF + LGG and BLF alone saw reduced NEC incidence, it is likely that the probiotic LGG was not the critical intervention ingredient.

A second double-blind, randomized controlled trial included 1099 infants from hospitals in Australia and New Zealand.\textsuperscript{13} A 1.5 gram powder probiotic combination of *Bifidobacterium infantis*, *Streptococcus thermophilis* and *Bifidobacterium lactis* was administered daily to infants <1500 grams birth weight and younger than 32 completed weeks gestational age who were randomized to the intervention group until discharged or term corrected age. The group found a reduced incidence ≥ stage 2 NEC in the intervention group (2% vs 4.4%, RR = 0.46, 95% CI 0.23-0.93, p=0.03).

A retrospective cohort study from the United States found similarly positive results.\textsuperscript{14} Hunter et al. compared infants who were born before a departmental protocol change of introduction of 0.1 ml of the probiotic *Lactobacillus reuteri* with those infants born after protocol change who weighed...
≤1000 grams at birth. The incidence of NEC was significantly lower in the neonates given probiotics versus the controls (2.5% versus 15.1%, p=0.0475). No adverse events related to probiotic use were noted.

A Canadian prospective cohort study by Janvier et al. studied whether routine administration of a 0.5 gram probiotic mixture (Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, and Lactobacillus rhamnosus GG) would reduce the incidence of NEC. All infants before 48 hours of age and <32 weeks gestational age received probiotics beginning in August 2011, from the first feed until the infant reached 34 weeks gestational age. Study participants admitted prior to August 2011 served as the control group. After adjusting for confounding factors such as gestational age, intrauterine growth restriction, and gender, a significant reduction in NEC was found in the probiotics group (OR 0.51, 95% CI 0.26-0.98). There was no effect of probiotics on health care-associated infection. However, it is notable that when stratified to only infants <1001 grams birth weight, all results were non-significant, including reduction in NEC between groups. In addition, the proportion of NEC within the first two weeks of life, ‘early cases’, was increased in the probiotic group, although not significantly. Overall, this study did find a reduced frequency in NEC in this NICU with use of a probiotic mixture.

In Germany, Hartel et al. conducted an observational cohort study using 5351 infants divided into three groups based on probiotic use in the facility caring for the infant: 1) no prophylactic probiotic use in the facility, 2) facility change of probiotic nonuser to user during observational period, or 3) probiotic use currently in facility. Lactobacillus acidophilus and Bifidobacterium infantis were used, however the dosages and inclusion criteria differed between centers. Most centers administered 1 capsule once per day of probiotics and included infants with birth weight <1500 grams, although some restricted to <1000 grams. The use of probiotics was found to be protective against NEC surgery (OR 0.58, 95% CI 0.37-0.91). Duration of hospitalization was also significantly lower in the probiotic group (38 vs. 46 days, p=0.022). None of the positive blood cultures grew L.reuteri and no other adverse events were attributed to L.reuteri administration.

Serce et al. orchestrated a placebo controlled trial from Turkey of 208 infants ≤32 weeks gestational age and weighing ≤1500 grams at birth. These newborns were supplemented with Saccharomyces boulardii dosed at 50 mg/kg every 12 hours, starting with the first feeding until discharged. It is notable that the rate of NEC in this hospital NICU prior to this study was 17%, higher than the average rate. The authors found no statistically significant
In the United States, Al-Hosni et al. conducted a double-blind, randomized controlled trial with 101 premature infants with birth weight 501-1000 grams who were ≤14 days old at time of feeding initiation. The intervention group was given a combination of *Lactobacillus rhamnosus GG* and *Bifidobacterium infantis* (500 colony forming units) added once daily to 0.5 ml of their first enteral feeding and continued until discharge or 34 weeks gestational age. Although the primary outcome of this study was infant weight, the incidences of NEC and mortality were similar between the intervention and control groups. However, when the risk of mortality or NEC was combined, fewer infants in the probiotic group died or developed NEC (RR 0.39, 95% CI 0.17-0.87).

A retrospective cohort study from the United States by Li et al. analyzed charts to determine if a three-strain probiotic blend of *Streptococcus thermophiles*, *Bifidobacterium infantis*, and *Bifidobacterium bifidum* had an effect on morbidity and mortality of NEC. Infants who weighed <1500 grams at birth and received ≥ 5 days of feeding or probiotics were analyzed. The incidence and mortality of NEC was similar between the two groups. The severity of NEC was higher in the probiotics group (2.13 vs. 2.57, p=0.075). It is notable that the probiotics group did have a statistically lower average gestational age than the control group; lower gestational age is a known NEC risk factor.

**CONCLUSIONS**

Effectiveness of probiotics in assisting to prevent NEC

Among the ten studies collected for this analysis, four found statistically significant results in the reduction of incidence of NEC in neonate, one found a protective effect against the need for NEC surgery, and five studies did not conclude any significant findings (Table 1). Of the randomized controlled trials, viewed as the ‘gold-standard’ of research in this field, two found statistically significant results and four did not.

**Ideal gestational age or birth weight for intervention**

Inclusion criteria varied among the five studies with statistically significant findings. The majority of these studies used birth weight of <1500 grams and gestational age of <32 or <33 weeks to determine study participants. However, Janvier et al. found a loss of significance in the reduction of NEC for neonates <1001 when their results were stratified by infant birth weight. Thus, from this analysis, <32-33 weeks gestational age and birth weight of 1000-1500 grams appears to be the ideal inclusion criteria for use of probiotics to reduce NEC...
incidence or severity in premature infants.

Most effective probiotics

Summarizing findings from studies that use probiotics in an attempt to prevent NEC can be challenging due to the variety of probiotics that are administered. Within these ten studies, 12 different probiotics were used. It is difficult to determine whether individual probiotics are more effective, or if probiotics have a more profound effect when working in combination with other probiotics. Among the five studies that found significantly reduced NEC incidence or risk, *Bifidobacterium infantis* and *Lactobacillus rhamnosus* were the most common strains. Four of these studies used combination probiotics, while one study used just a single strain.

Safety of probiotics

No adverse events related to the use of probiotics were found in any of the studies included in this analysis. In fact, many of the studies found other positive results from probiotic use not related to NEC. Janvier et al. found that the duration of intravenous nutrition was significantly shorter in the probiotic period of their cohort study. The randomized controlled trial by Al-Hosni et al. found a higher calculated growth velocity in their probiotic intervention group, which is an especially important parameter in premature infants. A second randomized controlled trial found the time to reach full enteral feedings and the duration of hospitalization were significantly lower in their probiotic intervention group.

Overall, half of the studies in this analysis found promising reductions in incidence or severity of NEC in premature neonates. However, of the six randomized controlled trials, only two found positive results. Importantly, no adverse events were found in any of the studies related to probiotic administration. Additionally, other benefits of probiotics were identified without any harmful side effects. The administration of probiotics in neonates <33 weeks gestational age and 1001-1500 grams birth weight is a safe strategy to assist in preventing NEC.

LIMITATIONS AND FUTURE DIRECTIONS

While this review does provide important insights into the most recent evidence on the use of probiotics to assist in NEC prevention, some important limitations should be considered. This review only included studies from January 2012 – August 2014 and was unable to pool data into a meta-analysis. Further research into the use of probiotics to assist in preventing NEC is warranted. While important to note that the administration of probiotics appears to be safe, the efficacy of probiotics in general is not clear. The studies in this field contain a diverse array of probiotic strains, inclusion criteria, administration amounts and methods, and duration times. To maximize resources, additional studies comparing the promising strains of *Bifidobacterium infantis* and *Lactobacillus rhamnosus* identified in this analysis, should be performed. Varying inclusion criteria should continue, and analyses should be stratified according to gestational age and weight to continue to narrow the neonatal population that may most benefit from the use of probiotics to assist in the prevention of NEC.

*Note: In September 2014, after beginning this review paper, a Cochrane review was published that analyzed randomized controlled trials from several databases from 1966 to 2013. This review found evidence to support using probiotics to
reduce occurrence of NEC in premature infants with birth weight less than 1500 grams, while evidence for effectiveness in infants with birth weight less than 1000 grams is not conclusive.
REFERENCES


# Articles published January 2012 - August 2014 focused on probiotic use to reduce incidence or severity of NEC in preterm and/or low birth weight neonates

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study design</th>
<th>Subjects</th>
<th>Probiotic used</th>
<th>Significantly reduced NEC incidence or severity?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Hosni et al. 2012 USA</td>
<td>RCT</td>
<td>N=101</td>
<td>Lactobacillus rhamnosus GG + Bifidobacterium infantis</td>
<td>No Incidence of NEC similar between groups</td>
</tr>
<tr>
<td>Fernandez-Carrocera et al. 2013 Mexico</td>
<td>RCT</td>
<td>N=150 &lt;1500 g</td>
<td>Lactobacillus acidophilus + Lactobacillus casei + Lactobacillus planatarum + Bifidobacterium infantis + Streptococcus thermophilus</td>
<td>No Incidence of developing NEC similar between groups</td>
</tr>
<tr>
<td>Hartel et al. 2014 Germany</td>
<td>Retrospective cohort</td>
<td>N=5351 &lt;1500 g &gt;22+6 and &lt;32+0 GA</td>
<td>Lactobacillus acidophilus + Bifidobacterium infantis</td>
<td>Yes Protective for NEC surgery</td>
</tr>
<tr>
<td>Hunter et al. 2012 USA</td>
<td>Retrospective cohort</td>
<td>N=311 (79 probiotic, 232 comparison) ≤1000 g</td>
<td>Lactobacillus reuteri DSM 17938</td>
<td>Yes Reduced incidence of NEC</td>
</tr>
<tr>
<td>Jacobs et al. 2013 Australia/New Zealand</td>
<td>RCT</td>
<td>N=1099 &lt;1500 g &lt;33 weeks GA</td>
<td>Bifidobacterium infantis + Streptococcus thermophilis + Bifidobacterium lactis</td>
<td>Yes Reduced incidence of NEC</td>
</tr>
<tr>
<td>Janvier et al. 2014 Canada</td>
<td>Prospective cohort</td>
<td>N=611 (294 probiotic, 317 comparison) &lt;32 weeks GA</td>
<td>Lactobacillus rhamnosus GG + 4 types Bifidobacterium: breve, bifidum, infantis, longum</td>
<td>Yes Reduced incidence of NEC (when unstratified for weight)</td>
</tr>
<tr>
<td>Li et al. 2013 USA</td>
<td>Retrospective cohort</td>
<td>N=580 (291 probiotic, 289 comparison) &lt;1500 g</td>
<td>Streptococcus thermophilus + Bifidobacterium infantis + Bifidobacterium bifidum</td>
<td>No Incidence and mortality of NEC similar between groups</td>
</tr>
<tr>
<td>Manzoni et al. 2014 Italy/New Zealand</td>
<td>RCT</td>
<td>N=743 &lt;1500 g</td>
<td>Bovine Lactoferrin ± Lactobacillus rhamnosus GG</td>
<td>Yes Incidence of NEC significantly lower</td>
</tr>
<tr>
<td>Oncel et al. 2014 Turkey</td>
<td>RCT</td>
<td>N=400 ≤1500 g ≤32 weeks GA</td>
<td>Lactobacillus reuteri DSM 17938</td>
<td>No Incidence of NEC similar between groups</td>
</tr>
<tr>
<td>Serce et al. 2013 Turkey</td>
<td>RCT</td>
<td>N=208 ≤1500 g ≤32 weeks GA</td>
<td>Saccharomyces boulardii</td>
<td>No Incidence of NEC similar between groups</td>
</tr>
</tbody>
</table>