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Routine Supplementation of *Lactobacillus rhamnosus* GG and Risk of Necrotizing Enterocolitis in Very Low Birth Weight Infants

Andrea F. Kane, MD^{1,2}, Anisha D. Bhatia, MD^{1,2,3}, Patricia W. Denning, MD^{1,2}, Andi L. Shane, MD MPH MSc^{1,2}, and Ravi Mangal Patel, MD MSc^{1,2}

¹Department of Pediatrics, Emory University School of Medicine, Atlanta, GA

²Children's Healthcare of Atlanta, Atlanta, GA

³Department of Pediatrics, University of Alabama at Birmingham School of Medicine, Birmingham, AL

Abstract

Objective—To evaluate if routine supplementation of *Lactobacillus rhamnosus* GG ATCC 53103 (LGG) is associated with a decreased risk of NEC in very low birth weight (VLBW) infants.

Study design—Retrospective observational cohort study of VLBW (<1500 g) infants at a single center from 2008–2016. LGG supplementation with Culturelle at a dose of 2.5 to 5 × 10⁹ CFU/d began in 2014. We used multivariable logistic regression to evaluate the association between LGG supplementation and NEC (modified Bell Stage 2A or greater), after adjusting for potential confounders. We also compared changes in NEC incidence before and after implementation of LGG using statistical process control charts.

Results—We evaluated 640 VLBW infants with a median gestational age of 28.7 weeks (IQR 26.3–30.6); 78 (12%) developed NEC. The median age at first dose of LGG was 6 days (IQR 3–10) and duration of supplementation was 32 days (IQR 18–45). The incidence of NEC in the epoch before LGG implementation was 10.2% compared with 16.8% after implementation. In multivariable analysis, LGG supplementation was associated with a higher risk of NEC (adjusted odds ratio 2.10, 95 % CI 1.25–3.54, *P* = .005). We found no special cause variation in NEC after implementation of LGG supplementation. There were no episodes of *Lactobacillus* sepsis during 5558 infant days of LGG supplementation.

Corresponding author and reprint requests: Ravi Mangal Patel, MD, MSc, 2015 Uppergate Dr. NE, 3rd floor, Division of Neonatology, Emory University School of Medicine, Atlanta, GA 30322. rmpatel@emory.edu; Tel: (404) 727-5905; Fax: (404) 727-3236.

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Portions of this study were presented at the Pediatric Academic Societies annual meeting, April 30–May 3, 2016, Baltimore, Maryland.

Conclusions—In this study, routine LGG supplementation was not associated with a decreased risk of NEC. Our findings do not support the use of the most common probiotic preparation currently supplemented to VLBW infants in the US.

Keywords

bacteria; microbiome; morbidity; neonate; preterm; probiotic

Necrotizing enterocolitis is a major cause of morbidity and mortality in infants born prematurely^{1–3}. Between 4–7% of very low birth weight (VLBW, <1500 grams at birth) infants will develop NEC⁴ and 15% to 30% of VLBW infants with NEC will not survive.¹ Multiple randomized trials have studied the use of probiotics to prevent NEC with a variety of probiotic products. A meta-analysis of 25 trials, including 6,587 VLBW infants, demonstrate probiotics reduce both severe NEC (pooled relative risk [RR] 0.47; 95% CI 0.36–0.61) and all-cause mortality (RR 0.74; 95% CI 0.61–0.90)⁵. Despite the heterogeneity in preparations used in these trials, this meta-analysis did not demonstrate a difference in treatment effect for NEC by various species of probiotics, including *Lactobacillus*, *Bifidobacterium*, or multispecies products.

In a phone survey of neonatal intensive care units (NICUs) in the US, 14% of NICUs reported supplementing probiotics to VLBW infants, of which *Lactobacillus rhamnosus* GG (LGG) in the form of Culturelle was the most commonly used product⁶. However, randomized trials demonstrating the effectiveness of this probiotic product in decreasing the risk of NEC are lacking. In addition, there is uncertainty as to the appropriate dose and optimal timing of administration of probiotics. Implementation studies may provide data on the treatment effects of specific probiotic products in routine practice and help provide answers to these questions. We examined the association of routine supplementation with LGG and the risk of NEC in VLBW infants at a single center. We hypothesized that VLBW infants supplemented with LGG would have a lower risk of NEC compared with non-supplemented infants.

METHODS

We conducted this retrospective observational cohort study at a single, academically-affiliated level III neonatal intensive care unit in Atlanta, Georgia (Emory University Hospital Midtown). We included all consecutively admitted infants with a birth weight <1500 g who were admitted to the NICU between 8/1/2008 and 7/31/2016. We excluded infants with major congenital anomalies, those with a length of stay $\geq$ 3 days or those admitted after 1 week of age, as they would not have been eligible to initiate probiotic supplementation within the first week of life. We reviewed routinely collected clinical data, including physician and nursing documentation, laboratory results from hematology and microbiology, pediatric radiologists' interpretations of radiographic studies, and the medication administration record. The study was reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement⁷. This study was approved by the Emory University Institutional Review Board.

Definitions

The primary exposure was LGG supplementation, which we defined as the receipt of at least a single dose of LGG. LGG supplementation was implemented in February of 2014 through a standard protocol. LGG was supplemented once daily at a dose of 2.5×10^9 colony forming units (CFU) per day and then increased to 5×10^9 CFU per day once feedings were advanced using a single sachet of LGG powder (Culturelle, i-Health, Cromwell, Connecticut). For infants feeding 1–2 mL every 3 hours (or an equivalent hourly volume), LGG was mixed in sterile water. For infants feeding 3 mL every 3 hours or greater (or an equivalent hourly volume), LGG was mixed in either breast milk or formula. Supplementation was initiated once an infant was tolerating enteral feeding and continued until 35 weeks postmenstrual age. The primary outcome was NEC, defined as modified Bell Stage IIA or greater⁸. Isolated pneumoperitoneum without other radiographic or clinical evidence of NEC was considered to be a spontaneous intestinal perforation and not NEC. The modified Bell staging of all cases of NEC were adjudicated by the study team through unblinded review of clinical notes and abdominal radiograph reports interpreted by pediatric radiologists to identify staging criteria. Infants with possible NEC who underwent staging were identified through one of 3 methods: 1) Identifying all infants with NEC listed as a diagnosis (eg, ICD-9); 2) Concern for NEC noted anywhere in the summary of the infant's hospitalization; 3) review of all abdominal radiographs taken for each infant. If there was uncertainty regarding the staging, a third study team member (R.P.) reviewed the cases to reach consensus. Staging and adjudication was performed before statistical analysis and radiographic characteristics of staged cases were summarized. We specified denominators to indicate any missing data; no imputation was performed. We ascertained race and ethnicity based on documentation in the medical record. We defined small for gestational age as birth weight <10% percentile for gestational age using published sex-specific intrauterine growth curves⁹. We defined indomethacin prophylaxis as receipt of indomethacin within 1 day of birth.

Statistical Analyses

We used SPSS Version 23 (IBM, Armonk, New York) for all statistical analysis. We acquired data and performed statistical analysis from September 16, 2015 to October 4, 2017. We compared baseline maternal and neonatal characteristics between infants exposed and unexposed to LGG and with and without NEC. We described continuous variables using medians with interquartile ranges (IQR) reported as 25th–75th percentiles with comparisons using a Wilcoxon rank-sum test. We compared categorical variables using chi-square or Fisher exact tests.

For the primary analysis, we evaluated the association between exposure to LGG and the risk of NEC using multivariable logistic regression. We evaluated for potential confounding from differences in case-mix over time by including variables in the model based on available knowledge¹⁰ or variables associated with NEC in bivariable analysis at $P < 0.1$ with either the exposure or outcome. We included only exposures that occurred before the onset of NEC in multivariable models. We retained confounders for inclusion in the model by determining the change in the estimate of the association between LGG and NEC between full and reduced models with and without the potential confounder of interest.

Collinearity was assessed using correlation matrices, and variables with high collinearity (e.g. birth weight and gestational age) were not included in the models. We adjusted for gestational age, small for gestational age, multiple gestation, prolonged rupture of membranes > 18 hours, receipt of initial empiric antibiotics, and receipt of indomethacin prophylaxis. Additional variables that were individually evaluated but not included in the final model because they were not associated with NEC or inclusion did not change the point estimate of the association between LGG and NEC by more than 10% included: maternal race, maternal age, maternal receipt of tocolytic therapy, maternal receipt of antibiotics, maternal receipt of antenatal steroids, Apgar at 1 min, Apgar at 5 min, receipt of initial empiric antibiotics for > 2 days, receipt of inotropes, age at first feed, admission hemoglobin (Hb), and lowest Hb in 1st month. We did not adjust for human milk feeding given the overall high number of infants receiving any human milk (i.e. small number of unexposed). No tests for interaction were performed given the relatively low number of outcome events.

We performed four sensitivity analyses: 1) including only baseline covariates with complete data in the multivariable model to limit the effect of covariates with missing data; 2) evaluating NEC or death as a composite outcome to account for the competing outcome of death; 3) restricting analysis to a more contemporaneous cohort of infants born from 2011 onwards to limit the effect of changes in practice over time and; 4) comparing infant characteristics and the incidence of NEC between the pre- and post-LGG implementation epochs and assessing changes over time using a statistical process control P chart. We used all standard control rules in SPSS to detect special cause variation and identify a significant change in the incidence of NEC following the implementation of LGG supplementation that was unlikely from random variation.

RESULTS

Of the 733 infants with birth weight <1500 g born from 8/1/08 to 7/31/16, 640 infants met the selection criteria and were evaluated in this study (Figure 1; **available at www.jpeds.com**).

The median gestational age and birth weight of the study cohort was 28.7 weeks (IQR 26.3–30.6) and 1070 g (IQR 800–1290), respectively. The gestational age and birth weight were significantly lower among infants who developed NEC as well as those who received LGG (Table I). Infants with NEC were less likely to be the product of a multiple gestation pregnancy. Maternal characteristics, with the exception of maternal age, were similar among all groups. Apgar scores at 1 and 5 minutes were lower in infants with NEC compared with infants without NEC.

The median age at first dose of LGG was 6 days (IQR 3–10) and median duration of supplementation was 32 days (IQR 18–45) (Table II). Among infants receiving LGG, there was no difference in the age at initiation of LGG supplementation between infants with and without NEC. Receipt of initial empiric antibiotics and receipt of indomethacin prophylaxis were more frequent among infants with NEC compared with those without NEC. Receipt of

any human milk was common among all groups, although more frequent among infants receiving LGG and infants with NEC.

Clinical outcomes

Over the full study period, 78 (12%) infants developed NEC (Bell Stage IIA or greater), with definite pneumatosis and portal venous gas the two most commonly reported radiographic findings (Table III; [available at www.jpeds.com](http://www.jpeds.com)). Special cause variation, with an increase in the incidence of NEC, was noted in 2013 and LGG supplementation was implemented in 2014 (Figure 2; [available at www.jpeds.com](http://www.jpeds.com)). Of the 197 infants in the post-LGG implementation epoch, 175 (89%) received LGG (Table IV). The incidence of NEC among infants receiving LGG was 19% compared with 10% among infants not receiving LGG. Similarly, the incidence of NEC in the epoch after implementation of LGG supplementation was 17% compared with 10% in the epoch before implementation. The severity of NEC and distribution of modified Bell stages were similar in the pre- and post-LGG implementation epochs (Table V; [available at www.jpeds.com](http://www.jpeds.com)). There were no episodes of culture-positive *Lactobacillus* sepsis during 5558 infant days of LGG supplementation. In addition, there were no differences in culture-positive sepsis between the pre- and post-LGG implementation epochs (Table IV).

In bivariable analysis, LGG supplementation was associated with a higher risk of NEC (unadjusted odds ratio 2.17; 95% CI 1.33–3.53, $P=0.002$). In multivariable analysis, adjusting for potential confounders, LGG supplementation remained associated with a higher risk of NEC (adjusted odds ratio 2.10, 95% CI 1.25–3.54, $P=0.005$, Table VI). In this model, increasing gestational age and multiple gestation were independently associated with a lower risk of NEC. When limiting the model to inclusion of only baseline covariates with complete data ($n=640$), LGG supplementation remained associated with an increased risk of NEC (model 2) and an increased risk of NEC or death (model 3). We found similar results when limiting the analysis to a contemporaneous period from 2011–2016 (model 4). Finally, we detected no special cause variation in the risk of NEC after implementation of LGG supplementation, as shown in the control chart (Figure 2). As previously noted, the only period with special cause variation occurred in 2013 before the implementation of LGG supplementation.

DISCUSSION

In this study evaluating the routine clinical use of probiotics at a single center, we did not observe a reduction in the risk of NEC following implementation of LGG supplementation. Contrary to our expectations, we found LGG supplementation was associated with a higher risk of NEC. As this is an observational study, our results only support an association and not causation. Considering our findings in the context of 38 randomized trials of probiotic supplementation ($n=10,520$ infants) in which none of the studies demonstrated an increased risk of NEC in probiotic-supplemented infants compared with control infants⁵, the association between an increased risk of NEC among infants receiving LGG could be attributed to unmeasured differences in patient characteristics or clinical practice over time that were associated with both LGG supplementation and NEC. In addition, our use of LGG

in routine practice may have differed from the use in published clinical trials; the median age at initiation of LGG in our study of 6 days was later than several trials that initiated probiotic supplementation in the first 3 postnatal days⁵. However, the daily dose used in this study was within the range of common dosing regimens in trials of probiotics to prevent NEC¹¹. Importantly, we observed no clinical or laboratory instances of *Lactobacillus* sepsis during 5558 infant days of supplementation. Of note, we reviewed with staff the risk of LGG-associated sepsis, which has been previously reported as a complication of probiotic supplementation,¹² and separated probiotic preparation and administration with other nursing activities such as intravenous medication administration and emphasized hand hygiene before and after LGG administration.

Increased interest in methods to reduce NEC in our NICU began in 2013, following a significant increase in the incidence of NEC. Although the factors leading to this increase could not be determined, recent studies from the US,¹³ Sweden¹⁴ and Netherlands¹⁵ report increases in NEC or NEC-related deaths over time, potentially related to improving early survival of extremely preterm infants. As part of local quality improvement efforts to reduce the incidence of NEC, interventions including the use of donor human milk and reduction of acid-suppression medication use were introduced and continued before implementation of LGG supplementation. We selected our specific probiotic product containing LGG due to its use in other pediatric populations, the availability of this product on our hospital formulary and its preparation as a single dose sachet that could be easily dispensed by our pharmacy. Although LGG, in the form of Culturelle, is the most commonly supplemented probiotic product to VLBW infants in the US⁶, this is the first US study to evaluate the routine supplementation of LGG as part of efforts to reduce NEC. Our findings do not support the effectiveness of this specific LGG-containing product in decreasing the risk of NEC in VLBW infants.

Although a number of studies have evaluated probiotics and their impact on NEC⁵, few have looked specifically at the probiotic preparation used in this study. A recent study highlighted concerns regarding the quality of some commercially available probiotic preparations in the US, with many products not matching the species listed on the ingredient list¹⁶. In addition, an infant death has been associated with a multi-species probiotic product contaminated with mucormycosis¹⁷. The variability between product label and content suggest that each probiotic product should be considered individually rather than by bacterial strain and underscores the need for additional studies to guide the choice of probiotic products that are available, reliable, safe and effective in reducing NEC. A retrospective cohort study from Italy of 743 VLBW infants attested to the relative safety of enteral use of LGG in their population, in which active surveillance cultures were performed and no clinical sepsis episodes were attributable to LGG¹⁸. However, the LGG product was from a different manufacturer than the preparation used in our study. An observational study from France of 1,130 infants <32 weeks gestation reported a significant reduction in NEC from 5.3% to 1.2% with LGG supplementation using a different product than we used in our study¹⁹. A single-center randomized trial from the US of 101 infants with a birth weight of 501 to 1000 g used LGG in the same preparation as in our study in combination with *Bifidobacterium infantis* and found no effect on NEC as well as no probiotic-related adverse events²⁰.

When examining the effect of other probiotic strains, recent large, multicenter randomized controlled trials have shown varying results. The PiPS trial enrolled 1315 infants at 23–30 weeks' gestation in the UK and showed no effect on NEC with the use of *Bifidobacterium breve*²¹. By contrast, the ProPrems trial enrolled 1099 infants with a birth weight < 1500 g and gestational age < 32 weeks in Australia and New Zealand and reported a 54% relative risk reduction in NEC using a combination of *Bifidobacterium infantis*, *Streptococcus thermophilus* and *Bifidobacterium lactis* in the setting of high maternal breast milk usage²². However, NEC was not the primary outcome of the ProPrems trial. Several studies of routine probiotic supplementation as standard of care have demonstrated reductions in NEC and late-onset sepsis, as reported in a systematic review and meta-analysis of observational studies²³; however, these studies have reported on a variety of probiotic preparations with only one study from France demonstrating a significant reduction in NEC with routine supplementation of LGG¹⁹. The lack of a beneficial effect of probiotic supplementation in our study on NEC as well as culture-positive sepsis, along with the negative PiPs trial, highlights that each probiotic may need to be considered as individual agents and meta-analysis of trials using heterogeneous strains may not reflect the unique functional properties of individual probiotic strains.

Our study has several strengths. We measured a number of potential confounders, which allowed us to control for differences in patient characteristics that could have confounded the relationship between LGG supplementation and NEC. In addition, after LGG supplementation was implemented at our center, 89% of VLBW infants received LGG, which represents high uptake in routine clinical practice and reduces bias from highly variable use after implementation. Finally, we found consistent findings in the association between LGG and NEC in each of the sensitivity analyses.

We should also address the limitations of this study. The study was conducted over an 8 year period, and changes in clinical practice that could have impacted the incidence of NEC may have occurred that we did not measure. To account for this, we performed a sensitivity analysis limited to a contemporaneous period, which showed similar results. Although we ascertained if infants had received any human milk, we were unable to quantify the contribution of human milk to their entire nutritional intake. In addition, we did not assess the interaction between LGG supplementation and other variables in our analysis and therefore did not assess for differences in the association between LGG and NEC among subgroups of patients, such as those that received only human milk. However, given our findings of a significant association between LGG supplementation and increased risk of NEC, it is highly unlikely that we were underpowered to detect a favorable association between LGG and lower risk NEC. Finally, the adjudication of NEC cases was not performed by blinded reviewers. However, we found no significant differences in the distribution of NEC staging or severity between the pre- and post-LGG implementation epochs to indicate ascertainment bias between epochs.

In conclusion, routine supplementation with LGG at a dose of 2.5 to 5×10^9 CFU/d initiated at a median age of 6 days was not associated with a lower risk of NEC in our single-center cohort of VLBW infants. Given that these results are in contrast to results from pooled meta-analyses, our findings highlight the need for additional studies to evaluate individual

probiotic strains and products, with a focus on product quality, timing of initiation, as well as assessing any interaction with human milk feeding and antibiotic use to better understand the treatment effects of probiotics on NEC.

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Abbreviations

LGG	<i>Lactobacillus rhamnosus</i> GG
VLBW	very low birth weight
NICU	neonatal intensive care unit
IQR	interquartile range
Hb	hemoglobin
CI	confidence interval.

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Figure 1 (online only). Flow Diagram of Subject Selection

Figure 2 (online only). Control Chart of NEC Before and After LGG supplementation
 P chart with mean (solid straight line) and 3 sigma control limits (dotted lines) is shown. Special cause variation indicated by open square. Mean sample size per each quarterly period is 20 (range 11 to 29). Data for Q3 of 2016 not shown given small sample size (denominator n=2).
 Abbreviations: NEC, necrotizing enterocolitis; LGG, *Lactobacillus rhamnosus* GG; Q, quarter.

Table I

Baseline Characteristics

Characteristic	LGG (n=175)	No LGG (n=465)	NEC (n=78)	No NEC (n=562)
Gestational age, wk ^{a,b}	28.1 (26.1–30.0)	28.9 (26.4–30.7)	27.0 (25.4–28.7)	29.0 (26.7–30.7)
Birth weight, g ^{a,b}	970 (730–1230)	1085 (820–1310)	750 (640–1070)	1103 (850–1310)
Small for gestational age	37/175 (21%)	117/465 (25%)	21/78 (27%)	133/562 (24%)
Female sex	87/175 (50%)	246/465 (53%)	43/78 (55%)	290/562 (52%)
Multiple gestation ^b	47/175 (27%)	114/465 (25%)	12/78 (15%)	149/562 (27%)
Maternal Race				
White	17/174 (10%)	68/462 (15%)	6/78 (8%)	79/558 (14%)
Black	148/174 (85%)	364/462 (79%)	65/78 (83%)	447/558 (80%)
Hispanic	5/174 (3%)	10/462 (2%)	2/78 (3%)	13/558 (2%)
Other	4/173 (2%)	20/462 (4%)	5/78 (6%)	19/558 (3%)
Maternal age in years ^a	31 (26–35)	28 (22–33)	29 (22–33)	29 (24–33)
Maternal receipt of tocolytic	58/175 (33%)	134/465 (29%)	24/78 (31%)	168/562 (30%)
Maternal antihypertensive treatment	53/175 (30%)	128/465 (28%)	28/78 (36%)	153/562 (27%)
Maternal receipt of antibiotics	55/173 (32%)	182/464 (39%)	28/78 (36%)	209/559 (37%)
Maternal receipt of antenatal steroids	146/174 (84%)	349/460 (76%)	62/78 (79%)	433/556 (78%)
Vaginal delivery	51/175 (29%)	167/465 (36%)	27/78 (35%)	191/462 (34%)
Rupture of membranes > 18 hr	42/166 (25%)	100/449 (22%)	13/76 (17%)	129/539 (24%)
Apgar at 1 min ^b	5 (3–8)	5 (2–7)	4 (2–7)	5 (3–7)
Apgar at 5 min ^b	8 (7–9)	8 (6–9)	7 (6–9)	8 (7–9)

Categorical variables reported as n/N (%) and continuous variables reported as median (IQR).

Abbreviations: LGG, *Lactobacillus rhamnosus* GG; NEC, necrotizing enterocolitis.

^aP<0.05 by unadjusted comparisons of LGG and non-LGG infants using the Wilcoxon rank sum test for continuous variables and chi-square test for categorical variables.

^bP<0.05 by unadjusted comparisons of NEC and non-NEC infants using the Wilcoxon rank sum test for continuous variables and chi-square test for categorical variables.

Table II

Clinical Characteristics

Characteristic	LGG (n=175)	No LGG (n=465)	NEC (n=78)	No NEC (n=562)
Indomethacin prophylaxis ^{a,b}	89/175 (51%)	172/465 (37%)	51/78 (65%)	210/562 (37%)
Receipt of initial empiric antibiotics ^b	145/175 (83%)	377/465 (81%)	70/78 (90%)	452/562 (80%)
Treatment > 2d	61/175 (35%)	196/465 (42%)	34/78 (44%)	223/562 (40%)
Early onset sepsis	2/175 (1%)	4/464 (1%)	0/78 (0%)	6/561 (1%)
Age at first feed, d ^b	2 (1–3)	2 (1–3)	2 (2–3)	2 (1–3)
Admission Hb, g/dL	14.9 (13.2–16.4)	14.7 (13.3–16.3)	14.7 (13.2–15.9)	14.8 (13.3–16.4)
Lowest Hb in 1 st month, g/dL ^b	9.5 (8.6–10.8)	9.4 (8.3–11.0)	8.9 (8.1–10.6)	9.5 (8.3–11.0)
Number of RBC transfusions ^b	2 (0–5)	1 (0–3)	4 (2–7)	1 (0–3)
Age at first LGG dose, d ^c	6 (3–10)	-	7 (3–12)	6 (3–9)
Duration of LGG supplementation, d ^{b,c}	32 (18–45)	-	19 (11–27)	35 (22–46)
Receipt of any breastmilk ^{a,b}	173/175 (99%)	407/460 (89%)	76/78 (97%)	504/557 (91%)
Donor milk ^{a,b}	58/175 (33%)	11/465 (2%)	17/78 (22%)	52/562 (9%)
Maternal milk ^a	168/175 (96%)	402/460 (87%)	72/78 (92%)	498/557 (89%)
Patent ductus arteriosus	17/94 (18%)	99/453 (22%)	11/61 (18%)	105/486 (22%)
Indomethacin treatment	6/94 (6%)	55/453 (12%)	6/61 (10%)	55/486 (11%)
Surgical ligation	3/94 (3%)	17/452 (4%)	1/60 (2%)	19/486 (4%)
Oxygen need at 28 d ^b	102/166 (61%)	227/432 (53%)	58/66 (88%)	271/532 (51%)
Oxygen need at 36 weeks PMA ^b	66/153 (43%)	144/416 (35%)	36/56 (64%)	174/513 (34%)
Receipt of any inotropes ^b	38/175 (22%)	74/460 (16%)	25/78 (32%)	87/557 (16%)
Receipt of gastric acid suppression ^{a,b}	8/175 (5%)	92/465 (20%)	5/78 (6%)	95/562 (17%)
Death ^b	9/175 (5%)	21/465 (5%)	14/78 (18%)	16/562 (3%)

Categorical variables reported as n/N (%) and continuous variables reported as median (IQR).

Abbreviations: LGG, *Lactobacillus rhamnosus* GG; NEC, necrotizing enterocolitis; Hb, hemoglobin; RBC, red blood cell; PMA, postmenstrual age.

^aP<0.05 by unadjusted comparisons of LGG and non-LGG infants using the Wilcoxon rank sum test for continuous variables and chi-square or Fisher's exact test for categorical variables.

^bP<0.05 by unadjusted comparisons of NEC and non-NEC infants using the Wilcoxon rank sum test for continuous variables and chi-square or Fisher's exact test for categorical variables.

^cOnly evaluated among infants who received LGG.

Table III (Online only)

Reported radiographic findings for ascertainment of NEC

Radiographic findings	Modified Bell Stage ^a			p ^b
	IA or IB (n=20)	IIA or IIB (n=47)	IIIA or IIIB (n=31)	
Description of pneumatosis				
Definite^c	0 (0%)	34 (72%)	24 (77%)	<0.001
Possible	2 (10%)	3 (6%)	3 (10%)	0.83
Questionable	5 (25%)	0 (0%)	0 (0%)	<0.001
Difficult to exclude^d	6 (30%)	5 (11%)	1 (3%)	0.02
Other reported findings				
Ileus	4 (20%)	4 (9%)	0 (0%)	0.04
Ascites	0 (0%)	1 (2%)	4 (13%)	0.05
Portal venous gas	0 (0%)	10 (21%)	13 (42%)	0.002
Free air	0 (0%)	0 (0%)	7 (23%)	<0.001
Gasless abdomen	1 (5%)	1 (2%)	0 (0%)	0.47
Fixed loop	0 (0%)	0 (0%)	1 (3%)	0.34

^aThe study definition of NEC was modified Bell stage IIA or greater^bUnadjusted comparisons performed using the chi-square test.^cIncludes cases where pneumatosis is clearly described.^dIncludes reporting of bubbly or mottled lucencies that may represent stool versus pneumatosis.

Table IV

Infant Characteristics and Outcomes Before and After Implementation of LGG supplementation

Outcome	Pre-LGG implementation epoch, 2008–2014 (n=443)	Post-LGG implementation epoch, 2014–2016 (n=197)	<i>p</i> ^a
Gestational age, wk	28.7 (26.4–30.6)	28.3 (26.3–30.6)	0.26
Birth weight, g	1080 (820–1300)	1000 (740–1270)	0.10
Receipt of any initial antibiotics	366/443 (83%)	156/197 (79%)	0.30
Receipt of prophylactic indomethacin	164/443 (37%)	97/197 (49%)	0.004
Receipt of any human milk	387/438 (88%)	193/197 (98%)	<0.001
Age at first feed	2 (1–3)	2 (1–3)	0.86
NEC stage IIA or greater	45/443 (10%)	33/197 (17%)	0.02
NEC stage IIIA or IIIB	20/443 (5%)	11/197 (6%)	0.56
Death	17/443 (4%)	13/197 (7%)	0.13
NEC or death	53/443 (12%)	41/197 (21%)	0.004
Blood culture-positive sepsis	86/440 (20%)	47/196 (24%)	0.20
LGG-associated sepsis	0 (0%)	0 (0%)	-

Categorical variables reported as n/N (%) and continuous variables reported as median (IQR).

Abbreviations: LGG, *Lactobacillus rhamnosus* GG; NEC, necrotizing enterocolitis.^aUnadjusted comparisons performed using the Wilcoxon rank sum test for continuous variables and chi-square test for categorical variables.

Table V (online only)

Ascertainment of NEC in the Pre- and Post-LGG implementation epochs.

Variable	Pre-LGG implementation epoch, 2008–2014 (n=58)	Post-LGG implementation epoch, 2014–2016 (n=40) ^a	p ^b
Ascertainment of NEC by modified Bell stage ^c			0.51
IA or IB	13/58 (22%)	7/40 (18%)	
IIA or IIB	25/58 (43%)	22/40 (55%)	
IIIA or IIIB	20/58 (34%)	11/40 (28%)	
Death or surgery ^d			
Stage IA or IB	0 (0%)	0 (0%)	NA
Stage IIA or IIB	0 (0%)	0 (0%)	NA
Stage IIIA or IIIB	14/20 (70%)	8/11 (73%)	>0.99

Abbreviations: NEC, necrotizing enterocolitis; LGG, *Lactobacillus rhamnosus* GG.

^a All 40 infants received LGG supplementation.

^b Unadjusted comparisons performed using the chi-square or Fisher's exact test.

^c The study definition of NEC was modified Bell stage IIA or greater.

^d Infants permanently transferred to another hospital had limited follow-up for these outcomes.

Table VI

Association between LGG supplementation and Risk of NEC

Variable	Odds ratio for NEC	95% CI	P
Model 1 - Primary model (n=615)			
LGG supplementation	2.10	1.25 –3.54	0.005
Gestational age (per 1 wk increase)	0.81	0.71 –0.93	0.002
Small for gestational age	1.64	0.89 –3.04	0.11
Multiple gestation	0.39	0.20 –0.76	0.006
Rupture of membranes > 18 hr	0.54	0.28 –1.04	0.07
Receipt of initial empiric antibiotics	1.80	0.80 –4.06	0.16
Receipt of prophylactic indomethacin	1.41	0.70 –2.83	0.34
Model 2 –Reduced model (n=640)			
LGG supplementation	1.97	1.18 –3.29	0.009
Gestational age (per 1 wk increase)	0.81	0.71 –0.92	0.001
Small for gestational age	1.65	0.91 –3.00	0.10
Multiple gestation	0.41	0.21 –0.79	0.008
Receipt of prophylactic indomethacin	1.46	0.73 –2.90	0.28
Model 3 - Model 2 fitted to outcome of NEC or death (n=640)			
LGG supplementation	1.70	1.04 –2.78	0.03
Model 4 –Model 2 with analysis limited to years 2011–2016 (n=424) ^a			
LGG supplementation	1.71	0.97 –3.02	0.06

Abbreviations: LGG, *Lactobacillus rhamnosus* GG; NEC, necrotizing enterocolitis; CI, confidence interval.^aExcludes infants born from 2008–2010, all of whom were LGG unexposed (N=216).