Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial[1–4]

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ABSTRACT

Background: Although recent reports suggest that supplementation with probiotics may enhance intestinal function in premature infants, the mechanisms are unclear, and questions remain regarding the safety and efficacy of probiotics in extremely low-birth-weight infants.

Objective: The objective was to evaluate the efficacy of probiotics on the digestive tolerance to enteral feeding in preterm infants born with a very low or extremely low birth weight.

Design: In a bicentric, double-blind, randomized controlled clinical trial that was stratified for center and birth weight, 45 infants received enteral probiotics (*Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* GG; BB536-LGG) and 49 received placebo. The primary endpoint was the percentage of infants receiving >50% of their nutritional needs via enteral feeding on the 14th day of life. A triangular test was used to perform sequential analysis.

Results: The trial was discontinued after the fourth sequential analysis concluded a lack of effect. The primary endpoint was not significantly different between the probiotic (57.8%) and placebo (57.1%) groups ($P = 0.95$). However, in infants who weighed >1000 g, probiotic supplementation was associated with a shortening in the time to reach full enteral feeding ($P = 0.04$). Other than colonization by the probiotic strains, no alteration in the composition of intestinal microbiota or changes in the fecal excretion of calprotectin was observed. No colonization by probiotic strains was detected in infants who weighed ≤1000 g, presumably because of more frequent suspensions of enteral feeding, more courses of antibiotic treatment, or both.

Conclusions: Supplementation with BB536-LGG may not improve the gastrointestinal tolerance to enteral feeding in very-low-birth-weight infants but may improve gastrointestinal tolerance in infants weighing >1000 g. This trial was registered at clinicaltrials.gov as NCT 00290576. *Am J Clin Nutr* 2009;89:1828–35.

INTRODUCTION

In neonatal intensive care units, the immaturity of intestinal function, frequent use of broad-spectrum antibiotics, delay in initiating enteral feeding, infection control procedures, and sterilization of milk limit the exposure of preterm infants to normal commensal microorganisms. As a consequence, very-low-birth-weight (<1500 g) preterm infants experience a delayed and abnormal pattern of gut colonization, particularly with regard to bifidobacteria and lactobacilli, normally dominant in healthy full-term infants (1–5). This impaired intestinal colonization may predispose preterm infants to necrotizing enterocolitis (6–9) and increase the risk of bacterial translocation (10).

Probiotics, defined as live microbial supplements providing health benefits to the host (11, 12), might help modulate the intestinal microbiota in preterm infants. Two meta-analyses (13, 14), based on an aggregate of 7 (15–21) and 9 (15–20, 22–24) clinical trials concluded that probiotic supplementation reduces the incidence of necrotizing enterocolitis in preterm infants. Shortening the time required to reach full enteral feeding might be an additional beneficial effect, of particular relevance in extremely low-birth-weight infants; the latter was observed in one study (15) and was found to be significant in a meta-analysis (13, 14). Several questions, however, remain unanswered. For instance, it is unclear whether colonization with the probiotic strains is required to achieve a beneficial effect (12), and the safety and efficacy of probiotics in extremely low-birth-weight infants (≤1000 g) remain to be proven (12, 14).

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We therefore designed a bicentric, randomized, double-blind controlled clinical trial to evaluate the efficacy of combined supplementation with *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* GG (BB536-LGG) on the digestive tolerance to enteral feeding in preterm infants born with a very-low (<1500 g) or extremely low (≤1000 g) birth weight. The primary outcome chosen was the percentage of infants receiving >50% of their overall nutritional needs enterally on the 14th day of life. The study was powered to detect a 20% increase in that proportion of infants. Secondary endpoints were the time to reach full enteral feeding, the effects of oral probiotics on morbidity and mortality until hospital discharge, the composition of intestinal microbiota, and the fecal excretion of calprotectin—a putative marker of intestinal inflammation.

SUBJECTS AND METHODS

Study population

Two centers (Mère-Enfant Hospital, Nantes, France, and Institut de Puériculture, Paris, France) participated in this trial. The protocol was approved by the Medical Ethics Committee of Nantes. Written informed parental consent was obtained for each infant before inclusion. To be eligible for enrollment in the present study, the infants had to meet the following inclusion criteria: a gestational age <32 wk, a birth weight <1500 g, a postnatal age ≤2 wk, the absence of any disease other than those linked to prematurity, and the start of enteral feeding before inclusion.

Procedures

The infants were randomly assigned to the placebo or the probiotic group with the aid of in-house software (Nantes University Hospital, Nantes, France), and randomization was stratified on the basis of neonatal intensive care unit (NICU) center (Nantes or Paris) and birth weight category (≤1000 g and >1000 g). Infants were fed human (own mother's expressed milk or bank milk) and/or preterm formula and were randomly assigned to receive 4 daily capsules of a supplement containing either maltodextrin alone (placebo group) or 10^8 lyophilized cells per unit of the probiotics *L. rhamnosus* GG (Valio, Ltd) and *B. longum* BB536 (Morinaga Milk Industry Co, Ltd, Tokyo, Japan) and maltodextrin (probiotic group) beginning on the day when enteral feeding started until discharge. Placebo and probiotics were prepared by Nestec Research Center (Vers-Chez-Les-Blancs, Switzerland) and stored in closed capsules that were kept at 4°C until used. Capsules were opened and mixed with 1mL of sterile water immediately before administration to infants who were receiving enteral feeding on the day of the supplementation.

Stool samples were collected from the first 24 infants enrolled in each NICU for the follow-up of intestinal microbiota and fecal calprotectin. Samples were frozen immediately after collection and kept at −80°C until analyzed. This procedure prevents the degradation of DNA. For culture, samples were collected in 0.5 mL brain heart infusion medium, which contains glycerol as a cryoprotectant, and then immediately stored at −80°C. This method is known to prevent significant changes in the microbiota. Stool samples were collected weekly from birth until hospital discharge. Intestinal microbiota was analyzed weekly by culture, allowing the isolation of the main genera of microbiota found in the preterm infants' feces. In parallel, polymerase chain reaction–temporal temperature gradient gel electrophoresis was used to analyze the dominant intestinal microbiota. The most prevalent molecular species were identified after sequencing by comparing bacterial 16S rRNA gene sequences with entries in databases, using appropriate software such as BIBI, Blast, Multalin, and ClustalW software. The 2 probiotic strains used in our study were detected specifically in stool samples by a culture–polymerase chain reaction method. Fecal calprotectin concentrations were measured at 2-wk intervals in duplicate with a commercial enzyme-linked immunoassay (Calprest, Eurospital, Trieste, Italy).

Statistical analysis

The primary outcome was the percentage of infants receiving >50% of their overall nutritional needs enterally at a postnatal age of 14 d. The sample size estimation for the analysis of primary outcome was based on an expected rate of 50% in the placebo group compared with 70% in the probiotic group. We estimated that 104 patients per group were required to detect such a difference with an 80% power and a 5% α risk. To avoid exposing an excess number of extremely premature infants to a putative risk of probiotics in the event of a potential harmful effect (25, 26), we carried out a sequential trial using the Whitehead triangular test (27, 28). Inspection and interim analysis of the data were planned for every 20 patients and were performed by using Pest 3.0 software. Data were analyzed based on intention to treat. The final statistical analysis was performed using SPSS 15.0 software. Student's *t* test, or the Mann-Whitney *U* test when appropriate, was used for comparison of continuous variables, and a chi-square test, or Fisher's exact test when appropriate, was used for comparison of categorical variables. The "time to reach full enteral feeding" curves were computed according to the Kaplan-Meier method, and statistical comparisons were made by using the log-rank test. The Cox regression model was performed to adjust for the potential confounders gestational age, center, and type of enteral feeding. A logistic regression was performed to analyze whether factors were associated with the colonization by probiotics. All tests were 2-tailed. *P* values <0.05 were considered significant.

RESULTS

The trial began in April 2005 and was initially planned to last for 3 y. However, as the fourth sequential analysis showed no statistically significant difference regarding our primary endpoint, we had to stop inclusions in January 2007. A total of 94 preterm infants were enrolled: 45 were randomly assigned to the probiotic group and 49 to the placebo group (**Figure 1**). The baseline characteristics of the infants and their mothers were similar in the 2 study groups. Although lactate concentrations differed slightly between the probiotic (3.2 ± 1.7 mmol/L) and placebo (4.2 ± 2.9 mmol/L) groups ($P = 0.04$; **Table 1**), the clinical significance of the difference was questionable.

Enteral feeding was initiated at a similar postnatal age in the probiotic (3.2 ± 1.5 d) and placebo (3.9 ± 1.2 d) groups ($P = 0.12$) and consisted of human (bank or own mother's) milk in all infants in both groups. Oral supplementation with probiotics or placebo began in parallel with enteral feeding (within <24 h): 3.5 ± 1.6 and 4.3 ± 2.4 d of life, respectively ($P = 0.06$). Although the

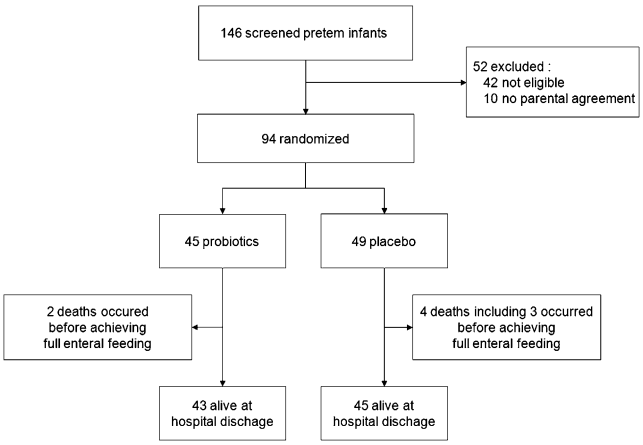

FIGURE 1. Trial profile.

initiation of treatment occurred slightly sooner in the probiotic group (at 3.5 ± 1.6 d) than in the placebo group (4.3 ± 2.4 d), the trend was not significant ($P = 0.06$).

One infant in the probiotic group received the placebo treatment during 1 wk because of a dispensing error. During the stay in the NICU, the incidence of feeding with human milk was not significantly different between the probiotic (75.6%) and placebo (81.6%) groups ($P = 0.47$).

The primary outcome did not differ between groups (**Table 2**). During the stay in the NICU, 5 preterm infants died before achieving full enteral feeding, 2 of whom were in the probiotic group. The time to full enteral feeding was not significantly different between the 2 study groups (**Figure 2**). Crude and adjusted hazard ratios were not significantly different between groups. Median daily weight gain was 11.3 $g \cdot kg^{-1} \cdot d^{-1}$ (25th-75th percentiles: 10.2–12.5) in the placebo group compared with 11.5 $g \cdot kg^{-1} \cdot d^{-1}$ (10.2–12.7) in the placebo group (NS). The difference in weight gain, expressed as z scores, was also equivalent in the probiotic group (−0.16; −1.11–0.98) and the placebo group (−0.41; −1.26–0.9) ($P = 0.18$). Hospital course, morbidity, and mortality were not significantly different between groups (Table 2). Six deaths were observed. Four deaths (one in the probiotic group) were attributed to neurological lesions. One death was due to documented necrotizing enterocolitis in the probiotic group, and one was attributed to septic shock in the placebo group.

Because randomization was stratified for center and birth weight (≤1000 g and >1000 g), an analysis by subgroup was performed. Differences were observed between centers. In center A (Nantes), time to reach full enteral feeding tended to be lower in the probiotic group than in the placebo group and was significantly lower after adjustment for gestational age, z score of birth weight, and type of enteral feeding ($P = 0.03$). In center B (Paris), we observed no such significant difference.

Stratification for birth weight revealed other differences. Among infants who weighed ≤1000 g at birth, the time to reach full enteral feeding did not differ ($P = 0.12$) between the probiotic (34 d; 18–59 d) and placebo (32 d; 23–38 d) groups. In contrast, among infants who weighed >1000 g, the time to reach full enteral feeding was significantly shorter ($P = 0.04$) in the probiotic group (16 d; 13–20 d) than in the placebo group (19 d; 15–26 d) (Figure 2; **Table 3**).

Yet, even within the subpopulation of 56 infants who weighed >1000 g, no difference in outcome was found between the probiotic and placebo groups on the basis of the number of infections, the number of days on antibiotic treatment, the duration of ventilatory support, or the length of hospital stay (data not shown).

Regarding safety, no unexpected adverse events were observed during the course of the study. There was no significant difference in the incidence of nosocomial infection in the probiotic group. Among the infants who weighed ≤1000 g, 12 of 16 infants (75%) in the probiotic group developed an infection compared with 14 of 22 infants (64%) in the placebo group; the difference was not significant ($P = 0.51$).

The monitoring of intestinal microbiota and fecal calprotectin excretion was planned for the first 48 infants included, but was actually performed in 46 infants because 1 infant died before fecal sampling and fecal samples were not collected until week 5 in another infant. The baseline characteristics of the 46 infants (ie, 23 infants in each group) were not significantly different from

TABLE 1
Baseline characteristics of the infants

	Probiotic group ($n = 45$)	Placebo group ($n = 49$)	P value
Multiple pregnancies [n (%)]	23 (48.9)	31 (63.3)	0.29
Pregnancy-induced hypertension [n (%)]	6 (13.3)	9 (18.4)	0.51
Rupture of membranes >12 h [n (%)]	8 (17.8)	5 (10.2)	0.31
Antenatal corticoid treatment [n (%)]	35 (77.8)	41 (83.7)	0.47
Antenatal antibiotic treatment [n (%)]	14 (31.1)	13 (26.5)	0.62
Perinatal antibiotic treatment [n (%)]	9 (20.0)	9 (18.4)	0.84
Cesarean section [n (%)]	28 (62.2)	35 (71.4)	0.34
Gestational age (wk)	28.1 ± 1.9[1]	28.1 ± 1.8	0.87
Birth weight (g)	1115 ± 251	1057 ± 260	0.28
Birth weight ≤1000 g [n (%)]	15 (33.3)	20 (40.8)	0.45
Intrauterine growth restriction [n (%)]	1 (2.2)	3 (6.1)	0.35
Male sex [n (%)]	28 (62.2)	26 (53.1)	0.37
z score of birth weight	−0.16 ± −0.83	−0.41 ± −0.92	0.18
Apgar score at 1 min	6.8 ± 2.7	6.9 ± 3.4	0.85
Apgar score at 5 min	9.1 ± 1.4	8.8 ± 1.8	0.33
Lactate at <12 h of life (mmol/L)	3.2 ± 1.7	4.2 ± 2.9	0.04

[1] Mean ± SD (all such values).

TABLE 2
Efficacy of treatment

	Probiotic group ($n = 45$)	Placebo group ($n = 49$)	P value
Nutrition on day 14			
>50% of calories received enterally [n (%)]	26 (57.8)	28 (57.1)	0.95
Total calories delivered enterally (%)	59.8 ± 36.7[1]	57.3 ± 31.3	0.73
Other outcome measures			
Nosocomial infections [n (%)]	21 (46.7)	26 (53.1)	0.54
Sepsis with positive blood culture [n (%)]	15 (33.3)	13 (26.5)	0.47
Duration of antibiotic use (d)	11.7 ± 14.4	10.2 ± 9.7	0.55
Necrotizing enterocolitis [n (%)]	2 (4.4)	1 (2.0)	0.51
Duration of ventilatory support (d)	10.1 ± 2.4	10.2 ± 1.7	0.98
Duration of continuous positive airway pressure (d)	20.9 ± 2.2	21.4 ± 2.1	0.88
Duration of oxygen therapy (d)	20.3 ± 4.5	14.6 ± 2.7	0.28
Systemic postnatal corticoid treatment [n (%)]	13 (28.9)	10 (20.4)	0.34
Duration of hospital stay (d)	60.7 ± 28.8	65.6 ± 30.0	0.43
Death [n (%)]	2 (4.4)	4 (8.2)	0.76

[1] Mean ± SD (all such values).

those of the 94 preterm infants included in the present study. A total of 83 stool samples were collected for fecal calprotectin measurement and 142 for intestinal microbiota analysis during the first 4 wk. Fecal calprotectin concentrations were not significantly different between the placebo and probiotic groups ($P = 0.31$), between all 46 infants (**Figure 3**), or between the 26 infants who weighed >1000 g (154 ± 84 μg/g feces in the probiotic group compared with 103 ± 90 μg/g in the placebo group; $P = 0.17$). When the entire patient population was considered as a whole, the incidence of gastrointestinal colonization by bifidobacteria (except for week 4) and lactobacilli was significantly higher in the probiotic group. Among infants colonized by bifidobacteria and lactobacilli, levels were similar in both groups, except for bifidobacteria at week 2, which was significantly greater in the probiotic group ($P = 0.01$). In contrast, the incidence and levels of colonization by the other bacterial genera were not significantly different between groups (**Figure 4**). Nevertheless, the incidence and levels of clostridia tended to be lower at week 1 in the probiotic group, the levels of clostridia tended to be lower at week 1 ($P = 0.06$), and the levels of enterococci were significantly lower in the probiotic group at week 2 ($P = 0.02$). Regarding the microbiota analysis by temporal temperature gradient gel electrophoresis, no significant difference was found between the probiotic and placebo groups, except for the presence of *B. longum* BB536 and *L. rhamnosus* GG in the probiotic group ($P = 0.01$).

Because a method designed to specifically detect the probiotics was available, we were able to determine whether the infants were successfully colonized by the strains. Probiotic strains were detected at least twice at a 1-wk interval in 19 of 23 infants in the probiotic group and in 3 of 23 infants in the placebo group. The primary outcome did not differ between those infants who were successfully colonized by probiotics and those who were not: 64% compared with 50%, respectively ($P = 0.40$). Nevertheless, the time to full enteral feeding was significantly lower ($P = 0.04$) in the probiotic group (median: 16; interquartile range: 13–25) than in the placebo group (median: 26; interquartile range: 20–35). Probiotic strain colonization was dependent on birth weight (odds ratio: 1.6; 1.1–2.4 per 100-g increment; $P = 0.02$) with a lower incidence of colonization in infants who weighed ≤1000 g than in those who weighed >1000 g ($P = 0.02$). However, colonization by probiotic strains was no longer dependent on birth weight (odds ratio: 1.3; 0.8–2.1 per 100-g increment; $P = 0.20$) once the number of days with postnatal antibiotic treatment or without enteral feeding was included in the logistic regression model, but was instead dependent on the latter factors (odds ratio: 0.9; 0.8–1.0 per day; $P = 0.03$). Fecal calprotectin concentrations did not differ significantly between infants colonized by probiotic strains and those who were not ($P = 0.40$).

DISCUSSION

The present prospective, double-blind, randomized trial was designed to determine whether oral supplementation with BB536-LGG improved the gastrointestinal tolerance to enteral feeding in very-low-birth-weight infants. When the group was considered as a whole, BB536-LGG failed to accelerate weaning from parenteral nutrition and had no significant effect on the composition of intestinal microbiota (except for colonization by the probiotic strains) or on the excretion of fecal calprotectin—a presumed marker of inflammation. We further showed that this overall negative outcome may have resulted from the presence of 2 different populations among the infants studied, defined on the basis of birth weight.

In infants with an extremely low birth weight (≤1000 g), BB536-LGG did not improve the gastrointestinal tolerance to enteral feeding. This finding was explained by the fact that the probability to be colonized by probiotic strains diminished with decreasing birth weight. This observation is consistent with that of an earlier report (20). We further observed that probiotic strain colonization was negatively affected by the number of days of postnatal antibiotic treatment or without enteral feeding. Infants who weighed ≤1000 g received antibiotic treatment more frequently and had more frequent interruptions of enteral feeding than did infants who weighed >1000 g. These findings collectively suggest that the suspension of enteral feeding and antibiotics may impair colonization by probiotic strains and, consequently, the ability of BB536-LGG to enhance intestinal function in extremely low-birth-weight infants.

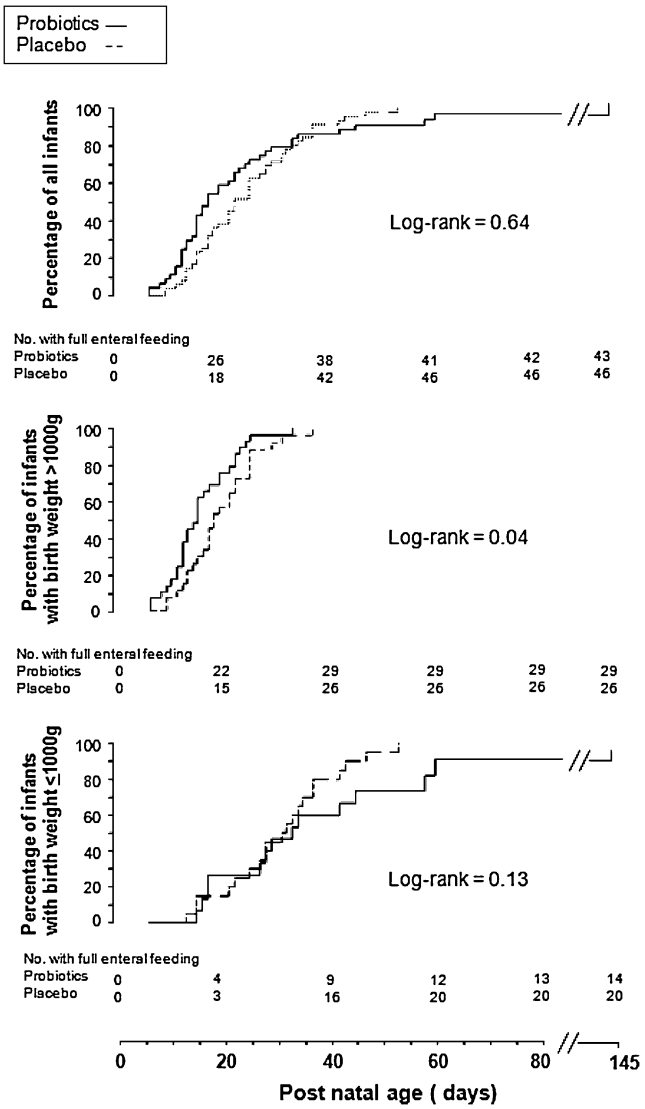

FIGURE 2. Kaplan-Meier curves indicating the time to reach full enteral feeding in the probiotics and placebo groups. Data are shown for all infants, infants with a birth weight >1000 g, and infants with a birth weight ≤1000 g.

In contrast, in infants who weighed >1000 g at birth, oral supplementation with *B. longum* BB536 and *L. rhamnosus* GG shortened the time required to achieve full enteral feeding. Such an improvement in gastrointestinal tolerance is consistent with an earlier report (15) and with the conclusion from a meta-analysis (13, 14). In center A, among the 32 infants who weighed >1000 g, the lactate concentration was slightly, but not significantly, lower in the probiotic group than in the placebo group (3.1± 1.3 compared with 3.8 ± 1.8 mmol/L; $P = 0.47$). When the logistical regression was adjusted for lactate concentrations, the hazard ratios were not modified. Similarly, in the same subgroup, the 5-min Apgar score was slightly, but not significantly, higher in the probiotic group than in the placebo group (9.5 ± 0.8 compared with 8.9 ± 2.0; $P = 0.31$). Thus, the benefit of probiotic supplementation is unlikely to arise from a difference in the severity of stress between the treatment and placebo groups. Despite randomization, among infants in the upper weight class (>1000 g), those who received BB536-LGG tended to have a better tolerance to enteral feeding from the start, because enteral feeding happened to start slightly, albeit not significantly, sooner than in the placebo group (3.2 ± 1.5 compared with 3.9 ± 1.2 d; $P = 0.12$; Figure 2). Thus, an amplification effect due primarily to an earlier feeding cannot be ruled out.

In contrast with a previous study (21), probiotic supplementation was not associated with a modification of intestinal microbiota, except for colonization by the probiotic strains. The mechanism by which probiotics enhance intestinal function in very premature infants remains to be elucidated. A variety of mechanisms have been hypothesized, including decreased adherence of pathogenic bacteria to gut mucosa, improved intestinal barrier function, protection against ischemic injury, and a decrease in the nuclear transcription factor κB–mediated inflammatory response (29). Calprotectin was unchanged even in the group that benefited from probiotics. Even though the use of calprotectin as a marker of inflammation has not been validated in this population of patients, this finding does not support an antiinflammatory effect of probiotics. However, other mechanisms may be involved, because *Lactobacillus GG* may protect intestinal epithelial cells from oxidative stress by inducing the expression of heat shock chaperone protein and activating signal transduction pathways in enterocytes (30). Furthermore, *L. acidophilus* was recently shown to modulate abdominal pain through the induction of opioid and cannabinoid receptors in intestinal cells (31).

Safety is a major concern when the use of probiotics is considered in frail populations. The design of the present study involved a sequential analysis by triangular test to avoid exposing an excessive number of extremely premature infants with a birth weight as small as 550g to a potential harmful effect of probiotics (25, 26). As a matter of fact, *Lactobacillus* sepsis was reported previously in an infant and in a 6-y old child who received probiotic supplementation (25). In a recent study, sepsis was observed in 9 of 33 preterm infants who weighed <750 g and received *L. acidophilus* and *Bifidobacterium bifidum* as compared with 1 of 18 infants who received placebo ($P = 0.08$) (32). For safety reasons, we elected to use a total daily dose of 4×10^8 cells for each strain, ie, the same range (between 10^8 and 6×10^9) used in earlier trials. No deleterious effects were observed, and we found no difference in the incidence of sepsis between the probiotic and placebo groups, even in the subgroup that weighed ≤1000 g, and detected no BB536-LGG in any blood culture in the relatively small population studied. Another concern was the potential for a long-term effect of any manipulation of intestinal microbiota in early life. Because the neonatal period is a time when bacterial colonization is initiated, and as the pattern of initial colonization might permanently alter gut flora through adulthood, the long-term safety of neonatal probiotic supplementation remains to be explored. The fact that we observed no deleterious effects after short-term supplementation with BB536-LGG does not resolve all safety-related issues of short- and long-term supplementation.

In contrast with earlier studies (18, 19), supplementation with BB536-LGG was not associated with a reduction in the incidence of sepsis in the present study. Differences in the probiotic strains used might have contributed to this discrepancy. Differences in the source of sepsis might also have played a role.

TABLE 3
Time to achieve full enteral feeding in infants with a birth weight >1000 g

	Probiotic group[1]	No. of subjects	Placebo group[1]	No. of subjects	*P*
	d		*d*		
Center A, Nantes ($n = 32$)	13.5 (11.2–21.5)	16	23.0 (18.0–26.0	16	0.007
Center B, Paris ($n = 24$)	16.0 (13.5–21.5)	13	16.0 (14.0–19.0)	11	0.53
Pooled population ($n = 56$)	16.0 (12.5–21.0)	29	19.0 (15.0–26.0)	27	0.038

[1] All values are medians (25th–75th percentiles).

We speculate that probiotics might be effective when bacterial translocation from the gut is the predominant source of sepsis and ineffective when the source of sepsis is not bacterial translocation from the gut but rather nosocomial sources, eg, catheter-related sepsis. The specific bacterial milieu in each NICU may determine the potential efficacy of probiotics.

Our choice of primary outcome deserves comment. Although prevention of necrotizing enterocolitis is of utmost interest, it may not be a relevant objective when the incidence of necrotizing enterocolitis is low, as was the case in the centers participating in this trial. We indeed observed only 3 cases of necrotizing enterocolitis, 2 of which were in the probiotic group. Although the present study was not powered to detect a difference in the incidence of necrotizing enterocolitis as a primary endpoint, it is tempting to speculate that when the baseline incidence of necrotizing enterocolitis is low in an NICU, the use of probiotics is unlikely to decrease it further.

In contrast, we believe that enhancing the gastrointestinal tolerance to feeds—thus shortening the time by which full enteral feeding is achieved—is a desirable and relevant objective because virtually all infants with a very low birth weight routinely receive intravenous nutrition for several weeks. Thus, a very large number of infants are exposed to intravenous nutrition, a technique associated with a high risk of complications, including catheter-related sepsis, thrombosis, and cholestasis. A putative reduction in the duration of parenteral nutrition would thus potentially benefit a very large number of extremely low-birth-weight infants. Such was the rationale for the choice of the primary outcome measure in the present study.

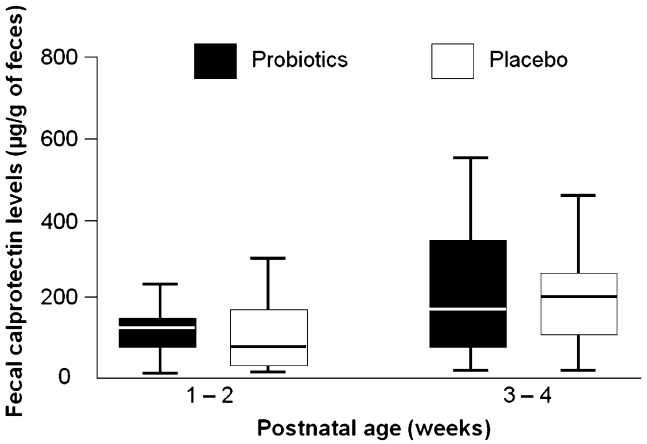

FIGURE 3. Fecal calprotectin concentrations in fecal samples collected at 2-wk intervals during the first month of supplementation in 47 preterm infants. The boxplot shows the median (central horizontal line) and includes the 25th (lower box border) to 75th (upper box border) percentiles. No statistically significant differences were observed between groups.

The efficacy of probiotic supplementation may depend on the specific strain of bacteria, as shown in children treated for acute diarrhea (33). Many studies of *B. longum* BB536 have been conducted in human term and premature infants (16) and have shown health benefits to the host. *L. rhamnosus* GG has been used successfully in premature infants to modulate intestinal microbiota (20, 34, 35). Moreover, because we observed a positive effect with this strain in the present study among infants who weighed >1000 g, it is unlikely that the choice of this strain per se was responsible for the lack of effect among the extremely premature infants.

From a theoretical standpoint, the type of feeding may affect the response to probiotics. In the present study, a larger proportion (94%) of the infants who weighed ≤1000 g received human milk exclusively (own mother's plus expressed milk), whereas only 80% of the infants who weighed >1000 g did so ($P = 0.047$). However, when included in a Cox model, exclusive human milk feeding did not play a significant role.

In summary, in the present double-blind, randomized trial, oral supplementation with *B. longum* BB536 and *L. rhamnosus* GG failed to improve the gastrointestinal tolerance to enteral feeding in extremely very-low-birth weight infants, whereas a beneficial effect was observed in infants who weighed >1000 g . Although several earlier trials of probiotics have addressed the efficacy of probiotics in very-low-birth-weight infants, the present study is first to our knowledge to show evidence of a clear-cut difference in the efficacy of BB536-LGG probiotics between 2 subgroups based on birth weight and to have investigated the actual colonization of probiotic strains in the gut of preterm infants and its potential relation with the efficacy of this treatment. The present findings suggest that the efficacy of oral probiotics may be limited in this context by frequent postnatal antibiotic treatment and the frequent need to withhold enteral feeding in extremely low-birth-weight infants. Finally, no deleterious effects were observed in the relatively small population studied in the present study; therefore, the use of BB536-LGG probiotics, albeit ineffective, appeared to be safe in the short term. Long-term effects were not assessed. Thus, the present study did not resolve all short- and long-term safety-related issues associated with use of probiotics in very-low-birth-weight infants.

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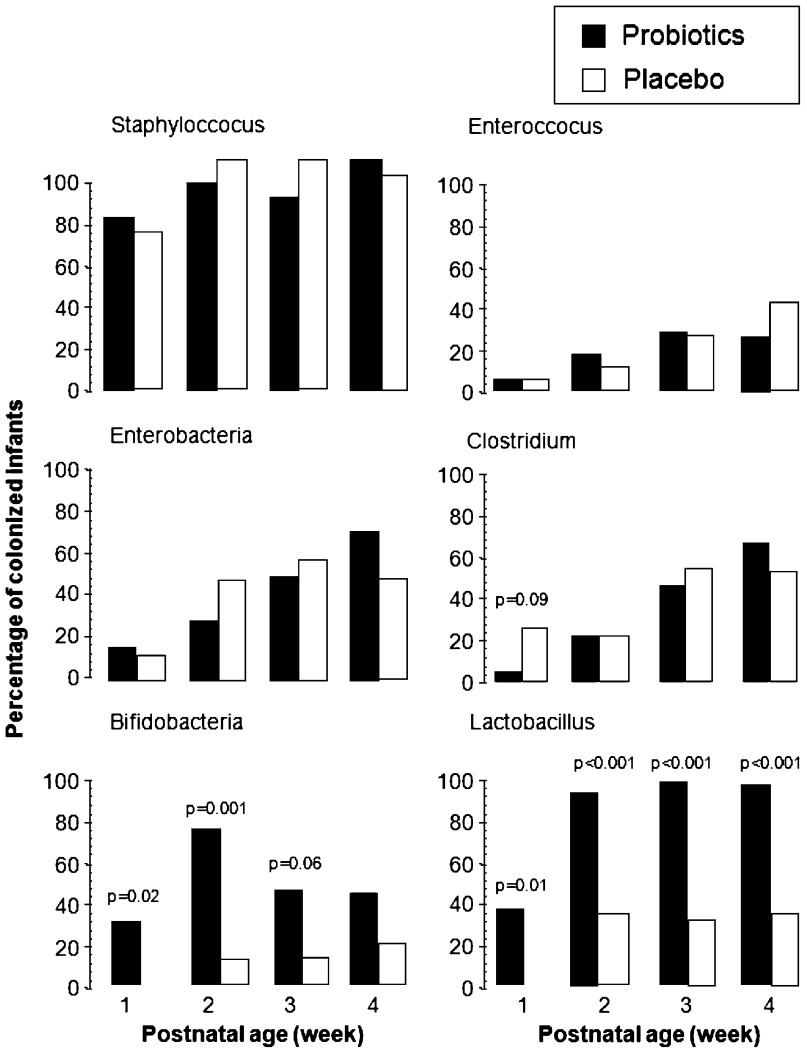

FIGURE 4. Incidence of intestinal colonization by selected bacterial strains in 47 preterm infants in the probiotic and placebo groups at 1-wk intervals during the first month of supplementation.

AL, M-FdlC, J-MN, M Vodovar, M Voyer, DD, and J-CR: data analysis and interpretation; CR and J-CR: draft of the manuscript; CR, HP, M-JB, CDR, DD, and J-CR: critical revision of the manuscript; J-MN and J-CR: statistical analysis; and HP, M Voyer, DD, and JCR: overall supervision of the study. BB and FR are employed by Nestec Research Center. None of the grant suppliers had any involvement in the design of the study, analysis of the data, or interpretation of the results. None of the authors had any conflicts of interest.

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