

# Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial

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## KEY WORDS

probiotics, sepsis, necrotizing enterocolitis, infant, preterm

## ABBREVIATIONS

CI—confidence interval

CoNS—coagulase-negative staphylococci

NEC—necrotizing enterocolitis

RCT—randomized controlled trial

RR—relative risk

Dr Garland conceptualized and designed the study and was responsible for the molecular microbiology components of the study. Together with Dr Jacobs, she contributed equally to the coordination of the trial, assisted with interpretation of the data, drafted the initial manuscript, and critically reviewed and revised the manuscript. Dr Jacobs assisted with study design and development of the operational protocol, was a site investigator at one site and supervised recruitments and data collection at 8 of the 10 sites. Dr Opie assisted with development of the operational protocol, was a site investigator and supervised recruitment and data collection at one site, and reviewed the manuscript. Ms Donath was the trial statistician, performed all the analyses, and reviewed the manuscript. Dr Tabrizi designed the assays for molecular microbiology of fecal samples and coordinated the testing of these samples, and critically reviewed and revised the manuscript. Dr Pirotta assisted with study design, and reviewed the manuscript. Dr Morley conceptualized and assisted with study design, and critically reviewed and revised the manuscript. Dr Tobin wrote the submission together with Drs Garland and Morley to the National Health and Research Medical Council for funding, developed the operational protocol, obtained ethical approval for the study, was involved in the initial coordination of the study including probiotic logistics, and reviewed the manuscript. All authors approved the final manuscript as submitted.

This trial has been registered with the Australia and New Zealand Clinical Trials Register (identifier ACTRN012607000144415).

(Continued on last page)

## WHAT'S KNOWN ON THIS SUBJECT:

Late-onset sepsis is a frequent complication of prematurity, contributing to morbidity and mortality. Although evidence is accumulating that administration of probiotics to very preterm infants reduces necrotizing enterocolitis (NEC) and all-cause mortality, the effect on late-onset sepsis is less clear.

## WHAT THIS STUDY ADDS:

The probiotic combination *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium lactis* reduced NEC in very preterm infants, but not mortality or late-onset sepsis. Probiotics may be of greatest global value in neonatal settings with high rates of NEC.

## abstract

**BACKGROUND AND OBJECTIVE:** Late-onset sepsis frequently complicates prematurity, contributing to morbidity and mortality. Probiotics may reduce mortality and necrotizing enterocolitis (NEC) in preterm infants, with unclear effect on late-onset sepsis. This study aimed to determine the effect of administering a specific combination of probiotics to very preterm infants on culture-proven late-onset sepsis.

**METHODS:** A prospective multicenter, double-blinded, placebo-controlled, randomized trial compared daily administration of a probiotic combination (*Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium lactis*, containing  $1 \times 10^9$  total organisms) with placebo (maltodextrin) in infants born before 32 completed weeks' gestation weighing  $<1500$  g. The primary outcome was at least 1 episode of definite late-onset sepsis.

**RESULTS:** Between October 2007 and November 2011, 1099 very preterm infants from Australia and New Zealand were randomized. Rates of definite late-onset sepsis (16.2%), NEC of Bell stage 2 or more (4.4%), and mortality (5.1%) were low in controls, with high breast milk feeding rates (96.9%). No significant difference in definite late-onset sepsis or all-cause mortality was found, but this probiotic combination reduced NEC of Bell stage 2 or more (2.0% versus 4.4%; relative risk 0.46, 95% confidence interval 0.23 to 0.93,  $P = .03$ ; number needed to treat 43, 95% confidence interval 23 to 333).

**CONCLUSIONS:** The probiotics *B. infantis*, *S. thermophilus*, and *B. lactis* significantly reduced NEC of Bell stage 2 or more in very preterm infants, but not definite late-onset sepsis or mortality. Treatment with this combination of probiotics appears to be safe. *Pediatrics* 2013;132:1055–1062

Late-onset sepsis occurring more than 48 hours after birth is a frequent complication of prematurity and is associated with significant mortality and morbidity. In 2009, the Australian and New Zealand Neonatal Network reported late-onset sepsis in 15.7% of infants born at <32 weeks and weighing <1500 g.<sup>1</sup> A recent large study from 313 North American NICUs reported an overall mortality of 15.1% in infants born <1500 g diagnosed with late-onset sepsis, compared with 8.5% in infants assessed for sepsis in whom cultures were negative.<sup>2</sup>

In contrast to term infants, preterm infants largely acquire their colonizing gastrointestinal bacteria from the NICU environment, rather than their mother's genital tract flora, skin, or breast milk.<sup>3</sup> In addition, gastrointestinal colonization with normal bacterial flora (eg, *Bifidobacterium* spp and *Lactobacillus* spp) is delayed and lacks biodiversity, whereas colonization with potentially pathogenic bacteria is increased (eg, *Escherichia coli*, *Enterococcus* spp, and *Klebsiella pneumoniae*).<sup>3,4</sup> This is exacerbated by treatment with broad-spectrum antibiotics that alter the composition of the intestinal flora and may predispose very preterm infants to both late-onset sepsis and necrotizing enterocolitis (NEC).<sup>4,5</sup>

Probiotics are live microorganisms that when administered in adequate numbers confer health benefits.<sup>6</sup> Probiotic effects are dose, condition, and strain-specific.<sup>7</sup> Meta-analyses and systematic reviews of randomized controlled trials (RCTs) of a variety of different probiotic strains and combinations administered to preterm infants of varying gestational ages and birth weights report a significant reduction in NEC and all-cause mortality, but not late-onset sepsis.<sup>8–12</sup> However, RCTs that reported late-onset sepsis were limited by lack of power and inconsistent definitions of late-onset sepsis.<sup>13,14</sup>

We therefore designed a trial to evaluate the effect of a combination of 3 probiotics administered daily to very preterm infants on the incidence of definite late-onset sepsis.<sup>15</sup>

## METHODS

### Patients and Study Design

The ProPrems trial was a prospective, multicentered, double-blind, placebo-controlled, randomized trial in which supplementing very preterm infants with probiotics was compared with placebo. The trial was conducted in perinatal hospitals in Australia ( $n = 8$ ) and New Zealand ( $n = 2$ ) and approved by the Human Research Ethics Committees at each center. Written informed consent was obtained from parents or guardians within 72 hours of birth.

Infants, born <32 completed weeks' gestation and weighing <1500 g, were eligible for enrollment within 72 hours of birth. Infants were excluded if they had major congenital or chromosomal anomalies, if death was considered likely within 72 hours of birth, or if the mother was taking nondietary probiotic supplements.

### Randomization

The Clinical Epidemiology and Biostatistics Unit at the Murdoch Children's Research Institute, who were independent of the study investigators, constructed the randomization sequence using Stata 9.0 (Stata Corp, College Station, TX) statistical software. Randomization was stratified by center; allocation was 1:1 and random block sizes of 2, 4, and 6 were used. Infants from multiple births were randomized individually. This schedule was provided to the pharmacist at RWH the Royal Women's Hospital who made up individual bottles for each randomized infant, coded by sequential study number. Apart from the pharmacist, all staff and parents were blinded to the randomized allocation.

## Study Intervention

The intervention was the probiotic combination *Bifidobacterium infantis* ( $BB-02\ 300 \times 10^6$ ), *Streptococcus thermophilus* ( $TH-4\ 350 \times 10^6$ ) and *Bifidobacterium lactis* ( $BB-12\ 350 \times 10^6$ ) (ABC Dophilus Probiotic Powder for Infants; Solgar, Leonia, New Jersey) with  $1 \times 10^9$  total organisms per 1.5 g, in a maltodextrin base powder. This is registered with the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) as BB-12 15954, BB-02 96579, Th-4 15957. It was the only probiotic combination available at the time that had been previously evaluated in preterm infants.<sup>14</sup> It was imported under license from the Australian Therapeutic Goods Administration Clinical Trial Notification Scheme. Taxonomy and quality of the probiotic organisms were confirmed independently. The placebo was maltodextrin powder (Professional Compounding Chemists of Australia, Sydney, Australia and/or Biotech Pharmaceuticals, Melbourne, Australia), identical in color and texture to the probiotic powder. Each new batch of probiotic and maltodextrin powder was analyzed for the presence and quantitation of the probiotic organisms with real-time polymerase chain reaction techniques (as they are not cultivatable readily by standard microbiological methods), and for purity using standard microbiological culture techniques. Polymerase chain reaction on fecal specimens confirmed colonization with ingested probiotic strains. The intervention was administered only when an infant was receiving at least 1 mL of milk every 4 hours. The intervention was withheld during periods when infants were nil orally. The daily dose was two 1-mL spoons (leveled by a wooden spatula), equivalent to 1.5 g of study powder, reconstituted with 3 mL breast milk or formula. When an infant received <3 mL milk per feed,

one 1-mL spoon of powder was mixed with 1.5 mL milk and given twice daily. The dose was the same irrespective of the infant's current weight or postnatal age and was administered daily by gastric tube or mouth, until discharge from hospital or term corrected age.

### Primary and Secondary Outcomes

The primary outcome was the incidence of at least 1 episode of definite late-onset sepsis before 40 weeks' postmenstrual age or discharge home, whichever occurred first. An episode of late-onset sepsis was defined as either the first episode  $>48$  hours after birth,<sup>1</sup> or a subsequent episode occurring  $\geq72$  hours after antibiotic treatment was stopped.

Definite sepsis was diagnosed when a pathogen was isolated from blood, urine (suprapubic aspirate or catheter specimen), or cerebrospinal fluid, and the infant was treated with antibiotics for  $\geq5$  days, or a postmortem culture of organ tissue grew a pathogen with concomitant histology of infection. When coagulase-negative staphylococcal species (CoNS) were isolated from blood, definite sepsis was diagnosed either when there were 2 time-separated cultures of the same species and the infant had been treated with antibiotics for  $\geq5$  days, or when a single CoNS species was isolated in association with raised blood markers of sepsis (C-reactive protein more than 10 mg/L and/or immature to total neutrophil ratio more than 0.2), and treatment with  $\geq5$  days of antibiotics.

Clinical sepsis was diagnosed either when a blood culture was negative, but the C-reactive protein was  $>10$  mg/L and/or the immature-to-total neutrophil ratio was  $>0.2$  and the infant was treated with antibiotics for  $\geq5$  days.

Secondary outcomes were the incidence of definite or clinical sepsis, as well as the composite outcome of definite or clinical late-onset sepsis, the number of

courses and duration of antibiotic treatment, the incidence of definite sepsis with a probiotic species, mortality, the incidence of NEC and NEC Bell stage 2 or greater,<sup>16,17</sup> duration of primary hospitalization and intravenous nutrition, time to enteral feeds of 120 mL/kg per day for  $\geq3$  days, breast milk feeding rates, days to regain birth weight, weight at 28 days of age and at discharge, patent ductus arteriosus treated with either medication or surgery, intraventricular hemorrhage grade 3 or 4 or cystic periventricular leukomalacia, retinopathy of prematurity  $\geq$ grade 3, oxygen treatment and/or respiratory support at 28 days of life and at 36 weeks' postmenstrual age.

### Sample Size and Statistical Analysis

The sample size was estimated on the baseline rate of 23% of at least 1 episode of late-onset sepsis in infants born  $<32$  weeks' gestation and  $<1500$  g from the Australian and New Zealand Neonatal Network database in 2003.<sup>18</sup> A 33% reduction was considered clinically important; therefore, 1100 infants (550 per arm) were required to have at least 80% power to detect a difference of 7% (23% vs 16%) between the 2 groups with a 0.05 2-sided significance level.

Differences between the 2 study groups were assessed using  $\chi^2$  and 95% confidence intervals (CIs) of relative risk (RR) for the primary outcome and other categorical outcome variables, tests, and 95% CI of difference of means for continuous unskewed outcome variables, and rank-sum tests for continuous outcome measures with skewed distributions. Main analysis was by intention to treat. Subgroup analyses were undertaken within prespecified gestational age strata ( $<28$  weeks) and birth weight ( $<1000$  g) for predefined outcomes, definite late-onset sepsis, and NEC  $\geq$ Bell stage 2, with logistic regression used to assess evidence for

interaction between treatment and subgroup. For the primary and main secondary outcomes, intention-to-treat analyses using logistic regression adjusting for stratification factor (site), gestational age  $<28$  weeks, gender, and commencement of study powder before day 7 of life, as well as per-protocol analyses excluding infants who did not receive the allocated intervention were also performed. Stata version 12.1 (Stata Corp) was used for all analyses.

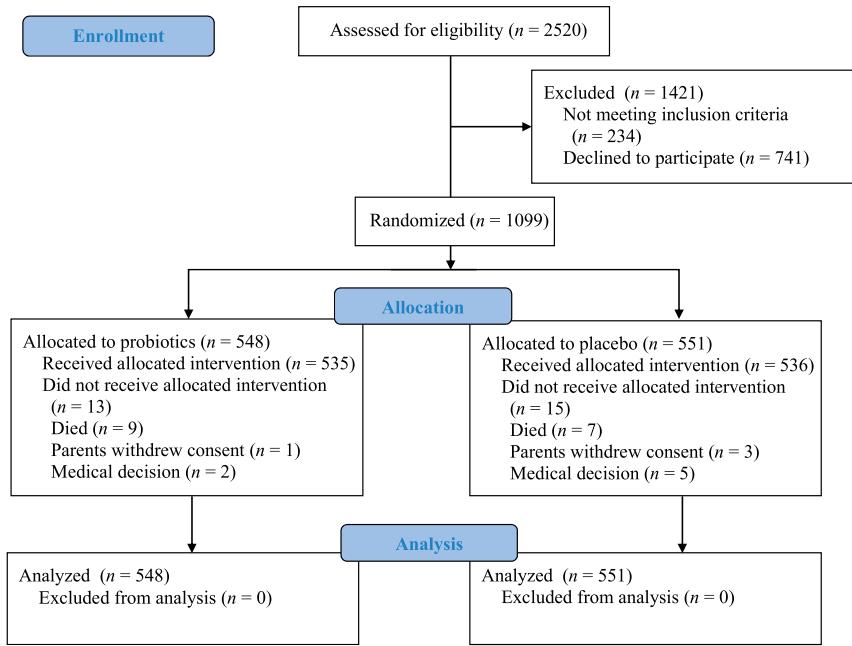
The independent Data Monitoring Committee assessed interim analyses of prespecified outcomes, late-onset sepsis, NEC, and mortality, and adverse events after 100, 200, 350, and 700 enrolled infants had reached term.

The trial is registered with the Australia and New Zealand Clinical Trials Register, number ACTRN012607000144415.

## RESULTS

Between October 5, 2007, and November 11, 2011, 2520 infants born  $<32$  weeks' gestation weighing  $<1500$  g were assessed for eligibility, with 1099 infants enrolled from 10 centers and randomized to the probiotics group ( $n = 548$ ) or the control group ( $n = 551$ ) (Fig 1). The baseline characteristics of the 2 groups are shown in Table 1.

There was no significant difference in the number of infants with at least 1 episode of definite late-onset sepsis (13.1% vs 16.2%; RR 0.81; (95% CI 0.61 to 1.08),  $P = .16$ ) with the probiotic combination (Table 2). This was unchanged when the analysis was adjusted for the prespecified confounding variables. In the planned subgroup analyses, there was a differential effect on late-onset sepsis for gestational age ( $P$  value for interaction .03), but not for birth weight ( $P = .24$ ). A significant reduction in definite late-onset sepsis was seen in the probiotic subgroup  $\geq 28$  weeks' gestation, but not for those  $<28$  weeks.



**FIGURE 1**

Trial profile.

There was no difference in the number of episodes of definite late-onset sepsis between the groups, or between groups in the number of episodes where conventional pathogens or CoNS as pathogens were isolated (Table 2). There was also no difference in the number of infants with  $\geq 1$  episode of clinical late-onset sepsis, the number with the composite outcome of definite or clinical sepsis, or in the number of episodes of clinical sepsis. There were no episodes of definite late-onset sepsis with the administered probiotic species *B infantis*, *S thermophilus*, and *B lactis*.

NEC of Bell stage 2 or more was significantly reduced in the probiotic group compared with controls (NEC of Bell stage 2 or more 2.0% vs 4.4%; RR 0.46; 95% CI 0.23 to 0.93;  $P = .03$ ), an absolute risk reduction of 2.4%, and number needed to treat of 43 (95% CI 23 to 333) (Table 3). This remained significant after logistic regression adjusting for stratification (site) and confounding variables. In the subgroup analysis for NEC of Bell stage 2 or more, there was no significant differential effect of the probiotic combination for birth weight ( $P$  value for interaction .08); these sub-

group results are therefore presented without  $P$  values. A  $P$  value for interaction for gestation could not be estimated, as the number of NEC cases in the  $\geq 28$  weeks subgroup was too small. The composite outcome of mortality or NEC of Bell stage 2 or more was not significantly different between groups.

There was no significant difference found in all-cause mortality between the groups during the study period, or the primary hospitalization (Table 3). There were no significant differences in mortality from NEC of Bell stage 2 or more or definite late-onset sepsis.

No significant differences were found in duration of intravenous nutrition or in days to establish full enteral feeds between the groups. More than 95% of the infants received breast milk (Table 3). The probiotic group weighed 48.9 g more at 28 days of life than the controls, but there was no significant difference at hospital discharge. There was no significant difference in duration of hospitalization between the groups, or any neonatal morbidities, including oxygen treatment or respiratory support at 36 weeks' postmenstrual age (Table 4).

## DISCUSSION

This large, multicenter, RCT in Australia and New Zealand found no significant effect of the probiotic combination *B infantis*, *S thermophilus*, and *B lactis* on definite late-onset sepsis in preterm infants born at  $<32$  weeks' gestation weighing  $<1500$  g. This was true for culture-proven sepsis with either conventional pathogens or CoNS species, as well as for clinical late-onset sepsis. Although a statistically significant reduction in NEC of Bell stage 2 or more was found in the probiotic group, the absolute reduction was only 2.4%. The study was not powered to detect a differential NEC reduction by birth weight or gestation; although we found an apparent benefit in NEC prevention in the groups that weighed  $\geq 1000$  g and were

**TABLE 1** Baseline Characteristics

	Probiotic Group, n = 548	Control Group, n = 551
Gestational age, wk, mean (SD)	27.9 (2.0)	27.8 (2.0)
<28 wk, n (%)	219 (40.0)	235 (42.6)
Birth weight, g, mean (SD)	1063 (259)	1048 (260)
<1000 g, n (%)	235 (42.9)	239 (43.4)
Male, n (%)	272 (49.6)	300 (54.4)
5-min Apgar score, median (IQR)	8 (7–9)	8 (7–9)
Multiple births, n (%)	197 (35.9)	193 (35.0)
Antenatal steroids (any), n (%)	502 (91.6)	500 (90.7)
Maternal antibiotics, n (%)	262 (47.8)	270 (49.0)
Maternal infection (chorioamnionitis), n (%)	47 (8.6)	48 (8.7)
Cesarean delivery, n (%)	359 (65.5)	377 (68.4)
Age at enrollment, d, mean (SD)	2.0 (0.9)	2.1 (0.9)
Age commenced study powder, d, median (IQR)	5 (4–7)	5 (4–7)

IQR, interquartile range (25–75).

**TABLE 2** Late-onset Sepsis Outcomes

	Probiotic Group, n = 548	Control Group, n = 551	RR (95% CI)	P Value
Primary outcome				
Infants with at least 1 episode of definite late-onset sepsis, n (%)	72 (13.1)	89 (16.2)	0.81 (0.61 to 1.08)	.16
Subgroup analyses:				
Gestational age			.03 <sup>a</sup>	
<28 wk, n (%)	54 (24.7)	55 (23.4)	1.05 (0.76 to 1.46)	.75
≥28 wk, n (%)	18 (5.5)	34 (10.8)	0.51 (0.29 to 0.88)	.01
Birth weight			.24 <sup>a</sup>	
<1000 g, n (%)	53 (22.6)	58 (24.3)	0.93	
≥1000 g, n (%)	19 (6.1)	31 (9.9)	0.61	
Secondary outcomes				
Infants with at least 1 episode of definite late-onset sepsis with pathogens, n (%)	38 (6.9)	48 (8.7)	0.80 (0.53 to 1.20)	.27
Infants with at least 1 episode of definite late-onset sepsis with CoNS, n (%)	40 (7.3)	43 (7.8)	0.94 (0.62 to 1.42)	.75
Infants with at least 1 episode of definite late-onset sepsis with probiotic species, n (%)	0	0		
Infants with clinical late-onset sepsis, n (%)	75 (13.7)	83 (15.1)	0.91 (0.68 to 1.21)	.52
Infants with late-onset sepsis (definite or clinical), n (%)	129 (23.5)	146 (26.5)	0.89 (0.72 to 1.09)	.26
Courses of antibiotics, median (IQR)	1 (0–1)	1 (0–1)		.78
Days of antibiotic treatment, median (IQR)	2 (0–7)	2 (0–8)		.64

Late-onset sepsis >48 h after birth and before discharge home or term postmenstrual age.

Pathogens isolated included the following:

Pathogens: *Staphylococcus aureus* (n = 21), *Escherichia coli* (n = 20), Group B *Streptococcus* (n = 11), *Enterococcus faecalis* (n = 10), *Klebsiella* spp (n = 7), *Enterobacter* spp (n = 6), *Candida* spp (n = 5), *Pseudomonas aeruginosa* (n = 4), *Pseudomonas* spp (n = 2), *Stenotrophomonas maltophilia* (n = 2), *Serratia* spp (n = 2), *Enterococcus* spp (n = 1), *Bacillus cereus* (n = 1), *Clostridium perfringens* (n = 1), group A *Streptococcus* (n = 1), *Streptococcus viridans* (n = 1). Coagulase-negative *Staphylococcus* spp (CoNS spp) (n = 87; not speciated CoNS [n = 57]), *Staphylococcus epidermidis* (n = 24), *Staphylococcus capitis* (n = 5), *Staphylococcus warneri* (n = 1). IQR, interquartile range (25–75).

<sup>a</sup> Interaction P value.

born at ≥28 weeks' gestation, these results should be interpreted cautiously, as they may be chance findings. There was no significant difference in mortality or the composite outcome of death or NEC.

Our finding of a lack of effect of the probiotic combination on definite late-onset sepsis is consistent with most,<sup>14,19–29</sup> but not all,<sup>13,28,30–32</sup> RCTs, as well as published systematic reviews and meta-analyses.<sup>8–10</sup> Interestingly, our study suggests a differential benefit of this probiotic combination on late-onset sepsis and possibly NEC of Bell stage 2 or more in infants born at 28 to 31<sup>6</sup> weeks' gestation. However, given the small number of affected infants in the subgroups (late-onset sepsis ≥28 weeks, n = 52; NEC of Bell stage 2 or more ≥28

weeks, n = 7; and ≥1000 g, n = 11), the data must be interpreted with caution.

Consistent with other trials and meta-analyses, the probiotics *B. infantis*, *S. thermophilus*, and *B. lactis* significantly reduced NEC of Bell stage 2 or more, although the absolute difference in our population studied was only 2.4%. Although our trial specifically targeted the smallest and most immature preterm infants, the baseline incidence of NEC of Bell stage 2 or more was very low at 4.4% in controls. Consequently, between as few as 23 to as many as 333 preterm infants would need treatment with this probiotic combination, in Australia and New Zealand, to prevent 1 case of NEC of Bell stage 2 or more. In countries with higher rates, the effect may be greater.

In contrast to most other published trials and meta-analyses, the ProPrems study found no significant effect of probiotic supplementation with *B. infantis*, *S. thermophilus*, and *B. lactis* on all-cause mortality.<sup>8,9,13,14,21,28,29</sup> An RCT of 750 infants ≤2000 g also found no effect of *Lactobacilli reuteri* on mortality or nosocomial sepsis.<sup>29</sup> We found an absolute risk reduction in late-onset sepsis of 3.1%. Although this may be of clinical relevance, a much larger study would have been required to detect such a small difference with confidence. The rates of definite late-onset sepsis, mortality, and NEC of Bell stage 2 or more were low in the control group and may explain the lack of effect of the probiotic combination used on late-onset sepsis and mortality in this trial. Breast milk feeding initiation rates were high (96.9%) in both groups, compared with other studies.<sup>13,14,22–24,33</sup> Our findings emphasize the importance of performing well-powered trials of probiotic administration in very preterm infants in different settings.

Although some might contend that the explanation for the lack of effect of the probiotic combination used on mortality in this trial is related to the choice of probiotic combination, probiotic strains or dose used, or that probiotics were not started on the first day of life, the finding that probiotic supplementation with *B. infantis*, *S. thermophilus*, and *B. lactis* is beneficial to our very preterm infants by reducing NEC of Bell stage 2 or more may negate these factors. Moreover, no one has determined which is the most effective probiotic, combination of probiotics, when they should be started, what dosage should be used, or the duration of administration.<sup>34–37</sup>

No significant adverse effects of the probiotics *B. infantis*, *S. thermophilus*, and *B. lactis* were found in this trial. In particular, there were no episodes of definite late-onset sepsis from the

**TABLE 3** Other Secondary Outcomes

	Probiotic Group, n = 548	Control Group, n = 551	RR (95% CI)	P Value
<b>NEC</b>				
NEC (Bell stage 2 or more), n (%)	11 (2.0)	24 (4.4)	0.46 (0.23 to 0.93)	.03
Subgroup analyses:				
Gestational age				<sup>a</sup>
<28 wk, n (%)	11 (5.0)	17 (7.2)	0.69	
≥28 wk, n (%)	0	7 (2.2)		
Birth weight				<sup>b</sup>
<1000 g, n (%)	10 (4.3)	14 (5.9)	0.73	
≥1000 g, n (%)	1 (0.3)	10 (3.2)	0.10	
Age at NEC (Bell stage 2 or more), d, median (IQR)	20.5 (15.5–34.5)	21 (17.0–30.5)		.99
<b>Mortality</b>				
Death, n (%)	27 (4.9)	28 (5.1)	0.97 (0.58 to 1.62)	.91
Age at death, d, mean (SD)	21.7 (18.5)	23.3 (16.7)		.75
Death during primary hospitalization, n (%)	30 (5.5)	31 (5.6)	0.97 (0.60 to 1.58)	.91
Age at death during primary hospitalization, d, median (IQR)	21 (7–40)	24.5 (10–42.5)		.49
Causes of death, n (%)				
Late-onset sepsis	6 (1.1)	4 (0.7)	1.55 (0.43 to 5.32)	.52
NEC	4 (0.7)	11 (2.0)	0.37 (0.12 to 1.14)	.07
Composite of death or NEC (Bell stage 2 or more), n (%)	33 (6.0)	41 (7.4)	0.81 (0.52 to 1.26)	.35
<b>Other secondary outcomes</b>				
Length of primary hospital admission, d, median (IQR)	71 (54–92)	74 (58–93)		.09
Duration on parenteral nutrition, d, median (IQR)	12 (8–17)	12 (8–18)		.29
Days to full enteral feeds, median (IQR)	12 (9–16)	12 (10–17)		.31
Days to regain birth weight, mean (SD)	11.1 (4.5)	11.7 (4.8)	−0.5 (−1.1 to 0)	.06
Weight at 28 d, g, mean (SD)	1495.0 (401.2)	1446.0 (379.2)	48.9 (2.0 to 95.9)	.04
Weight at discharge, g, mean (SD)	2870.5 (748.8)	2864.0 (738.9)	6.5 (−84.2 to 97.2)	.89
Any breast milk, n (%)	520 (95.6)	532 (96.9)	0.99 (0.96 to 1.01)	.25
Exclusive breast milk at discharge home, n (%)	266 (51.6)	292 (56.5)	0.91 (0.82 to 1.02)	.11
Any breast milk at discharge home, n (%)	370 (71.7)	379 (73.3)	0.98 (0.91 to 1.05)	.56

Secondary outcomes during study period (before discharge or term postmenstrual age, whichever occurs sooner), unless specified otherwise. IQR, interquartile range (25–75).

<sup>a</sup> Interaction P value unable to be calculated.

<sup>b</sup> Interaction P value.

administered probiotic strains. This is similar to other probiotic trials, although isolated cases of probiotic sepsis in NICU infants have been reported.<sup>38,39</sup>

The strengths of the ProPrems trial are the blinded randomized design, the participant target population of very preterm infants derived from neonatal

units with high breast milk feeding rates, and the largest sample size of RCTs to date. The precise and reproducible definition of late-onset sepsis is also a strength and underpins our finding that there is no significant effect of probiotic administration with this combination on definite late-onset sepsis in very preterm infants. Molecular studies are ongoing to detail the intestinal microbiota of a subgroup of enrolled infants, in both groups and in those with and without NEC,<sup>40</sup> as well as to further describe colonization and cross-colonization of infants enrolled in this study. Surviving children are undergoing allergy evaluation, neurodevelopmental assessments after 2 years of age, and an economic evaluation of the probiotics *B. infantis*, *S. thermophilus*, and *B. lactis*.

## CONCLUSIONS

This large, multicenter, double-blinded, placebo-controlled, randomized trial demonstrated no evidence that the probiotic combination of *B. infantis* BB-02, *S. thermophilus* Th-4, and *B. lactis* BB-12, administered from soon after birth, reduced culture-proven, definite late-onset sepsis in very preterm infants. The rate of NEC of Bell stage 2 or more was halved, although the baseline rate was very low and so translates to as many as 333 infants requiring probiotic administration with this probiotic combination to prevent 1 case of NEC of Bell stage 2 or more. Although this probiotic combination did not affect all-cause mortality, it appears to be safe, cheap, and readily implemented. These results may assist neonatal units considering using

**TABLE 4** Morbidities

	Probiotic Group, n = 548	Control Group, n = 551	RR (95% CI)	P Value
PDA treated, n (%)	159 (29)	171 (31)	0.93 (0.78 to 1.12)	.47
IVH grade 3 or 4 or cystic PVL, n (%)	22 (4.0)	16 (2.9)	1.38 (0.73 to 2.60)	.31
ROP ≥grade 3, n (%)	28 (5.1)	30 (5.4)	0.94 (0.57 to 1.55)	.80
CLD at 28 d, n (%)	281 (53.1)	284 (53.3)	1.0 (0.89 to 1.12)	.96
BPD at 36 wk, n (%)	165 (31.6)	161 (30.7)	1.03 (0.86 to 1.23)	.74

BPD, bronchopulmonary dysplasia; CLD, chronic lung disease; IVH, intraventricular hemorrhage; PDA, patent ductus arteriosus; PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.

probiotics for very preterm infants. Probiotics may be of greatest value globally in neonatal settings with high rates of NEC, mortality, and late-onset sepsis.

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## **Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial**

Susan E. Jacobs, Jacinta M. Tobin, Gillian F. Opie, Susan Donath, Sepehr N. Tabrizi, Marie Pirotta, Colin J. Morley and Suzanne M. Garland

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