Prenatal probiotic administration can influence *Bifidobacterium* microbiota development in infants at high risk of allergy

To the Editor:

Presence of an altered intestinal *Bifidobacterium* microbiota during infancy is associated with an increased risk of atopic eczema and atopic sensitization in later life.[1-4] This provides a rationale for therapies to prevent allergic disease by modifying the early intestinal microbiota. Administration of probiotics from the prenatal period through to the first months of life may lead to reduced incidence of eczema[5,6] and may also influence the development of infants' *Bifidobacterium* microbiota.[7] Here, we demonstrate that short-term administration of a probiotic strain *Lactobacillus rhamnosus* GG (LGG) to mothers during late pregnancy can influence intestinal colonization by particular *Bifidobacterium* species in infants, but does not lead to infant colonization with the administered probiotic.

Our study population consisted of 122 mothers and infants participating in a randomized, double-blind, placebo-controlled trial assessing the efficacy of prenatally administered probiotics for the prevention of eczema in infants at high risk of developing allergic diseases (Probiotic Eczema Prevention Study registered with the Cochrane Skin Group). Details of the study set-up are available at www.nottingham.ac.uk/ongoingskintrials, trial no. 36. The study was approved by the Human Research Ethics Committees of both the Royal Children's Hospital and the Mercy Hospital for Women, Melbourne. Participating women were instructed to take capsules of LGG (1.8×10^{10} colony-forming units) or maltodextrin (LGG and placebo kindly provided by Dicofarm SpA, Rome, Italy) once daily from 36 weeks of gestation until delivery. Treatment compliance was assessed by capsule count of returned unused capsules. The following samples were collected: maternal rectal swabs at enrolment and at birth, maternal vaginal swabs at 38 weeks of gestation, infant fecal samples at 3, 7, 28, 90, and 180 days, and breast milk samples at 7 and 28 days. Bacterial DNA was extracted from fecal samples as described previously.[8] *Bifidobacterium* quantity and species composition of the samples were analyzed by quantitative PCR and terminal restriction fragment length polymorphism, respectively. The presence of LGG was analyzed by cultivation combined with strain-specific PCR. Details on the microbiological methods are available in this article's Methods text and Table E1 in the Online Repository at www.jacionline.org. All statistical analyses were performed according to the intention-to-treat principle. Prevalence ratios with 95% CIs were used to compare proportions between groups for dichotomous outcomes. To minimize the number of comparisons performed on correlated data, formal statistical comparisons were performed at 2 predefined time points only (7 and 90 days). Prevalences for all time points are depicted graphically along with associated 95% CIs derived from saturated generalized estimating equation models. Analyses were performed using Stata statistical software, version 10.0 (StataCorp LP, College Station, Tex).

The median compliances were 99% for the placebo group and 99.6% for the probiotic group. Demographic characteristics were similar between the 2 groups (Table I). Fig 1 shows the prevalence of different *Bifidobacterium* species in the groups randomized to placebo or probiotics at the 4 time points of data collection, and statistical comparisons for the time points 7 days and 90 days are presented in Table II. At 90 days, the *Bifidobacterium longum* group was detected more frequently in the probiotic group (82%) than in the placebo group (61%; $P = .01$; prevalence ratio, 1.35; 95% CI, 1.06-1.72). In addition, there were trends toward increased prevalence of *B breve* (prevalence ratio, 1.39; 95% CI, 0.88-2.21) and decreased prevalence of *B adolescentis* (prevalence ratio, 0.64; 95% CI, 0.35-1.19) and *B angulatum* (prevalence ratio, 0.68; 95% CI, 0.30-1.53) in the probiotic group at 90 days. There was no evidence that the species colonization at 7 days or the total concentration of *Bifidobacterium* at any time after birth differed between the 2 groups. Further details of the effects of duration of breast-feeding (Table E2), method of delivery (Tables E3 and E4), maternal LGG colonization (Table E5), and maternal *Bifidobacterium* colonization (Table E6) on the prevalence of *Bifidobacterium* species and LGG in infants are presented in this article's Online Repository at www.jacionline.org.

At enrolment, LGG was detected in 1.7% and 5.0% of the mothers in the probiotic group and the placebo group, respectively. At birth, 66.7% of the mothers in the probiotic group were colonized with LGG, compared with 11.8% of the mothers in the placebo group ($P < .001$; prevalence ratio, 5.67; 95% CI, 2.19-14.64). All breast milk samples ($n = 123$ at 7 days and $n = 46$ at 28 days) and all but 3 vaginal swabs ($n = 3/59$) were negative for LGG. Intention-to-treat analysis did not provide evidence of excess LGG colonization at any time point in infants whose mothers were assigned to receive probiotics. For example, at 90 days, 12.7% of infants in the probiotic group were colonized with LGG, compared with 8.8% of the placebo group infants ($P = .50$; prevalence ratio, 1.45; 95% CI, 0.49-4.30).

The composition of the fecal *Bifidobacterium* microbiota in infants from the placebo group would be expected to reflect that typical of Australian infants at elevated risk of developing allergic disease. Infants in the placebo group were found to be frequently colonized by the genus *Bifidobacterium* (91.7% at 7 days; 94.6% at 90 days). The dominant *Bifidobacterium* species at all time points evaluated was *B longum*. The prevalence of the different *Bifidobacterium* species at different time points is presented in Fig 2.

Infants with allergy in Western societies are reported to be less frequently colonized with infant-type *Bifidobacterium* species such as the *B longum* group (consisting of *B longum* biotype *infantis*, *B longum* biotype *longum*, and *B bifidum*) and *B breve*, and more frequently colonized by *B adolescentis* and other species typical of the adult intestinal microbiota than infants without allergy.[1,9] Here, we demonstrate that at 90 days of age, infants whose mothers received LGG during late pregnancy were more often colonized with species belonging to the *B longum* group, the most abundant group of *Bifidobacterium* microbiota of healthy infant intestine and human breast milk, than infants whose mothers received placebo. The direction of the effects reported in this study, in particular the higher prevalence of *B longum* and *B breve* and the lower prevalence of *B adolescentis*, is consistent with the development of healthy breast-fed infant-type *Bifidobacterium* microbiota. These results suggest that probiotic administration to women during late pregnancy can have beneficial effects on the early development of infant intestinal

TABLE I. Demographic characteristics of the 2 randomization groups

	Treatment allocation	
	Placebo (maximum, n = 57)	**Probiotic (maximum, n = 59)**
<2 Parents affected by allergies, n (%)	35 (61)	39 (66)
Vaginal delivery, n (%)	37 (66)	43 (75)
Male, n (%)	35 (63)	30 (53)
Percent compliance,* median (IQR)	99 (88, 100)	100 (88, 100)
Breast-fed exclusively for ≥3 months, n (%)	26 (46)	30 (54)
Yogurt consumption during pregnancy (g/wk), median (IQR)	300 (100, 900)	400 (50, 600)
Accidental ingestion of LGG-containing product during pregnancy, n (%)	2 (4)	6 (10)
Mother's yogurt consumption during first 3 months after delivery (g/wk), median (IQR)	200 (0, 600)	200 (0, 500)
LGG present in mother at baseline, n (%)	3 (5)	1 (2)

*Calculated as 100 × (number of capsules taken/number of capsules that would have been taken if mother took 6 capsules per day from enrollment to day of giving birth). *IQR,* Interquartile range.

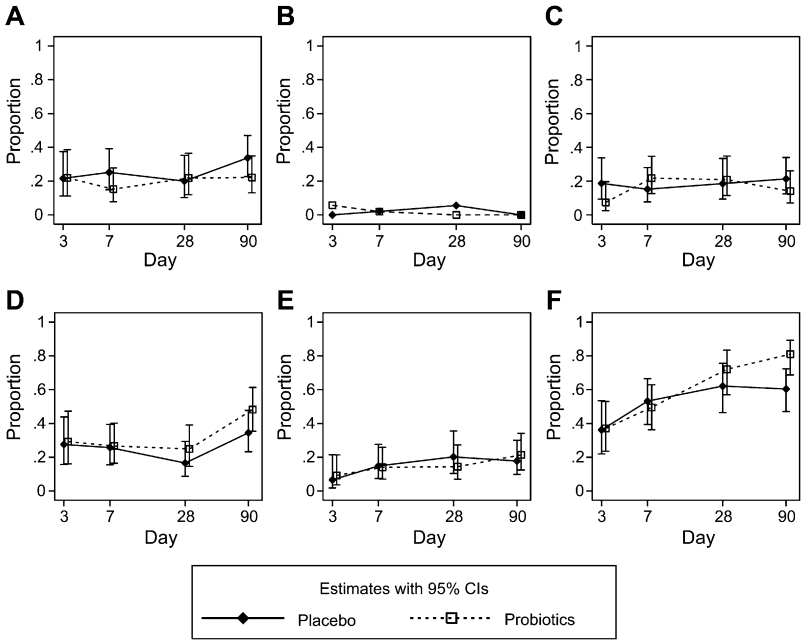

FIG 1. Prevalence of *Bifidobacterium* species in the placebo and the probiotic groups over time. **A,** *B adolescentis* group. **B,** *B animalis*. **C,** *B angulatum*. **D,** *B breve*. **E,** *B catenulatum* group. **F,** *B longum* group. The 95% CIs provided are adjusted for clustering by infant. No CI is provided for *B animalis* because of low numbers.

microbiota. Gueimonde et al[7] have reported an increased prevalence of infant-type *Bifidobacterium* microbiota in infants whose mothers received LGG from late pregnancy through to 6 months after delivery. In this study, we have demonstrated that well timed short-term prenatal probiotic administration may be sufficient to confer beneficial effects on infant intestinal microbiota. Limited administration of probiotic supplements to mothers during pregnancy would be preferred to more extended administration to pregnant mothers and then to infants in the first 6 months of life, and is more feasible as a public health intervention to prevent allergic disease.

The mechanisms by which maternal LGG supplementation might have influenced infant bifidobacterial colonization are unclear. In contrast with a small observational study by Schultz et al,[10] our data suggest that prenatal treatment with LGG does not result in intestinal LGG colonization in infants. The observed modulation of infant *Bifidobacterium* microbiota composition does not appear to be associated with LGG colonization of infant intestinal microbiota, breast milk, or maternal vaginal microbiota.

In conclusion, the administration of probiotics to mothers during late pregnancy was associated with higher prevalence of species belonging to the *B longum* group and the development of healthy infant-type microbiota. Our study is the first to demonstrate that prenatal administration of probiotics to mothers alone is sufficient to confer beneficial effects on the development of the infant intestinal microbiota. Although we investigated several factors which might influence infant *Bifidobacterium* populations, the mechanism by which prenatal LGG affects the composition of the infant intestinal microbiota remains unknown.

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TABLE II. Effects of prenatal administration of LGG probiotic on *Bifidobacterium* spp composition in the infant intestinal microbiota at age 7 days and 90 days

	Placebo, n (%)	Probiotic, n (%)	Effect of probiotic treatment, prevalence ratio (95% CI)	*P* value
Bifidobacterium present at 7 days				
Any	44 (91.7)	43 (82.7)	0.90 (0.78, 1.05)	.18
B adolescentis group	12 (25.0)	8 (15.4)	0.62 (0.28, 1.38)	.23
B angulatum	7 (14.6)	11 (21.2)	1.45 (0.61, 3.44)	.39
B animalis	1 (2.1)	1 (1.9)	0.92 (0.06, 14.35)	.95
B breve	12 (25.0)	14 (26.9)	1.08 (0.55, 2.09)	.83
B catenulatum group	7 (14.6)	7 (13.5)	0.92 (0.35, 2.44)	.87
B longum group	26 (54.2)	25 (48.1)	0.89 (0.60, 1.30)	.54
Bifidobacterium present at 90 days				
Any	53 (94.6)	55 (100.0)	1.06 (0.99, 1.12)	.08
B adolescentis group	19 (33.9)	12 (21.8)	0.64 (0.35, 1.19)	.16
B angulatum	12 (21.4)	8 (14.5)	0.68 (0.30, 1.53)	.35
B animalis	0 (0.0)	0 (0.0)	—	—
B breve	19 (33.9)	26 (47.3)	1.39 (0.88, 2.21)	.15
B catenulatum group	10 (17.9)	12 (21.8)	1.22 (0.58, 2.59)	.60
B longum group	34 (60.7)	45 (81.8)	1.35 (1.06, 1.72)	.01

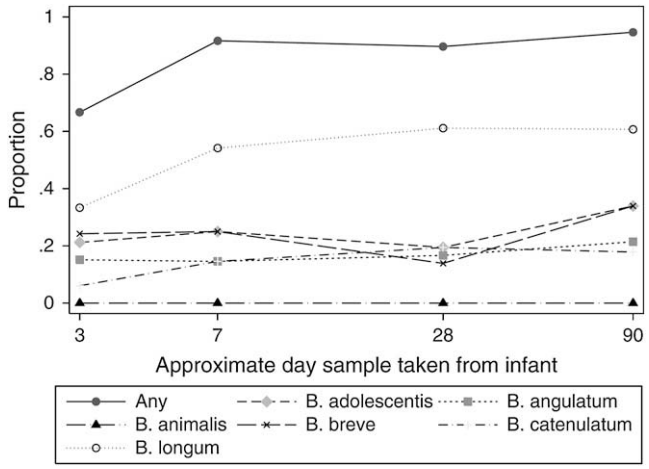

FIG 2. The *Bifidobacterium* spp composition of the early intestinal microbiota in Australian infants at high risk of allergic disease.

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METHODS

Identification of *Bifidobacterium* species based on terminal restriction fragment length polymorphism peaks

Bifidobacterium species composition of the fecal samples was analyzed by terminal restriction fragment length polymorphism (T-RFLP).[E1] Bacterial DNA was extracted as described previously[E2] and stored at –20°C until analysis. *Bifidobacterium* genus specific primers[E3] labeled at the 5′ end with 6′-carboxyfluorescein (Geneworks, Thebarton, Australia) were used to amplify a *Bifidobacterium* 16S rDNA fragment,[E4] which was isolated by gel electrophoresis and extracted with the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). Purified DNA was subjected to enzymatic restriction by incubating 60 ng DNA in the presence of 10 U *Alu*I (New England Biolabs, Ipswich, Mass) at 37°C for 4 hours. DNA was precipitated with 1:10 (vol/vol) 3 mol/L sodium acetate (pH 5.5) and 3 volumes of ethanol at –20°C overnight. DNA pellets were washed with cold 70% ethanol and air-dried. The digested PCR products were analyzed on a model 377 DNA sequencer (Applied Biosystems, Foster City, Calif) at the Australian Genome Research Facility, Melbourne, Australia, as described previously.[E5] The 12 *Bifidobacterium* strains used to obtain the reference peak values for *Bifidobacterium* species identification are presented in the Table E1. The reference strains were grown in reinforced clostridial medium (Oxoid, Basingstoke, United Kingdom) anaerobically at 37°C until the stationary growth phase, and 200 μL of each culture was subjected to DNA extraction. The identity of the cultures of the reference *Bifidobacterium* strains was confirmed by species-specific PCR as described.[E6,E7] Based on the reference peaks, the T-RFLP peaks derived from the stool samples were allocated into 6 groups: the *Bifidobacterium adolescentis* group (*B adolescentis* and *B dentium*), *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium breve*, the *Bifidobacterium catenulatum* group (*B catenulatum* and *B pseudocatenulatum*), and the *Bifidobacterium longum* group (*B bifidum, B longum* biotype *infantis* and *B longum* biotype *longum*). Peaks representing an area of <1% of the total area of peaks were excluded from the analysis.

Quantification of total genus *Bifidobacterium* from infant stool samples

Total levels of *Bifidobacterium* were determined in stool samples at 3, 7, 28, and 90 days from a subset of 86 infants by quantitative real-time PCR as described previously.[E8] To obtain a standard curve for the *Bifidobacterium* quantification, *B infantis* DSM 20088 (Deutsche Sammlung von Mikroorganismen und Zellkulturen DSMZ, Germany) was grown in reinforced clostridial medium anaerobically for 20 hours at 37°C. The actively growing culture was subjected to DNA extraction as described and concurrently plated on reinforced clostridial medium supplemented with agar. Plates were incubated anaerobically at 37°C for 3 days, and the colonies were counted. Serial dilutions of the DNA and the corresponding plate counts were used as standards in the real-time PCR. The change in total *Bifidobacterium* concentration over time was analyzed by using a generalized estimating equation approach with a normal distribution and an identity link. Because the distribution of concentrations was positively skewed, data were $\log_{10}$-transformed before analysis.

Detection of LGG in samples and capsules

For LGG detection, fecal samples were thawed, homogenized in PBS, and spread on de Mann, Rogosa, and Sharpe (Oxoid) agar plates supplemented with 0.5 g/L L-cysteine-hydrochloride. Thawed rectal and vaginal swabs and breast milk samples were plated directly on de Mann, Rogosa, and Sharpe plates. Plates were incubated aerobically for 3 days at 37°C. Suspected LGG colonies were identified visually on the basis of their typical creamy white, opaque appearance. Three suspected LGG colonies from each plate were subjected to strain-specific PCR as described previously.[E9] The stability of live probiotic in the treatment capsules was ensured by enumerating LGG from a total of 74 distributed and returned unused capsules representing 3 production batches by plate counting as described while maintaining blinding.

RESULTS

Effect of the extent of breast-feeding on the colonization of LGG and *Bifidobacterium* species in infants

Descriptive results for duration of breast-feeding and the colonization of *Bifidobacterium* species and LGG in infants are presented in Table E2. Because very few infants were breast-fed for less than 3 months, it was not possible to perform formal subgroup analyses to assess associations between *Bifidobacterium* species or LGG colonization and different combinations of duration of the breast-feeding and randomization groups. All of the 18 infants colonized by LGG at any time point were breast-fed for at least 3 months.

Effect of the mode of delivery on the colonization of LGG and *Bifidobacterium* species in infants

Because very few infants were colonized by LGG over the course of the trial, it was not possible to perform formal subgroup analyses to assess associations between LGG colonization and different combinations of delivery method and randomization group. For this reason, these results are reported descriptively. In the probiotic group, the majority (10/11) of the infants colonized with LGG at any time point were vaginally delivered, whereas in the placebo group, most colonized infants (5/7) were born by cesarean section (Table E3). It was observed that within the placebo group, infants born by cesarean section had a lower prevalence of *B longum* and a higher prevalence of *B adolescentis* than vaginally delivered infants, whereas in the probiotic group, the colonization of these species was similar between infants born by vaginal delivery or cesarean section (Table E4).

Effect of maternal LGG colonization on the colonization of LGG and *Bifidobacterium* species in infants

Because maternal colonization with LGG lies on the presumed causal pathway between LGG administration and infant LGG colonization, the association between maternal and infant LGG colonization was also assessed without considering randomization allocation. Weak evidence was found for an increase in infant LGG colonization at any time point where women were colonized by LGG at birth, irrespective of randomization allocation (prevalence ratio, 3.33; 95% CI, 1.01-10.95; Fisher exact test P value = .04; Table E5).

Effect of maternal *Bifidobacterium* on the colonization of infants with *Bifidobacterium* species

Descriptive results of the *Bifidobacterium* species colonization of mothers at the time of delivery are presented in Table E6. Because the numbers of mothers colonized with each *Bifidobacterium* species were comparable between the 2 randomization groups at the time of delivery, no formal statistical comparisons were made between the 2 groups to avoid multiple comparisons issues.

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TABLE E1. *Bifidobacterium* strains used as reference in the species identification, and the respective average lengths of the genus-specific PCR products and the terminal restriction fragments *(T-RFs)*

Species	Strain	PCR product (bp)	T-RF, bp (range)
B adolescentis	JCM 1275	511.8	424.5 (424.3-424.7)
B angulatum	JCM 7096	510.8	113.7 (113.6-113.8)
B animalis subsp *lactis*	Bb-12	521.1	433.5 (433.5-433.6)
B bifidum	DSM 20456	507.8	422.5 (422.4-422.5)
B breve	JCM 1192	513.1	427.0 (426.9-427.0)
B catenulatum	DSM 16992	510.8	510.8 (510.7-510.9)
B dentium	DSM 20436	511.5	424.4 (424.0-424.7)
B longum biotype *infantis*	DSM 20088	507.6	422.4 (422.3-422.4)
B longum biotype *infantis*	DSM 20090	507.7	422.5 (422.2-422.7)
B longum biotype *longum*	DSM 20219	507.7	422.4 (422.3-422.5)
B longum biotype *longum*	DSM 14579	507.8	422.5 (422.4-422.5)
B pseudocatenulatum	JCM 1200	510.9	510.9 (510.9)

Each value represents an average of 3 or more independent T-RLFP analyses, each based on an independent pure culture.

TABLE E2. Colonization of infants with *Bifidobacterium* species and LGG by randomization group and duration of breast-feeding

	Placebo <3 mo	Placebo >3 mo	Probiotic <3 mo	Probiotic >3 mo	Total
LGG present at any time point, n (column %)					
No	2 (100)	20 (74)	1 (100)	19 (63)	42 (70)
Yes	0 (0)	7 (26)	0 (0)	11 (37)	18 (30)
Total	2 (100)	27 (100)	1 (100)	30 (100)	60 (100)
B longum present in infant on day 90, n (column %)					
No	4 (67)	17 (37)	2 (40)	7 (16)	30 (30)
Yes	2 (33)	29 (63)	3 (60)	37 (84)	71 (70)
Total	6 (100)	46 (100)	5 (100)	44 (100)	101 (100)
B breve present in infant on day 90, n (column %)					
No	4 (67)	30 (65)	3 (60)	22 (50)	59 (58)
Yes	2 (33)	16 (35)	2 (40)	22 (50)	42 (42)
Total	6 (100)	46 (100)	5 (100)	44 (100)	101 (100)
B adolescentis present in infant on day 90, n (column %)					
No	2 (33)	32 (70)	2 (40)	35 (80)	71 (70)
Yes	4 (67)	14 (30)	3 (60)	9 (20)	30 (30)
Total	6 (100)	46 (100)	5 (100)	44 (100)	101 (100)
B catenulatum present in infant on day 90, n (column %)					
No	5 (83)	38 (83)	1 (20)	36 (82)	80 (79)
Yes	1 (17)	8 (17)	4 (80)	8 (18)	21 (21)
Total	6 (100)	46 (100)	5 (100)	44 (100)	101 (100)

TABLE E3. Colonization of infants with LGG by randomization group and mode of delivery

	Placebo vaginal	Placebo cesarean	Probiotic vaginal	Probiotic cesarean	Total
LGG present in infant at any time point, n (column %)*					
No	17 (89)	6 (55)	16 (62)	6 (86)	45 (71)
Yes	2 (11)	5 (45)	10 (38)	1 (14)	18 (29)
Total	19 (100)	11 (100)	26 (100)	7 (100)	63 (100)
Day 3, n (column %)					
No	22 (100)	11 (100)	25 (96)	8 (100)	66 (99)
Yes	0 (0)	0 (0)	1 (4)	0 (0)	1 (1)
Total	22 (100)	11 (100)	26 (100)	8 (100)	67 (100)
Day 7, n (column %)					
No	22 (100)	11 (100)	26 (90)	10 (100)	69 (96)
Yes	0 (0)	0 (0)	3 (10)	0 (0)	3 (4)
Total	22 (100)	11 (100)	29 (100)	10 (100)	72 (100)
Day 28, n (column %)					
No	24 (100)	11 (92)	28 (93)	10 (100)	73 (96)
Yes	0 (0)	1 (8)	2 (7)	0 (0)	3 (4)
Total	24 (100)	12 (100)	30 (100)	10 (100)	76 (100)
Day 90, n (column %)					
No	35 (95)	16 (84)	34 (83)	13 (100)	98 (89)
Yes	2 (5)	3 (16)	7 (17)	0 (0)	12 (11)
Total	37 (100)	19 (100)	41 (100)	13 (100)	110 (100)
Day 180, n (column %)					
No	36 (100)	16 (84)	36 (86)	12 (92)	100 (91)
Yes	0 (0)	3 (16)	6 (14)	1 (8)	10 (9)
Total	36 (100)	19 (100)	42 (100)	13 (100)	110 (100)

*Defined for the 63 infants who had LGG colonization status recorded at all of the 5 time points (n = 57) or were recorded as colonized by LGG at least 1 time point (n = 18).

TABLE E4. Colonization of infants with *Bifidobacterium* species by randomization group and mode of delivery

	Placebo vaginal	Placebo cesarean	Probiotic vaginal	Probiotic cesarean	Total
B longum present in infant on day 90, n (%)					
No	12 (32)	10 (56)	8 (20)	2 (15)	32 (29)
Yes	25 (68)	8 (44)	33 (80)	11 (85)	77 (71)
Total	37 (100)	18 (100)	41 (100)	13 (100)	109 (100)
B breve present in infant on day 90, n (%)					
No	24 (65)	12 (67)	23 (56)	5 (38)	64 (59)
Yes	13 (35)	6 (33)	18 (44)	8 (62)	45 (41)
Total	37 (100)	18 (100)	41 (100)	13 (100)	109 (100)
B adolescentis present in infant on day 90, n (%)					
No	27 (73)	9 (50)	32 (78)	10 (77)	78 (72)
Yes	10 (27)	9 (50)	9 (22)	3 (23)	31 (28)
Total	37 (100)	18 (100)	41 (100)	13 (100)	109 (100)
B catenulatum present in infant on day 90, n (%)					
No	30 (81)	15 (83)	32 (78)	10 (77)	87 (80)
Yes	7 (19)	3 (17)	9 (22)	3 (23)	22 (20)
Total	37 (100)	18 (100)	41 (100)	13 (100)	109 (100)

TABLE E5. Colonization by randomization group and maternal colonization with LGG at delivery

	Placebo, mother not colonized	Placebo, mother colonized	Probiotic, mother not colonized	Probiotic, mother colonized	Total
LGG present at any time point, n (column %)					
No	17 (89)	1 (50)	5 (83)	11 (61)	34 (76)
Yes	2 (11)	1 (50)	1 (17)	7 (39)	11 (24)
Total	19 (100)	2 (100)	6 (100)	18 (100)	45 (100)
B longum present in infant on day 90, n (column %)					
No	13 (43)	2 (50)	3 (30)	4 (17)	22 (33)
Yes	17 (57)	2 (50)	7 (70)	19 (83)	45 (67)
Total	30 (100)	4 (100)	10 (100)	23 (100)	67 (100)
B breve present in infant on day 90, n (column %)					
No	21 (70)	3 (75)	5 (50)	11 (48)	40 (60)
Yes	9 (30)	1 (25)	5 (50)	12 (52)	27 (40)
Total	30 (100)	4 (100)	10 (100)	23 (100)	67 (100)
B adolescentis present in infant on day 90, n (column %)					
No	20 (67)	2 (50)	8 (80)	17 (74)	47 (70)
Yes	10 (33)	2 (50)	2 (20)	6 (26)	20 (30)
Total	30 (100)	4 (100)	10 (100)	23 (100)	67 (100)
B catenulatum present in infant on day 90, n (column %)					
No	25 (83)	3 (75)	10 (100)	19 (83)	57 (85)
Yes	5 (17)	1 (25)	0 (0)	4 (17)	10 (15)
Total	30 (100)	4 (100)	10 (100)	23 (100)	67 (100)

TABLE E6. Maternal *Bifidobacterium* colonization at birth by randomization group

	Placebo (n = 31)	Probiotic (n = 33)	Total (n = 64)
Maternal *B adolescentis*, n [frequency] (%)			
No	16 (52)	21 (64)	37 (58)
Yes	15 (48)	12 (36)	27 (42)
Maternal *B angulatum*, n [frequency] (%)			
No	21 (68)	26 (79)	47 (73)
Yes	10 (32)	7 (21)	17 (27)
Maternal *B breve*, n [frequency] (%)			
No	28 (90)	30 (91)	58 (91)
Yes	3 (10)	3 (9)	6 (9)
Maternal *B catenulatum*, n [frequency] (%)			
No	12 (39)	13 (39)	25 (39)
Yes	19 (61)	20 (61)	39 (61)
Maternal *B lactis*, n [frequency] (%)			
No	30 (97)	31 (94)	61 (95)
Yes	1 (3)	2 (6)	3 (5)
Maternal *B longum*, n [frequency] (%)			
No	9 (29)	7 (21)	16 (25)
Yes	22 (71)	26 (79)	48 (75)