A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial

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Background: The role of probiotics in prevention of allergic disease is still not clearly established, although early reports suggested *Lactobacillus GG* halved the risk of eczema at 2 years.
Objective: To determine whether probiotic supplementation in early life could prevent development of eczema and atopy at 2 years.
Methods: Double-blind, randomized placebo-controlled trial of infants at risk of allergic disease. Pregnant women were randomized to take *Lactobacillus rhamnosus* HN001 (*L rhamnosus*), *Bifidobacterium animalis* subsp *lactis* strain HN019 or placebo daily from 35 weeks gestation until 6 months if breast-feeding, and their infants were randomized to receive the same treatment from birth to 2 years (n = 474). The infant's cumulative prevalence of eczema and point prevalence of atopy, using skin prick tests to common allergens, was assessed at 2 years.
Results: Infants receiving *L rhamnosus* had a significantly (*P* = .01) reduced risk of eczema (hazard ratio [HR], 0.51; 95% CI, 0.30-0.85) compared with placebo, but this was not the case for *B animalis* subsp *lactis* (HR, 0.90; 95% CI, 0.58-1.41). There was no significant effect of *L rhamnosus* (HR, 0.74; 95% CI, 0.46-1.18) or *B animalis* subsp *lactis* (HR, 0.82; 95% CI, 0.52-1.28) on atopy. *L rhamnosus* (71.5%) was more likely than *B animalis* subsp *lactis* (22.6%) to be present in the feces at 3 months, although detection rates were similar by 24 months.
Conclusion: We found that supplementation with *L rhamnosus*, but not *B animalis* subsp *lactis*, substantially reduced the cumulative prevalence of eczema, but not atopy, by 2 years. Understanding how Lactobacilli act to prevent eczema requires further investigation. (J Allergy Clin Immunol 2008;122:788-94.)

Key words: *Probiotics, eczema, atopy, allergic disease, infants, allergy prevention, randomized controlled trial*

In 1989, Strachan[1] suggested that decreased exposure to infections could explain the increasing prevalence of allergic disease in Western countries. This has become known as the hygiene hypothesis. Since then, investigations have progressed from the examination of the indirect markers of exposure to infections, such as family position and child care attendance, to measuring direct exposure to microbes and microbial products, such as lactic acid–producing bacteria and endotoxin. The prevalence of lactobacilli was shown to be higher in the feces of infants at 1 year in Estonia, where there is a low prevalence of allergic disease compared with Sweden, which has a higher prevalence of allergic disease.[2] *In vitro* and animal studies have also lent support for a role for organisms such as lactobacilli in immunological maturation.[3,4] Such observations have led to human experimental studies investigating the effect of probiotics on the development of allergic disease. The first of these was a small Finnish study showing that prenatal and postnatal exposure for 6 months to *Lactobacillus rhamnosus GG* halved the frequency of eczema at 2, 4, and 7 years but had no effect on atopic sensitization.[5-7] Since then, 4 other studies have been reported in which lactobacilli were administered to infants from birth to 6 months, but the species used differed between the studies, as have the findings. Kukkonen et al[8] used a combination of 4 probiotics, including 2 *Lactobacillus* species, along with prebiotic galacto-oligosaccharides. This study demonstrated a reduction in eczema that was stronger for the subgroup with atopic eczema. Another Scandinavian study used *Lactobacillus reuteri*[9] and found no overall effect on the cumulative incidence of eczema despite a reduction in

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Abbreviations used
HR: Hazard ratio
OR: Odds ratio
SCORAD: SCORing Atopic Dermatitis
SPT: Skin prick test

IgE-associated eczema. Other studies have found no effect of *Lactobacillus acidophilus*[10] *or L rhamnosus GG*[11] on atopic dermatitis, with 1 of these studies finding that *L acidophilus* supplementation actually increased the risk of atopic sensitization.[10] The different organisms used and whether there was a prenatal intervention may have influenced the divergent findings.

In this study, we tested the hypothesis that prenatal and postnatal supplementation with *L rhamnosus* strain HN001 or *Bifidobacterium animalis* subsp *lactis* strain HN019 can reduce the prevalence of eczema and allergy during the first 2 years of life in a population of high-risk New Zealand infants. Our study is unique in combining prenatal and postnatal probiotic supplementation, continued use of probiotics for 2 years postnatally, comparison of 2 different probiotics, and fecal sample analysis.

METHODS

Participants

Pregnant women in Auckland and Wellington, New Zealand, were recruited to the study through maternity care providers, antenatal classes, and advertisements. They were invited to take part in the study if they or the infant's father had a history of treated asthma, eczema, or hay fever. Women were ineligible for the study if they planned to move from the study center in the next 2 years, were already taking probiotic supplements long-term, or intended to use these in the child. They were not able to continue in the study if they delivered before 37 weeks gestation, they had not taken the study capsules for $\geq$2 weeks before birth, their infant's weight was <3rd percentile for sex and gestation, or their infant was placed in the neonatal unit for more than 48 hours or had serious congenital abnormalities at birth. If there were twins, only the heavier was included in the study.

Study design

The study was a 2-center, double-blind, randomized, placebo-controlled trial of the effects of probiotic supplementation on the development of eczema and atopic sensitization in infants (Australian New Zealand Clinical Trials Registry: ACTRN12607000518460). There were 2 treatment groups who received either *L rhamnosus* HN001 (6×10^9 colony-forming units/d) or *B animalis* subsp *lactis* HN019 (9×10^9 colony-forming units/d; Fonterra Co-operative Group, Auckland, New Zealand).

The probiotic supplements were manufactured by using aseptic fermentation, concentration, and freeze-drying. The growth media contained skim milk powder, yeast extract, and glucose. After growth, cells of the HN001 and HN019 strains were concentrated by centrifugation and washed twice with sterile saline. During prototype development of the low-allergenic probiotic supplements, the separate ingredients were tested by skin prick test (SPT) on several patients with cow's milk allergy. This work established that after 2 washes, the material had no reaction in the patients with cow's milk allergy. The final washed cells had a cryoprotectant solution, maltodextrin, mixed with the cells. This mix was frozen on trays and freeze-dried. The resulting powder had a particle size of 200 μm or less and was tested for the presence of pathogens before dispatch to a registered pharmaceutical packaging company. The placebo group received a capsule identical in appearance and smell containing dextran, salt, and a yeast extract (Fonterra Co-operative Group). The yeast extract used in the probiotics and the placebo contained no viable cells.

All batches of capsules were tested monthly to ensure viability of the probiotics. Shelf life was managed to ensure minimum cell counts were maintained. In addition, capsules returned from the field were tested for their viability. With very few exceptions, the viability was higher than the minimum required.

At 35 weeks gestation, pregnant women were randomized to receive one of the probiotics or placebo daily, to continue while they were breast-feeding for as long as 6 months postpartum. Infants started the capsules between 2 and 16 days postbirth (median, 6 days), continuing until age 2 years. The capsule powder was either given undiluted to the infant or mixed with water, breast milk, or formula and given via a teaspoon or syringe until solid food was started, when it was sprinkled on food.

Randomization and allocation of supplements were performed by a clinical trials pharmacist at Auckland City Hospital who had no contact with the participants. Randomization was stratified by study center and performed in blocks of 15 according to a computer-generated randomization list. At enrollment, a research study nurse assigned the next study number and provided the participant with the appropriate capsules. All study nurses and participants were blind to treatment assignment for the duration of the study. To evaluate the efficacy of the blinding, the final questionnaire asked participants to indicate whether they believed they were in a probiotic or placebo group.

Information collected at baseline included parental history of allergic disease; sex; ethnicity; household smoking; pet exposure; and length, weight, and head circumference at birth. Eczema prevalence and severity were assessed at follow-up visits at 3, 6, 12, and 18 months and 2 years, and SPTs performed at 2 years to assess atopic sensitization. History of antibiotic use was also collected at these visits.

Ethical approval was granted by a national multiregion ethics committee, covering both study centers.

Outcome measures

Eczema prevalence from birth to 2 years was defined using the UK Working Party's Diagnostic Criteria for atopic dermatitis[12] modified for use in infants. Eczema was determined to be present at each visit if there was a history of scratching or rubbing and 2 or more of the following occurring since birth or the previous visit: (1) a history of involvement of outer arms or legs, (2) a history of a generally dry skin, or (3) visible atopic eczema present on the cheeks or outer arms or legs with no axillary involvement. The research staff were trained in determining eczema by using an internationally recognized training manual for defining atopic eczema.[13]

Eczema severity from birth to 2 years was assessed by using SCORing Atopic Dermatitis (SCORAD)[14] in all children regardless of their eczema diagnosis (as defined). SCORAD was analyzed dichotomously using a cutoff $\geq$10 to exclude those with trivial rash. All staff were trained to apply SCORAD in a standardized way.

After training in the use of a standardized protocol,[15] the study nurse performed SPTs at 2 years to egg white, peanut, cow's milk, cat pelt, *Dermatophagoides pteronyssinus*, and mixed grass pollen (Hollister-Stier, Spokane, Wash). This panel of allergens has been shown to identify 90% of atopic children at 15 months who were tested to a wider range of allergens.[16] Antihistamine medication was withheld for an appropriate period. The allergens and positive (histamine 10 mg/mL) and negative control were applied to the child's arm and pricked vertically for 1 second using Dome-Hollister-Stier lancets (United Kingdom). The histamine response was read at 10 minutes, and allergens and negative control at 15 minutes. A 3-mm or greater mean wheal diameter to 1 or more allergens after subtraction of the negative control wheal diameter and with a positive response to histamine was considered positive. For safety reasons, 6 children who had previously had a severe allergic reaction to a food and a positive SPT response for that food were not retested for the food but considered positive on the basis of the previous test. IgE-associated eczema was defined as eczema plus a positive SPT response, and non–IgE-associated eczema as eczema plus a negative SPT response.

Fecal sample collection

Fecal samples were collected from infants soon after birth and at 3, 12, and 24 months of age. The samples were held in the home freezer until

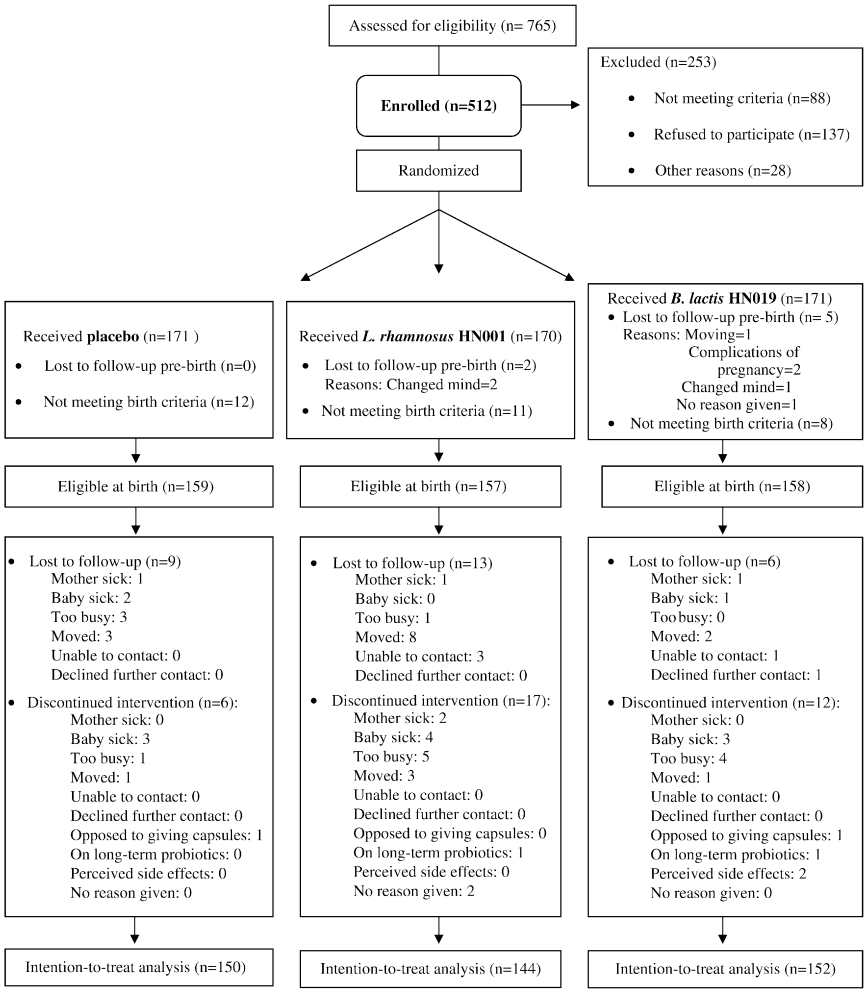

FIG 1. Diagram showing the flow of participants in the placebo, *L rhamnosus* HN001, and *B animalis* subsp *lactis* HN019 groups.

transportation to the research center for storage at –80°C. Bacterial DNA was extracted from feces by using a previously described method.[17] Bifidobacterial DNA was amplified by using PCR primers targeting the transaldolase gene,[18] and *Lactobacillus* amplicons were obtained by using PCR primers targeting the 16S ribosomal RNA gene.[19] Denaturing gradient gel electrophoresis was performed by using a Bio-Rad DCode universal mutation detection system (Bio-Rad, Hercules, Calif). Gradient concentrations and electrophoretic conditions have been described previously.[18,19] PCR amplicons generated from DNA extracted from pure cultures of *B animalis* subsp *lactis HN019* and *L rhamnosus* HN001 were used as markers in relation to fecal profiles in gels. Visual comparisons of fecal profiles with strain markers thus permitted the detection of *B animalis* subsp *lactis* and *L rhamnosus* in the fecal samples. Detection was at the species level because strain-specific PCR primers were not available.

Compliance

Bottles of capsules were replaced every 3 months and counted by a member of staff who had no participant involvement.

Sample size

Sample size calculation was based on a 50% cumulative prevalence of eczema by 2 years in the control group. To detect an 18% absolute reduction in eczema caused by probiotics, with 80% power at the 5% significance level, 127 were needed in each study group. To allow for a 25% loss because of ineligibility at birth or subsequent withdrawal, we planned to enroll 170 mothers in each group.

Statistical analysis

Analysis was undertaken by using SAS version 9.0 (SAS Institute, Cary, NC). Differences between study groups in the cumulative prevalence of eczema or SCORAD ($\geq$10) at each age were summarized by using Kaplan-Meier curves and proportional hazard models. Proportional hazard models were also used to assess differences in study groups in the point prevalence of atopy, and variables dependent on atopy, at 2 years. The persistence of *L rhamnosus* and *B animalis* subsp *lactis* in fecal samples over the study period was defined as detection of these bacteria on 2 or more occasions versus detection on 1 occasion only or absence of detection to limit the effect of adventitious exposure from food and other environmental sources. Odds ratios were used to assess associations between the persistence of each bacterium in feces and the 2-year prevalence of eczema and SCORAD $\geq$10, and the point prevalence of atopic sensitization at 2 years. The presence or absence of each probiotic species in fecal samples was also analyzed by study group at each time point. All children who completed the study were included in an intention-to-treat analysis regardless of their compliance. The χ^2 test was used to compare differences between groups and differences at baseline, with $P < .05$ considered statistically significant. Because baseline differences were small, these variables were not adjusted for in the analysis of the outcome variables.

TABLE I. Prevalence of study characteristics of eligible children in the placebo (n = 159), *L rhamnosus* HN001 (n = 157), and *B animalis* subsp *lactis* HN019 (n = 158) groups

	Placebo, n (%)	*L rhamnosus* HN001, n (%)	*B animalis* subsp *lactis* HN019, n (%)	*P* value*
Female	76 (47.8)	79 (50.3)	73 (46.2)	.76
Ethnicity				
Maori	18 (11.5) *(157)*	15 (9.6) *(157)*	15 (9.6) *(156)*	.83
European	121 (77.1) *(157)*	129 (82.2) *(157)*	124 (79.5) *(156)*	
Other	18 (11.5) *(157)*	13 (8.3) *(157)*	17 (10.9) *(156)*	
Birth				
Cesarean	50 (31.5)	46 (29.3)	57 (36.1)	.42
Birth weight (kg), mean (SD)	3.48 (0.4) *(157)*	3.48 (0.4) *(157)*	3.47 (0.5) *(156)*	1.00
Birth length (cm), mean (SD)	51.5 (2.0) *(156)*	51.7 (1.9) *(157)*	51.5 (2.0) *(156)*	.63
Head circumference (cm), mean (SD)	35.4 (1.3) *(157)*	35.6 (1.2) *(157)*	35.5 (1.3) *(156)*	.55
Breast-feeding				
Breast-feeding ever	152 (95.6)	153 (97.5)	154 (97.5)	.55
Mean duration (SD) (mo)	9.9 (5.7) *(147)*	9.8 (5.5) *(143)*	9.6 (6.1) *(151)*	.89
Environmental exposures				
Smoking in pregnancy	4 (2.5)	5 (3.2)	7 (4.4)	.57
Any smoking inside or outside	19 (12.0)	25 (15.9)	18 (11.4)	.61
Any pet	77 (48.4)	70 (44.6)	83 (52.5)	.37
Family history				
Family history of eczema†	119 (74.8)	114 (72.6)	119 (75.3)	.84
Maternal history of allergic disease‡	134 (84.3)	132 (84.1)	133 (84.2)	1.00
Paternal history of allergic disease‡	104 (65.4)	111 (70.7)	107 (67.7)	.60
Antibiotic use during study	129 (86.0)	118 (81.9)	129 (84.9)	.35

Sample sizes are shown in *italics* if different from those shown in the heading.
*P value χ^2 test for difference among the 3 study groups.
†Restricted to those treated by a doctor.
‡Defined as asthma, eczema, or hay fever with at least 1 disease treated by a doctor.

Compliance was calculated as the number of capsules taken as a proportion of the number of days in the study period.

RESULTS

Participants were recruited from January 2004 to May 2005 at an average rate of 7 per week. Among randomized participants who received treatment, 87.7%, 84.7%, and 88.9% in the placebo, *L rhamnosus* HN001, and *B animalis* subsp *lactis* HN019 groups, respectively, completed the study (Fig 1). Among participants who were eligible at birth, 94.3%, 91.7%, and 96.2% in the placebo, *L rhamnosus* HN001, and *B animalis* subsp *lactis* HN019 groups, respectively, completed the study (Fig 1). Of these, there were 6 participants in the placebo group, 17 in the *L rhamnosus* HN001 group, and 12 in the *B animalis* subsp *lactis* HN019 group who discontinued treatment but who continued to be followed up until the end of the study. One mother in the placebo group and 3 mothers in the *B animalis* subsp *lactis* HN019 group gave their reasons for discontinuing treatment as a result of perceived side effects of, or opposition to, taking study capsules. All these participants provided outcome data at each time point and were included in an intention-to-treat analysis. An additional 9, 13, and 6 in the placebo, *L rhamnosus* HN001, and *B animalis* subsp *lactis* HN019 groups, respectively, withdrew from the study completely and could not be included in an intention-to-treat analysis. None of these withdrawals was a result of perceived side effects of study treatment.

There were no significant differences between the groups in the proportion of participants who took more than 75% of the study capsules. Defined this way, the compliance rates were 77.3%, 73.6%, and 78.3%, in the placebo, *L rhamnosus* HN001, and *B animalis* subsp *lactis* HN019 groups, respectively.

There were no significant differences between study groups in baseline characteristics (Table I).

Infants receiving *L rhamnosus* HN001 had a significantly reduced risk of developing eczema by 2 years (14.8%) compared with infants in the placebo group (26.8%; Table II; Fig 2). The number needed to treat was 8.3. The hazard ratio was similar for those with IgE-associated eczema and those whose eczema was not IgE-associated, although not statistically significant in the latter group (Table II). There was no statistically significant effect of either probiotic on the likelihood of having a positive skin test result for any allergen, or for food allergens, at 2 years (Table II). The risk of developing SCORAD $\geq$10 was significantly reduced by 2 years in the *L rhamnosus* HN001 group (Table II; Fig 3). In contrast, there was no effect of *B animalis* subsp *lactis* HN019 on eczema prevalence or SCORAD $\geq$10 by 2 years (Table II; Figs 2 and 3).

Fifty-eight infants were exposed to commercially available nonstudy probiotics short-term either directly or through the mother's breast milk during the course of the study. After excluding these infants, the associations with eczema prevalence by 2 years for *L rhamnosus* HN001 (hazard ratio [HR], 0.45; 95% CI, 0.26-0.78; $P = .004$) and *B animalis* subsp *lactis* HN019 (HR, 0.87; 95% CI, 0.56-1.38; $P = .56$) strengthened slightly.

B animalis subsp *lactis* and *L rhamnosus* were detected in the feces of infants in the placebo and alternative probiotic group at birth, pointing to the adventitious inoculation of the alimentary tract with these bacteria from environmental sources[20-23] (Fig 4, *A and B*). However, administration of a probiotic resulted in markedly increased detection rates for that probiotic in fecal samples at 3, 12, and 24 months compared with the other groups ($P <$ .0001). Detection of *B animalis* subsp *lactis* in fecal samples

TABLE II. HRs (95% CIs) for the 2-year cumulative prevalence of eczema and SCORAD ≥10, and point prevalence of atopy in infants taking *L rhamnosus* HN001 and *B animalis* subsp *lactis* HN019

	Placebo (N = 159)	*L rhamnosus* HN001 (N = 157)	*P* value	*B animalis* subsp *lactis* HN019 (N = 158)	*P* value	*P* value*
Eczema	1.00 (26.8%)	0.51 (0.30-0.85) (14.8%)	.01	0.90 (0.58-1.41) (24.2%)	.64	.03
SCORAD ≥10	1.00 (38.7%)	0.57 (0.38-0.87) (24.0%)	.009	0.99 (0.69-1.43) (37.3%)	.97	.02
Eczema prevalence + SCORAD ≥10	1.00 (22.9%)	0.52 (0.30-0.91) (12.8%)	.02	0.98 (0.61-1.57) (22.2%)	.93	.047
	N = 146	N = 141		N = 149		
Atopy to any allergen	1.00 (28.8%)	0.74 (0.46-1.18) (21.3%)	.21	0.82 (0.52-1.28) (23.5%)	.38	.42
Atopy to food allergens	1.00 (21.2%)	0.74 (0.43-1.27) (15.6%)	.22	0.70 (0.40-1.20) (14.8%)	.19	.35
IgE-associated eczema	1.00 (18.5%)	0.51 (0.27-0.97) (9.9%)	.04	0.69 (0.38-1.24) (12.8%)	.21	.11
Non–IgE-associated eczema	1.00 (8.9%)	0.52 (0.21-1.30) (5.0%)	.16	1.28 (0.62-2.63) (11.4%)	.57	.13

*P value χ^2 test for difference among the 3 study groups.

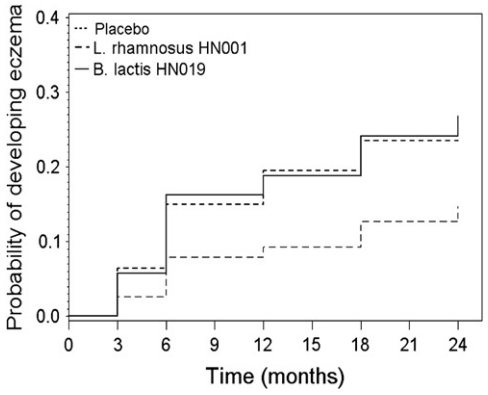

FIG 2. Kaplan-Meier plot showing the 2-year cumulative prevalence of eczema in infants taking placebo, *L rhamnosus* HN001, or *B animalis* subsp *lactis* HN019.

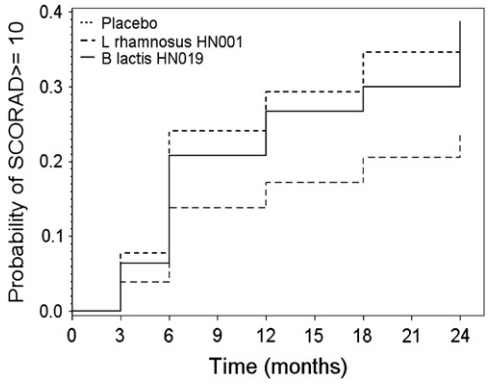

FIG 3. Kaplan-Meier plot showing the 2-year cumulative prevalence of SCORAD ≥10 in infants taking placebo, *L rhamnosus* HN001, or *B animalis* subsp *lactis* HN019.

increased progressively over the course of the study from 22.6% at 3 months to 53.1% at 24 months among infants administered HN019 (Fig 4, *A*). In contrast, detection of *L rhamnosus* was greatest at 3 months at 71.5% and was slightly lower at 24 months at 62.3% among infants administered this probiotic (Fig 4, *B*).

There was no relationship between the persistence (2 or more samples positive) of *B animalis* subsp *lactis* in feces and the development of eczema (odds ratio [OR], 1.21; 95% CI, 0.63-2.33; $P = .58$), atopic sensitization (OR, 1.01; 95% CI, 0.52-1.94; $P = .98$), or eczema severity (OR, 1.04; 95% CI, 0.57-1.88; $P = .90$) by 2 years. There was a trend toward a lower prevalence of eczema (OR, 0.65; 95% CI, 0.38-1.11; $P = .12$) and atopic sensitization (OR, 0.73; 95% CI, 0.44-1.21; $P = .22$) that reached significance for SCORAD ≥10 (OR, 0.56; 95% CI, 0.35-0.90; $P = .02$) among infants with persistent *L rhamnosus* in feces.

At the end of the study, parents were asked whether they thought they were in a probiotic or placebo group. More than half the respondents in each study group could not offer an opinion, 14.7% of the placebo group participants thought they had received a placebo, and 23.7% of the *B animalis* subsp *lactis* HN019 group and 25.7% of the *L rhamnosus* HN001 group thought they were in a probiotic group.

DISCUSSION

In this study, treatment with *L rhamnosus* HN001 for the first 2 years of life was associated with a reduction in the prevalence of any eczema by about a half. Although there was a similar reduction in IgE-associated and non-IgE-associated eczema, the effect was only significant for IgE-associated eczema, perhaps because of small numbers in the non–IgE-associated group. *L rhamnosus* HN001 also showed a strong protective effect against having a SCORAD value ≥10. Neither probiotic, however, showed a statistically significant protective effect against atopic sensitization, although for both probiotics the ORs were less than 1.

Our findings for eczema are consistent with 2 recent meta-analyses[24,25] and similar to those in the original study of Kalliomaki et al.[5] These authors did not analyze their findings according to whether the child had IgE-associated eczema, but they also found no effect on allergic sensitization measured by RAST or SPTs. Later studies have reported results both for all cases of eczema and for IgE-associated eczema and have either found slightly stronger protective effects for IgE-associated eczema[8] or protection against IgE-associated disease only.[9] No studies have found a significant protective effect on sensitization measured by using RAST or SPTs. Indeed, a recent Australian study found no effect on eczema prevalence at 6 or 12 months of age but found an increase in atopic sensitization in the probiotic group at 12 months.[10] Interestingly, this study was the only one in which there was no prenatal exposure to the intervention. A recent study[26] provides evidence of the presence of not only small quantities of viable bacteria but also a range of bacterial DNA signatures in human breast milk and maternal PBMCs. This suggests

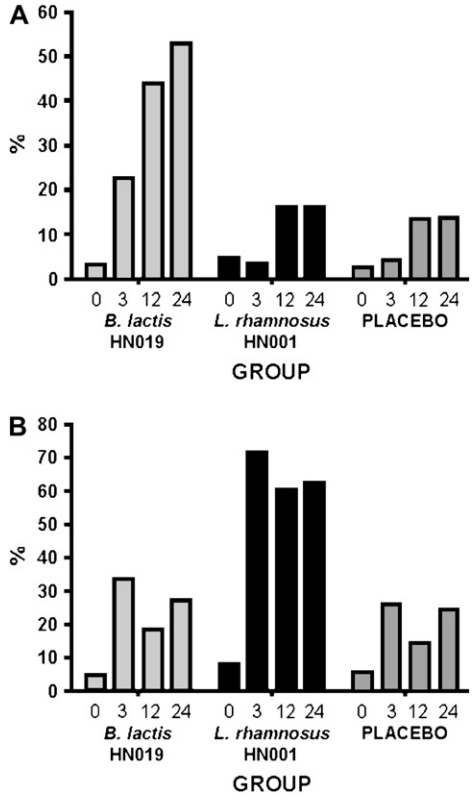

FIG 4. A, For each infant group (administered *B animalis* subsp *lactis* HN019, *L rhamnosus* HN001, or placebo), the percentage in which *B animalis* subsp *lactis* was detected at each time point (in months). **B,** For each infant group (administered *B animalis* subsp *lactis* HN019, *L rhamnosus* HN001, or placebo), the percentage in which *L rhamnosus* was detected at each time point (in months).

a potential mechanism by which the neonate might be influenced by maternal probiotic ingestion. Interestingly, in the study by Kopp et al,[10] exposure of neonates to *Lactobacillus GG* for the first 3 months was only through breast milk, followed by 3 months of direct supplementation to the infant. The lack of an effect on atopic dermatitis at 2 years may be a result of a delay in direct infant supplementation during a critical period of immune maturation and suggests that ingestion via breast milk may be less important than direct supplementation in influencing the development of allergic disease.

Responses may also be affected by dose and viability of the probiotic, with these factors in turn potentially modified by the host environment.

Despite some disparities between studies, the weight of evidence suggests a protective role for at least some *Lactobacillus* species in the pathogenesis of eczema, but there is little evidence overall that this is mediated through effects on allergic sensitization. The suggestion of Kukkonen et al[8] that probiotics regulate the pathway from sensitization to clinical disease is not supported by our findings, which show the effect of *L rhamnosus* HN001 is similar for sensitized and nonsensitized eczema.

A number of immunologic pathways have been shown to be affected by probiotics, involving several different mechanisms. Probiotic influences may be local, and potentially include reduction of permeability and systemic penetration of antigens; alteration of local inflammation or tolerance induction; anti-inflammatory effects mediated by Toll-like receptors; activation of tolerogenic dendritic cells; T_H1 skewing of responses; alteration of T-regulatory function; and increased local IgA production. Systemic effects with increased monocytes and effects on T cells, B cells, and stem cells have also been suggested.[27] Some strains of lactobacilli and bifidobacteria have been shown to modulate IL-10 production, possible enhancing regulatory or tolerance-inducing mechanisms.[28]

There have been no previous studies examining the effects of bifidobacteria species on the primary prevention of eczema, other than in combination with other probiotics.[8] Previous studies that have shown an effect on eczema have included a species of *Lactobacillus* in the intervention. A strength of our study is that we investigated the effects of 2 different probiotics but found a protective effect only for *L rhamnosus* HN001. It is also of interest that in the current study, cord blood IFN-γ levels were higher and more often detectable among the probiotic groups, but this was statistically significant only for the *L rhamnosus* HN001 group.[29]

We looked for bifidobacteria and lactobacilli in the feces of participants because it has rarely been established whether probiotic cultures can pass through the infant gastrointestinal tract and reach the colon when administered in trials of long duration.[23] We found different distributions in the detection rate of each probiotic between birth and 24 months. This might be a result of the changing ecosystem within the infant bowel as the characteristic shifts in bacterial community composition occur over time.[23] *B animalis* subsp *lactis* was detected at relatively low frequency during the first 3 months of administration. This may explain the lack of impact of this probiotic on the prevalence of eczema in our study. An alternative explanation for the lack of effect with *B animalis* subsp *lactis* HN019 is that it is less effective as an immunomodulatory agent. *L rhamnosus* detection in the feces was boosted by HN001 administration, but even so, after subtraction of background exposure to this species, less than half of the infants had detectable DNA from this species in their feces. A recent report from Singapore[23] showed that peak detection (after subtraction of background exposure) of a *Bifidobacterium longum* strain in infant feces occurred at 3 days after intervention commenced (44% of infants), falling to 26% and 16% after 1 and 3 months, respectively, of probiotic administration. In that study,[23] detection of *L rhamnosus* GG was 83% after 3 days of administration, then 77% and 69%, respectively, after 1 and 3 months of administration. Analytical methods of detection of fecal bacteria based on bulk-extracted DNA do not provide information about viability of the bacteria in the bowel with DNA potentially derived from active, quiescent, or dead bacterial cells. Nevertheless, the bacteriologic results of the study are notable because they reveal the relative abilities of the bifidobacteria and lactobacilli to transit the gastrointestinal tract.

There is some evidence that our sample population was not representative of the general New Zealand population. For example, the proportion of Maori participants (10.2%) and the baseline rate of household smoking (13.1%) in our study were both lower than population-based proportions, in which Maori make up 14.6% of the population[30] and the prevalence of individual smoking is 23.5%.[31] Nevertheless, we do not believe this seriously compromises the generalizability of our findings to all infants at risk of allergic disease.

There has been controversy about whether probiotics prevent the development of eczema. Our study provides further evidence that *L rhamnosus* is indeed an effective intervention for reducing

the prevalence of eczema among high-risk children. By comparing 2 different probiotics, we were able to demonstrate that not all probiotics are equally effective. Given the uncertainty about how probiotics exert their effects on allergic disease, future studies investigating their modes of action are required.

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Clinical implications: This study suggests that the probiotic *L rhamnosus* HN001 might be effective in preventing the development of eczema in high-risk infants.

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