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Safety aspects of probiotic bacterial strains *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* HN019 in human infants aged 0–2 years

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ABSTRACT

Given the relatively immature state of the neonatal gut and gut-associated immune system, the safety of probiotic strains for use as ingredients in infant milk formulae must be demonstrated in infant populations. As part of a double-blind placebo-controlled clinical trial of two commercially available probiotic strains in the reduction of risk for infant eczema, a number of safety outcomes were measured. Infants received daily doses of *Lactobacillus rhamnosus* HN001 (6×10^9 cfu day^{-1}) or *Bifidobacterium animalis* subsp. *lactis* HN019 (9×10^9 cfu day^{-1}), or placebo from birth to 24 months. Mothers received the same treatment from 35 weeks gestation, for up to 6 months postnatally while breastfeeding. No statistically significant differences were observed between the treatment groups for study withdrawal, incidence of adverse events, morphometric data, wheeze, and antibiotic use over the treatment period. We conclude that probiotics strains HN001 and HN019 were safe and well tolerated in infants, and did not affect normal growth.

1. Introduction

Probiotic bacteria, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002), reach the consumer via a wide range of dairy products, including yoghurts and other fermented milks, cheese, non-fermented milks and milk powders. There is also an increasing trend for probiotic bacteria to be supplied as a functional ingredient in infant milk formulae, based on the growing recognition of the heath benefits that probiotics offer (Parracho et al., 2007). Although probiotic bacteria have an excellent safety record, human infants represent a population that may be especially vulnerable to any adverse effects of ingesting relatively large numbers of live bacteria. At birth the infant gut and associated immune system have not fully developed, and unlike the adult gastrointestinal tract, the infant does not receive the probiotics in the context of a stable and established microflora.

Although the neonatal gastrointestinal tract is considered to be sterile at birth, colonisation by microorganisms occurs very rapidly, with the first exposure taking place during the process of birth itself, via the maternal vaginal microflora. While maternal breast milk appears to be an important source of bacterial species found in the developing gut microbiota (Gueimonde et al., 2007; Martin et al., 2007), maternal faecal microflora and bacteria from care givers or the local environment also appear to act as sources of colonising bacteria (Tapiainen et al., 2006). Added to this complexity is the role of external factors, such as the transition from milk to solid food, the role of bacteria derived from food (such as fermented dairy products), and episodes of antibiotic use and/or hospitalization (Penders et al., 2006) Thus, compared with the usually stable adult microflora, the developing microbiota of the infant is highly variable in nature, with bacterial species that transiently inhabit the gastrointestinal tract, and rapid and constant changes in both the make-up and relative proportions of a wide range of bacterial species (Favier et al., 2002; Heilig et al., 2002).

The unpredictability and complexity of the infant microbiota have made it difficult to determine the long-term impacts of the

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deliberate addition of probiotic bacteria. Most probiotic bacteria belong to the *Lactobacillus* or *Bifidobacterium* genera. Species of both genera can be found in human breast milk (Gueimonde et al., 2007; Martin et al., 2007), are common gut commensals in humans and other animals, and are commonly isolated from dairy and other foods. Lactobacilli are also considered ubiquitous in the environment. Thus, exposure of infants to lactic acid bacteria such as lactobacilli and bifidobacteria can be regarded as a natural event. Indeed, studies of premature infants at risk of necrotising enterocolitis have shown that the presence of strains of lactobacilli and bifidobacteria are protective against this serious gastrointestinal disorder (Hoyos, 1999; Hung-Chih et al., 2005), suggesting that at least some strains of lactic acid bacteria may be required for normal gut and immune development.

The aim of the present report was to examine the safety and tolerance of two commercial probiotic bacterial strains in human infants aged 0–2 years, and is based on a randomized, double-blind, placebo-controlled trial that investigated the impacts of probiotics on the development of childhood eczema and atopy in infants at high risk of allergic disease. The study consisted of three treatment groups: one group was fed *Lactobacillus rhamnosus* HN001 (HN001); one group was fed *Bifidobacterium animalis* subsp. *lactis* HN019 (HN019); the remaining group was fed a placebo. Mothers were given the same treatment daily from the 35th week of their pregnancy and while breastfeeding for up to 6 months after the birth of their infant; the infants were treated daily from birth for 24 months.

While the clinical outcomes of probiotic supplementation on eczema prevalence and atopy have been reported elsewhere (Wickens et al., 2008), the study also collected data on a range of safety outcomes to examine whether long-term feeding of infants with HN001 or HN019 was associated with any deleterious impacts on growth or general health. Presented here are the findings from the safety data, including results of standard morphometric measures (height, weight and head circumference), the frequency and reason for any potential adverse events that occurred in either the mother or infant over their respective treatment periods, and antibiotic use by the infants over the treatment period.

2. Materials and methods

2.1. Overall study design

The aim of the original study was to determine whether dietary supplementation of human infants with probiotic strains HN001 and HN019 was associated with a reduced prevalence of atopy and eczema in the first 2 years of life (Australian New Zealand Clinical Trials Registry: ACTRN12607000518460). The trial was conducted as a two-centre randomized, double-blind, placebo-controlled trial, with the objective of recruiting a total of 510 subjects with at least one parent with physician-diagnosed allergic disease. Following recruitment, mothers were randomly assigned into one of the three study arms, two treatment groups (that received either *L. rhamnosus* HN001, or *B. animalis* subsp. *lactis* HN019), and a placebo group, giving approximately 170 subjects in each group, with equal numbers recruited from Auckland and Wellington, New Zealand.

The study was conducted over a period of 3 years, with recruitment over a period of one year and children remaining in the study until 2 years of age. Seven visits by trained research nurses to each study participant were scheduled: recruitment of mothers by 35 weeks gestation, when the infant was born, and at 3, 6, 12, 18 and 24 months after birth.

Inclusion criteria for the study were asthma, hay fever or eczema treated by a doctor in either biological parent. Exclusion criteria were: intention to move from the area during the 2-year study period; gestation greater than 37 weeks; birth weight of the infant lower than the 3rd percentile for gender and gestation; infant admission to a neonatal unit for more than 48 h; serious congenital abnormalities; long-term probiotic use by the mother or intention to treat the infant with probiotics; and the mother receiving less than 2 weeks of treatment prior to giving birth.

At recruitment, mothers received a 7-week supply of capsules for themselves and at birth a 3-month supply for themselves and their baby. Every 3 months after that point, each mother received further supplies of capsules for herself up to 6 months after the birth if still breastfeeding, and for the baby until 2 years of age. All capsules were stored at 4 °C prior to distribution, and then kept in home refrigerators. Old capsules were collected and returned for analysis of probiotic viability and to assess compliance.

Capsules were taken daily, with mothers taking one capsule per day from 35 weeks gestation and up until the baby was 6 months old while still breastfeeding. Infants were given the contents of one capsule per day starting between days 2 and 16 post-birth, with a median of 6 days. Capsule contents were dissolved in approximately 2 mL of breast milk or water and given orally via a syringe (or teaspoon), as demonstrated by research nurses using a placebo capsule. After solid food was commenced, the contents of the capsules were sprinkled on food.

2.2. Capsule preparation

Probiotic bacterial strains *L. rhamnosus* strain HN001 and *B. animalis* subsp. *lactis* HN019 (Fonterra NZ, Palmerston North, New Zealand) were identified to the species level by a number of methods including sugar fermentation patterns, pulse field electrophoresis, 16S rRNA sequence analysis, and species specific probes as described in Prasad et al. (1998). Biologically pure cultures of HN001 and HN019 have been deposited with the Patent Culture Collection at the Australian Government Analytical Laboratory (AGAL) under deposit numbers NM97/09514 and NM97/01925, respectively. In addition, the genomes of both HN001 and HN019 have been sequenced, and sequences deposited on GenBank (accession numbers NZ_ABWJ00000000 and NZ_ABOT00000000, respectively).

Treatment groups received capsules that contained *L. rhamnosus* strain HN001 (6×10^9 cfu), *B. animalis* subsp. *lactis* HN019 (9×10^9 cfu), or placebo.

HN001 and HN019 were grown by aseptic fermentation in growth media containing skim milk powder, yeast extract and glucose. Bacterial cells were then concentrated approximately 15 times using a centrifuge and washed twice with sterile saline. After the final centrifugation (10,000 x g, 10 min), a cryoprotectant solution containing maltodextrin was added to the washed cells, and then frozen on trays. Following freeze-drying, the resultant powder was milled to 90% less than 200 μm and tested for the absence of pathogens before dispatch to a registered pharmaceutical packaging company.

Placebo capsules were identical in appearance and smell, and contained dextran, salt and yeast extract (Fonterra NZ, Palmerston North, New Zealand).

Prior to manufacture of the capsules, ingredients were tested by skin prick test on several cows' milk-allergic patients, and results confirmed that the sterile saline washing procedure used during manufacture of the capsules resulted in probiotic supplements that gave no reaction in cows' milk-allergic patients.

To ensure the viability of HN001 and HN019 present in the capsules, all batches of capsules were tested monthly. In addition, samples of capsules were returned from the study participants each month and re-tested for viability. Apart from a few exceptions, the viability of HN001 and HN019 met the required counts.

2.3. Safety data

Morphometric measurements of the infants were taken at set times during the intervention period. Head circumference, length and weight measurements were taken by research nurses according to the World Health Organisation (WHO), Physical Status: the use and interpretation of anthropometry; report of a WHO expert committee (WHO, 1995). Head circumference was measured soon after birth, at 3, 12 and 24 months, while length and weight were measured soon after birth, and at 12 and 24 months.

Adverse event information was collected from all infants over the duration of the trial, regardless of whether the study capsules were still being taken. Adverse event information was collected by questionnaires completed by research staff using standard questions. Calendars were provided to mothers at recruitment or the birth of their child to help parental recall of illness episodes when completing the questionnaires at each visit. The presence of wheeze in infants was assessed according to the "International study of asthma and allergies in childhood" (ISAAC) (Asher et al., 1995).

Serious adverse events (usually requiring hospitalization) were recorded for both mothers and their infants over the duration of the respective treatment periods. De-identified copies of serious advent forms were forwarded monthly to the Wellington Ethics Committee for review. Reasons for hospitalization were coded according to the International Classification of Disease version 10 (ICD10) at the 3 digit level.

The term "diarrhoea" was not defined clinically for adverse event data collected by interview, such that the term included events such as episodes of loose watery stools. To obtain a measure of clinically-defined diarrhoea and vomiting recorded from serious adverse event data, ICD10 codes A09 ("diarrhoea and gastroenteritis of presumed infectious origin") K52 ("other non-infective gastroenteritis and colitis"), R11 ("nausea and vomiting") and R19 ("other symptoms and signs involving digestive system and abdomen") were combined.

2.4. Loss to follow-up/withdrawal from study

If subjects chose to stop taking the study capsules during the study, they were encouraged to remain in the study, and were included in an intention-to treat analysis. If they chose to completely withdraw, reasons for withdrawal were collected.

2.5. Ethical considerations

The study was approved by a multi-regional ethics committee covering both Wellington and Auckland (New Zealand). The protocol was discussed with the Maori Health Unit at Capital Coast Health Ltd (Wellington, New Zealand) and was reviewed by the Maori Research Review Committee of the Auckland District Health Board (Auckland, New Zealand). Written informed consent was gained from all mothers at recruitment.

2.6. Statistics

Data were analysed using SAS version 9.0 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was used to assess differences between study groups in mean height, weight and head circumference. The χ^2 statistic was used to test differences between study groups in the number of hospitalization episodes by reason for hospitalization, and differences in gastrointestinal adverse events. Reasons for hospitalization were coded according to ICD10 categories at the 3 digit level, but analysed at the highest level because of small numbers of events in each 3 digit level category. A p value <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of study participants

Of the 765 pregnant mothers originally assessed for eligibility for enrolment into the study, 512 were randomized to one of three treatment arms at approximately the 35th week of pregnancy, with 170 in the HN001 group, and 171 each in the HN019 and placebo groups (Table 1). There were no statistically significant associations between reason for withdrawal from the study at any stage and treatment group.

At the close of the trial, there were 144 infants in the HN001 group, 152 in the HN019 group and 150 in the placebo group (Table 1). Compared with the numbers initially randomized into each treatment group, this gave completion rates of 84.7% for the HN001 group, 88.9% for the HN019 group and 87.7% in the placebo groups. There was no statistically significant association between the numbers completing the study and treatment group.

A number of mother/infant pairs discontinued the treatment, but were still available for assessment of the study outcomes, with 17 in the HN001 group, 12 in the HN019 group and 6 in the placebo group (Table 1). These cases were still included in the final analyses on an "intention to treat" basis.

Analysis of various characteristics of the study participants, as summarised in Table 2, showed no statistically significant differences between the study groups with respect to gender, ethnicity, delivery method, gestational age, breastfeeding or environmental exposures.

3.2. Morphometric data

Morphometric data were collected from the infants at birth and at various times throughout the 2-year treatment period to determine if probiotic treatment had any impact on normal growth

Table 1
The numbers of mother/infant pairs included at each stage of the study, the numbers withdrawing from the study, and the reasons for the withdrawal.

Treatment group	HN019	HN001	Placebo
Randomized	171	170	171
Lost to follow-up pre-birth:			
Changed mind	1	2	–
Moving	1	–	–
Complications of pregnancy	2	–	–
No reason given	1	–	–
Assessed for eligibility at birth	166	168	171
Failed to meet birth criteria:			
<3rd percentile	–	–	1
Premature	3	5	6
Congenital abnormality	1	–	–
Neonatal ICU >48 h	2	3	2
Insufficient study treatment	2	1	3
Underweight	–	2	–
Lost to follow-up:			
Mother sick	1	1	1
Baby sick	1	–	2
Too busy	–	1	3
Moved	2	8	3
Declined further contact	1	–	–
Unable to contact	1	3	–
"Intention to treat"	152	144	150
Discontinued intervention:			
Mother sick	–	2	–
Baby sick	3	4	3
Too busy	4	5	1
Moved	1	3	1
Perceived side-effects	2	–	–
Refused to use capsules	1	–	1
Taking other probiotics	1	1	–
No reason given	–	2	–

Table 2
Characteristics of infants in the placebo ($n = 159$), *L. rhamnosus* HN001 ($n = 157$) and *B. lactis* HN019 ($n = 158$) groups (adapted from Wickens et al., 2008).

	B. lactis HN019[a]	*L. rhamnosus* HN001[a]	Placebo[a]	*p* value[b]
Female	73 (46.2)	79 (50.3)	76 (47.8)	0.76
Ethnicity				
Maori (%)	15 (9.6) [*156*]	15 (9.6) [157]	18 (11.5) [*157*]	0.83
European (%)	124 (79.5) [*156*]	129 (82.2) [157]	121 (77.1) [*157*]	
Other (%)	17 (10.9) [*156*]	13 (8.3) [157]	18 (11.5) [*157*]	
Birth				
Caesarean (%)	57 (36.1)	46 (29.3)	50 (31.5)	0.42
Mean gestational age in weeks (SD)	39.4 (1.3)	39.7 (1.3)	39.6 (1.2)	0.21
Breastfeeding				
Breastfeeding ever (%)	154 (97.5)	153 (97.5)	152 (95.6)	0.55
Mean duration in months (SD)	9.6 (6.1) [*151*]	9.8 (5.5) [*143*]	9.9 (5.7) [*147*]	0.89
Environmental exposures				
Smoking in pregnancy (%)	7 (4.4)	5 (3.2)	4 (2.5)	0.57
Any smoking inside or outside (%)	18 (11.4)	25 (15.9)	19 (12.0)	0.61
Any pet (%)	83 (52.5)	70 (44.6)	77 (48.4)	0.37

[a] Percentages are given in parenthesis; sample sizes are given in square brackets and are shown in italics if different from those shown in the heading.
[b] *p* value chi sq test for difference between the 3 study groups.

(Table 3). No statistically significant differences were observed between the treatment groups in terms of weight, height, or head circumference.

3.3. Adverse events

Serious adverse event information was recorded for both mothers and infants during their respective intervention periods, and reasons for hospitalization classified according to ICD10 codes at the 3-digit level. The resultant data (summarised in Tables 3 and 4 using higher level codes) revealed no significant associations between treatment group and serious adverse event for infants, $p = 0.90$ (Table 4), or for mothers, $p = 0.29$ (Table 5).

The occurrence of less serious adverse events (i.e., not requiring hospitalization) was collected by questionnaire for each infant (Table 6). Results indicated that there were no statistically significant differences in the frequency of reported diarrhoea, vomits, reflux or spilling, or abdominal pain between the three treatment groups.

The presence of wheeze in infants was assessed at 3, 6, 12, 18 and 24 months by research staff using ISAAC criteria (Asher et al., 1995). Based on the 465 infants in the study at 3 months, by the end of the study 81 of 156 infants (51.9%) in the placebo group had experienced wheezing, compared with 81 of 155 infants (52.3%) in the HN019 group and 70 of 154 infants (45.5%) in the HN001 group. The finding of a lower proportion of children experiencing wheeze in the HN001 group was not statistically significant with respect to the placebo group ($p = 0.26$) or to the HN019 group ($p = 0.23$).

3.4. Antibiotic use

Lastly, as a further indicator of general health impact, the use of physician-prescribed antibiotics by the infants over the 2-year feeding period was monitored. A total of 129 infants in the HN019 group (84.9%) were prescribed antibiotics by a physician at some point over the 2-year study period, compared with 118 infants (81.9%) in the HN001 group and 129 (86.0%) in the placebo group. Despite the slight decrease in antibiotic use by infants treated with HN019 or HN001 compared with the placebo group, this difference was not statistically significant ($p = 0.35$).

4. Discussion

Lactic acid bacteria, including strains of lactobacilli and bifidobacteria, are normally found in breast milk and may play important roles in normal gut development and function. There is also increasing evidence that particular probiotic strains offer demonstrable health benefits to infants. However, to be accepted as useful ingredients to infant milk powders, probiotic bacteria must be shown to be safe as well as effective. Although the safety record of probiotics in healthy adult populations is impressive, given the special characteristics of infant physiology and the developing infant microbiota, it is important to establish probiotic safety in infants.

The probiotic strains HN019 and HN001 have both been shown by numerous studies to be safe in elderly, adult and child populations, and offer various immune and gut health benefits (Dekker et al., 2007; Gopal et al., 2005; Sanders, 2006). Both strains were originally isolated from dairy sources, have been the subjects of extensive safety testing in animal models (Prasad et al., 1998; Zhou & Gill, 2005; Zhou et al., 2000a, b, 2001, 2005), and have been shown to be safe and well tolerated in various human studies (Ahmed et al., 2007; Arunachalam et al., 2000; Chiang et al., 2000; Gill & Rutherfurd, 2001a, b; Gill et al., 2001a, b; Gopal et al., 2003).

While strains of bifidobacteria and lactobacilli are common early residents of the infant gut microbiota, comprehensive and direct

Table 3
Mean (range) weight, length and head circumference at birth, 12 and 24 months among children completing the study.

	B. lactis HN019 ($n = 152$)	*L. rhamnosus* HN001 ($n = 144$)	Placebo ($n = 150$)	*p* value
Birth				
Weight (kg)	3.48 (2.45–4.88)	3.48 (2.60–4.70)	3.48 (2.40–4.50)	1.00
Length (cm)	51.5 (45.6–56.5)	51.7 (46.9–57.0)	51.6 (46.0–57.0)	0.50
Head circumference (cm)	35.5 (30.9–39.1)	35.6 (32.5–38.9)	35.4 (32.0–38.8)	0.49
12 months				
Weight (kg)	9.85 (6.70–13.15)	9.86 (7.33–13.01)	9.87 (7.47–13.81)	0.99
Length (cm)	76.3 (68.0–84.0)	76.2 (68.0–83.0)	76.1 (68.5–85.0)	0.92
Head circumference (cm)	46.9 (43.0–50.3)	46.8 (42.4–50.1)	46.7 (43.5–49.9)	0.42
24 months				
Weight (kg)	12.82 (9.65–16.70)	12.92 (9.76–18.05)	12.82 (9.68–17.75)	0.77
Length (cm)	89.0 (78.8–98.0)	89.1 (78.5–98.0)	88.7 (80.0–100.0)	0.49
Head circumference (cm)	49.2 (46.1–53.0)	49.2 (46.0–52.2)	49.0 (46.0–53.2)	0.36

Table 4
Episodes of hospitalization among eligible infants during the treatment period (from birth to 24-months-old) by ICD10 category.

	HN019 $n = 158$	HN001 $n = 157$	Placebo $n = 159$	p value
Congenital malformation	3	3	2	0.86
Dermatological	4	3	0	0.15
Gastrointestinal	0	4	6	0.13
Genito-urinary	1	0	1	0.60
Infectious diseases	7	6	2	0.35
Neonatal	2	4	0	0.13
Neurology	1	0	0	0.37
Ophthalmology & Otology	5	4	6	0.60
Orthopaedics & Rheumatoid	0	0	1	0.37
Respiratory	6	9	16	0.47
Symptoms	4	7	4	0.21
Trauma & Injury	4	1	1	0.73
Diarrhoea or vomiting[a]	2	3	2	0.88
Any hospitalization	31	29	28	0.90
% of Total	19.6	18.5	17.6	

[a] Based on ICD10 codes: A09; K52; R11; and R19.

evidence of the safety of probiotic bacteria in human infants is still comparatively rare. In the present study, doses of HN019 or HN001 in the 10^9 cfu day^{-1} range were fed to infants from birth to 24-months-old as a part of a study to determine the impacts of probiotic bacteria on the development of eczema and atopy (Wickens et al., 2008). The study group was considered as being at risk of developing eczema based on a history of allergy from either parent, but were otherwise healthy.

Even though the probiotic feeding period was relatively long compared with similar studies (Kalliomaki et al., 2001; Taylor et al., 2007), there was no evidence of any adverse effects associated with HN019 or HN001consumption. This is based on several lines of evidence from safety outcomes examined as part of the clinical trial. First, similar dropout rates were observed for each treatment group, with no statistically significant relationship between the reason for no longer continuing with the study and treatment group. Completion rates in the treatment groups were all well over 80%, which compares well with similar studies. Second, standard morphometric measures of weight, height and head circumference collected at various time points during the study showed no evidence of any negative impact of receiving daily probiotics. Third, the prevalence of adverse events in the infant, both in terms of serious events resulting in hospitalization, or common symptoms of ill-health or discomfort during the first two years of life, showed no evidence of harm associated with probiotic treatment from birth. These findings were consistent with the observation that the level of antibiotic use also did not differ between the three treatment groups.

A recent study by Kopp and co-workers tested the use of *Lactobacillus rhamnosus* GG (LGG) to reduce the development of atopic eczema in at-risk infants (Kopp et al., 2008). While their study failed to show that LGG had any affect on atopic eczema, one concerning observation was the significantly increased prevalence of bronchial wheeze in the LGG-treated group at two years of age, compared with the placebo-treated group. Bronchial wheeze in infants can reflect a number of conditions, such as an early symptom of asthma, or a sign of respiratory infection. However, in the present study, the prevalence of wheeze up to 24 months did not differ between the three treatment groups.

Table 5
Episodes of hospitalization of mothers of eligible infants during the treatment period (from 35th week of pregnancy for up to 6 months post-birth) by ICD10 category.

	HN019 $n = 158$	HN001 $n = 157$	Placebo $n = 159$	p value
Cardio-vascular	0	1	0	0.36
Dermatological	0	0	1	0.37
Gastrointestinal	0	3	1	0.17
Genito-urinary	0	1	2	0.37
Infectious diseases	0	0	1	0.37
Obstetric	5	4	7	0.93
Psychiatric	0	1	0	0.36
Trauma & Injury	0	1	2	0.37
Any hospitalization	5	10	11	0.29
% of Total	3.2	6.4	6.9	

Table 6
Occurrence of any gastrointestinal adverse event, as recorded by questionnaire, from birth to 24 months by study group, among those infants in the study at 3 months.

	B. lactis HN019 (%) ($n = 155$)	*L. rhamnosus* HN001 (%) ($n = 154$)	Placebo (%) ($n = 156$)	p value
Diarrhoea after antibiotics	36.1	31.8	32.1	0.66
Other diarrhoea	76.1	76.0	75.0	0.97
Reflux or spilling	65.8	61.7	68.0	0.50
Abdominal Pain	52.3	48.7	49.4	0.80
Vomiting	78.7	70.1	73.1	0.22

5. Conclusion

Investigation into the effects of probiotic strains HN019 or HN001 on infant eczema included a number of safety endpoints designed to identify any negative health impacts arising from probiotic treatment. Analysis of the results showed that consumption of the probiotics daily from birth for 2 years had no effect on measures of general growth, health, and tolerance. The conclusion from the safety endpoints of the study were that both *B. animalis* subsp. *lactis* HN019 and *L. rhamnosus* HN001 were safe and well tolerated when given to infants from birth.

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