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Dietary Supplementation with Lactobacilli and Bifidobacteria Is Well Tolerated and Not Associated with Adverse Events during Late Pregnancy and Early Infancy[1–3]

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Abstract

Lactic acid bacteria and bifidobacteria are increasingly being administered to pregnant women and infants with the intention of improving health. Although these organisms have a long record of safe use, it is important to identify any adverse effects in potentially vulnerable populations. In a randomized, double-blinded, placebo-controlled trial, we evaluated the safety of a bacterial dietary supplement for the prevention of atopy in infants. Two strains of lactobacilli (*Lactobacillus salivarius* CUL61 and *Lactobacillus paracasei* CUL08) and bifidobacteria (*Bifidobacterium animalis subsp. lactis* CUL34 and *Bifidobacterium bifidum* CUL20) with a total of 1×10^{10} colony-forming units were administered daily to women during the last month of pregnancy and to infants aged 0–6 mo. Adverse events (AE) were classified according to WHO International Statistical Classification of Diseases criteria. Common symptoms were recorded by regular questionnaires. Baseline characteristics of 220 mother-infant dyads in the treatment and 234 in the placebo group were similar. Compliance with the trial interventions, loss to follow-up, symptoms, drug usage, infant growth, method of feeding, visits to the doctor, and mothers' assessment of infant health were similar in the 2 groups. Fifteen (6.8%) mothers and 73 (33.2%) infants in the treatment group and 21 (9.0%) mothers and 75 (32.1%) infants in the placebo group reported AE (P = 0.49 and P = 0.84, respectively). Severe AE occurred in 18 mothers and 63 infants with a similar frequency in each group. None of the AE were attributed to the intervention. Our findings support the safe use of this consortium of organisms during pregnancy and early infancy. J. Nutr. 140: 483–488, 2010.

Introduction

A range of microbial supplements are consumed by various groups of people with the aim of improving health. The term probiotics, although defined as live microorganisms which, when administered in adequate amounts, confer health benefits on the host (1), is often applied inaccurately to organisms in which no evidence for a clear health benefit exists. Probiotic organisms include the lactic acid bacteria (principally *Lactobacillus* and *Streptococcus*) and bifidobacteria.

Lactic acid bacteria and bifidobacteria have a long record of safe use (2). Clinical studies have provided evidence for their use in acute and antibiotic-associated diarrhea, necrotizing enterocolitis, and the prevention of atopy (3), and the safety of probiotics in children has recently been reviewed (4). However, there are rare case reports of infection associated with lactic acid bacteria administration, primarily in compromised hosts (5). Other potential concerns include horizontal gene transfer of virulence factors or antimicrobial resistance genes between probiotic organisms and other intestinal or food-borne microorganisms, the production of harmful metabolites, and possible adverse immunologic effects (6). The report of possible adverse effects of probiotics in acute pancreatitis (7) highlights the need to assess the safety of specific probiotic organisms in specific at-risk populations. In view of these concerns, many researchers have emphasized the need for careful monitoring for potential adverse effects (3–6).

We undertook a prospective, randomized, controlled trial of a microbial dietary supplement in the prevention of atopy. Two strains of lactobacilli and 2 strains of bifidobacteria were

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administered to mothers during the last month of pregnancy and to infants during the first 6 mo of life. Here, we report common symptoms and adverse effects that occurred in the study according to intervention group.

Materials and Methods

The study was approved by the Swansea Local Research Ethics Committee in February 2004 (International Standard Randomized Controlled Trial, ISRCTN 26287422).

Participants. Women aged 16 y or older with a normal singleton pregnancy attending antenatal clinics in hospitals or general practice surgeries were eligible to join the study. Most women were carrying a fetus at increased risk of atopy, defined as a fetus with a first-degree relative with either asthma or eczema diagnosed by a health professional or allergic rhinitis treated by a doctor. Some women carrying a fetus not at increased risk of atopy were also recruited. Exclusion criteria were any known adverse condition affecting the woman, fetus, or the likely outcome of the pregnancy; if a member of the fetus's sibship or household was already recruited to the study; or if the woman was unwilling to discontinue use of other live bacterial dietary supplements. Information regarding demographic factors and other factors that may be associated with adverse events (AE),[8] such as smoking during pregnancy and housing conditions, was collected at recruitment on standard forms.

Randomization. A computer-generated, random allocation sequence without blocks allocated the mother-infant dyad at 36 wk of gestation to either the treatment or placebo arm of the study on a 1:1 basis. The allocation sequence was generated by the independent statistician (D.W.) and was also held in sealed, opaque envelopes at the trial site for emergency access but was not available to any member of the research team. Research staff allocated dyads sequentially to the next number in the sequence.

Intervention. Women during the last month of pregnancy and their infants from birth to age 6 mo received daily vegetarian capsules composed of hydroxypropyl methylcellulose containing either the treatment consisting of *Lactobacillus salivarius* CUL61 [National Collection of Industrial, Food and Marine Bacteria (NCIMB) 30211] 6.25×10^9 colony-forming units (cfu), *Lactobacillus paracasei* CUL08 (NCIMB 30154) 1.25×10^9 cfu, *Bifidobacterium animalis subsp. lactis* CUL34 (NCIMB 30172) 1.25×10^9 cfu, and *Bifidobacterium bifidum* CUL20 (NCIMB 30153) 1.25×10^9 cfu or identical placebo capsules containing maltodextrin. The identity of the organisms was confirmed at the species and strain level by 16S rRNA gene sequencing, rep PCR fingerprinting and cluster analysis, and Random Amplified Polymorphic DNA typing. The products were independently tested at a United Kingdom Accreditation Service-accredited laboratory prior to release for the study.

Mothers were provided with 2 bottles each containing 30 capsules at recruitment. The mother either took the capsule by mouth or sprinkled the contents of the capsule onto food. For infants, 6 bottles of capsules were provided and the contents of the capsule were mixed with formula or expressed breast milk or sprinkled directly into the baby's open mouth.

Follow-up for AE. AE were defined as any untoward medical occurrence in a participant in the trial, including events that were not necessarily caused by or related to the intervention (8). Parents/caregivers were issued with a study card with the contact details of a named research nurse and were encouraged to maintain close contact by phone, to report any AE occurring in the mother or infant, and to discuss any concerns regarding the study. Parents and caregivers were asked to report their participation in the trial to hospital staff in the event of either the mother or infant requiring admission to hospital. If mothers or infants were admitted to the hospital, staff were alerted to their participation in the trial by a sticker placed on the cover of both the mother and baby case notes and encouraged to notify the research team. Throughout the course of the study, the hospital microbiology laboratory results were checked to identify lactobacilli or bifidobacteria infections.

In addition to spontaneous reporting, regular questionnaires collected information on common symptoms, compliance with trial interventions, feeding method, visits to general practitioners, medicines received, hospital admissions, and the mother's or main caregiver's assessment of the baby's overall health. Questionnaires were scheduled at ages 6, 12, 18, and 24 wk. At 6 wk, questionnaires were completed during a home visit by a research nurse, at 12 and 18 wk by telephone interviews, and at 24 wk during a research clinic or, if the infant was unable to attend, by telephone interview. Research staff also made additional visits to participants' homes to complete follow-up if required. Unused trial preparations were collected at home visits and the 6-mo clinic visit as a further assessment of compliance.

Categorization and independent review of AE. All AE were entered into an electronic database and reviewed at regular intervals by independent safety monitors who also provided guidance on the attribution of serious unexpected AE according to Directive 2001/20/EC (8). Symptoms and signs related to atopy have not been included as these were the primary outcome measures of the trial and will be reported separately. All other AE recorded by spontaneous reporting and questionnaires were included in the analysis. AE were categorized independently by 2 pediatricians (S.J.A. and G.M.) using WHO International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD10) criteria (9) and any discrepancies discussed. The severity of AE was graded according to Directive 2001/20/EC (8). All events where the participant was admitted overnight to hospital were classified as serious.

Data analysis. Once data collection and classification of AE was completed, the database was locked and sent for statistical analysis by intention to treat. Demographic variables and the frequency of AE in the 2 arms of the trial (treatment or placebo) were described. The number of spontaneously reported AE in infants was reported for the whole 6-mo period. Difficulties in contacting parents and caregivers, especially once many mothers had returned to work, often resulted in delayed or missed scheduled questionnaires. Information to the age of 8 wk was collated to assess the effects of exposure to the bacterial food supplement early in life and to ages 9–28 wk to assess effects of exposure later in infancy. Analysis was performed using SAS 9.1 (SAS Institute). Binary outcomes were compared with Fisher's exact test. Continuous variables at baseline are shown as mean and SD. Continuous outcomes tended to have skewed distributions, so were described using median values and ranges and analyzed by the Mann-Whitney U test. $P < 0.05$ was considered significant. Values in the text are medians (ranges) unless noted otherwise.

Results

From a total of 1419 pregnant women attending antenatal clinics who were assessed for eligibility to participate in the trial, 454 women were recruited, of whom 413 (91.0%) were carrying a fetus at increased risk of atopy. Two hundred and twenty women were allocated to the treatment group and 234 to the placebo group (**Supplemental Fig. 1**). Demographic variables and factors potentially associated with either AE or the reporting of AE were similar in the 2 arms of the study (**Table 1**). The number of respondents varied according to parents' and caregivers' ability to recall information.

Mothers. Median compliance with the trial intervention was 20 (range 0–60) d in 207 mothers in the treatment group and 20 (range 0–60) d in 213 in the placebo group ($P = 0.82$). Some

[8] Abbreviations used: AE, adverse event; CFU, colony-forming unit; ICD10, International Statistical Classification of Diseases and Related Health Problems, 10th Revision; NCIMB, National Collection of Industrial, Food and Marine Bacteria; SAE, serious adverse event.

TABLE 1 Baseline demographic and environmental factors that may be associated with AE or the reporting of AE

Variable	Treatment group, *n* = 220		Placebo group, *n* = 234	
	n		*n*	
Mother				
Age, *y*	220	29.0 ± 5.6	234	29.3 ± 6.0
Alcohol in pregnancy, *n (%)*	218	93 (42.7)	233	100 (42.9)
Current cigarette smoker, *n (%)*	220	33 (15.0)	234	40 (17.1)
Smoked cigarettes during pregnancy, *n (%)*	220	52 (23.6)	233	62 (26.6)
Education, *n (%)*				
University or college education	219	136 (62.1)	232	133 (57.3)
Other professional qualification	219	29 (13.2)	232	37 (15.9)
Currently working, *n (%)*	219	150 (68.5)	234	164 (70.1)
Father				
Education, *n (%)*				
University or college education	216	113 (52.3)	229	117 (51.1)
Other professional qualification	216	30 (13.9)	229	37 (16.2)
Currently working, *n (%)*	215	189 (87.9)	228	208 (91.2)
Housing				
Other children present, *n*	220	0.9 ± 0.9	234	1.0 ± 1.0
Adults present, *n*	220	2.1 ± 0.6	234	2.1 ± 0.6
House damp or condensation present, *n (%)*	218	57 (26.1)	232	56 (24.1)
House contains mold, *n (%)*	217	33 (15.2)	233	32 (13.7)
Cigarette smokers present, *n*	220	0.5 ± 0.7	233	0.6 ± 0.8

mothers mistakenly continued to take the trial interventions after delivery. In all, 36 mothers (7.9%) reported a total of 44 AE (**Table 2**). Eighteen were classified as serious AE (SAE), of which 13 SAE were categorized as ICD10 chapter XV (pregnancy, childbirth, and the puerperium) and consisted of pregnancy-induced hypertension (4 women), complications of Caesarean section (4), prolonged rupture of membranes (3), third-degree perineal tear (1), and pyrexia during delivery (1). There were 2 central nervous system SAE and both occurred in the treatment group. One woman developed acute inflammation of the central nervous system 6 d after delivery with persisting disability. Despite intensive investigation, no infectious or alternative cause was identified. One woman had transient demyelination of the pons occurring 16 mo after delivery with full recovery. There were 2 intrauterine deaths (ICD10 chapter XVI) and both occurred in the placebo group. One resulted from acute placental failure associated with severe pregnancy-induced hypertension and the other was unexplained but associated with adverse social circumstances. One mother in the placebo group collapsed 5 mo after delivery and made a full recovery; no cause was found (chapter XVIII). Categorized according to ICD10 chapters, the frequency of the AE was similar in the 2 groups (Table 2). No lactobacilli or bifidobacteria infections were identified and none of the AE were attributed to the trial interventions.

Infants. Birthweights did not differ ($P = 0.44$) and were 3.49 (2.1–4.9) kg in the treatment group ($n = 219$) and 3.55 (2.0–5.2) kg in the placebo group ($n = 233$). One infant in each group was delivered outside of the local National Health Service Trust and birthweight could not be ascertained. Loss to follow-up for infants in the 2 groups was similar at follow-up to 8 wk

TABLE 2 Number of mothers reporting AE classified according to ICD10 code chapter

ICD10 code chapter	Treatment group, *n* = 220	Placebo group, *n* = 234	*P*-value
	n (%)		
I: Certain infectious and parasitic diseases	0 (0.0)	1 (0.4)	0.61
III: Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	0 (0.0)	1 (0.4)	1.00
VI: Diseases of the nervous system	2 (0.9)	0 (0.0)	0.23
X: Diseases of the respiratory system	1 (0.5)	0 (0.0)	0.49
XI: Diseases of the digestive system	2 (0.9)	2 (0.9)	1.00
XV: Pregnancy, childbirth and the puerperium	10 (4.5)	13 (5.6)	0.50
XVI: Certain conditions originating in the perinatal period	0 (0.0)	3 (1.3)	0.25
XVIII: Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	0 (0.0)	2 (0.9)	0.50
XXII: Codes for special purposes	1 (0.5)	0 (0.0)	0.49
Total mothers[1]	15 (6.8)	21 (9.0)	0.49

[1] 6 mothers reported 2 AE and 1 mother reported 3 AE.

TABLE 3 Common symptoms, drug usage, and growth up to age 8 wk[1]

Variable	Treatment group, n = 220		Placebo group, n = 234		P-value
	n		n		
Age at follow-up, *d*	167	46 (26–60)	161	46 (33–62)	0.47
Common symptoms (yes), *n (%)*					
Colic	167	83 (49.7)	161	75 (46.6)	0.58
Regurgitation	167	123 (73.7)	161	128 (79.5)	0.24
Constipation	167	26 (15.6)	161	44 (27.3)	0.01
Diarrhea	167	23 (13.8)	161	21 (13.0)	0.87
High temperature	163	6 (3.7)	159	12 (7.5)	0.15
Drugs (yes), *n (%)*					
Antibiotic	166	31 (18.7)	161	25 (15.5)	0.47
Paracetamol	167	23 (13.8)	161	27 (16.8)	0.54
Weight,[2] *kg*	159	4.90 (3.6–7.1)	156	4.99 (3.6–6.9)	0.79
Weeks exclusively breast-fed, *n*	167	2 (0–8)	161	3 (0–8)	0.20
Visits to doctor, *n (%)*	166	1 (0–4)	161	1 (0–8)	1.00
Mother`s assessment of infant`s health, *n (%)*					1.00
Very healthy	166	145 (87.3)	160	139 (86.9)	
Occasionally unwell	166	20 (12.0)	160	20 (12.5)	
Nearly always unwell	166	1 (0.6)	160	1 (0.6)	

[1] Information collected either during home visit or by telephone interview. Data are medians (range) or *n* (%).
[2] Either weight measured during home visits or recent weight recorded by community midwife during telephone interviews

(P = 0.11) and between 9 and 28 wk (P = 0.29; Supplemental Fig. 1). The most common reason given for loss to follow-up was that parents and caregivers were too busy. Many parents and caregivers who could not be contacted before 8 wk did provide information at later follow-ups.

During the first 8 wk, compliance did not differ between groups (P = 0.99) and was 27 (0–60) d (n = 217) in the treatment group and 26 (0–61) d (n = 228) in the placebo group. Parents and caregivers in both groups reported that the trial interventions were easy to administer to infants: 109/142 (76.8%) in the treatment group and 110/139 (79.1%; P = 0.67) in the placebo group. Drug usage, infant weight, feeding practice, number of visits to the doctor, and mothers' assessments of their infants' health were also similar in the 2 groups (**Table 3**). Constipation occurred in significantly fewer infants in the treatment than the placebo group (Table 3). The frequency of other symptoms was similar in the 2 groups.

During 9–28 wk, compliance with the trial interventions was 106 (0–182) d (n = 206) in the treatment group and 105 (0–180) d (n = 216) in the placebo group (P = 0.82). There were no significant differences in the various factors between the 2 groups (**Table 4**).

In the first 6 mo, parents or caregivers reported AE in about 1 in 3 infants in each group (Table 5). Most AE were common illnesses in infancy and occurred at a similar frequency in each group. Overall, AE in 27 (12.3%) infants in the treatment group and 36 (15.4%) in the placebo group were classified as serious (P = 0.41). **Table 5** also shows specific common AE (reported in $\geq$5 infants in either group). One infant living in a family with adverse social circumstances presented with a sudden unexpected death in infancy. This mother and infant had been allocated to the placebo arm of the study. A total of 14 infants were classified as having "unspecified acute lower respiratory infection" (ICD10 code J22) and this occurred more frequently in the treatment than the placebo group. Nine of these had a respiratory illness that was treated with an antibiotic by their General Practitioners. Five were admitted to the hospital and were therefore SAE (4 in the treatment and 1 in the placebo group; P = 0.20) and all recovered. A specific infectious agent was not identified in any of these infants and it is likely that some had viral infections.

Five infants reported vomiting and/or diarrhea, none classified as serious, all in the treatment group (ICD10 code P93). In 2 infants with regurgitation, the mother considered that the trial intervention caused the symptoms and discontinued adminis-

TABLE 4 Common symptoms and drug usage recorded between ages 9 and 28 wk[1]

Variable	Treatment group, n = 220		Placebo group, n = 234		P-value
Common symptoms (yes), *n (%)*	*n*		*n*		
Colic	191	65 (34.0)	195	63 (32.3)	0.75
Regurgitation	191	135 (70.7)	195	145 (74.4)	0.43
Constipation	191	55 (28.8)	195	62 (31.8)	0.58
Diarrhea	191	63 (33.0)	195	55 (28.2)	0.32
High temperature	191	51 (26.7)	195	53 (27.2)	1.00
Drugs (yes), *n (%)*					
Antibiotic	192	39 (20.3)	195	48 (24.6)	0.33
Paracetamol	192	172 (89.6)	195	177 (90.8)	0.73
Growth[2]					
Weight, *kg*	138	7.90 (5.8–10.2)	141	7.90 (5.4–10.4)	0.32
Length, *cm*	137	68.0 (44.1–75.3)	141	68.1 (46.5–83.7)	0.94
Head circumference, *cm*	131	43.5 (38.2–49.7)	139	44.0 (39.0–48.7)	0.26
Weeks received cereals, *n (%)*	146	4 (0–12)	150	5 (0–12)	0.17
Trial intervention was easy to administer (yes), *n (%)*	130	109 (83.8)	130	110 (84.6)	1.00
Visits to doctor, *n (%)*	192	1 (0–11)	195	1 (0–9)	0.62
Mother`s assessment of baby`s health,[1] *n (%)*					0.62
Very healthy	146	126 (86.3)	151	135 (89.4)	
Occasionally unwell	146	16 (11.0)	151	14 (9.3)	
Nearly always unwell	146	4 (2.7)	151	2 (1.3)	

[1] Data are medians (range) or *n* (%).
[2] Measured or assessed at the clinic visit scheduled for 24 wk.

TABLE 5 Number of infants aged 0–6 mo with AE classified according to ICD10 chapter and specific common AE (≥5 in 1 group) by ICD10 code

ICD10 chapter or code	Treatment group, *n* = 220	Placebo group, *n* = 234	*P*-value
	n (%)		
I: Certain infectious and parasitic diseases	15 (6.8)	12 (5.1)	0.55
A09: Gastroenteritis	6 (2.7)	3 (0.9)	0.33
B34.9: Viral infection, unspecified	2 (0.9)	6 (2.1)	0.29
B37.0: Candidal stomatitis	5 (1.8)	2 (0.9)	0.27
IV: Endocrine, nutritional and metabolic diseases	0 (0.0)	1 (0.4)	1.00
VII: Diseases of the eye and adnexa	6 (2.7)	12 (5.1)	0.23
H10.0: Conjunctivitis	6 (2.7)	8 (3.4)	0.79
VIII: Diseases of the ear and mastoid process	3 (1.4)	3 (1.3)	1.00
X: Diseases of the respiratory system	24 (10.9)	16 (6.8)	0.14
J06: Upper respiratory tract infection	5 (2.3)	5 (2.1)	1.00
J21.9: Bronchiolitis	9 (3.6)	9 (3.8)	1.00
J22: Lower respiratory tract infection	11 (3.6)	3 (0.9)	0.03
XI: Diseases of the digestive system	8 (3.6)	14 (6.0)	0.28
K21: Gastro-esophageal reflux disease	4 (1.8)	6 (2.6)	0.75
XII: Diseases of the skin and subcutaneous tissue	12 (5.5)	12 (5.1)	1.00
L01.0: Impetigo	7 (3.2)	3 (1.3)	0.21
XIV: Diseases of the genitourinary system	0 (0.0)	1 (0.4)	1.00
XV: Pregnancy, childbirth and the puerperium	4 (1.8)	5 (2.1)	1.00
O98.9: Unspecified maternal infectious or parasitic disease complicating pregnancy, childbirth and the puerperium	4 (1.8)	5 (2.1)	1.00
XVI: Certain conditions originating in the perinatal period	10 (4.5)	18 (7.7)	0.18
P59.9: Neonatal jaundice, unspecified	0 (0.0)	6 (2.6)	0.03
P92.5: Neonatal difficulty in feeding at breast	3 (1.4)	7 (3.0)	0.34
P93: Reactions and intoxications due to drugs administered to fetus and newborn	5 (2.3)	0 (0.0)	0.026
XVII: Congenital malformations, deformations and chromosomal abnormalities	12 (5.5)	12 (5.1)	1.00
Q82.5: Congenital non-neoplastic naevus	1 (0.5)	5 (2.1)	0.22
XVIII: Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	7 (3.2)	4 (1.7)	0.37
XIX: Injury, poisoning and certain other consequences of external causes	2 (0.9)	2 (0.9)	1.00
XX: External causes of morbidity and mortality	0 (0.0)	2 (0.9)	0.50
Total number of infants reporting ≥1 AE	73 (33.2)	75 (32.1)	0.84

tration. One infant was receiving an antibiotic and this was considered the likely cause of the symptoms. In 2 infants, the relationship with the trial intervention was unclear. Six infants had physiological neonatal jaundice (ICD10 code P59.9) and all occurred in the placebo group. All 6 infants with a SAE classified as a disease of the digestive system (ICD10 chapter XI) were in the placebo group ($P = 0.03$). Two were surgical cases (obstructed inguinal hernia, umbilical hernia repair) and 4 were admitted with a variety of symptoms, including colic and constipation. With these exceptions, the frequency of SAE was similar in the 2 groups ($P > 0.05$), with SAE categorized either according to ICD10 chapter or specific code. Apart from the 2 possible adverse reactions listed above, no AE was considered to be associated with this supplement. In addition, no lactobacilli or bifidobacteria infections were identified. All life-threatening and fatal AE in both mothers and infants were reviewed carefully by the research team and the external safety monitors and none were considered to be attributable to the supplement.

Discussion

Administration of 2 strains of lactobacilli and 2 strains of bifidobacteria to mothers during the last month of pregnancy and infants during the first 6 mo of life, most of whom were at increased risk of atopic disease, was not associated with an increase in AE. Only a small proportion of women reported 1 or more AE, which were mostly events related to pregnancy, delivery, and the puerperium. It was not possible to identify a specific cause in the 2 women in the treatment group who developed neurological illnesses after delivery. However, we are not aware of any previous reports of similar illnesses attributed to ingestion of lactobacilli or bifidobacteria. In keeping with the findings in this study, many studies of probiotics administered during pregnancy have not identified adverse outcomes and a recent systematic review reported no adverse effects of lactobacilli and bifidobacteria during pregnancy (10).

In infants, lower respiratory tract infections were reported more frequently in the treatment than the placebo group. Although these infants received antibiotic treatment, it is likely that many were suffering from viral rather than bacterial infection. Also, the difference between the 2 groups may have arisen by chance given the large number of comparisons reported. Regurgitation may have been an adverse reaction to the dietary supplement in 2 infants, but this seems unlikely. The questionnaires identified regurgitation as the most common symptom and it occurred with similar frequency in each group. The supplement appeared to reduce the frequency of constipation in early infancy.

However, no effect on constipation was observed later in infancy and this early difference may have arisen by chance given the large numbers of comparisons reported. The greater frequency of physiological jaundice in the placebo than the treatment group was also likely to be a chance finding.

A strength of the current study was the identification of AE both by spontaneous reporting as well as regular questionnaire and clinical follow-up in a double-blind study. A weakness was loss to follow-up of ~15% overall. Many mothers were working and were too busy; domestic difficulties were common with some women living in a refuge. Although loss to follow-up was similar in both groups, the possibility that the bacterial supplement caused AE in some of these participants cannot be excluded.

Many different bacterial preparations have been administered to large numbers of healthy infants in research studies without reported adverse effects and some authors consider that there is no evidence that probiotics are harmful to children (11). However, the increased use of bacterial dietary supplements, their inclusion in infant formula milks, and the fact that AE may be strain specific (5) have prompted closer scrutiny for possible adverse effects. In reports focusing on safety, AE were not attributable to the supplemented organisms in infants administered *Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis* subsp. lactis HN019 (12), combinations of *Bifidobacterium longum* BL999, *Lactobacillus rhamnosus* LPR and *Lactobacillus paracasei* ST11 (13), and *Bifidobacterium lactis* BB-12 and *Lactobacillus reuteri* ATCC 55730 (14). Similarly, adverse outcomes have not been associated with infant formula milks containing 1 or more bacterial species with a prebiotic; organisms have included *Bifidobacterium longum* BL999 (15), *Lactobacillus rhamnosus* GG and LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* spp. *shermanii* (16), and *Lactobacillus paracasei* ssp. *paracasei* and *Bifidobacterium animalis* ssp. *lactis* (17). Finally, the administration of *Bifidobacterium lactis* with long-chain PUFA was not associated with detrimental effects (18).

Several other randomized trials have evaluated probiotics in the prevention of atopy in infants at increased risk of atopy. Most have shown favorable outcomes, but Taylor et al. (19) reported increased allergen sensitization in infants who received *Lactobacillus acidophilus* (LAVRI-A1). Symptoms and signs of atopy are the primary clinical endpoints in this current study and these outcomes will be reported.

In conclusion, this study assessed the safety of a dietary supplement of *L. salivarius* CUL61, *L. paracasei* CUL08, *B. animalis subsp. lactis* CUL34, and *B. bifidum* CUL20 in mothers during the last month of pregnancy and infants during the first 6 mo of life. There was no evidence that these strains were associated with adverse effects in these women or their infants.

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