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Specific probiotics in reducing the risk of acute infections in infancy – a randomised, double-blind, placebo-controlled study

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A randomised, double-blind, placebo-controlled study was conducted to determine whether probiotics might be effective in reducing the risk of infections in infancy. Infants requiring formula before the age of 2 months were recruited from community well-baby clinics. Infant formula supplemented with the probiotics *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12 or placebo was administered daily until the age of 12 months. Incidence of early infections (before the age of 7 months) and incidence of recurrent (three or more) infections during the first year of life were recorded as the main outcome measures of the study. During the first 7 months of life, seven out of thirty-two (22 %) infants receiving probiotics and twenty out of forty (50 %) infants receiving placebo experienced acute otitis media (risk ratio (RR) 0·44 (95 % CI 0·21, 0·90); $P=0·014$) and antibiotics were prescribed for ten out of thirty-two (31 %) infants receiving probiotics and twenty-four out of forty (60 %) infants receiving placebo (RR 0·52 (95 % CI 0·29, 0·92); $P=0·015$). During the first year of life, nine out of thirty-two (28 %) infants receiving probiotics and twenty-two out of forty (55 %) infants receiving placebo encountered recurrent respiratory infections (RR 0·51 (95 % CI 0·27, 0·95); $P=0·022$). These data suggest that probiotics may offer a safe means of reducing the risk of early acute otitis media and antibiotic use and the risk of recurrent respiratory infections during the first year of life. Further clinical trials are warranted.

Acute otitis media: Infants: Probiotics

Infectious disease is the most important cause of morbidity in infants. In developed countries, infants experience three to six respiratory tract infections during the first year of life[1,2] and 40 % of them suffer from at least one episode of acute otitis media (AOM), the most common reason for antibiotic use[3]. Widespread use of antibiotics has led to increasing microbial resistance. In addition to increased caution in prescribing antimicrobial agents, new means of reducing the risk of infectious disease in infancy are called for.

The infant's developing immune system is dependent on contact with environmental antigens to achieve adequate and appropriate immune competence. Breast-feeding and the intestinal microbiota are considered the most important sources of maturational stimuli[4]. On this basis, specific strains from the healthy intestinal microbiota have been used as probiotics, defined as 'specific live or inactivated microbial cultures that have documented targets in reducing the risk of human disease or in their nutritional management'[5].

Breast milk provides crucial protection against microbial invaders in the form of antibodies and non-specific antimicrobial factors[6]. In addition, breast milk may contain viable lactobacilli[7] and factors that promote the growth of bifidobacteria in the infant's intestine[8,9], which may contribute to healthy immunological maturation. We therefore hypothesised that administering specific probiotics may promote the maturing effect of gut microbiota in infants devoid of the recommended exclusive breast-feeding. Such nutritional intervention could reduce the incidence of acute infections and need for antibiotic treatment during the first year of life.

Methods

The infants participating in this randomised, double-blind, placebo-controlled clinical trial were recruited in Turku, Finland, between September 2000 and May 2002.

At recruitment, reliable data regarding the occurrence of respiratory tract infections or AOM in infants who were not exclusively breast-fed were not available. Based on a rough estimate for statistical power, our initial aim was to recruit 200 infants. Due to the baby-friendly hospital initiative and the strong policy of promoting breast-feeding, which is of utmost importance in well-baby clinics, advertising the study with formula supplementation at an early age was not acceptable. Thus information regarding the study was given only when the need of formula was imminent. Recruitment was therefore slow and a pragmatic deadline for enrolment was set at the end of May 2002. All families who were interested in participating and contacted the research nurse during the recruitment period were assessed for eligibility (Fig. 1).

Abbreviation: AOM, acute otitis media.

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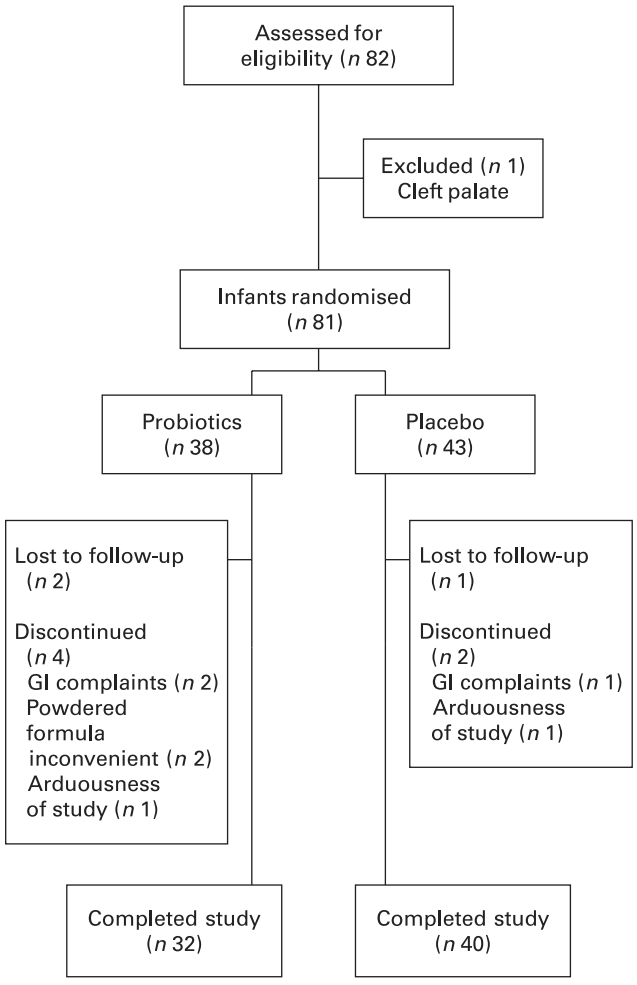

Fig. 1. The study profile. GI, gastrointestinal.

The sole inclusion criterion for the study was need for infant formula before the age of 2 months. Infants with chronic disease were excluded. The study was found ethically acceptable by the Turku University Central Hospital Ethical Committee. Written informed consent was obtained from the infants' parents.

In all, eighty-one infants were randomised by block randomisation with individual codes to receive either 1×10^{10} colony-forming units of both *Lactobacillus rhamnosus* (*Lactobacillus* GG, American type culture collection 53103; Valio Ltd, Helsinki, Finland) and *Bifidobacterium lactis* Bb-12 (Chr. Hansen A/S, Hoersholm, Denmark) or placebo (microcrystalline cellulose) daily until the age of 12 months. These strains were chosen because of documented safety properties and a long history of safe use both in infant studies and in food products. In addition, we have previously demonstrated that these two strains have synergistic effects *in vitro*, with *Lactobacillus* GG increasing the adherence of *B. lactis* Bb-12[10]. The random allocation was generated independently from the investigators by the manufacturer of the capsules (Chr. Hansen A/S). All investigations were performed double blind; the code was opened after all the infants had completed the study and data had been edited. The daily probiotics or placebo were provided in visually identical capsules (Chr. Hansen A/S), the contents of which were supplemented to infant formula (Enfamil; Mead Johnson Nutritionals, Evansville, IN, USA) given at one feeding. Administration of the supplemented formula was commenced after recruitment when the need of formula arose and the formula was to be used as the sole infant formula during the study period. At recruitment, maternal use of commercially available products containing probiotics was discouraged. Such products were not available for infants in Finland at the time of the study.

To ascertain that the probiotics remained viable during storage, microbiological analysis of a random sample of capsules was performed by a microbiologist in a blinded fashion. Viable counts between 1×10^9 and 1×10^{10} per capsule were found for both *Lactobacillus* GG and *B. lactis* Bb-12.

The infants were followed up until the age of 12 months. Clinical examination of the infants was performed at scheduled visits at the ages of 3, 7 and 12 months. The follow-up was completed by seventy-two of the eighty-one (89 %) infants enrolled (Fig. 1). All infections during the study period were recorded in special diaries by the family or the family physician. Respiratory infections, doctor-diagnosed AOM, gastrointestinal infections and the number of treatments with antibiotic agents were separately recorded in detail. AOM was defined as an acute, short-course inflammation in the middle ear that can be clinically verified, accompanied by at least one of the following features: rhinitis, cough, fever, sore throat, earache, hypacusis, restlessness at night, irritability, loss of appetite, vomiting or diarrhoea. Purulent otorrhoea through a perforated tympanic membrane or tympanostomy tube was also considered to constitute AOM.

The primary outcome measure for the study was the incidence of early respiratory infections, doctor-diagnosed AOM and gastrointestinal infections defined as infections occurring before the age of 7 months. The incidence of recurrent (defined as three or more occurrences) respective infections during the first year of life was considered a secondary outcome measure. Early or recurrent need for antibiotics was interpreted to reflect suspected early or recurrent bacterial infections, respectively, and thus recorded. In Finland, antibiotics are available only through prescription by licensed physicians. Tympanostomy before the age of 12 months was interpreted to indicate frequent ear infections.

All adverse effects were recorded in detail and the infants in whom symptoms were suspected to be related to the study products were subjected to an elimination and re-exposure procedure.

The data are expressed as means with range and risk ratios with 95 % CI to accurately depict statistical power[11]. The comparisons between the groups were conducted using the χ^2 test. Subjects lost to follow-up were excluded from the analyses. A complete intention-to-treat approach was not possible because the mean age of the nine subjects lost to follow-up was 2·9 (range 1·5–7·0) months and no evaluation after randomisation was performed in eight out of nine of these infants. Imputation techniques were not considered satisfactory solutions to estimate the missing outcome variables due to the proportion of lost-to-follow-up cases and relatively small sample size.

Results

The baseline characteristics were similar in infants receiving probiotics and placebo (Table 1).

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Table 1. Baseline characteristics and history
(Mean values and ranges or numbers and percentages)

	Probiotics (*n* 32)		Placebo (*n* 40)	
	Mean	Range	Mean	Range
Boys				
n	16		19	
%	50		48	
Gestational age (weeks)	39·8	36·7–42·1	39·9	35·1–42·3
Birth-weight (g)	3440	2300–4100	3540	2140–4580
Older siblings				
n	15		24	
%	47		60	
Parental smoking				
n	18		22	
%	56		55	
Exclusive breast-feeding (weeks)	1·9	0·0–6·0	1·9	0·0–6·0
Total breast-feeding (months)	2·0	0·25–12·0	2·4	0·25–7·5
Age at start of complementary feeding (months)	3·0	1·5–5·0	3·0	2·0–4·0
Age at start of intervention (d)	38	6–65	35	2–59

The effect of probiotics on the incidence of early and recurrent infections

The study probiotics reduced the risk of early AOM and need for antibiotic treatment during the first 7 months of life. The results are presented in detail in Table 2. Probiotics also reduced significantly the incidence of recurrent respiratory infections during the first 12 months of life (Table 3). None of the infants in the study experienced more than two gastrointestinal infections during the study period. In addition, the administration of probiotics tended to reduce the need for tympanostomy, performed either to prevent recurrent AOM or to treat secretory otitis media: none of the infants receiving probiotics required tympanostomy during the first year of life, whereas the procedure was performed on four out of forty (10 %) infants receiving placebo ($P=0{\cdot}066$).

Adverse effects

No serious adverse effects resulting from probiotic supplementation were detected during the study. In addition to the infants who discontinued the intervention (Fig. 1), gastrointestinal symptoms that the parents related to the study product were reported in four infants who completed the study. One infant experienced vomiting after accidental ingestion of a several-fold dosage of the study product daily. The vomiting subsided when supplementation was discontinued and the infant was able to continue the intervention with the appropriate dosage shortly thereafter. The infant was discovered to have received probiotics when the follow-up was completed and the code opened. The remaining three out of four infants with gastrointestinal symptoms, including vomiting, flatulence and increased fussing, were subjected to an elimination and re-exposure procedure and two out of three of these infants subsequently continued with the intervention. When the code was opened after the follow-up was completed, all three of these infants were discovered to have received placebo.

Discussion

A significant reduction in the incidence of early and recurrent infections and the use of antibiotics during the first year of life was achieved by probiotic supplementation in the present study. The effect was most prominent with regard to respiratory infections and AOM, the most prevalent infections in infancy. In contrast, no statistically significant effect was detected on the occurrence of gastrointestinal infections. The relatively small number of patients is an obvious limitation of the study and was reflected in the wide CI of the results. The possibility that all true differences between the infants receiving probiotics and placebo do not display statistical significance cannot be excluded. Nonetheless, probiotics appeared to confer significant protection against early infections, the importance of which culminates in the fact that the children developing frequent infections, including AOM, experience their first infection early[12,13].

According to a recent survey, only 51 % of infants in Finland are exclusively breast-fed at the age of 2 months, and the proportion of those exclusively breast-fed declines rapidly thereafter[14]. Lack of breast-feeding and parental smoking are among the most important risk factors for recurrent infections in infancy in developed countries[13]. The present study demonstrates the tendency for risk factors to cluster in certain families, as at least one of the parents smoked in more than half of the families participating in the study. This may restrict the applicability of the present results to the general population on the one hand and underscores the importance of continuing counselling with regard to health-promoting lifestyle on the other.

The efficacy of *Lactobacillus* GG[15,16] and bifidobacteria[17,18] in reducing the risk of infectious disease has previously been most comprehensively documented in the context of viral diarrhoea. There are limited data on the effects of probiotics in reducing the risk of respiratory tract infections and the present study is the first to suggest protection against AOM. In an older age group, a modest

Table 2. Incidence of infections during the first 7 months of life

	Probiotics (*n* 32)		Placebo (*n* 40)				
	n	%	*n*	%	RR	95 % CI	*P*
Respiratory infection	22	69	31	78	0·89	0·67, 1·18	0·40
AOM	7	22	20	50	0·44	0·21, 0·90	0·014
Gastrointestinal infection	1	3	6	15	0·21	0·03, 1·64	0·091
Antibiotic use	10	31	24	60	0·52	0·29, 0·92	0·015

RR, risk ratio; AOM, acute otitis media.

reduction in the severity of respiratory tract infections and absence from day care was achieved with milk supplemented with *Lactobacillus* GG whereas the occurrence of infections was not significantly reduced[19]. In a recent study, the combination of *Lactobacillus* GG and three other probiotic bacteria failed to reduce the occurrence of AOM but appeared to protect against recurrent respiratory tract infections in children with a history of recurrent AOM[20]. In the present study, probiotic supplementation was commenced in the first months of life before the first occurrence of AOM. According to our interpretation, it is crucial that children are subjected to intervention in early infancy, as the maturing immune system might be more amenable to probiotic modification. Nonetheless, it has been reported that *B. lactis* Bb-12 or *L. reuteri* (ATCC 55730) do not confer protection against respiratory tract infections in infancy, but it should be noted that the follow-up in the study only lasted 12 weeks[21].

There are numerous potential mechanisms through which probiotics might mediate their protective effect against infectious disease. Specific probiotics have direct antipathogenic effects by promoting mucosal barrier functions[22] and inhibiting mucosal pathogen adherence by inducing mucin secretion[23]. Lactobacilli have been shown to be potent inducers of innate immune responses against microbial pathogens[24]. Both *Lactobacillus* GG and *B. lactis* Bb-12 have been documented to augment humoral immune responses[25,26], especially during viral infections[27]. Indeed, contact with environmental and intestinal microbes appears to be essential for normal immunological maturation[4].

It is conceivable that the protection against AOM provided by probiotics in the present study may have been mediated *via* both reduction of colonisation by pathogens by local inhibition and immunomodulation throughout the common mucosa-associated immune system. Nasopharyngeal colonisation by the predominant causative agents of AOM, *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis*[28], before the age of 3 months is associated with an increased risk of AOM[29]. Probiotics, such as *Lactobacillus* GG and bifidobacteria, have been shown to reduce nasal colonisation with pathogenic bacteria, including *S. pneumoniae* in adults[30]. The effect may be immunologically mediated, as no indication of nasal colonisation by the probiotic strains was detected in the study in contrast to a previous study indicating that ingested *Lactobacillus* GG briefly colonise the mouth[31]. Indeed, we have previously demonstrated that ingestion of *Lactobacillus* GG leads to favourable changes at mucosal sites beyond the intestine[32]. According to a recent study, however, *Lactobacillus* GG in combination with three other probiotics does not reduce nasopharyngeal carriage of *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* in infants suffering from recurrent AOM[20]. It is not known whether probiotics have the potential to reduce initial nasal colonisation by these pathogens in early infancy.

Respiratory infections are a major cause of infant morbidity and still a prominent cause of infant death in many areas. AOM is the most common reason for consulting a doctor in children and more prescriptions for antibiotics are written for AOM than any other disease in infancy[13], thus potentially contributing to the rise in microbial resistance to antibiotic agents. Based on the present study, the probiotic combination of *Lactobacillus* GG and *B. lactis* Bb-12 may offer a safe and inexpensive means of reducing the continuing burden of these infections and the need for antibiotic treatment in full-term infants who require formula at an early age. Clinical trials with larger numbers of infants are required to corroborate these results.

Table 3. Incidence of recurrent infections (three or more episodes) and use of medical interventions during the first 12 months of life

	Probiotics (*n* 32)		Placebo (*n* 40)				
	n	%	*n*	%	RR	95 % CI	*P*
Respiratory infection	9	28	22	55	0·51	0·27, 0·95	0·022
AOM	4	13	10	25	0·50	0·17, 1·45	0·183
Antibiotic use	10	31	16	40	0·78	0·41, 1·48	0·44
Tympanostomy	0	0	4	10	0·31	0·04*, 2·66	0·066

RR, risk ratio; AOM, acute otitis media.
*Calculated assuming one positive case in probiotic group.

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