Papers

Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial

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Abstract

Objective To examine whether long term consumption of a probiotic milk could reduce gastrointestinal and respiratory infections in children in day care centres.

Design Randomised, double blind, placebo controlled study over seven months.

Setting 18 day care centres in Helsinki, Finland.

Participants 571 healthy children aged 1-6 years: 282 (mean (SD) age 4.6 (1.5) years) in the intervention group and 289 (mean (SD) age 4.4 (1.5) years) in the control group.

Intervention Milk with or without *Lactobacillus* GG. Average daily consumption of milk in both groups was 260 ml.

Main outcome measures Number of days with respiratory and gastrointestinal symptoms, absences from day care because of illness, respiratory tract infections diagnosed by a doctor, and course of antibiotics.

Results Children in the *Lactobacillus* group had fewer days of absence from day care because of illness (4.9 (95% confidence interval 4.4 to 5.5) *v* 5.8 (5.3 to 6.4) days, 16% difference, P = 0.03; age adjusted 5.1 (4.6 to 5.6) *v* 5.7 (5.2 to 6.3) days, 11% difference, P = 0.09). There was also a relative reduction of 17% in the number of children suffering from respiratory infections with complications and lower respiratory tract infections (unadjusted absolute % reduction −8.6 (−17.2 to −0.1), P = 0.05; age adjusted odds ratio 0.75 (0.52 to 1.09), P = 0.13) and a 19% relative reduction in antibiotic treatments for respiratory infection (unadjusted absolute % reduction −9.6 (−18.2 to −1.0), P = 0.03; adjusted odds ratio 0.72 (0.50 to 1.03), P = 0.08) in the *Lactobacillus* group.

Conclusions *Lactobacillus* GG may reduce respiratory infections and their severity among children in day care. The effects of the probiotic *Lactobacillus* GG were modest but consistently in the same direction.

Introduction

Children attending day care centres have a 1.5-3.0 times higher risk of gastrointestinal and respiratory tract infections than children cared for at home or in small family care groups.[1–6]

Probiotic bacteria, which beneficially affect the host by improving the intestinal microbial balance, may affect the immune response. *Lactobacillus rhamnosus* GG, ATCC 53103, a probiotic strain of human origin with widely documented health effects,[7] influences immune response, both specifically by stimulating antibody production[8] and non-specifically by enhancing the phagocytic activity of the blood leucocytes.[9] It promotes recovery from rotavirus diarrhoea[8] and reduces the incidence of diarrhoea associated with use of antibiotics in children.[10 11] However, most studies of probiotic bacteria have been short term trials. Over a seven month winter period we examined the effect of consumption of milk containing probiotic bacteria on the incidence of gastrointestinal and respiratory infections in children attending day care centres.

Methods

Participants—This randomised, double blind, placebo controlled clinical study was carried out in 18 municipal day care centres, in similar socioeconomic areas in north, west, and north east Helsinki. Children aged 1-6 years were recruited through meetings with parents. We excluded children with allergy to cows' milk, lactose intolerance, severe food allergy, and other severe chronic diseases. The study protocol was approved by the ethics committee of Helsinki City Health Department.

Intervention—The intervention lasted seven months over the winter. The *Lactobacillus* milk (Gefilus, Valio, Riihimäki, Finland) contained 1% fat and 5-10x10^5 colony forming units/ml of strain *Lactobacillus rhamnosus* GG. The control milk had the same composition but without *Lactobacillus*. The day care staff served the study milks three times a day, five days a week. In both groups children consumed at least 200 ml of milk on 60% of the days in the study.

Data collection—During the study, parents recorded daily in a symptom diary any respiratory symptoms (fever, runny nose, sore throat, cough, chest wheezes, earache) and gastrointestinal symptoms (diarrhoea, vomiting, stomach ache). They also reported absences

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from the day care centre, doctors' diagnoses, and prescriptions of antibiotics. A questionnaire at the end of the study collected information on general health and the use of other products that contained lactic acid bacteria during the study.

Sample size and randomisation—We estimated that, with a power of 90% and at a significance level of 0.05, we needed 250 children per group to show a 20% difference in respiratory infections between the groups. Each child was randomly allocated to the *Lactobacillus* or the control group.

Outcome measures—The primary outcome measures were the number of days with respiratory and gastrointestinal symptoms or days with any illness; absences from day care centre because of illness; number of children with upper respiratory tract infections with complications (acute otitis media and sinusitis) and lower respiratory tract infections (acute bronchitis and pneumonia) as diagnosed by a doctor; and antibiotic treatments during the seven month intervention. A secondary outcome was the correlation between the amount of milk consumed and the number of days with symptoms.

Statistical analysis—The distribution of the number of days of illness, days with respiratory and gastrointestinal symptoms, and days of absence due to illness were logarithmically transformed. The results are given as geometric means with 95% confidence intervals. We used the *t* test for independent samples to compare the groups. To control for differences in age distribution, we used analysis of covariance. We used Kaplan-Meier survival curves for the time without respiratory symptoms or diarrhoea and the log rank test to compare the groups. We calculated medians with 95% confidence intervals to describe the time without symptoms. Cox's regression analyses were performed to adjust for confounding factors. The number of upper respiratory infections with complications, lower respiratory infections, and antibiotic treatments were dichotomised (none/one or more) and analysed with the χ^2 test or Fisher's exact test. We used logistic regression analyses to control for age. All analyses were based on the intention to treat population.

Results

Of the 594 children randomised, 571 started the study, receiving either milk containing *Lactobacillus* GG (n = 282) or control milk (n = 289). Fifty eight children did not complete the follow up period. The table details characteristics of the children before treatment. Randomisation resulted in a similar distribution of children in the age groups under 3 years and 3 years and over. However, detailed analysis of age distribution showed that there were some differences between the groups. Also, there were more children in the control group who had had five or more respiratory infections during the preceding 12 months. Age and preceding infections both have an effect on the incidence of infections and as they were strongly correlated we adjusted only for age in comparisons of treatment.

Symptoms of illness as reported by parents—There were no significant differences between the groups in the number of days with respiratory and gastrointestinal symptoms, though the actual number of days with symptoms was lower in the *Lactobacillus* group. In the *Lactobacillus* group there were fewer days of absence because of illness (4.9 days (4.4 to 5.5) *v* 5.8 (5.3 to 6.4), P = 0.03)—a *Lactobacillus*:control ratio of 0.85 (95% confidence interval 0.73 to 0.98). The time without respiratory symptoms was significantly longer in the *Lactobacillus* group compared with the control group (5 (4.1 to 5.9) *v* 4 (3.5 to 4.6) weeks, P = 0.03). Time without diarrhoea was not significantly different (25 (24 to 26) *v* 24 (23 to 25) weeks, respectively, P = 0.20.

Characteristics before treatment of children allocated to probiotic milk (*Lactobacillus* GG) and control groups. Figures are means (ranges) or numbers (percentage) of children

Characteristic	*Lactobacillus* GG (n=282)	Control (n=289)
Age (years)	4.6 (1.3-6.8)	4.4 (1.3-6.7)
1	16 (6)	17 (6)
2	35 (12)	38 (13)
3	42 (15)	65 (23)
4	62 (22)	46 (16)
5	66 (23)	81 (28)
6	61 (22)	42 (14)
Male	151 (54)	140 (48)
Siblings	1.1 (0-4)	1.0 (0-4)
Duration of breast feeding (months)	6.8 (0-32)	7.1 (0-30)
Duration of day care (months)	22 (0.5-60)	20 (0.2-66)
House area (m^2)	90 (42-300)	93 (34-330)
Smoking in household	91 (32)	97 (34)
Health in past 12 months:		
Respiratory infections:		
0-2	140 (50)	134 (47)
3-4	99 (36)	86 (30)
⩾5	38 (14)	64 (23)
Gastrointestinal infections:		
0-1	218 (79)	211 (75)
⩾2	58 (21)	69 (25)
Antibiotic treatments:		
0-1	180 (65)	179 (64)
⩾2	95 (35)	101 (36)
History of allergy (diagnosed by doctor):		
Atopic diseases*	59 (21)	64 (22)
Allergic eye infection (%)	25 (9)	21 (7)
Food allergy (%)	24 (9)	30 (10)

*Allergic rhinitis, atopic eczema, or asthma.

Upper and lower respiratory tract infections diagnosed by doctor—The number of children with respiratory infections (otitis media, sinusitis, bronchitis, and pneumonia) was significantly lower in the *Lactobacillus* group (97/252 *v* 123/261, absolute reduction –8.6% (–17.2 to –0.1), P = 0.05). There were also fewer children in the *Lactobacillus* group who were prescribed antibiotics for respiratory infections (111/252 *v* 140/261, absolute reduction –9.6% (–18.2 to –1.0), P = 0.03).

Age adjusted results—Adjustment for age reduced the difference between the groups in the number of days of absence (5.1 days (4.6 to 5.6) *v* 5.7 (5.2 to 6.3), P = 0.09). After age adjustment the odds ratio for the *Lactobacillus* group was 0.89 (0.77 to 1.02). Time without respiratory symptoms and time without diarrhoea were not significantly different between the groups, but for the *Lactobacillus* group the estimated odds ratios were 0.86 (0.70 to 1.06, P = 0.16) for respiratory symptoms and 0.87 (0.64 to 1.28, P = 0.36) for diarrhoea. There was a significant negative correlation between the amount of *Lactobacillus* milk consumed and the number of days without gastrointestinal symptoms ($r = -0.17$, P = 0.007). The numbers of children

with respiratory tract infections diagnosed by a doctor and being given antibiotic treatments for these were not significantly different between the groups. However, the age adjusted odds ratios for the *Lactobacillus* group were 0.75 (0.52 to 1.09, P = 0.13) for all respiratory infections and 0.72 (0.50 to 1.03, P = 0.08) for antibiotic treatment for respiratory infection.

Other effects—Mean daily milk consumption was 260 ml in both groups (range 110-520 ml in the *Lactobacillus* and 100-600 ml in the control group). Neither *Lactobacillus* nor the control milk affected stool frequency or consistency. There was no difference between the groups in abdominal pain or allergic symptoms and no apparent side effects.

Discussion

This randomised, double blind, placebo controlled study is the first to examine the long term effects of probiotic bacteria on infections in normally healthy children. The intervention lasted seven months during the season in which the infection rate is usually highest.[12]

Age is strongly associated with the incidence of infection.[13] We noticed that despite the age stratified randomisation, there were differences in the age distributions between the study groups. Because of this unintentional imbalance we adjusted the analyses for age, which reduced the differences between the groups. However, the results came close to conventional significance, and the differences were consistently in favour of the *Lactobacillus* GG group. The compliance in our study was good. Because *Lactobacillus* GG products are widely consumed in Finland, the children in the control group may unwittingly have consumed *Lactobacillus* during the study. At the end of the study, *Lactobacillus* GG-like bacteria were found in 15% of the children in the control group, which could have reduced the actual differences between the groups.

Lactobacillus GG may influence the incidence of infections by stimulating non-specific immunity or enhancing humoral and cellular immunity.[14] This immunostimulatory effect of bacteria has previously been shown to prevent recurrent infections in children attending day care centres.[15]

Respiratory infections in children have a major impact on families and on society in general. In 1985 and 1986, the annual cost to society in general due to illness in children attending Finnish day care centres was about £650 to £2300 per child, depending on age.[16] Thus a 10-20% reduction in the incidence of infections and absences from day care centres, which our results suggest is possible, could have important clinical, public health, and economic consequences.

Alternative programmes for preventing respiratory tract infections in children are much needed. The administration of probiotic milk products is an easy and acceptable method, with no adverse effects. Our results show that probiotic milk containing *Lactobacillus* GG may slightly reduce respiratory infections and their complications among children attending day care centres.

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What is already known on this topic

Children attending day care centres are at high risk of respiratory and gastrointestinal infection

The successful prevention of respiratory infections could be extremely useful for families and for society in general

Short term use of probiotic bacteria has been shown to reduce the severity of rotavirus diarrhoea and the incidence of diarrhoea associated with the use of antibiotics

What this study adds

In a double blind, randomised, long term study milk containing *Lactobacillus* GG slightly reduced the incidence of respiratory infections and antibiotic treatment in children

Contributors: KH designed the protocol and the questionnaires, participated in the creation of the database, and wrote the paper. ES supervised the study and revised the manuscript. TP was responsible for the data analysis and wrote the sections on statistical methods. AP, JHM, and LN participated in the planning of the study and revised the manuscript. RK and MS initiated the study, participated in the planning, were responsible for the management of the study, and revised the manuscript. KH and RK are the guarantors of the paper.

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Competing interests: KH has been employed by Valio Research Centre for two of the past five years. MS and RK are employed by Valio Research Centre. ES has given two educational presentations on *Lactobacillus* GG for Valio, and TP has received consulting fees from Valio.

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