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Perinatal probiotics decreased eczema up to 10 years of age but at 5–10 years allergic rhino-conjunctivitis was increased

Condensed title: Perinatal probiotics decreased eczema up to 10 years of age

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Research letter

Perinatal use of *Lactobacillus GG* (LGG) has been associated with fewer allergic diseases, mainly eczema in high-risk children at the age of two years [1,2], a phenomenon still seen at 6–9 years [2-5]. We reported earlier that in high-risk children pre- and postnatal use of **a mixture of probiotics** reduced the incidence of atopic eczema at two years of age, and during the first five years atopic eczema reduction was seen in Caesarean-delivered children but not in the whole cohort [1,6]. Mothers were recruited during pregnancy and randomized at 35 weeks of gestation to the probiotic or placebo group. Prematurity (less than 37 weeks of gestation), infants having major malformations or infants born as B-twins were excluded. From gestational week 36 the mothers twice daily received one capsule containing a mixture of probiotics: LGG (ATCC 53103; 5×10^9 colony-forming units [cfu]), *L. rhamnosus* LC705 (DSM 7061; 5×10^9 cfu), *Bifidobacterium breve* Bb99 (DSM 13692; 2×10^8 cfu) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076; 2×10^9 cfu), or placebo. Their infants were given the same capsules opened and mixed with 20 drops of syrup containing 0.8 g of galacto-oligosaccharides (prebiotics) once daily from birth, continuing to six months after birth. In the placebo group mothers received capsules containing microcrystalline cellulose and the infants received the same capsules with sugar syrup without prebiotics. Valio (Helsinki, Finland) supplied the capsules and syrups and they looked, smelled and tasted identical. The study paediatrician examined the children at six months, two years and five years and the families answered questionnaires at three, six and 12 months and then yearly until five years of age. Double blinding (participants/parents and investigators) was maintained until five years of age. Questions concerning allergic diseases between 5–10 years of age, and allergic diseases at any time were based on questions from the International Study of Asthma and Allergies in Childhood (ISAAC) (<http://isaac.auckland.ac.nz/>), with added questions on allergic diseases, infections, use of antibiotics and probiotics, pet contacts, and environmental tobacco smoke.

We asked the parents if their child had ever had an itchy rash (eczema lasting for more than six months), allergic rhinitis or asthma (via ISAAC questions) or if the child had ever been diagnosed with eczema, allergic rhinitis or asthma by a doctor, in order to calculate the cumulative incidence of allergic diseases and doctor-diagnosed allergic diseases during the follow-up time.

The child was considered to have had eczema at 5–10 years of age if the parents answered affirmatively to the following three ISAAC-based questions: 1) “Has your child ever had an itchy rash that kept coming and going for at least six months?”, 2) “Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, the front of the ankles, under the buttocks, and around the neck, ears, or eyes?”, and 3) “Has your child had this itchy rash at any time in the past five years?”.

The child was considered to have had allergic **rhino-conjunctivitis** at 5–10 years of age if the parents responded positively to the following three ISAAC-based questions: 1) “Has your child ever had sneezing, or a runny or blocked nose when he/she did not have a cold or flu?”, 2) “In the past five years has your child had a problem with sneezing, or a runny or blocked nose when he/she did not have a cold or flu?” and 3) “Has this nose problem been accompanied by itchy/watery eyes?”.

The child was considered to have had asthma during the past five years if the parents answered yes to the following two ISAAC-based questions: 1) “Has your child had wheezing or whistling in the chest in the past five years?”, and 2) “Has your child ever had asthma?” (<http://isaac.auckland.ac.nz/>).

Sample-size calculation was based on an expected 40% cumulative incidence of allergic diseases at age five. To detect a 10% absolute reduction (odds ratio 0.64) via use of probiotics at the 5% significance level and with 90% power, the estimated size of each group was 597, which allowed a 20% dropout rate. The χ^2 test was used to compare differences between groups in the 10-year study, with $p < 0.05$ considered as being statistically significant. Multivariate logistic regression analysis was used to adjust the outcomes for potential confounders (sex, study group, parental smoking, use of probiotics

regularly during the previous year, i.e. weekly or more often in the year or regular use in the winter, university-educated parent, use of antibiotics, breast feeding for ≥ 6 months, mode of delivery and cat/dog at home), and unadjusted and adjusted results are given as odd ratios with 95% confidence intervals (CIs). **Caesarean delivery/vaginal delivery was used as an interaction term.** The data was analysed by using SPSS software, version 22. The Ethics Committee of the Hospital for Children and Adolescents, Helsinki University, approved the study. The clinical trials registration number is NCT00298337.

Our primary outcome measure was the lifetime prevalence of any allergic disease (eczema, allergic rhinitis or asthma) at five years of age. Secondary outcomes were lifetime prevalence of eczema, food allergy, allergic rhinitis and asthma. We here report the 10-year results obtained by using questionnaires.

The questionnaire was returned by 407 (79.5%) participants in the probiotic group and 400 (79.1%) in the placebo group (Fig. 1). Children in the probiotic group had used probiotics or products containing probiotics regularly during the previous 12 months more often than children in the placebo group (33.2% vs. 26.3%, OR 1.39; 95% CI 1.03–1.89; $p=0.03$). Otherwise, baseline characteristics (mode of delivery, age of the mother, sex, birth weight, siblings, parental atopy, parental education, cat/dog at home, exclusive breast feeding over 3 or 5 months, parental smoking, no use of antibiotics) of the groups were identical. Children answering the 10-year questionnaire were representative of the whole cohort as regards baseline characteristics (Supplementary Table). The initial baseline characteristics of non-responders were largely identical compared with those of responders, but non-responders had more often been breastfed for more than three months and they more often had siblings than responders (Supplementary Table). This might have resulted in some selection bias.

The lifetime **prevalence** of any allergic disease was similar in the probiotic and placebo groups. However, the lifetime **prevalence** of eczema was lower in the probiotic group (35.2% vs. 41.7%, adjusted OR 0.74; 95% CI 0.55–1.00; $p<0.05$). The lifetime **prevalences** of allergic rhinitis (45.5% vs.

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41.8%) and asthma (14.3% vs. 16.5%) were comparable in the probiotic and placebo groups. The **prevalence** of any doctor-diagnosed allergic disease was similar in the two groups. However, the **prevalence** of doctor-diagnosed food allergy was significantly lower in the probiotic group (24.4% vs. 30.5%, adjusted OR 0.71; 95% CI 0.52–0.97; $p=0.03$), but there were no other differences in the separate **prevalences** of doctor-diagnosed allergic diseases. The prevalence of any allergic disease at age 5–10 years (based on responses to ISAAC questions) did not differ in the probiotic and placebo groups. However, children given probiotic supplements had more allergic rhino-conjunctivitis at this age (36.5% vs. 29.0%, OR 1.43; 95% CI 1.06–1.94; $p=0.03$). The difference was seen in vaginally delivered children (38.6% vs. 25.8%, OR 1.80; 95% CI 1.29–2.51; $p<0.01$), but not in Caesarean-delivered children. No significant difference was found in the prevalences of other allergic diseases at age 5–10 years. At this age, the prevalence of frequent (≥ 4 per year) upper respiratory tract infections (URTIs – common flu, flu with fever or ear infection) was lower among Caesarean-delivered children in the probiotic group vs. those in the placebo group (13.0% vs. 33.3%, OR 0.30; 95% CI 0.13–0.7; $p<0.01$) and they were less likely to have received antibiotics (17.4% vs. 6.7%, adjusted OR 3.19; 95% CI 1.02–9.97; $p<0.05$). No significant difference was found in the prevalence of frequent ($\geq 1/5$ years) lower respiratory tract infections (LRTIs – bronchitis or pneumonia) among Caesarean-delivered children in the probiotic vs. the placebo group (Table 1).

Our study shows a lack of a preventive effect of the **study probiotics on the prevalence** of any allergic disease when classified as any type or doctor-diagnosed disease in the total cohort of children up to 10 years of age. The present results, however, indicate that in high-risk children perinatally received probiotics may reduce the prevalence of eczema up to the age of 10 years [1,6]. Use of *Lactobacillus rhamnosus* HN001 prenatally and up to the age of two years reduced the cumulative prevalence of eczema in high-risk children up to the age of six years in New Zealand [2]. In addition, maternal supplementation with a mixture of probiotics (*Lactobacillus rhamnosus* GG, *L. acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12) prenatally and for three months after delivery reduced the cumulative incidence of atopic dermatitis in their offspring up to the age of six years in a general

Norwegian population [4]. In our work, the strongest allergy-preventive effect seen at earlier follow-up time points (at two years [1] and partly at five years [6]) concerned eczema in particular. Most cases of allergic eczema became manifest at up to two years of age. At 5–10 years eczema prevalence in the probiotic group remained lower than in the control group, albeit non-significantly. The probiotic effect seems to taper off.

We found, surprisingly, that use of **the study probiotics** was associated with more allergic rhinoconjunctivitis at age 5–10 years in vaginally delivered children, but this was not seen as regards allergic rhinitis ever or doctor-diagnosed allergic rhinitis ever. The results should thus be interpreted with caution. In the Panda study, pre- and 1-year postnatal use of a probiotic mixture (*Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Lactococcus lactis*) was associated with a tendency towards a higher prevalence of allergic rhinitis at six years of age in high-risk children, but no significant difference in the prevalence of allergic diseases (**asthma diagnosis was not always based on objective lung measures**) or sensitization was found. [7]. Kalliomaki et al. also reported that allergic rhinitis and asthma (diagnosis based on reimbursement for asthma medication) tended to be more common in their probiotic (**LGG**) group, vs. controls [3]. **The prevalence of asthma at six years of age was comparable in the probiotic and control groups when *Lactobacillus rhamnosus* HN001 was received prenatally and up to two years of age [2] as well as at the age of 8–9 years among children receiving *Lactobacillus paracasei* ssp *paracasei* F19 from 4 to 13 months of age [5]. Asthma diagnosis was based on fractional exhaled nitric oxide (FeNo) results and spirometry [2,5]. Abrahamsson et al. reported no significant difference in the prevalence of asthma (diagnosis based on spirometry and FeNo) at seven years of age between their probiotic and control groups when using *L. reuteri* ATCC 55730 prenatally and for one year after delivery [8].**

We strived to avoid mistaking viral rhinitis for allergic rhinoconjunctivitis by asking the parents if the child ever had sneezing or a blocked/runny nose when he/she did not have a cold or flu **and if the child had watery/itchy eyes at the same time as the nasal symptoms**, but we cannot totally exclude

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response bias. A higher prevalence was seen in vaginally born children, whereas we previously found an IgE-associated allergy-decreasing effect at five years of age in Caesarean-born children [6], highlighting the fact that probiotic intervention has long-lasting effects on allergy prevalences, which appear different depending on delivery mode, i.e. initial microbial load in newborn infants. In our previous reports there was no difference in the prevalence of allergic rhinitis between intervention groups and controls at two or five years of age [1,6]. Children in the probiotic group had significantly more often regularly used probiotic products (dairy products containing probiotics or probiotics bought at a grocery store or pharmacy) during the previous year compared with children in the placebo group, but before the age of five years there was no difference in use of probiotics. Double blinding was maintained until five years of age, and it is possible that parents who were told that they and their children received probiotics, and that probiotics can prevent allergic diseases, used them more frequently than subjects in the placebo group. We cannot exclude the possibility that regular use of probiotics in the previous year had an effect on allergy outcomes.

We also showed that study probiotics used prenatally and up to six months after birth can diminish the number of URTIs (common flu, flu with fever or ear infection) in the subgroup of Caesarean-delivered children. Hatakka et al. showed that healthy children aged 1–6 years receiving LGG in day-care had fewer days of sickness than a control group during seven months of intervention, a finding that is in line with our results, although in that study infections and days of sickness were investigated during the LGG treatment [9], not years after the treatment, as in our present study.

These 10-year results relied on reported data, while previous reports (2- and 5-year reports) also relied on clinical examination and measurements of IgE-sensitization, which is a study limitation. A strength of our study is that the number of families answering the 10-year questionnaire was high (80%). Another advantage of our study was the perinatal administration of probiotics leading to colonization of mothers who transfer these to their infants at vaginal birth. We have also shown that the given probiotics lead to immune effects in vivo [10]. We met all the children and parents at six months and

at two and five years and collected data on children and their allergies prospectively by way of regular questionnaires, keeping compliance high. Another limitation is that double blinding was kept for five years and during the last five years of follow-up parents were aware of the child's intervention group. This might have resulted in parental bias.

Our results indicate that early use of probiotics remains associated with a reduction in the incidence of eczema at 10 years, **but may be associated with more allergic rhino-conjunctivitis** at 5–10 years. Explanations for varying effects on different allergy phenotypes should be sought in long-term follow-up studies.

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Table 1.

	Probiotic		Placebo		Unadjusted OR (95% CI)	p-value*	Adjusted OR (95% CI)	Adjusted p-value**
Total cohort	(n)	(%)	(n)	(%)				
Any allergic disease ever (ISAAC-based)	241/406	59.4%	258/397	65.0	0.79(0.59–1.05)	0.100	0.79(0.59–1.07)	0.123
Any doctor-diagnosed allergic disease ever	220/405	54.3%	232/399	58.1	0.86(0.65–1.13)	0.274	0.86(0.65–1.15)	0.318
Any allergic disease at age 5–10 years (ISAAC-based)	203/405	50.1%	202/396	51.0	0.97(0.73–1.27)	0.802	0.97(0.72–1.29)	0.806
Eczema ever (ISAAC-based)	143/406	35.2%	165/396	41.7	0.76(0.57–1.01)	0.061	0.74(0.55–1.00)	0.049
Eczema, doctor-diagnosed ever	134/406	33.0%	150/399	37.6	0.82(0.61–1.09)	0.173	0.83(0.61–1.11)	0.211
Eczema at age 5–10 years (ISAAC-based)	106/405	26.2%	121/395	30.6	0.80(0.59–1.09)	0.162	0.79(0.58–1.09)	0.146
Allergic rhinitis ever (ISAAC-based)	185/407	45.5%	167/400	41.8	1.16(0.88–1.54)	0.289	1.21(0.91–1.62)	0.191
Allergic rhinitis, doctor-diagnosed ever	110/407	27.0%	98/400	24.5	1.14(0.83–1.57)	0.412	1.61(0.84–1.61)	0.369
Allergic rhinitis at age 5–10 years (ISAAC-based)	148/406	36.5%	116/400	29.0	1.40(1.05–1.89)	0.024	1.43(1.06–1.94)	0.020
Asthma ever (ISAAC-based)	58/407	14.3%	66/399	16.5%	0.84(0.57–1.23)	0.367	0.86(0.58–1.27)	0.441
Asthma, doctor-diagnosed ever	58/407	14.3%	62/399	15.5	0.90(0.61–1.33)	0.607	0.91(0.61–1.36)	0.649
Asthma at age 5–10 years (ISAAC-based)	36/407	8.8%	47/400	11.8	0.73(0.46–1.15)	0.174	0.73(0.46–1.17)	0.194
Upper respiratory tract infections, ≥4/year	89/407	21.9%	103/400	25.8	0.81(0.58–1.12)	0.195	0.79(0.56–1.10)	0.161
Lower respiratory tract infections, ≥1/5years	82/406	20.2%	90/400	22.5	0.87(0.62–1.22)	0.425	0.90(0.63–1.28)	0.552
No antibiotics during last 5 years	72/407	17.7%	59/400	14.8	1.24(0.85–1.81)	0.257	1.21(0.84–1.78)	0.330
Doctor-diagnosed food allergy ever	99/405	24.4%	122/400	30.5	0.74(0.54–1.01)	0.054	0.71(0.52–0.97)	0.034
Caesarean-delivered								

Any allergic disease ever (ISAAC-based)	44/68	64.7%	56/74	75.7	0.59(0.29–1.22)	0.152	0.64(0.30–1.38)	0.254
Any doctor-diagnosed allergic disease ever	42/69	60.9%	46/75	61.3	0.98(0.50–1.92)	0.955	1.09(0.54–2.22)	0.807
Any allergic disease at age 5–10 years (ISAAC-based)	35/68	51.5%	46/74	62.2	0.65(0.33–1.26)	0.199	0.70(0.35–1.43)	0.330
Eczema ever (ISAAC-based)	29/68	42.6%	34/74	45.9	0.88(0.45–1.7)	0.693	0.84(0.42–1.67)	0.611
Eczema, doctor-diagnosed ever	29/69	42.0%	27/75	36.0	1.29(0.66–2.52)	0.458	1.34(0.66–2.70)	0.414
Eczema at age 5–10 years (ISAAC-based)	21/68	30.9%	27/74	36.5	0.78(0.39–1.57)	0.481	0.88(0.42–1.81)	0.717
Allergic rhinitis ever (ISAAC-based)	29/69	42.0%	42/75	56.0	0.57(0.29–1.10)	0.094	0.74(0.36–1.52)	0.416
Allergic rhinitis, doctor-diagnosed ever	19/69	27.5%	24/75	32.0	0.81(0.39–1.65)	0.559	0.99(0.47–2.10)	0.977
Allergic rhinitis at age 5–10 years (ISAAC-based)	18/69	26.1%	32/75	42.7	0.47(0.23–0.96)	0.037	0.51(0.24–1.06)	0.071
Asthma ever (ISAAC-based)	12/69	17.4%	13/74	17.6	0.99(0.42–2.34)	0.978	1.12(0.45–2.78)	0.803
Asthma, doctor-diagnosed ever	10/69	14.5%	11/74	14.9	0.97(0.38–2.45)	0.950	1.10(0.42–2.89)	0.850
Asthma at age 5–10 years (ISAAC-based)	7/69	10.1%	9/75	12.0	0.83(0.29–2.36)	0.723	0.88(0.30–2.60)	0.818
Upper respiratory tract infections, ≥ 4 /year	9/69	13.0%	25/75	33.3	0.30(0.13–0.70)	0.004	0.29(0.12–0.72)	0.007
Lower respiratory tract infections, $\geq 1/5$ years	9/69	13.0%	17/75	22.7	0.51(0.21–1.24)	0.134	0.62(0.25–1.59)	0.321
No antibiotics during last 5 years	12/69	17.4%	5/75	6.7	2.95(0.98–8.86)	0.046	3.19(1.02–9.97)	0.046
Doctor-diagnosed food allergy ever	13/69	18.8%	24/75	32.0	0.49(0.23–1.07)	0.071	0.54(0.25–1.20)	0.133
Vaginally delivered								
Any allergic disease ever (ISAAC-based)	197/338	58.3%	202/323	62.5	0.84(0.61–1.14)	0.264	0.83(0.59–1.15)	0.249
Any doctor-diagnosed allergic disease ever	178/336	53.0%	186/324	57.4	0.84(0.62–1.14)	0.252	0.82(0.59–1.13)	0.217
Any allergic disease at age 5–10 years (ISAAC-based)	168/337	49.9%	156/322	48.4	1.06(0.78–1.44)	0.718	1.05(0.76–1.44)	0.783
Eczema ever (ISAAC-based)	114/338	33.7%	131/322	40.7	0.74(0.54–1.02)	0.064	0.72(0.52–1.00)	0.046
Eczema, doctor-diagnosed ever	105/337	31.2%	123/324	38.0	0.74(0.54–1.02)	0.066	0.73(0.52–1.01)	0.060
Eczema at age 5–10 years (ISAAC-based)	85/337	25.2%	94/321	29.3	0.82(0.58–1.15)	0.242	0.77(0.54–1.10)	0.149
Allergic rhinitis ever (ISAAC-based)	156/338	46.2%	125/325	38.5	1.37(1.01–1.87)	0.045	1.38(1.00–1.91)	0.050
Allergic rhinitis, doctor-diagnosed ever	91/338	26.9%	74/325	22.8	1.25(0.88–1.78)	0.216	1.23(0.85–1.77)	0.274
Allergic rhinitis, at age 5–10 years (ISAAC-based)	130/337	38.6%	84/325	25.8	1.80(1.29–2.51)	<0.001	1.87(1.33–2.63)	<0.001
Asthma ever (ISAAC-based)	46/338	13.6%	53/325	16.3	0.81(0.53–1.24)	0.330	0.80(0.52–1.24)	0.322
Asthma, doctor-diagnosed ever	48/338	14.2%	51/325	15.7	0.89(0.58–1.36)	0.590	0.87(0.56–1.35)	0.543
Asthma at age 5–10 years (ISAAC-based)	29/338	8.6%	38/325	11.7	0.71(0.43–1.18)	0.184	0.70(0.42–1.18)	0.183
Upper respiratory tract infections, ≥ 4 /year	80/338	23.7%	78/325	24.0	0.98(0.69–1.40)	0.920	0.97(0.67–1.40)	0.865

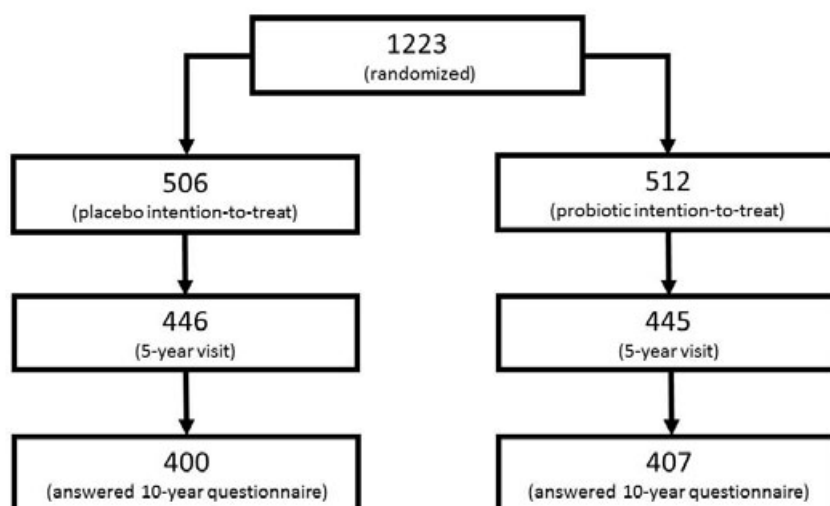
Lower respiratory tract infections, $\geq 1/5$ years	73/337	21.7%	73/325	22.5	0.96(0.66–1.38)	0.804	0.95(0.64–1.40)	0.785
No antibiotics during last 5 years	60/338	17.8%	54/325	16.6	1.08(0.72–1.62)	0.698	1.06(0.70–1.60)	0.791
Doctor-diagnosed food allergy ever	86/336	25.6%	98/325	30.2	0.80(0.57–1.12)	0.191	0.75(0.53–1.07)	0.112

Values in boldface are statistically significant.

Caesarean delivery/vaginal delivery used as an interaction term

*p-value (χ^2 test) for difference between the two study groups

**Adjusted by means of multivariate logistic regression for study group, sex, parental smoking, use of probiotics regularly (weekly or more throughout the year or regular use in winter), university-educated parent, use of antibiotics, breast feeding at ≥ 6 months, exposure to cat/dog at home and delivery by means of Caesarean section



Supplementary Table 1. Initial characteristics of study children

Initial characteristics of study children	Intention-to-treat children	10 year children		
	(1018)	Responder (807)	Non-Responder (211)	p-value*
Male	48.1%	50.9%	50.6%	0.363
Birth weight (g), mean(SD)	3594(483)	3591(480)	3612(499)	
Mother's age (y), mean (SD)	31.0(4.75)	31.2(4.77)	30.4(4.60)	
Mother smoking	12.3%	12.0%	13.3%	0.622
Father smoking	28.9%	30.2%	23.7%	0.062
University-educated parent	43.3%	46.5%	49.0%	0.587
Vaginal delivery	77.9%	82.2%	88.4%	0.062
Maternal atopy	81.0%	80.3%	83.9%	0.236
Paternal atopy	58.2%	58.6%	57.4%	0.765
Both parents atopic	39.2%	38.8%	40.8%	0.601
Siblings	44.8%	42.9%	53.9%	0.005
Cat/dog in home	15.1%	15.5%	19.6%	0.212
Exclusive breast feeding > 3 mo	42.9%	46.8%	28.0%	<0.001
Exclusive breast feeding > 5 mo	3.3%	3.3%	3.3%	0.984

Values in boldface are statistically significant.

*p-value, χ^2 test for difference between the 2 study groups