

Original Investigation

Prophylactic Use of a Probiotic in the Prevention of Colic, Regurgitation, and Functional Constipation

A Randomized Clinical Trial

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IMPORTANCE Infantile colic, gastroesophageal reflux, and constipation are the most common functional gastrointestinal disorders that lead to referral to a pediatrician during the first 6 months of life and are often responsible for hospitalization, feeding changes, use of drugs, parental anxiety, and loss of parental working days with relevant social consequences.

OBJECTIVE To investigate whether oral supplementation with *Lactobacillus reuteri* DSM 17938 during the first 3 months of life can reduce the onset of colic, gastroesophageal reflux, and constipation in term newborns and thereby reduce the socioeconomic impact of these conditions.

DESIGN A prospective, multicenter, double-masked, placebo-controlled randomized clinical trial was performed on term newborns (age <1 week) born at 9 different neonatal units in Italy between September 1, 2010, and October 30, 2012.

SETTING Parents were asked to record in a structured diary the number of episodes of regurgitation, duration of inconsolable crying (minutes per day), number of evacuations per day, number of visits to pediatricians, feeding changes, hospitalizations, visits to a pediatric emergency department for a perceived health emergency, pharmacologic interventions, and loss of parental working days.

PARTICIPANTS In total, 589 infants were randomly allocated to receive *L reuteri* DSM 17938 or placebo daily for 90 days.

INTERVENTIONS Prophylactic use of probiotic.

MAIN OUTCOMES AND MEASURES Reduction of daily crying time, regurgitation, and constipation during the first 3 months of life. Cost-benefit analysis of the probiotic supplementation.

RESULTS At 3 months of age, the mean duration of crying time (38 vs 71 minutes; $P < .01$), the mean number of regurgitations per day (2.9 vs 4.6; $P < .01$), and the mean number of evacuations per day (4.2 vs 3.6; $P < .01$) for the *L reuteri* DSM 17938 and placebo groups, respectively, were significantly different. The use of *L reuteri* DSM 17938 resulted in an estimated mean savings per patient of €88 (US \$118.71) for the family and an additional €104 (US \$140.30) for the community.

CONCLUSIONS AND RELEVANCE Prophylactic use of *L reuteri* DSM 17938 during the first 3 months of life reduced the onset of functional gastrointestinal disorders and reduced private and public costs for the management of this condition.

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Functional gastrointestinal disorders (FGIDs) are defined as a variable combination of chronic or recurrent gastrointestinal symptoms not explained by structural or biochemical abnormalities.¹

Since FGIDs in childhood are age dependent, the Rome Foundation established 2 different pediatric committees to identify the criteria for FGID diagnosis: the Infant/Toddler Committee (age up to 4 years)² and the Child/Adolescent Committee (aged 4-18 years).³

Infantile colic, gastroesophageal reflux, and constipation are the most common FGIDs that lead to referral to a pediatrician during the first 6 months of life and are often responsible for hospitalization, feeding changes, use of drugs, parental anxiety, and loss of parental working days with relevant social consequences.⁴

Although FGIDs have been considered self-limited processes, a low-grade mucosal inflammation and immune or motor alteration has been found in infants affected by colic, regurgitation, and constipation. This early traumatic insult to the intestine may represent a risk factor for the development of irritable bowel syndrome and psychologic problems later in life.⁵⁻⁷ Recent work indicates a crucial role of the intestinal microbiota in the pathogenesis of gastrointestinal disorders as in FGIDs,⁸ and many studies target probiotic therapy for specific conditions such as colic,⁹ regurgitation,¹⁰ and constipation.¹¹ The effect of a probiotic could play a crucial role in the modulation of intestinal inflammation.

We performed a prospective, multicenter, double-masked, placebo-controlled randomized clinical trial to evaluate whether oral supplementation with *Lactobacillus reuteri* DSM 17938 during the first 3 months of life can reduce the onset of colic, gastroesophageal reflux, and constipation in term newborns and thereby reduce the socioeconomic impact of these conditions.

Methods

Enrollment

The study was carried out between September 1, 2010, and October 30, 2012, in 9 Italian pediatric units. The ethics committees of each participating institution approved the study protocol, and written informed consent was obtained from parents before inclusion of participants.

Eligibility

Inclusion criteria were (1) gestational age more than 37 to less than 41 weeks, (2) age less than 1 week on entry into the study, (3) birth weight adequate for gestational age, (4) Apgar score of more than 8 at 10 minutes, (5) no congenital disorders and/or clinical or physical alterations at clinical examination, and (6) no antibiotic or probiotic administration before inclusion.

Study Design

Infants were randomly allocated to receive either *L reuteri* DSM 17938 or placebo. The active study product consisted of freeze-dried *L reuteri* suspended in a mixture of pharmaceutical-grade sunflower and medium-chain triglyceride oils sup-

plied in a dark bottle fitted with a dropper cap. Five drops of the formulation, delivering a dose of 1×10^8 colony-forming units of *L reuteri* DSM 17938, were administered to the newborns each day for 90 days. Parents were instructed to deliver the drops directly in the mouth of the infants. The placebo consisted of an identical formulation of oils supplied in an identical bottle, except that the live bacteria were excluded. There were no differences in smell or taste between the 2 formulations. Analysis of total *Lactobacillus* counts was performed in our laboratory on a sample probiotic bottle to ensure the viability of the live bacteria. The investigators assessed compliance through a weekly telephone call to record missed or refused probiotic administration. Both the *L reuteri* DSM 17938 and placebo study products were manufactured and donated by BioGaia AB.

Sample Size

With an α level of 0.05 and a power of 90% to detect an absolute difference of 15% between the proportion of infants with FGIDs in the placebo (assumed to be 30%) and probiotic groups, a sample size of 460 (230 in each group) was needed.

Randomization

An independent statistician generated the random allocation sequence, which was stratified for sex and gestational age. The study personnel, health care workers, and parents were masked to the study group allocation. Random allocation was performed using Stata 9 software (StataCorp LP).

Masking

Both parents and investigators were masked to the intervention and remained unaware of group assignments during the study.

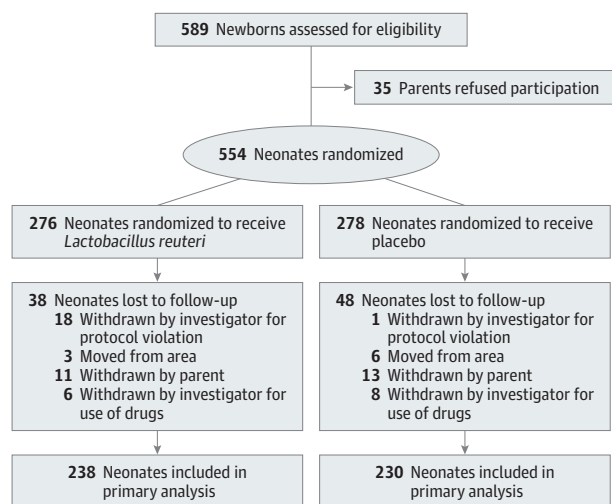
Symptom and Data Evaluation

Parents were asked to record daily the number of episodes of regurgitation (defined as the passage of refluxed gastric contents into the oral pharynx), periods of inconsolable crying (minutes per day as already described in the literature¹²), and the number of evacuations. Using a structured diary, parents were asked to record the number of visits to pediatricians, feeding changes, hospitalizations, access to a pediatric emergency department for a perceived health emergency, pharmacologic interventions, and loss of parental working days. The parents recorded the data from recruitment up to age 3 months. Adverse events related to the protocol were monitored by prompted questions on the telephone. To promote uniform documentation by the parents and to confirm that the infants were given the study products correctly, 1 investigator for each center (A. Di Mauro, E.C., C.I., M.B., L.C., E.B., and A. Del Vecchio) was always available by telephone to help parents. All families received a weekly telephone call or personal meeting with one of the investigators, and all infants were observed at visits each month.

Primary and Secondary Outcomes

The primary outcome was to evaluate if *L reuteri* DSM 17938 supplementation from the first days of life can reduce incon-

Figure. Flowchart of the Participants' Progression Through the Study



Data represent children included in the study and the total number evaluated.

solable crying, improve regurgitation, and modify bowel movements in neonates during the first 3 months of life.

Secondary outcomes included a cost-benefit evaluation of the supplementation, calculated with the number of primary care pediatrician visits, feeding changes, hospitalizations, access to a pediatric emergency department, loss of parental working days, and the use of simethicone, cimetropium bromide, and natural or herbal products to control gastrointestinal symptoms.

Statistical Analysis

Quantitative data (daily time crying, bowel movements, and regurgitation) were expressed as mean (SD) differences. The *t* test for unpaired samples was used to compare groups. For pediatrician visits, feeding changes, hospitalizations, access to a pediatric emergency department, loss of parental working days, and use of drugs, the χ^2 test was used to compare the percentages between groups. For all tests, $P < .05$ was considered significant.

Cost assessment was made by analyzing the costs for the family and community related to the frequency of each parameter in the treated and nontreated groups. Costs were as follows:

1. Medical examination in the pediatric emergency department (€20.66 [US \$27.87] for each examination, as provided by the Italian Ministry of Health)
2. Work loss days (€43.15 [US \$58.21] for each day, as provided by the Italian Ministry of Welfare)
3. Drugs (at cost, defined in the list from the National Drug Authority)
4. *L. reuteri* (€60.48 [US \$81.59] for the complete treatment)

Community costs were based on factors 1 and 2, while costs for the family were based on factors 3 and 4. This calculation was made since the National Health System in Italy covers all costs for pediatrician visits, and the National Welfare System finances all work loss expenses.

Results

Using the inclusion criteria, 589 newborns were initially included in the study, while 468 completed the 3-month study. Of these, 238 received *L. reuteri* DSM 17938 supplementation and 230 had placebo (Figure). Participants were lost to follow-up because of voluntary withdrawal, withdrawal by the investigator for protocol violation, relocation from the study area, and use of antibiotics, proton-pump inhibitors, or other antacids. Demographic characteristics at baseline are described in Table 1. No clinically relevant differences between the 2 groups were seen at baseline, and the randomization procedure was successful.

After 1 month of intervention, infants receiving *L. reuteri* DSM 17938 displayed a significant decrease in crying time, a significant increase in evacuation frequency, but no significant difference in regurgitation episodes compared with those given the placebo (Table 2). At the end of the 3-month intervention, significantly decreased crying time and significantly increased evacuation frequency were still evident (Table 3). Furthermore, infants who received *L. reuteri* DSM 17938 showed significantly decreased regurgitation frequency compared with those who received the placebo (Table 3). There were no adverse events reported that were related to the trial.

At the end of the study, there were significantly less pediatric emergency department visits, lost parental working days, and use of agents to promote gastrointestinal comfort in the infants who received *L. reuteri* DSM 17938 (Table 4). The frequency of feeding changes during the study was unaffected by the intervention (Table 4). The mean cost per child for each family related to these parameters was €150 (US \$202.35) and €238 (US \$321.06) for infants who received *L. reuteri* DSM 17938 and placebo, respectively, while the mean cost per child for the community was €133 (US \$179.42) and €237 (US \$319.71) for infants who received *L. reuteri* DSM 17938 and placebo, respectively. Thus, the prophylactic use of *L. reuteri* DSM 17938 resulted in an estimated mean savings per participant of €88 (US \$118.71) for the family, considering also the cost of the probiotic supplementation (Table 4) and an additional €104 (US \$140.30) for the community. The assessment model used is straightforward and conservative and does not take into account indirect costs (such as those related to parental anxiety, exhaustion and stress, and increased number of visits to the family pediatrician), all of which increase the costs in the untreated group, augmenting the expected benefit of treatment.

Discussion

Daily administration of *L. reuteri* DSM 17989 early in life decreased the reported incidence of inconsolable crying, regurgitation, and functional constipation in the first 3 months of life. There were no adverse events, and the supplementation was well tolerated. The socioeconomic impact of supplementation was a savings of €88 (US \$118.71) for the family and €104

Table 1. Clinical and Demographic Data at Baseline

Characteristic	<i>Lactobacillus reuteri</i> DSM 17938 (n = 238)	Placebo (n = 230)	P Value
Gestational age, mean (SD), wk	39.4 (19.0)	39.1 (1.4)	.05
Birth weight, mean (SD), g	3378 (413)	3302 (392)	.04
Male/female sex, No. (%)	122 (51.3)/116 (48.7)	120 (52.2)/110 (47.8)	.84
Vaginal/cesarean delivery, No. (%)	159 (66.8)/79 (33.2)	141 (61.3)/89 (38.7)	.21
Breast/formula feeding, No. (%)	132 (55.5)/106 (44.5)	121 (52.6)/109 (47.4)	.53

Table 2. Primary Outcome at 1 Month of Life

Characteristic	Mean (SD) [95% CI]		P Value
	<i>Lactobacillus reuteri</i> DSM 17938	Placebo	
Colic, min/d	45 (12) [43.5-46.5]	96 (34) [91.6-100.4]	<.01
Regurgitation, No./d	2.7 (1.5) [2.5-2.9]	3.3 (2.3) [3.0-3.6]	.35
Evacuation, No./d	4.01 (1.1) [3.9-4.1]	2.8 (0.6) [2.7-2.9]	<.01

Table 3. Primary Outcome at 3 Months of Life

Characteristic	Mean (SD) [95% CI]		P Value
	<i>Lactobacillus reuteri</i> DSM 17938	Placebo	
Colic, min/d	37.7 (33.8) [33.4-42.0]	70.9 (51.9) [64.2-77.6]	<.01
Regurgitation, No./d	2.9 (1.1) [2.7-3.0]	4.6 (3.2) [4.2-5.0]	
Evacuation, No./d	4.2 (1.8) [4.0-4.4]	3.6 (1.8) [3.4-3.8]	

Table 4. Secondary Outcome at 3 Months of Life

Characteristic	<i>Lactobacillus reuteri</i> DSM 17938	Placebo	P Value	<i>Lactobacillus reuteri</i> DSM 17938 vs Placebo, %
Pediatric visits due to the symptoms, mean (SD), No.	1.3 (0.6)	2.3 (0.7)	<.05	-44.5
Emergency department accesses, mean (SD), No.	0.52 (0.72)	1.78 (1.11)	<.05	-71.3
Loss of parents' working days, mean (SD), d	0.54 (0.62)	2.89 (1.3)	<.05	-82.5
Use of simeticone, yes/no, %	20	37	<.05	-46.1
Use of cimetropium bromide, yes/no, %	18	56.7	<.05	-67.9
Natural or herbal products, yes/no, %	21.3	37	<.05	-42.5
Feeding changes, yes/no, %	23.9	19.7	.23	...

(US \$140.30) for the community. To our knowledge, this is the first randomized clinical trial to address the use of a probiotic to prevent FGIDs in neonates. The study was adequately powered, and to serve as a proof of concept, we chose to perform a multicenter study that included a sufficient number of patients to increase the generalizability of our results.

Although infantile colic is not considered a serious problem, it is the cause of 10% to 20% of all pediatrician visits in the first 4 months of life and can lead to parental anxiety, exhaustion, and stress. Nearly 50% of all healthy infants aged 3 months and younger regurgitate at least once a day, with infant regurgitation representing 25% of pediatric consultations and 3% of gastrointestinal pediatric consultations. Constipation is generally responsible for 3% of all pediatric visits and can have several implications on the quality of life for both the child and the family.⁴

Functional gastrointestinal disorders in the neonatal period are often considered self-limiting clinical conditions, even though numerous studies in the literature reported these conditions as an early traumatic event predictive of different disease later in life.^{7,13} Furthermore, a recent study showed a significant association between migraine and history of infantile colic, where for children with migraine, the odds of having had colic as an infant were significantly increased.⁶

Our group performed a retrospective study of 3000 children diagnosed with irritable bowel syndrome according to Rome III criteria in our pediatric gastroenterology outpatient unit and matched them with healthy controls. The healthy children were evaluated through each child's personal national health booklet and a parental interview. The results show that children diagnosed with irritable bowel syndrome had a higher percentage of neonatal infantile

colic, regurgitation, and constipation than those without irritable bowel syndrome.¹³

The role of early life events in consequent FGIDs later in life is still controversial, even though some noxious stimulations at birth (ie, gastric suction or alteration of intestinal microbiota) might promote the development of long-term visceral hypersensitivity and cognitive hypervigilance, leading to functional disorders.¹⁴⁻¹⁶ Early life events could increase visceral sensitivity and mucosal permeability, alter the balance of the enteric microflora, and increase mucosal and neurogenic low-grade inflammation, altering the signal of the gut-brain-microbiota axis.¹⁷

In support of this broad concept, several studies have found associations between maternal stress and anxiety during pregnancy and high amounts of infant crying in the weeks after birth.¹⁸⁻²⁰

Driving a change of colonization during the first weeks of life through giving lactobacilli may promote an improvement in intestinal permeability; visceral sensitivity and mast cell density and probiotic administration may represent a new strategy for preventing these conditions, at least in predisposed children. A recent article by de Weerth et al²¹ indicates the presence of microbial signatures in the first weeks of life in infants who later develop colic. These microbial signatures may be used to understand the excessive crying and offer opportunities for early diagnostics as well as for developing specific therapies.

The use of *L reuteri* DSM 17938 was cost-effective. Symptoms related to FGIDs are common from birth to 6 months of age.¹⁰ These conditions often cause numerous visits to the pe-

diatrician, changes in feeding patterns, parental anxiety, and loss of parental working days with important socioeconomic consequences. In our study, daily crying time decreased during the first month of treatment, whereas regurgitation was significantly reduced only after the third month of treatment, possibly explained by the different temporal presentation of these symptoms in infants. Regurgitation actually appears more often in the second and third months of life, while constipation and crying have their peak at 4 to 6 weeks of life.^{22,23} Finally, a prophylactic approach using *L reuteri* can save money for both family and society and provide a helpful psychologic effect on the parents. Indeed, it is well known that supporting parents by teaching them to manage the crying and, consequently, acting on parental vulnerability can have a dramatic influence on parent-infant interactions and outcomes in these conditions.

Our study has some limitations. There was a loss to follow-up of 16.8% of the study population, although the numbers lost in each group were similar and therefore should not significantly affect the results. Furthermore, an unselected general population was recruited, and the possible risk of overtreating normal neonates cannot be excluded.

Conclusion

Given the considerable burden of morbidity and the socioeconomic impact of early-life FGIDs, new research should urgently be initiated not only to validate our results but also to tailor optimal schemes of intervention.

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