Lactobacillus reuteri DSM 17938 for the Management of Infantile Colic in Breastfed Infants: A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective To determine whether administration of *Lactobacillus reuteri* (*L reuteri*) DSM 17938 is beneficial in breastfed infants with infantile colic.

Study design Eighty infants aged <5 months with infantile colic (defined as crying episodes lasting 3 or more hours per day and occurring at least 3 days per week within 7 days prior to enrollment), who were exclusively or predominantly (>50%) breastfed were randomly assigned to receive *L reuteri* DSM 17938 (10^8 colony-forming units) (n = 40) or an identically appearing and tasting placebo (n = 40), both orally, in 5 drops, 1 time daily, for 21 days. The primary outcome measures were the treatment success, defined as the percentage of children achieving a reduction in the daily average crying time ≥50%, and the duration of crying (minutes per day) at 7, 14, 21, and 28 days after randomization.

Results The rate of responders to treatment was significantly higher in the probiotic group compared with the placebo group at day 7 (P = .026), at day 14 (relative risk (RR) 4.3, 95% CI 2.3-8.7), at day 21 (RR 2.7, 95% CI 1.85-4.1), and at day 28 (RR 2.5, 95% CI 1.8-3.75). In addition, throughout the study period, the median crying time was significantly reduced in the probiotic group compared with the control group.

Conclusion Exclusively or predominantly breastfed infants with infantile colic benefit from the administration of *L reuteri* DSM 17938 compared with placebo. *(J Pediatr 2013;162:257-62)*.

The criteria for infantile colic includes all of the following in infants from birth to 4 months of age: paroxysms of irritability, fussing, or crying that start and stop without obvious cause; episodes lasting 3 or more hours per day and occurring at least 3 days per week for at least 1 week; and no failure to thrive.[1] The crying typically peaks at approximately 6 weeks of life and ends around the fourth month. Possible causes of colic include painful intestinal contractions, lactose intolerance, food hypersensitivity, altered gut microbiota, gas, parental misinterpretation of the normal crying pattern, or various combinations of the above.[2] A number of therapies have been tried, including use of hydrolyzed formulas, sucrose, herbal teas, soy formula, lactose-reduced formula, and fiber-enriched formulas, increased carrying, music, vibration or massage, and spinal manipulation; however, none has been proven to be effective.[3] It has been suggested that colic in infancy increases the susceptibility to recurrent abdominal pain, allergic diseases, and psychological disorders in childhood.[4] Recent evidence suggests that probiotics might offer some benefit. First, an open randomized controlled trial (RCT) performed in 83 breastfed infants documented that compared with simethicone, administration of *Lactobacillus reuteri* (*L reuteri*) ATCC 55730 may reduce the crying time.[5] However, it was considered that methodological limitations of the study, including no allocation concealment, no blinding, and no intention-to-treat analysis, as well as the lack of a true placebo group, might invalidate the results. Furthermore, the *L reuteri* ATCC 55730 strain was found to carry potentially transferable resistance traits for tetracycline and lincomycin and was replaced by a new strain, *L reuteri* DSM 17938, with no unwanted plasmid-borne resistances.[6] More recently, Savino et al[7] showed in a double-blind, RCT that compared with placebo, *L reuteri* DSM 17938 administered to 46 breastfed infants improved symptoms of infantile colic.

There is no consensus regarding the use of *L reuteri* DSM 17938 for the management of infantile colic.[8] The importance of repeat studies in different populations and by independent investigators before firm conclusions can be drawn has been highlighted in the literature.[9] Thus, we undertook this clinical study to compare the effectiveness of *L reuteri* DSM 17938 with placebo in the treatment of breastfed infants with infantile colic in a double-blind, RCT.

Methods

The standards from the guidelines of the Consolidated Standards of Reporting Trials (CONSORT) were followed for this RCT. The trial was registered at

L reuteri	*Lactobacillus reuteri*
RCT	Randomized controlled trial
RR	Relative risk
VAS	Visual analog scale

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Funded by the Medical University of Warsaw, which received a donation from the manufacturer of *L reuteri* DSM 17938, BioGaia AB, Lund, Sweden. The manufacturer had no role in the conception, design, or conduct of the study, or in the analysis or interpretation of the data. The authors declare no conflict of interest.

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ClinicalTrials.gov (NCT01046617). All infants were eligible for recruitment after written informed consent was obtained from their parents. The study was approved by the Ethics Committee of the Medical University of Warsaw.

The study was carried out between January 2010 and December 2011 in a family primary care practice in Warsaw, Poland. To be eligible for entry, participants had to be full-term infants aged <5 months with infantile colic (defined as crying episodes lasting 3 or more hours per day and occurring at least 3 days per week within 7 days prior to enrollment), who were exclusively or predominantly (>50%) breastfed. Exclusion criteria included acute or chronic illness, gastrointestinal disorders, or use of any antibiotics and/or probiotic pharmaceutical products within 7 days prior to the study.

Investigators at the Medical University of Warsaw used computers to generate independent allocation sequences and a randomization list (StatsDirect statistical software; StatsDirect Ltd, Altrincham, Cheshire, United Kingdom). To avoid disproportionate numbers of patients in each group, randomization was performed in blocks of 6 subjects (3 receiving the probiotic product and 3 receiving the placebo). To ensure allocation concealment, an independent person prepared the randomization schedule and oversaw the packaging and labeling of the study products. All study personnel, parents, and guardians were unaware of the group assignments. Randomization codes were secured until all data were analyzed.

All participants and investigators were blinded throughout the study. Both study products, *L reuteri* DSM 17938 and the placebo, were manufactured and supplied by BioGaia AB (Lund, Sweden) as a fluid in identical bottles and kept refrigerated until use. The manufacturer had no role in the conception, design, or conduct of the study, or in the analysis or interpretation of the data. The unblinding was done when all data were analyzed.

All infants were eligible for screening. If an infant appeared to meet the criteria for enrollment and caregivers expressed interest in the study, caregivers were asked to record symptoms of colic for 1 week. Children fulfilling the inclusion criteria were asked to participate in the study. Eligible infants were randomly assigned to receive either *L reuteri* DSM 17938, administered orally at a dose of 10^8 colony-forming units, or placebo. The placebo consisted of an identical formulation in all respects except that the live probiotic bacteria were excluded. Both the active treatment and placebo were taken orally, in 5 drops, 1 time daily, for 21 days. Parents were given a diary and were asked to record the times of administration of study products, the daily duration of crying time, parental perceptions of colic severity, and family quality of life, as well as any adverse events. The visits after the enrollment were scheduled for 7, 14, 21, and 28 days after the initiation of the administration of study products. The end of the treatment visit was scheduled for day 28 to evaluate the effect of the intervention 1 week after its termination. At that visit, diaries and unused study products were returned. However, no specific measures to assess compliance were taken. The same study physician (E.G.) examined all study infants at all visits. Parents were encouraged to contact the same physician whenever needed. Parents were also encouraged to keep their infants in the study for follow-up visits even in cases of discontinuation of the study products. Only the study physician was in contact with the parents. The analyses of the diaries were done independently, first by the study physician (E.G.), and then by 2 other investigators. All members of the study team interpreting the diaries were blinded from treatment allocation.

The primary outcome measures were: (1) the treatment success (defined as the percentage of children achieving a reduction in the daily average crying time ≥50% during the study); and (2) the duration of crying (minutes per day). The secondary outcome measures were as follows: a reduction in the daily average crying time, from baseline until the end of the treatment period (day 21), to <3 h/d (the cutoff value proposed by Wessel et al[3]); persistence of infantile colic after the intervention; parental perceptions of colic severity; and parental/family quality of life. To assess the 2 latter outcomes, a 10-cm visual analog scale (VAS) was used. The possible scores ranged from 0 to 10. For the parent's perception of colic severity, 0 indicated no pain and 10 indicated the worst pain. For the parental/family quality of life, 0 indicated no effect and 10 indicated a very good effect.[10] Parents were instructed how to use the VAS scale prior to the study. In addition, adverse effects (ie, vomiting, constipation, and other symptoms spontaneously reported) were recorded by the caregivers.

We estimated that with 33 infants per group, we would be able to detect an absolute increase of 35% in the rate of treatment success from 15% in the control group to 50% in the intervention group with 80% power ($\alpha = 0.05$). In total, we planned to enroll 80 infants to account for possible 20% follow-up losses.

Statistical Analyses

The statistical analyses were conducted with the computer software StatsDirect v. 2.7.8. The Student *t* test was used to compare mean values of continuous variables approximating a normal distribution. For non-normally distributed variables, the Mann–Whitney U test was used. The χ^2 test or Fisher exact test was used, as appropriate, to compare percentages. The same computer software was used to calculate the relative risk (RR), number needed to treat, and median difference, all with a 95% CI. The difference between study groups was considered significant when the *P* value was <.05, when the 95% CI for RR did not include 1.0, or when the 95% CI for mean difference did not include 0. All statistical tests were two tailed and performed at the 5% level of significance. All analyses were conducted on an intention-to-treat basis, including all patients in the groups to which they were randomized for whom outcomes were available.

Results

The Figure is a flow diagram showing the subjects' progression through the study. The intention-to-treat population included 80 infants—40 were assigned to the