# *Lactobacillus reuteri* for Infants with Colic: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial

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**Objective** To assess the safety of probiotic *Lactobacillus reuteri* strain Deutsche Sammlung von Mikroorganismen (DSM) 17938 with daily administration to healthy infants with colic and to determine the effect of *L reuteri* strain DSM 17938 on crying, fussing, inflammatory, immune, and microbiome variables.

**Study design** We performed a controlled, double-blinded, phase 1 safety and tolerability trial in healthy breast-fed infants with colic, aged 3 weeks to 3 months, randomly assigned to *L reuteri* strain DSM 17938 ($5 \times 10^8$ colony-forming units daily) or placebo for 42 days and followed for 134 days.

**Results** Of 117 screened infants, 20 were randomized to *L reuteri* strain DSM 17938 or placebo (sunflower oil) (in a 2:1 ratio) with 80% retention. Eleven of the 20 (55%) presented with low absolute neutrophil counts (<1500/mm$^3$), which resolved in all subjects by day 176. *L reuteri* strain DSM 17938 produced no severe adverse events and did not significantly change crying time, plasma bicarbonate, or inflammatory biomarkers. Fecal calprotectin decreased rapidly in both groups. In the infants with dominant fecal gram negatives (*Klebsiella*, *Proteus*, and *Veillonella)*, resolution of colic was associated with marked decreases in these organisms.

**Conclusions** Daily administration of *L reuteri* strain DSM 17938 appears to be safe in newborn infants with colic, including those with neutropenia, which frequently coexists. A placebo response of 66% suggests that many infants with colic will have resolution within 3 weeks. *(J Pediatr 2017;■■:■■-■■).*

**Trial registration** ClinicalTrials.gov: NCT01849991.

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Colic is defined as inexplicable and severe crying in an otherwise-healthy newborn. Despite 40 years of research, little is known about its pathogenesis. Colic appears to represent abdominal pain, as manifested by abdominal distension and tenderness. In the original review by Wessel et al,[1] colonic hyperperistalsis was emphasized, and the use of enemas was suggested. Of babies with colic, 92% were reported to cry mainly after feedings,[2] also consistent with a problem in the gastrointestinal tract. Two meta-analyses have suggested that *Lactobacillus reuteri* strain Deutsche Sammlung von Mikroorganismen (DSM) 17938 significantly reduces infant crying and fussing time in breast-fed infants with colic.[3,4]

An abnormal fecal microbial community in babies with colic was first postulated by Savino et al, who showed increased *Escherichia coli* and reduced *Lactobacilli*.[5] Our previous study suggested increased *Klebsiella* and reduced microbial diversity in these infants.[6] Therefore, we postulated that children with colic may have an abnormal gut microbiome; the intestine may be inflamed in colicky babies, based on a high fecal calprotectin,[6] and *L reuteri* strain DSM 17938 may reduce gut inflammation associated with this dysbiosis.[7-9] During the review of our proposal, the Food and Drug Administration (FDA) asked whether an immunosuppresive

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| AE | Adverse event |
| ANC | Absolute neutrophil count |
| FDA | Food and Drug Administration |
| IL | Interleukin |
| NCCIH | National Center for Complementary and Integrative Health |
| RR | Rate ratio |

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effect of *L reuteri* strain DSM 17938, potentially produced by increased regulatory T cells,[7] could predispose newborn infants to more infections, lactic acidosis, or even lactobacillus bacteremia.

The aim of this study was therefore to demonstrate the safety of a liquid probiotic *L reuteri* strain DSM 17938, given over a 42-day period in infants with colic. In addition, we sought to investigate biomarkers that might give insight into the mechanism of action of *L reuteri* strain DSM 17938 related to infant colic.

## Methods

This trial was a single center, randomized, double-blind, placebo-controlled trial, ClinicalTrials.gov: NCT01849991. Protocol and amendments were approved by the institutional review board at the University of Texas Health Science Center at Houston (HSC-MS-11-0203) and the FDA (investigational new drug: 13561); reviewed by the National Center for Complementary and Integrative Health (NCCIH) (5R34AT006727) and overseen by the Office of Clinical Research Affairs.

Screening included parent/guardians signing of the informed consent, a physical examination by one of the research clinicians, and a clinical blood draw. Barr diaries[10] required for eligibility had to show greater than 2 of 3 days of 3 hours' daily of crying and fussing (nonconsecutive) at age 21-90 days, with a checked box stating that on the days with >3 h/d "this was a typical day." Subjects were required to have no previous or continuous probiotic use; no history of antibiotic exposure; and to be otherwise healthy and exclusively breast-fed. Five of the children in the study were on acid blockers (4 on lansoprazole, 1 on ranitidine); they were not disqualified.

Clinical and basic science laboratory assessments were conducted at screening, baseline, and follow-up visits (days 21, 42, 92, and 176). Clinical and basic science laboratory blood draws were collected at screening and end of treatment (day 42). Safety laboratory assessments included complete blood count, comprehensive metabolic panel (consisting of electrolytes, aspartate and alanine aminotransferases, urea nitrogen, creatinine, calcium, glucose, total protein, albumin, and C-reactive protein). Clinical laboratory results generally were considered abnormal if they were 2 times the upper limit in the Memorial Hermann Laboratory Directory of Services for aspartate aminotransferase and alanine aminotransferase; >20% for complete blood count and electrolytes; or >30% for glucose or kidney tests based on healthy infants.[11]

At the baseline visit, eligible subjects randomly were assigned to probiotic (*L reuteri* strain DSM 17938) or placebo (sunflower oil). Vials of sunflower oil and sunflower oil (placebo) with probiotic looked identical. Dose administration was explained to parents (5 drops once daily for 42 days). Physical examinations and laboratory values were completed at each visit. Stool also was collected for microbiota analysis and fecal calprotectin at baseline (day 1), at the end of treatment (day 42), and during observation period (day 92). Crying and fussing times were graded via the Barr diary, 2 diaries per week until day 92.[2] Case report forms were completed during each clinic visit. Weekly communications were completed through telephone calls or via email. Clinical visits were performed at Memorial Hermann Hospital/University of Texas Health Clinical Research Unit, Houston. Adverse events (AEs) were monitored strictly based on the FDA Adverse Events Response System and a clinical severity index.[12]

The biostatistician developed a block randomization with block size of 6 for allocation to each group. Randomization was implemented by research pharmacists. To detect potential differences in safety, subjects were randomized via a ratio of 2:1 (treatment to placebo).

The dose of *L reuteri* strain DSM 17938 was approximately $5 \times 10^8$ colony-forming units (given as 5 drops) or placebo (sunflower oil) (provided by BioGaia AB, Stockholm, Sweden). All *L reuteri* strain DSM 17938 vials contained $\sim 5 \times 10^8$ colony-forming units per day during treatment, documented by anaerobic cultures of every fifth returned vial.

Safety (primary outcome) was defined by strict monitoring of AEs and severe AEs throughout the study. A daily diary card was completed by the each study subject's parent, 2 days per week until the fifth visit. Secondary outcomes allowed us to estimate the effect sizes of biomarkers for future studies, which included crying and fussing time, immunologic, microbiologic, and hematologic findings.

The independent medical monitor and data safety monitoring board examined progress throughout the trial, convening after enrollment and follow up every 12 subjects. Study data were collected and managed with REDCap (Research Electronic Data Capture).[13] Our data management system allowed logic checks to ensure data quality. All errors or discrepancies were corrected with a Web-based query program.

Peripheral blood mononuclear cells were isolated from whole blood and processed by flow cytometry.[14]

Plasma levels of interleukin (IL)-1$\beta$, IL-2, IL-10, and tumor necrosis factor-$\alpha$, tissue inhibitor of metalloproteinase-1, and osteoprotegerin were assessed by the use of human single or multiplex panel kits from Meso Scale Discovery (Meso Scale Diagnostics LLC, Rockville, Maryland); plasma tumor necrosis factor–like weak inducer of apoptosis was assessed by using a human enzyme-linked immunosorbent assay kit from eBioscience (a division of Thermo Fisher Scientific, Waltham, Massachusetts).

Stool samples were prepared and analyzed per manufacturer's instructions by fecal calprotectin enzyme-linked immunosorbent assay kit (Eagle Biosciences, Nashua, New Hampshire) as described.[14]

Parents were instructed to collect a stool sample within 48 hours of the visit; stool samples were subdivided and stored at –80°C until analyzed. DNA extraction, polymerase chain reaction amplification, pyrosequencing, and taxonomic identification of 16S rRNA gene sequences in stool specimens were performed as previously described[15] with QIIME[16] and the R statistical package R (R version 3.3.1, R Foundation for Sta-



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