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Lactobacillus reuteri in the management of infantile colic

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ABSTRACT

Objective: To determine if *Lactobacillus reuteri* is an effective treatment for infantile colic by reviewing existing literature and performing a systematic analysis. **Design:** Systematic literature review.
Methods: The PubMed database was searched using the terms "*Lactobacillus reuteri*" and "colic." The yielded results were refined to only include human clinical trials published within the past 10 years.
Results: Chau et al., Savino et al., and Szajewska et al. each found that the average crying times were significantly shorter for the *L. reuteri* group than they were for the probiotic group on days 7, 14, and 21. All three trials also found that the *L. reuteri* group had a significantly higher number of infants who had ≥ 50% reduction in their crying times by the end of the study.
Conclusion: The studies included in this review consistently showed therapeutic benefit in administering *L. reuteri* to colicky breastfed infants. The subjects receiving *L. reuteri* had significant reductions in crying times when compared to those who received placebo. These findings support the use of *L. reuteri* in the management of infantile colic. However, these trials were slightly limited by their relatively small sample sizes and further studies with larger sample sizes are necessary in order to assess the strength of this conclusion.

INTRODUCTION

Infantile colic (IC) is generally known as excessive crying with an unknown cause. A more structured definition is outlined by "the rule of three": more than 3 hours of crying per day, over 3 days per week, for over 3 weeks, in an otherwise healthy, well-fed infant.[1] IC is fairly common, affecting as many as 1 in 4 newborns which equates to approximately 1.2 million infants in the United States.[2] It typically begins in the first 2 weeks of life and usually goes away without intervention by 3 or 4 months of age. While this is not a particularly long affliction, it has significant impacts on the infant, the parents, and even clinicians, who often have no concrete explanations or solutions for their distressed patient.[3,4]

Having a colicky infant causes parental stress, and studies have shown that parental stress contributes to colic, thus creating a vicious cycle. This makes infantile colic difficult to combat, and while it usually subsides by 4 months, the degree of suffering the family experiences through this period is quite significant. Studies have found colic to be associated with maternal depression, shaken baby syndrome, and early cessation of breastfeeding.[5] In order to prevent these possible ramifications of colic, there needs to be an understanding of its cause, but unfortunately the pathophysiology behind colic has long been elusive.

An underlying organic cause for colic is found in less than 5 percent of these infants and its pathogenesis remains unclear.[4] However, over the past 2 decades there has been increasing evidence that gut microbiota is intimately tied to health and disease, and promise has been found in exploring the connection between gut microbiota and IC. When compared to non-colicky infants, the gut microbiota of infants with colic have less bacterial diversity and lower concentrations of protective, anti-inflammatory bacteria, such as lactobacilli.[6] Studies have revealed correlation of intestinal dysbiosis with IC, which seems to stem from the chronic inflammatory response brought on by lack of microbiota diversity and high populations of pathogenic bacteria in the gut.[2,7]

Due to the suspected involvement of gut microbiota on the pathophysiology behind colic, and the protective effects of bacteria, such as lactobacilli, there have been numerous randomized controlled trials (RCTs) evaluating the usefulness of probiotics in the treatment of IC. While their findings have been mixed, evidence suggests that certain strains of probiotics, specifically *Lactobacillus reuteri*, can lead to the resolution of infant colic. The development of a safe treatment would drastically decrease numerous pediatric visits, parental burden, and potential long-term consequences for the infant. Therefore, it needs to be determined if *Lactobacillus reuteri* is truly a solution to this problem.

PICO

Population: breastfed infants less than 6 months old who have been diagnosed with colic

Intervention: *Lactobacillus reuteri*
Comparison: placebo
Outcome: reduction in crying time

CLINICAL QUESTION

Is *Lactobacillus reuteri* more effective than placebo in reducing crying time in breastfed infants with colic?

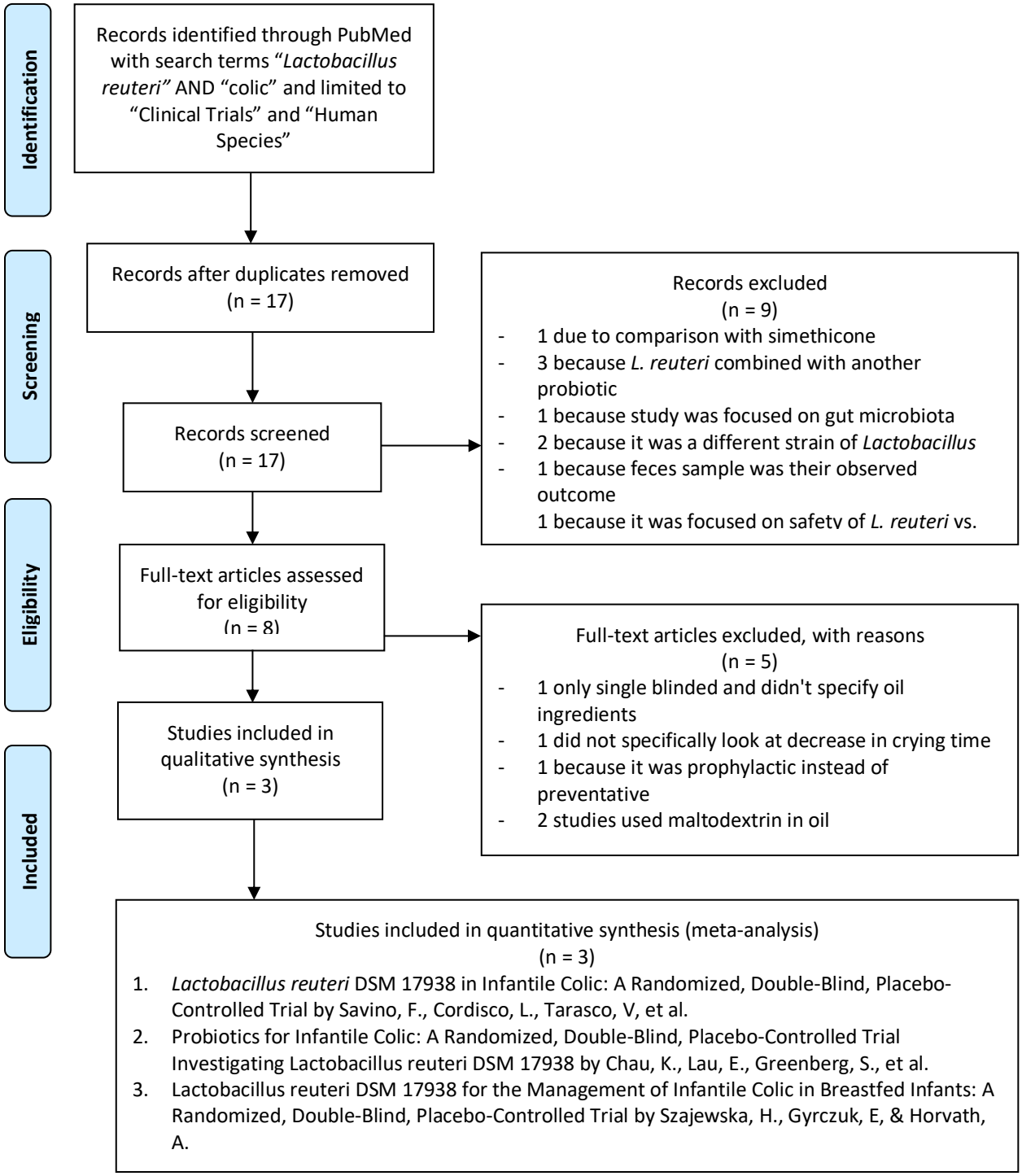

Figure 1. PRISMA Flow Diagram depicting process of study selection

METHODS

A PubMed search was completed in September 2019 using the terms "*Lactobacillus reuteri"* AND "colic". Results were filtered to only include clinical trials involving the human species that were published in the last 10 years. This yielded 17 articles. Upon evaluation of the studies, several did not meet the predetermined inclusion and exclusion criteria seen in Table 1. Subsequent studies were ruled out for the following reasons: *L. reuteri* compared with simethicone rather than placebo, use of multiple probiotics, primary focus was gut microbiota, a different strain of *Lactobacillus* was used, feces samples were the observed outcome, and the focus was on safety rather than efficacy. This process is demonstrated in the PRISMA Flow Diagram seen in Figure 1. The remaining 3 articles were selected for qualitative analysis in this study.

Table 1. Criteria for Selection of Studies for Analysis

Inclusion Criteria	**Exclusion Criteria**
Randomized control trials	Different strain of lactobacillus
Predominantly breastfed infants	Solely formula fed infants
L. reuteri as only probiotic strain	
Evaluating infant colic	

RESULTS

Study 1

Probiotics for Infantile Colic: A Randomized, Double-Blind, Placebo-Controlled Trial Investigating Lactobacillus reuteri DSM 17938. Chau et al.

Objective: Investigated *Lactobacillus reuteri* DSM 17938 for treating infant colic vs. placebo in Canadian infants

Study Design

This was a randomized, double-blind, placebo-controlled trial involving 55 infants with colic, as defined by the modified Wessel criteria. Participants were recruited from The Hospital for Sick Children and from pediatric practices in Toronto, Ontario, Canada. Table 2 outlines the inclusion and exclusion criteria. The 55 infants were randomly assigned using a 2-treatment randomization schedule. This schedule was computer generated by personnel from the Independent Research Support Pharmacy, and these individuals were not participating in the study.

Participants received *L. reuteri* or placebo for 21 days with the purpose of assessing reduction in crying time. The *L. reuteri* group was given a suspension of freeze-dried *L. reuteri* DSM 17938 in a combination of medium-chain triglyceride oil, sunflower oil and silicon dioxide. The concentration of the probiotic in the oil was 1×10^8 CFU per 5 drops. The placebo had the exact same ingredients, but without *L. reuteri*. The primary outcome evaluated the average length of crying times, from baseline (day 0) to the end of treatment (day 21). The secondary outcome measured the number of participants who responded to treatment, meaning those infants with reduction in daily average crying times of more than 50% from baseline, on days 7, 14, and 21. During the duration of the treatment period, parents were instructed to record any adverse events, weekly weights, bowel changes, and digestive discomfort.

On enrollment day, guardians were interviewed, and the referring pediatrician performed an examination and recorded infant growth parameters. Caregivers in both groups were told to give 5 drops orally, at the same time once daily for 21 days and to refrain from "other modes of therapy or methods to

console their infant".[8] The potential modes and methods of consolation were not defined. Parents keep a maternal diary to record colic episodes, daily crying time, feeding schedule, stool frequency and characteristics, and any adverse events, including how often and how long each adverse lasted.

Follow-up visits were scheduled for day 7, 14, and 21 by the same referring pediatrician and a study investigator to monitor infant progress. Colic symptoms, weight, and any adverse events were reported. On the last day of the study, day 21, the same referring pediatrician performed an examination and a study investigator collected any extra study product and the diary. The diaries were reviewed independently by the pediatrician and 2 study team investigators for completion. Data were entered independently by 2 study investigators, and then reviewed by another investigator to ensure accurate diary data transfer.

Table 2. Inclusion and Exclusion Criteria for Study 1

Inclusion Criteria	Exclusion Criteria
Diagnosis of infantile colic defined by a modified definition of Wessel criteria at study commencement	Major medical problem or acute illness, including gastroesophageal reflux, as determined by a pediatrician
Age 3 weeks to 6 months at study commencement	History of antibiotic treatment before or during study
Exclusively breastfed	History of probiotic or *L. reuteri* supplementation
Term delivery (≥37 weeks gestation at birth)	Allergies to any of the ingredients in the probiotic
5-minute Apgar score ≥7	Concurrent participation in another clinical trial
Birth weight ≥2500 g	

Study Results

A total of fifty-two infants (24 from the *L. reuteri* group and 28 from the placebo group) completed the study and their results were analyzed. Using intention-to-treat approach, the total average crying time in minutes from day 0 to day 21 of treatment was shorter in the *L. reuteri* group versus the placebo group: 1719 +/- 750 minutes, 2195 +/- 764 minutes, respectively; p = .028. Compared to the placebo group, the daily average crying times in the *L. reuteri* group were significantly lower on day 7, 14, and 21. The average daily crying times at the end of the study were 60 minutes for the *L. reuteri* group and 102 minutes for the placebo group; p = .045. Additionally, by the end of the study 70.8% of the *L. reuteri* group had a ≥50% reduction in their crying times, while only 21.4% of the placebo group had a ≥50% reduction in their crying times. While the *L. reuteri* group had more members with ≥50% reduction in their crying times by day 7 and 14, the differences from the placebo group were not statistically significant. There were no adverse events reported by any of the participants and there were no growth differences seen between the two groups.

Study Critique

This study had a lot of strengths. It was a randomized, double-blind, placebo-controlled trial with inclusion criteria of 3 weeks to 6 months to capture any infants with delayed onset of colic. The placebo group received the exact same formulation as the study group, minus the live bacteria, and this ensured blinding was not compromised. In an effort to avoid confounding variables, caregivers of the participants were explicitly told to not try any other forms of consolation. However, they did not define what they meant by "other modes of therapy or methods" for consoling their infant. This could have an impact on the

results if parents were instructed to not even hold their infants during times of colic. Another strength of this study was that parents recorded various aspects of the infants life during the clinical trial, including feeding schedule, stool frequency and characteristics, and any adverse events experienced.

The pediatrician who performed the original medical examination of the infants on enrollment (day 0) also performed the medical examination at the end of the study on day 21, which allowed for thorough consistency in evaluation of the infants. The data from the diaries the caregivers filled out were reviewed independently by the pediatrician and 2 study team investigators, then they were reviewed by a third investigator to ensure that the data was accurately transferred.

A limitation to this study is that the data relied on accurate recording by the caregiver. The researchers provided important aspects to be recorded, but there is no way to definitively know that each caregiver followed through with accurate reporting. There is also no way to know that the parent administered the 5 oral drops every day and that they were given at the same time each day. Another limitation was that some of the patients were recruited from The Hospital for Sick Children, but no clarification was given on what these patients were at the hospital for. While exclusion criteria did omit any infants with major medical conditions or acute illnesses, if the patients had been admitted for any reason this could have impacted their response to the treatment.

Study 2

Lactobacillus reuteri DSM 17938 in Infantile Colic: A Randomized, Double-Blind, Placebo-Controlled Trial. Savino et al.

Objective: To test the efficacy of Lactobacillus reuteri in treating infantile colic and to evaluate its relationship to gut microbiota.

Study Design

This was a randomized, double-blind, placebo-controlled trial, involving 50 exclusively breastfed infants (29 boys and 21 girls) that had previously been diagnosed with infantile colic according to modified Wessel's criteria. The infants were recruited from the Department of Pediatrics at the Regina Margherita Children Hospital in Turin, Italy and an independent statistician randomly assigned infants using a computer-generated randomization list. Inclusion and exclusion criteria are listed in Table 3.

Table 3. Inclusion and Exclusion Criteria for Study 2

Inclusion Criteria	Exclusion Criteria
Infants born "at term"	Mother consuming cow's milk
Adequate for gestational age	Chronic illness or gastrointestinal disorders
Aged 2-16 weeks	Intake of probiotics in the week prior to recruitment
Exclusively breastfed	Use of antibiotics in week prior to recruitment
	Any formula feeding
	Use of acid-blocking medication

The experimental group received a suspension of freeze-dried *L. reuteri* in sunflower oil and medium-chain triglyceride oil, with a concentration of 1×10^8 CFU in 5 drops. The placebo group received

the same oil mixture without the *L. reuteri*. The oils were the same in taste and appearance and both were provided in identical 5mL dropper bottles. Parents were instructed to keep dropper bottles refrigerated in between uses. For 21 days, both groups were administered 5 oral drops daily, 30 minutes before their first feeding of the day. A structured diary was kept by the parents and daily duration of crying, stool characteristics, and any gastrointestinal disturbances or adverse effects were recorded.

The measured outcomes included a primary outcome of reduction in daily crying time to less than 3 hours by day 21, and a secondary outcome of ≥50% reduction from the baseline duration of daily crying at days 7, 14, and 21. Infants who had ≥50% reduction in their crying time were referred to as "responders." At day 0, the infants were medically examined, and a baseline was set for growth to be compared with growth at day 21. Patients had follow-up appointments with the same pediatrician on day 7 and day 21.

Study Results

Of the 50 infants enrolled, four were removed from the study by day 7: one due to diagnosis of gastroesophageal reflux, one due to development of fever, and two for parents' failure to record in the diary. All four of these patients were in the placebo group. Statistically significant differences were found in the daily crying times of the probiotic group versus the placebo group, $p = .022$. At day 21, the *L. reuteri* group was found to have a significant reduction in their daily crying time from baseline, while the placebo group did not. The probiotic group's average daily crying times decreased from 370 minutes at baseline to 35 minutes at day 21, while the placebo group's daily crying times only decreased from 300 at baseline to 90 at day 21. Secondarily, there were significantly more responders in the *L. reuteri* group versus the placebo group on days 7 ($p = .006$), 14 ($p = .007$), and 21 ($p = .036$). An intention-to-treat analysis (in which all 4 of the lost placebo participants were counted as responders) still showed that there were significantly more responders in the *L. reuteri* group versus the placebo group.

Study Critique

One of the major strengths of this study is that it was double-blinded, randomized, and placebo-controlled. There were no identifiable differences in the appearance and taste of the *L. reuteri* oil versus the placebo oil and this lent further to the blinding of the study. There was an intention to treat analysis performed, which strengthens the validity of the findings. Another potential strength of this study is that the infants' feces were evaluated for colonization with *L. reuteri*. This confirms adequate *L. reuteri* concentrations in the gastrointestinal tract of the experimental group and verifies the consistent administration of the probiotic.

This study failed to explicitly define the gestational age at birth in the inclusion criteria. The infants were described as being "born at term." This is an outdated designation that has been replaced with "early term" and "late term." "Term" can refer to anywhere from 37 weeks to 42 weeks.[9] This is a considerable amount of time in the life of a newborn. This is a possible flaw in the study, as gestational age could have impacts on the study's findings. A major limitation of this study was that findings were dependent on the parents accurately reporting daily crying times. While explicit instructions were given to the parents, there was no way of verifying the accuracy of their reports. The subjectiveness of the outcomes could potentially impact the validity of the study's findings.

Study 3

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

Objective: To determine if the administration of *Lactobacillus reuteri* is beneficial in the treatment of breastfed infants with infantile colic.

Study Design

This was a randomized, double-blind, placebo-controlled trial, involving 80 infants under 5 months old that were exclusively or predominantly breastfed. Infants were recruited from a primary care practice in Warsaw, Poland and suffering from infantile colic, defined as 3 or more hours of crying per day, 3 or more days per week, within 7 days of enrollment in the study. Table 4 outlines the inclusion criteria.

Table 4. Inclusion and Exclusion Criteria for Study 3

Inclusion Criteria	Exclusion Criteria
Full-term infants aged <5 months with infantile coli	Acute or chronic illness
Exclusively or predominantly (>50%) breastfed	Gastrointestinal disorders
	Use of antibiotics or probiotics within a week of the study

Study Results

Only one patient was lost to follow-up, a patient in the probiotic group, due to no diary return and discontinuation of product administration. The crying times were significantly reduced in the probiotic group compared with the placebo group at all points throughout the study period. The probiotic group had significantly more infants with a ≥50% reduction in their crying time when compared to the placebo group on days 7, 14, 21, and 28. The average crying time of the *L. reuteri* group was also significantly lower than the probiotic group throughout the 21-day period. The number needed to treat (NNT) for a ≥50% reduction in crying time was 7 at day 7, and only 2 at days 14 and 21.

Study Critique

This study had many strengths. It was a randomized, double-blind, placebo-controlled trial conducted with 80 infants with infantile colic <5 months old, so all the infants were of similar age. While 80 test subjects is not a significantly large sample size, it is still considerably larger than the other two studies, therefore this was considered a strength. Participants were exclusively or predominantly breastfed, which fit the inclusion criteria for this analysis, however, it would have been preferred if the participants were exclusively breastfed. As with the other two studies, the placebo was identical in taste and appearance to the *L. reuteri* product, and this helped to ensure proper blinding. This study not only followed up with participants multiple times during the treatment period, but also 7 days after treatment in an effort to evaluate for residual effects. The same pediatrician who did the patient evaluation at the beginning of the study did every subsequent evaluation, and this helped prevent any variation in provider evaluation. This consistency allowed for a better determination of change from baseline for each patient.

Another strength was that all caregivers were given explicit directions or filling out their diary, including the exact aspects related to colic to record, the time the product was given, quality of family life, and any adverse events. However, there were no measures taken to assess the compliance of the diary recording. It was a strength that these diaries were analyzed independently by a study physician and two other investigators, all of whom were blinded from treatment assignment. As with the other studies, the use of a diary as the only means of measuring the infants' colic makes the measurements completely subjective. This could affect the validity of the findings. A major limitation to this study is that there was no assessment of compliance of the diary recordings or proper administration of the product. Without ensuring strict compliance of the participants, the results could be invalid.

DISCUSSION

The primary outcome being investigated in this review was the overall reduction infant crying time. Each study examined a reduction of crying time ≥50% on day 7, 14 and 21, with the exception of the study done by Szajewska, et al. that included follow-up day 28. While the studies also looked at overall reduction in crying-time minutes, this was not a measure that could be evaluated for number needed to treat (NNT) due to the fact that the minutes were given for the groups as a whole. Therefore, this review focuses on the findings related to a ≥50% reduction in crying-time. The studies concluded similar results, insinuating that probiotic use can be implemented as a tool to help with infant colic. Table 5 summarizes the results of each study.

Table 5. Comparison Chart of Studies

	Study 1: Chau, et al.[8]	**Study 2: Savino, et al.[10]**	**Study 3: Szajewska, et al.[11]**
Objective	Investigated *Lactobacillus reuteri* DSM 17938 for treating infant colic vs. placebo in Canadian infants	To test the efficacy of Lactobacillus reuteri in treating infantile colic and to evaluate its relationship to gut microbiota	To determine if the administration of *Lactobacillus reuteri* is beneficial in the treatment of breastfed infants with infantile colic
Study Design	Double-blind, Placebo controlled RCT	Double-blind, Placebo controlled RCT	Double-blind, Placebo controlled RCT
Test Number	24	25	40
Control Number	28	21	40
Probiotic Treatment	Suspension of freeze-dried *L. reuteri* DSM 17938 $1x10^8$ per 5 drops in a mixture of sunflower oil, medium-chain triglyceride oil, and silicon dioxide	Suspension of freeze-dried *L. reuteri* DSM 17938 $1x10^8$ per 5 drops in a mixture of sunflower oil & medium-chain triglyceride oil	Suspension of freeze-dried *L. reuteri* DSM 17938 $1x10^8$ per 5 drops in a mixture of sunflower oil & medium-chain triglyceride oil with vitamin D3 added
Placebo Treatment	Combination of sunflower oil, medium-chain triglyceride oil, and silicon dioxide	Combination of sunflower oil and medium-chain triglyceride oil	Combination of sunflower oil and medium-chain triglyceride oil, with vitamin D3 added
Age of Participants	<5 months	2 - 16 weeks	3 weeks to 6 months
Gestational Age at Delivery	≥37 weeks	Term	Full-term
Feeding Type	Exclusively breastfed	Exclusively breastfed	Predominantly breastfed
Follow-up Period	Day 7, 14, and 21	Day 7, 14, and 21	Day 7, 14, 21, and 28

Conclusion	Infants in the *L. reuteri* group experienced a reduction in crying time compared to placebo	*L. reuteri* improves symptoms of infantile colic in breastfed infants with colic	*L. reuteri* reduces crying time in predominantly breast-fed infants with colic
NNT: Day 7, 14, 21, 28	8, 3, 2, N/A	2, 3, 4, N/A	7, 2, 2, 3

The studies were similar in many aspects, including overall objective, study design, methods, probiotic type and follow-up period. All three studies focused on reduction in crying time over a 21-day period of treatment. They each identified colic using the modified Wessel's criteria, which is defined as "crying and/or fussing ≥3 hours/day for ≥3 days/week for one week."[9] This is important because the goal of this research was to determine if *L. reuteri* was effective in overall reduction of crying-time in infants with colic. If there was not a consistent definition of colic, this would have significant impacts on the ability to compare the studies' results. All three studies were randomized control trials and double-blinded, which are considered the best tests to judge efficacy. They each used *L. reuteri* DSM 17938 suspended in a combination of sunflower oil and medium-chain triglyceride oil. The placebos used in all three studies were oil mixtures identical to the experimental product in taste and appearance but did not contain *L. reuteri.* They all had a similar method for data collection, which involved caregivers recording various aspects of the infant's symptoms into a diary. Each study had follow-ups on day 7, 14 and 21, however, the Szajewska et al. study included a follow-up on day 28, a week after the conclusion of the treatment, to further assess the effect of the intervention.

While these studies were very similar, they did have aspects that set them apart from one another. Savino et al. and Chau et al. had a primary outcome of reduction in average crying time and a secondary outcome of a decrease in daily average crying time of ≥50% from baseline. However, the Szajewska et al. study had these outcomes reversed; the primary outcome was evaluating a decrease in daily average crying time of ≥50% from baseline, and the secondary outcome was a reduction of average crying time. Another difference was that only Chau et al. and Szajewska et al. explicitly identified the gestational age at delivery. Chau et al. used "infants ≥37 weeks" and Szajewska, et al. used "full term" infants, which is defined as ≥39 weeks. However, Savino et al. simply identified their subjects as "term" infants, which is a gestational age at birth of anywhere from 37 weeks to 42 weeks.[8] While no obvious differences were seen in the studies, this discrepancy between the subjects' gestational age at birth could potentially impact the results. Savino, et al. was also the only study that told mothers to avoid consuming cow's milk during treatment and Szajewska, et al. was the only study that used "predominantly breastfed" infants as opposed to exclusively breastfed infants. While these differences may not have had dramatic impact on the outcomes of the studies, it would have been preferable if all three studies had the exact same requirements.

Each study had their share of strengths and weaknesses. Chau et al. is a randomized, double-blind, placebo-controlled trial with an age range of up to 5 months old, and an almost equal number of males to females. However, this study was limited due to its small sample size. Savino et al. was also a double-blind, placebo-controlled trial, but this study used a smaller age range for study participants, with only up to 4 months of age. This study had just as small a sample size as Chau et al. Like the other two studies, Szajewska et al. was a randomized, double-blind, placebo-controlled trial but it had the largest sample size of all of the studies. Each study had the limitation of having no objective measure to ensure compliance of probiotic and placebo administration and accurate records. Chau et al. and Szajewska et al. used the same physician for initial medical examination and the final medical examination, whereas Savino et al. did not specify. Due to the larger sample size, additional day of participant follow-up, as well as using full-term infants, it seems that Szajewska et al. may be the most thorough study out of the three.

All three studies provide evidence that the use of probiotics, specifically *L. reuteri*, can be used as a treatment method for infant colic. Duration of crying time in the *L. reuteri* groups was significantly reduced on day 21 in every study. Chau et al. found that when compared to placebo, the *L. reuteri* group had a significantly greater reduction in average crying time at days 7, 14, and 21, but only a significantly higher number of responders by day 21. Savino et al. found that when compared to placebo, the *L. reuteri* group only had a significantly greater reduction in average crying by day 21, but had a significantly greater number of responders on days 7, 14 and 21. Szajewska et al. had a statistically significant reduction in average crying times and reduction in crying time ≥50% from day 7 to day 21. A reduction in crying time of ≥50% was measured in all studies, and from this data NNT was calculated for each study (as seen in Table 5).

Some limitations of this systematic review were that these studies were very similar, and subsequently the studies often referenced one another. It is possible that they were modeled after one another, and while this is not a flaw, it may have been more informative to have additional studies referenced that were not already a part of this analysis. Also, none of the studies had an impressive sample size or used a measure other than subjective recording of symptoms by the parents. The small sample sizes are understandable considering that it is notoriously difficult to recruit large numbers of infants for RCTs, but nonetheless, larger sample sizes would have provided more weight to the findings of the studies. Further studies need a more direct way of making sure infants are all receiving the correct dosage and parents are recording symptoms accurately.

CONCLUSION

The findings of these three studies substantiate the claim that *L. reuteri* is significantly more effective than placebo in reducing crying-time in infants with colic. There were also no findings to suggest that giving an *L. reuteri* supplement would have any adverse effects on an infant. Therefore, this analysis supports the use of *L. reuteri* in the treatment of breastfed infants with colic. Unfortunately, the findings of this analysis can only truly be applied to breastfed infants and it will be necessary for further studies to address formula-fed infants. These future studies should control the supplied formula, as many formulas already contain probiotics, and this could confound the results. It could be beneficial to explore other supplied forms of *L. reuteri*, for instance, having solely formula-fed infants randomly assigned to either formula that has been fortified with *L. reuteri*, or an identical placebo formula without any probiotic.

Improvements should be made to future studies regarding sample size, and verification of diary tracking and product administration. Despite the well-known difficulty in accomplishing large RCTs with infants, larger sample sizes should be completed. A possible method of verifying diary compliance would be to make the diary submissions online, with daily or weekly submission requirements. More studies should employ fecal testing, as this could further verify product administration and ensure proper *L. reuteri* concentrations in the test subjects.

It also may be valuable to have studies focus solely on early-term or preterm infants so that analyses can be completed on this population independent of full-term infants. Lastly, there has been intriguing exploration into the value of giving *L. reuteri* prophylactically. While preliminary findings have been positive and suggest that *L. reuteri* supplementation could prevent the development of colic,[12,13] more studies are needed in this area in order to draw any conclusions. In the meantime, based on the findings of these three studies, our recommendation is that breastfed infants with established colic should be treated promptly with *L. reuteri*.

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