



Probiotic interventions in infantile colic

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Purpose of review

Up until 2014, the use of probiotics in infantile colic has shown promise. However, the past year has seen the publication of controversial results and rigorous debate on whether probiotics are effective in infantile colic. It is time to review the evidence and discuss whether probiotics should be used for infants with colic.

Recent findings

Despite previous trials indicating the probiotic *Lactobacillus reuteri* DSM 17938 to be effective in treating infantile colic in predominantly breastfed infants, a recent larger trial controversially concluded it to be ineffective for both breastfed and formula-fed infants. A further smaller trial indicated it to be effective, yet again, for treating breastfed infants with colic. Meanwhile, *L. reuteri* DSM 17938 has been suggested, for the first time, to be possibly effective in preventing infantile colic.

Summary

L. reuteri DSM 17938 may be effective for certain subgroups of breastfed infants with colic, and this will be clarified by an ongoing individual participant data meta-analysis. At this stage, the probiotic cannot be recommended for treating infantile colic in formula-fed infants, nor can it be routinely used to prevent infantile colic.

Keywords

crying, infantile colic, probiotics

INTRODUCTION

Infantile colic, or excessive infant crying with no medical cause, is a burdensome condition affecting up to 20% of infants less than 3 months old. Despite decades of research, its aetiology remains elusive and effective management options limited. Recently, the use of probiotics to treat infantile colic has shown promise. Probiotics are live microorganisms that, when consumed in adequate amounts, confer a health benefit on the host. Two systematic reviews published in 2013 concluded the probiotic *Lactobacillus reuteri* to be promising in treating breastfed infants with colic, but its effects in treating formula-fed infants with colic remained unknown [1,2]. Both meta-analyses included three small randomized trials of exclusively or predominantly breastfed infants with colic from Italy and Poland. Both reviews identified methodological limitations of all three trials, including lack of blinding [3], unclear sequence generation and allocation concealment [3], unbalanced baseline characteristics [4], restricted generalizability to infants whose mothers were on elimination diets [3,5] and lack of objective measures of infant crying [3–5]. One of the reviews also identified that there was insufficient evidence for the use of probiotics to prevent infant crying [1].

NEW CONTROVERSIAL RESULTS ON THE EFFECTIVENESS OF *LACTOBACILLUS REUTERI* DSM 17938 IN TREATING INFANTILE COLIC

In 2014, the *British Medical Journal (BMJ)* published results from the largest randomized trial [6**] that did not support the use of *L. reuteri* DSM 17938 in treating infantile colic. The publication generated heated discussions around its controversial negative results. The trial, conducted in Melbourne, Australia, recruited 167 breastfed and formula-fed infants with Wessel's definition of infantile colic. Eighty-five infants were randomized to receive *L. reuteri* DSM 17938 at an oral daily dose of 1×10^8 cfu as five drops per day for 1 month, and 82 infants were allocated placebo. The primary outcome was infant cry/fuss duration at 1 month. The trial found no differences in infant cry/fuss duration between the two groups at days 7, 14 and 21

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KEY POINTS

- Probiotics cannot be routinely recommended for treating all infants with colic.
- *L. reuteri* DSM 17938 may benefit certain subgroups of breastfed infants with colic; this will be clarified by ongoing research.
- *L. reuteri* DSM 17938 cannot be recommended for treating formula-fed infants with colic.
- There is promising evidence that *L. reuteri* DSM 17938 may be able to prevent infantile colic; however, there is currently insufficient evidence to support its routine use for all infants.

postintervention. Remarkably, at 1 month, the probiotic group significantly cried or fussed 49 min/day more than the placebo group [95% confidence interval (CI) 8–90, $P=0.02$]. In fact, when cry duration and fuss duration were analysed separately, infants in the probiotic group fussed significantly more than those in the placebo group at all time points during the study, but cry duration did not differ between the two groups at all time points. This was particularly the case with formula-fed infants ($n=99$). There were no group differences in cry or fuss duration amongst exclusively breastfed infants ($n=68$) at 1 month.

Shortly after the *BMJ* publication of the Australian trial's negative results, a Canadian study indicated *L. reuteri* DSM 17938 to be effective, once again in breastfed infants with colic only [7]. The trial recruited 52 exclusively breastfed infants with Wessel's criteria of infantile colic, with 24 infants assigned *L. reuteri* DSM 17938 in the same form and dose as all the previous studies, and 28 infants assigned placebo. The primary outcome was defined as 'a reduction in the duration of average crying and fussing times, from baseline (day 0) to the end of treatment (day 21), to less than 3 h per day'. This was not reported in the article, however, and the sample size calculation was based on detecting a difference between groups in mean crying and fussing times. The trial reported the probiotic group to exhibit a significantly greater reduction in daily crying and fussing time compared with the placebo group at day 21 (median difference -42 min/day, 95% CI -74 to -10 , $P=0.045$). A higher proportion of infants in the probiotic group responded to treatment (i.e. reduction in cry/fuss time by at least 50% from baseline) at day 21 compared with the placebo group (17 responders versus six responders, relative risk 3.3, 95% CI 1.55–7.03, $P=0.035$).

Table 1 summarizes the median daily infant cry/fuss durations reported in the existing probiotic trials for infantile colic management.

POSSIBLE REASONS FOR THE CONTRADICTION RESULTS

To date, all randomized trials of probiotics for treating infantile colic, except for the first one in 2007, have used *L. reuteri* DSM 17938 versus placebo in the same formulation and dosing supplied by the same manufacturer. The 2007 Italian study used *L. reuteri* ATCC 55730 (that is no longer manufactured because of its potential to confer antibiotic resistance) and simethicone rather than a true placebo as the comparator [3]. The Australian study was the only study that concluded *L. reuteri* DSM 17938 to be definitively ineffective in both breastfed and formula-fed infants with colic, and contradicts the positive results of the other four trials in breastfed infants. The editorial accompanying the *BMJ* publication suggested the trial 'represent(s) the most definitive and well designed study to date on (the) controversial topic' [8]. The trial was rigorously carried out according to the Consolidated Standards of Reporting Trials guidelines, with adequate sequence generation, allocation concealment and blinding of participant families and study investigators. Randomization effectively ensured baseline characteristics were similar between the probiotic and placebo groups.

However, other authors argued that the Australian trial was not 'rigorous' as it included infants on antireflux medications, probiotic-containing formulae and hypoallergenic formulae. The perception of a lesser degree of rigour essentially reflects the tension between 'explanatory' and 'pragmatic' therapeutic designs. The Australian trial was pragmatically designed to reflect 'real life practice' to ensure results were generalizable to most, if not all, infants with colic. In contrast, the other trials were explanatory studies to seek answers in a controlled environment, and their results can only be applied to selected infants who were not on formula feeding, and were not 'diagnosed' with gastro-oesophageal reflux. In reality, many infants with colic are formula-fed, with many parents choosing to cease breastfeeding early to try different infant formulae, including probiotic-containing formulae. In addition, many infants with colic are unnecessarily prescribed antireflux medications for presumed 'gastro-oesophageal reflux', when there is conclusive evidence that antireflux medications do not reduce infant crying, and that gastro-oesophageal reflux is not associated with infant crying.

Table 1. Comparison of median daily infant cry/fuss duration between existing trials

Median daily infant cry/fuss time (min/day)	Probiotic/Placebo					
	Savino <i>et al.</i> [3] ^a	Savino <i>et al.</i> [5] ^a	Szajewska <i>et al.</i> [4] ^a	Sung <i>et al.</i> [6] ^a	Sung <i>et al.</i> [6] ^b	Chau <i>et al.</i> [7] ^b
Baseline	197/197	370/300	240/240	176/160	322/350	131/122
Day 7	159/177	95/185	180/180	105/128	239/268	90/120
Day 14	95/153	60/150	105/150	88/103	231/245	75/103
Day 21	74/154	35/90	75/128	70/88	188/195	60/102
Day 28	51/145	–	52/120	56/63	203/166	–

^aInfant cry duration.^bInfant cry/fuss duration.

The different study designs give rise to several possible reasons that may explain the contradictory results. Table 2 lists some of the essential similarities and differences between the five randomized trials to date. First, the Australian trial was the largest study of the five – its sample size was more than double that of any other trial. The sample size of breastfed infants alone was comparable to the other four trials. Large rigorous trials may reveal ‘true effects’ of an intervention that are contrary to pre-existing smaller trials, because estimates of treatment effects may be more precise and/or the potential for bias less.

Second, the primary outcome measures were different amongst the studies. The Australian trial was the only one that measured infant fussing separate to infant crying. The Italian and Polish trials did not measure infant fussing, and the Canadian trial measured infant fussing and crying together. The Australian trial used a validated and relatively objective method to measure its primary outcome – the Baby Day Diary. It prospectively records detailed infant behaviour over a 24-h period, including time periods when infants were ‘awake and fussy’, ‘awake and crying’, ‘unsoothable crying’, ‘awake and content’, ‘sleeping’ and ‘feeding’. The Canadian study

used a modified version of the Baby Day Diary to record infant crying or fussing, without recording other infant behaviours for the full 24-h period. The other three studies measured infant crying by asking parents to recall how much their infants may have cried over the past day through an interview or diary. This latter method may be subject to recall bias. It is, therefore, difficult to accurately compare the severity of infantile colic at baseline or at the end of intervention between the five studies. Nevertheless, even when examining ‘infant crying’ alone without fussing, the Australian study demonstrated no differences between the probiotic and placebo groups.

Third, geographical differences in baseline infant gut microbiota or their susceptibility to probiotic effects may have contributed to the differences in results. There is evidence that gut microbiota of 6-week-old infants varied according to different geographical locations in Europe. Studies of infants at risk of allergy have shown geographical differences in gut microbiota, even amongst countries with Westernized lifestyles. This is interesting considering that the Australian study had the highest prevalence of family history of atopy compared with the other studies. However,

Table 2. Comparable baseline characteristics of recruited infants in different *L. reuteri* randomized trials for treating infant colic

Baseline characteristic	Savino <i>et al.</i> [3]	Savino <i>et al.</i> [5]	Szajewska <i>et al.</i> [4]	Sung <i>et al.</i> [6 ^{aa}]	Chau <i>et al.</i> [7]
Overall sample size	83	46	80	167	52
Sample size of exclusively breastfed infants	83	46	80 ^a	68/109 ^b	52
Median infant age (weeks)	4.5	4.4	4.9	7.4	5.9
Family history of atopy (%)	47	42	35	60	39
Caesarean delivery (%)	35	38	15	40	21
Males (%)	53	58	60	51	48

^aPredominantly (>50%) breastfed.^bPartially breastfed.

infant colic is not culture-specific, and its prevalence seems to be consistent across countries and continents. Nevertheless, it is possible that infants from Melbourne, Australia, may have had gut microbiota that were not as sensitive to probiotic effects as infants from Europe or North America.

Fourth, the Australian trial recruited infants who were, in general, older in age at study commencement, even though its upper age limit was 3 months as opposed to the Polish study that included infants up to 5 months, and the Canadian study that included infants up to 6 months old. Because the natural course of infant crying is improvement after around 8 weeks of age, and self-resolution by 3–4 months, it is conceivable that any possible probiotic benefits may have been dampened in the Australian study. However, even when considering infants less than 6 weeks old ($n=53$) in a subgroup analysis in the Australian study, the probiotic was not effective. In fact, infants less than 6 weeks old assigned to the probiotic group cried or fussed 88 min per day more than those assigned the placebo at 1 month (adjusted mean difference 88 min/day, 95% CI 16–160, $P=0.02$). Thus, the older infant age in the Australian trial was unlikely to explain the different results when compared with the other trials.

Finally, the Australian trial is the only one that did not exclude infants with a diagnosis of ‘gastro-oesophageal reflux’ and measured the use of antireflux medications. Some authors may propose that the use of antireflux medications for presumed gastro-oesophageal reflux may alter gut microbiota and hence confound the probiotic’s effects in the Australian study. However, as mentioned above, there is good evidence that gastro-oesophageal reflux is not associated with infant crying, and antireflux medications have been shown to be conclusively ineffective in reducing crying. In addition, the use of antireflux medications in the Australian study was balanced between the probiotic and placebo groups.

NEW TRIAL INDICATING *LACTOBACILLUS REUTERI* DSM 17938 TO SHOW PROMISE IN PREVENTING INFANTILE COLIC

Early last year, *JAMA Pediatrics* published the results of the first trial on the use of *L. reuteri* DSM 17938 to prevent infantile colic. Until then, no trial had been designed to investigate the use of probiotics to prevent infantile colic as its primary outcome. This large multicentre study aimed to determine whether oral *L. reuteri* DSM 17938 supplementation during the first 3 months of life could reduce the onset of infantile colic, gastro-oesophageal reflux and constipation [9]. Five hundred and eighty-nine term

newborns were recruited through nine neonatal units in Italy within 1 week of birth, and randomized to receive 1×10^8 cfu of *L. reuteri* DSM 17938 or placebo for 90 days. Parents recorded on a daily diary the duration of inconsolable crying, number of episodes of regurgitation and number of stool evacuations for 3 months. The retention rate was 79% to 3 months. At 1 month, the probiotic group displayed significantly shorter mean inconsolable crying duration than the placebo group (probiotic: 45 min/day, 95% CI 43.5–46.5, versus placebo: 96 min/day, 95% CI 91.6–100.4, $P<0.01$). The effect was sustained at 3 months (probiotic: 38 min/day, 95% CI 33.4–42.0, versus placebo: 80 min/day, 95% CI 64.2–77.6, $P<0.01$). The study authors concluded that the probiotic was effective in reducing crying time in term newborn infants.

This Italian trial is the largest and first to indicate *L. reuteri* DSM 17938 to be effective in preventing infantile colic. However, there are several methodological limitations that should be cautiously noted. First, the sample size calculation was based on detecting ‘an absolute difference of 15% between the proportion of infants with functional gastrointestinal disorders (infantile colic, regurgitation and constipation) in the placebo and probiotic groups’. However, the authors did not report relative risk reduction for the difference between treatment groups, nor did they define infantile colic. It is, therefore, unclear whether the study truly had adequate power to detect clinical meaningful outcomes. Second, most of those lost to follow-up in the probiotic group were because of protocol violations that were unexplained. Third, the trial measured ‘inconsolable crying’ likely by daily parental recall, rather than prospective recording through a validated measure such as the Baby Day Diary. Finally, the study did not report baseline inconsolable crying duration. It is unclear whether inconsolable crying at baseline was similar between groups, and whether this was adjusted for in the 1 and 3-month analyses.

CONCLUSION

Although four of five randomized trials of *L. reuteri* suggest the probiotic to be effective in treating breastfed infants with colic, the largest, most generalizable and pragmatically designed trial refutes this. At this stage, it is clear that probiotics cannot be routinely recommended for all infants with colic, and that there is no role for its use in formula-fed infants with colic. However, it is likely that *L. reuteri* DSM 17938 may be effective in certain subgroups of breastfed infants with colic. This requires further clarification. Currently, an individual participant

data meta-analysis (IPDMA) is underway to pool raw data from all available trials [10]. An IPDMA will yield more reliable estimates of treatment effects, and provide sufficient power to allow subgroup analyses to reach more definitive conclusions about its use for treating infantile colic.

As for the use of *L. reuteri* DSM 17938 in preventing infantile colic, results are promising but not yet conclusive. The Italian multicentre study needs to be replicated in other parts of the world, with future trials designed to be sufficiently powered to analyse infantile colic as a well defined primary outcome using validated measures of infant cry/fuss duration. Furthermore, the long-term effects of routine probiotic supplementation are unknown. We are still very far from being able to recommend probiotics for preventing infantile colic.

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