

PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

***Lactobacillus reuteri* to Treat Infant Colic: A Meta-analysis**

Valerie Sung, Frank D'Amico, Michael D. Cabana, Kim Chau, Gideon Koren,
Francesco Savino, Hania Szajewska, Girish Deshpande, Christophe Dupont, Flavia
Indrio, Silja Mentula, Anna Partty and Daniel Tancredi

Pediatrics 2018;141;

DOI: 10.1542/peds.2017-1811 originally published online December 26, 2017;

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

<http://pediatrics.aappublications.org/content/141/1/e20171811>

Pediatrics is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since . Pediatrics is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2018 by the American Academy of Pediatrics. All rights reserved. Print ISSN:

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



***Lactobacillus reuteri* to Treat Infant Colic: A Meta-analysis**

Valerie Sung, Frank D'Amico, Michael D. Cabana, Kim Chau, Gideon Koren, Francesco Savino, Hania Szajewska, Girish Deshpande, Christophe Dupont, Flavia Indrio, Silja Mentula, Anna Party and Daniel Tancredi

Pediatrics 2018;141;

DOI: 10.1542/peds.2017-1811 originally published online December 26, 2017;

Updated Information & Services	including high resolution figures, can be found at: http://pediatrics.aappublications.org/content/141/1/e20171811
Supplementary Material	Supplementary material can be found at: http://pediatrics.aappublications.org/content/suppl/2017/12/19/peds.2017-1811.DCSupplemental
References	This article cites 45 articles, 10 of which you can access for free at: http://pediatrics.aappublications.org/content/141/1/e20171811.full#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Community Pediatrics http://classic.pediatrics.aappublications.org/cgi/collection/community_pediatrics_sub Public Health http://classic.pediatrics.aappublications.org/cgi/collection/public_health_sub
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: https://shop.aap.org/licensing-permissions/
Reprints	Information about ordering reprints can be found online: http://classic.pediatrics.aappublications.org/content/reprints

Pediatrics is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since . Pediatrics is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2018 by the American Academy of Pediatrics. All rights reserved. Print ISSN:

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



Lactobacillus reuteri to Treat Infant Colic: A Meta-analysis

Valerie Sung, PhD,^a Frank D'Amico, PhD,^{b,c} Michael D. Cabana, MD,^d Kim Chau, PhD,^e Gideon Koren, MD,^e Francesco Savino, PhD,^f Hania Szajewska, MD,^g Girish Deshpande, MSc,^h Christophe Dupont, PhD,ⁱ Flavia Indrio, MD,^j Silja Mentula, PhD,^k Anna Partty, PhD,^l Daniel Tancredi, PhD^m

abstract

CONTEXT: *Lactobacillus reuteri* DSM17938 has shown promise in managing colic, but conflicting study results have prevented a consensus on whether it is truly effective.

OBJECTIVE: Through an individual participant data meta-analysis, we sought to definitively determine if *L reuteri* DSM17938 effectively reduces crying and/or fussing time in infants with colic and whether effects vary by feeding type.

DATA SOURCES: We searched online databases (PubMed, Medline, Embase, the Cumulative Index to Nursing and Allied Health Literature, the Database of Abstracts of Reviews of Effects, and Cochrane), e-abstracts, and clinical trial registries.

STUDY SELECTION: These were double-blind randomized controlled trials (published by June 2017) of *L reuteri* DSM17398 versus a placebo, delivered orally to infants with colic, with outcomes of infant crying and/or fussing duration and treatment success at 21 days.

DATA EXTRACTION: We collected individual participant raw data from included studies modeled simultaneously in multilevel generalized linear mixed-effects regression models.

RESULTS: Four double-blind trials involving 345 infants with colic (174 probiotic and 171 placebo) were included. The probiotic group averaged less crying and/or fussing time than the placebo group at all time points (day 21 adjusted mean difference in change from baseline [minutes] -25.4 [95% confidence interval (CI): -47.3 to -3.5]). The probiotic group was almost twice as likely as the placebo group to experience treatment success at all time points (day 21 adjusted incidence ratio 1.7 [95% CI: 1.4 to 2.2]). Intervention effects were dramatic in breastfed infants (number needed to treat for day 21 success 2.6 [95% CI: 2.0 to 3.6]) but were insignificant in formula-fed infants.

LIMITATIONS: There were insufficient data to make conclusions for formula-fed infants with colic.

CONCLUSIONS: *L reuteri* DSM17938 is effective and can be recommended for breastfed infants with colic. Its role in formula-fed infants with colic needs further research.



^aMurdoch Children's Research Institute, The Royal Children's Hospital, and the University of Melbourne, Melbourne, Victoria, Australia; ^bDepartment of Mathematics, Duquesne University, Pittsburgh, Pennsylvania; ^cUniversity of Pittsburgh Medical Center St. Margaret's Hospital, Pittsburgh, Pennsylvania; ^dDepartment of Pediatrics, Epidemiology and Biostatistics, School of Medicine, University of California, San Francisco, San Francisco, California; ^eUniversity of Toronto and The Hospital for Sick Children, Toronto, Ontario, Canada; ^fOspedale Infantile Regina Margherita, Citta della Salute e della Scienza di Torino, Torino, Italy; ^gDepartment of Paediatrics, The Medical University of Warsaw, Warsaw, Poland; ^hDepartment of Neonatology, Nepean Hospital and Sydney Medical School Nepean, University of Sydney, Sydney, New South Wales, Australia; ⁱDepartment of Pediatric Gastroenterology, Paris Descartes University and Necker-Enfants Malades Hospital, Paris, France; ^jDepartment of Pediatrics, University of Bari, Bari, Italy; ^kBacteriology Unit, National Institute for Health and Welfare, Helsinki, Finland; ^lDepartment of Pediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland; and ^mDepartment of Pediatrics, University of California Davis Health System, Sacramento, California

Dr Sung is the guarantor, had final responsibility for the decision to submit for publication, takes overall responsibility for all aspects of the study and this manuscript, conceptualized and designed the study, was 1 of the project coordinators, contributed data for pooling, drafted the initial manuscript, and reviewed and

To cite: Sung V, D'Amico F, Cabana MD, et al. *Lactobacillus reuteri* to Treat Infant Colic: A Meta-analysis. *Pediatrics*. 2018;141(1):e20171811

Infant colic, or excessive crying of unknown cause, is a burdensome condition affecting ~1 in 5 infants <3 months old.¹ It is most commonly defined by the modified Wessel's criteria of crying and/or fussing >3 hours per day for ≥3 days per week.² It is acutely associated with abusive head trauma,^{3,4} maternal depression,^{5,6} and premature cessation of breastfeeding.⁷ Although it is believed to be a self-resolving condition, there is emerging evidence of its long-term adverse effects on child behavior, sleep, and allergy outcomes.^{8,9} Despite years of research, its etiology remains elusive, and management options are limited.

In the past decade, numerous studies have demonstrated differences in gut microbiota between infants with and without colic.^{10–21} The role of probiotics in colic has come into the limelight, with a handful of randomized controlled trials indicating *Lactobacillus reuteri* DSM17938 to show promise in managing colic. However, results have been conflicting. Three studies of breastfed infants with colic demonstrated the probiotic to be effective.^{22–24} In contrast, 1 study of both breastfed and formula-fed infants with colic, the largest so far, concluded it was ineffective even for those who were breastfed.²⁵

Several recent meta-analyses have indicated that *Lactobacillus reuteri* DSM17938 may be effective in breastfed infants with colic.^{26–30} However, such meta-analyses do not overcome the limitations and biases of individual trials because they generate a single best estimate through the pooling of treatment effect estimates. In contrast, an individual participant data meta-analysis (IPDMA) pools raw data from individual studies to create sufficient power to produce more reliable estimates of treatment effects, allow for subgroup analyses, and provide more definitive conclusions. Because probiotic effects

and mechanisms of action are strain specific, this IPDMA is restricted to a single strain of the probiotic *L reuteri* DSM17398.

Our objectives in this IPDMA were to determine if *L reuteri* DSM17398 effectively reduces crying and/or fussing in infants with colic when compared with a placebo at 21 days postrandomization and whether its effects are modified by 4 specific characteristics specified a priori (eg, feeding type, proton pump inhibition exposure, hypoallergenic formula exposure for formula-fed infants, and maternal dairy elimination diets for breastfed infants).

METHODS

The details of our methodology are described in the published protocol.³¹ The IPDMA is registered through the international prospective register of systematic reviews (CRD42014013210) and approved by the Royal Children's Hospital Human Research Ethics Committee (34081).

Eligibility Criteria and Search Methods

The IPDMA included registered double-blind randomized controlled trials of the probiotic *L reuteri* DSM17398 versus a placebo, delivered orally to infants with modified Wessel's definition of infant colic as recorded by diaries, questionnaires, or parental interviews. We included studies published electronically by June 2017. Reported outcomes had to include infant crying and/or fussing duration. Studies in which researchers evaluated *L reuteri* ATCC 55730, the mother strain of *L reuteri* DSM 17938, were excluded. We identified studies through searching online databases using the following medical subject headings³² terms and keywords and limiting to all infants (birth to 23 months old): (1) medical subject headings terms included "colic or crying or irritable mood, and

probiotics or *L reuteri*"; (2) keywords included "colic* or cry* or irritab*, and probiotic* or *lactobacillus reut**". We searched PubMed (1966 to December 2014), Medline (Ovid, 1946 to December 2014), Embase (Ovid, 1980 to December 2014), the Cumulative Index of Nursing and Allied Health Literature (1980 to December 2014), the Database of Abstracts of Reviews of Effects (1994 to December 2014), Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com; through December 2014), e-abstracts from the Pediatric Academic Societies meetings, reference lists from articles, and Internet searches for non-peer-reviewed articles. PubMed was searched by using keywords only to retrieve e-pubs and items not indexed in Medline. The Medline search strategy was adapted for use in other databases. We also searched for ongoing trials through clinical trial registries (metaRegister of Controlled Trials; see <http://www.isrctn.com/page/mrct>, <https://clinicaltrials.gov>, <http://www.who.int/ictrp/en/>, and www.bioportfolio.com).

We assessed the risk of bias for each study by using the criteria outlined by Cochrane. We also assessed for publication bias by identifying registered trials that were not published and through funnel plot analysis.

All authors of eligible trials were contacted initially by e-mail then connected by teleconference, and they participated in a face-to-face meeting in June 2014 at the International Scientific Association for Probiotics and Prebiotics (ISAPP) Meeting in Aberdeen, United Kingdom. The project coordination and data management teams (V.S., M.C., F.D., and D.T.) corresponded regularly by e-mail and teleconference throughout 2014–2016. Through e-mail correspondence, statistician F.D. communicated with participating

authors and collected data into a single, secure electronic database. Statisticians F.D. and D.T. independently inspected the data with respect to range, internal consistency, and missing items by checking them against published reports. The authors met again at the 2016 ISAPP meeting in Turku, Finland, to finalize the results. In Fig 1, we demonstrate the baseline variables collected from the included studies. In Table 1, we show the baseline characteristics.

Main Outcomes

As described in our protocol, the primary outcomes of the IPDMA were infant crying and/or fussing duration (minutes per day) and treatment success, which is defined as $\geq 50\%$ reduction in crying and/or fussing time from baseline and at 21 days postintervention. Secondary outcomes were infant crying and/or fussing duration (minutes per day)

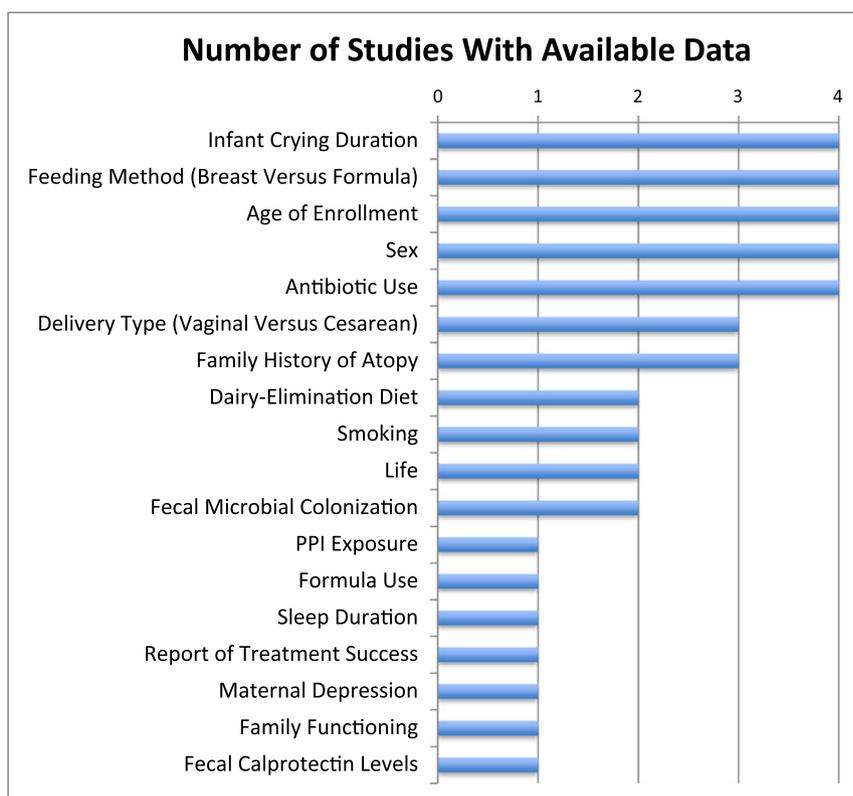


FIGURE 1 Variables available from the 4 studies.

TABLE 1 Baseline Characteristics of Included Trials

Study	Total N	Feeding Method	Median Infant Age, wks	Cesarean Delivery, %	Family History of Atopy, %	Boys, %	Median Baseline Crying and/or Fussing Duration, min per d	Measure of Crying and/or Fussing Duration
Italy ^{a22}	46	Breast (exclusive), with maternal dairy elimination	4.4	41	43	63	350	Diary completed at the end of each day
Poland ²³	80	Breast (predominant)	4.9	NA	NA	60	240	
Australia ²⁵	167	Breast and formula	7.1	40	60	50	330	Baby's Day Diary ³³
Canada ²⁴	52	Breast (exclusive)	5.9	21	39	48	126	(completed every few h in intervals of 5 min)
IPDMA	345	Breast and formula	5.9	32	49	54	260	

NA, not applicable.

^a Industry sponsored and/or funded.

and treatment success at 7 and 14 days postintervention. Infant crying and/or fussing duration (minutes per day) was available from all included studies at baseline and 7, 14, and 21 days postintervention.

Sample Size Calculation

As detailed in the IPDMA protocol, we estimated that ~120 infants per treatment group would be sufficient

for detecting a mean difference in treatment groups of 80 minutes per day (power = 0.80; 2-tailed $\alpha = .05$) as well as provide 80% power for detecting a difference of 20 percentage points (2-tailed $\alpha = .05$) in the treatment's success rates.³¹

Statistical Analysis

The analyses were conducted by 2 independent statisticians

(F.D. and D.T.). Individual participant data from all studies were modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of observations within participants and of participants within studies by using PROC GLIMMIX in SAS (SAS Institute, Inc, Cary, NC).³⁴ Models were specified with fixed main effects

and interaction terms for each individual participant's binary indicator treatment assignment (probiotic versus control) and study time point (7, 14, and 21 days) to permit estimating time point-specific treatment effects. Random intercepts for participants were used to account for residual within-participant correlation. Standard choices of link and variance functions were specified according to type of outcome, with linear-normal models used to estimate between-arm differences in adjusted mean changes in crying duration and adjusted proportion differences in treatment success (for use in computing numbers needed to treat), and log-Poisson models were used to estimate adjusted treatment success incidence ratios. SE estimates for confidence interval (CI) estimation and test statistics were based on robust empirical sandwich estimators with small-sample correction.³⁵

Of the 4 candidate effect modifiers selected a priori for subgroup analyses, only feeding type was available with sufficient completeness to examine heterogeneity. Based on available data, feeding type was classified as exclusively or predominantly breastfed versus formula fed. The latter group came exclusively from the Australian study. Heterogeneity of treatment effects was formally assessed by respecifying regression models with interaction terms for the binary treatment indicator with the candidate effect modifier and conducting formal hypothesis testing (with a statistical significance threshold reset to 0.10 to help offset the low statistical power associated with testing interaction terms). Confounders identified a priori included (1) family history of atopy, (2) delivery type (vaginal versus cesarean), and (3) enrollment age. All included studies excluded infants with a history of antibiotic use, eliminating the need to use this

variable as a covariate. We analyzed by intention to treat, specifically, the binary treatment term corresponded to assigned treatment.

RESULTS

The search yielded 282 abstracts, of which 194 were screened after duplicates were removed, and 17 full-text articles were assessed for eligibility (Supplemental Fig 3). Four double-blind randomized controlled trials involving 345 infants with colic (174 probiotic and 171 placebo) met eligibility criteria and were included in this IPDMA. All 4 studies provided individual participant data. Their baseline characteristics are described in Table 1, and the risk of bias assessment is in Supplemental Fig 4. In assessing for publication bias, we identified only 1 potentially eligible registered trial (NCT01855269) that was completed but unpublished. We performed funnel plot analysis, and the data from the 4 studies appeared symmetric, suggesting publication bias was unlikely (data not shown). However, with <10 studies, the usefulness of funnel plots for revealing patterns is limited.³⁶ Selective reporting of outcomes is not a concern because we verified that the study outcomes analyzed were properly listed on the relevant trial registration, and our outcome is based on a single and widely used standard measure: crying and/or fussing duration.

All 4 trials included breastfed infants with colic; only 1 trial included formula-fed infants with colic.²⁵ In the Italian trial, all mothers of breastfed infants were on a dairy-elimination diet.²² All studies reported no adverse events. Within each trial, there were no differences between treatment groups in baseline variables, and all data supplied by the authors matched precisely the results published in their individual articles. In Fig 1, we show the different types of data

available from the 4 studies. All 4 trials collected data on infant crying and/or fussing duration, feeding method, age of enrollment, and sex, and all 4 studies excluded infants who used antibiotics. Two studies used diaries, in which parents estimated their infants' crying duration at the end of the day,^{22,23} whereas 2 studies used the Baby's Day Diary,³³ which records crying and fussing duration every 5 minutes, with mothers filling in their diaries every few hours.^{24,25} Three of the 4 trials had available data on delivery type (vaginal versus cesarean) and family history of atopy; 1 study reported these variables but did not have available individual participant data. One study was industry sponsored or funded.²²

In Fig 2A, we show the actual mean crying and/or fussing durations by treatment group at baseline and at days 7, 14, and 21. The crying and/or fussing duration was reduced in both the probiotic and placebo groups but was significantly shorter in the probiotic group at all follow-up time points ($P < .05$). In Fig 2B, we depict the adjusted mean between-arm (probiotic versus placebo) differences in changes in crying and/or fussing duration from baseline to each of the 3 follow-up time points. The probiotic group experienced a significantly greater reduction in crying and/or fussing duration at 7 and 21 days than the placebo group (mean difference in change from baseline between groups was -21.0 [95% CI: -42.0 to -0.05] minutes per day at day 7 and -25.4 [95% CI: -47.3 to -3.5] minutes per day at day 21; Table 2). Furthermore, the probiotic group was almost twice as likely as the placebo group to experience treatment success at all time points (adjusted incidence ratios were 2.08 [95% CI: 1.26 to 3.42], 1.98 [95% CI: 1.46 to 2.70], and 1.71 [95% CI: 1.35 to 2.15] for days 7, 14, and 21, respectively; Table 3).

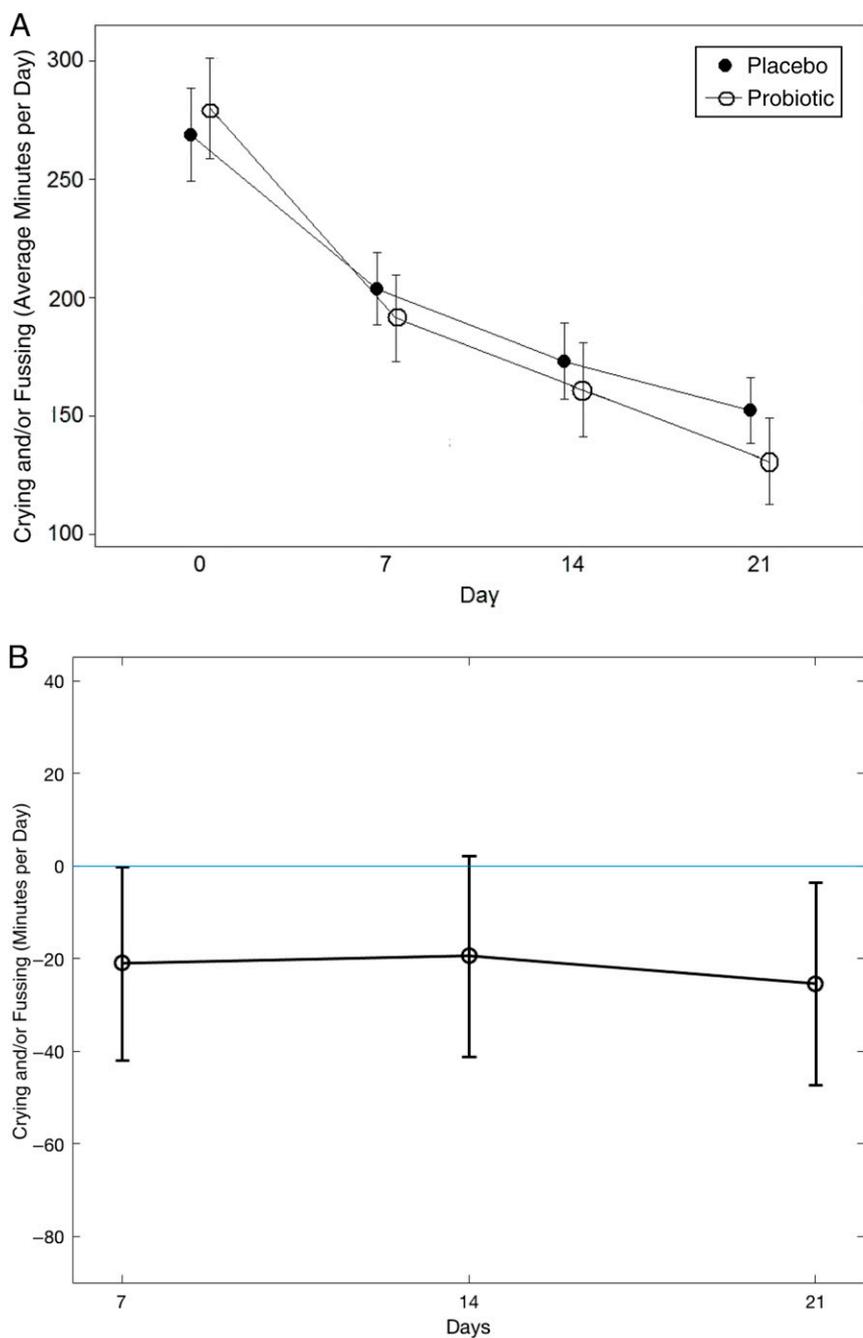


FIGURE 2
 A, Mean crying and/or fussing duration (95% CI) by days postrandomization and treatment group. B, Mean difference in crying and/or fussing duration from baseline between groups (95% CI). In panel B, the data represent the predicted mean difference between treatment groups in the change in crying and/or fussing duration from baseline (probiotic minus placebo). A negative difference implies the probiotic group had more of a reduction in crying and/or fussing duration from baseline than the placebo had. For example, at day 21, the probiotic group had ~25 minutes per day more reduction in crying and/or fussing duration from baseline than the placebo had.

The positive effect was seen more dramatically in the breastfed infants and was not seen at all in the formula-fed infants. For infants with colic who were breastfed,

the probiotic group's reduction in crying and/or fussing duration from baseline was 46 minutes per day more than the placebo group's (adjusted mean difference in change

from baseline was -46.4 [95% CI: -67.2 to -25.5] at day 21 (Table 2). This beneficial effect was statistically significant at days 7 and 14 as well. For breastfed infants with colic, the probiotic group was 2 to 3 times more likely than the placebo group to experience treatment success (adjusted incidence ratios were 3.03 [95% CI: 1.68 to 5.48], 2.27 [95% CI: 1.63 to 3.15], and 2.07 [95% CI: 1.60 to 2.68,] at days 7, 14, and 21, respectively; Table 3). The number needed to treat (NNT) for day 21 success in breastfed infants was 2.6 (95% CI: 2.0 to 3.6). This is in contrast to the formula-fed infants ($n = 78$), with the probiotic group experiencing less reduction in crying and/or fussing as compared with the placebo group at all time points and being less likely to experience treatment success (Table 3).

DISCUSSION

This is the first IPDMA and the most definitive method to assess the effectiveness of *L reuteri* DSM17938 in managing infants with colic. The pooled data suggest that *L reuteri* DSM17938 effectively reduces crying and/or fussing in breastfed infants with colic. Although the results of this study help clarify the debate on the usefulness of *L reuteri* DSM 17938 in improving colic symptoms in breastfed infants, its role in formula-fed infants with colic needs further clarification.

The study has many strengths. Its main strength is its large sample size from the pooling of data from existing studies, which provides enough power to detect treatment effects and subgroup differences. All included studies were double-blind randomized placebo-controlled trials of high quality. Lower-quality studies were excluded, such as the 2015 Mi et al³⁷ study, which was single-blind, and the 2015 Ashraf et al³⁸ and 2017 Martinelli et al³⁹ studies, which were nonblinded.

TABLE 2 Change in Daily Mean Crying and/or Fussing Duration (Minutes Per Day) and Subgroup Analyses by Feeding Type

Subgroup	Day 7			Day 14			Day 21		
	N	Mean Change Within Group (95% CI)	Mean Difference in Change From Baseline Between Groups (95% CI) ^a	N	Mean Change Within Group (95% CI)	Mean Difference in Change From Baseline Between Groups (95% CI) ^a	N	Mean Change Within Group (95% CI)	Mean Difference in Change From Baseline Between Groups (95% CI) ^a
All	150	-64.3 (-80.2 to -48.5)	-21.0 (-42.0 to -0.05)*	144	-93.8 (-110.0 to 77.6)	-19.4 (-41.1 to 2.3)	144	-114.9 (-131.8 to -98.0)	-25.4 (-47.3 to -3.5)*
Probiotic	159	-89.2 (-107.2 to 71.2)		151	-116.0 (-136.6 to 95.3)		149	-146.0 (-166.4 to 125.6)	
Breastfed ^{b**}	113	-56.8 (-72.2 to -41.3)	-33.7 (-53.9 to -13.6)*	112	-87.5 (-104.6 to 70.4)	-28.6 (-50.1 to -7.2)*	112	-101.9 (-120.3 to -83.6)	-46.4 (-67.2 to -25.5)*
Probiotic	118	-95.8 (-116.2 to 75.4)		114	-123.7 (-147.7 to 99.8)		115	-156.0 (-178.1 to 133.9)	
Formula fed ^{b**}	37	-87.5 (-131.8 to 43.2)	15.3 (-41.7 to 72.3)	32	-115.7 (-158.5 to 73.0)	8.4 (-50.2 to 66.9)	32	-160.4 (-199.1 to 121.8)	41.0 (-20.1 to 102.2)
Probiotic	41	-70.2 (-108.7 to 31.8)		37	-92.0 (-133.8 to 50.3)		34	-112.1 (161.8 to -62.4)	

^a Analyses adjusted for sex, age at enrollment, birth weight, birth type (vaginal versus cesarean), family history of atopy, and feeding type (except for subgroup analyses of feeding-type-specific effects).

^b Treatment effects were statistically and significantly heterogeneous by feeding type across the 3 time points: $F(3,880) = 2.65$.

* $P < .05$.

** $P = .048$.

The intervention groups in all 4 studies received the same probiotic (*L reuteri* DSM17398) manufactured by the same company (BioGaia, Stockholm, Sweden) in the same dose (0.2×10^8 colony-forming units per drop, 5 drops orally per day), with the control groups all receiving the same placebo (maltodextrin in oil suspension). All 4 included studies collected the same primary outcome measure of infant crying and/or fussing duration as well as measures of important potential confounders. We identified confounders a priori and were able to adjust for them in our analyses. Intention-to-treat analyses were conducted by statisticians independent of the 4 included trials to eliminate bias. We were able to analyze outcomes by taking into account within-infant changes, thus adjusting for between-infant differences, such as infant age. In addition, the IPDMA is up to date. We are aware of only 1 unpublished double-blind randomized placebo-controlled trial from the United States that is small and focused on safety and biomarkers rather than efficacy ($n = 20$, breastfed infants with colic, personal communication with J.M.R.).¹³ Adding this trial to the IPDMA is unlikely to change this study's conclusions. The IPDMA results have now yielded more definitive conclusions for the role of *L reuteri* DSM17938 in breastfed infants with colic.

However, there are several limitations to the study. First, it included studies with differing methods of defining infant colic and measuring outcomes. Two studies used a nonvalidated diary involving recall of infant crying duration at the end of the day,^{22,23} whereas 2 studies used the Baby's Day Diary,³³ which is a validated measure of infant crying and involves less degree of recall bias, with mothers filling it in every few hours throughout the day.^{24,25} The latter 2 studies included fussing, defined as a

TABLE 3 Treatment Success and Subgroup Analyses by Feeding Type

Subgroup		Day 7			Day 14			Day 21		
		N	No. (%) Successes	Incidence Ratio for Treatment Success (95% CI) ^a	N	No. (%) Successes	Incidence Ratio for Treatment Success (95% CI) ^a	N	No. (%) Successes	Incidence Ratio for Treatment Success (95% CI) ^a
All ^b	Placebo	150	17 (11)	2.08 (1.26 to 3.42)*	144	36 (25)	1.98 (1.46 to 2.70)*	144	56 (39)	1.71 (1.35 to 2.15)*
	Probiotic	159	39 (25)		151	76 (50)		149	99 (66)	
Breastfed ^b ^{c**}	Placebo	113	10 (9)	3.03 (1.68 to 5.48)*	112	28 (25)	2.27 (1.63 to 3.15)*	112	41 (37)	2.07 (1.60 to 2.68)*
	Probiotic	118	33 (28)		114	66 (58)		115	87 (76)	
Formula fed ^{c**}	Placebo	37	7 (19)	0.75 (0.27 to 2.15)	32	8 (25)	1.05 (0.45 to 2.47)	32	15 (47)	0.73 (0.39 to 1.36)
	Probiotic	41	6 (15)		37	10 (27)		34	12 (35)	

Treatment success is a $\geq 50\%$ reduction in crying and/or fussing time from baseline.

^a Analyses adjusted for sex, age at enrollment, birth weight, birth type (vaginal versus cesarean), family history of atopy, and feeding type (except for subgroup analyses of feeding-type-specific effects).

^b NNT estimates were computed by inverting adjusted differences in treatment success proportions and 95% CIs from linear-normal mixed effects models with robust SEs. The linear-normal model was used to overcome numerical convergence difficulties arising with a binomial or Poisson model specification. The NNT overall group is as follows: day 7 NNT = 7.9 (95% CI: 4.9 to 20.5); day 14 NNT = 4.0 (95% CI: 2.9 to 6.6); and day 21 NNT = 3.7 (95% CI: 2.6 to 6.0). The breastfed subgroup is as follows: day 7 NNT = 5.4 (95% CI: 3.7 to 10.0); day 14 NNT = 3.1 (95% CI: 2.3 to 4.8); and day 21 NNT = 2.6 (95% CI: 2.0 to 3.6).

^c Treatment effects were statistically and significantly heterogeneous by feeding type across the 3 time points: $F(3574) = 3.64$.

* $P < .05$.

** $P = .01$.

state of transition characterized by intermittent vocalizations and less intense and nonrhythmic motor activity^{40,41} in their outcome measures, whereas the former 2 studies did not. The Australian study was a pragmatic trial to reflect a more real-life situation, including infants on antireflux medications and hypoallergenic and probiotic and/or prebiotic formulae,²⁵ whereas the other studies were explanatory trials with strict inclusion criteria.^{22–24} Second, we are unable to make definitive conclusions regarding the effectiveness of the probiotic for formula-fed infants with colic because only 1 study included a limited number of formula-fed infants. Third, we were not able to determine if the probiotic's effects differed according to proton pump inhibitor (PPI) exposure, hypoallergenic formula exposure, or maternal dairy-elimination diets because such information was not available from all studies. For the same reason, we were unable to report on secondary outcomes, such as infant sleep duration, parental report of treatment success, maternal depression, quality of life, family functioning, adverse effects, stool colonization, or fecal calprotectin levels.

Our study results are more definitive than (and in keeping with) the previous published meta-analyses on this topic.^{27,28,42} The difference in the effects of *L reuteri* DSM17938 on breastfed as compared with formula-fed infants with colic is interesting and worthy of further investigation. Breastfed and formula-fed infants have different gut microbiota compositions, with *Bifidobacteria* reported to dominate in breastfed infants, whereas formula-fed infants have more diverse gut microbiota.^{43,44} The unique composition of breast milk or possibly the direct effects of microbes or oligosaccharides in breast milk may contribute to these differences.^{43,45,46} It is also possible that particular ingredients in formula may negate the effects of the probiotic. However, feeding type has not been shown to affect the occurrence of infant colic.^{1,47} Of note, the Australian trial included 99 formula-fed infants, of which $\sim 33\%$ were on probiotic-containing formulae without *L reuteri* DSM17938, 17% were on prebiotic-containing formulae (unpublished data), and 15% were on hypoallergenic formulae without clinical symptoms suggestive of cow's milk protein allergy. In addition, $\sim 30\%$ were on PPIs, which

are known to be ineffective for infant crying. It is possible that PPIs, concurrent probiotics, or prebiotics may alter the gut microbiome and hence affect *L reuteri*'s effectiveness; however, the proportions of infants taking these different formulae and PPIs were equally distributed between the probiotic and placebo groups and, therefore, would unlikely contribute to the negative results. The effectiveness of *L reuteri* DSM17938 in infant colic seems to be linked to breastfeeding, but the exact mechanism remains unknown. Comparison analyses using advanced sequencing techniques of available fecal samples collected in this IPDMA's participants would be useful in gaining an insight into whether differences in microbiota composition may play a part.

There is a pressing need for more rigorous randomized controlled trials of *L reuteri* DSM17938 in formula-fed infants with colic. However, in the era of widespread addition of probiotic mixtures to standard infant formula, such trials may be difficult to implement. Nevertheless, the collaboration formed through this IPDMA will be the platform to conduct future IPDMAs for the probiotic management and prevention of infant colic. The

collaboration will be particularly interested in conducting a similar IPDMA to investigate the probiotic's effectiveness in formula-fed infants with colic if such trials are being planned or conducted.

CONCLUSIONS

L reuteri DSM17938 is effective in breastfed infants with colic and should be considered in this subgroup of

infants with colic. The evidence of the probiotic's effectiveness in formula-fed infants is limited.

ACKNOWLEDGMENT

We thank Mary Ellen Sanders for organizing all funding arrangements from ISAPP and organizing the ISAPP meetings in Aberdeen, United Kingdom, and Turku, Finland, in June 2014 and 2016, respectively.

ABBREVIATIONS

CI: confidence interval
IPDMA: individual participant data meta-analysis
ISAPP: International Scientific Association for Probiotics and Prebiotics
NNT: number needed to treat
PPI: proton pump inhibitor

revised the manuscript; Drs Tancredi and D'Amico conceptualized and designed the study, were the data managers, collated the data, performed all statistical analyses, and reviewed and revised the manuscript; Dr Cabana conceptualized and designed the study, was 1 of the project coordinators, and reviewed and revised the manuscript; Drs Chau, Koren, Savino, and Szajewska contributed to the design of the study, contributed data for pooling, and reviewed and revised the manuscript; Drs Deshpande, Dupont, Indrio, Mentula, and Partry contributed to the design of the study and reviewed and revised the manuscript; and all authors had full access to all of the data (including statistical reports and tables) in the study, take responsibility for the integrity of the data and the accuracy of the data analysis, approved the final manuscript as submitted, and agree to be accountable for all aspects of the work.

This individual participant data meta-analysis has been registered at PROSPERO (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42014013210) (identifier CRD42014013210), and the protocol has been published at <http://bmjopen.bmj.com/content/4/12/e006475>.

DOI: <https://doi.org/10.1542/peds.2017-1811>

Accepted for publication Sep 20, 2017

Address correspondence to Valerie Sung, PhD, Centre for Community Child Health, Royal Children's Hospital, 50 Flemington Rd, Parkville, VIC 3052, Australia. E-mail: valerie.sung@rch.org.au

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Copyright © 2018 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: All authors declare support from the International Scientific Association for Probiotics and Prebiotics for the submitted work (as detailed in the Funding section). Other than the items listed under Potential Conflicts of Interest; the authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: This work was supported by the International Scientific Association for Probiotics and Prebiotics (ISAPP). ISAPP contributed to the costs of all teleconferences and facilitated the meetings for this collaboration in June 2014 in Aberdeen, United Kingdom, and in June 2016 in Turku, Finland. ISAPP had no role in the design or writing of this manuscript nor in the decision to submit this article for publication. Each individual trial has received funding from its own respective funding bodies. Dr Sung is supported by a National Health and Medical Research Council Early Career Fellowship (APP1125687), a Royal Australasian College of Physicians Cottrell Research Establishment Fellowship, and has been supported by a Melbourne Children's Clinician-Scientist Fellowship. The Murdoch Children's Research Institute is supported by the Victorian Government's Operational Infrastructure Support Program.

POTENTIAL CONFLICT OF INTEREST: Dr Sung reports personal fees from Mead Johnson Nutrition outside the submitted work; Dr Tancredi reports personal fees from Pfizer Consumer Healthcare outside the submitted work; Dr Cabana reports personal fees from BioGaia outside the submitted work; Dr Szajewska reports personal fees from BioGaia outside the submitted work; and Dr Dupont reports on the following outside the submitted work: Nutricia Research's Scientific Advisory Board, Nestle Health Science Scientific Advisory Board, Sodilac Consulting, and Novalac clinical trials. Dr Savino reports the following outside the submitted work: received fees for scientific consultancy from Nestlé Italia (Assago, Milan), HiPP GmbH & Company Vertrieb KG (Germany), and Danone Trading BV (Amsterdam); he receives royalties from Springer for the book *Nutrizione Parenterale in Pediatria* and declares that the book does not cover any interventions investigated in the article and is outside the submitted work; he has received travel grants, accommodations, and meeting expenses from Cana Pharmaceutical Laboratories (Iraklio, Attica), Radiotelevisione Italiana (Rome), Nestlé Italy, and Nestlé France; he has received personal fees from Mead Johnson Nutrition Italy, Cana Laboratories (Thessaloniki, Greece), Nutricia (part of Groupe Danone; Dubai, United Arab Emirates, and Kuwait); HiPP GmbH & Company Vertrieb KG (Germany), and Menarini Farmaceutica Internazionale (Firenze); and he declares that none of these companies had any input or involvement in any aspect of the manuscript conducted by him or have a real or vested interest in the findings of the article. Dr Indrio reports, outside of the submitted work, being a consultant for BioGaia for an educational program for its Web site and being a speaker for the bureaus for the Nestle Nutrition Institute, Abbot Danone, and BioGaia at scientific events and lectures; and the other authors have indicated they have no potential conflicts of interest to disclose.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2017-3445.

REFERENCES

1. Lucassen PL, Assendelft WJ, van Eijk JT, Gubbels JW, Douwes AC, van Geldrop WJ. Systematic review of the occurrence of infantile colic in the community. *Arch Dis Child*. 2001;84(5):398–403
2. Wessel MA, Cobb JC, Jackson EB, Harris GS Jr, Detwiler AC. Paroxysmal fussing in infancy, sometimes called colic. *Pediatrics*. 1954;14(5):421–435
3. Barr RG, Trent RB, Cross J. Age-related incidence curve of hospitalized shaken baby syndrome cases: convergent evidence for crying as a trigger

- to shaking. *Child Abuse Negl.* 2006;30(1):7–16
4. Lee C, Barr RG, Catherine N, Wicks A. Age-related incidence of publicly reported shaken baby syndrome cases: is crying a trigger for shaking? *J Dev Behav Pediatr.* 2007;28(4):288–293
 5. McMahon C, Barnett B, Kowalenko N, Tennant C, Don N. Postnatal depression, anxiety and unsettled infant behaviour. *Aust N Z J Psychiatry.* 2001;35(5):581–588
 6. Smart J, Hiscock H. Early infant crying and sleeping problems: a pilot study of impact on parental well-being and parent-endorsed strategies for management. *J Paediatr Child Health.* 2007;43(4):284–290
 7. Howard CR, Lanphear N, Lanphear BP, Eberly S, Lawrence RA. Parental responses to infant crying and colic: the effect on breastfeeding duration. *Breastfeed Med.* 2006;1(3):146–155
 8. Savino F, Castagno E, Bretto R, Brondello C, Palumeri E, Oggero R. A prospective 10-year study on children who had severe infantile colic. *Acta Paediatr Suppl.* 2005;94(449):129–132
 9. Hemmi MH, Wolke D, Schneider S. Associations between problems with crying, sleeping and/or feeding in infancy and long-term behavioural outcomes in childhood: a meta-analysis. *Arch Dis Child.* 2011;96(7):622–629
 10. Lehtonen L, Korvenranta H, Eerola E. Intestinal microflora in colicky and noncolicky infants: bacterial cultures and gas-liquid chromatography. *J Pediatr Gastroenterol Nutr.* 1994;19(3):310–314
 11. de Weerth C, Fuentes S, Puylaert P, de Vos WM. Intestinal microbiota of infants with colic: development and specific signatures. *Pediatrics.* 2013;131(2). Available at: www.pediatrics.org/cgi/content/full/131/2/e550
 12. Mentula S, Tuure T, Koskenala R, Korpela R, Könönen E. Microbial composition and fecal fermentation end products from colicky infants - a probiotic supplementation pilot. *Microb Ecol Health Dis.* 2008;20:37–47
 13. Rhoads JM, Fatheree NY, Norori J, et al. Altered fecal microflora and increased fecal calprotectin in infants with colic. *J Pediatr.* 2009;155(6):823.e1–828.e1
 14. Roos S, Dicksved J, Tarasco V, et al. 454 pyrosequencing analysis on faecal samples from a randomized DBPC trial of colicky infants treated with *Lactobacillus reuteri* DSM 17938. *PLoS One.* 2013;8(2):e56710
 15. Savino F, Bailo E, Oggero R, et al. Bacterial counts of intestinal *Lactobacillus* species in infants with colic. *Pediatr Allergy Immunol.* 2005;16(1):72–75
 16. Savino F, Cordisco L, Tarasco V, Calabrese R, Palumeri E, Matteuzzi D. Molecular identification of coliform bacteria from colicky breastfed infants. *Acta Paediatr.* 2009;98(10):1582–1588
 17. Savino F, Cordisco L, Tarasco V, et al. Antagonistic effect of *Lactobacillus* strains against gas-producing coliforms isolated from colicky infants. *BMC Microbiol.* 2011;11:157
 18. Savino F, Cresi F, Pautasso S, et al. Intestinal microflora in breastfed colicky and non-colicky infants. *Acta Paediatr.* 2004;93(6):825–829
 19. Pärtty A, Lehtonen L, Kalliomäki M, Salminen S, Isolauri E. Probiotic *Lactobacillus rhamnosus* GG therapy and microbiological programming in infantile colic: a randomized, controlled trial. *Pediatr Res.* 2015;78(4):470–475
 20. Akbarian-Rad Z, Zahedpasha Y, Ahmadpour-Kacho M, Rajabnia R, Tohidi FT. Intestinal lactobacillus species: Is it equal in colicky and non-colicky breastfed infants? *Iran J Neonatol.* 2013;4(2):1–4
 21. Savino F, Quartieri A, De Marco A, et al. Comparison of formula-fed infants with and without colic revealed significant differences in total bacteria, Enterobacteriaceae and faecal ammonia. *Acta Paediatr.* 2016;106(4):573–578
 22. Savino F, Cordisco L, Tarasco V, et al. *Lactobacillus reuteri* DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics.* 2010;126(3). Available at: www.pediatrics.org/cgi/content/full/126/3/e526
 23. Szajewska H, Gyrczuk E, Horvath A. *Lactobacillus reuteri* DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double-blind, placebo-controlled trial. *J Pediatr.* 2013;162(2):257–262
 24. Chau K, Lau E, Greenberg S, et al. Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating *Lactobacillus reuteri* DSM 17938. *J Pediatr.* 2015;166(1):74–78
 25. Sung V, Hiscock H, Tang MLK, et al. Treating infant colic with the probiotic *Lactobacillus reuteri*: double blind, placebo controlled randomised trial. *BMJ.* 2014;348:g2107
 26. Anabrees J, Indrio F, Paes B, Alfaleh K. Probiotics for infantile colic: a systematic review. *BMC Pediatr.* 2013;13:186
 27. Sung V, Collett S, de Gooyer T, Hiscock H, Tang M, Wake M. Probiotics to prevent or treat excessive infant crying: systematic review and meta-analysis. *JAMA Pediatr.* 2013;167(12):1150–1157
 28. Xu M, Wang J, Wang N, Sun F, Wang L, Liu XH. The efficacy and safety of the probiotic bacterium *Lactobacillus reuteri* DSM 17938 for infantile colic: a meta-analysis of randomized controlled trials. *PLoS One.* 2015;10(10):e0141445
 29. Schreck Bird A, Gregory PJ, Jalloh MA, Risoldi Cochrane Z, Hein DJ. Probiotics for the treatment of infantile colic: a systematic review. *J Pharm Pract.* 2017;30(3):366–374
 30. Gutierrez-Castrellon P, Indrio F, Bolio-Galvis A, Jimenez-Gutierrez C, Lopez-Velazquez G. Efficacy of *Lactobacillus reuteri* DSM 17938 for infantile colic: systematic review with network meta-analysis (NMA). *J Pediatr Gastroenterol Nutr.* 2016;63:S96–S97
 31. Sung V, Cabana MD, D’Amico F, et al. *Lactobacillus reuteri* DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis. *BMJ Open.* 2014;4(12):e006475

32. Srinivasan R, Meyer R, Padmanabhan R, Britto J. Clinical safety of *Lactobacillus casei shirota* as a probiotic in critically ill children. *J Pediatr Gastroenterol Nutr.* 2006;42(2):171–173
33. Barr RG, Kramer MS, Boisjoly C, McVey-White L, Pless IB. Parental diary of infant cry and fuss behaviour. *Arch Dis Child.* 1988;63(4):380–387
34. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ.* 2010;340:c221
35. Mancl LA, DeRouen TA. A covariance estimator for GEE with improved small-sample properties. *Biometrics.* 2001;57(1):126–134
36. Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ.* 2011;343:d4002
37. Mi GL, Zhao L, Qiao DD, Kang WQ, Tang MQ, Xu JK. Effectiveness of *Lactobacillus reuteri* in infantile colic and colicky induced maternal depression: a prospective single blind randomized trial. *Antonie van Leeuwenhoek.* 2015;107(6):1547–1553
38. Ashraf MW, Ayaz SB, Ashraf MN, Matee S, Shoaib M. Probiotics are effective in alleviating infantile colic; results of a randomized controlled trial held at Benazir Bhutto hospital, Rawalpindi, Pakistan. *Rawal Medical Journal.* 2015;40(3):277–280
39. Martinelli M, Ummano D, Giugliano FP, et al. Efficacy of a standardized extract of *Matricaria chamomilla* L., *Melissa officinalis* L. and tyndallized *Lactobacillus acidophilus* (HA122) in infantile colic: an open randomized controlled trial [published online ahead of print June 30, 2017]. *Neurogastroenterol Motil.* doi:10.1111/nmo.13145
40. Barr R, Geertsma M. Colic: the pain perplex. In: Schechter N, Berde C, Yaster M, eds. *Pain in Infants, Children and Adolescents.* 2nd ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2003:751–764
41. Wolff P. *The Development of Behavioral States and the Expression of Emotions in Early Infancy: New Proposals for Investigation.* Chicago, IL: University of Chicago Press; 1987
42. Harb T, Matsuyama M, David M, Hill RJ. Infant colic-what works: a systematic review of interventions for breast-fed infants. *J Pediatr Gastroenterol Nutr.* 2016;62(5):668–686
43. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol.* 2012;9(10):565–576
44. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr.* 2000;30(1):61–67
45. de Weerth C, Fuentes S, de Vos WM. Crying in infants: on the possible role of intestinal microbiota in the development of colic. *Gut Microbes.* 2013;4(5):416–421
46. Chichlowski M, German JB, Lebrilla CB, Mills DA. The influence of milk oligosaccharides on microbiota of infants: opportunities for formulas. *Annu Rev Food Sci Technol.* 2011;2:331–351
47. Clifford TJ, Campbell MK, Speechley KN, Gorodzinsky F. Infant colic: empirical evidence of the absence of an association with source of early infant nutrition. *Arch Pediatr Adolesc Med.* 2002;156(11):1123–1128