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Title

Lactobacillus reuteri DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis

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Abstract

Introduction

Infant colic, or excessive crying of unknown cause in infants less than three months old, is common and burdensome. Its aetiology is undetermined, and consensus on its management is still lacking. Recent studies suggest a possible link between infant colic and gut microbiota, indicating probiotics to be a promising treatment. However, only a few strains have been tested, and results from randomised controlled trials are conflicting. It is important to clarify whether probiotics are effective for treating infant colic in general, and identify whether certain subgroups of infants with colic would benefit from particular strains of probiotics.

Methods and analysis

Through an individual participant data meta-analysis (IPDMA), we aim to identify whether the probiotic *Lactobacillus reuteri* DSM17938 is effective in the management of infant colic, and clarify whether its effects differ according to feeding method (breast versus formula versus combined), proton pump inhibitor exposure, and antibiotic exposure. The primary outcomes are infant crying duration and treatment success (at least 50% reduction in crying time from baseline) at 21 days post-intervention. Individual participant data from all studies will be modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies. Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants.

Ethics and dissemination

Approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). Results will be reported in a peer-reviewed journal in 2015.

Registration

PROSPERO CRD42014013210

Strengths and limitations of the study

- This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics.
- While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Combining raw data from individual trials via an IPDMA can yield more reliable estimates of treatment effects with universal applicability. This

is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

- The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation.

Introduction

Infant colic, or excessive crying of unknown cause, is a common, burdensome condition affecting up to 20% of infants less than three months old.[1] Although colic self-resolves beyond the first three months of life, it is associated with potentially significant adverse effects, such as maternal depression,[2 3] child abuse,[4 5] and early cessation of breastfeeding.[6] There is also some evidence of long-term adverse outcomes, such as behaviour and sleep problems.[7 8] The aetiology of infant colic remains unresolved, and effective treatment options are limited.[9-11] Most clinical guidelines recommend reassurance and offering support to affected families.[12 13] However, many families, when faced with a crying baby, find such a management approach insufficient. Meanwhile, health professionals often resort to prescribing non-evidence based therapies that have been proven to be ineffective, and even possibly harmful.[14-16] There is a need to find a safe and effective treatment option for infant colic.

Recent research has focused on the role of gut microbiota in the pathophysiological pathway to infant colic, with numerous studies revealing differences in gut microbiota between infants with and without colic.[17-26] At the same time, a handful of studies have examined the role of probiotics – live microorganisms believed to confer a health benefit – in the management of infant colic. The first study of its kind was an open-label randomised trial of *Lactobacillus reuteri* ATCC55730 versus simethicone, given to exclusively breastfed Italian infants with colic whose mothers were on dairy-exclusion diets.[27] This study was replicated as a double-blind randomised trial of *Lactobacillus reuteri* DSM17938, a daughter strain of ATCC55730, versus placebo, in exclusively breastfed Italian infants with colic whose mothers were on dairy-exclusion diets.[28] A third study in Poland was a double-blind randomised trial of *Lactobacillus reuteri* DSM17938 versus placebo in predominantly breastfed infants with colic.[29] All three trials showed the probiotic to be effective.

Two systematic reviews and meta-analyses that included these three trials concluded probiotics to be likely effective in select samples of infants with colic – those who are predominantly exclusively breastfed and those whose mothers are on dairy exclusion diets.[30 31] However, a more recent fourth study from Australia, a randomised controlled trial that compared *Lactobacillus reuteri* DSM17938 versus placebo in both breast- and formula-fed infants with colic using the same dose and form, did not demonstrate an effect of probiotic supplementation in managing colic.[32] Two other randomised controlled trials using different mixtures of probiotic strains were also ineffective in *managing* colic.[19 33] The reasons for such conflicting evidence are unclear, and there is a need to explore the reasons behind such controversial results, particularly with increasing probiotic marketing, variety of strains used, and addition of probiotics to infant formulae. It would be helpful to clearly identify which subgroups of infants with colic benefit from probiotics.

Currently there are some ongoing trials examining the role of probiotics in managing and preventing infant colic, using similar designs, participants, interventions, comparators and

outcome measures.[34] While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Ultimately, such meta-analyses often do not overcome limitations and biases of individual trials by generating a single best estimate through pooling of treatment effect estimates.[35] In contrast, combining raw data from individual trials via an individual participant data meta-analysis (IPDMA) can yield more reliable estimates of treatment effects with universal applicability.[35-39] This is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

The pooling of data into an IPDMA for analysis will ultimately provide more definitive answers as to whether the probiotic *Lactobacillus reuteri* DSM17938 is effective for infant colic, and determine which subgroups of infants would benefit from which particular probiotic strain. In general, probiotic strains are *not* homogenous, as individual probiotic strains may be effective for colic via different potential mechanisms and differ significantly.[23 25 28 40 41] Due to the heterogeneity of these therapies, it is not clear if these different interventions can be combined for analysis to determine their individual effectiveness. This IPDMA will therefore only include the most commonly studied probiotic strain used for the management of infant colic, and will form the protocol basis for further IPDMAs involving other probiotic strains for the management or prevention of infant colic.

The aims of this IPDMA are:

1. To determine whether the probiotic *Lactobacillus reuteri* DSM17398 is effective in the management of infant colic, and
2. To determine whether the effects of *Lactobacillus reuteri* DSM17398 on infants with colic differ according to
    1. Type of feeding (exclusively breastfed versus partially breastfed versus exclusively formula-fed),
    2. Proton pump inhibitor exposure,
    3. Hypoallergenic formula exposure for formula-fed infants, and
    4. Maternal dairy elimination diets for breastfed infants.

Methods and analysis

Individual participant data meta-analysis (IPDMA).

Search methods for identification of studies

We will search for completed and ongoing randomized controlled trials by identification of published papers and protocols through the online databases Medline, Embase, CINAHL, Database of Abstracts of Reviews of Effects (DARE), Cochrane Central Register for Controlled

Trials (CENTRAL), and clinical trial registries (eg. metaRegister of Controlled Trials). Reference lists from articles will be explored to identify other potential trials. We will also perform internet searches for non-peer reviewed articles, media articles and other relevant publications using Google, and approach presenters at relevant conferences and meetings. This IPDMA will be undertaken according to the methods recommended by the Cochrane Collaboration,[42] with reporting following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[43]

Eligibility criteria for included RCTs

The IPDMA will include registered randomized controlled trials of the probiotic *Lactobacillus reuteri* DSM17398 versus placebo, delivered orally to infants with modified Wessel's definition of infant colic (crying for more than three hours of the day, for more than three days of the week, for at least one week, as recorded by diaries, questionnaires or parental interviews).

Studies evaluating *L reuteri* ATCC 55730, the mother strain of *Lactobacillus reuteri* DSM 17938, will be excluded. *L reuteri* ATCC 55730 was found to carry potentially transferable resistance traits for tetracycline and lincomycin. Hence, it was replaced by *L reuteri* DSM 17938, a strain without unwanted plasmid-borne resistance.[44] It remains a matter of debate whether or not *L reuteri* DSM 17938, the strain with antibiotic resistance plasmids removed, and the original *L reuteri* ATCC 55730 strain can be regarded as equal. Moreover, only *L reuteri* DSM 17938 is commercially available.

Main outcomes

The primary outcomes of the IPDMA are

- Infant crying duration (minutes per day) at 21 days post-intervention, and
- Treatment success at 21 days post-intervention, defined as at least 50% reduction in crying time from baseline.

Secondary outcomes include:

- Infant crying duration (minutes per day) at days 7, 14 and 28 post-intervention, and
- Treatment success (at least 50% reduction in crying time) at days 7, 14 and 28 post-intervention.
- Infant sleep duration (minutes per day) per 24 hours at 7,14,21 and 28 days duration (post treatment baseline)
- Adverse effects: diarrhoea, constipation, vomiting, apnoea and apparent life threatening events (ALTE)
- Stool colonisation analysis

Sample size and power calculation

Abstracting data from published randomized trials, estimates of the standard deviations in crying time (min/day) at baseline and day 21 were collected and pooled to provide an estimated standard deviation of 210 (min/day). From this information, it is estimated that approximately 120 infants per treatment group would be sufficient for detecting a mean difference in treatment groups of 80 min per day (power = 0.80, alpha =0.05, two-tailed). Additionally, approximately 120 per group would also provide 80% power for detecting a difference of 20 percentage points (alpha = 0.05, two-tailed) in the treatment success rates. Treatment success is defined as (yes/no) with "yes" corresponding to at least 50% reduction in crying time from baseline to day 21.

For subgroup analysis to compare whether treatment effects differed by patient characteristics, hypothesis testing will be based on the comparison of treatment effects between subgroups, with a two-tailed alpha of 0.10 used to offset the decreased precision available for estimating interaction effects (i.e. differences in differences). We specified that it would be clinically significant to detect between-subgroup differences in treatment effects of 150 min/day on the crying time outcome and of 50 percentage points on the treatment success outcome, assuming that one subgroup consists of between 33% to 66% of the full sample and the other subgroup consists of the remainder. For example, if treatment group differences truly are 180 min/day in a pre-specified subgroup with one third of the patients and only 30 min/day for the remaining patients, the difference in treatment effects would correspond to 150 min/day. Again, a sample size of approximately 120 infants provides at least 80% power to detect such clinically important differences.

At the time of this writing, authors of four trials have agreed to participate. These four trials include three that assessed *Lactobacillus reuteri* strain DSM 17398, comprise a total of 293 patients with 150 randomized to probiotic and 143 to placebo. Thus it is projected that this IPDMA will have sufficient power for detecting clinically relevant differences in both the average crying times and success rates of at least 50% reduction from baseline to day 21 between probiotic and placebo groups.

Statistical analysis

The analysis will be conducted with individual participant data from all studies modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies.[36] Models will be specified with fixed-effects terms for the individual participant's binary indicator treatment-assignment (probiotic versus control), a parsimonious set of pre-specified participant-level characteristics, and the study identifier. This model specification will be straightforwardly extended to account for when longitudinally assessed outcomes are the units of analysis (one record per time point per participant), by including fixed effects terms for time (main effects as well as interaction terms with the binary treatment indicator) and random effects for the participant to account

for residual within-participant correlation. Standard choices of link and variance functions will be specified, according to type of outcome, with linear-normal models used for suitably (i.e., homogeneous) continuous outcomes and logit-binomial and log-Poisson models used for binary and count outcomes, respectively.

Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants. Heterogeneity of treatment effects will be 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). These characteristics are identified a priori and include: 1) feeding method (exclusively breastfed versus partially breastfed versus exclusively formula-fed), 2) proton pump inhibitor exposure, 3) hypoallergenic formula exposure for formula-fed infants, and 4) maternal dairy elimination diets for breastfed infants. Confounders identified a priori will include 1) family history of atopy, 2) delivery type (vaginal versus caesarian), and 3) enrolment age.

Analysis will be by intention-to-treat; specifically the binary treatment term will correspond to assigned treatment. Missing data a will be described and explored and their potential impact on the primary analysis by sensitivity analyses in which varying assumptions are made about missing data. The primary analysis will be conducted at the 0.05 level of significance.

Ethics and dissemination

Ethics committee approval

The IPDMA has been approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). The IPDMA is registered at PROSPERO, the International Prospective Register of Systematic Reviews, at the University of York (CRD42014013210).

Project management and data collection

Membership to the collaboration for this IPDMA will include representatives from each trial contributing data to the project, a project coordination team, and a data management team consisting of two independent statisticians (FD, DT). The collaboration will collect the minimum de-identified data required to answer the research questions. We will store data in a secure, centralized, customized database, accessible only by unique passcode known only to the project coordination team, data management team, and managers of each individual study contributing data. The two independent statisticians will inspect the data with respect to range, internal consistency, and missing items by checking them against published reports, trial protocols and if necessary, data collection sheets. The statisticians will discuss any

inconsistencies or missing data with individual trial managers, and any problems will be resolved by consensus using original raw data.

Data ownership and confidentiality

All included trials must have been given ethical approval by their respective Human Research Ethics Committee. Participants in individual trials must have consented to their participation in their respective trial. Each study manager remains the custodian of their own data and retain the right to withdraw their data from the analysis at any time. Data must be de-identified before being shared for this IPDMA. The pooled data can be accessed by the project coordination team, data management team, and managers of each individual study contributing data. The project intellectual property (IP) will be owned by the parties as tenants in common in proportion to their respective contributions to that project IP (including, without limitation, contributions and inventorship).

Data monitoring procedures

Each individual trial will follow its own data monitoring procedures. The collaboration plans to update the IPDMA data at regular intervals if further relevant individual trials are completed with available data.

Risks and benefits

The main risk for this study is the discovery of discrepant data, or results that are inconsistent with published manuscripts; however, all the studies have been published in peer-reviewed, scientific journals. In addition, this risk will be minimised by careful handling of the data, involvement of two independent statisticians in data analysis, having a unified plan for management of missing data, and the plan for open discussions to resolve any issues regarding any conflicting information. Participation in this study requires prior consent and approval of all trial managers in sharing each study's data and subjecting individual data to re-analysis.

The combination of data from multiple, similar trials via an IPDMA can yield more reliable estimates of treatment effects, especially for small subgroups of patients.[36-39]. The patients enrolled in each of the individual studies may have had particular patient characteristics or exposures that may have affected the effectiveness of the probiotic intervention. In addition, the different studies may have varied in the types of patients they recruited or varied slightly in their recruitment criteria. As a result, in addition to making more definitive conclusions as to whether probiotics are effective for infant colic, this IPDMA will be able to help determine if there are subgroups of infants who might benefit from a probiotic intervention for colic, in general, or to a particular probiotic strain.

Publication plan

Each individual trial will have the right to publish its main results before publication of this IPDMA. Study results from this meta-analysis will be reported in a peer-reviewed journal in

2015. Before publication of any IPDMA manuscripts, drafts will be circulated for comment, revision and approval by a nominated representative of each of the participating trials. Publications using these data will be authored on behalf of the IPDMA collaboration, with specific named authors (including a representative of each participating trial, the project coordination team and data management team) according to the amount of contribution to each manuscript, and names of other participating collaborators listed in the Acknowledgements.

Strengths and limitations of the study

This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics. The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation. The collaboration formed through this IPDMA will be the platform to conduct future IPDMAs for the probiotic management and prevention of infant colic.

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Authors' contributions

VS, MDC, FD and DT conceptualised the study protocol. VS and MDC are the project coordinators of this collaboration. FD and DT are the data managers of this study. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to the design of the study protocol. VS produced the first draft of the manuscript. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to writing the manuscript, read and approved the final manuscript.

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Competing interests statements

VS, MDC, FD, GD, FI, SM, FS, HS and DT have received travel reimbursement to attend the ISAPP meeting in Aberdeen, United Kingdom, in June 2014. All teleconference calls have been reimbursed by the ISAPP. MDC is a board member of the ISAPP and has received grant funding from Nestec. MDC has served as a paid consultant for Nestle, Mead Johnson and Pfizer Nutrition. FD and DT will receive funding from ISAPP for their work in the statistical analysis. In the past five years, DT has also received travel reimbursement to attend annual ISAPP meetings (2009-2014) and scientific consulting fees (2012). ISAPP is independent of the study, and played no role in the study protocol's design, in the writing of the manuscript, or in the decision to submit the manuscript for publication. HS and FI served as speakers for BioGaia, the manufacturer of *L reuteri* DSM 17938. CD received honoraria from Sodilac for a clinical trial.[33] FS reports travel grant from Nestlè Italy, personal fees from Mead Johnson Nutrition, Italy, personal fees from Cana S.A.S. Tessaloniki, Greek, personal fees from Nutricia -Part of Group Danone, Dubai Kuwait, travel grants and other from BioGaia AB , Stockholm I Sweden, personal fees from HiPP GmbH & Co Vertrieb KG Germany, travel grant from Nestlé France SAS, Paris, travel grants and other from Noos, srl , Roma Italy, personal fees from A. MENARINI IFR s.r.l, Firenze Italy, outside the submitted work. They have had no input or involvement in any aspect of the review process during this or previous systematic reviews carried out by FS. AP has no competing interests to declare.

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List of abbreviations

IPDMA	individual participant data meta-analysis
PRISMA	preferred reporting items for systematic reviews and meta-analyses
IP	intellectual property
ISAPP	The International Scientific Association for Probiotics and Prebiotics
PROSPERO	The International Prospective Register of Systematic Reviews

BMJ Open

Lactobacillus reuteri DSM 17938 for managing infant colic - protocol for an individual participant data meta-analysis

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Title

Lactobacillus reuteri DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis

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Colic, crying, infant, probiotics, review, meta-analysis, microbiota

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Abstract

Introduction

Infant colic, or excessive crying of unknown cause in infants less than three months old, is common and burdensome. Its aetiology is undetermined, and consensus on its management is still lacking. Recent studies suggest a possible link between infant colic and gut microbiota, indicating probiotics to be a promising treatment. However, only a few strains have been tested, and results from randomised controlled trials are conflicting. It is important to clarify whether probiotics are effective for treating infant colic in general, and identify whether certain subgroups of infants with colic would benefit from particular strains of probiotics.

Methods and analysis

Through an individual participant data meta-analysis (IPDMA), we aim to identify whether the probiotic *Lactobacillus reuteri* DSM17938 is effective in the management of infant colic, and clarify whether its effects differ according to feeding method (breast versus formula versus combined), proton pump inhibitor exposure, and antibiotic exposure. The primary outcomes are infant crying duration and treatment success (at least 50% reduction in crying time from baseline) at 21 days post-intervention. Individual participant data from all studies will be modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies. Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants.

Ethics and dissemination

Approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). Results will be reported in a peer-reviewed journal in 2015.

Registration

PROSPERO CRD42014013210

Strengths and limitations of the study

- This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics.
- While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Combining raw data from individual trials via an IPDMA can yield more reliable estimates of treatment effects with universal applicability. This

is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

- The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation. It is also limited by inclusion of studies with potentially different methods of defining infant colic and measuring outcomes.

Introduction

Infant colic, or excessive crying of unknown cause, is a common, burdensome condition affecting up to 20% of infants less than three months old.[1] Although colic self-resolves beyond the first three months of life, it is associated with potentially significant adverse effects, such as maternal depression,[2 3] child abuse,[4 5] and early cessation of breastfeeding.[6] There is also some evidence of long-term adverse outcomes, such as behaviour and sleep problems.[7 8] The aetiology of infant colic remains unresolved, and effective treatment options are limited.[9-11]

Recent research has focused on the role of gut microbiota in the pathophysiological pathway to infant colic, with numerous studies revealing differences in gut microbiota between infants with and without colic.[12-21] At the same time, a handful of studies have examined the role of probiotics – live microorganisms believed to confer a health benefit – in the management of infant colic. One study of *Lactobacillus reuteri* ATCC55730[22] and two studies of *Lactobacillus reuteri* DSM17938[23 24] in breastfed infants with colic were effective, but a subsequent study of both breastfed and formula fed infants with colic indicated *Lactobacillus reuteri* DSM17938 to be ineffective.[25] Two other studies using different mixtures of probiotic strains were also ineffective in *managing* colic.[14 26] The reasons for such conflicting evidence are unclear, and there is a need to explore the reasons behind such controversial results, particularly with increasing probiotic marketing, variety of strains used, and addition of probiotics to infant formulae.

Currently there are some ongoing trials examining the role of probiotics in managing and preventing infant colic, using similar designs, participants, interventions, comparators and outcome measures.[27] While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Ultimately, such meta-analyses often do not overcome limitations and biases of individual trials by generating a single best estimate through pooling of treatment effect estimates.[28] In contrast, combining raw data from individual trials via an individual participant data meta-analysis (IPDMA) can yield more reliable estimates of treatment effects with universal applicability.[28-32] This is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

The pooling of data into an IPDMA for analysis will ultimately provide more definitive answers as to whether the probiotic *Lactobacillus reuteri* DSM17938 is effective for infant colic, and determine whether certain subgroups of infants would benefit from it. As the effects of probiotics are strain specific,[33] this IPDMA will only include the most commonly studied probiotic strain used for the management of infant colic, and will form the protocol basis for

further IPDMAs involving other probiotic strains for the management or prevention of infant colic.

The aims of this IPDMA are:

1. To determine whether the probiotic *Lactobacillus reuteri* DSM17398 is effective in the management of infant colic, and
2. To determine whether the effects of *Lactobacillus reuteri* DSM17398 on infants with colic differ according to
    1. Type of feeding (exclusively breastfed versus partially breastfed versus exclusively formula-fed),
    2. Proton pump inhibitor exposure,
    3. Hypoallergenic formula exposure for formula-fed infants, and
    4. Maternal dairy elimination diets for breastfed infants.

Methods and analysis

Individual participant data meta-analysis (IPDMA).

Search methods for identification of studies

We will search for completed and ongoing randomized controlled trials by identification of published papers and protocols through the online databases Medline, Embase, CINAHL, Database of Abstracts of Reviews of Effects (DARE), Cochrane Central Register for Controlled Trials (CENTRAL), and clinical trial registries (eg. metaRegister of Controlled Trials). Reference lists from articles will be explored to identify other potential trials. We will also perform internet searches for non-peer reviewed articles, media articles and other relevant publications using Google, and approach presenters at relevant conferences and meetings. This IPDMA will be undertaken according to the methods recommended by the Cochrane Collaboration,[34] with reporting following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[35]

Eligibility criteria for included RCTs

The IPDMA will include registered randomized controlled trials of the probiotic *Lactobacillus reuteri* DSM17398 versus placebo, delivered orally to infants with modified Wessel's definition of infant colic (crying for more than three hours of the day, for more than three days of the week, for at least one week, as recorded by diaries, questionnaires or parental interviews).

Studies evaluating *L reuteri* ATCC 55730, the mother strain of *Lactobacillus reuteri* DSM 17938, will be excluded. *L reuteri* ATCC 55730 was found to carry potentially transferable resistance traits for tetracycline and lincomycin. Hence, it was replaced by *L reuteri* DSM 17938, a strain without unwanted plasmid-borne resistance.[36] It remains a matter of

debate whether or not *L reuteri* DSM 17938, the strain with antibiotic resistance plasmids removed, and the original *L reuteri* ATCC 55730 strain can be regarded as equal. Moreover, only *L reuteri* DSM 17938 is commercially available.

All authors of eligible trials have been contacted and invited to participate in this IPDMA. As more trials satisfying eligibility criteria become published, the relevant authors will be approached and invited to participate, as long as their trials are published within the timeframe of conducting this IPDMA.

Main outcomes

The primary outcomes of the IPDMA are

- Infant crying duration (minutes per day) at 21 days post-intervention, and
- Treatment success at 21 days post-intervention, defined as at least 50% reduction in crying time from baseline.

Secondary outcomes include:

- Infant crying duration (minutes per day) at days 7, 14 and 28 post-intervention,
- Treatment success (at least 50% reduction in crying time) at days 7, 14 and 28 post-intervention,
- Infant sleep duration (minutes per day) per 24 hours at 7, 14, 21 and 28 days duration (post treatment baseline),
- Parental report of treatment success, maternal depression, quality of life, and family functioning at the end of the intervention period,
- Adverse effects: diarrhoea, constipation, vomiting, apnoea and apparent life threatening events (ALTE),
- Stool colonisation analysis, and
- Faecal calprotectin levels.

We anticipate that not all included studies will have all secondary outcomes available for analysis, and will analyse only data that are available.

Sample size and power calculation

Abstracting data from published randomized trials, estimates of the standard deviations in crying time (min/day) at baseline and day 21 were collected and pooled to provide an estimated standard deviation of 210 (min/day). From this information, it is estimated that approximately 120 infants per treatment group would be sufficient for detecting a mean difference in treatment groups of 80 min per day (power = 0.80, alpha =0.05, two-tailed). Additionally, approximately 120 per group would also provide 80% power for detecting a difference of 20 percentage points (alpha = 0.05, two-tailed) in the treatment success rates.

Treatment success is defined as (yes/no) with "yes" corresponding to at least 50% reduction in crying time from baseline to day 21.

For subgroup analysis to compare whether treatment effects differed by patient characteristics, hypothesis testing will be based on the comparison of treatment effects between subgroups, with a two-tailed alpha of 0.10 used to offset the decreased precision available for estimating interaction effects (i.e. differences in differences). We specified that it would be clinically significant to detect between-subgroup differences in treatment effects of 150 min/day on the crying time outcome and of 50 percentage points on the treatment success outcome, assuming that one subgroup consists of between 33% to 66% of the full sample and the other subgroup consists of the remainder. For example, if treatment group differences truly are 180 min/day in a pre-specified subgroup with one third of the patients and only 30 min/day for the remaining patients, the difference in treatment effects would correspond to 150 min/day. Again, a sample size of approximately 120 infants provides at least 80% power to detect such clinically important differences.

At the time of this writing, authors of four trials have agreed to participate. These four trials include three that assessed *Lactobacillus reuteri* strain DSM 17398, comprise a total of 293 patients with 150 randomized to probiotic and 143 to placebo. Thus it is projected that this IPDMA will have sufficient power for detecting clinically relevant differences in both the average crying times and success rates of at least 50% reduction from baseline to day 21 between probiotic and placebo groups.

Statistical analysis

The analysis will be conducted with individual participant data from all studies modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies.[29] Models will be specified with fixed-effects terms for the individual participant's binary indicator treatment-assignment (probiotic versus control), a parsimonious set of pre-specified participant-level characteristics, and the study identifier. This model specification will be straightforwardly extended to account for when longitudinally assessed outcomes are the units of analysis (one record per time point per participant), by including fixed effects terms for time (main effects as well as interaction terms with the binary treatment indicator) and random effects for the participant to account for residual within-participant correlation. Standard choices of link and variance functions will be specified, according to type of outcome, with linear-normal models used for suitably (i.e., homogeneous) continuous outcomes and logit-binomial and log-Poisson models used for binary and count outcomes, respectively.

Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants. Heterogeneity of treatment effects will be 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). These characteristics are identified a priori and include: 1) feeding method (exclusively breastfed versus partially breastfed versus exclusively formula-fed), 2) proton pump inhibitor exposure, 3) hypoallergenic formula exposure for formula-fed infants, and 4) maternal dairy elimination diets for breastfed infants. Confounders identified a priori will include 1) family history of atopy, 2) delivery type (vaginal versus caesarian), 3) enrolment age, and 4) antibiotic use.

Analysis will be by intention-to-treat; specifically the binary treatment term will correspond to assigned treatment. Missing data a will be described and explored and their potential impact on the primary analysis by sensitivity analyses in which varying assumptions are made about missing data. The primary analysis will be conducted at the 0.05 level of significance.

Ethics and dissemination

Ethics committee approval

The IPDMA has been approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). The IPDMA is registered at PROSPERO, the International Prospective Register of Systematic Reviews, at the University of York (CRD42014013210).

Project management and data collection

Membership to the collaboration for this IPDMA will include representatives from each trial contributing data to the project, a project coordination team, and a data management team consisting of two independent statisticians (FD, DT). The collaboration will collect the minimum de-identified data required to answer the research questions. We will store data in a secure, centralized, customized database, accessible only by unique passcode known only to the project coordination team, data management team, and managers of each individual study contributing data. The two independent statisticians will inspect the data with respect to range, internal consistency, and missing items by checking them against published reports, trial protocols and if necessary, data collection sheets. The statisticians will discuss any inconsistencies or missing data with individual trial managers, and any problems will be resolved by consensus using original raw data.

Data ownership and confidentiality

All included trials must have been given ethical approval by their respective Human Research Ethics Committee. Participants in individual trials must have consented to their participation in their respective trial. Each study manager remains the custodian of their own data and retain the right to withdraw their data from the analysis at any time. Data must be de-identified before being shared for this IPDMA. The pooled data can be accessed by the project coordination team, data management team, and managers of each individual study

contributing data. The project intellectual property (IP) will be owned by the parties as tenants in common in proportion to their respective contributions to that project IP (including, without limitation, contributions and inventorship).

Data monitoring procedures

Each individual trial will follow its own data monitoring procedures. The collaboration plans to update the IPDMA data at regular intervals if further relevant individual trials are completed with available data.

Risks and benefits

The main risk for this study is the discovery of discrepant data, or results that are inconsistent with published manuscripts; however, all the studies have been published in peer-reviewed, scientific journals. In addition, this risk will be minimised by careful handling of the data, involvement of two independent statisticians in data analysis, having a unified plan for management of missing data, and the plan for open discussions to resolve any issues regarding any conflicting information. Participation in this study requires prior consent and approval of all trial managers in sharing each study's data and subjecting individual data to re-analysis. There is also a risk of inadequate representation of all trial participants due to authors who do not consent to their data being pooled into the IPDMA.

The combination of data from multiple, similar trials via an IPDMA can yield more reliable estimates of treatment effects, especially for small subgroups of patients.[29-32]. The patients enrolled in each of the individual studies may have had particular patient characteristics or exposures that may have affected the effectiveness of the probiotic intervention. In addition, the different studies may have varied in the types of patients they recruited or varied slightly in their recruitment criteria. As a result, in addition to making more definitive conclusions as to whether probiotics are effective for infant colic, this IPDMA will be able to help determine if there are subgroups of infants who might benefit from a probiotic intervention for colic, in general, or to a particular probiotic strain.

Publication plan

Each individual trial will have the right to publish its main results before publication of this IPDMA. Study results from this meta-analysis will be reported in a peer-reviewed journal in 2015. Before publication of any IPDMA manuscripts, drafts will be circulated for comment, revision and approval by a nominated representative of each of the participating trials. Publications using these data will be authored on behalf of the IPDMA collaboration, with specific named authors (including a representative of each participating trial, the project coordination team and data management team) according to the amount of contribution to each manuscript, and names of other participating collaborators listed in the Acknowledgements.

Strengths and limitations of the study

This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics. The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation. The study is also limited by inclusion of studies with differing methods of defining infant colic and measuring outcomes. The collaboration formed through this IPDMA will be the platform to conduct future IPDMAs for the probiotic management and prevention of infant colic.

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Authors' contributions

VS, MDC, FD and DT conceptualised the study protocol. VS and MDC are the project coordinators of this collaboration. FD and DT are the data managers of this study. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to the design of the study protocol. VS produced the first draft of the manuscript. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to writing the manuscript, read and approved the final manuscript.

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This work was supported by the International Scientific Association for Probiotics and Prebiotics (ISAPP). ISAPP has contributed to the costs of all teleconferences and has facilitated the first meeting for this collaboration in June 2014 in Aberdeen, United Kingdom. ISAPP will also offer support for the statistical work involved in this IPDMA. ISAPP has no role in the design or writing of this protocol, nor in the decision to submit this protocol for publication, although it has contributed to the fees for submission of this manuscript for publication. Each individual trial has received funding from their own respective funding bodies.

Competing interests statements

VS, MDC, FD, GD, FI, SM, FS, HS and DT have received travel reimbursement to attend the ISAPP meeting in Aberdeen, United Kingdom, in June 2014. All teleconference calls have been reimbursed by the ISAPP. MDC is a board member of the ISAPP and has received grant funding from Nestec. MDC has served as a paid consultant for Nestle, Mead Johnson and Pfizer Nutrition. FD and DT will receive funding from ISAPP for their work in the statistical analysis. In the past five years, DT has also received travel reimbursement to attend annual ISAPP meetings (2009-2014) and scientific consulting fees (2012). ISAPP is independent of the study, and played no role in the study protocol's design, in the writing of the manuscript, or in the decision to submit the manuscript for publication. HS and FI served as speakers for

BioGaia, the manufacturer of *L reuteri* DSM 17938. CD received honoraria from Sodilac for a clinical trial.[26] FS reports travel grant from Nestlè Italy, personal fees from Mead Johnson Nutrition, Italy, personal fees from Cana S.A.S. Tessaloniki, Greek, personal fees from Nutricia -Part of Group Danone, Dubai Kuwait, travel grants and other from BioGaia AB , Stockholm I Sweden, personal fees from HiPP GmbH & Co Vertrieb KG Germany, travel grant from Nestlé France SAS, Paris, travel grants and other from Noos, srl , Roma Italy, personal fees from A. MENARINI IFR s.r.l, Firenze Italy, outside the submitted work. They have had no input or involvement in any aspect of the review process during this or previous systematic reviews carried out by FS. AP has no competing interests to declare.

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List of abbreviations

IPDMA	individual participant data meta-analysis
PRISMA	preferred reporting items for systematic reviews and meta-analyses
IP	intellectual property
ISAPP	The International Scientific Association for Probiotics and Prebiotics
PROSPERO	The International Prospective Register of Systematic Reviews

Title

Lactobacillus reuteri DSM 17938 for managing infant colic: protocol for an individual participant data meta-analysis

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Colic, crying, infant, probiotics, review, meta-analysis, microbiota

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Abstract

Introduction

Infant colic, or excessive crying of unknown cause in infants less than three months old, is common and burdensome. Its aetiology is undetermined, and consensus on its management is still lacking. Recent studies suggest a possible link between infant colic and gut microbiota, indicating probiotics to be a promising treatment. However, only a few strains have been tested, and results from randomised controlled trials are conflicting. It is important to clarify whether probiotics are effective for treating infant colic in general, and identify whether certain subgroups of infants with colic would benefit from particular strains of probiotics.

Methods and analysis

Through an individual participant data meta-analysis (IPDMA), we aim to identify whether the probiotic *Lactobacillus reuteri* DSM17938 is effective in the management of infant colic, and clarify whether its effects differ according to feeding method (breast versus formula versus combined), proton pump inhibitor exposure, and antibiotic exposure. The primary outcomes are infant crying duration and treatment success (at least 50% reduction in crying time from baseline) at 21 days post-intervention. Individual participant data from all studies will be modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies. Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants.

Ethics and dissemination

Approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). Results will be reported in a peer-reviewed journal in 2015.

Registration

PROSPERO CRD42014013210

Strengths and limitations of the study

- This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics.
- While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Combining raw data from individual trials via an IPDMA can yield more reliable estimates of treatment effects with universal applicability. This

is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

- The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation. It is also limited by inclusion of studies with potentially different methods of defining infant colic and measuring outcomes.

Introduction

Infant colic, or excessive crying of unknown cause, is a common, burdensome condition affecting up to 20% of infants less than three months old.[1] Although colic self-resolves beyond the first three months of life, it is associated with potentially significant adverse effects, such as maternal depression,[2 3] child abuse,[4 5] and early cessation of breastfeeding.[6] There is also some evidence of long-term adverse outcomes, such as behaviour and sleep problems.[7 8] The aetiology of infant colic remains unresolved, and effective treatment options are limited.[9-11] ~~Most clinical guidelines recommend reassurance and offering support to affected families.~~[12 13] ~~However, many families, when faced with a crying baby, find such a management approach insufficient. Meanwhile, health professionals often resort to prescribing non-evidence-based therapies that have been proven to be ineffective, and even possibly harmful.~~[14-16] ~~There is a need to find a safe and effective treatment option for infant colic.~~

Recent research has focused on the role of gut microbiota in the pathophysiological pathway to infant colic, with numerous studies revealing differences in gut microbiota between infants with and without colic.[17-26] At the same time, a handful of studies have examined the role of probiotics – live microorganisms believed to confer a health benefit – in the management of infant colic. One study of *Lactobacillus reuteri* ATCC55730[27] and two studies of *Lactobacillus reuteri* DSM17938[28 29] in breastfed infants with colic were effective, but a subsequent study of both breastfed and formula fed infants with colic indicated *Lactobacillus reuteri* DSM17938 to be ineffective.~~The first study of its kind was an open-label randomised trial of *Lactobacillus reuteri* ATCC55730 versus simethicone, given to exclusively breastfed Italian infants with colic whose mothers were on dairy-exclusion diets.~~[27] ~~This study was replicated as a double-blind randomised trial of *Lactobacillus reuteri* DSM17938, a daughter strain of ATCC55730, versus placebo, in exclusively breastfed Italian infants with colic whose mothers were on dairy-exclusion diets.~~[28] ~~A third study in Poland was a double-blind randomised trial of *Lactobacillus reuteri* DSM17938 versus placebo in predominantly breastfed infants with colic.~~[29] ~~All three trials showed the probiotic to be effective.~~

~~Two systematic reviews and meta-analyses that included these three trials concluded probiotics to be likely effective in select samples of infants with colic – those who are predominantly exclusively breastfed and those whose mothers are on dairy exclusion diets.~~[30 31] ~~However, a more recent fourth study from Australia, a randomised controlled trial that compared *Lactobacillus reuteri* DSM17938 versus placebo in both breast- and formula-fed infants with colic using the same dose and form, did not demonstrate an effect of probiotic supplementation in managing colic.~~[32] Two other ~~randomised controlled trials~~studies using different mixtures of probiotic strains were also ineffective in *managing* colic.[19 33] The reasons for such conflicting evidence are unclear, and there is a need to explore the reasons behind such controversial results, particularly with increasing probiotic

marketing, variety of strains used, and addition of probiotics to infant formulae. ~~It would be helpful to clearly identify which subgroups of infants with colic benefit from probiotics.~~

Currently there are some ongoing trials examining the role of probiotics in managing and preventing infant colic, using similar designs, participants, interventions, comparators and outcome measures.[34] While individual trials can provide important data, and meta-analyses of randomised controlled trials can give important conclusions, there can be problems with interpreting such conclusions. Ultimately, such meta-analyses often do not overcome limitations and biases of individual trials by generating a single best estimate through pooling of treatment effect estimates.[35] In contrast, combining raw data from individual trials via an individual participant data meta-analysis (IPDMA) can yield more reliable estimates of treatment effects with universal applicability.[35-39] This is particularly important when there is significant chance that particular strains of probiotics may work for particular subgroups of infants with colic, an effect that cannot be detected by individual studies with limited sample sizes.

The pooling of data into an IPDMA for analysis will ultimately provide more definitive answers as to whether the probiotic *Lactobacillus reuteri* DSM17938 is effective for infant colic, and determine ~~which~~ whether certain subgroups of infants would benefit from ~~which particular probiotic strain~~it. ~~In general, probiotic strains are *not* homogenous, as individual probiotic strains may be effective for colic via different potential mechanisms and differ significantly.~~As the effects of probiotics are strain specific,[40] ~~Due to the heterogeneity of these therapies, it is not clear if these different interventions can be combined for analysis to determine their individual effectiveness. T~~this IPDMA will ~~therefore~~ only include the most commonly studied probiotic strain used for the management of infant colic, and will form the protocol basis for further IPDMAs involving other probiotic strains for the management or prevention of infant colic.

The aims of this IPDMA are:

1. To determine whether the probiotic *Lactobacillus reuteri* DSM17398 is effective in the management of infant colic, and
2. To determine whether the effects of *Lactobacillus reuteri* DSM17398 on infants with colic differ according to
    1. Type of feeding (exclusively breastfed versus partially breastfed versus exclusively formula-fed),
    2. Proton pump inhibitor exposure,
    3. Hypoallergenic formula exposure for formula-fed infants, and
    4. Maternal dairy elimination diets for breastfed infants.

Methods and analysis

Individual participant data meta-analysis (IPDMA).

Search methods for identification of studies

We will search for completed and ongoing randomized controlled trials by identification of published papers and protocols through the online databases Medline, Embase, CINAHL, Database of Abstracts of Reviews of Effects (DARE), Cochrane Central Register for Controlled Trials (CENTRAL), and clinical trial registries (eg. metaRegister of Controlled Trials). Reference lists from articles will be explored to identify other potential trials. We will also perform internet searches for non-peer reviewed articles, media articles and other relevant publications using Google, and approach presenters at relevant conferences and meetings. This IPDMA will be undertaken according to the methods recommended by the Cochrane Collaboration,[41] with reporting following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[42]

Eligibility criteria for included RCTs

The IPDMA will include registered randomized controlled trials of the probiotic *Lactobacillus reuteri* DSM17398 versus placebo, delivered orally to infants with modified Wessel's definition of infant colic (crying for more than three hours of the day, for more than three days of the week, for at least one week, as recorded by diaries, questionnaires or parental interviews).

Studies evaluating *L reuteri* ATCC 55730, the mother strain of *Lactobacillus reuteri* DSM 17938, will be excluded. *L reuteri* ATCC 55730 was found to carry potentially transferable resistance traits for tetracycline and lincomycin. Hence, it was replaced by *L reuteri* DSM 17938, a strain without unwanted plasmid-borne resistance.[43] It remains a matter of debate whether or not *L reuteri* DSM 17938, the strain with antibiotic resistance plasmids removed, and the original *L reuteri* ATCC 55730 strain can be regarded as equal. Moreover, only *L reuteri* DSM 17938 is commercially available.

All authors of eligible trials have been contacted and invited to participate in this IPDMA. As more trials satisfying eligibility criteria become published, the relevant authors will be approached and invited to participate, as long as their trials are published within the timeframe of conducting this IPDMA.

Main outcomes

The primary outcomes of the IPDMA are

- Infant crying duration (minutes per day) at 21 days post-intervention, and
- Treatment success at 21 days post-intervention, defined as at least 50% reduction in crying time from baseline.

Secondary outcomes include:

- Infant crying duration (minutes per day) at days 7, 14 and 28 post-intervention, ~~and~~
- Treatment success (at least 50% reduction in crying time) at days 7, 14 and 28 post-intervention,
- ~~.~~
- Infant sleep duration (minutes per day) per 24 hours at 7, 14, 21 and 28 days duration (post treatment baseline),
- Parental report of treatment success, maternal depression, quality of life, and family functioning at the end of the intervention period,
- Adverse effects: diarrhoea, constipation, vomiting, apnoea and apparent life threatening events (ALTE),
- Stool colonisation analysis, and
- Faecal calprotectin levels.

- We anticipate that not all included studies will have all secondary outcomes available for analysis, and will analyse only data that are available.

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Sample size and power calculation

Abstracting data from published randomized trials, estimates of the standard deviations in crying time (min/day) at baseline and day 21 were collected and pooled to provide an estimated standard deviation of 210 (min/day). From this information, it is estimated that approximately 120 infants per treatment group would be sufficient for detecting a mean difference in treatment groups of 80 min per day (power = 0.80, alpha =0.05, two-tailed). Additionally, approximately 120 per group would also provide 80% power for detecting a difference of 20 percentage points (alpha = 0.05, two-tailed) in the treatment success rates. Treatment success is defined as (yes/no) with "yes" corresponding to at least 50% reduction in crying time from baseline to day 21.

For subgroup analysis to compare whether treatment effects differed by patient characteristics, hypothesis testing will be based on the comparison of treatment effects between subgroups, with a two-tailed alpha of 0.10 used to offset the decreased precision available for estimating interaction effects (i.e. differences in differences). We specified that it would be clinically significant to detect between-subgroup differences in treatment effects of 150 min/day on the crying time outcome and of 50 percentage points on the treatment success outcome, assuming that one subgroup consists of between 33% to 66% of the full sample and the other subgroup consists of the remainder. For example, if treatment group differences truly are 180 min/day in a pre-specified subgroup with one third of the patients and only 30 min/day for the remaining patients, the difference in treatment effects would

correspond to 150 min/day. Again, a sample size of approximately 120 infants provides at least 80% power to detect such clinically important differences.

At the time of this writing, authors of four trials have agreed to participate. These four trials include three that assessed *Lactobacillus reuteri* strain DSM 17398, comprise a total of 293 patients with 150 randomized to probiotic and 143 to placebo. Thus it is projected that this IPDMA will have sufficient power for detecting clinically relevant differences in both the average crying times and success rates of at least 50% reduction from baseline to day 21 between probiotic and placebo groups.

Statistical analysis

The analysis will be conducted with individual participant data from all studies modeled simultaneously in multilevel generalized linear mixed-effects regression models to account for the nesting of participants within studies.[36] Models will be specified with fixed-effects terms for the individual participant's binary indicator treatment-assignment (probiotic versus control), a parsimonious set of pre-specified participant-level characteristics, and the study identifier. This model specification will be straightforwardly extended to account for when longitudinally assessed outcomes are the units of analysis (one record per time point per participant), by including fixed effects terms for time (main effects as well as interaction terms with the binary treatment indicator) and random effects for the participant to account for residual within-participant correlation. Standard choices of link and variance functions will be specified, according to type of outcome, with linear-normal models used for suitably (i.e., homogeneous) continuous outcomes and logit-binomial and log-Poisson models used for binary and count outcomes, respectively.

Subgroup analyses of both participant level and intervention level characteristics will be undertaken on the primary outcomes to assess if the intervention effect differs between certain groups of infants. Heterogeneity of treatment effects will be 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). These characteristics are identified a priori and include: 1) feeding method (exclusively breastfed versus partially breastfed versus exclusively formula-fed), 2) proton pump inhibitor exposure, 3) hypoallergenic formula exposure for formula-fed infants, and 4) maternal dairy elimination diets for breastfed infants. Confounders identified a priori will include 1) family history of atopy, 2) delivery type (vaginal versus caesarian), ~~and~~ 3) enrolment age, and 4) antibiotic use.~~.~~

Analysis will be by intention-to-treat; specifically the binary treatment term will correspond to assigned treatment. Missing data a will be described and explored and their potential impact on the primary analysis by sensitivity analyses in which varying assumptions are made about missing data. The primary analysis will be conducted at the 0.05 level of significance.

Ethics and dissemination

Ethics committee approval

The IPDMA has been approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 34081). The IPDMA is registered at PROSPERO, the International Prospective Register of Systematic Reviews, at the University of York (CRD42014013210).

Project management and data collection

Membership to the collaboration for this IPDMA will include representatives from each trial contributing data to the project, a project coordination team, and a data management team consisting of two independent statisticians (FD, DT). The collaboration will collect the minimum de-identified data required to answer the research questions. We will store data in a secure, centralized, customized database, accessible only by unique passcode known only to the project coordination team, data management team, and managers of each individual study contributing data. The two independent statisticians will inspect the data with respect to range, internal consistency, and missing items by checking them against published reports, trial protocols and if necessary, data collection sheets. The statisticians will discuss any inconsistencies or missing data with individual trial managers, and any problems will be resolved by consensus using original raw data.

Data ownership and confidentiality

All included trials must have been given ethical approval by their respective Human Research Ethics Committee. Participants in individual trials must have consented to their participation in their respective trial. Each study manager remains the custodian of their own data and retain the right to withdraw their data from the analysis at any time. Data must be de-identified before being shared for this IPDMA. The pooled data can be accessed by the project coordination team, data management team, and managers of each individual study contributing data. The project intellectual property (IP) will be owned by the parties as tenants in common in proportion to their respective contributions to that project IP (including, without limitation, contributions and inventorship).

Data monitoring procedures

Each individual trial will follow its own data monitoring procedures. The collaboration plans to update the IPDMA data at regular intervals if further relevant individual trials are completed with available data.

Risks and benefits

The main risk for this study is the discovery of discrepant data, or results that are inconsistent with published manuscripts; however, all the studies have been published in peer-reviewed, scientific journals. In addition, this risk will be minimised by careful handling of the data, involvement of two independent statisticians in data analysis, having a unified plan for management of missing data, and the plan for open discussions to resolve any issues

regarding any conflicting information. Participation in this study requires prior consent and approval of all trial managers in sharing each study's data and subjecting individual data to re-analysis. There is also a risk of inadequate representation of all trial participants due to authors who do not consent to their data being pooled into the IPDMA.

The combination of data from multiple, similar trials via an IPDMA can yield more reliable estimates of treatment effects, especially for small subgroups of patients.[36-39]. The patients enrolled in each of the individual studies may have had particular patient characteristics or exposures that may have affected the effectiveness of the probiotic intervention. In addition, the different studies may have varied in the types of patients they recruited or varied slightly in their recruitment criteria. As a result, in addition to making more definitive conclusions as to whether probiotics are effective for infant colic, this IPDMA will be able to help determine if there are subgroups of infants who might benefit from a probiotic intervention for colic, in general, or to a particular probiotic strain.

Publication plan

Each individual trial will have the right to publish its main results before publication of this IPDMA. Study results from this meta-analysis will be reported in a peer-reviewed journal in 2015. Before publication of any IPDMA manuscripts, drafts will be circulated for comment, revision and approval by a nominated representative of each of the participating trials. Publications using these data will be authored on behalf of the IPDMA collaboration, with specific named authors (including a representative of each participating trial, the project coordination team and data management team) according to the amount of contribution to each manuscript, and names of other participating collaborators listed in the Acknowledgements.

Strengths and limitations of the study

This is the first IPDMA and the most definitive method to assess the effectiveness of *Lactobacillus reuteri* DSM17938 in managing infants with colic, and clarify which subgroups of infants with colic may benefit from probiotics. The study is limited by the number of participating authors who contribute data to the study, and cannot include data from authors who decline participation. The study is also limited by inclusion of studies with differing methods of defining infant colic and measuring outcomes. The collaboration formed through this IPDMA will be the platform to conduct future IPDMAs for the probiotic management and prevention of infant colic.

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Authors' contributions

VS, MDC, FD and DT conceptualised the study protocol. VS and MDC are the project coordinators of this collaboration. FD and DT are the data managers of this study. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to the design of the study protocol. VS produced the first draft of the manuscript. All authors (VS, MDC, FD, GD, CD, FI, SM, AP, FS, HS and DT) contributed to writing the manuscript, read and approved the final manuscript.

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Competing interests statements

VS, MDC, FD, GD, FI, SM, FS, HS and DT have received travel reimbursement to attend the ISAPP meeting in Aberdeen, United Kingdom, in June 2014. All teleconference calls have been reimbursed by the ISAPP. MDC is a board member of the ISAPP and has received grant funding from Nestec. MDC has served as a paid consultant for Nestle, Mead Johnson and Pfizer Nutrition. FD and DT will receive funding from ISAPP for their work in the statistical analysis. In the past five years, DT has also received travel reimbursement to attend annual ISAPP meetings (2009-2014) and scientific consulting fees (2012). ISAPP is independent of the study, and played no role in the study protocol's design, in the writing of the manuscript, or in the decision to submit the manuscript for publication. HS and FI served as speakers for BioGaia, the manufacturer of *L reuteri* DSM 17938. CD received honoraria from Sodilac for a clinical trial.[33] FS reports travel grant from Nestlè Italy, personal fees from Mead Johnson

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List of abbreviations

IPDMA	individual participant data meta-analysis
PRISMA	preferred reporting items for systematic reviews and meta-analyses
IP	intellectual property
ISAPP	The International Scientific Association for Probiotics and Prebiotics
PROSPERO	The International Prospective Register of Systematic Reviews