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To cite this version:

Andrea Horvath, Piotr Dziechciarz, Hania Szajewska. Systematic review and meta-analysis of randomized controlled trials: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood. Alimentary Pharmacology and Therapeutics, 2011, 33 (12), pp.1302. 10.1111/j.1365-2036.2011.04665.x . hal-00633997

HAL Id: hal-00633997

https://hal.science/hal-00633997

Submitted on 20 Oct 2011

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Alimentary Pharmacology & Therapeutics

Systematic review and meta-analysis of randomized controlled trials: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood

Journal:	*Alimentary Pharmacology & Therapeutics*
Manuscript ID:	APT-0287-2011.R1
Wiley - Manuscript type:	Meta-analysis
Date Submitted by the Author:	04-Apr-2011
Complete List of Authors:	Horvath, Andrea; The Medical University of Warsaw, Department of Paediatrics Dziechciarz, Piotr; The Medical University of Warsaw, Department of Paediatrics Szajewska, Hania; The Medical University of Warsaw, Department of Paediatrics
Keywords:	Functional GI diseases < Disease-based, Paediatric gastroenterology < Topics, Probiotics/prebiotics < Topics, Nutrition < Topics

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Systematic review and meta-analysis of randomized controlled trials: *Lactobacillus rhamnosus* GG for abdominal pain-related functional gastrointestinal disorders in childhood

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Short title: *Lactobacillus GG* and abdominal pain-related functional disorders

Key words: RCT, *Lactobacillus* GG, probiotics, microbiota, chronic abdominal pain.

Word count: 4190

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ABSTRACT

Background: A lack of reliable treatments for abdominal pain-related functional gastrointestinal disorders (FGD) prompts interest in new therapies.

Aim: To systematically evaluate the effect of *Lactobacillus rhamnosus* GG (LGG) for treating abdominal pain-related FGD in children.

Methods: MEDLINE, EMBASE, CINAHL, the Cochrane Library, trial registries, and proceedings of major meetings were searched for randomized controlled trials (RCTs) evaluating LGG supplementation in children with abdominal pain-related FGD based on the Rome II or Rome III criteria. Risk of bias was assessed for generation of the allocation sequence, allocation concealment, blinding, and follow-up.

Results: Compared with placebo, LGG supplementation was associated with a significantly higher rate of treatment responders (defined as no pain or a decrease in pain intensity) in the overall population with abdominal pain-related FGD (3 RCTs, n=290; relative risk, RR 1.31, 95% CI 1.08 to 1.59, number needed to treat, NNT 7, 95% CI 4 to 22) and in the irritable bowel syndrome (IBS) subgroup (3 RCTs, n=167; RR 1.70, 95% CI 1.27 to 2.27, NNT 4, 95% CI 3 to 8). However, no difference was found in the rate of treatment responders between children with functional abdominal pain or functional dyspepsia who received placebo or LGG. The intensity of pain was significantly reduced in the overall study population and in the IBS subgroup. The frequency of pain was significantly reduced in the IBS subgroup only.

Conclusion: The use of LGG moderately increases treatment success in children with abdominal pain-related FGD, particularly among children with IBS.

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INTRODUCTION

According to the Rome III criteria,[1,2] abdominal pain-related functional gastrointestinal disorders (FGD) in children may be categorized as functional dyspepsia (FD), irritable bowel syndrome (IBS), abdominal migraine, and functional abdominal pain (FAP). Because of their obscure pathophysiology, management of abdominal pain-related FGD remains difficult, prompting interest in new and safe treatment options.

Recently, probiotics, defined as *'live microorganisms which when administered in adequate amounts confer a health benefit on the host'*[3], have been proposed as treatment for FGD. While the exact mechanisms by which probiotics may exert their actions in patients with FGD are not fully understood, several mechanisms have been suggested. These include changes in colonic fermentation by displacement of gas-producing, bile salt-deconjugating bacteria strains, down-regulation of the local proinflammatory response, control of intestinal motor functions, and reducing visceral hypersensitivity by inducing the expression of u-opioid and cannabinoid receptors in human intestinal epithelial cells.[4]

Previously, 4 meta-analyses[5 6 7 8] and 1 systematic review[9] evaluated the effects of probiotics for the treatment of IBS, primarily in the adult population, and reached slightly different conclusions. For adults, despite the equivocal results, the American College of Gastroenterology recently concluded that *'in single organism studies, lactobacilli do not appear effective; bifidobacteria and certain combinations of probiotics demonstrate some efficacy.'*[10] Regarding the pediatric population, a Cochrane systematic review[11] (search date: December 2006) concluded that there is no evidence that lactobacillus supplementation is effective in the management of children with recurrent abdominal pain. New studies[12,13] have been published since this meta-analysis, prompting interest in re-evaluating the role of probiotics in the management of abdominal pain-related FGD in children. With this systematic review, we aimed to update and synthesize the available randomized controlled

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clinical trial evidence of the likely effects of *Lactobacillus rhamnosus* GG (LGG) compared with placebo or no intervention in children affected by abdominal pain-related FGD. In contrast to previous meta-analyses, whether performed in adults or in children, we focused on a single probiotic microorganism. This is because the beneficial effects of probiotics seem to be strain specific, thus, pooling data on different strains may result in misleading conclusions. The choice of the probiotic LGG was determined by the fact that it is available and commonly used in many countries, and it has demonstrated efficacy in the treatment of some gastrointestinal conditions in children.[14 15]

METHODS

Criteria for considering studies for this review

All relevant RCTs that compared the effects of LGG supplementation with the effects of placebo or no supplementation for treating abdominal pain-related FGD in children were considered for this review.

Search methods for identification of studies

The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library, Issues 4, 2010), MEDLINE (1966-2010), and EMBASE (1980-2010) were systematically searched up to December 2010. In addition, we searched 2 trial registries (ClinicalTrials.gov, www.clinicaltrials.gov, and EU Clinical Trials Register, www.clinicaltrialsregister.eu) and proceedings from major scientific gastrointestinal meetings such as ESPGHAN, NASPGHAN, UEGW, and DDW published in the last 3 years. The reference lists of identified studies and key review articles, including previously published reviews, were also searched. In all cases, the searches were for studies that assessed the effects of probiotic supplementation on abdominal pain-related FGD in children up to 18 years of age. No language restrictions were imposed. The search strategy included the use of a validated filter for identifying RCTs, which was combined with a topic-specific strategy using the following PubMed MeSH terms: ("probiotics"[MeSH Terms] OR "probiotics"[All Fields] OR "probiotic"[All Fields]) OR ("lactobacillus"[MeSH Terms] OR

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"lactobacillus"[All Fields]) OR (LGG [All Fields]) OR ("lactobacillus rhamnosus"[MeSH Terms] OR ("lactobacillus rhamnosus"[All Fields]) AND (recurrent[All Fields] AND ("abdominal pain"[MeSH Terms] OR ("abdominal"[All Fields] AND "pain"[All Fields]) OR "abdominal pain"[All Fields])) OR (functional[All Fields] AND ("abdominal pain"[MeSH Terms] OR ("abdominal"[All Fields] AND "pain"[All Fields]) OR "abdominal pain"[All Fields])) OR ("irritable bowel syndrome"[MeSH Terms] OR ("irritable"[All Fields] AND "bowel"[All Fields] AND "syndrome"[All Fields]) OR "irritable bowel syndrome"[All Fields]) OR (functional[All Fields] AND ("dyspepsia"[MeSH Terms] OR "dyspepsia"[All Fields]) OR (("abdomen"[MeSH Terms] OR "abdomen"[All Fields] OR "abdominal"[All Fields]) AND pain-related[All Fields] AND ("disease"[MeSH Terms] OR "disease"[All Fields] OR "disorders"[All Fields])) OR "Rome criteria"[All Fields]) AND ("child"[MeSH Terms] OR "child"[All Fields] OR "children"[All Fields]) OR ("adolescent"[MeSH Terms] OR "adolescent"[All Fields]). Two of the reviewers (PD, AH) searched the databases independently and screened bibliographies of retrieved studies and recent review articles. The primary outcome measure was the rate of responders to the treatment (defined as no pain or a clinically meaningful decrease in pain intensity). The secondary outcome measures were the frequency and intensity/severity of abdominal pain reported by patients and adverse events.

Selection of studies

We excluded studies if the title and abstract were not relevant; however, we obtained papers for all potentially relevant studies if the abstract contained insufficient information to warrant exclusion. All areas of disagreement were discussed by the researchers in order to achieve a consensus.

Data extraction and management

Data from each study were extracted by all of the reviewers using standardized data extraction forms. The data sought included baseline characteristics of the participants (age, diagnostic criteria), the LGG dose and duration of the intervention, follow-up duration, the nature of the placebo, and all outcome measures with their definitions,

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as reported by the authors. After extraction, all data were compared in order to minimize the possibility of errors.

Assessment of risk of bias in included studies

The reviewers independently, but without being blinded to the authors or journal, assessed the risk of bias in the studies that met the inclusion criteria. We used the Cochrane Collaboration's tool for assessing risk of bias, which includes the following criteria: adequacy of sequence generation, allocation concealment, and blinding of participants, personnel and outcome assessors; and incomplete outcome data are addressed. In all cases, an answer of 'yes' indicates a low risk of bias, and an answer of 'no' indicates a high risk of bias.[16]

Measures of treatment effect

The dichotomous outcomes, the results for individual studies, and pooled statistics are reported as the risk ratio (RR) between the experimental and control groups with 95% confidence intervals (CI). The standardized mean difference (SMD) between the treatment and control groups was selected to represent the difference in continuous outcomes (with 95% CI). For all outcomes, subgroup analyses based on the diagnosis (i.e., IBS, FAP, FD) were performed.

Dealing with missing data

We assessed pooled data using available case analysis, i.e., an analysis in which data are analyzed for every participant for whom the outcome was obtained, rather than intention-to-treat analysis with imputation.[17]

Assessment of heterogeneity

Heterogeneity was quantified by χ^2 and I^2, which can be interpreted as the percentage of the total variation between studies that is attributable to heterogeneity rather than to chance. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. If heterogeneity was not revealed, we present results of only the fixed effects model. If there was substantial heterogeneity (over 50%), all

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analyses were based on the random effects model if it was still considered appropriate to pool the data.

Data synthesis (Statistical methods)

The data were analyzed using Review Manager (RevMan) [Computer program. Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008]. The number needed to treat (NNT), with a 95% CI, was calculated using StatsDirect statistical software (version 2,7,8 [2010-03-15]). The weights given to each study were based on the inverse of the variance.

RESULTS

Included studies

The literature search initially yielded 45 articles, of which 3 RCTs met the inclusion criteria (**Figure 1**).[12,18,19] All were published in English. These trials randomized a total of 290 patients. **Table 1** summarizes the characteristics of the included studies. All 3 studies enrolled patients with IBS,[12,18,19] 2 RCTs enrolled patients with FAP,[12,19] and 1 RCT also enrolled children with FD.[19] In all of the studies, the diagnoses were made according to the Rome II criteria. The sample size ranged from 64 to 141 participants. In all of the studies, LGG was compared with placebo. The daily dose of LGG ranged from 10^9 CFU twice daily[18] to 3 x 10^9 CFU twice daily,[12,19] and supplementation lasted for 4 weeks[19], 6 weeks,[18] or 8 weeks[12]. Two RCTs were undertaken in the 2 European countries Poland (one RCT)[19] and Italy (one RCT),[12] and one RCT was conducted in the United States.[18]

Risk of bias in included trials

Table 2 shows the results of the methodological quality assessment. All of the studies had good methodological quality. The main limitation of the study by Bausserman *et al.* was incomplete outcome data (more than 20% lost to follow-up).[18]

Heterogeneity

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No heterogeneity for the primary outcome was found (I^2=0%). For the secondary outcomes, significant heterogeneity (I^2 ≥50%) was found for the overall study population for the frequency of pain (chi^2 =9.15, p=0.002, I^2=89%) and the intensity of pain (chi^2 =2.21, p=0.14, I^2=55%). In all cases, the observed statistical heterogeneity was not judged to be clinically relevant (i.e., studies consistently reported results in the same direction with clinically insignificant differences between the studies). However, there were too few studies to adequately determine heterogeneity.[20]

Effects of intervention

Primary outcome

All 3 RCTs reported responders to the treatment or treatment success defined as no pain or a decrease in pain intensity (**Figure 2)**. One RCT[19] reported both treatment success (defined as no pain) and improvement of symptoms (defined as a change in the Faces Pain Scale by at least 2 faces scores). For this review, data related to those 2 outcomes were combined into a single outcome.

For the overall study population with abdominal pain-related FGD, compared with placebo, LGG supplementation was associated with a significantly higher rate of responders to the treatment (defined as no pain or a decrease in pain intensity)(3 RCTs, n=290, RR 1.31, 95% CI 1.08 to 1.59, NNT 7, 95% CI 4 to 22). For a subgroup of children with IBS, those in the LGG group were more likely to respond to the treatment than those in the placebo group (3 RCTs, n=167, RR 1.70, 95% CI 1.27 to 2.27, NNT 4, 95% CI 3 to 8). For the FAP group (2 RCTs, n=103, RR 1.08, 95% CI 0.77 to 1.50), as well as for the FD group (1 RCT, n=20, RR 0.83, 95% CI 0.37 to 1.85), we found no evidence that LGG supplementation influenced the treatment response in these children.

Secondary outcomes

Frequency of pain

Only for a subgroup of children with IBS, the frequency of pain was reduced in those in the LGG group compared with those in the placebo group (2 RCTs, n=117; SMD -

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1.04, 95% CI -1.43 to -0.65). For the overall study population, as well as for the subgroups of children with FAP or FD, there were no differences in the frequency of pain between the groups that received placebo or LGG **(Figure 3).**

Intensity/severity of pain

Compared with placebo, the use of LGG was associated with a significant decrease in the perception of pain intensity in the overall study population with abdominal pain-related FGD (2 RCTs, n=240; SMD -0.44, 95% CI -0.82 to -0.05). Similarly, there was a reduction in pain intensity in the subgroup of children with IBS who received LGG compared with placebo (2 RCTs, n=117; SMD -0.60, 95% CI -0.97 to -0.23), but not in children with FAP and FD **(Figure 4).**

Adverse effects

The LGG was well tolerated and no adverse effects were reported.

DISCUSSION

Summary of main results

This meta-analysis provides a summary of current knowledge regarding the effects of a single probiotic microorganism, LGG, in children affected by abdominal pain-related FGD. With the limited evidence available, we found that the use of LGG increased the proportion of responders to the treatment (defined as no pain or a decrease in pain intensity) in children with abdominal pain-related FGD, particularly among children with IBS. Additionally, LGG reduced the frequency and intensity of pain, again particularly among children with IBS. Of note, although positive and statistically significant, the effects were clinically modest.

Overall completeness and applicability of evidence

Our search included 3 major databases, with no language restrictions. The searching, extraction of the data, and assessment of the validity of the studies were performed independently by 2 reviewers to decrease the likelihood of reviewer error or bias. Still, we cannot fully exclude the possibility of publication bias, which is an

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important threat to the validity of systematic reviews and is difficult to combat except through the registration of all RCTs. We did not perform a statistical test for the detection of publication bias, as we are aware that these tests have very low power in the meta-analysis of only a few trials.[21] One strength of our review, which distinguishes it from other reviews, is that it focuses on only one probiotic microorganism. This is because probiotic supplementation is not a homogeneous intervention. Pooling data from different genera, species, strains, and doses of probiotics may result in misleading conclusions.

Quality of evidence

Any systematic review is only as good as the constituent studies. In general, the included RCTs were of sound methodology with adequate randomization, allocation concealment, blinding, and follow-up. One potential limitation of the review is that it included a small number of trials with a small sample size, particularly when subgroups of patients with specific diagnoses were evaluated. However, to increase power is one of the reasons why a meta-analysis is performed within a systematic review.

In all of the included trials, the probiotic intervention lasted not less than 4 weeks, which is in line with the Rome Foundation document providing guidance for the design of treatment trials in patients with FGD.[22] However, there is a lack of data on the long-term effects of LGG treatment for abdominal pain-related FGD. Only one study[12] analyzed children for 8 weeks after cessation of the therapy.

According to the published recommendations,[22] the primary outcome measure for treatment studies in patients with FGD should be defined as 'adequate relief' or 'satisfactory relief'. However, studies assessing this outcome measure in the pediatric population are lacking. To our knowledge, no outcome measures for FGD are sufficiently validated to be recommended unequivocally for use as the primary outcome measure.[22] The studies included in our review used different scales and different definitions in assessing treatment success and treatment response. While in

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2 trials, validated tools (Faces Pain Scale and Visual Analog Scale) were used, the definition of treatment success was subjective.[12,19] In the remaining study,[18] the investigators used a psychometric scale that was not validated for the pediatric population.

All 3 RCTs were placebo controlled, which is considered an essential requirement for interventional studies of FGD. However, there were differences in the choice of placebo. Two of the 3 RCTs used inulin and maltodextrin as their placebos.[18] [19] As these are potential prebiotics, it could have affected the outcome. However, this was not observed in our analysis. Still, the choice of the placebo should be taken into account when designing future studies.

Agreement and disagreement with other studies or reviews

Compared with the previously published Cochrane review,[11] which concluded that there is no evidence that lactobacillus supplementation is effective in the management of children with recurrent abdominal pain, our review focuses on a single probiotic microorganism and also includes recent data. Thus, our results more precisely define the effects of LGG. In adults, as discussed in the Introduction, some meta-analyses[6] [7] [8] have shown that some probiotics are clinically more effective than placebo in the treatment of irritable bowel syndrome. However, there are no data on LGG given as a single probiotic.

Implications for practice

The results of our systematic review provide preliminary evidence that LGG may be useful for treating children with abdominal pain-related FGD, particularly in a subgroup of children with IBS. While it is too soon to recommend its routine use in clinical practice, given the lack of effective therapy for abdominal pain-related FGD and the generally good safety profile of LGG in an otherwise healthy population, this therapeutic option could be discussed with patients and/or caregivers.

Implications for research

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LGG seems to be a good candidate for a large multicenter trial in children with abdominal pain-related FGD, properly categorized into IBS, FAP, and FD. Due to the relapsing nature of the disease and that effects of probiotics may endure only for as long as the organism is administered, the treatment duration and follow-up period after cessation of treatment should allow for the adequate assessment of short- and long-term effects of LGG. There is also a strong need for a standardized, properly validated outcome assessments scale. Due to the fact that the exact mechanism of action of LGG is not known, experimental studies are desirable.

CONTRIBUTORS

AH and HS initially conceptualized this study. AH and PD were responsible for data collection. All authors were responsible for data analysis, interpretation, and preparation of the report. All authors contributed to the writing of this manuscript and agreed upon the final version.

ACKNOWLEDGMENTS, COMPETING INTERESTS, FUNDING

Authors' declaration of personal interest: Authors declare no conflict of interest. Declaration of funding interests: This study was funded in full by The Medical University of Warsaw.

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FIGURE LEGENDS

Figure 1. Identification process for eligible trials

Figure 2. Primary outcome: Effect of *Lactobacillus* GG on responder rates

Figure 3. Secondary outcome: Effect of *Lactobacillus* GG on frequency of pain

Figure 4. Secondary outcome: Effect of *Lactobacillus* GG on intensity/severity of pain

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Table 1. Characteristics of included studies

Study	Participants	Diagnostic criteria	LGG (dose)	Comparison	Primary outcome	Secondary outcomes
Bausserman et al. 2005[18]	Children & adolescents (mean age: 12 y, range: 6-17 y)	Rome II criteria for IBS	10^{10} CFU, twice daily, for 6 wk	Placebo (inulin)	Change in the abdominal pain severity score.	Number of responders vs. nonresponders in each group and changes in the symptoms on the Gastrointestinal Symptom Rating Scale (15-item GSRS) by syndrome. Responders were classified as patients with a decrease in abdominal pain severity (1 point or more on the 4-point Likert scale)
Francavilla et al. 2010[12]	Children (mean age: 6.4 y, range: 5-14 y)	Rome II criteria for IBS or FAP	3 x 10^9 CFU, twice daily, for 8 wk	Placebo (inert powder)	Change in abdominal pain (frequency/severity) according to the VAS score from baseline to the end of the treatment period.	(1) A decrease of at least 50% in the number of episodes and intensity of pain (treatment success); (2) A decrease in the perception of children's pain according to their parents; (3) Modification of intestinal permeability.
Gawronska et al. 2007[19]	Children (mean age: 11.6 y, range: 6-16 y)	Rome II criteria for IBS, FAP, FD	3 x 10^9 CFU, twice daily, for 4 wk	Placebo (maltodextrin)	Treatment success defined as no pain (a relaxed face, score of 0, on the Faces Pain Scale) at the end of the intervention.	Improvements defined as a change in: 1) the Faces Pain Scale by at least 2 faces scores; 2) self-reported severity of pain during the preceding week measured on the Faces Pain Scale; 3) self-reported frequency of pain during the preceding week; 4) use of medication for abdominal

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						pain; and 5) school absenteeism due to abdominal pain.

CFU, colony forming units; IBS, irritable bowel syndrome; FAP, functional abdominal pain; FD, functional dyspepsia

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Table 2. Methodological quality summary: review authors' judgments about each methodological quality item for each included study

Study ID	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete data addressed?
Bausserman et al. 2005[18]	Yes	Yes	Yes	No 50/64 (78%)
Gawronska et al. 2007[19]	Yes	Yes	Yes	Yes 104/104 (100%)
Francavilla et al. 2010[12]	Yes	Yes	Yes	Yes 136/141 (96%)

In all cases, an answer of '*yes*' indicates a low risk of bias, and an answer of '*no*' indicates a high risk of bias.

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Figure 1. Identification process for eligible trials

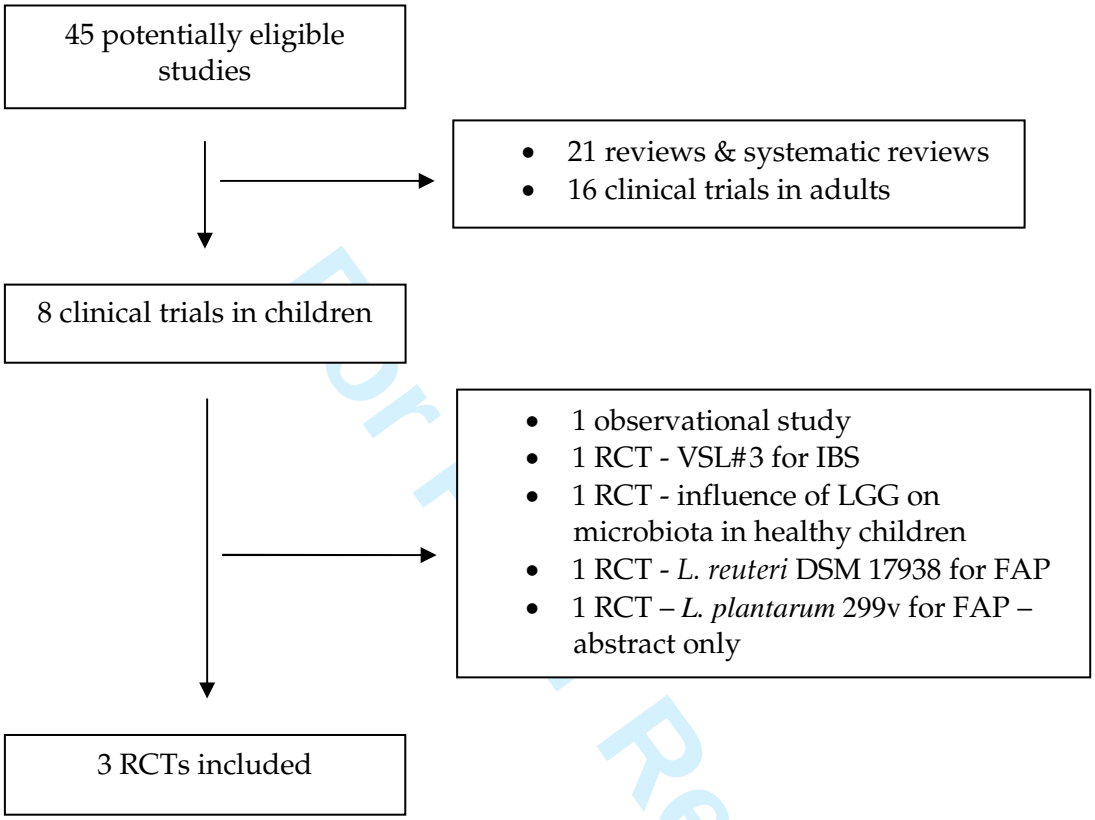

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Figure 2. Primary outcome: Effect of *Lactobacillus* GG on responder rates

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 Overall						
Bausserman 2005	11	25	10	25	13.4%	1.10 [0.57, 2.11]
Francavilla 2010	48	67	37	69	49.0%	1.34 [1.02, 1.74]
Gawronska 2007	38	52	28	52	37.6%	1.36 [1.00, 1.83]
Subtotal (95% CI)		**144**		**146**	**100.0%**	**1.31 [1.08, 1.59]**
Total events	97		75			
Heterogeneity: $Chi^2 = 0.35$, df = 2 (P = 0.84); $I^2 = 0\%$						
Test for overall effect: Z = 2.77 (P = 0.006)						
1.1.2 Irritable bowel syndrome						
Bausserman 2005	11	25	10	25	28.9%	1.10 [0.57, 2.11]
Francavilla 2010	33	42	17	38	51.5%	1.76 [1.19, 2.59]
Gawronska 2007	16	18	7	19	19.6%	2.41 [1.31, 4.44]
Subtotal (95% CI)		**85**		**82**	**100.0%**	**1.70 [1.27, 2.27]**
Total events	60		34			
Heterogeneity: $Chi^2 = 3.00$, df = 2 (P = 0.22); $I^2 = 33\%$						
Test for overall effect: Z = 3.56 (P = 0.0004)						
1.1.3 Functional abdominal pain						
Francavilla 2010	12	25	14	31	44.9%	1.06 [0.61, 1.87]
Gawronska 2007	17	24	15	23	55.1%	1.09 [0.73, 1.61]
Subtotal (95% CI)		**49**		**54**	**100.0%**	**1.08 [0.77, 1.50]**
Total events	29		29			
Heterogeneity: $Chi^2 = 0.00$, df = 1 (P = 0.95); $I^2 = 0\%$						
Test for overall effect: Z = 0.43 (P = 0.67)						
1.1.4 Functional dyspepsia						
Gawronska 2007	5	10	6	10	100.0%	0.83 [0.37, 1.85]
Subtotal (95% CI)		**10**		**10**	**100.0%**	**0.83 [0.37, 1.85]**
Total events	5		6			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.45 (P = 0.66)						

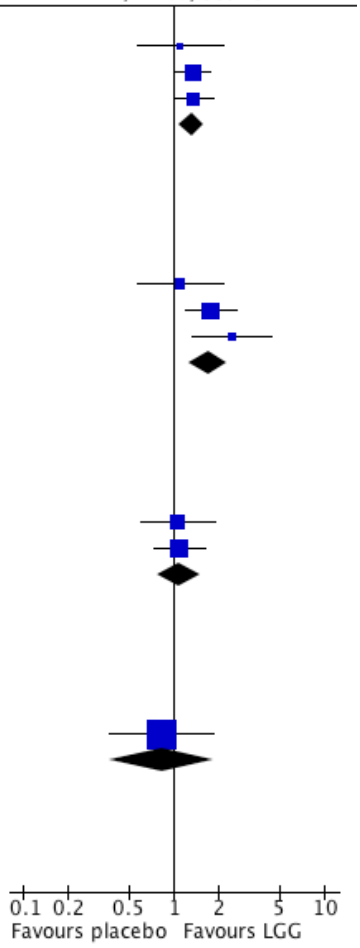

Figure 3. Secondary outcome: Effect of *Lactobacillus* GG on frequency of pain

Study or Subgroup	LGG Mean	LGG SD	LGG Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
2.1.1 Overall								
Francavilla 2010	1.1	0.8	67	2.2	1.2	69	50.4%	-1.07 [-1.43, -0.71]
Gawronska 2007	2.2	1.7	52	2.6	1.4	52	49.6%	-0.25 [-0.64, 0.13]
Subtotal (95% CI)			**119**			**121**	**100.0%**	**-0.67 [-1.46, 0.13]**
Heterogeneity: Tau² = 0.30; Chi² = 9.15, df = 1 (P = 0.002); I² = 89%								
Test for overall effect: Z = 1.63 (P = 0.10)								
2.1.2 Irritable bowel syndrome								
Francavilla 2010	1.6	0.8	42	3.2	1.9	38	67.4%	-1.11 [-1.58, -0.63]
Gawronska 2007	1.8	1.7	18	3.1	1.1	19	32.6%	-0.89 [-1.57, -0.21]
Subtotal (95% CI)			**60**			**57**	**100.0%**	**-1.04 [-1.43, -0.65]**
Heterogeneity: Tau² = 0.00; Chi² = 0.26, df = 1 (P = 0.61); I² = 0%								
Test for overall effect: Z = 5.24 (P < 0.00001)								
2.1.3 Functional abdominal pain								
Francavilla 2010	1.9	0.7	25	1.7	1.5	31	54.0%	0.16 [-0.37, 0.69]
Gawronska 2007	2.3	1.8	24	2.4	1.4	23	46.0%	-0.06 [-0.63, 0.51]
Subtotal (95% CI)			**49**			**54**	**100.0%**	**0.06 [-0.33, 0.45]**
Heterogeneity: Tau² = 0.00; Chi² = 0.32, df = 1 (P = 0.57); I² = 0%								
Test for overall effect: Z = 0.30 (P = 0.76)								
2.1.4 Functional dyspepsia								
Gawronska 2007	2.7	1.3	10	2	1.6	10	100.0%	0.46 [-0.43, 1.35]
Subtotal (95% CI)			**10**			**10**	**100.0%**	**0.46 [-0.43, 1.35]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.01 (P = 0.31)								

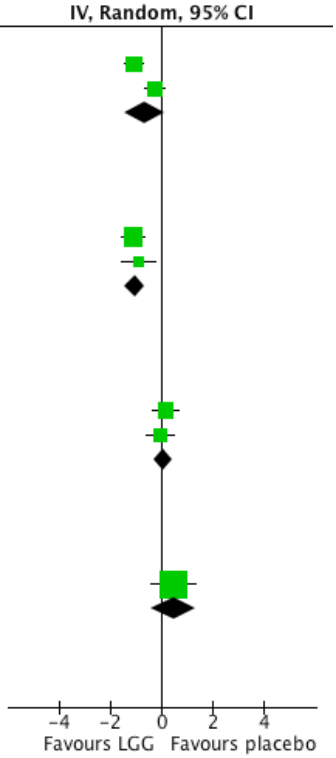

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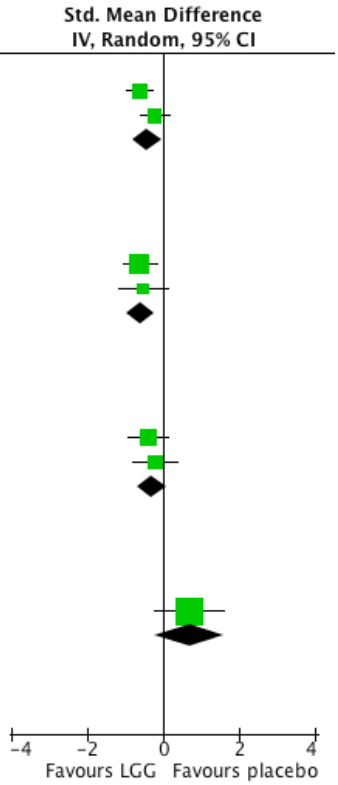
Figure 4. Secondary outcome: Effect of *Lactobacillus* GG on intensity/severity of pain

Study or Subgroup	LGG Mean	LGG SD	LGG Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
3.1.1 Overall								
Francavilla 2010	2.3	1.3	67	3.4	2.1	69	52.5%	-0.62 [-0.97, -0.28]
Gawronska 2007	2.5	1.9	52	2.9	1.5	52	47.5%	-0.23 [-0.62, 0.15]
Subtotal (95% CI)			**119**			**121**	**100.0%**	**-0.44 [-0.82, -0.05]**
Heterogeneity: Tau² = 0.04; Chi² = 2.21, df = 1 (P = 0.14); I² = 55%								
Test for overall effect: Z = 2.24 (P = 0.03)								
3.1.2 Irritable bowel syndrome								
Francavilla 2010	2.5	1.2	42	3.6	2.2	38	68.1%	-0.62 [-1.07, -0.17]
Gawronska 2007	2.2	2.1	18	3.2	1.5	19	31.9%	-0.54 [-1.20, 0.12]
Subtotal (95% CI)			**60**			**57**	**100.0%**	**-0.60 [-0.97, -0.23]**
Heterogeneity: Tau² = 0.00; Chi² = 0.04, df = 1 (P = 0.83); I² = 0%								
Test for overall effect: Z = 3.15 (P = 0.002)								
3.1.3 Functional abdominal pain								
Francavilla 2010	2.5	1.6	25	3.1	1.4	31	53.7%	-0.40 [-0.93, 0.14]
Gawronska 2007	2.6	2	24	3	1.5	23	46.3%	-0.22 [-0.80, 0.35]
Subtotal (95% CI)			**49**			**54**	**100.0%**	**-0.32 [-0.71, 0.07]**
Heterogeneity: Tau² = 0.00; Chi² = 0.19, df = 1 (P = 0.66); I² = 0%								
Test for overall effect: Z = 1.59 (P = 0.11)								
3.1.4 Functional dyspepsia								
Gawronska 2007	2.9	1.5	10	1.9	1.3	10	100.0%	0.68 [-0.23, 1.59]
Subtotal (95% CI)			**10**			**10**	**100.0%**	**0.68 [-0.23, 1.59]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.47 (P = 0.14)								



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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3,4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3,4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4,5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6 Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6,7 Tab. 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7 Tab. 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7,8 Fig.3 Fig.4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7,8 Fig.3 Fig.4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8,9
Limitations	25	Discuss limitations at study and outcomelevel (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9,10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	11

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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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