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META-ANALYSIS OF PROBIOTICS FOR THE PREVENTION AND TREATMENT OF ACUTE PEDIATRIC DIARRHEA

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ABSTRACT ***Pediatric diarrhea is the most common cause of global mortality in children under five years of age. The use of probiotics for this disease remains controversial. The objective of this study was to compare the efficacy of probiotics for the treatment and prevention of pediatric diarrhea based on published randomized, controlled clinical trials. Methods: PubMed, Google Scholar, NIH registry of clinical trials and Cochrane Central Register of Controlled Trials were searched from 1973-2005, unrestricted by language. Secondary searches of reference lists, authors, reviews, commentaries, associated diseases, books and meeting abstracts were also made. Inclusion criteria included: randomization, controlled, blinded, efficacy trials, in humans, peer-reviewed journals. Results: 39 (24%) of 160-screened studies met the inclusion and exclusion criteria and included a total of 41 probiotic treatment arms. The pooled relative risk found a significant reduction of diarrhea duration by probiotics (SMD =-0.56 days, 95% CI -0.73, -0.38) and a significant reduction of treatment failures (RR=0.38, 95% CI 0.28, 0.52). Probiotics were also effective in preventing pediatric diarrhea (RR 0.39, 95% CI 0.27, 0.55). Conclusion: Pooled estimates found that probiotics offer a safe and effective method to prevent and treat acute pediatric diarrhea. No serious adverse reactions were reported in the trials.***

KEY WORDS: Clinical trials, Diarrhea, Lactobacillus, Bifidobacterium, Pediatric, Randomized, Saccharomyces, Synbiotics

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INTRODUCTION

Diarrhea is common among children and the consequences of a severe case of acute pediatric diarrhea can be serious. In the United States, 16.5 million children below age 5 have at least one episode of diarrhea a year (Van Niel et al., 2002). Pediatric diarrhea also places a heavy burden on the US health-care system: 3 million physician visits and 163,000 hospitalizations occur per year (13% of all hospital visits for children younger than 5 are due to pediatric diarrhea) (Parashar et al., 1998; Chang et al., 2003). In developing countries, 3.2 million children die every year due to diarrhea and diarrhea can account for as much as 25% of their national healthcare costs (Ribeiro 2000). Risk factors for pediatric diarrhea include age under two years old, setting (hospital, daycare centers), season (higher in winter), country (underdeveloped) and type of etiology (McFarland et al., 2000a; Barros and Lunet 2003; Cardoso et al., 2003).

The consequences of pediatric diarrhea range from mild, self-limiting episodes to severe disease requiring hospitalization. Children are especially prone to severe dehydration, which can be life threatening, and may develop within just a few days. The median cost of a hospital visit for pediatric diarrhea was found to be $2,307/visit in one study (Zimmerman et al., 2001) and $2,428 in another study (Parashar et al., 1999).

Current therapies for pediatric diarrhea are limited to supportive and symptomatic care. Severe cases are usually given oral rehydration therapy. The use of antibiotic treatments is not useful in 85-95% of cases because the etiology is usually not known or is viral. Common etiologies include rotavirus, Salmonella, Shigella, *C. difficile,* Entamoeba, adenovirus, but studies report 31-48% of the etiologies remain undetermined after testing (Riberio 2000; McFarland 2000a; O'Ryan 2005; Kurugol 2005; Guandalini 2000).

Probiotics may offer an attractive supportive therapy for acute pediatric diarrhea as they are especially useful in diseases that are mediated by the disruption of the normally protective microflora. The normal flora possesses "colonization resistance", a complex phenomena that resists colonization of opportunistic pathogens that may invade the intestines after broad-spectrum antibiotic use, medications or surgery (McFarland 2000b). Once the normal

flora is disrupted, the body becomes susceptible to infection and recovery of the flora may take as long as eight weeks after antibiotics are discontinued. Probiotics are uniquely suited for this window of susceptibility, as they may act as a surrogate normal flora. Mechanisms for probiotics include production of antimicrobial substances, modification of toxins, interference with attachment, stimulation of the immune system or a combination of mechanisms (McFarland 2000b; Castagliuolo et al., 1999; Kaili et al., 1992). Most of the research on probiotics has been done using adults. A consensus has not been reached as to whether probiotics may be effective in acute pediatric diarrhea. The available research varies considerably by results, type of outcome analyzed, study population and quality. The objectives of these meta-analyses were to assess the efficacy and safety of probiotics for the treatment and the prevention of acute pediatric diarrhea.

MATERIALS AND METHODS

Criteria for study selection

Abstracts of all citations and retrieved studies were reviewed and rated for inclusion. Full articles were retrieved if specific treatments were given to either prevent or treat the disease of interest. Inclusion criteria include: randomized, controlled, and blinded efficacy trials in children (age under 18 years) published in peer-reviewed journals. Controlled trials included the use of placebo or a similar delivery vehicle without added probiotic (such as formula). Exclusion criteria include: pre-clinical studies, safety studies only, case reports or case series, phase 1 studies in volunteers, reviews, duplicate reports, trials of unspecified probiotics, not in the disease being studied (chronic pediatric diarrhea, inflammatory bowel disease, stimulation of immune response), or inconsistent outcome measures. Including only randomized, controlled trials strengthens external and internal validity.

Outcomes and definitions

As a wide variety of outcomes are reported in the pediatric literature, three of the most common were selected. To assess the efficacy of probiotics for the treatment of acute pediatric diarrhea, reduction in the duration of diarrhea and percent cured by study end were analyzed separately. To assess the efficacy for the prevention of acute pediatric diarrhea, the outcome analyzed was percent developing diarrhea by the end of the study. For this meta-analysis, pediatric diarrhea was limited to acute onset (<1 week). Documentation of diarrhea was based on clinical assessment and symptoms of ≥3 loose stools/day or ≥5 loose stools/48 hours (McFarland 2000b, McFarland 2000c, Costa-Riberio et al., 2003). If multiple outcomes were reported in one study, only one choice of outcome was selected for this analysis.

Data sources

PubMed and Google Scholar were searched from 1977-2005 for articles unrestricted by language. Non-English articles were translated. Three on-line clinical trial registers were searched: Cochrane Central Register of Controlled Trials (www.cochrane.org), metaRegister of Controlled Trials (www.controlled-trials.com/mrct) and National Institutes of Health (www.clinicaltrials.gov). Secondary and hand searches of reference lists, authors, reviews, commentaries, associated diseases, books and meeting abstracts also were performed. *A priori* search terms were defined for randomized controlled trials (RCT, human, blinding, phase 2, phase 3, efficacy) and terms for probiotics were also defined. Search terms included probiotic*, microflora, antibiotics, *Clostridium difficile*, colitis, PMC, diarrhea, Saccharomyces, Lactobacill*, Bifidobacter*, Enterococc*, Bacill*, VSL#3, synbiotics, Lactinex). Search strategies were broad-based initially, then narrowed to the disease of interest.(Shaw et al., 2004) The procedure for this meta-analysis was designed as suggested by Egger et al. and MOOSE guidelines using clearly delineated parameters, *a priori* inclusion and exclusion criteria and standardized data extraction methods. (Egger et al., 1997; Stroup et al., 2000).

Data extraction

Information on study design, methods, interventions, outcomes, adverse effects and treatments was extracted from each article. Data on patient inclusion and exclusion criteria, number of completed subjects, attrition, treatment dose and duration, and outcome was extracted into a standardized table. In some cases, the primary or secondary author was contacted for data not reported in the original article. Two reviewers independently selected trials for inclusion using the previously defined criteria. The studies were not blinded by authors, journal, results or conclusions of individual studies. A few trials had multiple probiotic arms and each probiotic arm was compared to a control group separately.

Assessment of methodological quality

Studies that met the inclusion criteria were graded for quality using a scale reported by the U.S. Preventive Services Task Force. (Harris et al., 2001). Quality of evidence is rated from 1-3 (poor, fair and good) based on randomization, study design, sample size, generalizability, study biases and outcome assessment. Study quality was not integrated with the model weights, as trials of poor quality were excluded from review and this practice is not uniformly recommended (Juni et al.,2001). Weights for this analysis are based solely on sample sizes.

Statistical analysis

Statistical analysis was performed using Stata software version 8.1 (Stata Corporation, College Station, Texas). Continuous outcomes (duration of diarrhea) are presented as standardized mean difference (SMD) between the probiotic treatment and controls with 95% confidence intervals. Discrete outcomes (number with diarrhea) are presented as relative risks with 95% confidence intervals. The weights given to each study are based on the inverse of the variance. Heterogeneity across trials was evaluated using Cochran Q test based on pooled relative risks by the Mantel-Haenszel method. If the studies were homogeneous, a fixed-effects model was used and a pooled relative risk was

calculated with the Mantel-Haenszel method for fixed effects. If the studies were heterogeneous, a random effects was employed and a pooled relative risk was calculated using the DerSimonian and Laird method (DerSimonian and Laird 1986). Subgroup analysis was performed to identify sources of heterogeneity. *A priori* subgroup analyses were by type of probiotic and therapeutic indication (treatment or prevention). A funnel plot as well as an adjusted rank correlation test using the Egger method was used to assess publication bias (Egger et al.,1997; Begg and Mazumdar 1994). Two-tailed P values less than 0.05 were considered significant.

RESULTS

Overview of included studies

The literature search yielded 940 citations on probiotics, of which 160 relating to pediatric diarrhea were selected for retrieval. Thirty-nine (24.4%) of the 160-screened articles met inclusion criteria and provided data on 5,422 enrolled subjects. Two trials had multiple probiotic treatment arms, resulting in a total of 41 probiotic treatments analyzed. Details of the included trials are presented in Tables 1-3. The number of patients in each of these studies was generally moderate (median, 71; range 15-928 subjects). Most of the studies were done in hospitalized children (59%), while 23% included outpatients or children in day-care centers and 18% did not specify the source of subjects. Most (69%) of the trials were done in developed countries, while 31% were done in developing countries. Most trials did not report the cause of the diarrhea (46%), 38% found mixed viral and bacterial etiologies and 15% found viral causes (usually rotavirus). All trials used a control group: 24 (61%) used a placebo and 15 (39%) used a non-probiotic containing vehicle, such as formula or cereal.

Table 1. Randomized. Controlled Trails of Various Probiotics for the Treatment of Pediatric Diarrhea Using Duration of Diarrhea as Outcome Measure

N	Subjects (age in months)	Probiotic	Dose per day	Duration (days)	Probiotic		Controls		Quality	Reference	+ or -
					#	days diarrhea	#	days diarrhea			
287	1-36 mon	*L. rhamnosus* GG	4×10^{10}	varied	147	2.4 ± 1.1	140	3.0 ± 1.5	3	Guandalini 2000	+
123	1-36 mon	*L. rhamnosus* GG	5×10^{9}	5	59	2.7 ± 2.2	64	3.8 ± 2.8	3	Shornikova 1997a	+
100	3-36 mon	*L. rhamnosus* GG	6×10^{9}	5	52	3.3 ± 2.9	48	5.9 ± 2.8	3	Guarino 1997	+
71	4-45 mon	*L. rhamnosus* GG	$2 \times 10^{10\text{-}11}$	5	47	1.4 ± 0.8	24	2.4 ± 1.1	2	Isolauri 1991	+
42	5-28 mon	*L. rhamnosus* GG	2×10^{10}	5	21	1.5 ± 0.7	21	2.3 ± 0.8	3	Isolauri 1994	+
39	7-37 mon	*L. rhamnosus* GG	$2 \times 10^{10\text{-}11}$	5	22	1.1 ± 0.6	17	2.5 ± 1.4	3	Kaila 1992	+
124	<24	*L. rhamnosus* GG	1×10^{10}	7	61	1.6 ± 0.1	63	1.6 ± 0.2	3	Costa-Ribeiro 2003	-
39	<24	*L. rhamnosus* GG	$2 \times 10^{10\text{-}11}$	2	20	1.9 ± 0.6	19	3.3 ± 2.3	2	Pant 1996	+
40	3-36	*L. reuteri*	$1 \times 10^{10\text{-}11}$	5	19	1.7 ± 1.6	21	2.9 ± 2.3	3	Shornikova 1997b	-
45	6-36	*L. reuteri*	1×10^{7}	5	20	1.9 ± 0.9	25	2.5 ± 1.5	3	Shornikova 1997c	+
46	6-36	*L. reuteri*	$1 \times 10^{10\text{-}11}$	5	21	1.5 ± 1.1	25	2.5 ± 1.5	3	Shornikova 1997c	+
73	3-24	*L. acidophilus* LB (killed)	0	3	37	1.8 ± 1.1	36	2.4 ± 1.5	3	Simakachorn 2000	+
100	6-60	LABI	6×10^{9}	4	50	3.1 ± 0.7	50	3.6 ± 0.8	2	Lee 2001	+
43	9-44	LRLR	4×10^{10}	5	24	3.2 ± 1.6	19	4.8 ± 3.5	3	Rosenfeldt 2002a	+
69	6-36	LRLR	4×10^{10}	5	30	3.4 ± 1.6	39	4.2 ± 2.0	3	Rosenfeldt 2002b	-
54	2-36	LALBSL	2×10^{9}	3	27	2.5 ± nr	27	2.8 ± nr	2	Chicoine 1973	-
94	<36	LALBST	$4\text{-}8 \times 10^{8}$	varied	53	2.7 ± 2.5	41	2.1 ± 1.6	2	Pearce 1974	-
65	6-12	Synbiotic1	6×10^{9}	180	33	1.4 ± 0.7	32	2.0 ± 1.2	3	Shamir 2005	+

Abbrevations: N= number with outcome evaluated; LABI=Lactobacillus acidophilus and Bifidobacterium infantis; LRLR=Lactobacillus rhamnosus 1970-2 and L reuteri DSM; LALBSL=Lacto. acidophilus + L bulgaricus + Strept. lactis; LALBST=Lacto. acidophilus + L bulgaricus + Strepto. thermophilus; Synbiotic 1 = Streptococcus thermophilus+ Bifidocterium lactis + Lacto. acidophilus + Zink (10 mg/d) + fructooligosaccharide (0.3 g)

Table 2. Randomized, Controlled Trials of Various Probiotics for the Treatment of Pediatric Diarrhea Using Percent Cured as Outcome Measure

N	Subjects (age in months)	Probiotic	Dose per day	Duration (days)	Probiotic-treated		Control group		Quality	Reference	+ or -
					# failed	# cured	# failed	# cured			
32	1-24	*L. rhamnosus* GG	$2 \times 10^{10-11}$	2	5 (31)	11	12 (75)	4	2	Raza 1995	+
46	1-48	*L. acidophilus* LB (killed)	0	varied	3 (12.5)	21	3 (13.6)	19	2	Boulloche 1994	–
130	3-36	*S. boulardii*	2×10^{10}	4	10 (15)	55	39 (60)	26	3	Cetina-Sauri 1994	+
38	0.5-30	*S. boulardii*	1×10^{10}	5	1 (5)	18	4 (21)	15	2	Chapoy 1985	+
15	11-35	*S. boulardii*	varied	30	3 (43)	4	6 (75)	2	2	Chouraqui 1995	+
200	3-84	*S. boulardii*	5×10^{9}	5	1 (1)	99	4 (4)	96	3	Kurugol 2005	+
100	2-29	*S. boulardii*	5×10^{9}	3	8 (16)	42	18 (32)	32	2	Urganci 2001	+
40	6-36	*S. boulardii*	1×10^{10}	30	6 (30)	14	12 (60)	8	3	Guillot 1995	+

Abbreviations:

N=number with outcome evaluated

Table 3. Prevention of Pediatric Diarrhea by Various Probiotics from Randomized Controlled Trials

N	Subjects (age in months)	Probiotic	Dose per day	Duration (days)	Probiotic (developing diarrhea)		Control group		Quality	Reference	+ or -
					# failed	# cured	# failed	# cured			
81	1-36	*L. rhamnosus* GG	1.2×10^{10}	varied	3 (6.7)	42	12 (33)	24	3	Szajewska 2001a	+
188	6-120	*L. rhamnosus* GG	2×10^{10}	10	7 (7)	87	25 (26)	69	3	Vanderhoof 1999	+
204	6-24	*L. rhamnosus* GG	3.7×10^{10}	450	5 (5.2)	94	6 (6)	99	3	Oberhelman 1999	+
220	1-18	*L. rhamnosus* GG	1×10^{10}	varied	29 (25.4)	85	32 (30.2)	74	3	Mastretta 2002	-
59	5-132	*L. rhamnosus* GG	8×10^{10}	7	6 (26)	17	8 (22)	28	2	Vaisanen 1998	-
239	12-36	*L. reuteri*	$1 \times 10^{6-10}$	98	29 (24)	90	43 (36)	77	2	Ruiz-Palacios 1999	+
928	6-24	*L. casei* DN114	1×10^{8}	60	74 (15.9)	390	102 (22)	362	3	Pedone 2000	+
354	12-216	*L. acidophilus*	2×10^{9}	varied	10 (4.6)	205	30 (21.6)	109	2	Pancheva-Dimitrova 2004	+
466	12-180	*S. boulardi*i	5×10^{9}	varied	14 (5.7)	230	42 (18.9)	180	2	Erdeve 2004	+
269	6-168	*S. boulardii*	1×10^{10}	14	4 (3)	115	22 (17.3)	105	3	Kotowska 2005	+
110	1-180	*Cl. butyricum* miyairi	$1\text{-}4 \times 10^{7}$	6	6 (7)	77	16 (59)	11	2	Seki 2003	+
128	4-10	*L. reuteri* 55730	1×10^{9}	84	2 (3)	66	18 (30)	42	3	Weizman 2005	+
133	4-10	*B. lactis* BB-12	1×10^{9}	84	9 (12)	64	18 (30)	42	3	Weizman 2005	+
18	1-36	*L. acidophius + Bifid. infantis*	6×10^{9}	7	3 (37.5)	5	8 (80)	2	2	Jirapinyo 2002	-
55	5-24	*Bifido. bifidum + Strept thermophilus*	2×10^{8}	varied	2 (7)	27	8 (31)	18	3	Saavedra 1994	+

Abbreviations: N=number with outcome evaluated

Excluded studies

Of the pediatric diarrhea studies, 121 failed to meet one or more of the inclusion criteria. Most were reviews or commentaries (n=46), epidemiologic (n=24), chronic diarrhea or non-diarrheal outcomes (n=18), mechanistic or studies of changes in microflora (n=12) or pre-clinical (n=3). Eighteen trials that passed initial screening were excluded due to lack of a control group (n=9) (Barone et al., 2000, Bellomoet al., 1980, Butset al., 1993, Giudiciet al., 1985; Gupta et al., 2000; Kanamon et al., 2000; Kanamon et al., 2002; Kanamon et al., 2004; Majamaa et al., 1995). unavailability of journal article and no response from original author (n=4) (Castanada 1995 Gaon et al., 2003; Sazawal et al., 2004;Tojo et al., 1987); poor study design (n=3), (Pedone et al., 1999; Hoyos 1999; Jouirou et al., 1990; Rautanen et al., 1998) or outcome inconsistent with other trials in category (n=1) (Duggan et al., 2003).

Study quality

The quality of the studies is presented in Tables 1-3, indicating generally good methodological quality. Most studies of poor quality were excluded from the data extraction in the preliminary steps of this study.

Efficacy studies

Of the 41 probiotic treatment arms providing adequate data regarding efficacy, 32 (78%) reported efficacy from the individual trials. Due to the differences in outcomes and therapy strategies, three different meta-analyses are presented. First, for the treatment of pediatric diarrhea trials that measured duration of diarrhea, 18 trials were analyzed. In the pooled estimate, probiotics were found to significantly reduce the duration of diarrhea compared to controls (SMD=-0.56 days, 95% CI -0.73, -0.38, z=6.20, P<0.001) in the pooled standardized mean difference random effect model (Figure 1). Significant heterogeneity was observed across the 18 trials (P=0.001). A subgroup analysis revealed that two studies were responsible for the majority of the heterogeneity (Pearce et al., 1974, Costa-Ribeiro et al., 2003). Both of these studies did not find a significant reduction in the duration of diarrhea by the probiotics tested. When these two studies were removed from the analysis, a homogenous group of 16 trials found the pooled estimate of the reduction of diarrhea duration was similar (SMD=-0.63 days, 95% CI -0.76, -0.50, P<0.0001), but the heterogeneity was reduced (P=0.30). A subgroup analysis restricted to eight trials using one type of probiotic (*L. rhamnosus* GG) found a similar pooled reduction in the duration of days of diarrhea (SMD=-0.70 days, 95% CI -0.99, -0.41, P<0.0001). No other subgroup analysis by type of probiotic was possible due to the diversity of probiotics tested. No significant publication bias was observed for these 18 trials as shown by the funnel plot in Figure 2 and confirmed by Egger's test (p=0.08).

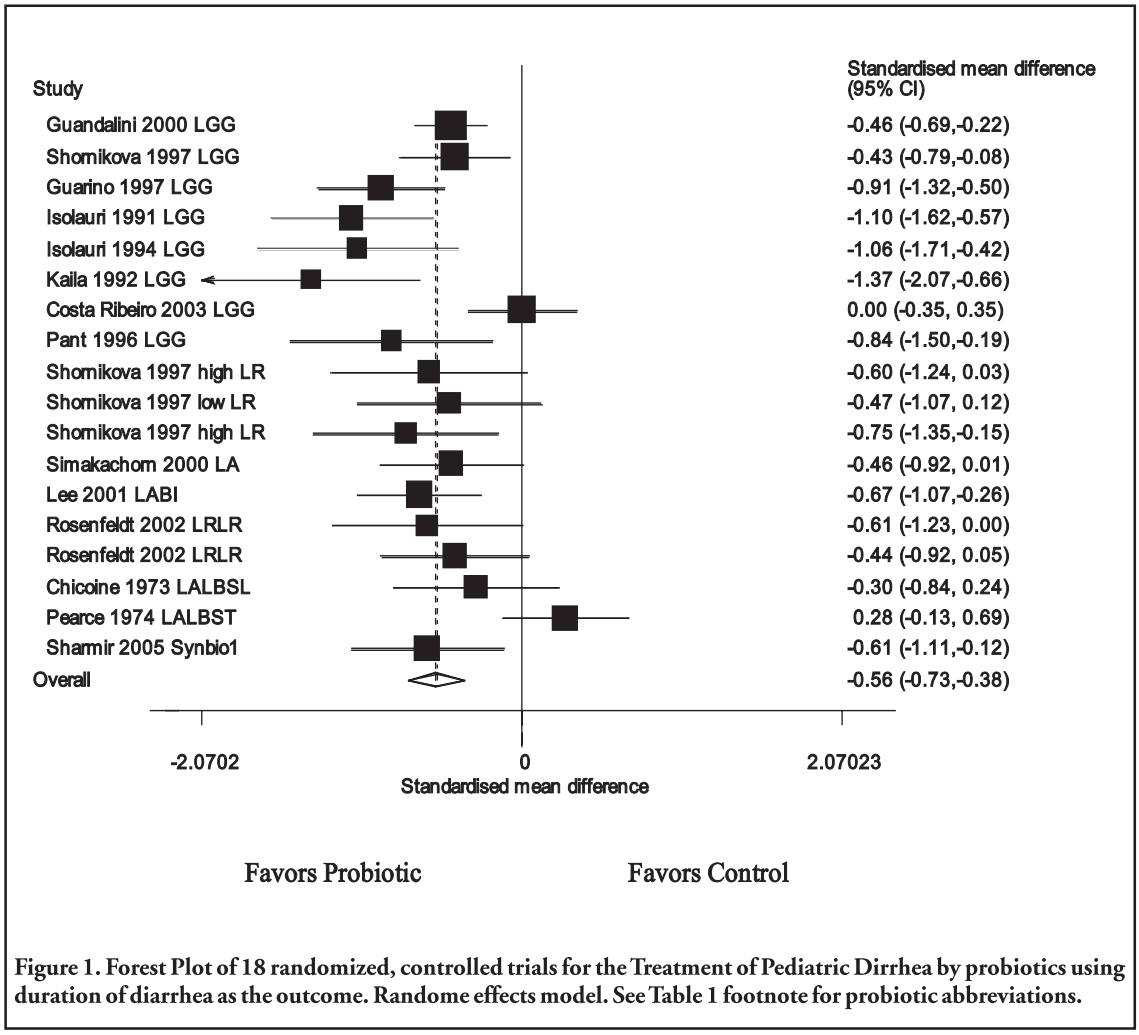

Figure 1. Forest Plot of 18 randomized, controlled trials for the Treatment of Pediatric Dirrhea by probiotics using duration of diarrhea as the outcome. Randome effects model. See Table 1 footnote for probiotic abbreviations.

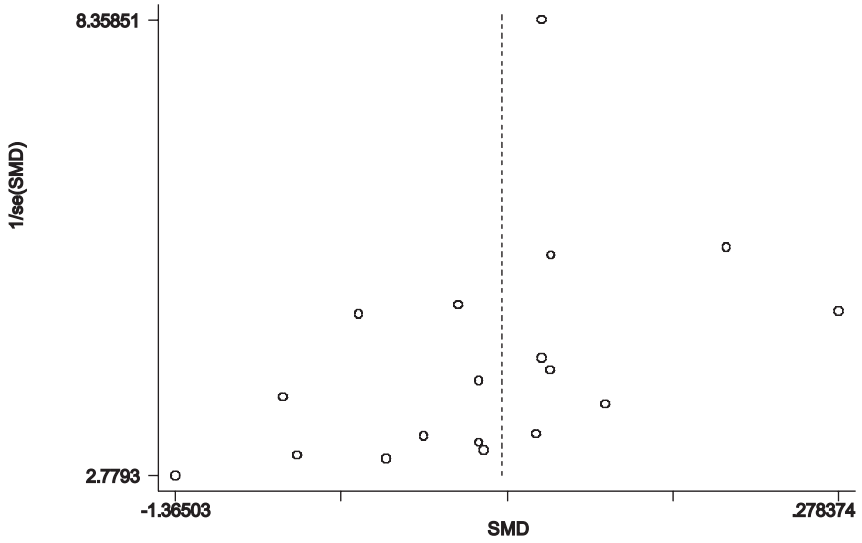

Figure 2. Funnel Plot of 18 Randomized Controlled Trails for Treatment of Pediatrics Diarrhea using Duration of Diarrhea as Outcome. Assessment of Publication Bias.

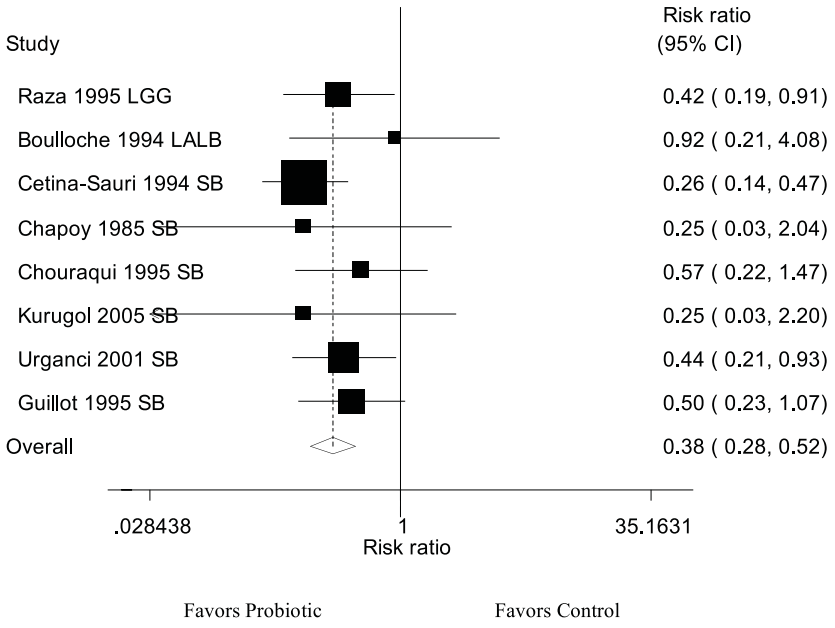

Figure 3. Forest Plot of 8 randomized, controlled trials for the treatment of Pediatric Diarrhea by probiotics using percent cured as the outcome. Fixed Effects Model. See Table 2 footnote for probiotic abbreviations.

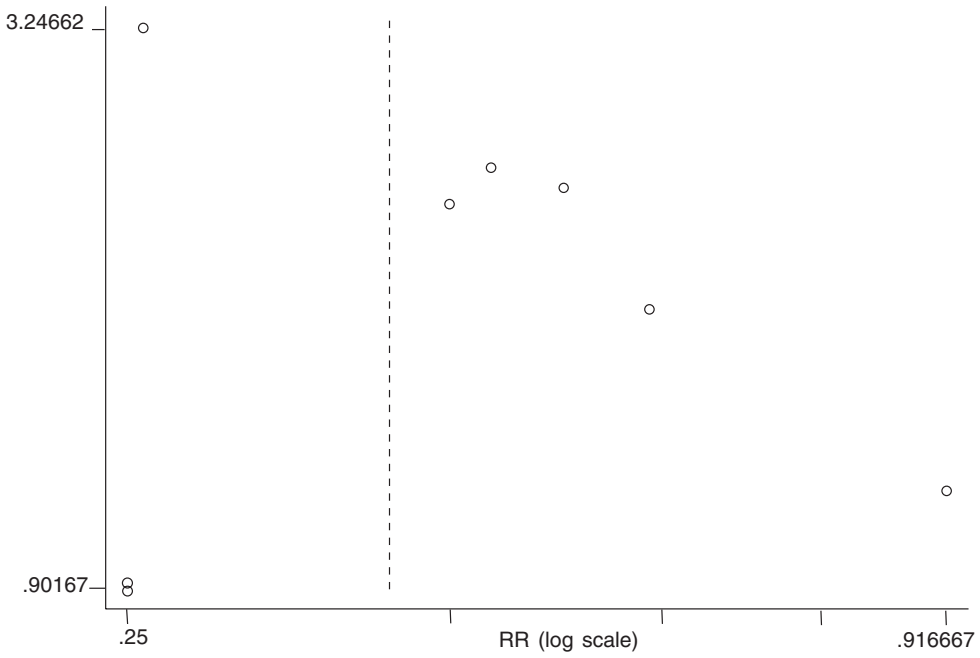

Figure 4. Funnel Plot of 8 randomized, controlled trials for the Treatment of Pediatric Diarrhea by probiotics using percent cured as the outcome. Assessing Publication bias.

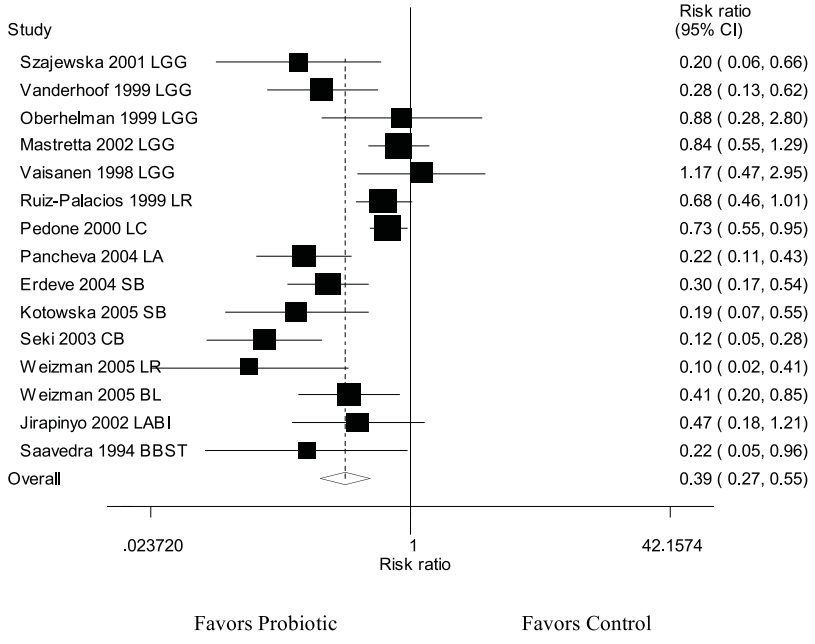

Figure 5. Forest Plot of 15 randomized, controlled trials for the Prevention of Pediatric Diarrhea by probiotics using percent failed as the outcome. Random Effects Model. See Table 3 footnote for probiotic abbreviations.

Second, for the treatment of pediatric diarrhea trials that measured the frequency of subjects who failed to respond to treatment, 8 trials were analyzed. In the pooled estimate, probiotics were found to significantly reduce the frequency of treatment failures compared to controls (RR=0.38, 95% CI 0.28, 0.52, z=5.9, p<0.001) in the pooled fixed effect model (Figure 3). Significant heterogeneity was not observed across the 8 trials (P=0.69). A subgroup analysis restricted to six trials using one type of probiotic (*Saccharomyces boulardii*) found a similar pooled reduction in the treatment failure risk (RR=0.35, 95% CI 0.25, 0.51, P<0.0001). No other subgroup analysis by type of probiotic was possible due to the diversity of probiotics tested. No significant publication bias was observed for these 8 trials as shown by the funnel plot in Figure 4 and confirmed by Egger's test (p=0.46).

Third, for the prevention of pediatric diarrhea trials that measured the frequency of subjects who developed new episodes of diarrhea by the end of the study, 15 trial arms were analyzed. In the pooled estimate, probiotics were found to significantly reduce the frequency of pediatric diarrhea compared to controls (RR=0.39, 95% CI 0.27, 0.55, z=5.3, P<0.0001) in the pooled random effect model (Figure 5). Significant heterogeneity was observed across the 15 trial arms (P<0.001). A subgroup analysis eliminating various trials did not significantly affect the degree of heterogeneity. To analyze another source of potential heterogeneity, trials were stratified by the type of probiotic given. A subgroup analysis restricted to five trials assessing *L. rhamnosus GG* found the pooled relative risk indicated no significant effect of this probiotic for the prevention of pediatric diarrhea (RR=0.57, 95% CI 0.30, 1.09, P=0.09). Pooling the trials that did not use *L. rhamnosus GG* did not result in a significantly different pooled risk estimate than the full robust model of 15 trials (RR=0.32, 95% CI 0.21, 0.50, P<0.001). No significant publication bias was observed, as shown by the funnel plot in Figure 6 and confirmed by Egger's test (p=0.73).

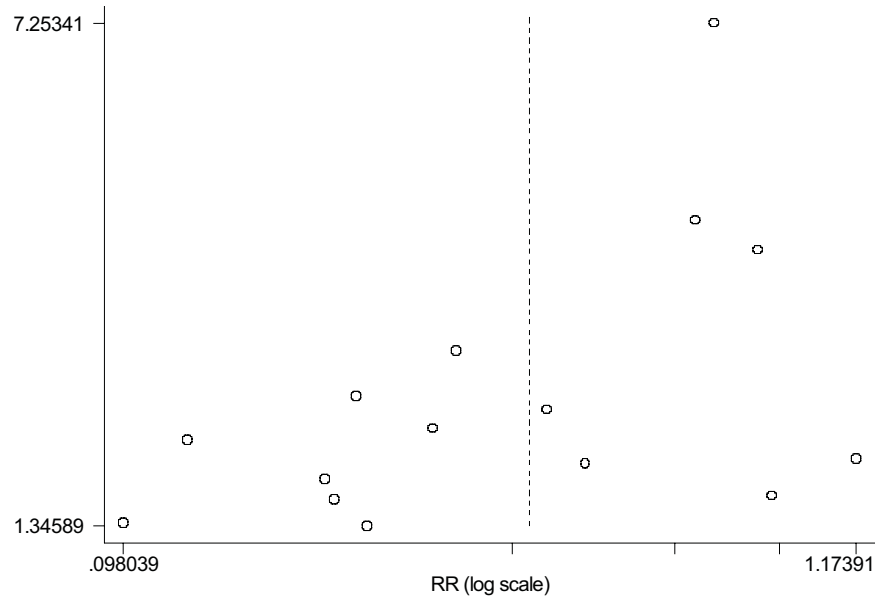

Figure 6. Funnel Plot of 15 randomized, controlled trials for the Prevention of Pediatric Diarrhea by probiotics using percent failed as the outcome. Assessing publication bias.

Adverse events

Only 12 (31%) of the 39 trials presented data on adverse reactions, and only one reported seizures (in one subject treated with *L. rhamnosus* GG and one control) (Raza et al., 1995). No serious adverse reactions including bacteremia or fungemia were associated with any of the probiotic treatments. Unfortunately, the majority of the trials (72%) did not report any safety data on adverse reactions during the trial.

DISCUSSION

This meta-analysis found probiotics are safe and effective for both the treatment and the prevention of acute pediatric diarrhea. The pooled risk estimates found probiotic reduced treatment failures by 38%, reduced mean duration of diarrhea by 13 hours and significantly reduced the risk of new cases of pediatric diarrhea to 43%. The main advantage of probiotic therapy for acute diarrhea, which is mediated through changes in intestinal microflora, is that probiotics are therapeutically active and yet do not disrupt the re-establishment of the protective normal microbial flora.

An important consideration when drawing conclusions from meta-analyses is that potential biases may be present due to publication bias and a variety of sources of heterogeneity. Sutton et al. reviewed 48 meta-analyses and found 30 (63%) made no reference to publication bias or reported funnel plots (Sutton et al., 2002). In these three meta-analyses, publication bias was minimized by conducting extensive searches through multiple databases and receiving original data from the authors. In addition, the funnel plot and adjusted rank correlation test indicated there was no significant publication bias in these datasets.

Another source of heterogeneity in pediatric diarrhea trials is the type of infectious etiology can vary and different types are often present in one study population. Most trials did not restrict inclusion into the trial based on etiology, 46% of the 39 trials did not determine the cause of the pediatric diarrhea and 39% of the trials found mixed types of diarrheal pathogens in their subject population. Other studies have reported a mix of bacterial and viral etiologies in their trials (Riberio 2000; O'Ryan et al., 2005).

Another source of heterogeneity for probiotic trials is the type of probiotic being assessed. Significant differences in effectiveness have been reported for different species and strains of similar species of bacteria and yeasts (McFarland and Elmer 2006a; Dunne et al., 2001). Examining the individual trials, the frequency of positive efficacy findings differed by the type of probiotic: *S. boulardii* (100% of 8 trials), *C. butyricum* (100% of 1 trial), *Bifido. lactis* (100% of 1 trial), *L. rhamnosus* GG (79% of 14 trials), other Lactobacilli probiotics (88% of 8 trials) and probiotic mixtures (44% of 9 trials). To determine which probiotics may be more effective than others, more trials are needed that are of similar study design and study populations so direct comparisons can be made. Testing several promising probiotics versus placebo in the same study would be ideal.

Another source of heterogeneity arises from the differences in probiotic dose, which ranged from 0 (for killed preparations) to a maximum of 2×10^{11} cfu/day. The differences in the results may have been due to sub-therapeutic doses of probiotics ($<10^{10}$ cfu/day). From studies in adults with diarrhea, a therapeutic threshold of $\geq 10^{10}$/day has been reported (McFarland 2006b, McFarland 2006c). In these trials, 19 (46%) of the treatment arms used doses $<10^{10}$/day. However, children may not require doses as high as adults, as the frequency of positive efficacy does not differ significantly (79% and 75%, respectively) if children were given low doses (1×10^7 to 6×10^9/day) compared with higher doses (1×10^{10}-2×10^{11}/day). This finding is in conflict with a dose-effect found by Van Niel et al. assessing the reduction of diarrhea duration in children treated with probiotics. (Van Niel et al., 2002) However, this finding was based on only eight trials and the range of doses was narrower (10^9-10^{11}).

Some studies noted differences in efficacy in various sub-samples of their study population, especially for breast-feeding (higher in breast-fed) and type of diarrhea (higher in non-bloody diarrheas) (Pant et al., 1996; Raza et al., 1995; Oberhelman et al., 1999; Mastretta et al., 2002). However, other differences did not significantly impact the efficacy of probiotics (hospitalization versus outpatient or day-care centers or developed versus underdeveloped countries) Some meta-analyses have only included placebo-controlled trials and excluded non-probiotic containing vehicle controls. As it can be difficult for children to comply with swallowing capsules, the use of cereals or formulas has been utilized. In our meta-analysis, the type of controls did not significantly affect efficacy: 71% of placebo-controlled trials showed a significant efficacy and 87% of the probiotic-free vehicle controlled trials were positive (P=0.44).

How can future trials better define benefit of probiotics in children? Several limitations can be found including differences in duration of treatment, doses, potency, lack of reported safety data and the lack of cost-effectiveness data. Comparing trials of adults with diarrhea to pediatric diarrhea, the duration of treatments ranged from 2 days to 6 months, but most were remarkably short (66% less than 4 weeks). Adult trials usually extend probiotic treatments to 6-8 weeks, allowing time for the normal intestinal microflora to be re-established (McFarland 2000b). However, intestinal flora recovery time has been based on animal models and pharmacokinetic studies in adults and the recovery time in children has not been well studied. Inconsistent efficacy results may also be due to the viability and stability of the probiotic product. Probiotics that are lyophilized are stable at room temperature for extended periods (such as *S. boulardii* and *L. rhamnosus* GG), but some products require refrigeration (such as Lactinex). Storage conditions and viable levels were not reported in most of these trials. One study did report premature termination of the trial (and a subsequent negative finding) due to the expiration of the probiotic (Jirapinyo et al., 2002). Viability of the probiotic is an important factor in efficacy, as one trial using a killed preparation did not show positive efficacy (Boulloche et al., 1994).

The safety of probiotics should also be considered. Although case reports and case series of bacteremia and fungemia have been reported in the literature, no cases occurred in patients enrolled in

the 38 trials reviewed for this meta-analysis. Caution should be exercised for patients who are severely ill and receiving nutrition or antibiotics through a potentially open portal (catheter or nasogastric tube). Infrequent blood-stream infections have been reported, most probably due to contamination of the environment as the probiotic capsule is opened at bedside and mixed with food (Hennequin et al., 2002). Rare complications including endocarditis and liver abscess have been associated with *L. rhamnosus* GG use (Rautio et al., 1999, Sipsas et al., 2002). Bacteremia and fungemia have been associated with probiotics, but respond well to antibiotics or anti-fungal medications (Munoz et al., 2005; Land et al., 2005; Surawicz and McFarland 1999). As many of the 39 trials did not report safety data or evaluate cost-effectiveness, these two issues should be considered in future trials of probiotic therapy.

The value of a meta-analysis is that it provides a tool to combine studies with the above differences and arrive at a pooled estimate of the efficacy of different probiotics. Three other meta-analyses on pediatric diarrhea have been published. Huang et al. reported a meta-analysis of 18 studies and found a pooled reduction in the mean duration of diarrhea of -0.8 days (95% CI -1.1, -0.6). (Huang et al., 2002). Two of the trials were abstracts from meetings that were not available as published articles and as such, the study data could not be properly examined. In addition, this meta-analysis reported incorrect outcome data for four of the studies. Van Niel et al. conducted a meta-analysis of nine trials and found a similar reduction in diarrhea duration (-0.7 days, 95% CI -0.3, -1.2) (Van Niel et al., 2002). Szajewska pooled 10 trials and found a mean reduction in the duration of diarrhea of -0.83 days (95% CI -1.09, -0.59) (Szajewska and Mrukowicz 2001b) These three meta-analysis did not conduct an analysis of trials for the prevention of pediatric diarrhea, nor did they present an analysis of safety reports. Despite of these limitations, all these meta-analyses demonstrated a reduction in symptoms among the probiotic treated children and validate our findings.

Future studies on the therapeutic potential of probiotics are necessary. To increase comparability, efforts should be made to standardize doses, duration of treatment and study populations. Direct comparisons of probiotics versus placebo are indicated. Adverse reaction data and cost-effectiveness analyses would be helpful. This meta-analysis included a large number of trials for several indications of pediatric diarrhea and found overall safety and efficacy for probiotics for both the treatment and prevention of pediatric diarrhea.

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