Probiotics for prevention of necrotizing enterocolitis in preterm infants (Review)

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TABLE OF CONTENTS

[Intervention Review]

Probiotics for prevention of necrotizing enterocolitis in preterm infants

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ABSTRACT

Background

Necrotizing enterocolitis (NEC) and nosocomial sepsis are associated with increased morbidity and mortality in preterm infants. Through prevention of bacterial migration across the mucosa, competitive exclusion of pathogenic bacteria, and enhancing the immune responses of the host, prophylactic enteral probiotics (live microbial supplements) may play a role in reducing NEC and associated morbidity.

Objectives

To compare the efficacy and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe NEC and/or sepsis in preterm infants.

Search strategy

For this update, searches were made of MEDLINE (1966 to October 2010), EMBASE (1980 to October 2010), the Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library,* Issue 2, 2010), and abstracts of annual meetings of the Society for Pediatric Research (1995 to 2010).

Selection criteria

Only randomized or quasi-randomized controlled trials that enrolled preterm infants < 37 weeks gestational age and/or < 2500 g birth weight were considered. Trials were included if they involved enteral administration of any live microbial supplement (probiotics) and measured at least one prespecified clinical outcome.

Data collection and analysis

Standard methods of the Cochrane Collaboration and its Neonatal Group were used to assess the methodologic quality of the trials, data collection and analysis.

Main results

Sixteen eligible trials randomizing 2842 infants were included. Included trials were highly variable with regard to enrollment criteria (i.e. birth weight and gestational age), baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics, and feeding regimens. Data regarding extremely low birth weight infants (ELBW) could not be extrapolated. In a meta-analysis of trial data, enteral probiotics supplementation significantly reduced the incidence of severe NEC (stage II or more) (typical RR 0.35, 95% CI 0.24 to 0.52) and mortality (typical RR 0.40, 95% CI 0.27 to 0.60). There was no evidence of significant reduction of nosocomial sepsis (typical RR 0.90, 95% CI 0.76 to 1.07). The included trials reported no systemic infection with the probiotics supplemental organism. The statistical test of heterogeneity for NEC, mortality and sepsis was insignificant.

Authors' conclusions

Enteral supplementation of probiotics prevents severe NEC and all cause mortality in preterm infants. Our updated review of available evidence supports a change in practice. More studies are needed to assess efficacy in ELBW infants and assess the most effective formulation and dose to be utilized.

PLAIN LANGUAGE SUMMARY

Probiotics for prevention of necrotizing enterocolitis in preterm infants

Necrotizing enterocolitis (NEC) is a serious disease that affects the bowel of premature infants in the first few weeks of life. Although the cause of NEC is not entirely known, milk feeding and bacterial growth play a role. Probiotics (dietary supplements containing potentially beneficial bacteria or yeast) have been used to prevent NEC. Our review of studies found that the use of probiotics reduces the occurrence of NEC and death in premature infants born less than 1500 grams. There is insufficient data with regard to the benefits and potential adverse effects in the most at risk infants less than 1000 grams at birth.

BACKGROUND

Description of the condition

Necrotizing enterocolitis (NEC) is the most common serious acquired disease of the gastrointestinal tract in preterm infants (Lee 2003). It is characterized by bowel wall necrosis of various length and depth. Bowel perforation occurs in one third of the affected infants (Kafetzis 2003). Although 5 to 25% of cases occur in term infants, it is primarily a disease of preterm infants, with the majority of cases occurring in very low birth weight infants (infants with birth weight < 1500 g) (Kosloske 1994). NEC is categorized into three different stages, with clinical symptoms varying from feeding intolerance to severe cardiovascular compromise, coagulopathy, and peritonitis with or without pneumoperitoneum (Bell 1978). The incidence of NEC varies among countries and neonatal centers. It has been reported to affect up to 10% of very low birth weight infants (VLBW) (Kosloske 1994).

The pathogenesis of NEC remains incompletely understood. NEC most likely represents a complex interaction of factors causing mucosal injury (Neu 1996). It is speculated that NEC occurs with the coincidence of two of the following three pathologic events; intestinal ischemia, colonization of the intestine by pathologic bacteria, and excess protein substrate in the intestinal lumen (Kosloske 1984; La Gamma 1994). Bacterial colonization is necessary for the development of NEC (Kosloske 1990; Musemeche 1986). When compared to term infants, VLBW infants at risk of NEC have abnormal fecal colonization, demonstrate a paucity of normal enteric bacterial species, and have delayed onset of bacterial colonization (Goldmann 1978; Gewolb 1999).

Nosocomial infection is also a frequent complication in VLBW infants. Data from the NICHD Network demonstrated that as many as 25% of these infants have at least one or more positive blood cultures, and 5% have positive cerebrospinal fluid cultures over the course of their hospitalization (Stoll 1996). Late onset sepsis is associated with an increased risk of death, neonatal morbidity and prolonged hospitalization (Stoll 2002a; Stoll 2002b).

Description of the intervention

Probiotic bacteria are live microbial supplements that colonize the

gastrointestinal tract and potentially provide benefit to the host (Millar 2003). The most frequently used probiotics are lactobacillus and bifidobacterium. There is increasing interest in the potential health benefits of proactive colonization of the gastrointestinal tract of preterm infants (Millar 2003).

How the intervention might work

Potential mechanisms by which probiotics may protect high risk infants from developing NEC and/or sepsis include increased barrier to migration bacteria and their products across the mucosa (Orrhage 1999; Mattar 2001), competitive exclusion of potential pathogens (Reid 2001), modification of host response to microbial products (Duffy 2000), augmentation of IGA mucosal responses, enhancement of enteral nutrition that inhibit the growth of pathogens, and up-regulation of immune responses (Link-Amster 1994).

Why it is important to do this review

VLBW infants with NEC have a mortality rate up to 20% (Caplan 2001; Holman 1997). Approximately 27 to 63% of affected infants require surgical intervention (Lee 2003). Strictures, primarily in the colon, occur in more than one third of affected infants (Ricketts 1994). Increased rate of total parenteral nutrition (TPN) related complications and extended hospitalization have been reported (Bisquera 2002). Recent data from the National Institute of Child Health and Human Development Network (NICHD) suggest an increase in neurodevelopmental impairment rates among infants with NEC and sepsis (Stoll 2004). There is a theoretical risk of bacteremia secondary to enterally administered probiotics strains, though few data support this concern. Bacillus species administered as probiotics were reported to be associated with invasive disease in target populations (Richard 1988).

OBJECTIVES

The primary objective was to compare the effectiveness and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe (stage II or more) NEC and/or sepsis in preterm infants.

The secondary objective was to conduct a subgroup analysis to investigate the effect of probiotics in extreme low birth weight infants (infants with birth weight < 1000 g).

METHODS

Criteria for considering studies for this review

Types of studies

Only randomized and quasi-randomized controlled trials were included.

Types of participants

Preterm infants < 37 weeks and/or birth weight < 2500 g.

Types of interventions

Enteral administration of any live microbial supplement (probiotics) at any dose for more than seven days compared to placebo or no treatment.

Types of outcome measures

Primary outcomes

- Severe NEC (stage II or more) as per Bell's criteria (Bell 1978; Walsh 1986), diagnosed prior to discharge.
- Nosocomial sepsis, defined as positive blood or cerebrospinal fluid cultures taken beyond 5 days of age.

Secondary outcomes

- All cause neonatal mortality.
- Any NEC (according Bell's criteria).
- The composite of nosocomial sepsis or NEC or death.
- Systemic infection with the supplemented organism.
- Duration of total parenteral nutrition (days).
- Time to establish full enteral feeds (days).
- Duration of hospitalization (days).
- Neurodevelopmental impairment i.e. rates of cerebral palsy, cognitive delay, deafness, blindness or their composite reported at 18 months corrected age or later.

Search methods for identification of studies

Electronic searches

Our search was updated from December 2006 to October 2010. We used the standard search strategy for the Cochrane Neonatal Review Group. Randomized and quasi-randomized controlled trials that compared enteral probiotics to placebo or no treatment in premature infants were identified from OVID MEDLINE-National Library of Medicine (1966 to October 2010) using the following subject headings (MeSH) and text word terms: "neonate(s),

newborn(s), infant(s), probiotics, lactobacillus, bifidobacterium, saccharomyces and publication type 'controlled trial'. No language restrictions were applied.
Other databases were searched including: EMBASE (1980 to October 2010), Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*, Issue 2, 2010). Authors performed the electronic database search independently.

Searching other resources

A manual search of the abstract books published from the Society of Pediatric Research (SPR) and the European Society of Pediatric Research (ESPR) for the period of 1998 to 2010 were performed. Additional citations were sought using references in articles retrieved from searches. Subject experts were contacted to identify the unpublished and ongoing studies. Authors of the published trials were contacted to clarify or provide additional information. Authors independently screened candidate articles to check the eligibility for inclusion in the review.
We also searched clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; controlled-trials.com; and who.int/ictrp)

Data collection and analysis

The standard methods of the Cochrane Neonatal Review Group Guidelines were employed in creating this update.

Selection of studies

Retrieved articles were assessed for eligibility independently by two review authors. Discrepancies were resolved by discussion and consensus.

Data extraction and management

Data was abstracted independently by two review authors. Discrepancies were resolved by discussion and consensus. Where data were incomplete, the primary investigator was contacted for further information and clarification.

Assessment of risk of bias in included studies

Standard methods of the Cochrane Collaboration and the Neonatal Review Group were used to assess the methodological quality (validity criteria) of the trials. For each trial, information was sought regarding the method of randomization, blinding and reporting of all outcomes of all the infants enrolled in the trial. Each criteria was assessed as yes, no, can't tell.
Two review authors separately assessed each study. Any disagreement was resolved by discussion. This information was added to the table 'Characteristics of Included Studies'.
In addition, for the update in 2010, the following issues were evaluated and entered into the Risk of Bias table:
1) Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?
For each included study, we categorized the method used to generate the allocation sequence as:
- adequate (any truly random process e.g. random number table; computer random number generator);
- inadequate (any non random process e.g. odd or even date of birth; hospital or clinic record number);
- unclear.
(2) Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?
For each included study, we categorized the method used to conceal the allocation sequence as:
- adequate (e.g. telephone or central randomization; consecutively numbered sealed opaque envelopes);
- inadequate (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear.
(3) Blinding (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study? At study entry? At the time of outcome assessment?
For each included study, we categorized the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or classes of outcomes. We categorized the methods as:
- adequate, inadequate or unclear for participants;
- adequate, inadequate or unclear for personnel;
- adequate, inadequate or unclear for outcome assessors.
(4) Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?
For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomized participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorized the methods as:
- adequate (< 20% missing data);
- inadequate ($\geq$ 20% missing data):
- unclear.
(5) Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?
For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found.

We assessed the methods as:
- adequate (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- inadequate (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported);
- unclear.
(6) Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?
For each included study, we described any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:
- yes; no; or unclear.
If needed, we planned to explore the impact of the level of bias through undertaking sensitivity analyses.

Measures of treatment effect

For dichotomous outcomes, relative risk (RR) risk difference (RD) and the number needed to treat (NNT) and its associated confidence interval were calculated. For continuous outcomes, treatment effect was expressed as mean difference and its calculated standard deviation.

Assessment of heterogeneity

Heterogeneity was defined as a significant test of heterogeneity ($p < 0.1$) and differences in the treatment effects across studies. Tests for between-study heterogeneity (including the I^2 test) were applied. If noticed, possible sources of heterogeneity were examined, including differences in the type or dose of probiotics used, the population under study (VLBW versus ELBW infants), and the quality of the study.

Data synthesis

If appropriate, meta-analysis of pooled data was performed assuming a fixed effect model. Review Manager 5.0.25 software was used for statistical analysis. For estimates of typical relative risk and risk difference, we used the Mantel-Haenszel method. For measured quantities, we used the inverse variance method. All meta-analyses were done using the fixed effect model.
A subgroup analysis to investigate the effect of probiotics in extreme low birth weight infants was conducted.

Subgroup analysis and investigation of heterogeneity

The secondary objective was to conduct a subgroup analysis to investigate the effect of probiotics in extreme low birth weight infants.

Sensitivity analysis

A sensitivity analysis was carried out to assess the effect of trials methodological quality on results of the meta-analysis. Studies were considered of high quality if allocation was concealed and adequately described.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies.
See tables 'Characteristics of included and excluded studies'. Our updated search in October 2010 yielded seven additional studies meeting our inclusion criteria. Therefore, a total of 16 randomized trials are included in our updated review. Excluded studies and reasons for exclusion are outlined in characteristics of excluded studies. The details of four identified ongoing studies are provided in the tables of ongoing studies.

Participants

Sixteen included studies reported outcomes on 1371 infants treated with probiotics and 1376 control infants. While all studies enrolled infants < 37 weeks and/or birth weight < 2500 g, entry criteria varied between studies. Li 2004, Reuman 1986, Kitajima 1997, Lin 2005, Lin 2008, Bin-Nun 2005, Manzoni 2009, and Manzoni 2006 enrolled infants based on birth weight criteria. On the other hand, Millar 1993, Mohan 2006, Stratiki 2007, and Costalos 2003 enrolled infants based on their gestational age. Dani 2002, Rougé 2009, Samanta 2009, and Sari 2010 utilized both criteria to enrolls infants. None of the included studies limited their enrolment to ELBW infants.

Intervention

Included studies randomized infants to different preparations and dosages of probiotics. While Reuman 1986, Millar 1993, Dani 2002, Manzoni 2006, Manzoni 2009, Rougé 2009, and Sari 2010 administered Lactobacillus species to the intervention groups; Kitajima 1997, Mohan 2006, Stratiki 2007 and Li 2004 utilized the Bifidobacterium species and Costalos 2003 utilized Saccharomyces boulardii. Lin 2005, Lin 2008, Samanta 2009 and Bin-Nun 2005 used a mixture of two to three species of probiotics (L acidophilus - B infantis, and Lactobacillus bifidus-streptococcus thermophillus-bifidobactrium infantis).
The time of initiation and duration of therapy was different among included studies. Probiotics were administered either during the

first 24 hours of life (Reuman 1986; Kitajima 1997; Li 2004), at the third day of life (Manzoni 2009), at the time of the first feed (Millar 1993; Dani 2002; Lin 2005; Lin 2008; Rougé 2009; Samanta 2009; Sari 2010), or during the first week when enteral feeds were tolerated (Costalos 2003; Manzoni 2006, Mohan 2006). The duration of probiotics administration varied from two weeks (Reuman 1986), four to six weeks (Kitajima 1997; Costalos 2003; Lin 2008; Manzoni 2009), or until discharge (Dani 2002; Li 2004; Lin 2005; Manzoni 2006; Rougé 2009; Samanta 2009, Sari 2010).

Outcomes

The major outcomes reported in included studies were severe stage II-III NEC (Dani 2002; Costalos 2003; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009; Kitajima 1997; Mohan 2006; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007), all causes mortality (Kitajima 1997; Reuman 1986; Dani 2002; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009; Rougé 2009; Samanta 2009) and sepsis (Millar 1993; Kitajima 1997; Costalos 2003; Dani 2002; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009, Rougé 2009, Samanta 2009, Sari 2010; Stratiki 2007). Weight gain was reported in three studies (Reuman 1986; Millar 1993; Costalos 2003; Sari 2010) using different measurement scales. Only one study reported data on apnea and long term neurosensory outcomes (Kitajima 1997).

Risk of bias in included studies

Details of included studies are presented in the table 'Characteristics of Included Studies'. The methodologic details of the studies were extracted from the published data and by contacting the primary author. However, a response was only received from one primary author (Dani 2002).

- Bin-Nun 2005: This was a single centre study. Infants less than 1500 g were randomized to receive either probiotics mixture (Lactobacillus bifidus, streptococcus thermophillus, and bifidobactrium infantis) or placebo. Information regarding allocation concealment was not specified, intervention was masked, and blinding of outcome assessment was not specified. Of note, this trial was published in an abstract form on two previous occasions at the Society of Pediatrics Research (SPR 2003, 2005) with different inclusion criteria and clinical outcomes, which suggests a change in the *a priori* specified criteria and multiple looks at the trials results.

- Costalos 2003: This was a single center study. Infants were randomized to receive either enteral probiotics (Saccharomyces boulardii) added to preterm formula or the same formula with maltodextrins. Allocation concealment was apparently adequate. Intervention and outcome assessment were masked. All infants were accounted for in the final results. There was a discrepancy with regard to the infants enrolled in both groups (51 in the treatment group and 36 in the control). The author presented no explanation of whether this discrepancy was a result of imbalance in the randomization process or a loss to follow-up.

- Dani 2002: This was a multicenter study. Infants were randomized to receive either enteral probiotics (Lactobacillus GG) or placebo. Allocation was adequately concealed. The intervention was masked. All enrolled infants were accounted for and outcome measurement was blinded.

- Kitajima 1997: This was a single center study. 91 infants were randomized to receive enteral probiotics (Bifidobacterium breve) or control. It was unclear whether allocation was concealed, intervention blinded, or the outcome assessment was blinded. Not all enrolled infants accounted for the final results (six infants excluded for various reasons).

- Li 2004: This was a single center study. Infants were randomized in three groups to receive either enteral probiotics (Bifidobacterium breve) (group A, B) or control (group C). Allocation concealment was not described. It was unclear whether the intervention or outcome assessment were blinded and whether all infants were included its final results.

- Lin 2005: This was a single centre study, infants less than 1500 g were randomized to either probiotics (Infloran® - L acidophilus and B infantis) or to a control group (breast milk only). Allocation was adequately concealed. Intervention was masked (except for investigators and breast milk team). All enrolled infants were accounted for. Outcomes measurement was blinded.

- Lin 2008: This was a multicenter trial, infants less than 1500g were randomized to either probiotics (217) were given Bifidobacterium bifidum and Lactobacillus acidophilus, added to breast milk or mixed feeding (breast milk and formula), twice daily for six weeks or to control (217) were fed with breast milk or mixed feeding. Allocation was adequately concealed. Intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Manzoni 2006: This was a single centre study, infants less than 1500 g were randomized to either probiotics (Dicoflor Lactobacillus casei) or to a control group (breast or donor milk only). Although authors utilized computer generated randomization, allocation concealment was not described. Intervention was masked from human bank and microbiology workers, however, it was unclear whether care givers are masked or not. All enrolled infants were accounted for. Blinding of outcomes measurement was reported.

- Manzoni 2009: This was a multicenter study, infants less than 1500 g younger than 3 days were randomized to either received BLF (100mg/d) (LF100; Dicofarm SpA, Rome, Italy) alone or BLF (bovine Lactoferrin) plus LGG (6109colony-forming units/d) (Dicoflor60;Dicofarm SpA); the control group

received placebo (2 mL of a 5% glucose solution). Treatment lasted 6 (birth weight 1000 g) or 4 (birth weight 1001-1500 g) weeks, unless neonates were discharged earlier. Drug administration began on the third day of life with 1 daily dose; all doses including placebo were diluted in prepared milk so as to maintain blinding. Allocation was adequately concealed. Intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Millar 1993: This was a single center study. Twenty infants were randomized to receive either enteral probiotics (Lactobacillus GG) or control. The intervention was masked. All infants enrolled were accounted for. It was unclear whether the outcome assessment was blinded or not.

- Mohan 2006: This was a single center study. Infants less than 37 weeks were randomized to probiotic (37) and placebo (32) groups. The formula-based placebo (Nestle´ FM 2000B) and verum (Nestle´ FM 2000A) preparations were supplied by Nestle´ , Konolfingen, Switzerland. The verum contained 2 X10 ^{9} cells of Bifidobacterium lactis Bb12 per gram of powder. The administration of the study preparation started on the first day after birth and continued for 21 days. The study ended at the 35th day after birth or when the infant was discharged from the hospital, if earlier. Allocation concealment was not described. The intervention was double masked; however it was unclear whether outcomes assessment was masked or not. All enrolled infants were accounted for. Of note, clinical data obtained through contact with the corresponding author were different from those recently published by Deshpande 2010.

- Reuman 1986: This was a single center study. Three groups of infants were randomized to receive either enteral probiotics (Lactobacillus) or control. Randomization and allocation concealment were clearly inadequate. The intervention was double masked. All infants enrolled were accounted for and outcome assessment was blinded.

- Rougé 2009: This trial was conducted in two centers, infants less than 1500 g and gestational age <32 weeks were randomized to either Probiotic group (45) 108 lyophilized cells per unit of the probiotics L. rhamnosus GG (Valio, Ltd) and B. longum BB536 (Morinaga Milk Industry Co, Ltd, Tokyo, Japan) and maltodextrin beginning on the day when enteral feeding started until discharge or Placebo group (49) Receive 4 daily capsules of a supplement containing either maltodextrin alone. Allocation was adequately concealed. Intervention was masked. All enrolled infants were accounted for. Outcomes measurement was blinded.

- Samanta 2009: This was a single center study. Infants <32 weeks and <1500 g started feed enterally and survived beyond 48 h of life were randomized to receive a probiotic mixture (Bifidobacteria infantis, Bifidobacteria bifidum, Bifidobacteria longum and Lactobacillus acidophilus, each 2.5 billion CFU) with expressed breast milk twice daily, the dosage being 125 g kg -1 till discharge or breast milk only (control). Allocation concealment, and blinding of Intervention and outcome was not adequately described. All enrolled infants were accounted for.

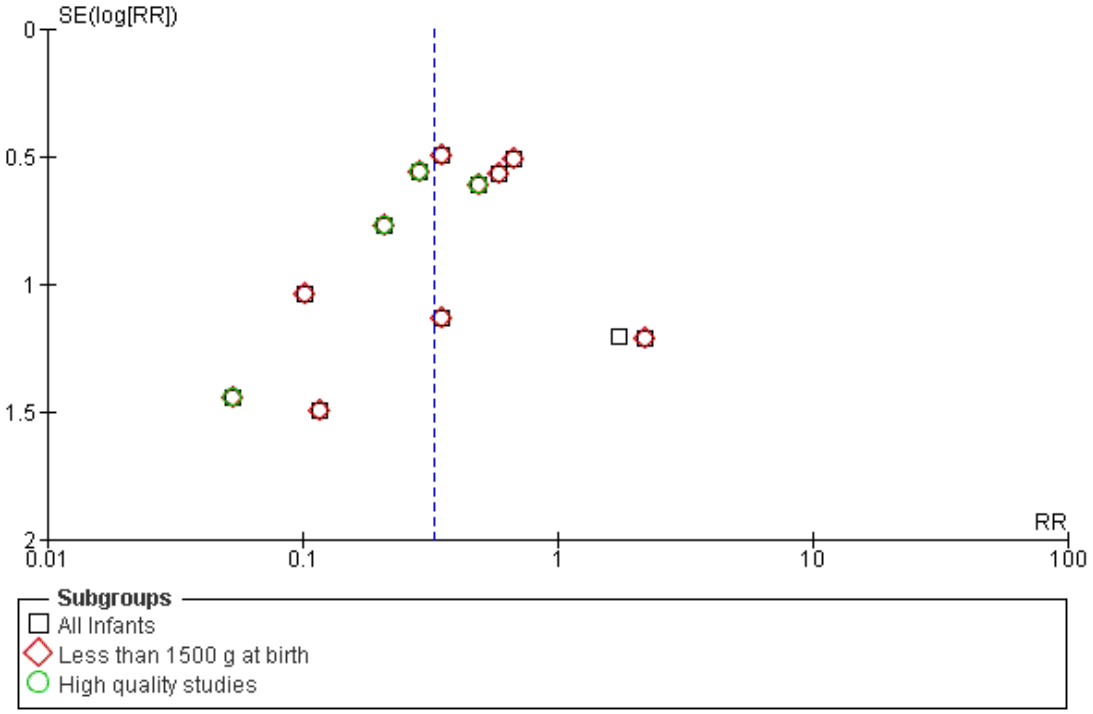
- Sari 2010: This was a single center study. Infants <33 weeks and <1500 g infants who survived to start enteral feeding were randomized into two groups. Infants in study group received L. sporogenes with a dose of 350.000.000 colony forming units added to breast milk or formula once a day starting with first feed until discharge. Infants in control group received no supplementation. Allocation concealment, blinding of Intervention and outcome was adequately described. All enrolled infants were accounted for.

- Stratiki 2007: This was a single center study. Infants (81 infants) with gestational age between 27 and 37 weeks, stable state, formula fed were randomized to group A given a BL supplemented preterm formula - Prenan Nestlé - (BLSPF) at a concentration of 2×107 cfu/g of milk powder or group B (control) received exactly the same formula but without the addition of BL. Allocation concealment was not described. The intervention and outcome assessment were blinded and all infants were included its final results.

Effects of interventions

PROBIOTICS VS. CONTROL (COMPARISON 1):
Severe stage II-III necrotizing enterocolitis (Outcome 1.1): Thirteen studies reported on severe stage II-III NEC (Dani 2002; Costalos 2003; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009; Kitajima 1997; Mohan 2006; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007). The administration of prophylactic probiotics significantly reduced the incidence of severe stage II -III NEC [typical RR 0.35 (95% CI 0.24 to 0.52); typical RD -0.04 (95% CI -0.06 to -0.02), NNT 25]. This effect is maintain even for subgroup of weight less than 1500 g at birth [typical RR 0.34 (95% CI 0.23 to 0.50)] and high quality studies [typical RR 0.25 (95% CI 0.13 to 0.49)]. Data pertaining to the most vulnerable infants (ELBW) could not be abstracted from the included studies. Figure 1

Figure 1. Funnel plot of comparison: 1 Probiotics vs. control, outcome: 1.1 Severe Necrotising Enterocolitis (stage II-III).



Mortality (Outcome 1.2):
Ten studies reported on mortality (Kitajima 1997; Reuman 1986; Dani 2002; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009; Rougé 2009; Samanta 2009). The number of deaths was significantly lower in the probiotics group [typical RR 0.40 (95% CI 0.27 to 0.60); typical RD -0.04 95% CI (-0.06 to -0.01), NNT 25]. Five studies (Bin-Nun 2005; Dani 2002; Kitajima 1997; Lin 2008; Sari 2010) reported NEC-related mortality. The number of NEC related deaths was also significantly lower in the probiotics group [typical RR 0.31 (95% CI 0.10 to 0.94).

Sepsis (Outcome 1.3):
Thirteen studies reported on sepsis (Millar 1993; Kitajima 1997; Costalos 2003; Dani 2002; Lin 2005; Lin 2008; Bin-Nun 2005; Manzoni 2006; Manzoni 2009; Rougé 2009; Samanta 2009; Sari 2010; Stratiki 2007). There was no significant difference among both groups in the rate of culture proven sepsis [typical RR 0.90 (95% CI 0.76, 1.07).

Days on total parenteral nutrition (Outcome 1.4):
Two studies reported this outcome. No statistical difference was found in either of the studies . Dani 2002 reported a mean of 12.8 (13.9) days in the probiotics group, and a mean of 14.7(18.7) days in the control group [WMD -1.9 (-4.6 to 0.77)]. Lin 2005 reported a mean of 14.7 (5.7) days in the probiotics group and 13.9 (5.0) days in the control group [WMD 0.80 (-0.3 to 1.9)]. Other studies report incomplete data to be pooled. Due to the significant test of heterogeneity, these results were not pooled.

Hospitalization days (Outcome 1.5):
Five studies reported this outcome (Lin 2005; Lin 2008; Reuman 1986; Rougé 2009; Samanta 2009). Pooled Data of five studies shows significant reduction in hospitalization days [typical WMD -6.08 (95% CI -7.08 to -5.09).

Weight gain (Outcome 1.6):
Four studies (Reuman 1986; Millar 1993; Costalos 2003; Sari 2010) reported weight gain results. No significant statistical difference in weight gain among study groups was observed. Due to the use of different scales i.e. g/week, g/day and g/kg/day, these results were not pooled.

Time to full enteral feeds (Outcome 1.7):
Three studies (Manzoni 2009; Samanta 2009; Sari 2010) reported time to full enteral feeds results. Pooled data of studies shows significant reduction in time to reach full enteral feeds [typical WMD -4.28 (-4.81 to -3.75)].

The composite of death or severe NEC or sepsis (Outcome

1.8):
Only one study reported this outcome (Lin 2005). Probiotics significantly reduced the incidence of this composite [typical RR 0.54 (95% CI 0.37 to 0.79)].
Systemic infection with the supplemented organism
None of the included studies reported a systemic infection caused by the supplemented probiotics organisms.
Long-term Outcomes (Outcome 1.9):
Kitajima 1997 reported mental retardation and cerebral palsy outcome at six years. No significant statistical difference among study groups was observed.
A subgroup analysis to demonstrate the effect of probiotics administration in ELBW infants was not performed since data pertains to this high risk group could not be extracted from the included studies.

DISCUSSION

Our updated review summarizes the evidence of probiotics efficacy in preterm infants. Sixteen randomized trials and more than 2700 preterm infants are included. Since the publication of our first review, we noted a tremendous increase in published studies, reviews of editorials addressing the efficacy and safety of probiotics utilization in the preterm host.

Our update shows with more robust data that enteral administration of probiotics reduces the incidence of severe NEC, mortality, and NEC related mortality. The administration of probiotic organisms also resulted in a shorten time to full feeds. Our data shows a trend toward a benefit in reduction of sepsis, however, this didn't reach statistical significance. We believe that based on the available evidence for probiotics use in the preterm infant, the number of included infants, the narrow confidence interval, that a change in practice is warranted at this stage. More studies to address the precise efficacy in ELBW infants, the optimal preparation, dosing and duration of therapy are still needed.

Four of our included trials were classified as high quality based on adequacy of allocation concealment procedures and blinding of intervention.

Although all included trials evaluated probiotics use in preterm infants, the trials were highly variable with regard to enrolment criteria (i.e. birth weight, and gestational age), baseline risk of NEC in control groups, timing, dose, formulation of probiotic used and feeding regimens. Most of included trials enrolled preterm infants less than 1500 g at birth; however, specific efficacy and safety data on most vulnerable infants (ELBW) couldn't be evaluated.

Case reports of systemic infections caused by probiotic organisms are reported in biomedical literature. None of our included studies reported this adverse effect. The use of probiotics was described as safe and well tolerated. Our update provide a more robust safety data of probiotics use.

This review utilized a very thorough and comprehensive search strategy. All attempts were made to minimize the potential of a publication bias. Only randomized or quasi-randomized controlled trials were included. To minimize the reviewer bias, all steps of this review were conducted independently by review authors. The validity of our review's results is potentially compromised by the following: included trials utilized different preparations and dosing regimens of the intervention under study; data on the highest risk population (ELBW infants) could not be retrieved.

Our updated review includes five more randomized controlled trials compared to the recent review by Deshpande and coworkers (Deshpande 2010). The results of our updated review are in line with the published data of Deshpande 2010, but allow for a more precise estimate of effect given the larger sample of trials. The issue of whether it is time to change practice and adopt the use of probiotics as a standard of care in preterm infants has been widely discussed in the medical literature of the last year. While some advocate a change in practice based on significant reduction in severe NEC and all cause mortality (Tarnow-Mordi 2010), others suggest to wait until further precise data of efficacy and safety in ELBW infants are available in addition to the determination of the most effective preparation and dosing to be utilized (Soll 2010). We believe that based on the available evidence and in comparison to other effective interventions in neonatal medicine such as induced hypothermia in hypoxic ischemic encephalopathy, a change in practice at this stage is warranted. Parents of preterm infants should be informed of the current evidence if placebo controlled trials are to continue.

Four ongoing studies are identified and will be included into updates of our review in the future.

AUTHORS' CONCLUSIONS

Implications for practice

Enteral supplementation of probiotics prevents severe NEC and all cause mortality in preterm infants. Our updated review of available evidence supports a change in practice.

Implications for research

Our updated review of available evidence supports a change in practice. More studies are needed to assess efficacy in ELBW infants and to assess the most effective formulation and dose to be utilized.

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Costeloe *{unpublished data only}*
The administration of probiotic to premature babies to prevent infection, severe intestinal complication (i.e. necrotising enterocolitis) and death. Ongoing study 01/12/2009.

Lozano *{unpublished data only}*
Prophylactic Probiotics for the Prevention of Sepsis and NEC in Premature Infants in Colombia. A Randomized Double-Blind, Multicenter Trial. Ongoing study Recruiting.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Bin-Nun 2005

Methods	Single centre randomized study Method of generating randomization sequence: not described Blinding of randomization: not described Blinding of intervention: yes Blinding of outcome measurement: yes Completeness of follow-up: not specified
Participants	145 infants less than 1500 g at birth Demographic data: Probiotics Group N=72, Gestational age (weeks) 29.2(2.6), birth weight 1152 (262) Placebo Group N=73, Gestational age (weeks) 29.3 (4.3), birth weight 1111 (278)
Interventions	Probiotics group (N=72) received mixture of Lactobacillus bifidus, streptococcus thermophillus, and bifidobactrium infantis added to 3 ml of expressed breast milk or premature formula enteral feeds. Control group (N=73) received 3 ml of expressed milk or premature formula with no supplements added.
Outcomes	Stage 2 or 3 NEC. Mortality NEC or mortality Sepsis Days to full feeds Days till TPN stopped
Notes	Israel Period of study: Sept 2001-Sept 2004 Published: Journal of Pediatrics 2005 Source of Funding: ABC Dophilus

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	Method of generating randomization sequence: not described
Allocation concealment?	Unclear	Blinding of randomization: not described
Blinding? All outcomes	Yes	Blinding of intervention: yes Blinding of outcome measurement: yes

Bin-Nun 2005 (*Continued*)

Incomplete outcome data addressed? All outcomes	Unclear	Completeness of follow-up: not specified
Free of selective reporting?	Yes	All clinically important outcomes are described

Costalos 2003

Methods	Single center randomized double blind study Method of generating randomization sequence:Cards in sealed envelopes Allocation concealment: Possibly adequate Blinding of intervention: Yes Blinding of outcome measurement: Not described Complete follow-up: Yes
Participants	87 infants, gestational age 28-32 weeks Exclusion criteria: Major anomalies, receiving antibiotics or anti -fungals, receiving breast milk Demographic data: Probiotics Group N=51, Gestational age (weeks) 31.1(2.5), birth weight 1651 (470) Placebo Group N=36, Gestational age (weeks) 31.8 (2.7), birth weight 1644 (348)
Interventions	Probiotics group (N=51) received preterm formula containing approximately 15 nmol/dl polyamines with added Saccharomyces boulardii 50mg/kg every 12 hours during the first week of life when enteral feed are tolerated for 30 days. Placebo group (N=36) received same formula with maltodextrins
Outcomes	NEC Weight gain Abdominal distension Vomiting Gastric retention Stool characteristics Sepsis
Notes	Greece Period of study: not specified Published: 2003 Source of Funding: Unclear

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: Cards in sealed envelopes

Costalos 2003 (*Continued*)

Allocation concealment?	Yes	Allocation concealment: Possibly adequate
Blinding? All outcomes	Unclear	Blinding of intervention: Yes Blinding of outcome measurement: Not described
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes

Dani 2002

Methods	Multicenter randomized double blind study (12 centers) Method of generating randomization sequence: not described Allocation concealment: Clearly adequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	585 infants, < 33 weeks gestation or <1500 g birth weight enrolled. Exclusion criteria: Congenital malformation and death within two weeks of birth Demographic data: Probiotics Group N=295, gestational age (weeks) 30.8(2.4), birth weight 1325 (361) Placebo Group N=290, gestational age (weeks) 30.7 (2.3), birth weight 1345 (384)
Interventions	Probiotics group (N=295) received standard milk with Lactobacillus GG (Dicoflor®, Dicofarm, Rome, Italy) with an added dose of 6×109 colony forming units (cfu) once a day until discharge, starting with first feed. Placebo group (N=290) received standard milk with placebo which was an indistinguishable dried powder of maltodextrins.
Outcomes	Severe NEC Incidence of PDA Duration of parenteral nutrition Urinary tract infection Bacterial sepsis (culture proven) Stage 2 and 3 NEC Single course of antibiotics treatment NEC related mortality
Notes	Italy Period of study: not specified in paper Published: 2002 Source of Funding: not specified in paper

Risk of bias

Item	**Authors' judgement**	**Description**

Dani 2002 (*Continued*)

Adequate sequence generation?	Unclear	Method of generating randomization sequence: not described
Allocation concealment?	Yes	Allocation concealment: clearly adequate
Blinding? All outcomes	Yes	Blinding of Intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Complete Follow-up: Yes
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Kitajima 1997

Methods	Single center randomized study Method of generating randomization sequence: Not described Allocation concealment: Not described Blinding of intervention: Not described Blinding of outcome measurement: Not described Complete follow-up: No (6 patients dropped)
Participants	91 infants, birth weight <1500 g enrolled. Exclusion criteria: Major anomalies, severe asphyxia, severe IUGR Demographic data: Probiotics Group N=45, gestational age (weeks) 28.3(2.3), birth weight 1026 (24) Placebo Group N=46, gestational age (weeks) 28.2 (2.1), birth weight 1026 (205)
Interventions	Probiotics group (N=45) received 1 ml supplement of Bifidobacterium breve with distilled water 0.5×109 of live B. breve within the 1st 24 hrs of life once per day for 28 days Control group (N=46) received distilled water
Outcomes	Colonization rate Mean aspired air volume Vomiting times/week Apnoea times/week Weight gain Mental retardation and cerebral palsy outcome at 6 years
Notes	Japan Period of study: May 1990-April 1991 Published: 1997 Source of Funding: Unclear

Kitajima 1997 (*Continued*)

Risk of bias		
Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Unclear	Method of generating randomization sequence: Not described
Allocation concealment?	Unclear	Allocation concealment: Not described
Blinding? All outcomes	Unclear	Blinding of Intervention: Not described Blinding of outcome measurement: Not described
Incomplete outcome data addressed? All outcomes	No	Complete Follow-up: No (6 patients dropped)
Free of selective reporting?	No	Important patient oriented outcomes are not included

Li 2004

Methods	Single center randomized study Method of generating randomization sequence: Unclear Allocation concealment: Not described Blinding of intervention: Not described Blinding of outcome measurement: Not described Complete follow-up: Unclear
Participants	30 infants, of low birth weight. Exclusion criteria: Major anomalies, chromosomal anomalies, intrauterine infection Demographic data: Probiotics Group A N=10, gestational age (weeks) 33.8(2.9), birth weight 1523 (490) Probiotics Group B N=10, gestational age (weeks) 33.8(3.2), birth weight 1354 (280) Control (C) Group N=10, gestational age (weeks) 32.4 (3.1), birth weight 1480 (237)
Interventions	Probiotics group (N=10) received through gastric tube Bifidobacterium breve twice a day with feeds till discharge. Group A within several hours of birth, while group B after the 1st 24 hrs. Control group (N=10) received no supplement
Outcomes	Colonization rate NEC Sepsis
Notes	Japan Period of study: Jan 2000- Aug 2002 Published: 2004

Li 2004 (*Continued*)

	Source of Funding: Morinaja Milk industry and Meiji Dairies

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	Method of generating randomization sequence:unclear
Allocation concealment?	Unclear	Allocation concealment: Not described
Blinding? All outcomes	Unclear	Blinding of intervention: Not described Blinding of outcome measurement: Not described
Incomplete outcome data addressed? All outcomes	Unclear	Complete follow-up: Unclear
Free of selective reporting?	No	Important patient oriented outcomes are not included

Lin 2005

Methods	Single centre randomized study Method of generating randomization sequence: Random-number table sequence. Allocation concealment: Clearly adequate Blinding of intervention: Yes, only investigators and breast milk team were unblinded. Blinding of Outcome measurement: Yes Completeness of follow up: Yes
Participants	367 infants less than 1500 g at birth, survived beyond 7 days of life, and started on enteral feed were enrolled Demographic data: Probiotics Group N=180, gestational age (weeks) 28.5(2.5), birth weight 1104 (242) Placebo Group N=187, gestational age (weeks) 28.2 (2.5), birth weight 1071 (243)
Interventions	Probiotics group (N=180) received Infloran® (L acidophilus and B infantis) obtained from the American Type Culture Collection in 1973, 125 mg/kg/dose twice daily with breast milk until discharge. All enrolled infants received maternal or banked breast milk. Control group (N=187) received breast milk without any addition (no placebo).
Outcomes	Death Stage 2 or 3 NEC Sepsis (culture proven) Composite outcomes of death+ NEC, sepsis+ NEC, death+ NEC+ Sepsis Duration of parenteral nutrition Hospitalization days

Lin 2005 (*Continued*)

Notes	Taiwan Period of study: July 1999- December 2003 Published: 2005 Source of Funding: supported by research department of China medical university hospital.

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Method of generating randomization sequence: Random-number table sequence.
Allocation concealment?	Yes	Allocation concealment: Clearly adequate
Blinding? All outcomes	Yes	Blinding of intervention: Yes, only investigators and breast milk team were unblinded Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Completeness of follow up: Yes
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Lin 2008

Methods	Multicenter trial Method of generating randomization sequence: Sequential numbers generated at the computer center Allocation concealment: Adequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Completeness of follow up: Yes
Participants	Very low birth weight infants (birth weight $\leq$1500 g) Demographic data: The study group N=217, birth weight 1028.9 (246) The control Group N=217, birth weight 1077 (214.4)
Interventions	Infants in the study group were given Bifidobacterium bifidum and Lactobacillus acidophilus, added to breast milk or mixed feeding (breast milk and formula), twice daily for 6 weeks. Infants in the control group were fed with breast milk or mixed feeding.

Lin 2008 (*Continued*)

Outcomes	Death or severe NEC NEC, ≥ stage2 Death not attributable to NEC Death attributable to NEC Sepsis CLD PVL IVH, ≥ grade3
Notes	7 NICUs in Taiwan Period of study: January 2005 - May 2007 Published: 2008 Sources of support: National Science Council of Taiwan

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: Sequential numbers generated at the computer center.
Allocation concealment?	Yes	Allocation concealment: Adequate
Blinding? All outcomes	Yes	Blinding of intervention: Yes. Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Completeness of follow up: Yes
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Manzoni 2006

Methods	Single randomized study Method of generating randomization sequence: Computer generated randomization Allocation concealment: Unclear Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell Completeness of follow up: Yes
Participants	80 infants less than 1500 g at birth, survived beyond 3 days of life, and started on human or donor milk enteral feed were enrolled Demographic data: Probiotics Group N=39, gestational age (weeks) 29.6 (5), birth weight 1212 (290) Placebo Group N=41, gestational age (weeks) 41(4), birth weight 1174 (340)

Manzoni 2006 (*Continued*)

Interventions	Probiotics group (N=39) received LGG [Diclofor 60;Dicofarm spa]; single dose (1/2 packet of Diclofor 60) daily mixed with human or donor milk till end of the sixth week or discharge. Control group (N=41) received human or donor milk without any addition (no placebo) .
Outcomes	Fungal colonization rates Stage 2 or 4 NEC Death Sepsis (culture proven) Time to full feeds
Notes	Italy Period of study: 12 months Published: 2006 Sources of support: non reported

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: computer generated randomization
Allocation concealment?	Unclear	Allocation concealment: Unclear
Blinding? All outcomes	Unclear	Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell
Incomplete outcome data addressed? All outcomes	Yes	Completeness of follow up: Yes
Free of selective reporting?	Yes	

Manzoni 2009

Methods	Multicenter trial Method of generating randomization sequence: using ralloc.ado version 3.2.5 in Stata 9.2 (Stata-Corp, College Station, Texas) Allocation concealment: Yes Blinding of intervention: Yes Blinding of outcome measurement: Yes Completeness of follow up: Yes

Manzoni 2009 (*Continued*)

Participants	VLBW neonates younger than 3 days Demographic data: Probiotics Group N=151, gestational age (weeks) 29.8 (23-35), birth weight 1138 (550-1500) Control Group N=153, gestational age (weeks) 29.5 (23-39), birth weight 1109 (437-1500)
Interventions	Infants received either BLF (Bovine Lactoferrin) (100mg/d) (LF100; Dicofarm SpA, Rome, Italy) alone or BLF plus LGG ($6X10^9$ colony-forming units/d) (Dicoflor60;Dicofarm SpA); the control group received placebo (2 mL of a 5% glucose solution). Treatment lasted 6 (birth weight 1000 g) or 4 (birth weight 1001-1500 g) weeks, unless neonates were discharged earlier. Drug administration began on the third day of life with 1 daily dose; all doses including placebo were diluted in prepared milk so as to maintain blinding.
Outcomes	First episode of late-onset sepsis Incidence of gram-positive/gram-negative bacterial and fungal sepsis Mortality prior to discharge Incidence of urinary tract infections, fungal colonization, progression from fungal colonization to invasive fungal infection Severe NEC Threshold ROP Severe (grade 3-4) IVH BPD Alteration of liver function Adverse effects or intolerance
Notes	11 Italian tertiary NICU Period of study: October 1, 2007, and July 31, 2008 Published: 2009 Source of Funding: Dicofarm SpA

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence:using ralloc.ado version 3.2.5 in Stata 9.2 (Stata-Corp, College Station, Texas)
Allocation concealment?	Yes	Allocation concealment: Yes
Blinding? All outcomes	Yes	Blinding of intervention: Yes Blinding of outcome measurement: Yes

Incomplete outcome data addressed? All outcomes	Yes	Completeness of follow up: Yes
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Millar 1993

Methods	Single center randomized blinded study Method of generating randomization sequence: Not described Allocation concealment: Not described Blinding of Intervention: Yes Blinding of outcome measurement: Unclear Complete follow-up: Yes
Participants	20 infants, < 33 weeks gestation enrolled. Demographic data: Probiotics Group N=10, gestational age (weeks) 30.5(26-33), birth weight 1445 (800-2560) Placebo Group N=10, gestational age (weeks) 30.0 (24-33), birth weight 1500 (830-2150)
Interventions	Probiotics group received milk feeds with Lactobacillus GG 10^8 (cfu) twice a day for 14 days, starting with first feed. Placebo group received unsupplemented milk
Outcomes	Weight gain Sepsis clinical or lab proven Antibiotics treatment Oxygen and ventilatory requirements Hospital stay Perineal candidal infection Duration of hospital stay
Notes	UK Period of study: Sept 1991-Jan 1992 Published: 1993 Source of Funding: Wessex Medical Trust

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Unclear	Method of generating randomization sequence: Not described
Allocation concealment?	Unclear	Allocation concealment: Not described

Millar 1993 (*Continued*)

Blinding? All outcomes	Unclear	Blinding of intervention: Yes Blinding of outcome measurement: Unclear
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	No	Important patient oriented outcomes are not included

Mohan 2006

Methods	A double-blind, placebo-controlled, randomized trial Method of generating randomization sequence: Randoma software version 4.3 Allocation concealment: Not described Blinding of intervention: Yes Blinding of outcome measurement: Unclear Complete follow-up: Yes
Participants	Gestational age of less than 37 weeks No demographic data were provided
Interventions	69 preterm infants The probiotic and placebo groups contained 37 and 32 preterm infants, respectively. The verum contained 2 $X10^9$ cells of Bifidobacterium lactis Bb12 per gram of powder. The concentration of Bb12 in 1 ml solution of verum in water was 4 $X10^8$. The verum group received 1.6 $X10^9$ cells on day 1 to 3 and 4.8 $X10^9$ cells from day 4 onward. Started on the first day after birth and continued for 21 days. The study ended at the 35th day after birth or when the infant was discharged from the hospital, if earlier.
Outcomes	No clinical outcomes were presented in the published data NEC and sepsis data were collected by contacting the corresponding author
Notes	The Ernst von Bergmann hospital, Potsdam, Germany Period of study: August 2003 - June 2005 Published: 2006 Source of funding: Not reported

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: Randoma software version 4.3
Allocation concealment?	Unclear	Allocation concealment: Not described

Mohan 2006 (*Continued*)

Blinding? All outcomes	Unclear	Blinding of intervention: Yes Blinding of outcome measurement: Unclear
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	No	Improtant patient oriented outcomes are not included

Reuman 1986

Methods	Randomized double blind study Method of generating randomization sequence: random number charts and the last digit of patient's chart number, the next matched infants is assigned to the opposite group Allocation concealment: clearly inadequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	45 infants, <2000 gm at birth weight who survived beyond first 24 hrs and are younger than 72 hrs Demographic data: Probiotics Group N=15, gestational age (weeks) 30.6(2.7), birth weight 1366 (302) Placebo Group N=15, gestational age (weeks) 30.5 (2.8), birth weight 1377 (344) Untreated group N=15, gestational age(weeks) 30.7(2.9), birth weight 1329(337)
Interventions	Probiotics group received at least 1 ml of formula containing lactobacillus. 5×10^{10} organisms/ml preparation diluted 100 times in infants formula. Placebo group received 1 ml of formula with no added lactobacillus Both groups started within 72 hrs of birth The untreated group received nothing per mouth for 2 weeks
Outcomes	Death Colonization rates Hospitalization duration Daily weight gain Hospital acquired infection
Notes	US Period of study: not specified in paper Published: 1986 Source of Funding: not specified in paper

Risk of bias

Item	**Authors' judgement**	**Description**

Reuman 1986 (*Continued*)

Adequate sequence generation?	No	Method of generating randomization sequence: random number charts and the last digit of patient's chart number, the next matched infants is assigned to the opposite group
Allocation concealment?	No	Allocation concealment: Clearly inadequate
Blinding? All outcomes	Yes	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	No	

Rougé 2009

Methods	Two centers Method of generating randomization sequence: In-house software (Nantes University Hospital, Nantes, France) Allocation concealment: Possibly adequate Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	Gestational age, <32 wk, a birth weight, <1500 g Demographic data: Probiotics Group N=45, gestational age (weeks) 28.1 (1.9), birth weight 1115 (251) Placebo Group N=49, gestational age (weeks) 28.1 (1.8), birth weight 1057 (260)
Interventions	Placebo group (N 49) Receive 4 daily capsules of a supplement containing maltodextrin alone Probiotic group (N 45) 10^8 lyophilized cells per unit of the probiotics L. rhamnosus GG (Valio, Ltd) and B. longum BB536 (Morinaga Milk Industry Co, Ltd, Tokyo, Japan) and maltodextrin beginning on the day when enteral feeding started until discharge.
Outcomes	The percentage of infants receiving more than 50% of their nutritional needs via enteral feeding on the 14th day of life. Nutrition on day 14 (more than 50% of calories received enterally and total calories delivered enterally) Nosocomial infections Sepsis with positive blood culture Duration of antibiotic use Necrotizing enterocolitis Duration of ventilatory support

Rougé 2009 (*Continued*)

	Duration of CPAP Duration of oxygen therapy Systemic postnatal corticoid treatment Duration of hospital stay Death
Notes	France Period of study: Aprill 2005 - January 2007 Published: 2009 Source of Funding:from the Programme Hospitalier de Recherche Clinique of the French Ministry of Health and the De´ le´ gation a‘ la Recherche Clinique, CHU de Nantes.

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: In-house software (Nantes University Hospital, Nantes, France)
Allocation concealment?	Yes	Allocation concealment: Possibly adequate
Blinding? All outcomes	Yes	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	Yes	
Free of other bias?	Yes	

Samanta 2009

Methods	Prospective randomized double-blind control trial Method of generating randomization sequence: Can't tell Allocation concealment: Can't tell Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell Complete follow-up: Yes
Participants	Gestational age <32 weeks and VLBW infants (<1500 g) started feed enterally and survived beyond 48 h of life Demographic data: Probiotics Group N=91, gestational age 30.12 (weeks) (1.63), birth weight 1172 (143) Control Group N=95, gestational age 30.14 (weeks) (1.59), birth weight 1210 (143)

Samanta 2009 (*Continued*)

Interventions	The probiotic group received a probiotic mixture (Bifidobacteria infantis, Bifidobacteria bifidum, Bifidobacteria longum and Lactobacillus acidophilus, each 2.5 billion CFU) with expressed breast milk twice daily, the dosage being 125 g kg $^{-1}$ till discharge. The control group was fed with breast milk only.
Outcomes	Feed tolerance in terms of days required to reach full enteral feeding Length of hospital stay NEC Sepsis Death due to NEC or sepsis
Notes	Neonatal Care Unit of Medical College and Hospital, Kolkata, India Period of study: October 2007 - March 2008 Published: 2009 Source of Funding: not specified in paper

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Unclear	Method of generating randomization sequence: Can't tell
Allocation concealment?	Unclear	Allocation concealment: Can't tell
Blinding? All outcomes	Unclear	Blinding of intervention: Can't tell Blinding of outcome measurement: Can't tell
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes

Sari 2010

Methods	Single Center Method of generating randomization sequence: Sequential numbers generated at the computer center of the NICU Allocation concealment: Can't tell Blinding of intervention: Can't tell Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	Gestational age <33 weeks or birth weight <1500 g Demographic data: Probiotics Group N=110, gestational age 29.5 (weeks) (2.4), birth weight 1231 (262) Control Group N=111, gestational age 29.7 (weeks) (2.4), birth weight 1278 (282)

Sari 2010 (*Continued*)

Interventions	VLBW infants who survived to start enteral feeding were randomized The study group were given L. sporogenes with a dose of 350.000.000 colony forming units added to breast milk or formula once a day starting with first feed until discharge. The control group were fed without L. sporogenes supplementation.
Outcomes	Death or severe NEC NEC (stage 2, 3, $\geq$ 2) Death (attributable to NEC, not attributable to NEC) Total parental nutrition Intraventricular hemorrhage, grade 3-4, Sepsis (culture proven, gram negative, gram positive, fungus) NICU stay Feeding (amount, full feeding, intolerance) Weight gain
Notes	Turkey Period of study: October 2008 and June 2009 Published: Unpublished Source of Funding: not specified in paper

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Yes	Method of generating randomization sequence: Sequential numbers generated at the computer center of the NICU
Allocation concealment?	Unclear	Allocation concealment: Can't tell
Blinding? All outcomes	Unclear	Blinding of intervention: Can't tell Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	Yes	

Stratiki 2007

Methods	Single Center Method of generating randomization sequence: Can't tell Allocation concealment: Can't tell Blinding of intervention: Yes Blinding of outcome measurement: Yes Complete follow-up: Yes
Participants	Gestational age between 27 and 37 weeks, stable state, formula fed Demographic data: Probiotics Group N=, gestational age (weeks), birth weight Control Group N=, gestational age (weeks), birth weight
Interventions	81 infants Group A (study group) was given a BL supplemented preterm formula - Prenan Nestlé - (BLSPF) at a concentration of 2×107 cfu/g of milk powder. Group B (control) received exactly the same formula but without the addition of BL.
Outcomes	Intestinal permeability Somatic growth Tolerance Sepsis Necrotizing enterocolitis
Notes	Greece Period of study: January 2004 - December 2005 Published: 2007 Source of Funding: not specified in paper (Nestlé Company, Vevey provide the B. lactis supplemented milk formula)

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Unclear	Method of generating randomization sequence: Can't tell
Allocation concealment?	Unclear	Allocation concealment: Can't tell
Blinding? All outcomes	Yes	Blinding of intervention: Yes Blinding of outcome measurement: Yes
Incomplete outcome data addressed? All outcomes	Yes	Complete follow-up: Yes
Free of selective reporting?	No	Important patient oriented clinical outcomes are not included

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Agarwal 2003	No clinical outcomes were presented
Stansbridge 1993	No clinical outcomes were presented
Uhlemann 1999	Data included full term infants

Characteristics of ongoing studies *[ordered by study ID]*

Braga

Trial name or title	The efficacy of probiotics for prevention of necrotising enterocolitis in very low birth weight infants: a randomised clinical trial
Methods	Randomised controlled trial
Participants	Infants with birth weight from 750 g to 1500 g admitted in the Neonatal intensive Care Unit of the Institute for Maternal/Infant Health (Instituto Materno Infantil de Pernambuco [IMIP]).
Interventions	The participants will be randomised into two groups of 315 infants: Control group: 3 ml of pasteurised human milk once a day on the second to the 30th day of life, or at the discharge if it happens before the 30th day. Intervention group: Lactobacillus casei and Bifidobacterium breve (Yakult - LB) diluted with 3 ml of pasteurised human milk once a day on the second to the 30th day of life, or at the discharge if it happens before the 30th day.
Outcomes	Primary: The frequency of the necrotising enterocolitis classified as higher or equal to 2 according to Bell's criteria Secondary: 1. The frequency of pathogenic bacteria in the faeces 2. The duration of birth weight recovery 3. Time to reach full enteral feeds 4. The hospital stay
Starting date	Not mentioned
Contact information	Prof Taciana Duque-Braga IMIP - UTI Neonatal Rua dos Coelhos, 300
Notes	Brazil ISRCTN67165178

Costeloe

Trial name or title	The administration of probiotic to premature babies to prevent infection, severe intestinal complication (i.e. necrotising enterocolitis) and death
Methods	Multi-centre double-blind placebo-controlled randomised trial
Participants	1. Both males and females, born before 31 completed weeks of gestation, i.e. up to and including 30 weeks + 6 days by the best estimate of Expected Date of Delivery 2. Less than 48 hours old 3. With written informed parental consent 4. Babies already on antibiotics for suspected or proven infection are eligible for recruitment to the study
Interventions	Bifidobacterium breve strain BBG (B breve BBG). The placebo is corn starch alone. Both products are manufactured in identical foil sachets each containing 1 gram of product. The intervention will be given once daily starting as soon as possible after randomisation and continuing until 36 completed weeks of post-menstrual age (36 weeks + 0 days) or death or discharge from hospital if sooner. 1,300 babies will be recruited over 30 months.
Outcomes	Primary: 1. Any baby with an episode of blood stream infection, with any organism other than a skin commensal 2. Necrotising enterocolitis, Bell stage II or III 3. Death before discharge Secondary: 1. Number of babies with the composite outcome of any or a combination of the 3 primary outcomes Outcomes 2 to 7 are for samples taken more than 72 hours after birth and before death or discharge home: 2. Number of babies with any positive blood culture with an organism recognised as a skin commensal e.g. CoNS or Corynebacteria 3. Number of babies with blood cultures taken 4. Number of blood cultures taken per baby 5. Number of babies with episodes of blood stream infection with organisms other than skin commensals by organism 6. Number of babies with isolates of organisms other than skin commensals from a normally sterile site other than blood 7. Number of babies with a positive culture of B breve BBG from any normally sterile site 8. Total duration of days of antibiotics and/or anti-fungals administered per baby after 72 hours and until death or discharge 9. The number of babies colonised with the administered probiotic strain 10. Stool flora 11. Age at achieving full enteral nutrition (defined as 150 ml/kg/day for 1 day) 12. Change of weight Z score from birth to 36 weeks post-menstrual age or discharge from hospital if sooner 13. Broncho-pulmonary dysplasia 14. Hydrocephalus and/or intraparenchymal cysts confirmed by cerebral ultrasound scan performed during the baby's in-patient stay 15. Worst stage of retinopathy of prematurity in either eye at discharge or death 16. Length of stay in intensive, high dependency and special care (British Association of Perinatal Medicine (BAPM) 2001: definitions)
Starting date	01/12/2009

Costeloe (*Continued*)

Contact information	Prof Kate Costeloe Barts and the London School of Medicine and Dentistry Neonatal Unit Homerton University Hospital Homerton Row
Notes	UK ISRCTN05511098

Lozano

Trial name or title	Prophylactic Probiotics for the Prevention of Sepsis and NEC in Premature Infants in Colombia. A Randomized Double-Blind, Multicenter Trial
Methods	Randomized Double-Blind, Multicenter Trial
Participants	Admission to the NICU Written parental consent Birth weight < 2000 grams Hemodynamically stable Less than 48 hours of age
Interventions	Lactobacillus reuteri DSM 17938 will be administered at a dose of ten to the eighth colony-forming units in 5 drops of a commercially available oil suspension once per day until discharge from the hospital. Dietary Supplement: Placebo 5 drops of an available oil suspension without Lactobacillus reuteri
Outcomes	Primary Outcome Measures: Number of deaths and episodes of nosocomial sepsis among probiotic exposed and non-exposed preterm infants. Secondary Outcome Measures: Number of episodes of necrotizing enterocolitis experienced by each premature infant randomized to probiotic exposure or to placebo.
Starting date	Recruiting
Contact information	Juan M Lozano, MD, Msc jmlozano@javeriana.edu.co Maria X Rojas, RN, Msc mxrojas@javeriana.edu.co
Notes	Colombia NCT00727363

Tobin

Trial name or title	The use of probiotics to reduce the incidence of sepsis in premature infants
Methods	Randomised placebo controlled trial
Participants	Infants born/transferred to participating hospital within 72 hrs of birth. The birthweight of the infant is < 1500 g and < 32 weeks gestation.

Tobin (*Continued*)

Interventions	Probiotic combination (ABC Dophilus Infant Powder). The intervention ABC Dophilus infant powder contains 1x10^9 of total organisms, consisting of 3 bacterial strains (Bifidobacterium infantis, Bifidobacterium bifidus, Streptococcus thermophilus). This is presented in a powder form in a jar, which is opened, 0.5 teaspoon mixed with 3ml feed and given daily by mouth/nasogastric tube, from the start of milk feeds until discharged home or term (40 weeks post menstrual age), whichever comes first. The placebo will appear identical to the probiotic and consists of maltodextrin.
Outcomes	Primary: The incidence of proven or probable late onset sepsis (>48 hrs after birth) Secondary: The incidence of necrotising enterocolitis, death, length of the primary hospital admission including proportion experiencing prolonged hospital stay, number of courses of antibiotics, number of days until full oral feeds established (120 ml/kg). Weight, length and head circumference A maternal questionnaire will be used to report atopic eczema, but will also note food allergies, and wheeze from term until 12 months corrected age.
Starting date	1/03/2007
Contact information	Dr Jacinta Tobin The Roayl Women's Hospital 132 Grattan Street Carlton VIC 3053
Notes	Australia ACTRN12607000144415

DATA AND ANALYSES

Comparison 1. Probiotics vs. control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Severe necrotising enterocolitis (stage II-III)	13		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 All Infants	13	2747	Risk Ratio (M-H, Fixed, 95% CI)	0.35 [0.24, 0.52]
1.2 Less than 1500 g at birth	12	2678	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.23, 0.50]
1.3 High quality studies	4	1705	Risk Ratio (M-H, Fixed, 95% CI)	0.25 [0.13, 0.49]
2 Mortality	11		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 All cause neonatal mortality	10	2331	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.27, 0.60]
2.2 NEC reported mortality	5	1476	Risk Ratio (M-H, Fixed, 95% CI)	0.31 [0.10, 0.94]
3 Sepsis	13	2706	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.76, 1.07]
3.1 Culture proven sepsis	13	2706	Risk Ratio (M-H, Fixed, 95% CI)	0.90 [0.76, 1.07]
4 Parenteral nutrition duration (days)	2		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 Dani 2002	1	585	Mean Difference (IV, Fixed, 95% CI)	-1.90 [-4.57, 0.77]
4.2 Lin 2005	1	367	Mean Difference (IV, Fixed, 95% CI)	0.80 [-0.30, 1.90]
5 Hospitalization days	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
5.1 Mean (SD)	5	1111	Mean Difference (IV, Fixed, 95% CI)	-6.08 [-7.08, -5.09]
6 Weight gain	4		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 g/week	1	87	Mean Difference (IV, Fixed, 95% CI)	7.20 [-0.06, 14.46]
6.2 g/day	1	30	Mean Difference (IV, Fixed, 95% CI)	1.0 [-3.35, 5.35]
6.3 g/kg/day	2	241	Mean Difference (IV, Fixed, 95% CI)	0.28 [-0.93, 1.49]
7 Time to full enteral feeds	3	726	Mean Difference (IV, Fixed, 95% CI)	-4.28 [-4.81, -3.75]
8 Death or severe NEC or sepsis	1	367	Risk Ratio (M-H, Fixed, 95% CI)	0.54 [0.37, 0.79]
9 Long-term outcomes	1	85	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.15, 6.94]
9.1 Mental retardation and Cerebral palsy	1	85	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.15, 6.94]

Analysis 1.1. Comparison 1 Probiotics vs. control, Outcome 1 Severe necrotising enterocolitis (stage II-III).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 1 Severe necrotising enterocolitis (stage II-III)

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 All Infants				
Bin-Nun 2005	1/72	10/73		0.10 [0.01, 0.77]
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Mohan 2006	2/37	1/32		1.73 [0.16, 18.20]
Roug 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	9/111		0.67 [0.25, 1.83]
Stratiki 2007	0/38	3/31		0.12 [0.01, 2.19]
Subtotal (95% CI)	**1371**	**1376**		**0.35 [0.24, 0.52]**
Total events: 32 (Probiotics), 90 (Control)				
Heterogeneity: Chi² = 11.08, df = 11 (P = 0.44); I² =1%				
Test for overall effect: Z = 5.20 (P < 0.00001)				
2 Less than 1500 g at birth				
Bin-Nun 2005	1/72	10/73		0.10 [0.01, 0.77]
Costalos 2003	5/51	6/36		0.59 [0.19, 1.78]
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Kitajima 1997	0/45	0/46		0.0 [0.0, 0.0]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2006	1/39	3/41		0.35 [0.04, 3.23]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]

(*Continued . . .*)

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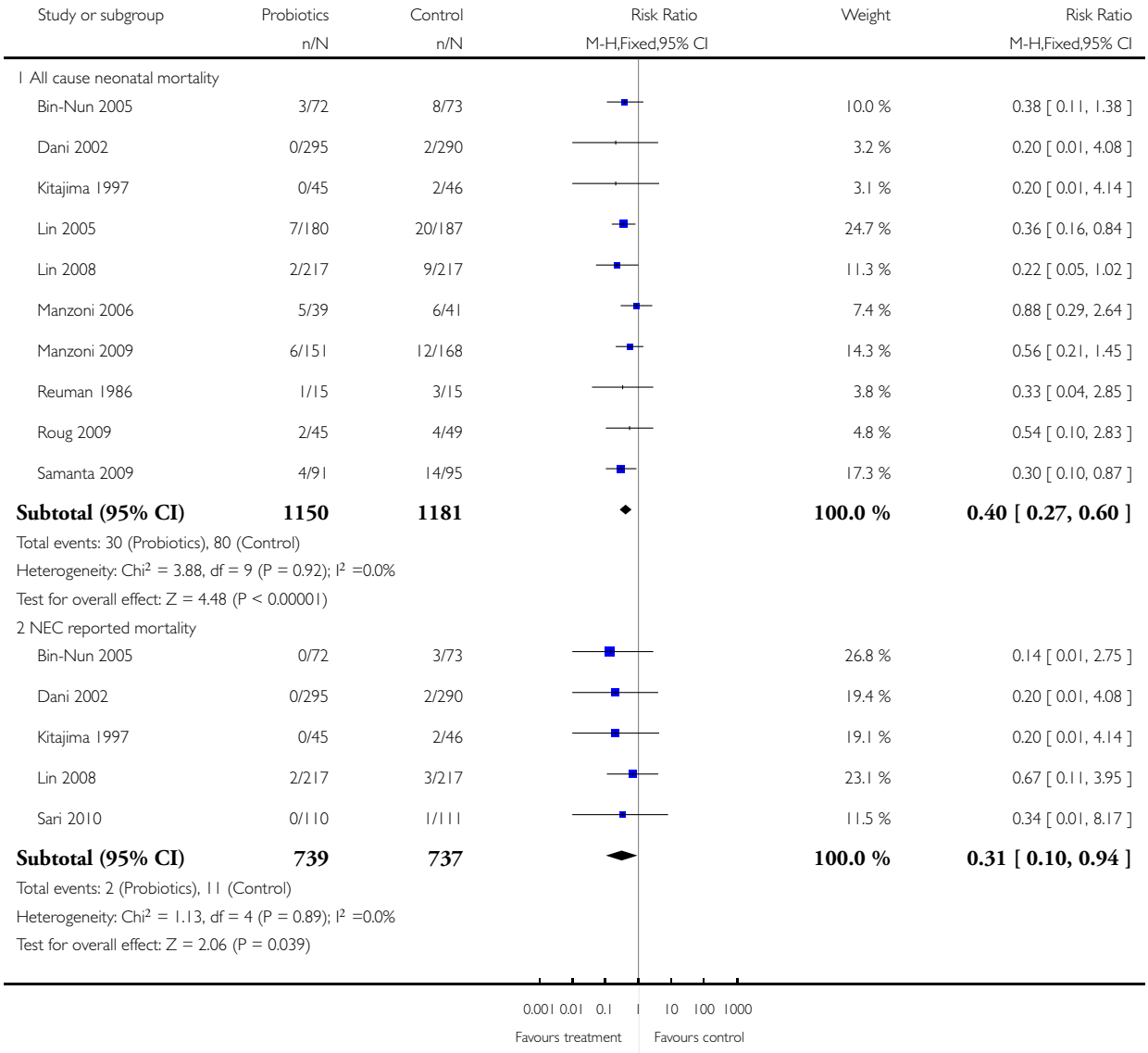
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Roug 2009	2/45	1/49		2.18 [0.20, 23.21]
Samanta 2009	5/91	15/95		0.35 [0.13, 0.92]
Sari 2010	6/110	9/111		0.67 [0.25, 1.83]
Stratiki 2007	0/38	3/31		0.12 [0.01, 2.19]
Subtotal (95% CI)	**1334**	**1344**		**0.34 [0.23, 0.50]**
Total events: 30 (Probiotics), 89 (Control)				
Heterogeneity: Chi² = 9.57, df = 10 (P = 0.48); I² =0.0%				
Test for overall effect: Z = 5.31 (P < 0.00001)				
3 High quality studies				
Dani 2002	4/295	8/290		0.49 [0.15, 1.61]
Lin 2005	2/180	10/187		0.21 [0.05, 0.94]
Lin 2008	4/217	14/217		0.29 [0.10, 0.85]
Manzoni 2009	0/151	10/168		0.05 [0.00, 0.90]
Subtotal (95% CI)	**843**	**862**		**0.25 [0.13, 0.49]**
Total events: 10 (Probiotics), 42 (Control)				
Heterogeneity: Chi² = 2.50, df = 3 (P = 0.48); I² =0.0%				
Test for overall effect: Z = 4.02 (P = 0.000059)				

Analysis 1.2. Comparison 1 Probiotics vs. control, Outcome 2 Mortality.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 2 Mortality



Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 All cause neonatal mortality					
Bin-Nun 2005	3/72	8/73		10.0 %	0.38 [0.11, 1.38]
Dani 2002	0/295	2/290		3.2 %	0.20 [0.01, 4.08]
Kitajima 1997	0/45	2/46		3.1 %	0.20 [0.01, 4.14]
Lin 2005	7/180	20/187		24.7 %	0.36 [0.16, 0.84]
Lin 2008	2/217	9/217		11.3 %	0.22 [0.05, 1.02]
Manzoni 2006	5/39	6/41		7.4 %	0.88 [0.29, 2.64]
Manzoni 2009	6/151	12/168		14.3 %	0.56 [0.21, 1.45]
Reuman 1986	1/15	3/15		3.8 %	0.33 [0.04, 2.85]
Roug 2009	2/45	4/49		4.8 %	0.54 [0.10, 2.83]
Samanta 2009	4/91	14/95		17.3 %	0.30 [0.10, 0.87]
Subtotal (95% CI)	**1150**	**1181**		**100.0 %**	**0.40 [0.27, 0.60]**
Total events: 30 (Probiotics), 80 (Control)					
Heterogeneity: Chi² = 3.88, df = 9 (P = 0.92); I² =0.0%					
Test for overall effect: Z = 4.48 (P < 0.00001)					
2 NEC reported mortality					
Bin-Nun 2005	0/72	3/73		26.8 %	0.14 [0.01, 2.75]
Dani 2002	0/295	2/290		19.4 %	0.20 [0.01, 4.08]
Kitajima 1997	0/45	2/46		19.1 %	0.20 [0.01, 4.14]
Lin 2008	2/217	3/217		23.1 %	0.67 [0.11, 3.95]
Sari 2010	0/110	1/111		11.5 %	0.34 [0.01, 8.17]
Subtotal (95% CI)	**739**	**737**		**100.0 %**	**0.31 [0.10, 0.94]**
Total events: 2 (Probiotics), 11 (Control)					
Heterogeneity: Chi² = 1.13, df = 4 (P = 0.89); I² =0.0%					
Test for overall effect: Z = 2.06 (P = 0.039)					

Analysis 1.3. Comparison 1 Probiotics vs. control, Outcome 3 Sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 3 Sepsis

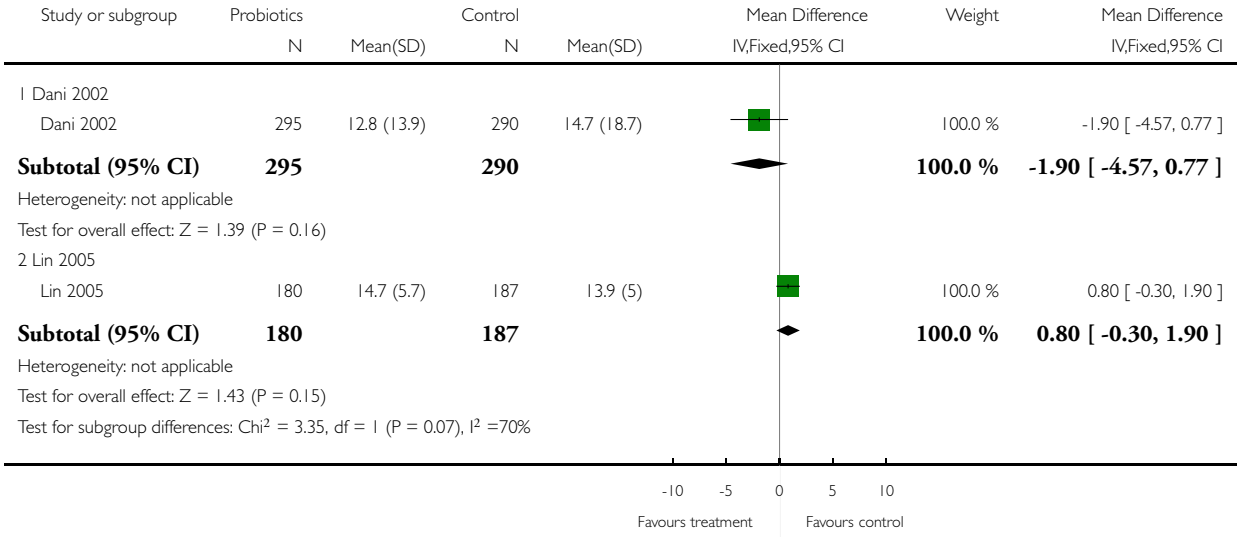
Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Culture proven sepsis				
Bin-Nun 2005	31/72	24/73		1.31 [0.86, 2.00]
Costalos 2003	3/51	3/36		0.71 [0.15, 3.30]
Dani 2002	14/295	12/290		1.15 [0.54, 2.44]
Kitajima 1997	1/45	0/46		3.07 [0.13, 73.32]
Lin 2005	22/180	36/187		0.63 [0.39, 1.04]
Lin 2008	40/217	24/217		1.67 [1.04, 2.67]
Manzoni 2006	19/39	22/41		0.91 [0.59, 1.40]
Manzoni 2009	7/151	29/168		0.27 [0.12, 0.60]
Millar 1993	0/10	0/10		0.0 [0.0, 0.0]
Roug 2009	15/45	13/49		1.26 [0.67, 2.34]
Samanta 2009	13/91	28/95		0.48 [0.27, 0.88]
Sari 2010	29/110	26/111		1.13 [0.71, 1.78]
Stratiki 2007	0/41	3/36		0.13 [0.01, 2.36]
Total (95% CI)	**1347**	**1359**		**0.90 [0.76, 1.07]**
Total events: 194 (Probiotics), 220 (Control)				
Heterogeneity: Chi^2 = 29.42, df = 11 (P = 0.002); I^2 =63%				
Test for overall effect: Z = 1.15 (P = 0.25)				

Analysis 1.4. Comparison 1 Probiotics vs. control, Outcome 4 Parenteral nutrition duration (days).

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 4 Parenteral nutrition duration (days)

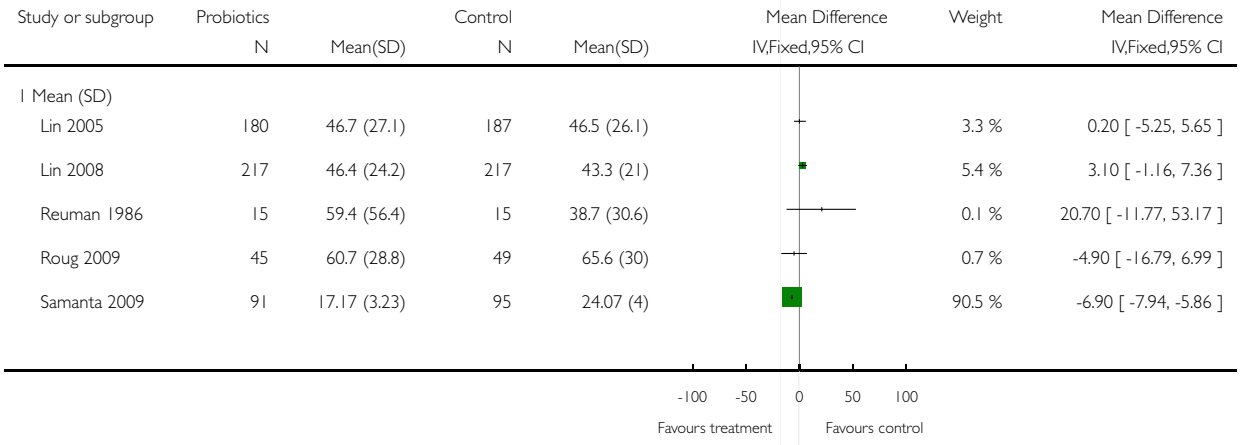


Analysis 1.5. Comparison 1 Probiotics vs. control, Outcome 5 Hospitalization days.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 5 Hospitalization days

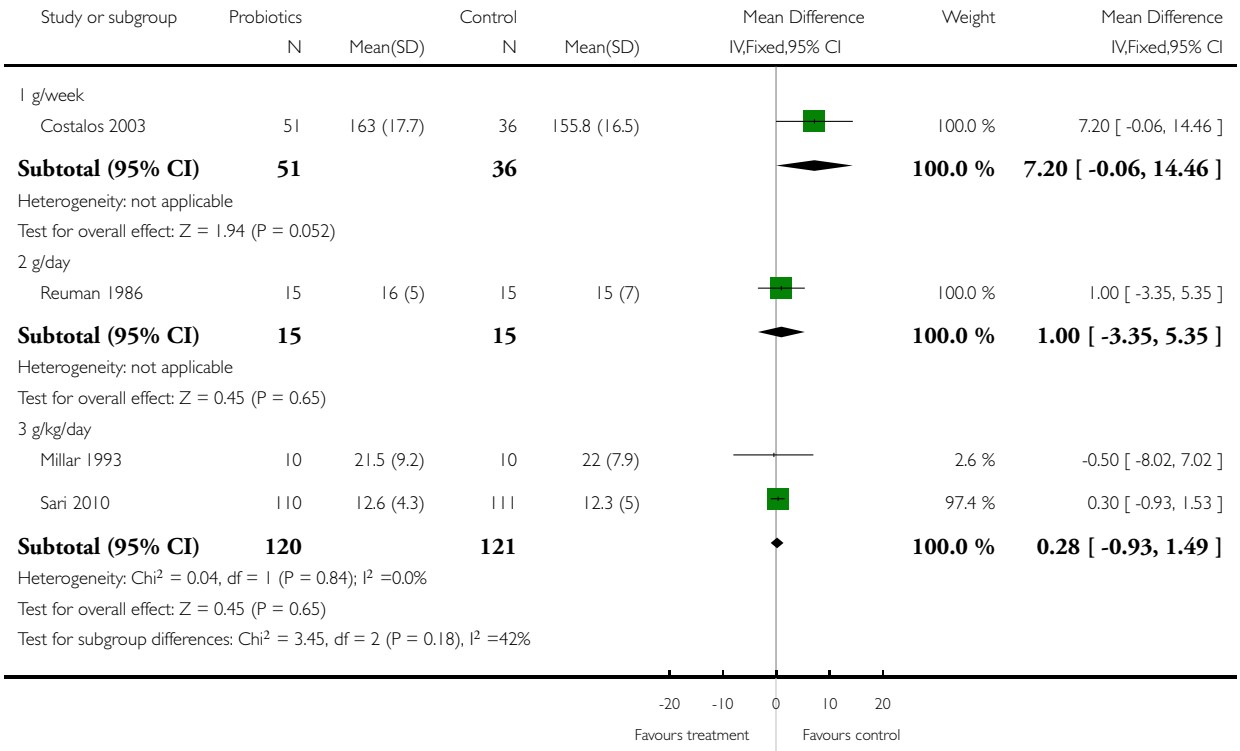


Analysis 1.6. Comparison 1 Probiotics vs. control, Outcome 6 Weight gain.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 6 Weight gain



Analysis 1.7. Comparison 1 Probiotics vs. control, Outcome 7 Time to full enteral feeds.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

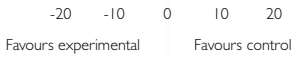
Comparison: 1 Probiotics vs. control

Outcome: 7 Time to full enteral feeds

Study or subgroup	Probiotics N	Mean(SD)	Control N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
Manzoni 2009	151	13.4 (5.1)	168	14.8 (4.7)		23.6 %	-1.40 [-2.48, -0.32]
Samanta 2009	91	13.76 (2.28)	95	19.2 (2.02)		71.7 %	-5.44 [-6.06, -4.82]
Sari 2010	110	17.3 (8.7)	111	18.3 (9.8)		4.6 %	-1.00 [-3.44, 1.44]
Total (95% CI)	**352**		**374**			**100.0 %**	**-4.28 [-4.81, -3.75]**

Heterogeneity: Chi^2 = 47.68, df = 2 (P<0.00001); I^2 =96%

Test for overall effect: Z = 15.97 (P < 0.00001)



Analysis 1.8. Comparison 1 Probiotics vs. control, Outcome 8 Death or severe NEC or sepsis.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 8 Death or severe NEC or sepsis

Study or subgroup	Probiotics n/N	Control n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Lin 2005	31/180	60/187		100.0 %	0.54 [0.37, 0.79]
Total (95% CI)	**180**	**187**		**100.0 %**	**0.54 [0.37, 0.79]**

Total events: 31 (Probiotics), 60 (Control)

Heterogeneity: not applicable

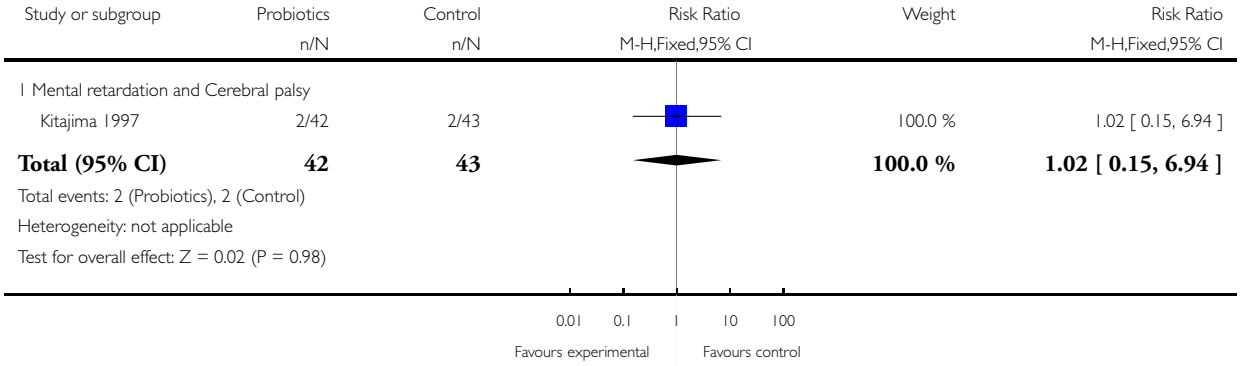
Test for overall effect: Z = 3.19 (P = 0.0014)

Analysis 1.9. Comparison 1 Probiotics vs. control, Outcome 9 Long-term outcomes.

Review: Probiotics for prevention of necrotizing enterocolitis in preterm infants

Comparison: 1 Probiotics vs. control

Outcome: 9 Long-term outcomes



FEEDBACK

Davies, 9 May 2008

Summary

I read with interest the review by AlFaleh and Bassler. It was a well conducted systematic review that revealed that the use of probiotics in preterm infants significantly reduces the incidence of NEC and death in preterm infants. I am not sure why the authors have concluded that probiotics should only be used for preterm infants with a birth weight greater than 1000 grams. If we assume that the data on birth weight from individual studies are normally distributed, we can surmise from the mean birth weight and standard deviations that approximately 25% of babies included in the studies that contribute to the two main meta-analyses (for the outcomes of severe NEC and mortality) had a birth weight of less than 1000 grams. Only about 3% or less had a birth weight of greater than 1500 grams. The authors conclusions imply that the use of probiotics is supported for infants who are preterm (born at <37 weeks gestational age) and who had a birth weight of >1500 grams (less than ~3% of the study population), but is not supported for infants who had a birth weight of <1000 grams (~25% of the study population). The results of the review and its meta-analysis are highly significant, both statistically and clinically. They should be applicable to the population of infants that contributed to the pooled data, i.e., preterm babies who were (almost all) <1500 grams at birth.

The authors should provide justification for their recommendation that extremely low birth weight infants should not be given this intervention that provides a 57% reduction in the risk of death. Also, if further large randomized controlled trial[s] are done they must include assessment of long-term

neurodevelopmental outcomes, not just important intermediate neonatal outcomes.

Reply

We first would like to thank you for your thoughtful comments on our recently published systematic review. Your question/comment was a one that we have thought of and discussed quite extensively prior to the publication of the review.

Although we agree that the efficacy of the probiotics in prevention of NEC or mortality holds true for the ELBW infant, we could not ensure the safety of this new intervention in a highly vulnerable group with the number of infants enrolled; especially with few cases of probiotics species sepsis reported in the literature.

Contributors

Khalid M. Al-Faleh, July 2008

WHAT'S NEW

Last assessed as up-to-date: 2 November 2010.

Date	Event	Description
3 November 2010	New citation required and conclusions have changed	With the addition of seven new trials to this update, it brings the total to sixteen eligible trials randomizing 2842 infants. The previous review included nine eligible trials, randomizing 1425 infants.
3 November 2010	New search has been performed	This updates the review "Probiotics for prevention of necrotizing enterocolitis in preterm infants" published in the Cochrane Database of Systematic Reviews (Al Faleh 2008). New authorship: Khalid AlFaleh, Jasim Anabrees, Dirk Bassler, Turki Al-Kharfi. Updated search identified seven new trials for inclusion in this review update.

HISTORY

Protocol first published: Issue 4, 2005

Review first published: Issue 1, 2008

Date	Event	Description
12 November 2008	Feedback has been incorporated	Feedback incorporated
22 July 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

KA developed the protocol.

KA and DB assessed trials for eligibility, quality and extracted the data independently for initial review.

KA wrote the manuscript with revisions made by DB.

KA, TA and JA updated the review.

DECLARATIONS OF INTEREST

None

SOURCES OF SUPPORT

Internal sources

- McMaster University Medical Center, Canada.

External sources

- No sources of support supplied

INDEX TERMS

Medical Subject Headings (MeSH)

Enterocolitis, Necrotizing [*prevention & control]; Infant, Newborn; Infant, Premature; Probiotics [*therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Humans