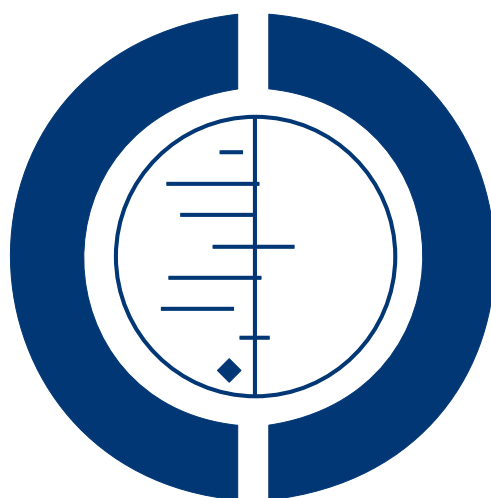


Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants (Review)

Venkatesh MP, Abrams SA



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[Intervention Review]

Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

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ABSTRACT

Background

Lactoferrin, a normal component of human colostrum, milk, tears and saliva can enhance host defence and may be effective in the prevention of sepsis and necrotizing enterocolitis (NEC) in preterm neonates.

Objectives

To assess the safety and effectiveness of oral lactoferrin in the prevention of sepsis and NEC in preterm neonates.

Search strategy

The Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library), MEDLINE and PREMEDLINE (1966 to Oct 2009), EMBASE (1980 to Oct 2009) and CINAHL (1982 to Oct 2009) were searched. Ongoing trials at www.clinicaltrials.gov and www.controlled-trials.com were searched. Conference proceedings of Pediatric Academic Societies (American Pediatric Society, Society for Pediatric Research and European Society for Pediatric Research) were searched for abstracts 1990 from the journal 'Pediatric Research' and 'Abstracts Online'.

Selection criteria

Randomized or quasi-randomized controlled trials evaluating oral lactoferrin at any dose or duration for the prophylaxis of sepsis or NEC in preterm neonates.

Data collection and analysis

Data collection and analysis were performed according to the standard methods of the CNRG.

Main results

One trial ([Manzoni 2008](#)) that randomized 472 very low birth weight infants was eligible. A statistically significant reduction in late-onset sepsis was observed in the groups that received either lactoferrin alone (RR 0.34, 95% CI 0.17, 0.70; RD -0.11, 95% CI -0.18, -0.05; NNT 9, 95% CI 5, 20) or in combination with *Lactobacillus rhamnosus* GG (RR 0.27, 95% CI 0.12, 0.60; RD -0.13, 95% CI -0.19, -0.06; NNT 8, 95% CI 5, 17).

In subgroup analyses, infants weighing less than 1000 g and those fed exclusively on maternal milk had significant reduction in late-onset sepsis after oral lactoferrin supplementation alone. In the group supplemented with oral lactoferrin and *Lactobacillus rhamnosus*, infants weighing less than 1000 g had a significant reduction in late-onset sepsis, but not exclusively maternal milk fed infants.

Prophylaxis with oral lactoferrin alone did not reduce the incidence of NEC (RR 0.33, 95% CI 0.09, 1.17; RD -0.04, 95% CI -0.08, 0.00), but a significant reduction in NEC with combination of lactoferrin with *Lactobacillus rhamnosus* GG was noted (RR 0.05, 95% CI 0.00, 0.90; RD -0.06, 95% CI -0.10, -0.02; NNT17, 95% CI 10, 50).

No adverse effects due to lactoferrin were observed in this study. Long-term neurological outcomes were not assessed in this trial.

Authors' conclusions

Oral lactoferrin prophylaxis reduces the incidence of late-onset sepsis in infants weighing less than 1500 g and most effective in infants weighing less than 1000 g. There is no evidence of efficacy of oral lactoferrin (given alone) in the prevention of NEC in preterm neonates.

Well designed, randomized trials should address dosing, duration, type of lactoferrin (bovine or human) prophylaxis in prevention of sepsis and NEC. The effect of exclusive maternal milk feeding should be clarified.

PLAIN LANGUAGE SUMMARY

Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Premature babies are at risk from blood infection (sepsis) and/or gastrointestinal injury (necrotizing enterocolitis or NEC). A number of babies with sepsis or NEC die or suffer from long-term brain and lung injury in spite of treatment with antibiotics. Lactoferrin, which is present in human milk, has been effective against infections when tested in animals and in the laboratory. Lactoferrin also enhances the ability of babies to fight infection. This review found one study conducted in Italy that used lactoferrin to prevent sepsis and NEC in preterm infants. In this study, supplementing lactoferrin in the milk of infants weighing less than 1500 g reduces infection after 72 hours of life, but not NEC. We recommend that the findings of this study be confirmed in future studies as regards safety, dosing, duration and type of lactoferrin in preventing infections and NEC in the preterm babies.

BACKGROUND

Description of the condition

Neonatal sepsis is the most common cause of neonatal deaths worldwide (Lawn 2006). The incidence of neonatal sepsis in the developed world is reported to be between one to four cases per 1000 live births (Stoll 2004b). In the developing world, the rate of neonatal sepsis is significantly higher (6.5 to 38 per 1000 live hospital births) (Zaidi 2005). Sepsis is a particular problem in very low birth weight infants (VLBW, birth weight < 1500 g); early onset sepsis (sepsis in infants < 72 hrs of life) occurs in about 1.5% and late-onset sepsis in about 21% of VLBW infants (Stoll 2005; Stoll 2002). Most infections are caused by *Staphylococci* and *Candida*. Mortality and morbidity (including patent ductus arteriosus, prolonged ventilation, prolonged need for intravascular access, bronchopulmonary dysplasia, necrotizing enterocolitis

and length of hospital stay) are significantly increased in infected infants. In a large cohort study of infants born weighing < 1000 g, infected infants had significantly higher incidence of adverse neurodevelopmental outcomes at follow-up when compared to uninfected infants (Stoll 2004a).

Necrotizing enterocolitis (NEC) occurs in 1% to 5% of admissions to the neonatal intensive care unit (Lin 2006). The most consistent risk factors are prematurity and low birth weight. Gastrointestinal immaturity, enteral feeding (especially formula feeding), presence of bacteria and inflammation in the gastrointestinal tract may all contribute to the development of NEC (Lin 2006). Host-pathogen interactions trigger inflammation in the gut, which may contribute to the pathogenesis of NEC and septic shock (Blackwell 1997; Neish 2004). NEC significantly increases mortality (attributable mortality of 15 to 30%) and morbidity (including surgery in 20% to 40% of infants and delayed neurodevelopment) (Bell 1978; Lin 2006; Stoll 2004a).

Mortality and morbidity due to sepsis and NEC remain high despite the use of potent antimicrobial agents (Stoll 2005; Stoll 2002). Increased use of antimicrobials has led to the emergence of antibiotic resistant strains of bacteria (Levy 1998). Adverse pulmonary and neurodevelopmental outcomes after sepsis or NEC may be due to inflammatory injury (Speer 1999; Adams-Chapman 2006). Agents that modulate inflammation and enhance host defences may improve the outcome of infants with neonatal sepsis or NEC.

Description of the intervention

The glycoprotein lactoferrin is a component of the innate immune response. It is found in significant concentrations in human colostrum and in lower concentrations in human milk, tears, saliva, seminal fluid and secondary granules of neutrophils. Lactoferrin has broad-spectrum antimicrobial activity against bacteria, fungi, viruses and protozoa resulting from either its ability to sequester iron or to a direct lytic effect on microbial cell membranes (Valenti 2005). Proteolysis of lactoferrin under acidic conditions (as would occur in the stomach or in the phagolysosomes of neutrophils) yields peptides called lactoferricins that have enhanced antimicrobial activity (Gifford 2005).

How the intervention might work

Lactoferrin inhibits the growth of *S. epidermidis* and *C. albicans in vitro* (Valenti 2005). It reduces the minimum inhibitory concentrations of vancomycin against *S. epidermidis* and antifungal agents such as azoles and amphotericin against *Candida* (Kuipers 1999; Leitch 1999). Lactoferrin and lactoferrin derived peptides are highly effective against antibiotic resistant *Klebsiella* and *S. aureus in vitro* (Nibbering 2001).

Lactoferrin prophylaxis is effective in animal models of systemic and intestinal infection. In mice infected with *E. Coli*, pretreatment with lactoferrin improved survival from 4% to 70% (Zagulski 1989). In neonatal rats, lactoferrin reduced the severity of blood and liver infection after enteral infection with *E. coli* (Edde 2001). Parenteral prophylaxis with lactoferrin enhanced survival in a neonatal rat model of polymicrobial infection with *C. albicans* and *S. epidermidis* (Venkatesh 2007). In a germ-free, colostrum deprived piglet model that is challenged with *E. coli* lipopolysaccharide, oral pretreatment with lactoferrin reduced mortality from 74% to 17% after challenge with *E. coli* lipopolysaccharide (Artym 2004). In animal colitis, lactoferrin reduced intestinal injury and inflammation (Togawa 2002). The systemic effects of oral lactoferrin are generally thought to be indirect and probably initiated by contact with intestinal epithelial cells and gut associated lymphoid tissues (GALT). Lactoferrin modulates cytokine and/or chemokine production by the GALT cells, which then enter the systemic circulation and influence circulating

leukocytes (Bellamy 1992; Tomita 2002). Lactoferrin and other similar products in milk (prebiotics) create an environment for the growth of beneficial bacteria in the gut, reducing colonization with pathogenic bacteria. The fact that intestinal receptors for lactoferrin have been demonstrated and that lactoferrin has the ability to modulate intestinal cell differentiation and proliferation (Buccigrossi 2007) makes lactoferrin a promising agent in the prevention or treatment of NEC.

In adult humans, oral recombinant human lactoferrin has been found to be safe and well tolerated. Oral lactoferrin has shown promise as an antitumor agent (Hayes 2006). Oral lactoferrin has been shown to reduce viremia in chronic hepatitis C infection (Tanaka 1999; Iwasa 2002). In patients with acute myeloid leukemia and neutropenia, lactoferrin reduced the incidence, duration and severity of bacteraemia due to enteric pathogens (Trumpler 1989). To date, no significant adverse effects have been reported in either animal or human studies.

Why it is important to do this review

The potential beneficial effects make lactoferrin a promising agent for prevention of neonatal sepsis and NEC. This review evaluates the role of oral lactoferrin in the prevention of neonatal sepsis or NEC.

OBJECTIVES

Primary objective:

To assess safety and effectiveness of oral lactoferrin in the prevention of mortality, sepsis and/or NEC in preterm neonates.

Secondary objectives:

1. To determine the effect of oral lactoferrin used for prophylaxis of neonatal sepsis and/or NEC on the duration of positive pressure ventilation, development of chronic lung disease (CLD), periventricular leukomalacia (PVL), length of hospital stay in survivors to discharge and adverse neurological outcome at two years of age or later;
2. To determine the adverse effects of oral lactoferrin in the prophylaxis of neonatal sepsis and/or NEC.

The following subgroups will be analyzed if data are available:

1. Gestational age: < 32 weeks, 32 to 36 weeks;
2. Birth weight: < 1000 g (ELBW infants) and birth weight < 1500 g (VLBW infants);
3. Type of feeding: breast milk and formula milk.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized or quasi-randomized controlled trials that have been completed (published or unpublished).

Types of participants

Preterm (< 37 completed weeks of gestation) neonates (< 28 days).

Types of interventions

Oral lactoferrin at any dosage or duration used to prevent neonatal sepsis or NEC compared with placebo or no intervention. Separate analyses were performed for oral lactoferrin given with or without additional probiotics.

Types of outcome measures

Primary outcomes

1. Confirmed or suspected sepsis during hospital stay.
Confirmed sepsis is defined as clinical signs and symptoms consistent with infection and microbiologically proven with a positive blood culture, CSF culture, urine culture (obtained by a suprapubic tap) or culture from a normally sterile site (e.g. pleural fluid, peritoneal fluid or autopsy specimens) for bacteria or fungi.
Suspected sepsis is defined as clinical signs and symptoms consistent with sepsis without isolation of a causative organism.
2. NEC Bell's stage II or III during hospital stay.
Necrotizing enterocolitis (NEC) (definite NEC and perforated NEC, Bell's stage II or III) (Bell 1978) during hospital stay.
3. 'All cause mortality' during hospital stay.

Secondary outcomes

Neurological outcome at two years of age or more (neurodevelopmental outcome assessed by a validated test).
Chronic lung disease (CLD) in survivors (CLD defined as oxygen requirement at 36 weeks postmenstrual age).

Adverse outcomes directly attributable to oral lactoferrin: increased gastric residuals (gastric aspirate greater than 10% of oral feed), vomiting and other GI disturbances during hospital stay.
Periventricular leukomalacia (PVL) [defined as necrosis of brain white matter in a characteristic distribution, i.e. in the white matter dorsal and lateral to the external angles of lateral ventricles

involving particularly the centrum semi ovale, optic and acoustic radiations and diagnosed by magnetic resonance imaging (MRI) or as periventricular cystic lesions by cranial ultrasound (Volpe 1995) at discharge or at neurodevelopmental follow-up].

Duration of assisted ventilation through an endotracheal tube measured in days during hospital stay.

Length of hospital stay measured in days for survivors to discharge.
Post hoc analyses were included regarding, bacterial infection, fungal infection, threshold retinopathy of prematurity and urinary tract infection.

Key subgroups will be based on:

a) Gestational age:

Preterm infants (32 to 36 weeks gestational age)

Preterm infants (< 32 weeks of gestational age)

b) Birth weight:

VLBW infants (birth weight < 1500 g)

ELBW infants (birth weight < 1000 g)

c) Feedings:

Breast milk feeding

Formula feeding

Search methods for identification of studies

The standard search method of the Cochrane Neonatal Review Group was used.

Electronic searches

Relevant trials were identified through:

1. The Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library);
2. Electronic journal reference databases: MEDLINE (1966 to present) and PREMEDLINE, EMBASE (1980 to present), CINAHL (1982 to present);
3. Ongoing trials were searched at the following web sites: www.clinicaltrials.gov and www.controlled-trials.com;
3. Abstract of conferences - proceedings of Pediatric Academic Societies (American Pediatric Society, Society for Pediatric Research and European Society for Pediatric Research) were searched from 1990 from the journal 'Pediatric Research' and 'Abstracts Online'. Search strategy for MEDLINE and PREMEDLINE. This strategy was adapted to suit EMBASE, CINAHL and the Cochrane Central Register of Controlled Trials.
1 explode 'sepsis' [all subheadings in MIME, MJME]
2 sepsis or septicemia
3 septic
4 NEC
5 'necrotizing enterocolitis'
6 # 1 or # 2 or # 3 or # 4 or # 5
7 explode 'infant - newborn' [all subheadings in MIME, MJME]
8 Neonat*
9 Newborn*

10 # 7 or # 8 or # 9

11 # 6 and # 10

12 'lactoferrin' [all subheadings on MIME, MJME]

13 Talactoferrin

14 # 10 or # 11

15 # 9 and # 12

No language restriction was applied. Randomized and quasi-randomized trials were searched from the results by reviewing the abstracts.

Searching other resources

Authors who published in this field were contacted for possible unpublished articles.

Additional searches were made from the reference list of identified clinical trials and in the review author's personal files.

Data collection and analysis

The standard methods of the Cochrane Neonatal Review Group (CNRG) for conducting a systematic review were used (<http://neonatal.cochrane.org/en/index.html>).

Selection of studies

The titles and the abstracts of studies identified by the search strategy were assessed for eligibility for inclusion in this review by the two review authors. If this could not be done reliably by title and abstract, then the full text version was obtained for assessment. Any differences were resolved by mutual discussion. Full text version of all eligible studies were obtained for quality assessment.

Data extraction and management

Forms were designed for trial inclusion/exclusion, data extraction and for requesting additional published information from authors of the original reports. Data extraction was done independently by the review authors using specifically designed paper forms.

Assessment of risk of bias in included studies

The standardized review methods of the CNRG were used to assess the methodological quality of the included study. The two review authors independently used the criteria developed by the CNRG and assessed the quality of the included study. The eligible trial was assessed for the criteria listed below and marked as: A) yes, B) can't tell or C) no.

A - Adequate allocation concealment

B - Uncertainty about whether the allocation was adequately concealed

C - Inadequate allocation concealment

D - No allocation concealment

Blinding of interventions, blinding of outcome assessment and completeness of follow-up of all randomized infants were reported. This information was added to the table 'Characteristics of Included Studies'.

In addition, the following issues were evaluated and entered into the the Risk of Bias table:

1. Sequence generation: Was the allocation sequence adequately generated?

2. Allocation concealment: Was allocation adequately concealed?

3. Blinding of participants, personnel and outcome assessors: Was knowledge of the allocated intervention adequately prevented during the study? At study entry? At the time of outcome assessment?

4. Incomplete outcome data: Were incomplete outcome data adequately addressed?

5. Selective outcome reporting: Are reports of the study free of suggestion of selective outcome reporting?

6. Other sources of bias: Was the study apparently free of other problems that could put it at a high risk of bias?

Measures of treatment effect

Statistical analyses were performed according to the recommendations of the CNRG. All randomized infants were analyzed on 'an intention to treat basis' irrespective of whether or not they received their allocated treatment. Treatment effects in the individual trials were analyzed. The statistical package (RevMan 5) provided by the Cochrane Collaboration was used. Relative risk (RR) and risk difference (RD) with 95% confidence interval (CI) were reported for dichotomous outcomes and weighted mean differences for continuous outcomes. NNT was calculated for statistically significant reduction in RD and reported.

Assessment of heterogeneity

Heterogeneity of treatment effects between trials was to be assessed using the I^2 statistic if more than one eligible trial was identified. The degree of heterogeneity would be graded as low (> 25%), moderate (> 50%) or high (> 75%). If we detected statistical heterogeneity, we planned to explore the possible causes (for example, differences in study quality, participants, intervention regimens or outcome assessments) using *post hoc* sub group analyses.

Data synthesis

If multiple studies were identified and meta-analysis was judged to be appropriate, the analysis would have been performed using Review Manager software (RevMan 5), supplied by the Cochrane Collaboration. For estimates of typical relative risk and risk difference, we planned to use the Mantel-Haenszel method. For measured quantities, we planned to use the inverse variance method. All meta-analyses were to be done using the fixed effect model.

Subgroup analysis and investigation of heterogeneity

Key subgroups will be based on:

a) Gestational age:

Preterm infants (32 to 36 weeks gestational age)

Preterm infants (< 32 weeks of gestational age)

b) Birth weight:

VLBW infants (birth weight < 1500 g)

ELBW infants (birth weight < 1000 g)

c) Feedings:

Breast milk feeding

Formula feeding

RESULTS

Description of studies

See: [Characteristics of included studies](#); [Characteristics of excluded studies](#); [Characteristics of studies awaiting classification](#); [Characteristics of ongoing studies](#).

Our search strategy identified one eligible trial of oral lactoferrin prophylaxis of sepsis or NEC \geq Stage II in premature neonates (Manzoni 2009).

Included Study

In eleven Italian neonatal intensive care units, Manzoni and coworkers (Manzoni 2009) randomized very low birth weight infants (birth weight < 1500 g) to oral bovine lactoferrin or bovine lactoferrin in combination with *Lactobacillus rhamnosus* GG or placebo. Late-onset sepsis defined as isolation of a pathogen in the blood, peritoneal fluid or CSF after three days of life was the primary outcome of interest. Secondary outcomes that were assessed were gram-positive, gram-negative or fungal sepsis, mortality prior to hospital discharge, urinary tract infection, fungal colonization, progression from fungal colonization to invasive fungal infection, bronchopulmonary dysplasia, severe intraventricular hemorrhage (grade III or IV), threshold retinopathy of prematurity, NEC \geq Stage II, alteration of liver functions and adverse effects. Please refer to table 'Characteristics of Included Studies' for more details.

Risk of bias in included studies

Selection bias (Grade B): Randomization was stratified by center and randomization sequences were generated by computer software. The pharmacy at each center prepared the interventions and diluted them in the milk feeds based on the random sequences. Allocation concealment is unclear as it is difficult to predict whether the pharmacy was aware of future allocations.

Performance bias: Interventions were diluted in feeds and clinical and research staff were blinded to the intervention. In the situation where the infant is not fed and interventions were administered by

the oro-gastric tube without milk, it is not clear whether blinding was adequate.

Detection bias: Not explicit regarding of blinding of outcome assessors.

Completeness of follow-up: All assessments were before hospital discharge and follow-up complete. Incomplete data was adequately accounted for.

Effects of interventions

Data for analyses for both comparisons and all outcomes are from one trial (Manzoni 2009) in which 472 infants were randomized to oral bovine lactoferrin or oral bovine lactoferrin in combination with *Lactobacillus rhamnosus* GG or placebo. Subgroup analyses using birth weight and type of milk subgroups for late-onset sepsis were performed for the outcome of 'late onset sepsis'. Data for subgroup analyses for other outcomes were not available.

LACTOFERRIN ALONE vs. PLACEBO (Comparison 1):

Late-onset sepsis (Outcome 1.1):

All Infants (Outcome 1.1.1):

Overall, nine of 153 infants in the lactoferrin group and 29 of 168 in the placebo group were diagnosed with late-onset sepsis. Diagnosis of late-onset sepsis was significantly lower in the group treated with oral bovine lactoferrin (RR 0.34, 95% CI 0.17, 0.70; RD -0.11, 95% CI -0.18, -0.05; NNT 9, 95% CI 5, 20).

Subgroup analyses:

Birth weight < 1000 grams (Outcome 1.1.2):

Six of 53 infants in the lactoferrin group and 22 of 60 in the placebo group were born weighing less than 1000 g and were diagnosed with late-onset sepsis. There was a significant difference in the diagnosis of late-onset sepsis in this subgroup after oral lactoferrin supplementation (RR 0.31, 95% CI 0.14, 0.70; RD -0.25, 95% CI -0.40, -0.10; NNT 4, 95% CI 2, 10).

Birthweight 1000 to 1500 grams (Outcome 1.1.3):

Three of 100 infants in the lactoferrin group and seven of 108 in the placebo group were born weighing 1000 to 1500 g and were diagnosed with late-onset sepsis. There was no statistical difference in the diagnosis of late-onset sepsis in this subgroup after oral lactoferrin supplementation (RR 0.46, 95% CI 0.12, 1.74; RD -0.03, 95% CI -0.09, -0.02).

Exclusively maternal milk fed infants (Outcome 1.1.4):

One of 42 infants in the lactoferrin group and seven of 37 in the placebo group were diagnosed with late-onset sepsis. There was a significant difference in the diagnosis of late-onset sepsis in this subgroup after oral lactoferrin supplementation (RR 0.13, 95% CI 0.02, 0.98; RD -0.17, 95% CI -0.30, -0.03; NNT 6 (3, 33)).

Exclusively formula fed infants (Outcome 1.1.5):

One of 24 infants in the lactoferrin group and four of 22 in the placebo group were diagnosed with late-onset sepsis. There was no statistical difference in the diagnosis of late-onset sepsis in this subgroup after oral lactoferrin supplementation (RR 0.23; 95% CI 0.03, 1.90; RD -0.14, 95% CI -0.32, -0.04).

Bacterial sepsis (Outcome 1.2):

Nine of 153 infants in the lactoferrin group and 20 of 168 in the placebo group were diagnosed with bacterial sepsis. There was no statistical difference in the diagnosis of bacterial sepsis after oral lactoferrin supplementation (RR 0.49; 95% CI 0.23, 1.05; RD -0.06, 95% CI -0.12, 0.00).

Fungal sepsis (Outcome 1.3):

None of 153 infants in the lactoferrin group and 9 of 168 in the placebo group were diagnosed with fungal sepsis. There was a significant difference in the diagnosis of fungal sepsis after oral lactoferrin supplementation (RR 0.06; 95% CI 0.00, 0.98; RD -0.05, 95% CI -0.09, -0.02; NNT 20, 95% CI 11, 50).

All cause mortality (Outcome 1.4):

Four of 153 infants in the lactoferrin group and 12 of 168 in the placebo group expired. There was no statistical difference in 'all cause mortality' after oral lactoferrin supplementation (RR 0.37, 95% CI 0.12, 1.11; RD -0.05; 95% CI -0.09, 0.00).

NEC \geq Stage II (Outcome 1.5):

Three of 153 infants in the lactoferrin arm and 10 of 168 in the placebo group were diagnosed with NEC stage \geq 2. There was no statistical difference in the diagnosis of NEC stage \geq 2 after oral lactoferrin supplementation (RR 0.33, 95% CI 0.09, 1.17; RD -0.04, 95% CI -0.08, 0.00).

Urinary tract infection (Outcome 1.6):

Four of 153 infants in the lactoferrin arm and 10 of 168 in the placebo group were diagnosed with urinary tract infection. There was no statistical difference in the diagnosis of urinary tract infection after oral lactoferrin supplementation (RR 0.44, 95% CI 0.14, 1.37; RD -0.03, 95% CI -0.08, 0.01).

Chronic lung disease (Outcome 1.7):

Manzoni 2009 et al, defined chronic lung disease as oxygen requirement more than 30% for 28 days, positive pressure ventilation at 36 weeks or both. We have requested data from the authors for infants who required oxygen at 36 weeks corrected age.

Based on study definition, four of 153 infants in the lactoferrin group and six of 168 in the placebo group were diagnosed with chronic lung disease. There was no statistical difference in chronic lung disease after oral lactoferrin supplementation (RR 0.73, 95% CI 0.21, 2.54; RD -0.01, 95% CI -0.05, 0.03).

Threshold retinopathy of prematurity (Outcome 1.8):

Six of 153 infants in the lactoferrin group and 19 of 168 in the placebo group were diagnosed with threshold retinopathy of prematurity. There was a significant statistical difference in the diagnosis of threshold retinopathy of prematurity after oral lactoferrin supplementation (RR 0.35, 95% CI 0.14, 0.85; RD -0.07, 95% CI -0.13, -0.02; NNT 14, 95% CI 8, 50).

ORAL BOVINE LACTOFERRIN + LACTOBACILLUS GG vs. PLACEBO (Comparison 2):**Late-onset sepsis (Outcome 2.1):****All infants (Outcome 2.1.1):**

Overall seven of 151 infants in the lactoferrin + Lactobacillus rhamnosus GG group and 29 of 168 in the placebo group were

diagnosed with late-onset sepsis. Diagnosis of late-onset sepsis was significantly lower in the group treated with oral bovine lactoferrin + Lactobacillus rhamnosus GG (RR 0.27, 95% CI 0.12, 0.60; RD -0.13, 95% CI -0.19, -0.06; NNT 8, 95% CI 5, 17).

Subgroup analyses:**Birth weight < 1000 grams (Outcome 2.1.2):**

Six of 54 infants in the lactoferrin + Lactobacillus rhamnosus GG group and 22 of 60 in the placebo group were born weighing less than 1000 g and were diagnosed with late-onset sepsis. There was a significant difference in this subgroup in the diagnosis of late-onset sepsis after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.30, 95% CI 0.13, 0.69; RD -0.26, 95% CI -0.40, -0.11; NNT 5, 95% CI 2, 9).

Birthweight 1000 to 1500 grams (Outcome 2.1.3):

One of 97 infants in the lactoferrin arm and seven of 108 in the placebo group were born weighing 1000 to 1500 g and were diagnosed with late-onset sepsis. There was no statistical difference in the diagnosis of late-onset sepsis in this subgroup after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.16, 95% CI 0.02, 1.27; RD -0.05, 95% CI -0.11, 0.0).

Exclusively maternal milk fed infants (Outcome 2.1.4):

Two of 32 infants in the lactoferrin arm and seven of 37 in the placebo group were exclusively maternal milk fed and were diagnosed with late-onset sepsis. There was no significant difference in the this subgroup in the diagnosis of late-onset sepsis after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.33, 95% CI 0.07, 1.48; RD -0.13; 95% CI -0.28, 0.02).

Exclusively formula fed infants (Outcome 2.1.5):

None of 26 infants in the oral lactoferrin + Lactobacillus rhamnosus GG group and four of 22 in the placebo group were exclusively formula fed and were diagnosed with late-onset sepsis. There was no statistical difference in this subgroup in the diagnosis of late-onset sepsis after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.09, 95% CI 0.01, 1.67; RD -0.18, 95% CI -0.35, -0.01).

Bacterial sepsis (Outcome 2.2):

Five of 151 infants in the lactoferrin + Lactobacillus rhamnosus GG group and 20 of 168 in the placebo group were diagnosed with bacterial sepsis. There was a significant difference in the diagnosis of bacterial sepsis after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.28, 95% CI 0.11, 0.72; RD -0.09, 95% CI -0.14, -0.03 ; NNT 11, 95% CI 7, 33).

Fungal sepsis (Outcome 2.3):

Two of 151 infants in the lactoferrin + Lactobacillus rhamnosus GG group and nine of 168 in the placebo group were diagnosed with fungal sepsis. There was no significant difference in the diagnosis of fungal sepsis after oral lactoferrin + Lactobacillus rhamnosus GG supplementation (RR 0.25, 95% CI 0.05, 1.13; RD -0.04 ; 95% CI -0.08, -0.00).

All cause mortality (Outcome 2.4):

Six of 151 infants in the oral lactoferrin + Lactobacillus rhamnosus GG group and 12 of 168 in the placebo group expired. There was

no statistical difference in all cause mortality after oral lactoferrin + *Lactobacillus rhamnosus* GG supplementation (RR 0.56, 95% CI 0.21, 1.45; RD -0.03, 95% CI -0.08, 0.02).

NEC \geq Stage II (Outcome 2.5):

None of 151 infants in the oral lactoferrin + *Lactobacillus rhamnosus* GG group and 10 of 168 in the placebo group were diagnosed with NEC stage \geq 2. There was a significant statistical difference in the diagnosis of NEC stage \geq 2 after oral lactoferrin + *Lactobacillus rhamnosus* GG supplementation (RR 0.05, 95% CI 0.00, 0.90; RD -0.06, 95% CI -0.10, -0.02; NNT17, 95% CI 10, 50).

Urinary tract infection (Outcome 2.6):

Six of 151 infants in the lactoferrin + *Lactobacillus rhamnosus* GG group and 10 of 168 in the placebo group were diagnosed with urinary tract infection. There was no significant difference in the diagnosis of urinary tract infection after oral lactoferrin + *Lactobacillus rhamnosus* GG supplementation (RR 0.67, 95% CI 0.25, 1.79; RD -0.02; 95% CI -0.07, 0.03).

Chronic lung disease (Outcome 2.7):

Study definition of chronic lung disease was oxygen requirement more than 30% for 28 days, positive pressure ventilation at 36 weeks or both. We have requested data from the authors for infants who required oxygen at 36 weeks corrected age.

Based on study definition, four of 151 infants in the lactoferrin + *Lactobacillus rhamnosus* GG group and six of 168 in the placebo group were diagnosed with chronic lung disease. There was no statistical difference in chronic lung disease after oral lactoferrin+LGG supplementation (RR 0.74, 95% CI 0.21, 2.58; RD -0.01, 95% CI -0.05, 0.03).

Threshold retinopathy of prematurity (Outcome 2.8):

Thirteen of 151 infants in the lactoferrin + *Lactobacillus rhamnosus* GG group and 19 of 168 in the placebo group were diagnosed with threshold retinopathy of prematurity. There was no significant difference in the diagnosis of threshold retinopathy of prematurity after oral lactoferrin + *Lactobacillus rhamnosus* GG supplementation (RR 0.76, 95% CI 0.39, 1.49; RD -0.03; 95% CI -0.09, 0.04).

Other outcomes

No adverse effects were reported in the study.

The following outcomes were not reported in the study and hence not analyzed in this review: neurological outcome at two years of age or more, periventricular leukomalacia, duration of assisted ventilation through an endotracheal tube or length of hospital stay.

DISCUSSION

Our search strategy identified one eligible trial (Manzoni 2009) that randomized 472 VLBW infants to oral bovine lactoferrin or lactoferrin in combination with *Lactobacillus rhamnosus* GG or placebo. A statistically significant reduction in late-onset sepsis was found in the groups that received either lactoferrin alone (RR

0.34, 95% CI 0.17, 0.70; RD -0.11, 95% CI -0.18, -0.05; NNT 9, 95% CI 5, 20) or lactoferrin in combination with *Lactobacillus rhamnosus* GG (RR 0.27, 95% CI 0.12, 0.60; RD -0.13, 95% CI -0.19, -0.06; NNT 8, 95% CI 5, 17). In this trial (Manzoni 2009), significant reduction in late-onset sepsis was found in the subgroup of infants < 1000 g (ELBW infants) with both oral lactoferrin supplementation (RR 0.31, 95% CI 0.14, 0.70; RD -0.25, 95% CI -0.40, -0.10; NNT 4, 95% CI 2, 10) and oral lactoferrin in combination with *Lactobacillus rhamnosus* GG (RR 0.30, 95% CI 0.13, 0.69; RD -0.26, 95% CI -0.40, -0.11; NNT 5, 95% CI 2, 9). However, in infants with birth weight between 1000 g to 1500 g, no such difference was noted. With current evidence from one trial, it appears the patient population that would benefit the most are infants with birth weight < 1000 g [extremely low birth weight infants (ELBW)]. A fixed dose of 100 mg/day of oral bovine lactoferrin was used in this trial and the authors point out that the ELBW infants had a higher dosing relative to their body weight and for longer periods (up to 30 days of life vs. up to 45 days of life) compared to infants weighing 1000 g to 1500 g. Whether higher birth weight groups would also benefit if given a higher dosage or longer duration of oral lactoferrin remains to be clarified in future trials.

Prophylaxis with oral lactoferrin alone did not reduce the incidence of NEC (RR 0.33, 95% CI 0.09, 1.17; RD -0.04, 95% CI -0.08, 0), but a significant reduction in NEC was noted with the combination of lactoferrin with *Lactobacillus rhamnosus* GG (RR 0.05, 95% CI 0.00, 0.90; RD -0.06, 95% CI -0.10, -0.02; NNT17, 95% CI 10, 50). This study was not powered for detection of a difference in NEC in the intervention and the placebo groups. Future adequately powered trials are needed to address NEC as a primary outcome.

The combination of oral lactoferrin and *Lactobacillus rhamnosus* GG was evaluated in the trial (Manzoni 2009) for possible synergistic effects. In newborn rats, recombinant human lactoferrin prevented *E. coli* colonization in the gut and combination with *Lactobacillus rhamnosus* did not confer a synergistic effect (Sherman 2004). Manzoni and coworkers did not compare the bovine lactoferrin only group to that of the bovine lactoferrin combined with *Lactobacillus rhamnosus* GG head to head. However, when these two groups were compared with placebo separately, the effects on late-onset sepsis was similar (although post hoc subgroup analysis showed significant differences in bacterial and invasive fungal sepsis), but combination of bovine lactoferrin with *Lactobacillus rhamnosus* GG showed a significant reduction in NEC (RR 0.05, 95% CI 0.00, 0.90; RD -0.06, 95% CI -0.10, -0.02; NNT17, 95% CI 10, 50). The interactions of lactoferrin and lactobacilli with each other and with the host that determines the outcomes of NEC and sepsis remain to be elucidated. In the Cochrane systematic review that evaluated the role of probiotics in the prevention of NEC and sepsis involving nine eligible trials that recruited 1425 preterm or low birth weight infants (birth weight < 2500

g), enteral probiotics reduced the incidence of severe NEC (stage II or more) [typical RR 0.32 (95% CI 0.17, 0.60)] and mortality [typical RR 0.43 (95% CI 0.25, 0.75)], but not nosocomial sepsis [typical RR 0.93 (95% CI 0.73, 1.19)]. The included trials reported no systemic infection with the probiotics supplemental organism (Alfaleh 2008).

The optimum timing of prophylaxis, at least for ELBW infants, appears to be within the first three days of life based on the trial by Manzoni and coworkers (Manzoni 2009). The duration of prophylaxis with oral lactoferrin that has optimal benefits without adverse effects is still not clear in preterm neonates. In the trial by Manzoni et al, ELBW infants received prophylaxis up to 45 days of life and infants weighing 1000 g to 1500 g only up to 30 days of life. It is also not clear whether increased duration of prophylaxis is more effective in preventing late-onset sepsis or NEC.

Exclusively maternal milk fed infants experienced a statistically significant difference in the incidence of late-onset sepsis after oral lactoferrin supplementation (RR 0.13, 95% CI 0.02, 0.98; RD -0.17 95% CI -0.30, -0.03; NNT 6, 95% CI 3, 33), but not with a combination of oral bovine lactoferrin and *Lactobacillus rhamnosus* GG (RR 0.33, 95% CI 0.07, 1.48; RD -0.13, 95% CI -0.28, 0.02). The exact effect of exclusively feeding with maternal milk and lactoferrin supplementation is not clear and needs to be explored in future trials.

Oral lactoferrin prophylaxis alone or in combination with *Lactobacillus rhamnosus* GG did not decrease 'all cause mortality' or chronic lung disease. No adverse effects due to lactoferrin were observed in this study by Manzoni and coworkers (Manzoni 2009). No hepatotoxicity or cholestasis as monitored by liver enzymes or increased bilirubin requiring phototherapy and no blood cultures positive for *Lactobacillus rhamnosus* GG in the intervention groups were observed. Long-term neurological outcomes, periventricular leukomalacia, length of hospital stay and duration of intubation were not assessed in this study.

In post-hoc analyses, we found oral lactoferrin prophylaxis reduced the incidence of fungal sepsis (RR 0.06; 95% CI 0.00, 0.98; RD -0.05, 95% CI -0.09, -0.02; NNT 20, 95% CI 11, 50) and threshold retinopathy of prematurity (RR 0.35, 95% CI 0.14, 0.85; RD -0.07, 95% CI -0.13, -0.02; NNT 14, 95% CI 8, 50), but not bacterial sepsis. Oral lactoferrin in combination with *Lactobacillus rhamnosus* GG significantly reduced bacterial sepsis (RR 0.28, 95% CI 0.11, 0.72; RD -0.09, 95% CI -0.14, -0.03 ; NNT 11, 95% CI 7, 33), but not fungal sepsis or threshold retinopathy of prematurity. Oral lactoferrin alone or in combination with *Lactobacillus rhamnosus* GG did not significantly reduce the incidence of urinary tract infection. In the trial by Manzoni (Manzoni 2009), no effect of lactoferrin alone or in combination with LGG was noticed on fungal colonization or invasive fungal infection. These post hoc analyses considered outcomes that are clinically significant, but were not powered in the trial by Manzoni and coworkers

(Manzoni 2009). The role of lactoferrin in the reduction of specific types of sepsis, bacterial or fungal, and threshold retinopathy of prematurity need to be explored in well designed, adequately powered future trials.

The main hurdle for the development of neonatal trials is the establishment of safety in premature neonates, especially VLBW infants who are at high risk of developing sepsis and necrotizing enterocolitis. In the recently published trial (Manzoni 2009), no adverse effects due to oral bovine lactoferrin were observed. Bovine lactoferrin has a 69% DNA sequence homology to human lactoferrin (Pierce 1991), but *in vitro* has been shown to have higher anti-microbial effects than its human counterpart. Whether human lactoferrin is as effective *in vivo* as bovine lactoferrin or higher doses of human lactoferrin can be tolerated needs to be confirmed in future trials. A phase I, multi-centre clinical study with recombinant human lactoferrin (talactoferrin; Agennix Incorporated; NCT00854633) is underway and is designed to determine the safety and efficacy of lactoferrin in the prevention of nosocomial infections in preterm neonates whose birth weight is between 750 g to 1500 g with a dose of 150 mg/kg every 12 hours.

A randomized, double-blind, placebo controlled study of oral talactoferrin (human recombinant lactoferrin) solution for prevention of nosocomial infection in preterm infants is ongoing (Malik 2009). Premature infants with birth weight from 750 g to 1500 g are enrolled in the first 24 hours of life and administered talactoferrin at 150mg/kg twice a day or placebo (n = 396). The primary outcome to be evaluated is late-onset sepsis and secondary outcomes are NEC, length of hospital stay and all cause mortality. Cleary and coworkers are conducting a community based, double-blind, randomized placebo controlled trial (n = 602) of lactoferrin in the prevention of diarrhea in children (12 to 18 months) in Lima, Peru (Cleary 2007). The primary outcome of interest is prevention of diarrhoea over six months of supplementation and the secondary outcome is evaluation of growth in this study. Greenough and coworkers are conducting a randomized, double-blind, placebo controlled study (n = 30) in patients who are 18 years or older, evaluating the efficacy of human recombinant lactoferrin (derived from rice, Ventria Bioscience) in managing post-antibiotic colonization and infection with *C. difficile* and intestinal inflammation (Greenough 2005). A randomized safety and efficacy study in Europe is underway that is evaluating a new adaptive feeding plan for newborn infants where newborn infants are randomized to the following arms: breast feeding, basic starter formula or basic starter formula supplemented with lactoferrin and probiotics (Picaud 2010). The primary outcome of interest is gut maturation as evaluated by protein excretion in stools and secondary outcomes are evaluation of gut microbiota and growth (n = 186). These trials will delineate the effects of lactoferrin on diarrhea, antibiotic associated colitis and gut maturation.

Current increase in interest in lactoferrin stems not only from improved understanding of its physiological functions, but also due

to its increased availability in various forms and sources. Lactoferrin processed from bovine and human milk is available commercially as a food supplement (Swedish Dairies Association, Tatua Co-operative Dairy Company in New Zealand, Lacto Bretagne Associates' in Belgium, Milei in Germany, Morinaga Industries, Japan, DoMO Food Ingredients a subsidiary of Friesland Dairy Foods in the Netherlands, etc.). In the United States, human recombinant lactoferrin (talactoferrin from Agennix, Inc) has an investigational new drug status for clinical research purposes. Lactoferrin expression in transgenic rice (Ventri Biosciences) and transgenic maize (Meristem therapeutics) are being researched. Bovine lactoferrin is less expensive than human lactoferrin and should be affordable even in developing countries.

Lactoferrin is a normal component of the innate immune system and has broad-spectrum antimicrobial and immunomodulatory effects. Lactoferrin's multimodal functions suggest great promise as a prophylactic agent for the prevention of neonatal sepsis and NEC. Current evidence for use of oral lactoferrin prophylaxis stems from one trial, conducted in eleven Italian neonatal intensive care units. Components of neonatal care as well as the type and frequency of sepsis vary between neonatal intensive care units, regionally and internationally. The efficacy of lactoferrin in preventing sepsis and NEC needs to be confirmed in well designed, adequately powered, multicenter randomized controlled trials that assess long-term neurodevelopmental outcomes.

AUTHORS' CONCLUSIONS

Implications for practice

Oral lactoferrin prophylaxis reduces the incidence of late-onset sepsis in infants weighing less than 1500 g and is most effective in infants weighing less than 1000 g based on findings of one single

study. There is no evidence of efficacy of oral lactoferrin (given alone) in the prevention of NEC in preterm neonates. While oral lactoferrin holds great promise in the prevention of neonatal sepsis and NEC, several questions regarding optimal dosage, timing of administration, optimal target population or whether lactoferrin should be regulated as a food additive or a medication still remain.

Implications for research

Further studies are necessary to reproduce and refine the findings of the single study in evaluating oral lactoferrin for the prevention of sepsis and NEC. Well designed, adequately powered, randomized multicenter trials are needed to address the dosing, duration and type of lactoferrin (e.g. bovine or human) that will be most effective in prevention of sepsis and NEC without causing adverse effects or intolerance. Clinical studies are needed to clarify the effect of exclusive maternal and formula milk feeding on prevention of sepsis and necrotizing enterocolitis in preterm neonates in conjunction with oral lactoferrin prophylaxis. Clinical randomized trials evaluating lactoferrin prophylaxis should assess not only short-term beneficial effects, but also long-term neurodevelopmental and pulmonary outcomes.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Manzoni 2009

Methods	Prospective, multicenter, double blind, placebo-controlled, randomized trial.
Participants	Premature neonates with birth weight < 1500 g within the first 3 days of life. Setting: Eleven Italian neonatal intensive care units, from October 1, 2007 through July 31, 2008.
Interventions	Bovine lactoferrin (100 mg/day) alone or bovine lactoferrin (100 mg/day) with Lactobacillus rhamnosus LGG (6×10^9 CFU/ml) or placebo. The interventions were diluted in milk feeds. If the infants were not being fed, the interventions were administered through an orogastric tube.
Outcomes	Primary: Late-onset sepsis defined as isolation of a pathogen from blood, peritoneal fluid or CSF after 3 days of life. Secondary: Gram-positive, gram-negative or fungal sepsis, mortality prior to hospital discharge, urinary tract infection, fungal colonization, progression from fungal colonization to invasive fungal infection, bronchopulmonary dysplasia, severe intraventricular hemorrhage (grade III or IV), threshold retinopathy of prematurity, NEC \geq Stage II, alteration of liver functions and adverse effects.
Notes	

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Randomly allocated to one of the 3 groups using computer generated allocation sequences.
Allocation concealment?	Yes	Randomized in pharmacy and allocation concealment is adequate.
Blinding? All outcomes	Unclear	The authors report that clinical and research staff were unaware of study group as the interventions and placebo were diluted in the milk. In a group of infants who were not fed, the interventions were administered by an orogastric tube, but blinding in that situation is not clear.
Incomplete outcome data addressed? All outcomes	Yes	9/472 infants had incomplete or missing data and this has been addressed. Intent to treat analyses performed.

Manzoni 2009 (Continued)

Free of selective reporting?	No	All outcomes addressed.
Free of other bias?	Yes	
Completeness of follow up?	Yes	Assessed in the hospital prior to discharge.
Blinding of outcome assessment?	Unclear	Blinding of outcome assessors not explicit e.g. detection of intraventricular hemorrhage, bronchopulmonary dysplasia.

Characteristics of excluded studies [ordered by study ID]

King 2007	Enrolled healthy, formula-fed infants greater than or equal to 34 weeks' gestation and less than or equal to 4 weeks of age. Infants received either formula supplemented with lactoferrin (850 mg/L) or commercial cow milk-based formula (102 mg/L) for 12 months. Growth parameters and information on gastrointestinal, respiratory, and colic illnesses were collected for the infants' first year.
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Characteristics of studies awaiting assessment [ordered by study ID]**van Zoeren-Grobben**

Methods	Prospective double-blind, randomized, placebo controlled study.
Participants	Preterm infants with gestational age from 26 to > 36 weeks.
Interventions	Infants will be randomized to: 1. Standard preterm formula; 2. Standard preterm formula with prebiotics (galacto-oligosaccharides 28.5%, lactose 9.5%, galactose 0.5%, minerals 3.5%, fat 1.5% and water 3%); 3. Standard preterm formula with dairy lactoferrin 1 mg/100ml.
Outcomes	Primary: Composition of gut flora at 6 weeks of full enteral feeds, incidence of infections, oxidative stress and iron status. Secondary: Growth (weight, length and head circumference), feeding intolerance and psychomotor development at 1 year of age.
Notes	Unpublished study completed in 2009. Author contacted for data.

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

Malik 2009

Trial name or title	Study of talactoferrin oral solution for nosocomial infection in preterm infants.
Methods	Phase I/II study - randomized, double-blind, placebo controlled study.
Participants	Premature infants with birth weight from 750 g to 1500 g enrolled in the first 24 hrs of life.
Interventions	Talactoferrin, enteral at 150 mg/kg twice a day or placebo.
Outcomes	Primary: Late-onset sepsis Secondary: NEC, length of stay, all cause mortality
Starting date	June 2009
Contact information	June 2013
Notes	Estimated 396 infants will be enrolled. clinical trials.gov identifier -NCT00854633

DATA AND ANALYSES

Comparison 1. Lactoferrin alone vs. placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Any late onset sepsis	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 All infants	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.17, 0.70]
1.2 Birth weight <1000g	1	113	Risk Ratio (M-H, Fixed, 95% CI)	0.31 [0.14, 0.70]
1.3 Birth weight 1000-1500g	1	208	Risk Ratio (M-H, Fixed, 95% CI)	0.46 [0.12, 1.74]
1.4 Maternal milk fed infants	1	79	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.02, 0.98]
1.5 Formula fed infants	1	46	Risk Ratio (M-H, Fixed, 95% CI)	0.23 [0.03, 1.90]
2 Bacterial sepsis	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.49 [0.23, 1.05]
3 Fungal infection	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.00, 0.98]
4 All cause mortality	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.37 [0.12, 1.11]
5 NEC ≥ Stage II	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.09, 1.17]
6 Urinary tract Infection	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.44 [0.14, 1.37]
7 Chronic lung disease	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.73 [0.21, 2.54]
8 Threshold retinopathy of prematurity	1	321	Risk Ratio (M-H, Fixed, 95% CI)	0.35 [0.14, 0.85]

Comparison 2. Lactoferrin + LGG vs. placebo

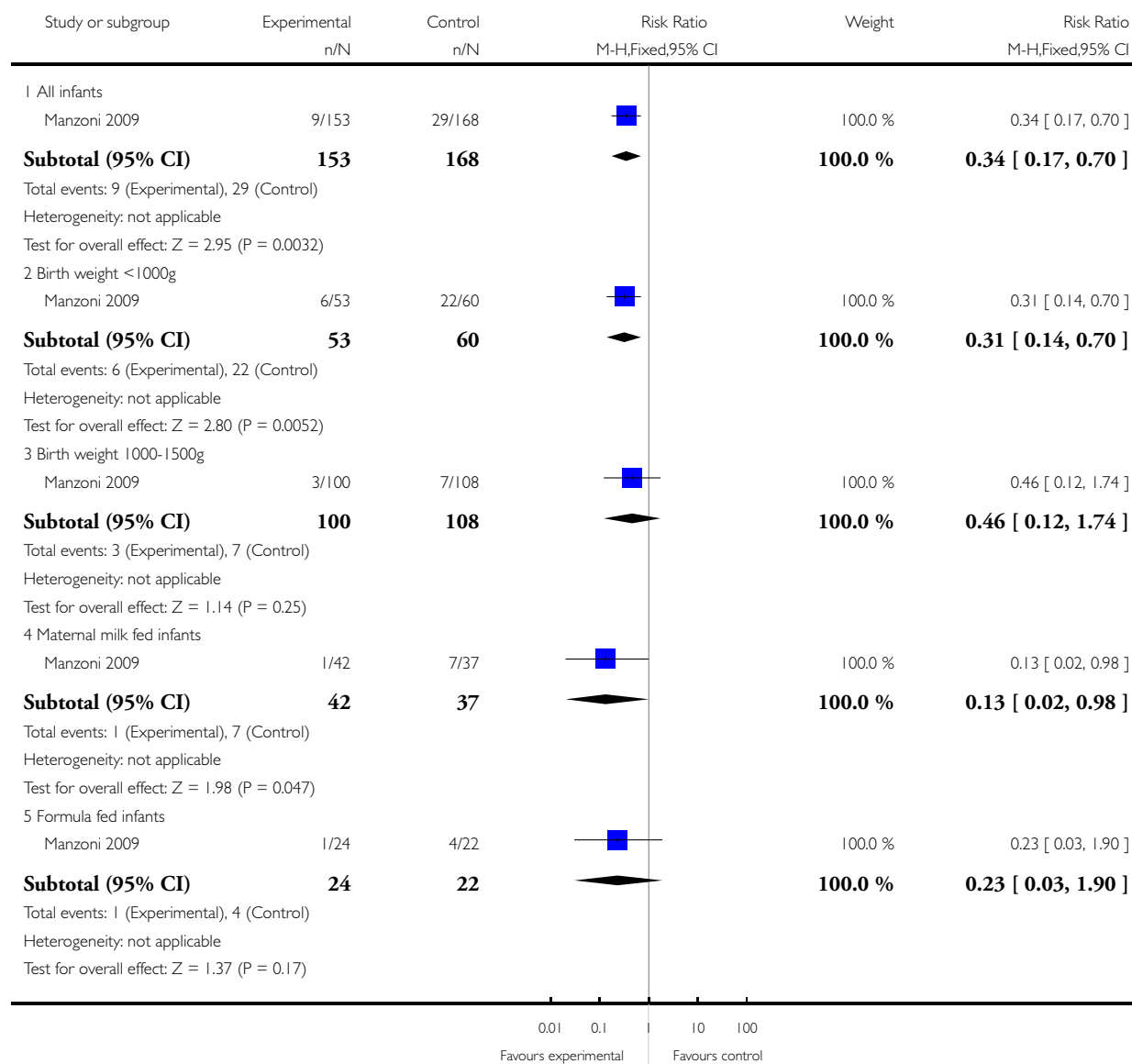
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Any late-onset sepsis	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 All infants	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.27 [0.12, 0.60]
1.2 Birth weight < 1000g	1	114	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.13, 0.69]
1.3 Birth weight 1000-1500g	1	205	Risk Ratio (M-H, Fixed, 95% CI)	0.16 [0.02, 1.27]
1.4 Maternal milk fed infants	1	69	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.07, 1.48]
1.5 Formula milk fed infants	1	48	Risk Ratio (M-H, Fixed, 95% CI)	0.09 [0.01, 1.67]
2 Bacterial sepsis	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.11, 0.72]
3 Fungal Infection	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.25 [0.05, 1.13]
4 All cause mortality	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.56 [0.21, 1.45]
5 NEC ≥ Stage II	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.05 [0.00, 0.90]
6 Urinary tract Infection	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.67 [0.25, 1.79]
7 Chronic lung disease	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.21, 2.58]
8 Threshold retinopathy of prematurity	1	319	Risk Ratio (M-H, Fixed, 95% CI)	0.76 [0.39, 1.49]

Analysis 1.1. Comparison 1 Lactoferrin alone vs. placebo, Outcome 1 Any late onset sepsis.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 1 Any late onset sepsis

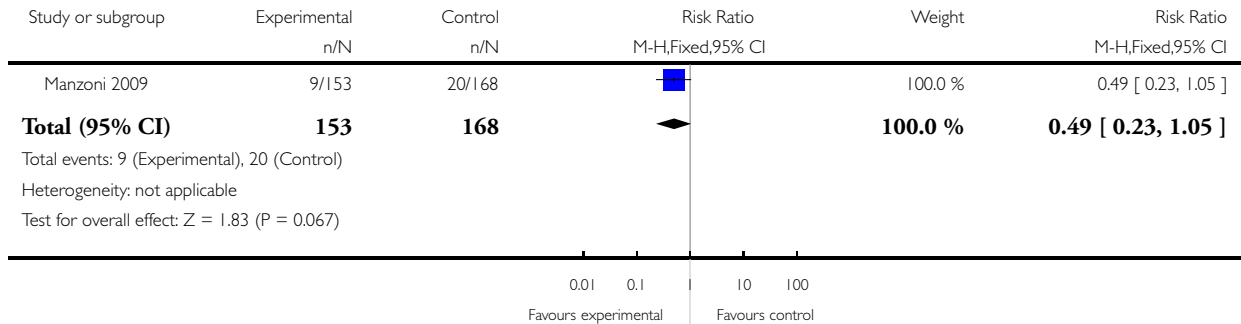


Analysis 1.2. Comparison 1 Lactoferrin alone vs. placebo, Outcome 2 Bacterial sepsis.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 2 Bacterial sepsis

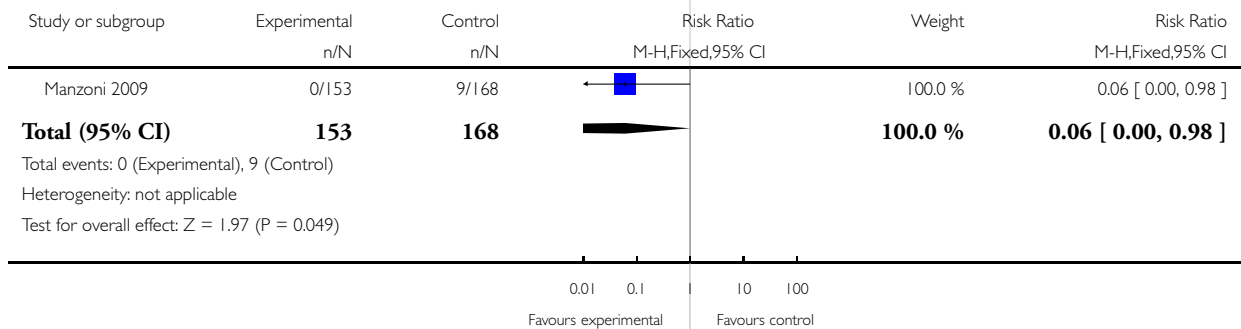


Analysis 1.3. Comparison 1 Lactoferrin alone vs. placebo, Outcome 3 Fungal infection.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 3 Fungal infection

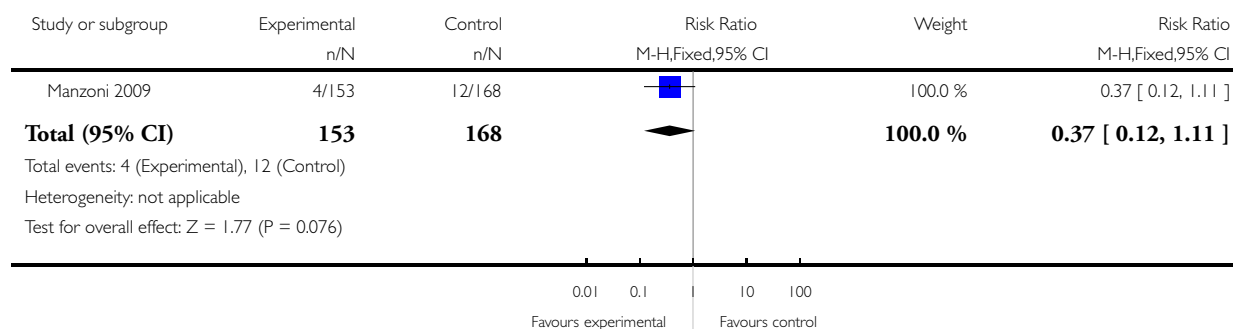


Analysis 1.4. Comparison 1 Lactoferrin alone vs. placebo, Outcome 4 All cause mortality.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 4 All cause mortality

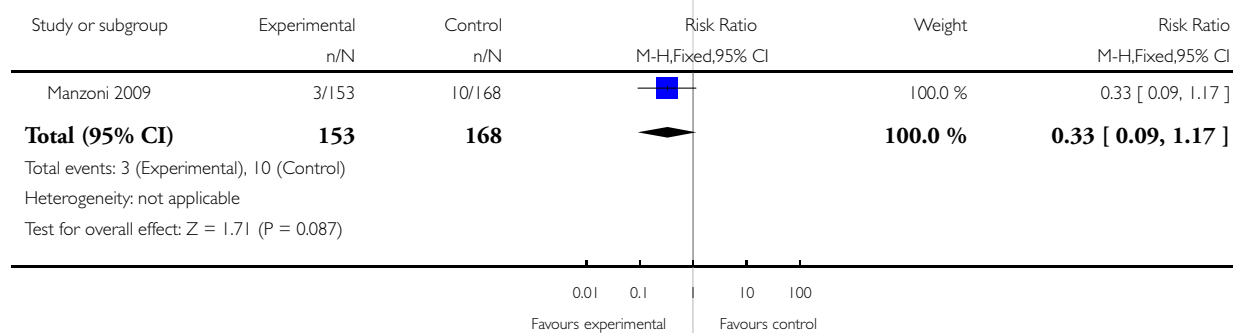


Analysis 1.5. Comparison 1 Lactoferrin alone vs. placebo, Outcome 5 NEC \geq Stage II.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 5 NEC \geq Stage II

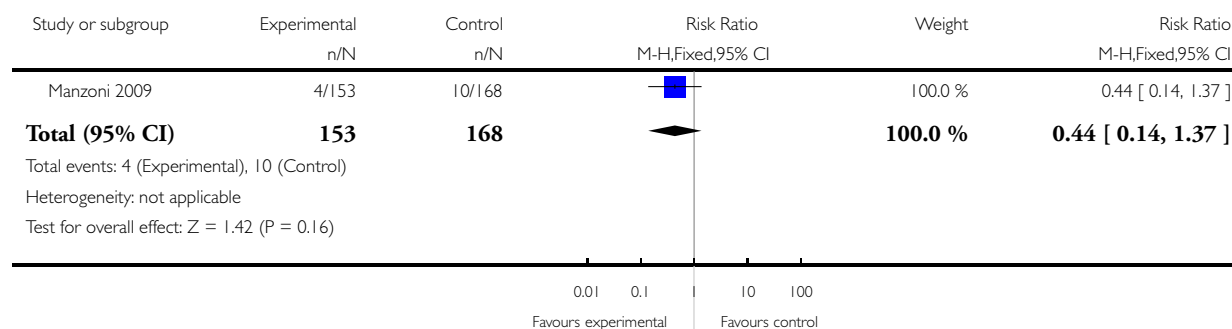


Analysis 1.6. Comparison 1 Lactoferrin alone vs. placebo, Outcome 6 Urinary tract Infection.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 6 Urinary tract Infection

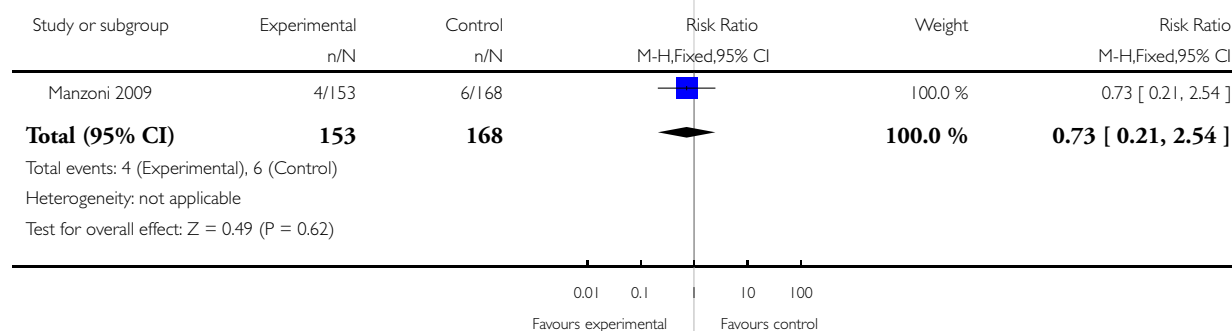


Analysis 1.7. Comparison 1 Lactoferrin alone vs. placebo, Outcome 7 Chronic lung disease.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 7 Chronic lung disease

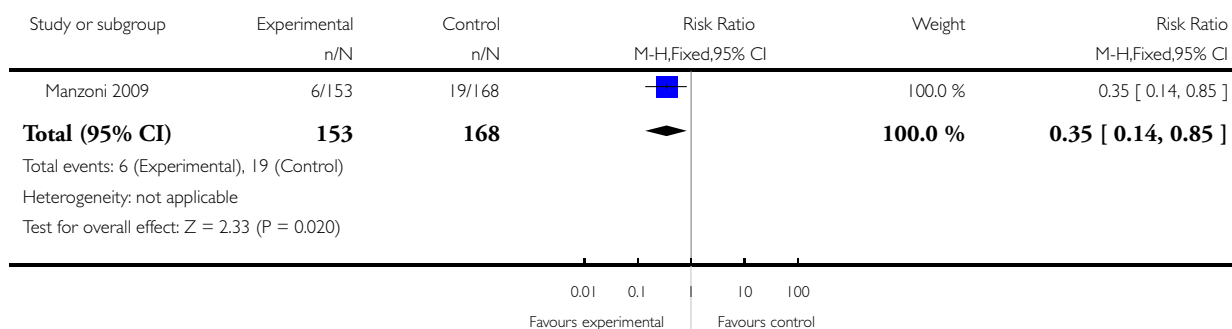


Analysis 1.8. Comparison 1 Lactoferrin alone vs. placebo, Outcome 8 Threshold retinopathy of prematurity.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 1 Lactoferrin alone vs. placebo

Outcome: 8 Threshold retinopathy of prematurity

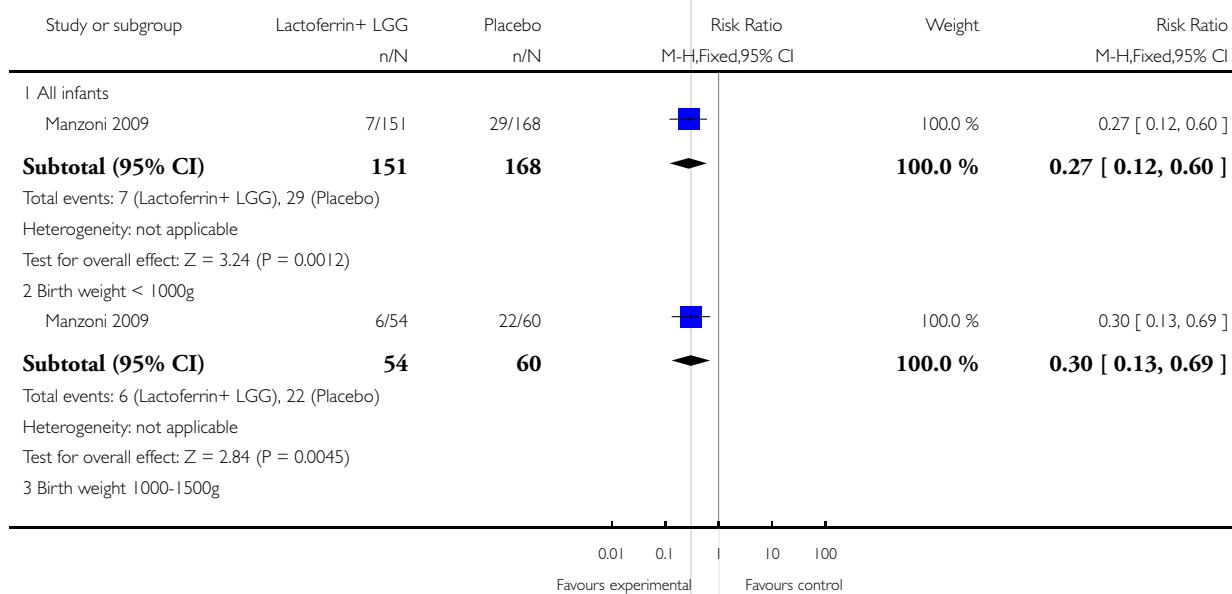


Analysis 2.1. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 1 Any late-onset sepsis.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

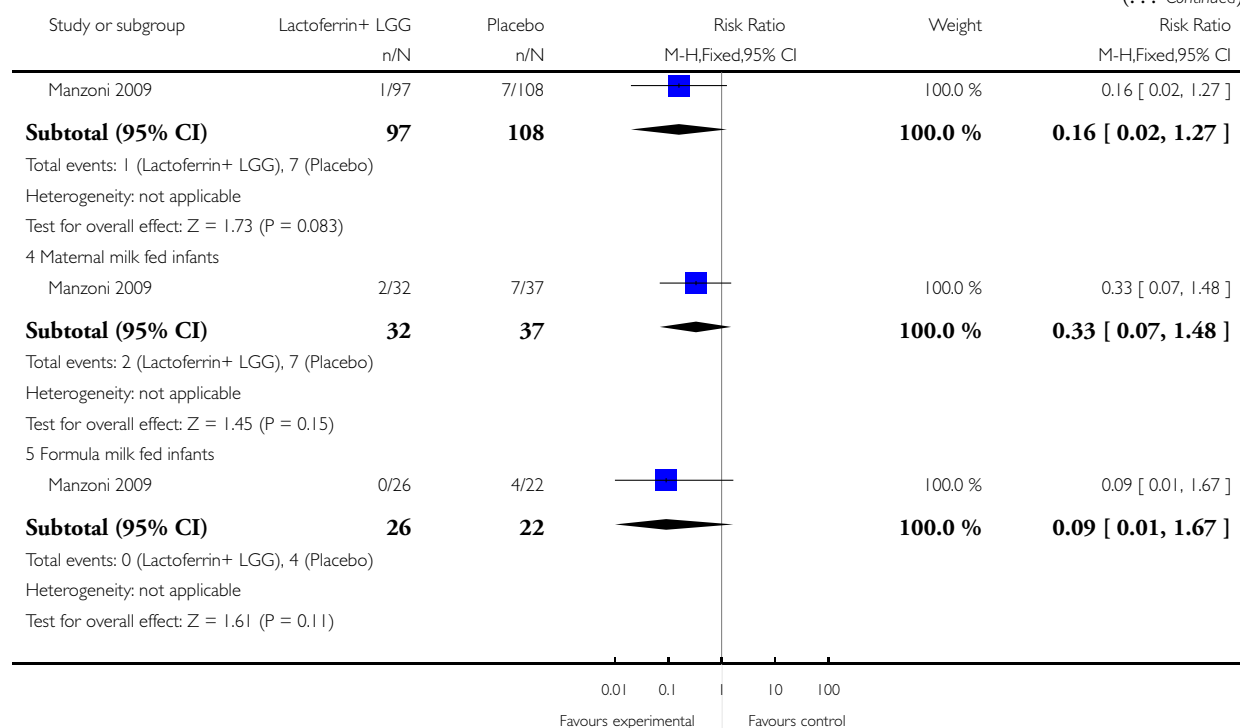
Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 1 Any late-onset sepsis



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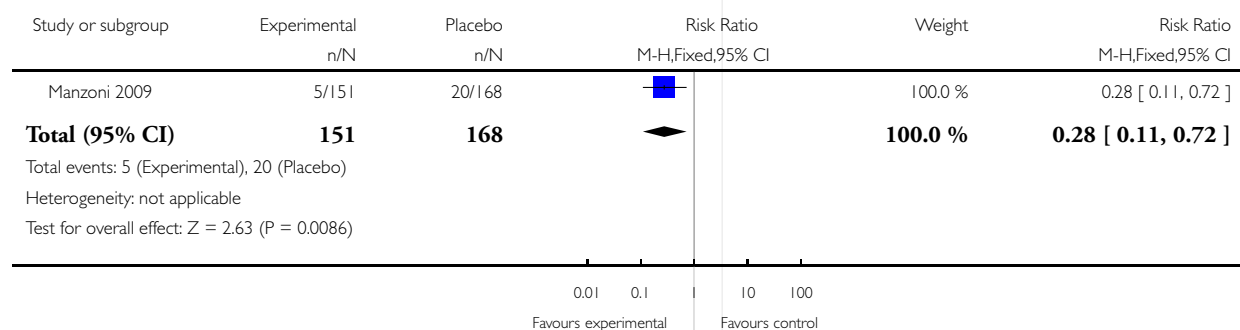


Analysis 2.2. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 2 Bacterial sepsis.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 2 Bacterial sepsis

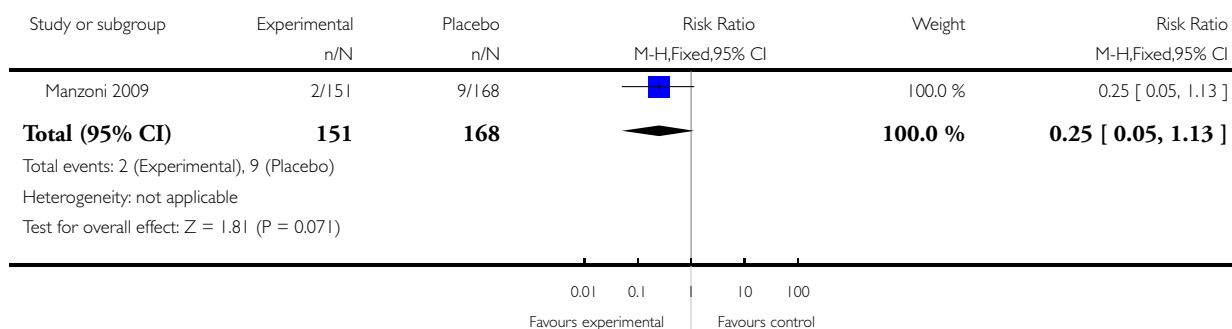


Analysis 2.3. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 3 Fungal Infection.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 3 Fungal Infection

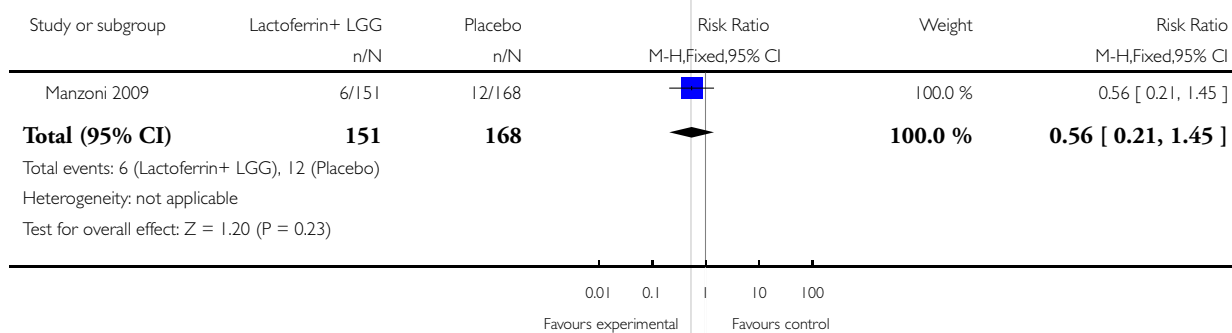


Analysis 2.4. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 4 All cause mortality.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 4 All cause mortality

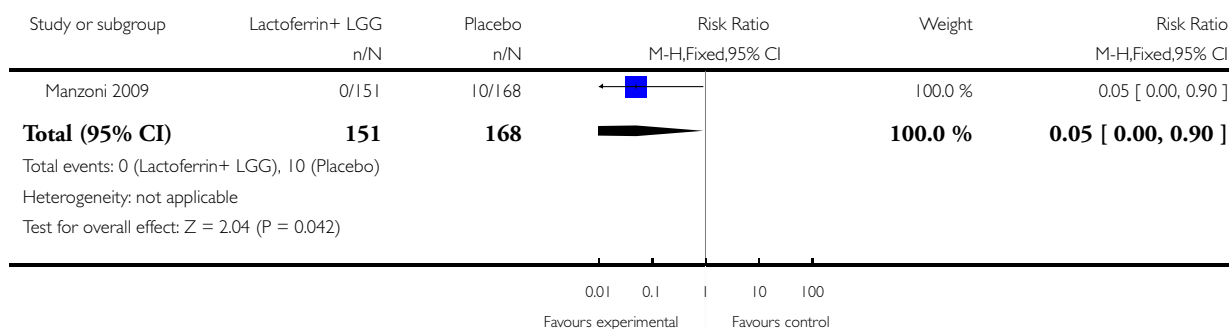


Analysis 2.5. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 5 NEC \geq Stage II.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 5 NEC \geq Stage II

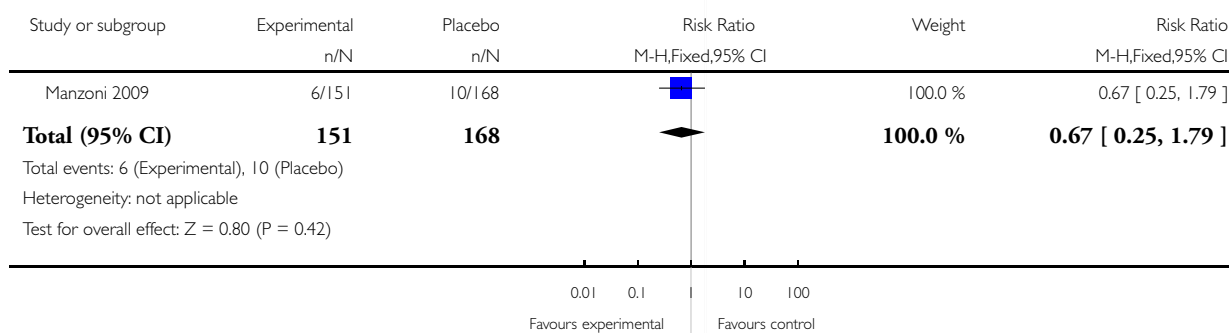


Analysis 2.6. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 6 Urinary tract Infection.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 6 Urinary tract Infection

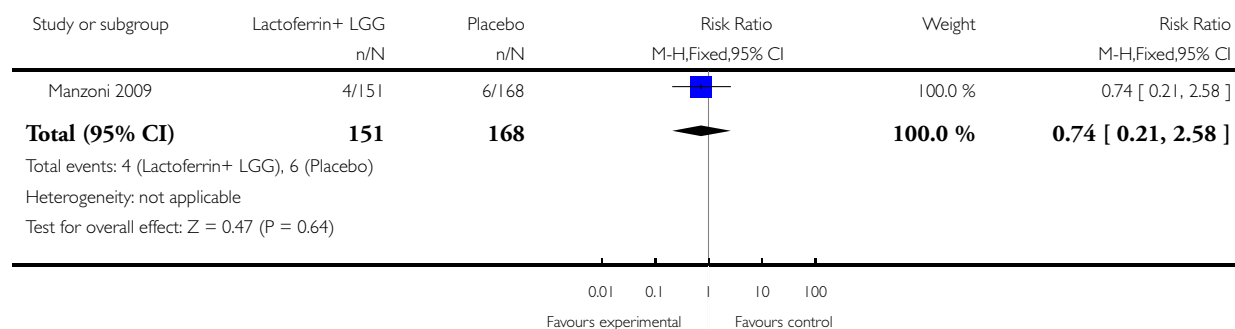


Analysis 2.7. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 7 Chronic lung disease.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 7 Chronic lung disease

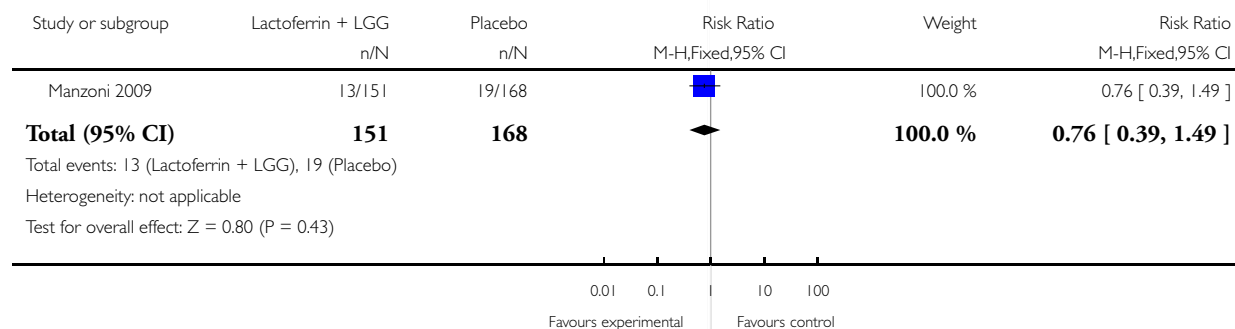


Analysis 2.8. Comparison 2 Lactoferrin + LGG vs. placebo, Outcome 8 Threshold retinopathy of prematurity.

Review: Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants

Comparison: 2 Lactoferrin + LGG vs. placebo

Outcome: 8 Threshold retinopathy of prematurity



HISTORY

Protocol first published: Issue 2, 2008

Review first published: Issue 5, 2010

7 July 2008	Amended	Protocol to review
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CONTRIBUTIONS OF AUTHORS

Mohan Venkatesh:

- Wrote the text of the protocol and the review
- Formulated the search strategy and performed literature search
- Is the corresponding author

Steve Abrams:

- Assisted in writing the protocol and review
- Assisted in incorporating peer-reviewed comments in the review

DECLARATIONS OF INTEREST

Human recombinant lactoferrin was donated for Dr. Mohan's laboratory research by Agennix Inc.

SOURCES OF SUPPORT

Internal sources

- None, Not specified.

External sources

- None, Not specified.