

ORIGINAL ARTICLE

Experimental Models of Allergic Disease

Prebiotics for the prevention of allergies: A systematic review and meta-analysis of randomized controlled trials

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Summary

Background: Prevalence of allergic diseases in infants is approximately 10% reaching 20 to 30% in those with an allergic first-degree relative. Prebiotics are selectively fermented food ingredients that allow specific changes in composition/activity of the gastrointestinal microflora. They modulate immune responses, and their supplementation has been proposed as an intervention to prevent allergies.

Objective: To assess in pregnant women, breastfeeding mothers, and infants (populations) the effect of supplementing prebiotics (intervention) versus no prebiotics (comparison) on the development of allergic diseases and to inform the World Allergy Organization guidelines.

Methods: We performed a systematic review of studies assessing the effects of prebiotic supplementation with an intention to prevent the development of allergies.

Results: Of 446 unique records published until November 2016 in Cochrane, MEDLINE, and EMBASE, 22 studies fulfilled a priori specified criteria. We did not find any studies of prebiotics given to pregnant women or breastfeeding mothers. Prebiotic supplementation in infants, compared to placebo, had the following effects: risk of developing eczema (RR: 0.68, 95% CI: 0.40 to 1.15), wheezing/asthma (RR, 0.37; 95% CI: 0.17 to 0.80), and food allergy (RR: 0.28, 95% CI: 0.08 to 1.00). There was no evidence of an increased risk of any adverse effects (RR: 1.01, 95% CI: 0.92 to 1.10). Prebiotic supplementation had little influence growth rate (MD: 0.92 g per day faster with prebiotics, 95% CI: 0 to 1.84) and the final infant weight (MD: 0.10 kg higher with prebiotics, 95% CI: -0.09 to 0.29). The certainty of these estimates is very low due to risk of bias and imprecision of the results.

Conclusions: Currently available evidence on prebiotic supplementation to reduce the risk of developing allergies is very uncertain.

KEYWORDS

allergy, prebiotics, prevention, systematic review

1 | INTRODUCTION

1.1 | Description of the condition

Allergies are a major health problem for patients and an important economic burden for healthcare systems.^{1,2} The prevalence of various allergies ranges between 2% and 40%, depending on the type of allergy and geographical location,^{3–6} from food allergy (10%),⁶ atopic dermatitis (20%),⁵ asthma (up to 37%)⁴ to allergic rhinitis/conjunctivitis (15%).³

1.2 | Description of the intervention

Prebiotics are defined as “selectively fermented food ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.”⁷

1.3 | How the intervention might work

Current evidence shows that gut microbiota is involved in modulation of immune response in early life. As a consequence, interventions that alter the microbiota (eg probiotics or prebiotics) have been suggested as preventive strategies.⁸ Supplementation of prebiotics has been regarded as a promising intervention for the prevention of allergies.^{9,10}

1.4 | Why it is important to do this review

Currently, most allergic diseases are not preventable and not curable, with debatable exception of some allergies for which specific allergen immunotherapy exists and new evidence suggesting probable pathways for prevention.¹¹ The management of allergies includes avoidance of the allergen, if possible, and treatment of symptoms. Therefore, primary prevention of the development of allergen sensitization and symptomatic allergy in the foetus and newborn is gaining attention.

This work aimed to inform the recommendations in the World Allergy Organization (WAO) clinical practice guidelines for the prevention of allergies or Guideline for Allergic Disease Prevention (GLAD-P) where we have previously addressed the use of probiotics¹² and vitamin D.¹³ For this, we conducted a systematic review to answer three clinical questions in relation to prebiotics:

1. Should supplementation of prebiotics versus no such supplementation be used in *pregnant women* to prevent development of allergy in their children?
2. Should supplementation of prebiotics versus no such supplementation be used in *breastfeeding mothers* to prevent development of allergy in their children?
3. Should supplementation of prebiotics versus no such supplementation be used in *infants* to prevent development of allergy?

2 | METHODS

2.1 | Criteria for considering studies for this review

2.1.1 | Types of studies

We included randomized trials with a minimum follow-up of 4 weeks, comparing any type of prebiotic against placebo or no prebiotics. We had no restriction on publication language. In case randomized studies were not available, we looked for observational studies (cohort and case-control studies) comparing prebiotics against no prebiotics.

2.1.2 | Types of participants

Studies must have included pregnant women, breastfeeding mothers, infants born at term, or children up to 9 years of age.

2.1.3 | Types of interventions

We included studies that used any type of prebiotic supplementation in any kind of formulation and dosage (capsules, oil droplets, suspension, or supplemented in infant formulas of any kind—including hypoallergenic formulas—or cereals). We excluded studies that used any type of co-interventions administered with prebiotics; for example, we did not include interventions that combined prebiotics with probiotics or with other supplemental nutrients (ie vitamins and other micronutrients).

2.1.4 | Types of outcome measures

The WAO guideline panel members in a formal process determined the outcomes of interest. The following outcomes in children were deemed critical to the decision whether to use prebiotics for prevention of allergies: development of eczema (defined as atopic eczema or any kind of eczema), development of asthma and/or recurrent wheezing, development of food allergy, development of allergic rhinitis, adverse events in children and/or mothers, and nutrition status, defined as the composite measure of either the final overall weight in kilograms at 12 months of age, the mean weight gained in grams per day from 1 to 12 months of age, or the adjusted increase in weight from baseline.

2.2 | Search methods

We searched Ovid MEDLINE, Ovid EMBASE, and the Cochrane Central Register of Controlled Trials. We ran a second search on March 2016 (no updated needed) and a second update on January 2017. We reviewed abstracts, conference proceedings, crosschecked references of included articles and contacted authors of identified studies and experts in the field to find any additional studies. We used a targeted search for non-randomized studies, looking only for cohort and case-control studies.

We present all search strategies in Table S1 in the supplementary material.

2.3 | Data collection and analysis

2.3.1 | Selection of studies

Four authors (CCG, JJYN, SG, and AA) independently screened in duplicate the titles and abstracts identified in database searches. The same authors assessed full-text publications for inclusion using pre-piloted screening forms. Any disagreements were resolved by consensus and/or by another review author (JLB).

2.3.2 | Data extraction

Four review authors (CCG, JJYN, GPM, and YZ) extracted the data using predefined and piloted data extraction forms. For each study, we obtained and assessed the following: sequence generation, concealment of allocation, masking, exclusions, patient dropout, loss to follow-up, participants' noncompliance, participants' characteristics, number of patients in the study, number of patients randomized, age, age range, sex, and inclusion and exclusion criteria. Characteristics of interventions included the type of prebiotic, dosage, placebo use, and duration of treatment. When one study was reported on several time-points, we used data closer to where most other studies reported their outcomes and addressed other time-points in the text. Any disagreements between the review assessors were solved by consensus and/or with the fifth author (JLB).

2.3.3 | Assessment of risk of bias in included studies

We assessed the risk of bias in the included studies using the Cochrane Collaboration risk of bias tool.¹⁴

2.3.4 | Measures of treatment effect

We estimated relative risks (RRs) and 95% confidence intervals (CI) for dichotomous outcomes and mean differences (MD) or standardized mean differences (SMD) with associated 95% CI for continuous outcomes.

2.3.5 | Dealing with missing data

We did not impute any missing data. We used the number of those randomized and analysed. When necessary, we contacted the study authors to obtain additional and/or missing information.

2.3.6 | Assessment of statistical heterogeneity and inconsistency

We assessed inconsistency between studies by visual examination of the forest plots, the Q statistic (with a P value of .05 or less as a

suggestion of important statistical heterogeneity), and the I^2 value.¹⁵ We made an a priori plan to explain inconsistency if found, based on the differences in study populations, prebiotic dosages, type, and forms of administration.

2.3.7 | Assessment of publication bias

We aimed to assess publication bias by means of visual assessment of funnel plot asymmetry in the main outcomes, but enough studies were not found (ie more than ten).

2.3.8 | Data synthesis

We used RevMan version 5.3.5 to perform meta-analyses using the random-effects model according to the methods suggested in the Cochrane Handbook for Systematic Reviews of Interventions.¹⁴

2.3.9 | Subgroup analysis and investigation of inconsistency

In some studies, prebiotics were used during pregnancy and breastfeeding as well as by mothers and children. Because we expected that prebiotics could be given to more than one population of interest, we planned to assess both direct (prebiotics given to one population only) and indirect evidence (prebiotics given to 2 or all populations). For instance, when infants were the population of interest and only they received the intervention, then we considered it as direct evidence; if the prebiotics were given to infants but also to breastfeeding mothers and/or to the mothers during pregnancy, then we considered it indirect evidence about the effects of supplementation in infants.

2.3.10 | Sensitivity analysis

We planned a sensitivity analyses to determine the effect of including or excluding the studies at higher (versus low) risk of bias.

2.3.11 | Certainty in the evidence (quality of evidence)

We graded the overall certainty of the body of evidence (also known as quality of the evidence or confidence in the effect estimates) for each outcome following the Grading of Recommendations Assessment Development and Evaluation (GRADE) approach.¹⁶ We used the GRADEpro Guideline Development Tool (www.grade-pro.org) to produce summary of findings in Table 1. As we performed this systematic review with the purpose to inform a clinical guideline, we did not produce or published a protocol.

3 | RESULTS

Our search strategy identified 446 unique records, of which 110 were selected for full-text assessment (Figure 1). We identified 25

TABLE 1 Summary of findings (GRADE)

Outcomes	Anticipated absolute effects (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)
	Risk with no prebiotics	Risk with prebiotics			
Eczema (general) Assessed as: physician-diagnosed eczema. Follow-up: range 3 to 24 mo	187 per 1000	127 per 1000 (75 to 215)	RR 0.68 (0.40 to 1.15)	2030 (6 RCTs)	⊕⊕○○ LOW ^{a,b,c}
Allergic rhinitis—not reported in any study	—	—	—	—	—
Asthma Defined as: “recurrent wheezing,” as reported by parents or physician-diagnosed. Follow-up: range 18 to 24 mo	174 per 1000	64 per 1000 (30 to 139)	RR 0.37 (0.17 to 0.80)	249 (2 RCTs)	⊕○○○ VERY LOW ^{c,d,e}
Food allergy Assessed with: symptoms of “self-reported food allergy,” by the parents. Follow-up: median 18 mo	170 per 1000	48 per 1000 (14 to 170)	RR 0.28 (0.08 to 1.00)	115 (1 RCT)	⊕○○○ VERY LOW ^{c,f,g}
Any allergy Assessed with: “any sign or symptom” of allergies reported together. ^h Follow-up: 12 mo	212 per 1000	295 per 1000 (193 to 450)	RR 1.39 (0.91 to 2.12)	264 (1 RCT)	⊕⊕○○ LOW ^{i,j}
Adverse events Assessed with: clinician-diagnosed and/or parent reporting Follow-up: range 1 to 12 mo	331 per 1000	334 per 1000 (304 to 364)	RR 1.01 (0.92 to 1.10)	2876 (9 RCTs)	⊕⊕○○ LOW ^{i,k}
Nutritional status Composite outcome defined as mean weight gained per day, final measurement of weight, or adjusted change from baseline as assessed by clinicians. Higher values indicate weight gain. Follow-up: range 1 to 12 mo	—	SMD 0.07 SD higher (0 to 0.15 higher)	—	2298 (15 RCTs)	⊕⊕○○ LOW ^{l,m}

CI, Confidence interval; RR, risk ratio; SMD, standardized mean difference.

The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). GRADE Working Group grades of evidence: high quality: we are very confident that the true effect lies close to that of the estimate of the effect. Moderate quality: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low quality: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low quality: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

^aFour studies with unclear risk of bias for random sequence allocation; five studies with concerns over an important number of patients lost to follow-up.

^bThere is statistical heterogeneity above 60% between studies, mostly explained by one study (Boyle 2016) with the highest weight and low risk of bias; the rest of the studies are small and chance could play a role; furthermore, the effect estimates point at the same direction and there is an overlap of confidence intervals.

^cNull effect is within the 95% confidence interval, which also does not exclude the threshold for an appreciable clinical benefit.

^dIncomplete outcome assessment in both studies. One study with concerns on blinded assessment of outcomes and other with poor description of the allocation concealment.

^eAsthma is measured as “recurring wheezing,” or self-reported or “proxy reported asthma,” indirect measurement.

^fConcerns over incomplete outcome data and blinded assessment.

^gIndirect assessment of “food allergy.” The authors provided limited information on how the outcome was measured, and considered it “self-reported outcome” rather than diagnosed food allergy per accepted diagnostic criteria.

^hOne single study (Sierra 2015) which reported “any sign or symptom related to allergy, wheezing, and atopic dermatitis” (clinically and by skin prick testing for atopic sensitization).

ⁱIncomplete outcome data (attrition bias) with lost to follow-up of 30% in the intervention group and 25% in the control group.

^j95% confidence interval does not exclude appreciable benefit or harm.

^kConcerns over varying degree of incomplete outcome data in all studies; random sequence and allocation concealment not described adequately in three studies; blinded assessment not performed in five studies; two studies with attrition bias.

^lBlind outcome assessment was not described in eight studies; other concerns were related to allocation concealment and number of patients lost to follow-up.

^mSMD used because of different ages of measurement of the outcome (from 1 to 12 mo of age) and different ways of measuring the weight gained.

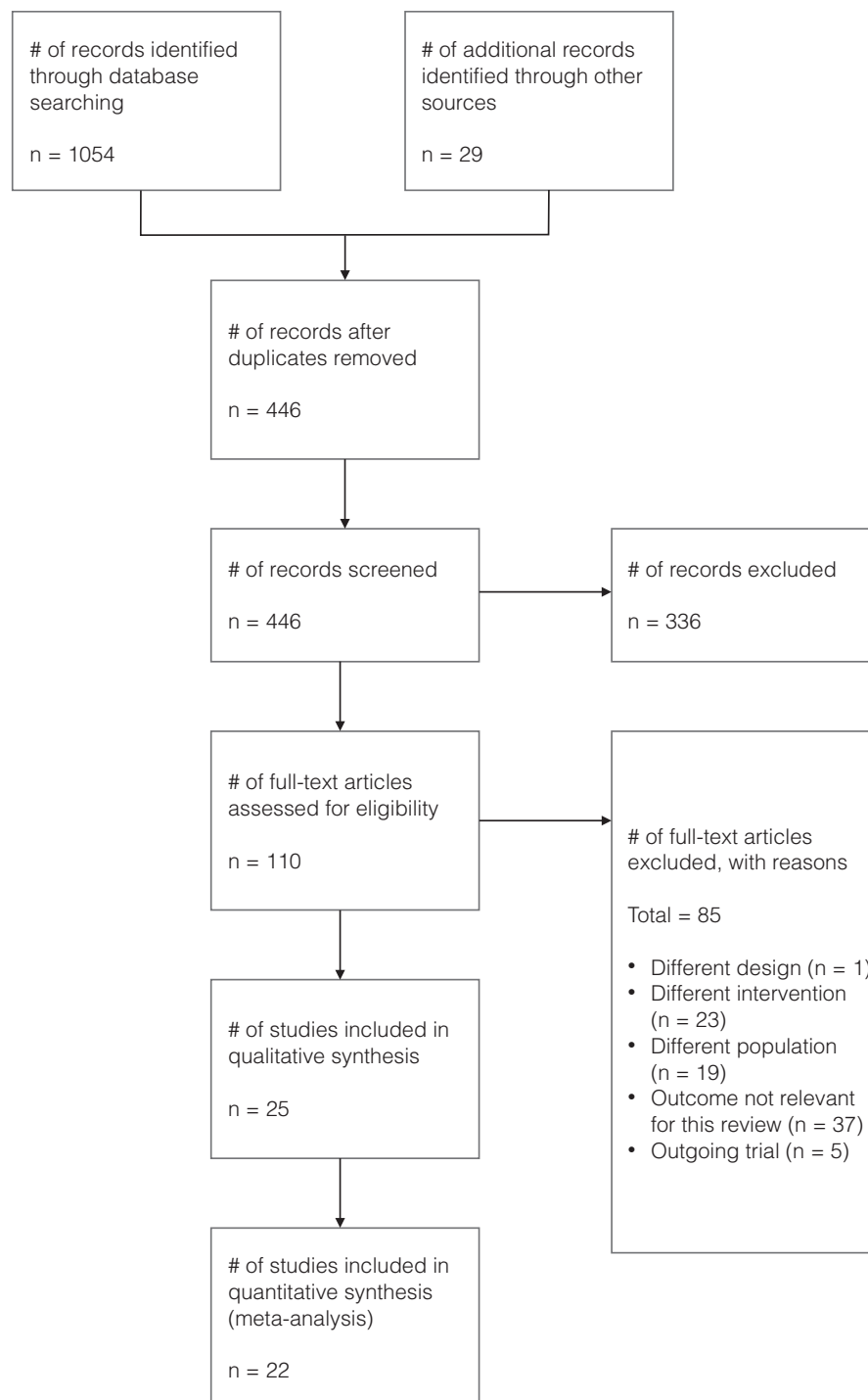


FIGURE 1 Flow chart of studies. Updated search (January 2017). Two published reports (Moro 2006 and Arslanoglu 2012) detailed follow-up data and are linked to one included study (Arslanoglu 2008). Another study (Bozensky 2015) is a randomized trial with complete results, but it was not possible to integrate into the meta-analysis

records that met our inclusion criteria comparing at least one prebiotic against placebo or no prebiotic, one of which is a study¹⁷ that was not pooled due to different outcome measures (described below), and two^{18,19} were follow-up reports. We included 22 studies in the quantitative analysis. We identified 593 records (after deduplication) from our search for observational studies. After title and

abstract screening, we did not find non-randomized studies eligible for inclusion (see Table S1).

Of the 22 included studies, three assessed infants at high risk of allergies, 16 with average risk, while three did not provide enough information to make a judgement. Most studies (19 of 22) used fructo-oligosaccharides (FOS) with galacto-oligosaccharides (GOS) as

the intervention; a minority (three studies) utilized a combination of inulin and polydextrose with GOS/FOS. The included studies used formula ($n = 16$), hypoallergenic formula ($n = 3$), cereal ($n = 2$), or capsules ($n = 1$) to deliver the intervention. Table S2 provides more details. Table S3 lists the studies that did not fulfil the inclusion criteria with the reasons for the exclusion.

3.1 | Risk of bias in included studies

Overall there was high risk of bias in 7 of 22 studies (31.8%; Figure 2). Six of 22 studies (27.3%) were at high risk of attrition bias. Generation of the random sequence was unclear in 4 studies, and in 8 studies, there was unclear description on how the allocation concealment was performed. The complete judgements of the risk of bias can be seen in Fig. S1 in the supplementary material.

3.2 | Effects of interventions

3.2.1 | Use of prebiotics during pregnancy and breastfeeding

We did not find any randomized controlled trials or observational studies of prebiotics as an intervention given to pregnant women or breastfeeding mothers for the prevention of allergies in their infants.

3.2.2 | Use of prebiotics in infants

Table 1 shows the summary of findings for the use of prebiotics in infants, and forest plots are shown in Figures 2 and 3.

Six studies assessed the development of *eczema*,²⁰⁻²⁵ where the overall effect of prebiotics was a reduction in the risk of eczema when compared to the control group, although the confidence interval did not exclude a null effect (RR, 0.68; 95% CI, 0.40 to 1.15; 2,030 participants; 6 trials). One study¹⁷ with low risk of bias was not included in the quantitative analysis because it reported eczema as a continuous variable (using the SCORAD system); the study included 103 children with high risk of allergies where at 6 months of follow-up found no clinical or statistical difference between prebiotic and control groups.

Two studies reported development of *wheezing/asthma*.^{20,22} Prebiotics were found to reduce the risk of asthma or wheezing when compared to the control group (RR, 0.37; 95% CI, 0.17 to 0.80; 249 participants; 2 trials), but precision was a concern due to a low number of events. Both studies used "recurrent wheezing" as their end-point of interest defining this outcome as "3 or more physician-diagnosed episodes in 2 years," and their data were obtained from both patients' self-reports and/or clinical records. There was considerable uncertainty to what extent this definition of an outcome reflected a true reduction in the risk of developing asthma. Furthermore, the study by Arslanoglu²⁰ also reported this outcome following participants at 5 years of age,¹⁹ where no effect of prebiotics on recurrent wheezing was found at this point.

Only one study reported the development of *food allergy*²² defining it as "gastrointestinal symptoms of food allergy" as reported by parents. In this trial, prebiotics reduced the risk of food allergy symptoms when compared to placebo (RR, 0.28; 95% CI 0.08 to 1; 115 participants; 1 trial), but it is also uncertain to what extent this outcome reflected a true food allergy.

One study²⁶ reported a composite of the development of any allergy with no difference between the groups (RR, 1.39; 95% CI 0.91 to 2.12; 264 participants; 1 trial). However, the number of events was small and the confidence interval did not exclude a possible large increase in the development of any allergy.

Nine studies reported *adverse events* associated with supplementation of prebiotics.^{24,25,27-33} Although definitions varied, most of the studies reported the number of patients with at least one adverse event assumed to be associated with the use of prebiotics. There were no differences between the prebiotic and comparison groups in the number of patients who reported any adverse events (RR, 1.01; 95% CI 0.92 to 1.10; 2,876 participants; 9 trials). Most of the reported events were gastrointestinal symptoms (such as diarrhoea, vomiting, retching, bloating), respiratory symptoms (cough, rhinorrhea), and rash.

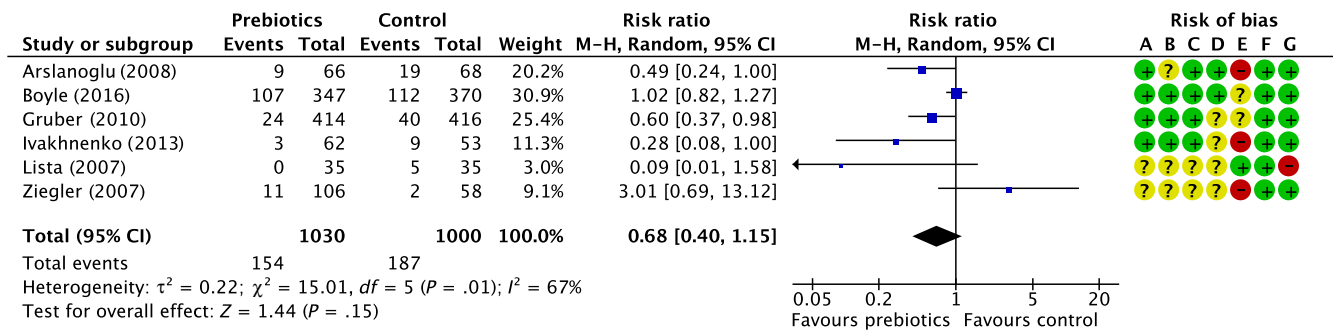
Fifteen studies assessed *nutrition status*^{24,26-29,31,33-41} of infants measured as either the mean weight gained per day during the supplementation of prebiotics,^{24,27,29,31,37} final overall weight in infants,^{26,34,38,39,41} or adjusted increase in weight from baseline. Prebiotic supplementation had little influence on the composite outcome of nutritional status (SMD: 0.07, 95% CI: 0 to 0.15; 2,670 participants; 15 trials; Fig. 3B) and in the individual outcomes of final infant weight (MD: 0.10 kg higher, 95% CI: -0.09 to 0.29; 613 participants; 5 trials; Fig. S2A) or growth rate (MD: 0.92 g per day faster, 95% CI: 0 to 1.84; 1481 participants; 5 trials; Fig. S2B).

4 | DISCUSSION

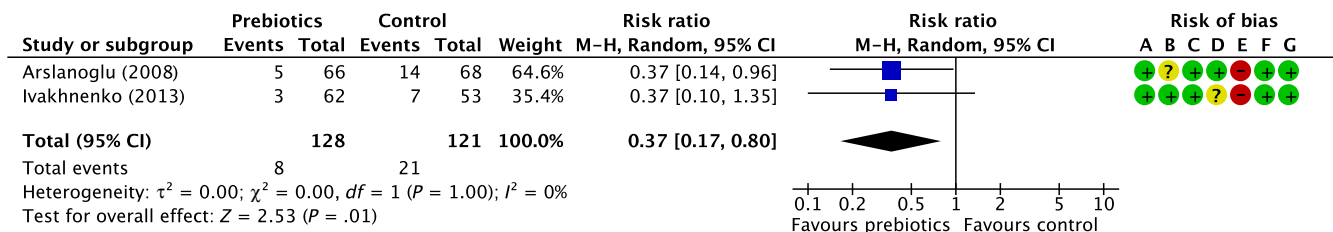
This review summarizes the available body of experimental evidence about the effects of supplementation of prebiotics for the prevention of allergies. We did not find enough evidence from randomized or observational trials to support or reject a beneficial or a harmful effect. We found evidence suggesting that prebiotics might reduce the risk of recurrent wheezing in infants; however, our certainty on these estimates is very low due to risk of bias, indirectness of the evidence, and imprecision due to low number of events of the estimated effect.

Randomized trials did not show an increased risk of adverse effects of prebiotics when compared to placebo. "Nutritional status" as the composite of the final weight at 12 months, mean weight gain, or the adjusted increase in weight from baseline improved at 1 to 12 months of follow-up, but the difference was small and unlikely to be noticed in most infants (differences of a few grams between groups in most studies).

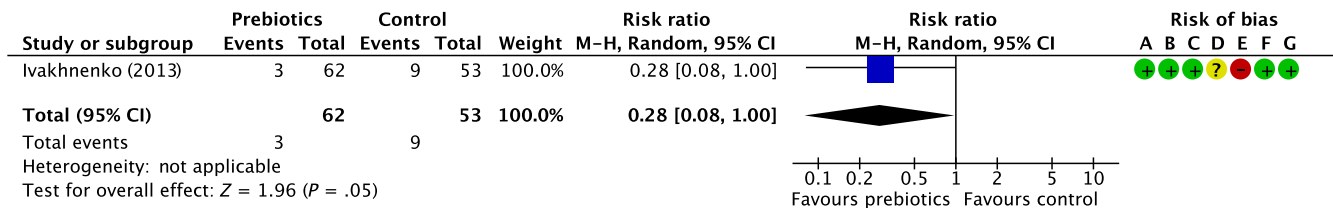
(A) Eczema



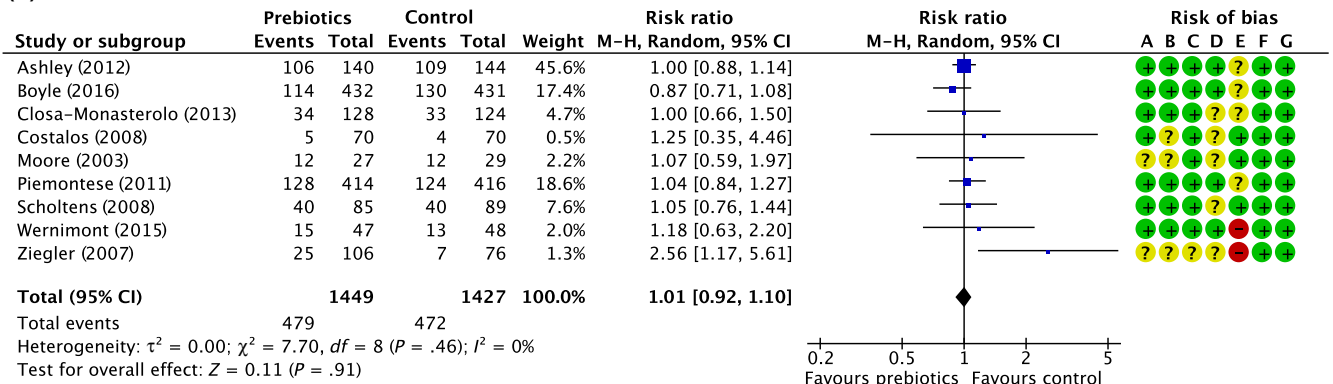
(B) Asthma/wheezing



(C) Food allergy



(D) Adverse events



Risk of bias legend

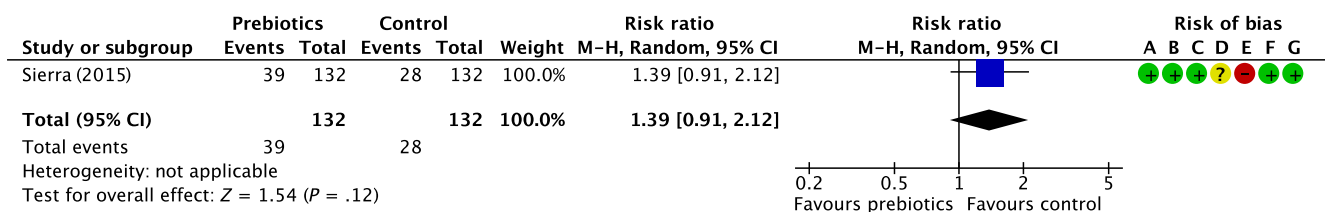
- (A) Random sequence generation (selection bias)
 (B) Allocation concealment (selection bias)
 (C) Blinding of participants and personnel (performance bias)
 (D) Blinding of outcome assessment (detection bias)
 (E) Incomplete outcome data (attrition bias)
 (F) Selective reporting (reporting bias)
 (G) Other bias

FIGURE 2 Forest plots for the effects of prebiotic supplementation versus no prebiotic supplementation on eczema, asthma/wheezing, food allergy, and adverse events

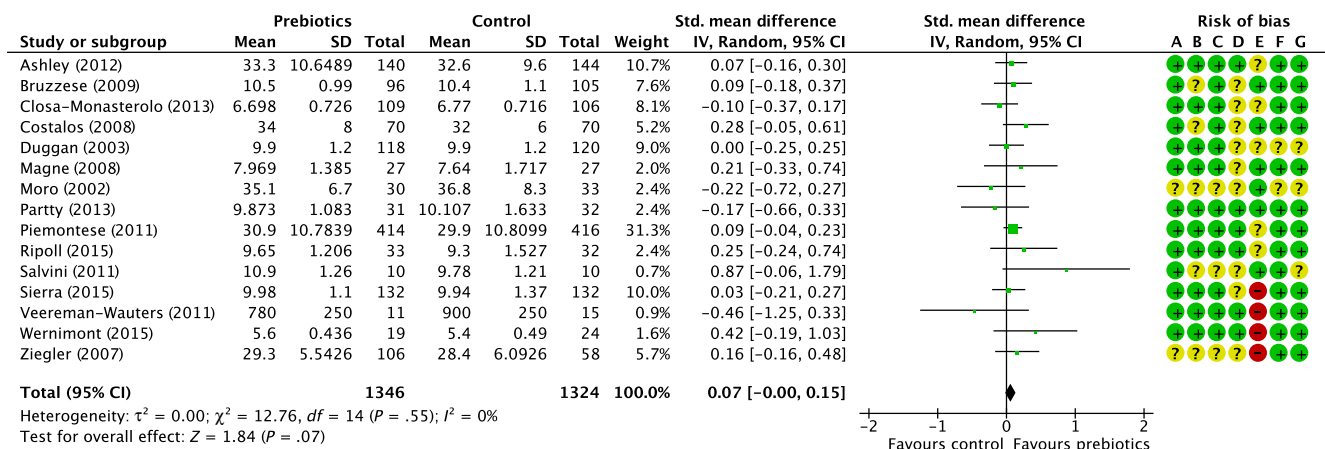
Among the strengths of our review is the comprehensive search for studies assessing the effect of prebiotics given to infants, during pregnancy, or to breastfeeding mothers. However, we found no studies in

which prebiotics were given to mothers emphasizing the need for relevant research to help parents and clinicians with decisions about the use of prebiotics in pregnancy and by breastfeeding mothers.

(a) Any allergy



(b) Nutritional status



Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

FIGURE 3 Forest plots for the effects of prebiotic supplementation versus no prebiotic supplementation on any allergy and nutritional status

Our review has several limitations; first, stemming from the limitations of the available evidence, for most outcomes there is only very low certainty of the estimated effects due to the risk of bias and imprecision of the results related to small number of observed events of development of allergy. Second, some limitations arise from the clinical heterogeneity and indirectness of populations and interventions; for instance, four studies^{17,20,23,25} included children with high risk of allergy, seven included children with average risk^{21,27,29,34,35} with the rest not specifying the population risk; this last situation precluded us from doing specific subgroup analyses based on these variables. Also, there are some inconsistencies on the prebiotic used as studies included GOS as the prebiotic of choice in the intervention groups, usually mixed with FOS, and polydextrose (see included studies in Table S2); with the low number of studies, it is difficult to ascertain an individual or class effect from prebiotics. Furthermore, different breastfeeding durations before inclusion (from 0 to 18 weeks of age) and different formulas were used in included infants in different studies—for example hypoallergenic formula vs standard formula. This may also account for the different results observed. Third, we detected and excluded a significant number of studies (37 of 110 assessed for full-text screening) due to indirectness of the outcomes (eg measurement of bacterial colonies or pH

values in faeces) which represent a 33% of the total studies with participants that could have been included in our review raising the possibility of selective reporting of outcomes. Fourth, 18 of 22 studies (81.9%) were funded by the manufacturer, although it is difficult to ascertain the real impact of this issue in our results. Fifth, the generalizability of our results may be compromised by the fact that the populations studied were mostly infants who were not breastfed (per eligibility criteria from individual studies) and results from these trials might be different in other settings where breastfeeding is common. One final issue is that this systematic review was made in parallel with the development of a clinical practice guideline, and we did not develop a protocol for this review.

A recent Cochrane review⁹ found four studies with an overall modest beneficial effect of prebiotics on development of eczema. Our results differ in that we decided not to include studies with pre-term infants to avoid clinical inconsistencies. We found five studies with differences between prebiotics and placebo groups on the risk of developing eczema, although the confidence interval could not exclude the null effect. In relation to asthma/wheezing, the Cochrane review also included premature infants showing a non-significant result. In contrast, we found two studies showing a risk reduction (Figure 2), yet with very low certainty. Our main

difference with this review derives from our consideration of not including preterm infants, including more recent studies, and the inclusion of other outcomes (eg allergic rhinitis, nutritional status, other allergies). The implications for these differences are less inconsistencies in our meta-analyses that result in less uncertainty.

5 | CONCLUSIONS

The currently available evidence from randomized trials shows a possible effect of prebiotic supplementation in infants on the reduction in the risk of asthma or wheezing. However, the certainty of that effect is very low. The effect on atopic eczema is present, but the result is imprecise (did not reject the null effect) and additional randomized trials, if done, are very likely to substantially contribute the certainty of the effects and may change the estimates of the effects themselves. We did not find evidence, including from non-randomized studies, of an effect on any other outcome of interest. We did not find studies that would assess the effect of diet supplementation with prebiotics in pregnant women and breastfeeding mothers. Currently available evidence is not enough to support or reject a beneficial or a harmful effect from prebiotics.

5.1 | Suggestions for future research

More well-designed and performed studies, ideally randomized trials, that would measure and report all properly defined outcomes of interest, including quality of life and adverse effects, are needed to assess the effect of supplementation of prebiotics on the prevention of allergies. Studies should be ideally performed in different populations, including newborns being breastfed, pregnant women, and breastfeeding mothers, with an a priori defined prebiotic and with an adequate duration of follow-up. As prebiotics are only a part of the equation of the possible mechanisms that the microbiota might have on atopic diseases, more research is needed analysing the full context of prebiotics, probiotics, and synbiotics for the prevention of allergies.

CONFLICT OF INTEREST

Carlos Cuello received support from WAO for development of GLAD-P guidelines. Alessandro Fiocchi received research support from Danone and has received travel support for meetings for presentation of DRACMA guidelines in South-East Asia from Danone Malaysia and support for travel and honorarium for speaking from Ordesa Group. Ruby Pawankar received support from Danone Asia Pacific for act as a speaker at a Food allergy Asia Pacific symposium 2012. Juan J Yepes-Nunez received support from WAO for development of GLAD-P guidelines. Gian Paolo Morgano received support from WAO for development of GLAD-P guidelines. Yuan Zhang received support from WAO for development of GLAD-P guidelines. Arnav Agarwal received support from WAO for development of GLAD-P guidelines. Shreyas Gandhi received support from

WAO for development of GLAD-P guidelines. Luigi Terracciano has been a Heinz-Plada Italy Medical consultant for website. Holger Schünemann received research support from WAO for development of DRACMA and GLAD-P Guidelines Travel support from WAO. Jan L. Brozek received support from WAO for development of GLAD-P guidelines. The authors have no other conflict of interest to declare.

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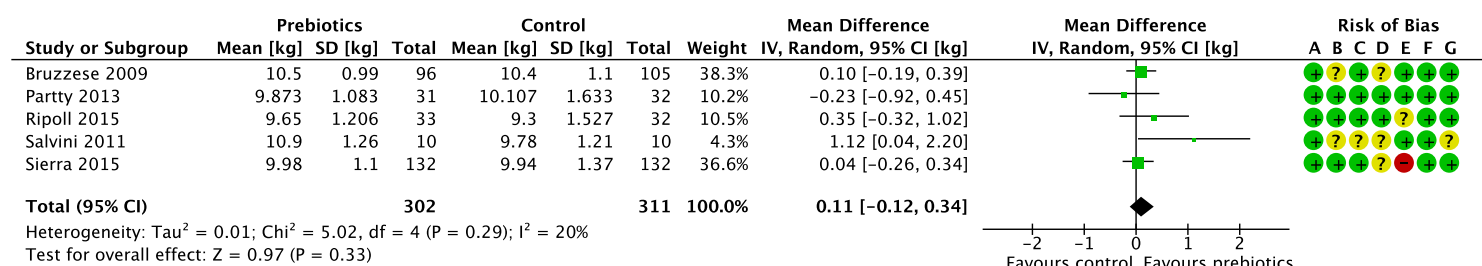
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

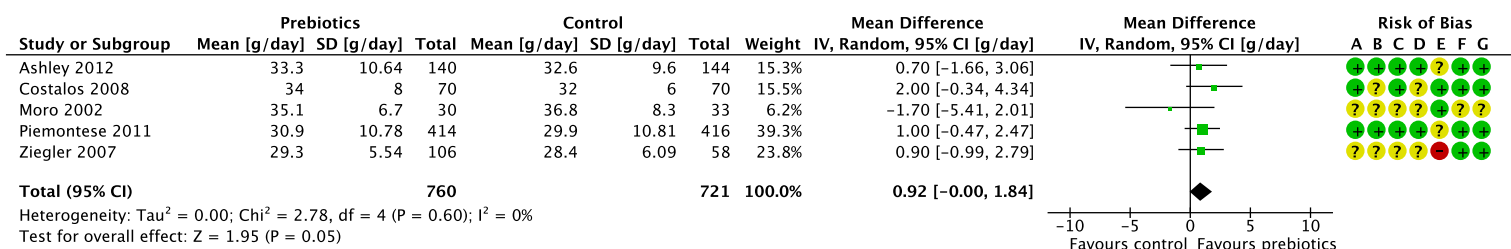
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	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Arslanoglu 2008	+	?	+	+	-	+	+
Ashley 2012	+	+	+	+	?	+	+
Boyle 2016	+	+	+	+	?	+	+
Bruzzese 2009	+	?	+	?	+	+	+
Closa-Monasterolo 2013	+	+	+	?	?	+	+
Costalos 2008	+	?	+	?	+	+	+
Duggan 2003	+	+	+	?	?	?	?
Gruber 2010	+	+	+	?	?	+	+
Ivakhnenko 2013	+	+	+	?	-	+	+
Lista 2007	?	?	?	?	+	+	-
Magne 2008	+	+	+	?	+	+	+
Moore 2003	?	?	+	?	+	+	+
Moro 2002	?	?	?	?	+	?	?
Partty 2013	+	+	+	+	+	+	+
Piemontese 2011	+	+	+	+	?	+	+
Ripoll 2015	+	+	+	+	?	+	+
Salvini 2011	+	?	?	?	+	+	?
Scholtens 2008	+	+	+	?	+	+	+
Sierra 2015	+	+	+	?	-	+	+
Veereman-Wauters 2011	+	+	+	+	-	+	+
Wernimont 2015	+	+	+	+	-	+	+
Ziegler 2007	?	?	?	?	-	+	+

A) Nutritional status – Final weight (in kilograms)



B) Nutritional status – Weight gain (in mean grams per day)



Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding of participants and personnel (performance bias)
- (D) Blinding of outcome assessment (detection bias)
- (E) Incomplete outcome data (attrition bias)
- (F) Selective reporting (reporting bias)
- (G) Other bias

Figure S2. Forest plots for the effects of prebiotic supplementation versus no prebiotic supplementation on A) final weight (in kilograms) measured at 12 months of age, and B) mean weight gain (in grams per day) measured through the first year.

SEARCH STRATEGIES - PREBIOTICS

RANDOMIZED STUDIES

MEDLINE (OVID)

- | | |
|----|-------------------------------------|
| 1 | randomized controlled trial.pt. |
| 2 | controlled clinical trial.pt. |
| 3 | randomized.ab. |
| 4 | placebo.ab. |
| 5 | clinical trials as topic.sh. |
| 6 | randomly.ab. |
| 7 | trial.ti. |
| 8 | 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 |
| 9 | exp animals/ NOT humans.sh. |
| 10 | 8 NOT 9 |
| 11 | exp prebiotics/ |
| 12 | oligosaccharide\$.ab,ti,tw. |
| 13 | fructo-oligosaccharide\$.ab,ti,tw. |
| 14 | galacto-oligosaccharide\$.ab,ti,tw. |
| 15 | inulin.ab,ti,tw. |
| 16 | lactulose.ab,ti,tw. |
| 17 | 11 OR 12 OR 13 OR 14 OR 15 OR 16 |
| 18 | infant\$.ab,ti,tw. |
| 19 | newborn.ab,ti,tw. |
| 20 | pediatric.ab,ti,tw. |
| 21 | paediatric.ab,ti,tw. |
| 22 | child\$.ab,ti,tw. |
| 23 | exp pregnancy/ |

24	exp breast feeding/
25	18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24
26	10 AND 17 AND 25

EMBASE (OVID)

1	random:.tw
2	placebo:.mp
3	double-blind:.tw
4	1 OR 2 OR 3
5	exp animals/ NOT humans.sh.
6	4 NOT 5
7	exp prebiotics/
8	oligosaccharide\$.ab,ti,tw.
9	fructo-oligosaccharide\$.ab,ti,tw.
10	galacto-oligosaccharide\$.ab,ti,tw.
11	inulin.ab,ti,tw.
12	lactulose.ab,ti,tw.
13	7 OR 8 OR 9 OR 10 OR 11 OR 12
14	infant\$.ab,ti,tw.
15	newborn.ab,ti,tw.
16	pediatric.ab,ti,tw.
17	paediatric.ab,ti,tw.
18	child\$.ab,ti,tw.
19	exp pregnancy/
20	exp breast feeding/
21	14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20
22	6 AND 13 AND 21

COCHRANE (CENTRAL)

1	PREBIOTICS
2	oligosaccharides
3	fructo-oligosaccharides
4	galacto-oligosaccharides
5	inulin
6	lactulose
7	1 OR 2 OR 3 OR 4 OR 5 OR 6
8	infant?
9	newborn?
10	pediatric?
11	paediatric?
12	child?
13	PREGNANCY
14	BREAST FEEDING
15	8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14
16	7 AND 15

SEARCH STRATEGY (AND FINDINGS) FOR OBSERVATIONAL STUDIES

We performed a targeted search for observational studies aimed at finding only cohort and case-control studies, with the following search terms:

MEDLINE

1. exp prebiotics/
2. oligosaccharide\$.ab,ti,tw.
3. fructo-oligosaccharide\$.ab,ti,tw.
4. galacto-oligosaccharide\$.ab,ti,tw.
5. inulin.ab,ti,tw.
6. lactulose.ab,ti,tw.
7. 1 or 2 or 3 or 4 or 5 or 6
8. infant\$.ab,ti,tw.
9. newborn.ab,ti,tw.
10. pediatric\$.ab,ti,tw.
11. paediatric\$.ab,ti,tw.
12. child\$.ab,ti,tw.
13. exp pregnancy/
14. exp breast feeding/
15. 8 or 9 or 10 or 11 or 12 or 13 or 14
16. epidemiologic studies/
17. exp case control studies/
18. exp cohort studies/
19. cross-sectional studies/
20. case control.ti,ab.
21. (cohort adj (study or studies or analys*)).ti,ab.
22. ((follow up or observational or uncontrolled or non randomi#ed or nonrandomi#ed or epidemiologic*) adj (study or studies)).ti,ab.
23. ((longitudinal or retrospective or prospective or cross sectional) and (study or studies or review or analys* or cohort*)).ti,ab.
24. 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25. 7 and 15 and 24

EMBASE

1. exp prebiotics/
2. oligosaccharide\$.ab,ti,tw.
3. fructo-oligosaccharide\$.ab,ti,tw.
4. galacto-oligosaccharide\$.ab,ti,tw.
5. inulin.ab,ti,tw.
6. lactulose.ab,ti,tw.
7. 1 or 2 or 3 or 4 or 5 or 6

8. infant\$.ab,ti,tw.
9. newborn.ab,ti,tw.
10. pediatric.ab,ti,tw.
11. paediatric.ab,ti,tw.
12. child\$.ab,ti,tw.
13. exp pregnancy/
14. exp breast feeding/
15. 8 or 9 or 10 or 11 or 12 or 13 or 14
16. exp case control study/
17. longitudinal study/
18. prospective study/
19. cohort analysis/
20. cohort*.ti,ab.
21. (cohort adj (study or studies or analys*)).ti,ab.
22. case control.ti,ab.
23. ((follow up or observational or uncontrolled or non randomi#ed or nonrandomi#ed or epidemiologic*) adj (study or studies)).ti,ab.
24. 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25. 7 and 15 and 24

COCHRANE LIBRARY (CENTRAL)

- #1 MeSH descriptor: [Prebiotics] explode all trees
- #2 oligosaccharides
- #3 fructo-oligosaccharides
- #4 galacto-oligosaccharides
- #5 inulin
- #6 lactulose
- #7 #1 or #2 or #3 or #4 or #5 or #6
- #8 infant?
- #9 newborn
- #10 pediatric?
- #11 paediatric?
- #12 child?
- #13 MeSH descriptor: [Pregnancy] explode all trees
- #14 MeSH descriptor: [Breast Feeding] explode all trees
- #15 #8 or #9 or #10 or #11 or #12 or #13 or #14
- #16 #7 and #15
- #17 MeSH descriptor: [Case-Control Studies] explode all trees
- #18 MeSH descriptor: [Cohort Studies] explode all trees
- #19 case control:ti,ab,kw
- #20 cohort:ti,ab,kw
- #21 non randomi?ed:ti,ab,kw
- #22 observational:ti,ab
- #23 #17 or #18 or #19 or #20 or #21 or #22
- #24 #16 and #23

ADDENDUM for observational studies:

After running the search strategy we proceeded with the screening of titles and abstracts of observational studies (only cohort and case-control studies were eligible). We screened 593 references found (after de-duplication); no observational studies were eligible for full-text screening for our questions.

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Arslanoglu 2008	Parallel randomized controlled trial	Age group: Infants Risk of allergy: high risk Inclusion criteria: gestational age between 37 and 42 weeks, birth weight appropriate for gestational age, and start of formula feeding within the first two weeks of life. Exclusion criteria: if breast-feeding after 6 weeks of inclusion; all else not described	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: hypoallergenic formula with 0.8 g GOS/FOS per 100 ml for 24 weeks Comparison: hypoallergenic formula with 0.8 g maltodextrin/100 ml as placebo	Atopic dermatitis at 2 yrs of age Wheezing at 2 yrs of age	Location: Italy Setting: Hospital Source of funding: partially supported by a grant from Numico Research Friedrichsdorf, Germany and the EARNEST program

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Ashley 2012	Parallel randomized controlled trial	Age group: newborns Risk of allergy: average risk Inclusion criteria: Eligible infants were singleton births at 37–42 weeks gestational age with birth weight $\geq$ 2500 g and solely formula-fed at least 24 h prior to randomization. Exclusion criteria: Exclusion criteria included history of underlying disease or congenital malformation likely to interfere with normal growth and development or participant evaluation; feeding difficulties or formula intolerance; weight at randomization $<$98% of birth weight; large for gestational age (defined as birth weight-for-age exceeding 90th	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: 4g/L in formula for 100 days. Investigational formulas were also supplemented with 4 g/L (1:1 ratio) of a blend of PDX (LitesseW Two Polydextrose; Danisco) and GOS (VivinalW GOS Galactooligosaccharide; Friesland Foods Domo) (PDX/GOS) or 4 g/L of GOS alone (GOS). Comparison: Formula with similar characteristics as placebo	Adverse events Nutritional status	Location: Multicentre from the United States Setting: Hospital Source of funding: Enfamil LIPIL, Mead Johnson Nutrition, Evansville, IN

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		percentile [15,16] born from a mother who was diabetic at childbirth; and immunodeficiency.			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Boyle 2016	Parallel randomized controlled trial	Age group: newborns Risk of allergy: High risk Inclusion criteria: Singleton infants born ≥ 36 weeks of gestational age and ≥ 2500 g were eligible for participation in the study if at least one of their parents had a documented history of allergic disease, confirmed by means of skin prick testing or a history of anaphylaxis. Exclusion criteria: Twins, infants with significant congenital abnormalities or severe neonatal illness, prematurity or low birth weight, consumption of cow's milk formula before randomization.	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: concentration was 0.8 g/100 ml (0.68 g/100 ml neutral; 0.12 g/100 ml acidic) in a formula HA - non-ultra-filtrated hydrolysed whey-based infant formula to which a specific mixture of neutral scGOS and lcFOS (ratio 9 : 1; Immunofortis Nutricia Cuijk BV, Cuijk, the Netherlands; 85 weight per cent) and acidic pAOS (15 weight per cent) were added. Comparison: Regular intact whey protein-	Eczema at 18 months of age. Adverse events	Location: Multicentre (UK, Ireland, Australia) Setting: Hospital Source of funding: Nutricia Research

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
			dominant infant formula without prebiotics. Both active and control formulae contained similar long-chain polyunsaturated fatty acids.		

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Bruzzese 2009	Parallel randomized controlled trial	Age group: Newborns and infants Risk of allergy: average risk Inclusion criteria: gestational age between 37 and 42 weeks, birth weight greater than 2500 g, and introduction of formula feeding after at least 15 days of exclusive feeding with maternal milk. Exclusion criteria: Exclusion criteria were twins, clinically significant illness of the mother, congenital immunodeficiency, any chronic or progressive diseases, proven or suspected allergy, and previous intake of pro- or prebiotics	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: 0.4 g/100 mL of infant formula for 52 weeks Comparison: formula without prebiotics as placebo	Nutrition status at 12 months (final weight)	Location: Italy Setting: Hospital Source of funding: Numico Research, Friedrichsdorf, Germany (partial support)

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Closa-Monasterolo 2013	Parallel randomized controlled trial	Age group: newborns and infants Risk of allergy: not described, probably average risk Inclusion criteria: healthy, born at term (>37 weeks gestational age), with normal birth weight Exclusion criteria: more than 4 weeks of life; antibiotic treatment; serious respiratory, neurological, gastrointestinal or metabolic disorders; and infections or other serious diseases that could hinder growth.	Prebiotic: oligofructose Dosage/presentation: SYN1 (0.8 g/dl) half oligofructose, half inulin Comparison: formula without prebiotics as control / placebo	Adverse events Nutrition status at 4 months	Location: Spain Setting: ambulatory Source of funding: funded by the BENEIO-Institute (an Initiative of BENEIO GmbH)

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Costalos 2008	Parallel randomized controlled trial	Age group: infants Risk of allergy: average Inclusion criteria: healthy term infants with birth weight between the 10th and 90th centiles, whose mothers had ceased breast feeding by the age of 14 days. Exclusion criteria: infants with major congenital abnormalities or chromosomal disorders or with disease requiring systemic antibiotic treatment, infants from multiple births and also adopted or fostered infants.	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: 0.4 g/100 mL in formula for 6 to 12 weeks Comparison: formula without prebiotics	Adverse events at 3 months Nutrition status at 3 months	Location: Greece Setting: Hospital Source of funding: none declared, although Numico provided the interventions

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Duggan 2003	Parallel randomized controlled trial	Age group: Infants Risk of allergy: average Inclusion criteria: Infants aged 6–12 mo were eligible for enrollment, provided that they had no known chronic illnesses or congenital anomalies that would interfere with the study outcomes and they were already consuming solid food (eg, purées, puddings, or cereals). Exclusion criteria: Excluded were those with severe acute malnutrition (weight-for-height z score <2), those who were exclusively breastfed (ie, not yet consuming solid foods), or those who were receiving chronic corticosteroids or	Prebiotic: oligofructose Dosage/presentation: 0.55 g/15g of cereal Comparison: cereal with similar characteristics but without prebiotics	Nutrition status at 6 months of follow-up	Location: Peru Setting: Ambulatory Source of funding: Gerber products

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		antibiotics (as defined by > 6 consecutive weeks of therapy before enrollment)			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Gruber 2010	Parallel randomized controlled trial	Age group: newborns and infants Risk of allergy: average risk Inclusion criteria: healthy, term infants (gestational age 37-42 weeks) with a normal birth weight, age up to 8 weeks when entering the study, without a positive history of allergic disease Exclusion criteria: mothers with hepatitis B, HIV, or group B streptococcal infection during pregnancy; mothers taking antibiotics during breast-feeding; infants with known congenital or postnatal diseases that could interfere with the study; and study prefeedings of the infants that could interfere	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: 8g/L with 6.8g/L neutral and 1.2g/L pAOS Comparison: placebo formula (non-hydrolyzed, as the intervention formula, but without prebiotics)	Atopic dermatitis (general cases without specifying sensitization) at 12 months of age Other outcomes assessed in Piemontese 2011	Location: Germany Setting: Ambulatory care Source of funding: Danone Research, Friedrichsdorf, Germany.

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		with the study.			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Ivakhnenko 2013	Parallel randomized controlled trial	Age group: newborns and infants Risk of allergy: not specified Inclusion criteria: Healthy term newborns with birth weight >2500 g appropriate for gestational age, Impossibility of breastfeeding Exclusion criteria: not described	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: in formula for 6 to 8 months; formula enriched with the specific mixture of oligosaccharides (scGOS/lcFOS; 9:1; 8 g/L) Comparison: formula without prebiotics	Atopic dermatitis/eccema at 18 months of age Asthma/wheezing at 18 months of age Food allergy at 18 months of age	Location: Ukraine Setting: ambulatory care Source of funding: Pediatrics Department of Lviv National Medical University

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Lista 2007	Parallel randomized controlled trial	Age group: newborns Risk of allergy: high Inclusion criteria: not described Exclusion criteria: not described	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: Hypoallergenic formula with dosage not specified during 13 weeks Comparison: hypoallergenic formula	Outcomes Atopic dermatitis / eczema at 3 months of age	Location: Italy Setting: not specified Source of funding: not specified

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Magne 2008	Parallel randomized controlled trial	Age group: infants Risk of allergy: average Inclusion criteria: Healthy, full-term infants, at age' s from 1 week to 3 months, taking breast milk and at least 300mL of formula feeding per 24 hours Exclusion criteria: Exclusive breast-feeding , history of family atopic diseases, full formula feeding from birth, previous episodes of diarrhoea, illnesses that required systemic antibiotic treatment or other diseases that would interfere with the study, systemic antibiotic treatment in the 2 months before inclusion, consumption of modulators of the intestinal microbiota in the	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: in formula (whey based) during 21 weeks, 5.4 g/L Comparison: whey based formula without prebiotics	Outcomes Nutrition status at the end of the 21 weeks	Location: Algeria Setting: ambulatory Source of funding: Numico Research, Wageningen, The Netherlands.

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		month before inclusion (eg, yogurt, fermented milk products), malnutrition, and twins or infants living in the same house			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Moore 2003	Parallel randomized controlled trial	Age group: infants Risk of allergy: not described, probably average Inclusion criteria: Healthy infants 4 to 12 months, birth weights >2500g and < 4200, tolerance of rice cereal and standard milk-based, lactose-containing infant formula. Exclusion criteria: Major congenital birth deformities, acute illness at enrolment, chronic conditions affecting food intake or metabolism, infants receiving more than one feeding of breast milk per day and infants participating in another clinical study.	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: 0.03 g FOS/g cereal (0.75 g FOS/25 g serving of cereal) Comparison: cereal without prebiotics and 0.03g of maltodextrin	Adverse events and nutrition status at month of follow-up.	Location: USA Setting: ambulatory care Source of funding: Nestle Nutrition Institute (Glendale, CA, USA).

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Moro 2002	Parallel randomized controlled trial	Age group: infants Risk of allergy: not described, probably average Inclusion criteria: infants not being breastfed Exclusion criteria: not described	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: formula supplemented with prebiotic at 0.4g/dL for 4 weeks Comparison: formula without prebiotics (maltodextrin as placebo)	Nutrition status at one month of follow-up	Location: Italy, Germany Setting: ambulatory Source of funding: none declared

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Partty 2013	Parallel randomized controlled trial	Age group: infants Risk of allergy: no description, probably average Inclusion criteria: Infants with a gestational age between 32 + 0 and 36 + 6 weeks, birth weight >1500 g Exclusion criteria: Absence of any congenital defects in the gastrointestinal system or other defects preventing enteral nutrition.	Prebiotic: Oligosaccharide (FOS/GOS) Dosage/presentation: capsules with 600 mg/d each day for 30 days, then 600 mg twice a day for 52 weeks Comparison: placebo capsules	Nutrition status at 1 year of age (weight)	Location: Finland Setting: ambulatory Source of funding: Supported by the Juho Vainio Foundation, the Paivikki and Sakari Sohlberg Foundation, the Foundation for Pediatric Research, an EVO grant from Turku University Hospital

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Piemontese 2011	Parallel randomized controlled trial (follow-up from Gruber 2010)	Age group: newborns and infants Risk of allergy: not declared, probably average risk Inclusion criteria: healthy, term infants (gestational age 37 to 42 weeks), with a normal birth weight (p10 and p90 for gestational age according to locally applicable growth charts), aged up to 8 weeks when entering the study, without a positive family history of allergic disease (hay fever, asthma or atopic dermatitis) and without a metabolic disorder requiring a special diet. Exclusion criteria: mothers suffering from hepatitis B, HIV	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: formula with 8 g/L with 6.8 g/L neutral and 1.2 g/L pectin acidic-oligosaccharides for 32 weeks Comparison: formula without prebiotics	Adverse events at 12 months Nutrition status at 12 months	Location: multicentre (Austria, Germany, Switzerland, others) Setting: ambulatory Source of funding: Danone research, Friedrichsdorf Germany

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		or Group B streptococcal infection during pregnancy; mothers taking antibiotics during breastfeeding; infants with known congenital or postnatal diseases			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Ripoll 2015	Randomized multi-centre controlled trial from different hospitals in Spain	Age group: Infants 4m of age at randomization Risk of allergy: average Inclusion criteria: 4 months (72 weeks) of age at randomization, not breastfed or who had stopped breastfeeding for at least 1 month prior to inclusion and who were available for 8 months following randomization. Exclusion criteria: Infants suffering from a chronic or severe disease susceptible to interfere with one of the evaluation criteria or suffering a gastro-intestinal disease (short bowel, cystic fibrosis, malabsorption, constipation, cow milk allergy), infants who had taken antibiotics during the 2 months preceding randomization, infants fed with	Prebiotic: Oligosaccharides (ScFOS) Dosage/presentation: formula with 5 g/L of prebiotics for 40 weeks Comparison: same formula without prebiotics	Nutritional status (weight) at 10 months of follow-up	Location: Spain multicentre Setting: ambulatory Source of funding: Supported by Syral Iberia S.A.U., Saragossa, Spain. The Ministry of Economy and Competitiveness approved and supported this IDI (Research, Development and Innovation) project during 2008 and 2009

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		FOS supplemented follow-on milk formula or regularly receiving yogurt or fermented dairy product before, or had a concomitant use of laxative or anti-secretory drugs that could modify intestinal flora and infant whose vaccination programme deviated from the one requested by the protocol			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Salvini 2011	Parallel randomized controlled trial	Age group: newborns and infants Risk of allergy: not described, probably average Inclusion criteria: Gestational age between 37 and 42 wk, a birth weight appropriate for gestational age, and exclusive formula feeding from the first day of life Exclusion criteria: Prematurity, breastfeeding, major malformations, and HCV infection, Infants who developed metabolic, endocrinologic, and immunologic disorders, lactose intolerance and/or allergy to cow' s milk as well as assumption of other pre- or	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: milk formula with 8 g/L of prebiotic for 52 weeks Comparison: formula without prebiotic	Nutrition status at 12 months of age	Location: multicentre, USA, Italy Setting: ambulatory Source of funding: Danone research

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
		probiotics			

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Scholtens 2008	Parallel randomized controlled trial	Age group: newborns and infants Risk of allergy: average and high Inclusion criteria: Healthy, term infants, with a normal birth weight (between the 5th and 95th percentiles) Exclusion criteria: Infants with a double heredity for atopic diseases, congenital abnormality or disease, gastrointestinal disease, a strongly suspected cow's milk intolerance, or with regular use of products with probiotic bacteria	Prebiotic: oligosaccharides (FOS/GOS) Dosage/presentation: 6 g/L scGOS/lcFOS in a ratio of 9:1 in formula for 6 months Comparison: formula without prebiotics	adverse events at 6 months	Location: Belgium Setting: ambulatory Source of funding: Numico Research, Wageningen, The Netherlands

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Sierra 2015	Randomized controlled trial (multicentre) in Spain	Term infants of less than 2 months of age. Unknown risk of allergy. Inclusion criteria: Gestational age of 37–42 weeks and a birth weight greater than 2,500 g and were exclusively formula fed for at least 15 days prior to enrolment Exclusion criteria: Infants that had previously consumed either prebiotics or probiotics and infants who themselves, or their mothers, had clinically significant diseases	Prebiotic: Galacto-oligosaccharide Dosage/presentation: formula (0.5 g/dL) Comparison: formula without prebiotics	Any allergy (as defined and detected by clinicians) including atopic dermatitis, ashtma/wheezing, and food allergy. Nutritional status (weight) at 12 months of follow-up	Location: Spain Setting: ambulatory Source of funding: Hero group (manufacturer)

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Veereman-Wauters 2011	Parallel randomized controlled trial	Age group: newborns Risk of allergy: not declared, probably average Inclusion criteria: Healthy babies with exclusive formula feeding and normal feeding behavior were included. Exclusion criteria: Born by cesarean section, and/or had respiratory, neurological, or GI problems, infections, use of antibiotics by the baby (including eye or nose drops) or by mother in the breast-fed group, fever, feeding problems, the need for therapeutic formulae (semi-elemental, hydrolyzed) or formula thickeners or cereals.	Prebiotic: Oligosaccharides (FOS/GOS) Dosage/presentation: GOS:FOS formula, 90:10 with long-chain inulin, 450-g tin cans, 0.8 g/l for 4 weeks Comparison: formula without prebiotics	Nutrition status at 1 month	Location: Belgium Setting: ambulatory Source of funding: Beneo-Orafti

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Wernimont 2015	Randomized controlled trial (multicentre)	Age group: newborns, 5 to 15 days of age Risk of allergy: not declared, probably average Inclusion criteria: All infants were required to have been either exclusively formula fed or human milk fed from birth Exclusion criteria: Infants that had previously consumed either prebiotics or probiotics and infants who themselves, or their mothers, had clinically significant diseases were excluded	Prebiotic: Oligosaccharides (FOS/GOS)^a Dosage/presentation: FOS formula, with 3 g/L for 8 weeks Comparison: Formula without prebiotics	Nutritional status (baseline adjusted weight) at 8 weeks of follow-up Adverse events related to feeding with prebiotics as judged and defined by clinicians	Location: United States Setting: ambulatory Source of funding: Nestlé, Wyeth, and DuPont

Table 2. Included studies

Study	Methods	Participants	Intervention arms	Outcomes	Notes
Ziegler 2007	Parallel randomized controlled trial	Age group: infants and newborns Risk of allergy: not described, probably average Inclusion criteria: Healthy infants who, had been born at a gestational age that exceeded 37 weeks, had a birth weight $\geq$ 2500 g, and were solely formula-fed for at least 24 hours before randomization Exclusion criteria: History of disease or congenital malformation, evidence of formula intolerance or poor intake, weight at visit 1 of $<$98% of birth weight, or born large-for-gestational age from a mother who was diabetic at childbirth	Prebiotic: PDX, GOS, and LOS Dosage/presentation: formula supplemented with 8 g/L of a prebiotic blend containing PDX, GOS, and LOS, 50:33:17 ratio for 17 weeks Comparison: control formula without prebiotics	Nutrition status at 4 months of age Adverse events at 4 months of age	Location: United States Setting: ambulatory Source of funding: Mead Johnson & Co

FOS, fructo-oligosaccharides; PDX, polydextrose; GOS, galacto-oligosaccharides; LOS, lacto-oligosaccharides; HCV, hepatitis-C virus; HIV, human immunodeficiency virus.

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
Aa 2011	Wrong intervention (Synbiotic)
Abrams 2005	Outcomes extremely indirect for our review
Abrams 2007	Outcomes extremely indirect for our review
Agostoni 2006	Ongoing trial, no results
Agustina 2007	Wrong intervention (synbiotics)
Alliet 2007	Outcomes extremely indirect for our review
Arslanoglu 2007	Outcomes extremely indirect for our review
Bakker-Zierikzee 2005	Outcomes extremely indirect for our review
Bakker-Zierikzee 2006	Outcomes extremely indirect for our review
Berg 2013	Population outside our objectives (preterm babies)
Berg 2013a	Population outside our objectives (preterm babies)
Bisceglia 2009	Population outside our objectives (preterm babies)
Boehm 2002	Population outside our objectives (preterm babies)
Boehm 2009	Protocol published
Bu 2007	Different population (constipation patients)

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
Chouraqui 2008	Different intervention (synbiotics)
Dasopoulou 2015	Different population (preterm babies)
Dilli 2013	Different population outside our objectives
Fanaro 2009	Outcomes extremely indirect for our review
Firmansyah 2009	Different intervention (synbiotics)
Firmansyah 2011	Different intervention (synbiotics)
Friedt 2010	Outcomes extremely indirect for our review
Gerasimov 2010	Population already with atopic dermatitis
Guimber 2010	Outcomes extremely indirect for our review
Herrera 2015	Outcomes extremely indirect for our review
Holscher 2012	Outcomes extremely indirect for our review
JPRN-UMIN000008142 2012	Ongoing trial, no results
Kapiki 2007	Different population (preterm babies)
Kajzer 2016	Different intervention and outcomes
Knol 2005	Outcomes extremely indirect

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
Kuitunen 2009	Intervention not relevant for our review (probiotics)
Kuitunen 2009a	Intervention not relevant for our review (synbiotic)
Kukkonen 2006	Outcomes extremely indirect for our review
Kukkonen 2007	Intervention not relevant for our review (synbiotic)
Kukkonen 2008	Intervention not relevant for our review (synbiotic)
Kukkonen 2011	Intervention not relevant for our review (probiotic)
Kukkonen 2011a	Intervention not relevant for our review (probiotic)
Kumar 2013	Population and intervention not relevant for our review (probiotics and infection prevention)
Modi 2010	Population not relevant for our review (preterm babies)
Moro 2003	Intervention not relevant for our review (synbiotic)
Nakamura 2010	Population not relevant for our review (undernourished children)
Niele 2013	Population not relevant for our review (preterm babies)
NTR1359 2008	Ongoing trial
NTR2521 2010	Ongoing trial

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
NTR3505 2012	Ongoing trial
Panigrahi 2008	Intervention and outcome not relevant for our review (synbiotics)
Perez 2010	Outcomes extremely indirect for our review
Pontes 2016	Different intervention and outcomes
Puccio 2007	Intervention not relevant for our review (synbiotic)
Raes 2010	Outcomes extremely indirect for our review
Ribeiro 2012	Outcomes extremely indirect for our review
Ringel-Kulka 2009	Intervention not relevant for our review (synbiotics)
Riskin 2010	Population not relevant for our review (preterm babies)
Rodriguez-Palmero 2013	Outcomes extremely indirect for our review
Rosenfeldt 2004	Outcomes extremely indirect for our review
Savino 2006	Outcomes extremely indirect for our review
Sazawal 2010	Intervention not relevant for our review (synbiotics)
Sazawal 2010a	Intervention not relevant for our review (synbiotics)

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
Scalabrin 2010	Outcomes extremely indirect for our review
Scalabrin 2010a	Outcomes extremely indirect for our review
Scalabrin 2010b	Outcomes extremely indirect for our review
Scalabrin 2010c	Outcomes extremely indirect for our review
Scalabrin 2012	Outcomes extremely indirect for our review
Schmelzle 2003	Intervention not relevant for our review (synbiotics)
Scholtens 2006	Outcomes extremely indirect for our review
Schouten 2011	Outcomes extremely indirect for our review
Shadid 2007	Outcomes extremely indirect for our review
Shibata 2009	Population not relevant for our review (infants with atopic dermatitis). This is a treatment study.
Stam 2011	Outcomes extremely indirect for our review
Underwood 2009	Intervention not relevant for our review (synbiotics)
Vaisman 2010	Population not relevant for our review (children with diarrhea)
van de Pol 2011	Intervention not relevant for our review (synbiotics)

Table 3. Characteristics of excluded studies

Study	Reason for exclusion
Van Der Aa 2009	Intervention not relevant for our review (synbiotics)
van Der Aa 2010	Intervention not relevant for our review (synbiotics)
van Der Aa 2012	Intervention not relevant for our review (synbiotics)
van Hoffen 2009	Outcomes extremely indirect for our review
van Stuijvenberg 2011	Outcomes extremely indirect for our review
van Stuijvenberg 2015	Outcomes extremely indirect for our review
Vivatvakin 2010	Interventions not relevant for our review (synbiotics)
Westerbeek 2008	Outcomes extremely indirect for our review
Westerbeek 2011	Preterm infants only. Different population.
Westerbeek 2011a	Preterm infants only. Different population.
Westerbeek 2011b	Preterm infants only. Different population.
Westerbeek 2013	Outcomes extremely indirect for our review
Yao 2010	Outcomes extremely indirect for our review