Web appendix: Supplementary data

Azad MA, Coneys JG, Kozyrskyj AL, Field CJ, Ramsey CD, Becker AB, Friesen C, Abou-Setta AM, Zarychanski R. **Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: A systematic review and meta-analysis.**

Table S1. Research question using PICOS structure.

Population	Fetuses carried to term, or healthy term infants under 1 year of age, without recurrent wheeze or asthma. **Since both prenatal and postnatal exposure to probiotics will be considered, the population may comprise mother-infant pairs, where the mother received probiotics during pregnancy.*
Intervention	Probiotics (live microorganisms consumed for their presumed health benefits): any strain, preparation or dose, administered with or without prebiotics.
Comparator	Any comparator including active comparator (non-probiotic), no intervention, or placebo.
Outcomes	*Primary Outcome:* • Incidence of doctor-diagnosed asthma. *Secondary Outcomes:* • Incidence of parent-reported asthma. • Incidence of wheeze (parent-reported or clinically-diagnosed). • Asthma Predictive Index score. • Incidence of hospitalization for asthma. • Incidence of asthma medication use. • Incidence of lower respiratory tract infection (rationale: these infections generally include wheeze). *Safety Outcomes:* • Severe gastrointestinal disturbances • Allergic reaction to probiotic • Withdrawal due to perceived side effects
Study design	Prospective randomized controlled trials.

Table S2. Study eligibility criteria.

Inclusion Criteria	1. Prospective, randomized, controlled trial. 2. Prenatal or postnatal administration of probiotics to mother or infant: any type, dose, route or frequency. 3. Majority of infants (>80%) must be under 1 year of age, or unborn, at randomization. 4. Majority of infants (>80%) must be healthy (not suffering from acute illness) at randomization. Infants with allergic disorders (other than wheeze or asthma) remain eligible.
Exclusion Criteria	1. Non-human studies. 2. Observational study designs, quasi-randomized, cross-over, or cluster randomized trials. 3. Study primarily enrolled infants with documented wheeze or asthma. 4. Majority of enrolled children were preterm infants (born at less than 36 completed weeks of gestation). 5. All subjects received probiotics. 6. No outcomes of importance to the review were reported, or available via contact with trial authors.

Table S3. PubMed / MEDLINE search strategy

1. probiotic* OR lactobacill* OR bifidobacteri*
2. probiotics[MeSH Terms]
3. infant* OR infancy OR newborn* OR neonat* OR pediatric* OR paediatric*
4. infant[MeSH Terms]
5. (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab]) NOT (animals [mh] NOT humans [mh])
6. (#1 OR #2) AND (#3 OR #4) AND #5

Table S4. Selection criteria associated with "allergic disease" in included trials.

Primary Article	Allergic disease-related selection criteria
West 2009	None (subgroup analyses performed, with infants classified as high risk for allergy when first-degree relative has "allergic disease")
Abrahamsson 2007	First-degree relative has eczema, asthma, gastrointestinal allergy, allergic urticaria, or ARC
Kalliomaki 2001	First-degree relative has AD, AR, or asthma
Niers 2009	AD, AR, food allergy, or asthma in either the mother, or the father plus an older sibling
Wickens 2008	Parent has history of treated asthma, eczema, or hay fever
Kukkonen 2007	Parent has diagnosed AR, AD or asthma.
Taylor 2007	Mother has diagnosed asthma, AR or eczema plus positive skin prick test
Gore 2012	Infant has diagnosed AD (SCORAD ≥ 10)
Ou 2012	Mother has diagnosed asthma, eczema, food allergy or AR and elevated total IgE
Dotterud 2010	None (subgroup analyses performed, with family history defined as: first-degree relative with AD, ARC, or asthma)
Kopp 2008	First-degree relative has AD, AR, or asthma and confirmed allergic sensitization against an inhalant allergen
van der Aa 2011	Infant has AD (SCORAD > 15)
Hol 2008	Infant has cow's milk allergy diagnosed with food challenge
Boyle 2011	First-degree relative has diagnosed AR, eczema, food allergy or asthma
Maldonado 2012	None
Chouraqui 2008	None
Gruber 2007	Infant has mild-to-moderate AD (SCORAD 15 – 40)
Allen 2010	First-degree relative has diagnosed asthma, eczema or AR (primarily; some women without family history were also recruited)
Hascoet 2011	None
Puccio 2007	None

AD, atopic dermatitis; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; SCORAD, index for SCORing Atopic Dermatitis.

Table S5. Summary risk of bias assessment.

Primary Article	OVERALL	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
West 2009	**HIGH**	Low	Low	Low	Low	Low (1y) High (8y)	Low	Low
Abrahamsson 2007	**HIGH**	Low	Low	Low (2y) High (7y)	Low (2y) High (7y)	High	Low	Low
Kalliomaki 2001	**UNCLEAR**	Low	Low	Low	Low	Unclear (2y) Unclear (4/7y)	Unclear (2y) Low (4/7y)	Low
Niers 2009	**HIGH**	Unclear	Low	Low (2y) High (6y)	Low	High (2y) High (6y)	Low	Low
Wickens 2008	**HIGH**	Low	Low	Low (2y) High (4y & 6y)	Low	Low (2y & 4y) Unclear (6y)	Low	Low
Kukkonen 2007	**UNCLEAR**	Low	Low	Low	Low	Low (2y) Unclear (5y)	Low	Low
Taylor 2007	**HIGH**	Low	Low	Low	Low	High (2.5y) High (5y)	Low	Low
Gore 2012	**UNCLEAR**	Low	Unclear	Low	Low	Low	Unclear	Unclear
Ou 2012	**HIGH**	Unclear	Low	Low	Low	High	Unclear	Low
Dotterud 2010	**HIGH**	Low	Low	Low	Low	High	Low	Low
Kopp 2008	**LOW**	Low	Low	Low	Low	Low	Low	Low
van der Aa 2011	**HIGH**	Low	Low	Low	Low	High	High	Low
Hol 2008	**UNCLEAR**	Low	Low	Low	Low	Unclear	Low	Low
Boyle 2011	**UNCLEAR**	Low	Low	Low	Low	Unclear	Unclear	Low
Maldonado 2012	**HIGH**	Low	Low	Low	Low	High	Low	Low
Chouraqui 2008	**UNCLEAR**	Low	Low	Low	Low	Unclear	Low	Unclear
Gruber 2007	**UNCLEAR**	Low	Unclear	Low	Unclear	Low	Unclear	Low
Allen 2010	**UNCLEAR**	Low	Low	Low	Low	Unclear	Low	Low
Hascoet 2011	**UNCLEAR**	Low	Low	Low	Low	Unclear	Low	Low
Puccio 2007	**HIGH**	Low	Low	Low	Low	High	Low	Low

Risk of bias assessed according to the Cochrance Collaboration Risk of Bias tool. Some domains were re-assessed after extended follow up (shown in brackets). Trials are listed according to follow-up duration, as in Table 1.

Table S6. Relevant outcome definitions from included trials.

Primary Article	Diagnosed Asthma	Wheeze	Lower Respiratory Tract Infection
West 2009	Age 1: Parent-reported doctor diagnosis; verified by examination of health records, and prescription of inhalant steroids. Age 8: Parent-reported doctor diagnosis, with wheeze that responded to bronchodilator therapy and/or a clinical history of wheeze and increase in FEV_1 > 12% from baseline following terbutalin inhalation.	Modified ISAAC questionnaire ("ever wheezing or whistling in the chest")	NR
Abrahamsson 2007	Age 2: ≥ 3 wheezing episodes, at least 1 verified by a physician Age 7: At least one of: 1) doctor diagnosis and asthma symptoms and/or medication during last 12 months; 2) wheeze or nocturnal cough and positive reversibility test and/or pathological FENO value. If the based on doctor diagnosis, medical records were reviewed to confirm GINA criteria	Modified ISAAC questionnaire (episode with obstructive airway symptoms)	NR
Kalliomaki 2001	Age 2: Chronic or recurrent cough, wheeze or shortness of breath, and effective antiasthma treatment Age 4: Chronic or recurrent cough, wheeze, or shortness of breath requiring regular inhaled corticosteroids Age 7: Qualified for asthma medication reimbursement	NR	NR
Niers 2009	Age 6: At least one of: physician diagnosed asthma active in past 12 months, parent reported wheeze in past 12months (modified ISAAC questionnaire), use of asthma medication in past 12 months and/or ≥9% reversibility in $FEV_{0.5}$ or FEV_1.	Age 2: Adapted British Medical Research Council questionnaire and European Community Respiratory Health Survey.	NR
Wickens 2008	Age 6: ISAAC questionnaire (history of asthma) plus wheeze or inhaler use in last 12 months	ISAAC questionnaire	NR
Kukkonen 2007	Age 2: ≥ 2 physician-diagnosed wheezing episodes with persistent cough or exercise-induced symptoms Age 5: as above, or reversible bronchial obstruction by oscillometry	NR	NR
Taylor 2007	Age 2.5: recurrent wheezing (≥ 3 episodes, at least 1 confirmed by a physician) and responded to bronchodilator therapy Age 5: recurrent wheeze (>2 episodes in last 12 months) and responded to bronchodilator therapy	Parents reporting "noisy breathing" were asked to give detailed descriptions. Only children with a convincing history of 'wheeze' (or physician documented wheeze) were classified.	Physician-confirmed chest infection (excludes 'upper respiratory infections' limited to coryzal symptoms in the absence of significant chest symptoms).
Gore 2012	Doctor-diagnosed asthma. (Reported in text only.	Interviewer-administered validated respiratory	NR

	Authors declined to provide data.)	questionnaire	
Ou 2012	≥ 3 episodes of wheezing/coughing, requiring bronchodilator treatment, and diagnosed by a physician	NR (authors clarified that in their report, "wheezing ever" actually refers to their asthma outcome, defined to the left)	NR
Dotterud 2010	≥ 3 wheezing episodes in last 12 months plus treatment by inhaled steroids, or signs of hyper-reactivity (cough or wheeze at excitement or impaired night sleep) without respiratory infection.	NR	NR
Kopp 2008	NR	≥ 5 episodes of wheezing bronchitis during the first 2 years (by parent report)	NR
van der Aa 2011	NR	≥3 episodes of wheezing, and wheezing apart from colds	NR
Hol 2008	NR	Structured interview	NR
Boyle 2011	NR	Not defined	NR
Maldonado 2012	NR	NR	Diagnosis by study pediatrician: mucosity and/or cough during ≥ 2 consecutive days with or without fever and presence of wheezing and/or crepitants, including acute bronchitis, bronchiolitis, and pneumonia.
Chouraqui 2008	NR	NR	Bronchiolitis as serious AE. AEs recorded by investigators or physician, coded using Medical Dictionary for Regulatory Activities, and considered serious if life threatening, caused permanent harm, required hospitalization, or was considered medically relevant.
Gruber 2007	NR	NR	LRTI as AE: no definition provided.
Allen 2010	NR	NR	Unspecified acute lower respiratory infection (ICD10 J22) or Bronchiolitis (J21.9), based on parent report and categorized independently by 2 pediatricians using ICD10 criteria.
Hascoet 2011	NR	NR	Lower Respiratory AE. AEs defined as illnesses or symptoms that occurred or worsened during the study. Recorded and evaluated by investigators.
Puccio 2007	NR	Parent-reported	NR

AE, adverse event; ICD10, International Classification of Diseases; FENO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; GINA, Global Initiative for Asthma; ISAAC, International Study of Asthma and Allergies in Childhood; LRTI, lower respiratory tract infection; NR, outcome not reported.

Table S7. Adverse events in included trials.

Primary Article	Severe Gastrointestinal Disturbance		Allergic Reaction		Withdrawal Due to Perceived Side Effects	
	Probiotic	Control	Probiotic	Control	Probiotic	Control
West 2009	NR	NR	NR	NR	NR	NR
Abrahamsson 2007	1/106 (0.9%)	1/103 (1.0%)	NR	NR	2/117 (1.7%)	1/115 (0.9%)
Kalliomaki 2001	NR	NR	NR	NR	NR	NR
Niers 2009	NR	NR	NR	NR	NR	NR
Wickens 2008	NR	NR	NR	NR	3/341 (0.9%)	1/171 (0.6%)
Kukkonen 2007	7/610 (1.1%)	12/613 (2.0%)	NR	NR	2/610 (0.3%)	4/613 (0.7%)
Taylor 2007	NR	NR	NR	NR	3/111 (2.7%)	1/115 (0.9%)
Gore 2012	NR	NR	NR	NR	NR	NR
Ou 2012	NR	NR	NR	NR	NR	NR
Dotterud 2010	NR	NR	NR	NR	NR	NR
Kopp 2008	NR	NR	NR	NR	NR	NR
van der Aa 2011	0/46 (0%)	0/44 (0%)	NR	NR	1/46 (2.2%)	1/44 (2.3%)
Hol 2008	NR	NR	NR	NR	0/59 (0%)	0/60 (0%)
Boyle 2011	NR	NR	NR	NR	1/125 (0.8%)	1/125 (0.8%)
Maldonado 2012	NR	NR	NR	NR	NR	NR
Chouraqui 2008	0/214 (0%)	2/70 (1.4%)	2/214 (0.9%)	0/70 (0%)	NR	NR
Gruber 2007	1/56 (1.8%)	0/50 (0%)	NR	NR	NR	NR
Allen 2010	NR	NR	NR	NR	NR	NR
Hascoet 2011	NR	NR	NR	NR	4/40 (10.0%)	1/40 (2.5%)
Puccio 2007	NR	NR	NR	NR	NR	NR

NR, not reported.

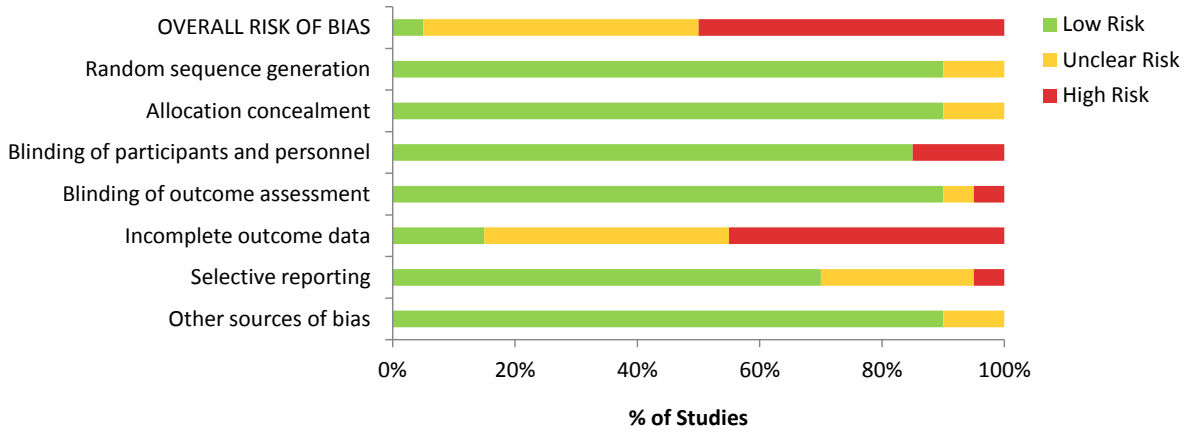

Figure S1. Summary risk of bias assessment. Included trials (n=20) were assessed for internal validity using the Cochrane Collaboration's Risk of Bias tool (see also Table S4), which evaluates 7 sources of bias across 6 domains. The percentage of trials adjudicated to be of low, unclear and high risk of bias, for each domain, are shown here.

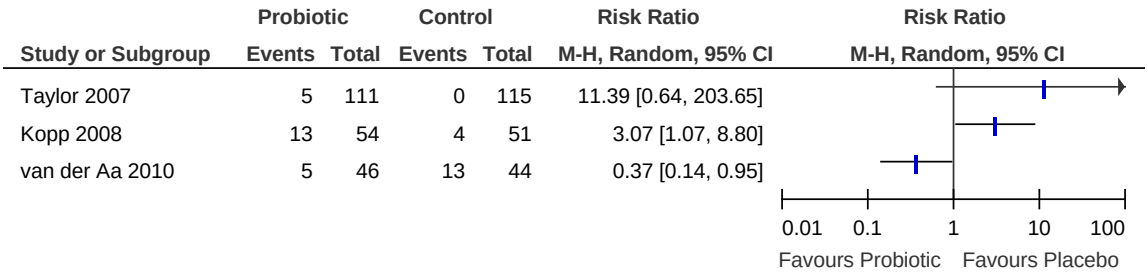

Figure S2. Probiotic supplementation during pregnancy or infancy and recurrent wheeze in children. The longest available follow up data (intention to treat) were extracted from each contributing trial. Trials are sorted in order of decreasing duration of follow up. Pooled effect estimates are not presented due to substantial statistical (I^2 = 83%) and clinical heterogeneity. CI, confidence interval; M-H, Mantel-Haenszel.

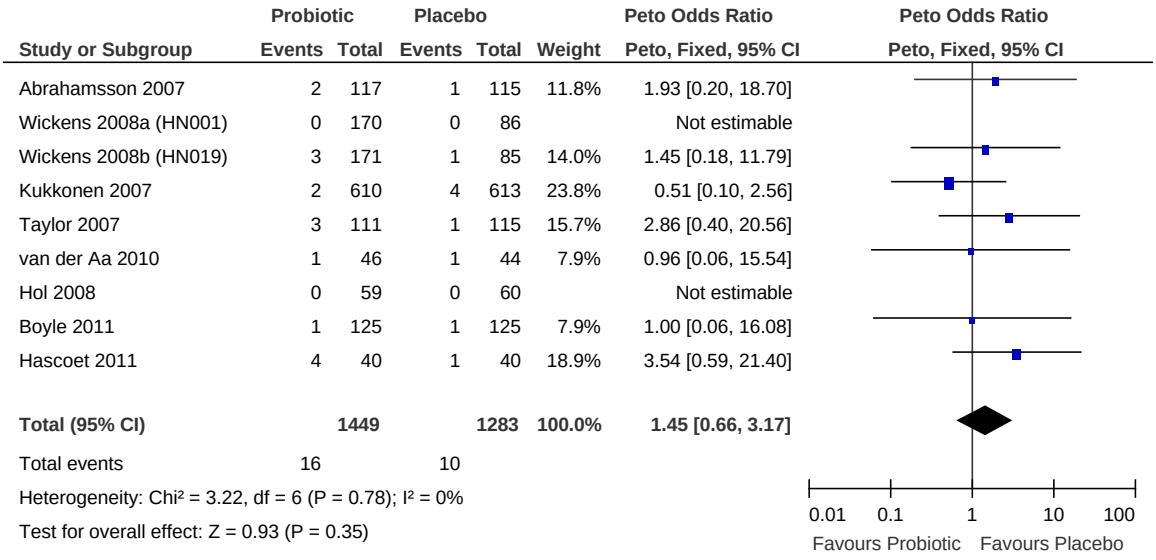

Figure S3. Probiotic supplementation during pregnancy or infancy and withdrawal due to perceived side effects. The longest available follow up data (intention to treat) were extracted from each contributing trial. Trials are sorted in order of decreasing duration of follow up. CI, confidence interval; df, degrees of freedom.

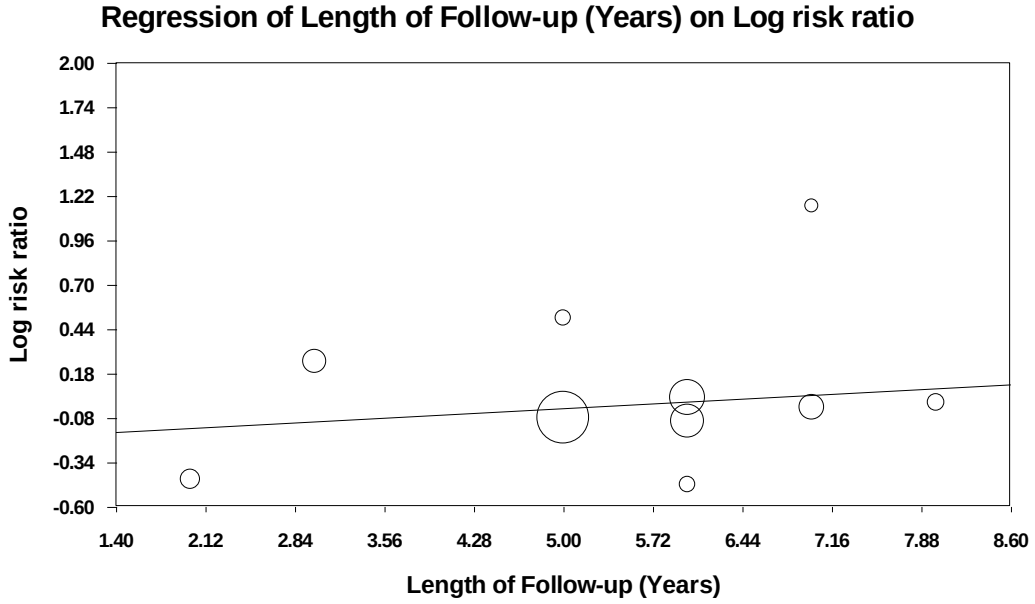

Figure S4. Meta-regression of time (duration of follow up) on log risk ratio for probiotic supplementation during pregnancy or infancy in the prevention of clinician diagnosed asthma (n = 9 trials). Mixed effects regression model (method of moments); p =0.65. One three-arm trial (Wickens et al.) evaluated two different probiotics, which are plotted separately.