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RESEARCH

Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: systematic review and meta-analysis

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Abstract

Objective To evaluate the association of probiotic supplementation during pregnancy or infancy with childhood asthma and wheeze.

Design Systematic review and meta-analysis of randomised controlled trials.

Data sources Medline, Embase, and Central (Cochrane Library) databases from inception to August 2013, plus the World Health Organization's international clinical trials registry platform and relevant conference proceedings for the preceding five years. Included trials and relevant reviews were forward searched in Web of Science.

Review methods Two reviewers independently identified randomised controlled trials evaluating probiotics administered to mothers during pregnancy or to infants during the first year of life. The primary outcome was doctor diagnosed asthma; secondary outcomes included wheeze and lower respiratory tract infection.

Results We identified 20 eligible trials including 4866 children. Trials were heterogeneous in the type and duration of probiotic supplementation, and duration of follow-up. Only five trials conducted follow-up beyond participants' age of 6 years (median 24 months), and none were powered to detect asthma as the primary outcome. The overall rate of doctor diagnosed asthma was 10.7%; overall rates of incident wheeze and lower respiratory tract infection were 33.3% and 13.9%, respectively. Among 3257 infants enrolled in nine trials contributing asthma data, the risk ratio of doctor diagnosed asthma in participants randomised to receive probiotics was 0.99 (95% confidence interval 0.81 to 1.21, I^2=0%). The risk ratio of incident wheeze was 0.97 (0.87 to 1.09, I^2=0%, 9 trials, 1949 infants). Among 1364 infants enrolled in six trials, the risk ratio of lower respiratory tract infection after probiotic supplementation was 1.26 (0.99 to 1.61, I^2=0%). We adjudicated most trials to be of high (ten trials) or unclear (nine trials) risk of bias, mainly due to attrition.

Conclusions We found no evidence to support a protective association between perinatal use of probiotics and doctor diagnosed asthma or childhood wheeze. Randomised controlled trials to date have not yielded sufficient evidence to recommend probiotics for the primary prevention of these disorders. Extended follow-up of existing trials, along with further clinical and basic research, are needed to accurately define the role of probiotics in the prevention of childhood asthma.

Systematic review registration PROSPERO (CRD42013004385).

Introduction

Over the past half century, there has been a sharp rise in the global prevalence of asthma, particularly in children.[1] About 300 million people worldwide are estimated to have asthma, and the prevalence has been increasing by 50% every decade.[1] As the most common chronic disease of childhood, asthma affects roughly one in five children in the United Kingdom and United States,[2] and is the leading cause of school absenteeism.[3]

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Web appendix: Supplementary data

The total annual cost of asthma to society has been estimated at €19bn (£16.2bn; $26.2bn) in Europe[4] and $56bn in the US.[5] Recurrent wheeze frequently precedes the diagnosis of asthma, and is estimated to occur in more than 20% of infants.[6 7]

The microflora hypothesis of allergic disease has been proposed to explain the rising incidence of asthma and other allergic disorders.[8] Commensal gut bacteria stimulate development of the neonatal immune system; therefore, disruption of the gut microbiota during early life may contribute to immune disorders later in childhood.[9] Indeed, prospective studies have shown that perturbation of the infant gut microbiota precedes development of atopic dermatitis (allergic eczema),[10-12] which is widely regarded as the first step in the progressive "atopic march" towards allergic rhinitis and asthma.[13] Moreover, early life factors that disrupt the gut microbiota (such as caesarean delivery, lack of breastfeeding, and use of antibiotics) increase the risk of asthma.[14-16]

In the light of this evidence, probiotics—live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host—have been proposed for the prevention and treatment of allergic disorders including asthma.[17 18] Although naturally present in fermented foods, probiotics are increasingly being produced and administered as supplements in preventative and therapeutic medicine.[19] A recent meta-analysis of 14 randomised controlled trials showed that probiotic supplementation during pregnancy or infancy decreased the incidence of atopic dermatitis by 21%.[20] Less clinical evidence exists for probiotics in the prevention of wheeze or asthma,[21] but animal studies have shown that perinatal use of probiotics can prevent airway inflammation and hyper-reactivity.[22 23] A 2007 Cochrane review of early life probiotics for prevention of allergic diseases reported no benefit for asthma prevention after probiotic supplementation,[24] based on findings from three trials[25-27] enrolling a total of 617 infants. All three existing trials have since published extended follow-up results,[28-31] and six additional trials enrolling 2308 infants have published new findings on probiotics for asthma prevention.[21 32-36] Prevention of related outcomes (wheeze or lower respiratory infection) was not covered in the 2007 Cochrane review.

The purpose of this systematic review was to identify, critically appraise, and meta-analyse data from prospective randomised trials evaluating the use of probiotic supplements for the primary prevention of asthma or childhood wheeze.

Methods

Using an a priori published protocol,[37] we conducted our systematic review using methodological approaches outlined in the *Cochrane Handbook for Systematic Reviewers*[38] and reported according to the criteria of preferred reporting items for systematic reviews and meta-analyses (PRISMA).[39] A technical panel of experts from multiple fields (nutrition, paediatric asthma, research methodology) formulated the review questions, reviewed the search strategies and review methods, and provided input throughout the review process.

Populations, interventions, comparators, outcome measures, settings, and study designs

We included only randomised controlled trials of pregnant women or healthy infants under 1 year of age (web appendix, table S1). Our primary research question was "In healthy infants under 1 year of age, are probiotics (administered prenatally or postnatally) safe and protective against the development of wheeze or asthma, compared to placebo, or to no intervention?"

The main outcome measure was doctor diagnosed asthma. Secondary outcomes included wheeze (incident or recurrent), asthma drug use, hospital admission for asthma, and the asthma predictive index score.[40] Lower respiratory tract infections were also included as a secondary outcome because these infections generally involve wheeze and could predispose for asthma.[41] Safety outcomes included gastrointestinal disturbances, allergic reaction to probiotics, and withdrawal due to perceived side effects. Table S2 presents inclusion and exclusion criteria (web appendix).

Search strategy for identification of studies

We searched Medline, Embase, and Central (Cochrane Library) databases from inception to August 2013 for relevant citations of published trials, using individualised search strategies for each database. Table S3 presents the Medline strategy (web appendix). We searched the World Health Organization's international clinical trials registry platform and relevant conference proceedings for the preceding five years to identify relevant planned, ongoing, or recently completed but unpublished trials (American Thoracic Society; American Academy of Allergy, Asthma, and Immunology; European Respiratory Society; European Academy of Allergy and Clinical Immunology; and the American Association for Parenteral and Enteral Nutrition). We performed forward searches of included trials and relevant reviews in the Web of Science to identify additional citations, and contacted study authors to request relevant unpublished data. Reference lists of narrative and systematic reviews and of the included trials were searched for additional citations. We performed reference management in EndNote X6 (Thomson Reuters).

Study selection

We used a two stage process for study screening and selection using standardised and piloted screening forms. Firstly, two reviewers (MBA and JGC) independently screened the titles and abstracts of search results to determine whether each citation met the inclusion criteria. Second, the full text versions of potentially relevant citations were reviewed independently with reference to the predetermined inclusion and exclusion criteria (web appendix, table S2). Discrepancies between the two reviewers were resolved through consensus by discussion with a third reviewer (AMA-S), as required.

Data abstraction and management

Two reviewers (MBA and JGC) independently extracted data from included trials, using standardised and piloted data extraction forms. Discrepancies between the two reviewers were resolved through consensus in discussion with a third reviewer (AMA-S), as required. Extracted data included funding sources, demographics of the enrolled mothers and infants (family history of allergic disease, mode of delivery, infant sex, gestational age or age at enrolment, and breastfeeding), details of probiotic intervention (organism, daily dose, timing, duration, and method of administration), and relevant outcomes as described above. Since the nature of asthma and wheeze can change over time,[42] outcome data were extracted for predefined time periods (age <3 years, 3 to <6 years, and ≥6 years). When a trial reported results for more than one time period, results from the longest follow-up were included in the main meta-analysis; results from earlier time periods were included in subgroup analyses. Data management was performed using Microsoft Excel 2007.

Assessment of methodological quality and potential risk of bias

We evaluated internal validity using the Cochrane Collaboration's risk of bias tool,[43] which assesses randomisation and allocation of participants; blinding of participants, personnel, and outcome assessors; incomplete or selective reporting; and other relevant sources of bias. If trial methodology was unclear from the published report, authors were contacted for clarification.

Measures of treatment effect

We analysed data from the included trials using Review Manager (RevMan 5.2, the Cochrane Collaboration).[44] A formal meta-analysis was conducted if the data were statistically and clinically homogeneous. Pooled dichotomous data were expressed as a risk ratio, or Peto odds ratio in the event of rare outcomes.[45] A risk ratio or odds ratio less than one suggests a lower rate of the outcome (for example, asthma) among participants randomised to receive probiotics than among the control group. We used the random effects model for all analyses, with the exception of the Peto odds ratio (fixed effect model). Statistical heterogeneity was explored and quantified using the I^2 statistic.[46] All tests of statistical inference reflect a two sided α value of 0.05.

Subgroup analyses and meta-regression

We performed subgroup analyses to determine summary effect estimates in several prespecified groups, including: the participant receiving the intervention (mother or infant), duration and timing of intervention (prenatal or postnatal), probiotic organism and dose, duration of follow-up or age at assessment, asthma risk, caesarean delivery rate, geographical area, and source of funding.[37] We conducted meta-regression to evaluate differences in effect according to duration of follow-up as a continuous variable, using Comprehensive Meta Analysis (Biostat).[47]

Results

Trial characteristics and study populations

Of 3011 citations identified from electronic and hand searches, we included 20 unique randomised trials enrolling a total of 4866 infants (fig 1⇓, table 1⇓). These trials were represented by 20 primary articles,[21-58] four companion articles,[59-62] 10 extended follow-up publications[28-31 63-68] plus one forthcoming report, and eight duplicate conference abstracts.[69-76] All were double blind, placebo controlled trials published in peer reviewed journals between 2001 and 2013. Sixteen trials were conducted in Europe, while four trials were conducted in Australia,[27 49] New Zealand,[36] and Taiwan.[34] Based on family history or existing allergic disease in the mother or infant (definitions provided in web appendix, table S4), 14 of 20 trials enrolled participants at high risk for asthma; the remaining six trials[21-57] were conducted in unselected populations. The caesarean delivery rate in study populations ranged from 0% to 45%, and was not reported in six trials.[21-52] Most trials (14 of 20) did not restrict infant feeding practices, although six trials[50-58] required exclusive formula feeding for enrolment. Nearly all trials (19 of 20) reported some degree of industry support (funding, salary support, or supplied products), including seven trials involving authors employed by the industry sponsor.[32-58]

Overall, most trials were adjudicated to be of unclear (nine of 20) or high risk of bias (10 of 20); only one trial[55] was considered to have a low risk of bias across all domains (web appendix, table S5; fig S1). Of 20 trials, most had adequate random sequence generation (n=18), allocation concealment (n=18), and blinding of outcome assessment (n=18). However, eight and nine trials were subject to unclear and high risk of attrition bias, respectively, owing to incomplete outcome data after substantial loss to follow-up. Three trials were at high risk of performance bias due to unblinding of study participants at extended follow-up assessments (including one trial with unpublished data).[28 68]

Probiotic interventions

Table 2⇓ presents details of the probiotic interventions administered in each trial. All trials evaluated probiotic supplements, rather than consumer food products. Supplements were delivered orally by various methods, including capsules; oil droplets; and suspensions in water, milk, or infant formula. One trial[49] exclusively evaluated prenatal maternal supplementation, 10 exclusively evaluated postnatal infant supplementation, while nine evaluated a combination of prenatal and postnatal supplementation. The total duration of intervention ranged from one to 25 months (median 6.3 months). Various probiotic organisms were tested in isolation or in combination, including four *Bifidobacterium* species (*B bifidum*, *B longum*, two strains of *B breve*, and four strains of *B lactis*), and six *Lactobacillus* species (*L acidophilus*, *L casei*, *L lactis*, *L reuteri*, two strains of *L paracasei*, and three strains of *L rhamnosus*). Six trials[21-54] evaluated combinations of multiple probiotic organisms, and five[32-58] evaluated probiotics in combination with prebiotics (selectively fermented compounds that facilitate changes in the composition or activity of the gut microbiota to confer benefits on host health).[77] The daily dose of probiotics ranged from 10^8 to 10^{11} colony forming units, and was not quantifiable in six trials using supplemented infant formulas that were fed without restraint.[50-58] Compliance was assessed by a variety of methods, including maternal interview or daily diaries, counting of unused supplements, and faecal analysis.

Outcomes

Among the included trials, the duration of follow-up ranged from four months to eight years, and the median age at final assessment was 24 months (table 1). Nine trials reported clinical asthma diagnosis, 11 reported wheezing outcomes, and six reported lower respiratory tract infections; table S6 provides individual study definitions for these outcomes (web appendix). One trial reported the asthma predictive index score,[49] two reported asthma drug use,[54 58] and none reported admission to hospital for asthma. Adverse events were inconsistently reported.

Primary outcome: asthma

Nine trials including 3257 children contributed asthma data for meta-analysis (fig 2⇓). Incidence of doctor diagnosed asthma at final assessment was 11.2% among patients randomised to receive probiotics and 10.2% among those receiving placebo (risk ratio 0.99, 95% confidence interval 0.81 to 1.21, I^2=0%). Results were similar when expressed as a Peto odds ratio for rare events.

Secondary outcomes: wheeze, lower respiratory tract infection, and adverse events

Nine trials including 1949 children contributed incident wheeze data for meta-analysis (fig 3⇓). Incident wheeze at final assessment was similar after supplementation with probiotics or placebo (35.0% *v* 31.1%; risk ratio 0.97, 95% confidence interval 0.87 to 1.09, I^2=0%). Three trials reported recurrent wheezing, and meta-analysis was not pursued owing to considerable statistical heterogeneity (I^2=83%). Two of these trials[29 55] reported an increased risk of recurrent wheeze after probiotic supplementation, whereas the third trial[58] reported a decreased risk (web appendix, fig S2).

Six trials including 1364 children contributed data on lower respiratory tract infections. The incidence of lower respiratory tract infection was 14.5% among children randomised to receive probiotics, and 13.2% among those who received placebo (risk ratio 1.26, 95% confidence interval 0.99 to 1.61, I^2=0%). Notably, four of six trials documented lower respiratory tract infections non-systematically as adverse events,[48-53] rather than as primary or secondary outcomes. Excluding these four trials, the pooled risk ratio of lower respiratory tract infection associated with probiotics was 1.11 (0.70 to 1.76, I^2=35%; fig 4⇓).

Most trials did not systematically screen for or report the incidence of relevant safety outcomes, including severe gastrointestinal disturbances or allergic reactions (web appendix, table S7). The Peto odds ratio associated with withdrawal due to perceived side effects was 1.45 (95% confidence interval 0.66 to 3.17, I^2=0%; eight trials, 2732 children; web appendix, fig S3).

Subgroup analysis

We evaluated the efficacy of probiotics for prevention of asthma in children according to predefined subgroups (table 3⇓). Subgroup analyses by participant type (mother, infant, or both), timing of intervention (prenatal, postnatal, or both), or duration of intervention (≤6 or >6 months) did not show significant differences. However, individual subgroups were subject to type II errors, owing to small sample sizes. Similarly, we observed no statistical differences according to baseline asthma risk, probiotic dose or organism, caesarean delivery rate, feeding restrictions, geographical area, risk of bias, or industry authorship. Differences were not observed according to duration of follow-up, whether classified according to predefined strata (table 3) or assessed as a continuous variable by meta-regression (web appendix, fig S4). We found no significant differences across subgroups for incident wheeze (table 3); subgroup analyses were not pursued for recurrent wheeze or lower respiratory tract infection because of the small number of trials reporting these outcomes.

Discussion

In this systematic review and meta-analysis of randomised controlled trials, we found no evidence to support a protective association between probiotic supplementation during pregnancy or early life, and subsequent development of childhood asthma or wheeze. Although inadequately reported, probiotic supplementation could be associated with clinically relevant increases in lower respiratory tract infections.

Comparison of results with other studies

Our review provides a timely update to the 2007 Cochrane review of probiotics for prevention of allergic diseases.[24] The Cochrane review reported no benefit for the prevention of asthma after probiotic supplementation, based on findings from three trials enrolling a total of 617 infants.[25-27] Our review evaluates updated long term findings from these three original trials,[28-31] and adds results from six new trials (2308 infants) reporting on probiotics for asthma prevention.[21-36] Furthermore, we have evaluated 11 additional probiotic trials (1976 infants) reporting asthma related outcomes (wheeze or lower respiratory infection) that were not analysed in the 2007 Cochrane review. After systematic evaluation of these new and extended trial results, involving over 4000 additional children, we conclude that there is still insufficient evidence to recommend probiotics for the primary prevention of asthma. Our findings further identify several unanswered questions and highlight opportunities for future research.

Opportunities for future research

Despite widespread enthusiasm for evaluating probiotics to prevent allergic disease[17] and recognition of asthma as a major allergic disease in childhood,[1 78] relatively few randomised trials have formally tested the use of probiotics for asthma prevention. Only nine probiotic trials have reported asthma diagnosis, and none were powered for asthma detection as the primary outcome. Long term follow-up is essential for asthma prevention studies because diagnosis is challenging before age 6 years.[79 80] However, among the 20 trials included in our review, the median age at last follow-up was 24 months, and only five trials reported outcomes at or beyond 6 years of age (including one trial with unpublished data).[28-68] Moreover, long term follow-up was frequently subject to high or unclear risk of bias owing to attrition or the unblinding of participants. Extended follow-up data from other established trials are highly anticipated, including planned adolescent assessments by Kalliomaki[26] and Kukkonen[32] and colleagues. Because of the paucity of long term follow-up data among probiotic trials, we also evaluated wheeze as an early presentation of asthma. However, only a minority of wheezing infants will ultimately develop asthma later in childhood.[81]

Thus, extended follow-up of existing studies, combined with novel, respiratory focused trials,[82-84] will be necessary to define the role of probiotics for asthma prevention. Owing to the dynamic nature of the gut microbiota, trials evaluating prolonged probiotic supplementation (beyond the first year of life) may also be needed. As West and colleagues have shown,[67] probiotics are transient colonisers of the intestine, indicating that prolonged supplementation may be required to achieve durable benefit.

Our findings also highlight a need to further consider the effect of probiotics on the incidence and severity of recurrent wheeze and lower respiratory tract infections. Recurrent wheeze during infancy is considered a better predictor of asthma than incident wheeze,[40] yet only three of 10 trials documenting wheeze reported variable measures of recurrence,[27-58] and statistical heterogeneity precluded meta-analysis. Reporting of lower respiratory tract infections was similarly incomplete and variably defined. In six trials reporting this outcome, we observed a trend toward increased infections in children who received probiotics. Future trials should systematically define and capture recurrent wheeze, along with lower respiratory tract infections and other relevant safety outcomes.

Our subgroup analyses indicated that the effect of probiotics was similar regardless of the timing of intervention (prenatal *v* postnatal *v* both) or the participant receiving the intervention (mother *v* infant *v* both). The efficacy of specific probiotic organisms was difficult to assess because of the large variety of strains, combinations, and doses tested, and requires further investigation. Different probiotic organisms probably have distinct effects on the gut microbiota and host physiology. A recent animal study found that four *Lactobacillus* species had markedly different immunomodulatory effects,[85] and at least one clinical study has shown strain specific anti-allergic effects.[36] Further basic and clinical research is also needed to characterise the mechanisms by which probiotics influence asthma development, including how specific organisms colonise the gut, modify the resident microbiota, and ultimately affect host immunity and health. Such knowledge will help optimise the selection of probiotic organisms and the design of intervention regimens for future study.

Finally, identifying infant populations most likely to benefit from probiotics is highly desirable. For example, one trial has shown that probiotics were protective against IgE associated allergic disease in infants delivered by caesarean section (whose gut microbiota is disrupted[86]), but not in their vaginally delivered counterparts.[64] With trial level data, we could not identify differential efficacy according to caesarean delivery rate, but this and other microbiota disrupting exposures (such as formula feeding and antibiotic treatment) warrant further study as possible indications for probiotic supplementation in the prevention of asthma and wheeze.

Strengths and weaknesses of the study

The strengths of this review included the completeness of the search strategy, which reviewed multiple citation databases, trial registries, and conference proceedings. By omitting outcome related search terms, we identified trials that were not primarily focused on asthma or allergic disease, but nevertheless reported relevant outcomes.[50-57] We focused on patient centred outcomes and evaluated efficacy in the context of relevant safety outcomes and adverse events. Finally, we used an a priori published protocol and followed established methodological guidelines in the conduct and reporting of this review. Limitations include pooling data from trials conducted in distinct populations (for example, infants at high risk for asthma, or unselected populations) receiving different probiotic formulations (various organisms with a 1000 times range in daily dose) through varying regimens (prenatal or postnatal supplementation, for 1-25 months). Subgroup analyses were susceptible to type II errors owing to relatively small sample sizes.

Conclusion

We found no evidence to support a protective association between perinatal administration of probiotics, and doctor diagnosed asthma or childhood wheeze. There is currently insufficient evidence to recommend probiotics for the primary prevention of these disorders, and further research is warranted to explore the potential association between probiotic supplementation and increased risk of lower respiratory tract infection. Extended follow-up of existing trials, along with further clinical and basic research, are needed to accurately define the role of probiotics in the prevention of childhood asthma.

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Contributors: MBA, AMA-S, and RZ conceived and designed the study with input from ABB, CJF, CDR, and ALK. CF provided guidance on search strategies. MBA and JGC screened and reviewed studies, extracted data, and assessed the quality of included trials. MBA analysed the data and all authors contributed to its interpretation. MBA drafted the manuscript and all authors participated in the revision process and have approved this submission for publication. MBA is guarantor, had full access to all of the data in the study, and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: As this study did not involve the collection of new data, but rather the analysis of data from previously published research, no ethics approval was sought.

Data sharing: The RevMan file used to generate summary effect measures will be made available on request.

MBA, as guarantor, affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned and registered have been explained.

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What is already known on this topic

Asthma is the most common chronic disease of childhood, and is frequently preceded by wheeze

Increases in asthma prevalence have been partly attributed to disruption of the commensal gut microbiota in early life

Perinatal probiotics have been shown to prevent atopic dermatitis, but uncertainty remains regarding their effectiveness in asthma prevention

What this study adds

We found no evidence to support a protective association between perinatal probiotics and childhood asthma or wheeze. Although inadequately reported, probiotic supplementation could be associated with increases in lower respiratory tract infections

Additional basic research and adequately powered long term clinical trials are needed to fully define the role of probiotics in the prevention of asthma

Probiotics cannot be recommended for primary prevention of childhood asthma or wheeze at this time

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Tables

Table 1| Characteristics of included trials and study populations

Primary article	Companion articles* and unpublished data	Country, enrolment period	Age at last follow-up	Number of participants†	Infant asthma risk	Feeding Restrictions	Caesarean delivery rate (%)	Relevant outcomes included‡		
								Asthma	Wheeze	LRTI
West 2009[35]	West 2008,[61] West 2013[67]	Sweden, 2000-03	8 years	179 infants	Unselected	None	0	Yes	Yes (I)	—
Abrahamsson 2007[25]	Abrahamsson 2013[28]	Sweden, 2001-03	7 years	239 mothers (209 infants)	High (family history)	None	13	Yes	Yes (I)	—
Kalliomaki 2001[26]	Kalliomaki 2003,[63] Kalliomaki 2007[30]	Finland, 1997-98	7 years	159 mothers (155 infants)	High (family history)	None	Not reported	Yes	—	—
Niers 2009[33]	Gorissen (unpublished data),** data provided	Netherlands, 2004-05	6 years	156 mothers (123 infants)	High (family history)	None	10	Yes	Yes (I)	—
Wickens 2008[36]	Wickens 2012,[66] Wickens 2013[68]	New Zealand, 2004-05	6 years	512 mothers (474 infants)	High (family history)	None	32	Yes	Yes (I)	—
Kukkonen 2007[32]	Kuitunen 2009,[64] data provided	Finland, 2000-03	5 years	1223 mothers (1018 infants)	High (family history)	None	17	Yes	—	—
Taylor 2007[27]	Prescott 2008,[31] Jensen 2012[29]	Australia, 2002-05	5 years	226 mothers (218 infants)	High (family history)	None	44	Yes	Yes (I, R)	Yes
Gore 2012[51]	None	UK, 2002-04	3 years	137 infants§	High (eczema)	FF required, BF permitted	Not reported	Yes¶	Yes (I)	—
Ou 2012[34]	None	Taiwan, 2002-06	3 years	191 mothers (151 infants)	High (family history)	None	Not reported	Yes	—	—
Dotterud 2010[21]	None	Norway, 2003-05	2 years	415 mothers (363 infants)	Unselected	BF required	Not reported	Yes	—	—
Kopp 2008[55]	None	Germany, 2002-04	2 years	105 mothers (104 infants)	High (family history)	None	19	—	Yes (R)	—
van der Aa 2011[58]	van der Aa 2010[60]	Netherlands, 2005-07	18 months	90 infants	High (eczema)	Exclusive FF at enrolment	18	—	Yes (R)	—
Hol 2008[54]	Data provided	Netherlands, 2004-07	16 months	119 infants	High (milk allergy)	Exclusive FF at enrolment	19	—	Yes (I)	—
Boyle 2011[49]	None	Australia, 2006-08	12 months	250 mothers (250 infants)	High (family history)	None	27	—	Yes (I)	—
Maldonado 2012[56]	None	Spain, 2008-09	12 months	215 infants	Unselected	Exclusive FF at enrolment	45	—	—	Yes
Chouraqui 2008[50]	None	France, 2004-05	12 months	284 infants	Unselected	Exclusive FF at enrolment	17	—	—	Yes
Gruber 2007[52]	None	Germany, not reported	10 months	106 infants	High (eczema)	None	Not reported	—	—	Yes
Allen 2010[48]	None	UK, not reported (2004)	6 months	454 mothers (454 infants)	High (family history)	None	Not reported	—	—	Yes
Hascoet 2011[53]	None	France, not reported (2006)	4 months	79 infants§	Unselected	Exclusive FF at enrolment	8	—	—	Yes
Puccio 2007[57]	Data provided	Italy, not reported	4 months	138 infants	Unselected	Exclusive FF at enrolment	40	—	Yes (I)	—

Trials are listed in order of decreasing duration of follow-up.

BF=breastfeeding; FF=formula feeding; LRTI=lower respiratory tract infection.

*Excludes conference abstracts from which no unique data were extracted.

†Indicates mothers or infants randomised; where recruitment occurred prenatally, includes number of infants eligible at birth (in brackets).

‡I=incident wheeze; R=recurrent wheeze.

§Trial included additional groups (for example, observational) not considered in this review.

Table 1 (continued)

Primary article	Companion articles* and unpublished data	Country, enrolment period	Age at last follow-up	Number of participants†	Infant asthma risk	Feeding Restrictions	Caesarean delivery rate (%)	Relevant outcomes included‡		
								Asthma	Wheeze	LRTI

¶This trial reported asthma outcomes in the text only, and the authors declined to provide data for meta-analysis.

**Unpublished data from a forthcoming report provided by Gorissen DMW, Rutten NBMM, Oostermeijer CMJ, Niers LEM, Hoekstra MO, Rijkers GT, et al. Preventive effects of selected probiotic strains on the development of asthma and allergic rhinitis in childhood. The PandA study.

Table 2| Characteristics of probiotic interventions in included trials

Primary article	Prenatal or postnatal intervention	Participant receiving probiotics	Duration of intervention (months)	Timing of intervention	Probiotic organism*	Added prebiotic	Total daily dose (CFU)
West 2009[35]	Postnatal	Infant	10	Age 4-13 months (infant)	*L paracasei* F19	No	1×10^{8} (minimum)
Abrahamsson 2007[25]	Prenatal and postnatal	Mother and infant	13	36 WG to delivery (mother), birth to 12 months (infant)	L reuteri	No	1×10^{8}
Kalliomaki 2001[26]	Prenatal and postnatal	Mother and infant (if not breastfed)	6.5	36-38 WG to 6 months postpartum if breastfeeding (mother), weaning to 6 months (infant)	*L rhamnosus* GG	No	2×10^{10}
Niers 2009[33]	Prenatal and postnatal	Mother and infant	14	24 WG to delivery (mother), birth to 3 months (infant)	*B bifidum* W23, *B lactis* W52, and *L lactis* W58	No	3×10^{9}
Wickens 2008[36]	Prenatal and postnatal	Mother and infant	25	35 WG to 6 months postpartum if breastfeeding (mother), birth to 2 years (infant)	2 probiotic groups: *L rhamnosus* HN001 or *B lactis* HN019	No	6×10^{9} (HN001) or 9×10^{9} (HN019)
Kukkonen 2007[32]	Prenatal and postnatal	Mother and infant	7	36-38 WG to delivery (mother), birth to 6 months (infant)	*L rhamnosus* GG, *L rhamnosus* LC705, *B breve* Bb-99, and *Propionibacterium freudenreichii* JS	Yes	2.4×10^{10} (mother), 1.2×10^{10} (infant)
Taylor 2007[27]	Postnatal	Infant	6	Birth to 6 months (infant)	*L acidophilus* LAVRI-A1	No	3×10^{9}
Gore 2012[51]	Postnatal	Infant	3	Age 5-8 months (infant)	2 probiotic groups: *L paracasei* or *B lactis*	No	1×10^{10}
Ou 2012[34]	Prenatal and postnatal	Mother and infant (if not breastfed)	10	24 WG to 6 months postpartum if breastfeeding (mother), weaning to 6 months (infant)	*L rhamnosus* GG	No	1×10^{10}
Dotterud 2010[21]	Prenatal and postnatal	Mother	4	36 WG to 3 months postpartum (mother)	*L rhamnosus* GG, *B lactis* Bb-12, and *L acidophilus* La-5	No	1.05×10^{11}
Kopp 2008[55]	Prenatal and postnatal	Mother and infant	7	34-36 WG to 3 months postpartum, if breastfeeding (mother), weaning to 6 months, or 3-6 months (infant)	*L rhamnosus* GG	No	1×10^{10}
van der Aa 2011[58]	Postnatal	Infant	3	Age 5-8 months (infant)	*B breve* M-16V	Yes	1.3×10^{6} per 100 mL†
Hol 2008[54]	Postnatal	Infant	12	Age 4-16 months (infant)	*L casei* and *B lactis* Bb-12	No	2×10^{7} per g†
Boyle 2011[49]	Prenatal	Mother	1	36 WG to delivery (mother)	*L rhamnosus* GG	No	1.8×10^{10}
Maldonado 2012[56]	Postnatal	Infant	6	Age 6-12 months (infant)	*L fermentum* CECT5716	Yes	2×10^{8} (average)
Chouraqui 2008[50]	Postnatal	Infant	4	Age 2 weeks to 4 months (infant)	3 probiotic groups: *B longum* BL999 and *L rhamnosus* LPR; BL999 and LPR plus prebiotic; or BL999 and *L paracasei* ST11 plus prebiotic	Yes	$5\text{-}8\times10^{8}$ per 100 mL†
Gruber 2007[52]	Postnatal	Infant	3	Age 7-10 months (infant)	*L rhamnosus* GG	No	1×10^{10}
Allen 2010[48]	Prenatal and postnatal	Mother and infant	7	36 WG to delivery (mother), birth to 6 months (infant)	*L salivarius* CUL61, *L paracasei* CUL08, *B lactis* CUL34, and *B bifidum* CUL20	No	1×10^{10}
Hascoet 2011[53]	Postnatal	Infant	4	Age 4 days to 4 months (infant)	*B longum* BL999	No	2×10^{7} per g†
Puccio 2007[57]	Postnatal	Infant	3.7	Age 2-16 weeks (infant)	*B longum* BL999	Yes	2×10^{7} per g†

CFU=colony forming units; WG=weeks' gestation.

*B=*Bifidobacterium*; L=*Lactobacillus*.

†Fed without restraint.

Table 3| **Subgroup analyses for probiotic supplementation during pregnancy or infancy and asthma or wheeze in children**

	Diagnosed asthma			Incident wheeze		
Subgroup	**No of Studies**	**Pooled risk ratio (95% CI)**	**I^2 (%)**	**No of Studies**	**Pooled risk ratio (95% CI)**	**I^2 (%)**
Overall	**9**	**0.99 (0.81 to 1.21)**	**0**	**9**	**0.97 (0.87 to 1.09)**	**0**
Participant receiving intervention						
Mother	1	0.64 (0.27 to 1.54)	—	1	0.93 (0.59 to 1.48)	—
Mother and infant	6	1.00 (0.80 to 1.23)	0	3	0.96 (0.83 to 1.11)	0
Infant	2	1.27 (0.61 to 2.66)	0	5	1.00 (0.78 to 1.28)	26
Timing of intervention						
Prenatal	0	—	—	1	0.93 (0.59 to 1.48)	—
Prenatal and postnatal	7	0.97 (0.79 to 1.19)	0	3	0.96 (0.83 to 1.11)	0
Postnatal	2	1.27 (0.61 to 2.66)	0	5	1.00 (0.78 to 1.28)	26
Duration of intervention						
≤median (6 months)	2	0.98 (0.39 to 2.45)	43	4	0.93 (0.73 to 1.19)	1
>median	7	0.99 (0.81 to 1.23)	0	5	0.98 (0.86 to 1.12)	2
Probiotic dose						
<median (10^{10} CFU daily)	5	0.98 (0.74 to 1.31)	0	5	1.04 (0.87 to 1.24)	23
≥median	4	1.06 (0.68 to 1.66)	38	2	0.79 (0.56 to 1.11)	0
Fed without restraint	0	—	—	2	0.97 (0.76 to 1.25)	0
Probiotic organism*						
Bifidobacterium species	1	1.04 (0.63 to 1.71)	—	3	0.96 (0.80 to 1.16)	3
Lactobacillus species	6	1.13 (0.82 to 1.55)	0	6	0.99 (0.80 to 1.23)	19
Combination	3	0.86 (0.63 to 1.16)	0	2	1.03 (0.78 to 1.37)	0
Follow-up duration†						
<3 years	6	0.90 (0.62 to 1.31)	0	8	0.98 (0.86 to 1.11)	0
3 to <6 years	4	1.04 (0.77 to 1.39)	0	3	0.91 (0.76 to 1.10)	18
≥6 years	5	1.01 (0.76 to 1.34)	0	2	0.99 (0.79 to 1.25)	47
Asthma risk						
High (infant atopic disease)	0	—	—	2	0.85 (0.65 to 1.12)	0
High (family history)	7	1.01 (0.82 to 1.25)	0	5	0.97 (0.85 to 1.12)	0
Unselected	2	0.78 (0.40 to 1.51)	0	2	1.19 (0.76 to 1.86)	40
Infant feeding						
Unrestricted	9	—	—	7	0.98 (0.84 to 1.15)	15
Exclusive formula feeding	0	—	—	2	0.97 (0.76 to 1.25)	0
Caesarean delivery rate						
≤median (19%)	4	0.92 (0.69 to 1.21)	0	4	1.11 (0.88 to 1.41)	0
>median	2	1.03 (0.73 to 1.45)	0	4	0.96 (0.84 to 1.09)	0
Not reported	3	1.25 (0.57 to 2.74)	53	1	0.64 (0.38 to 1.07)	0
Geographical area						
Europe	6	0.94 (0.72 to 1.22)	0	6	1.01 (0.83 to 1.23)	7
Asia or Oceania	3	1.07 (0.78 to 1.46)	0	3	0.95 (0.82 to 1.10)	0
Risk of bias						
Low or unclear	2	1.47 (0.45 to 4.77)	71	3	0.87 (0.69 to 1.10)	0
High	7	0.98 (0.76 to 1.26)	0	6	0.97 (0.87 to 1.09)	6
Industry authorship						
No or unclear	7	1.05 (0.82 to 1.36)	0	7	0.96 (0.84 to 1.08)	0
Yes	2	0.89 (0.65 to 1.23)	0	2	1.09 (0.80 to 1.49)	0

CFU=colony forming units. Tables 1 and 2 summarise the studies contributing data for each outcome and subgroup.

*Two trials compared *Lactobacillus* with *Bifidobacteria* in separate groups.

Table 3 (continued)

	Diagnosed asthma			Incident wheeze		
Subgroup	No of Studies	Pooled risk ratio (95% CI)	I^2 (%)	No of Studies	Pooled risk ratio (95% CI)	I^2 (%)

†With regard to subgroup analysis of follow-up duration, based on the number and duration of follow-up assessments, some trials contributed data to more than one predefined subgroup. For all other subgroup analyses, the longest available follow-up data from each trial were used.

Figures

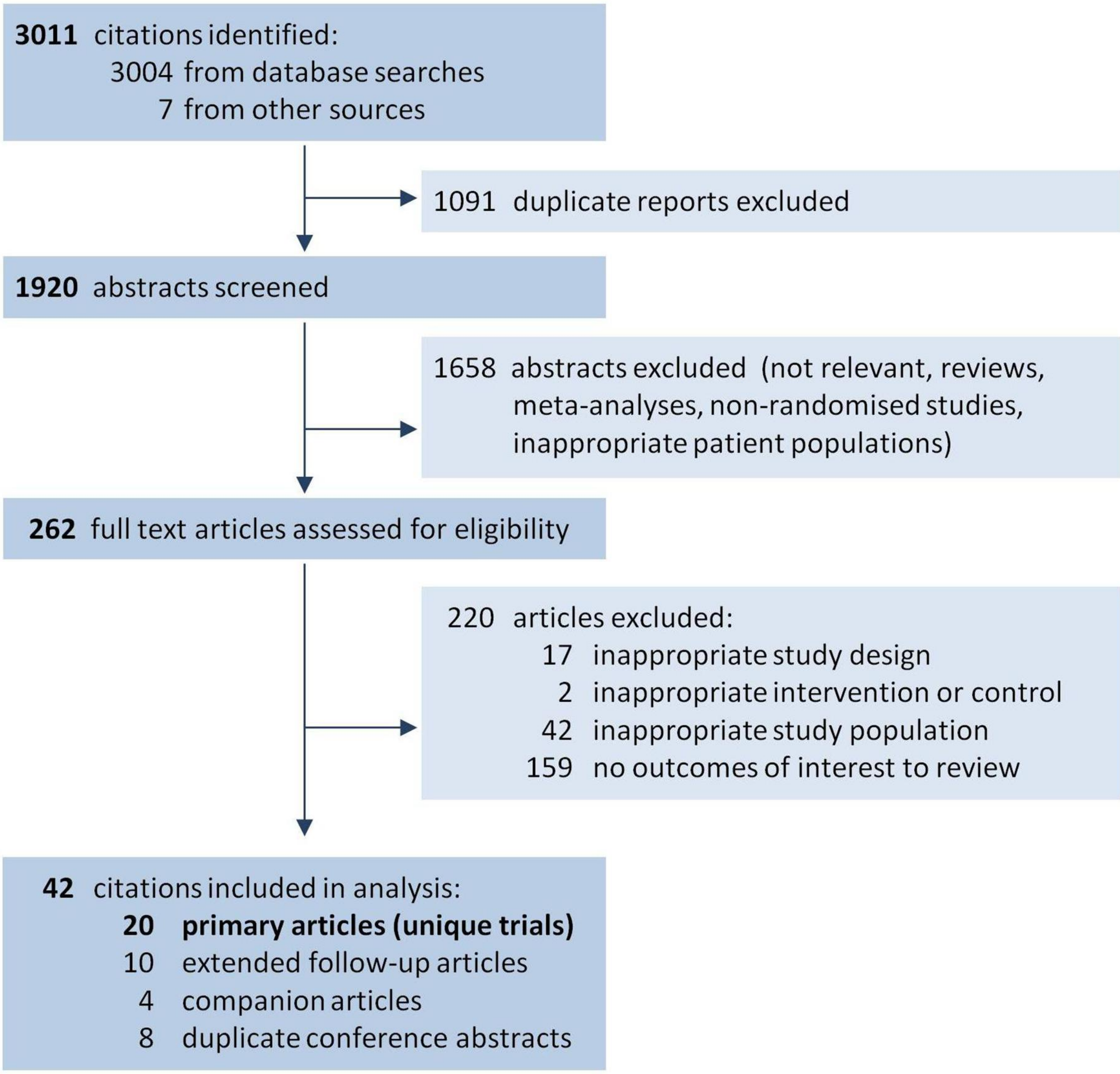

Fig 1 Study flow diagram, following PRISMA criteria with modifications[39]

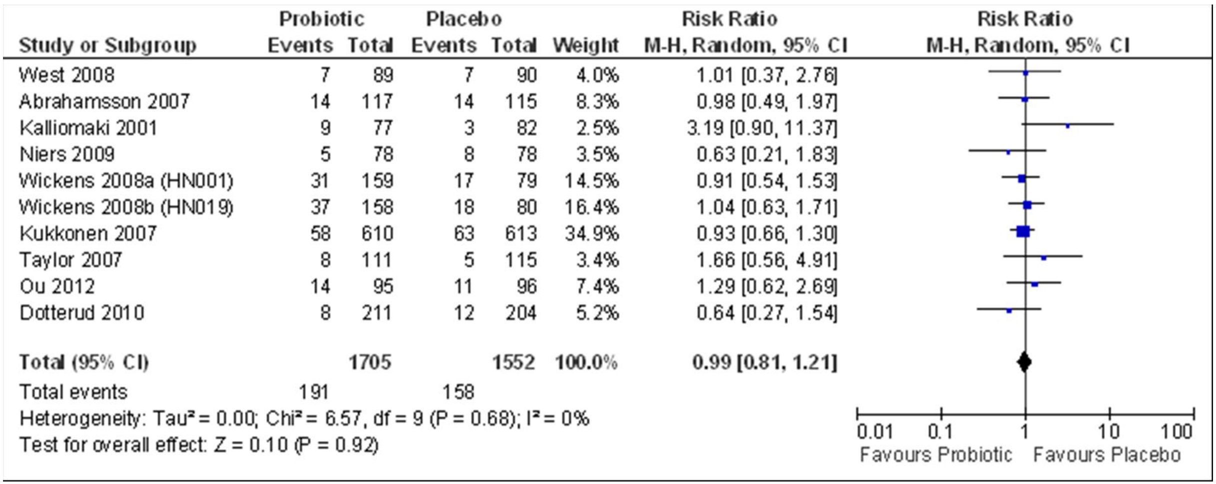

Fig 2 Probiotic supplementation during pregnancy or infancy and doctor diagnosed asthma in children. The longest available follow-up data (intention to treat) were extracted from each contributing trial. Trials are sorted in order of decreasing duration of follow-up. df=degrees of freedom; M-H=Mantel-Haenszel

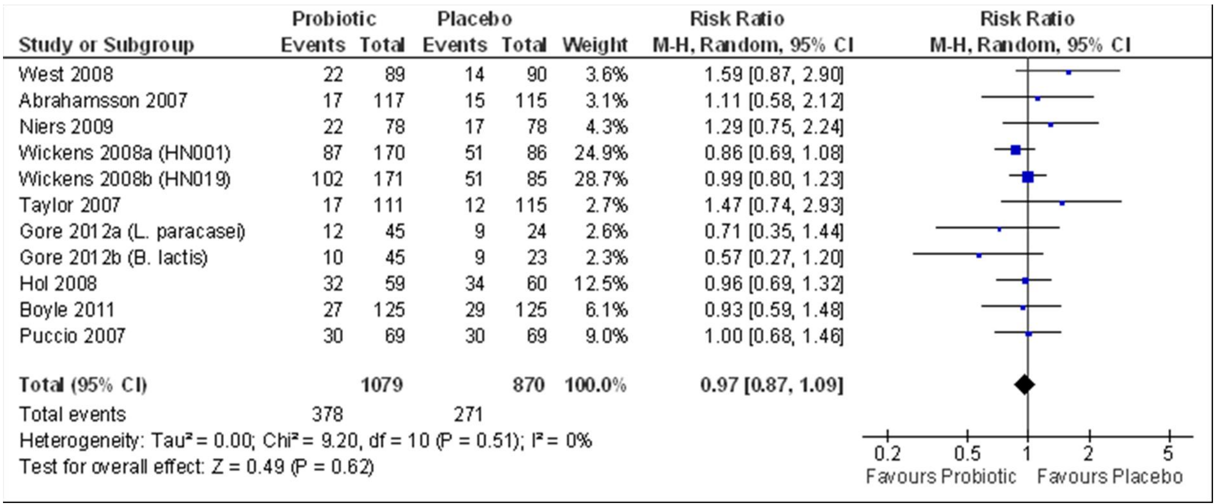

Fig 3 Probiotic supplementation during pregnancy or infancy and incident wheeze in children. The longest available follow-up data (intention to treat) were extracted from each contributing trial. Trials are sorted in order of decreasing duration of follow-up. df=degrees of freedom; M-H=Mantel-Haenszel

Study or Subgroup	Probiotic Events	Probiotic Total	Placebo Events	Placebo Total	Weight	Risk Ratio M-H, Random, 95% CI
4.3.1 LRTI reported as Primary or Secondary Outcome						
Taylor 2007	46	111	37	115	49.5%	1.29 [0.91, 1.82]
Maldonado 2012	12	117	13	98	10.9%	0.77 [0.37, 1.62]
Subtotal (95% CI)		**228**		**213**	**60.4%**	**1.11 [0.70, 1.76]**
Total events	58		50			
Heterogeneity: Tau² = 0.05; Chi² = 1.54, df = 1 (P = 0.21); I² = 35%						
Test for overall effect: Z = 0.44 (P = 0.66)						
4.3.2 LRTI reported as AE						
Chouraqui 2008	6	214	0	70	0.7%	4.29 [0.24, 75.26]
Gruber 2007	24	56	18	50	25.9%	1.19 [0.74, 1.92]
Allen 2010	20	220	12	234	12.3%	1.77 [0.89, 3.54]
Hascoet 2011	2	40	0	39	0.7%	4.88 [0.24, 98.47]
Subtotal (95% CI)		**530**		**393**	**39.6%**	**1.41 [0.96, 2.08]**
Total events	52		30			
Heterogeneity: Tau² = 0.00; Chi² = 2.32, df = 3 (P = 0.51); I² = 0%						
Test for overall effect: Z = 1.75 (P = 0.08)						
Total (95% CI)		**758**		**606**	**100.0%**	**1.26 [0.99, 1.61]**
Total events	110		80			
Heterogeneity: Tau² = 0.00; Chi² = 4.21, df = 5 (P = 0.52); I² = 0%						
Test for overall effect: Z = 1.89 (P = 0.06)						
Test for subgroup differences: Chi² = 0.62, df = 1 (P = 0.43), I² = 0%						

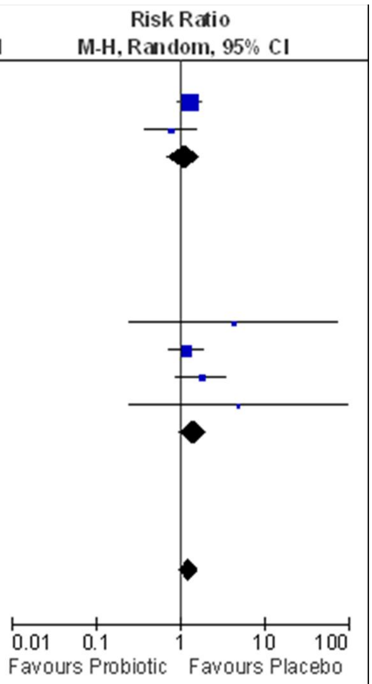

Fig 4 Probiotic supplementation during pregnancy or infancy and lower respiratory tract infection in children. The longest available follow-up data (intention to treat) were extracted from each contributing trial. Trials are sorted in order of decreasing duration of follow-up, and subgrouped according to whether the incidence of lower respiratory tract infection was systematically reported as a primary or secondary outcome, or as an adverse event. AE=adverse event; df=degrees of freedom; LRTI=lower respiratory tract infection; M-H=Mantel-Haenszel