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Title: Prenatal antibiotic exposure, asthma and the atopic march: A systematic review and meta-analysis

Short Title: Pregnancy antibiotics asthma meta-analysis

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Abstract:

Background Antibiotic use during pregnancy may increase the risk for asthma in children. We performed a meta-analysis assessing prenatal antibiotic exposure and the risk for childhood wheeze or asthma, as well as for diseases associated with the atopic march.

Methods: A systematic literature search protocol (PROSPERO-ID: CRD42020191940) was registered and searches were completed using Medline, Proquest, Embase, and the Cochrane central register of controlled trials. Screening for inclusion criteria: published in English, German, French, Dutch or Arabic, intervention (use of any antibiotic at any time point during pregnancy), and disease (reporting atopic disease incidence in children with a primary outcome of asthma or wheeze), and exclusion criteria: reviews, preclinical data, and descriptive studies, yielded 27 studies. Study quality was assessed using the Newcastle–Ottawa Assessment Scale. Quality of the evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.

Results: Our meta-analysis demonstrates that antibiotic use during pregnancy is associated with an increased relative risk (RR) of developing wheeze RR 1.51 (95% CI: 1.17-1.94) or asthma RR 1.28 (95% CI 1.22-1.34) during childhood. Assessment of the atopic march in association with asthma or wheeze revealed that antibiotic use during pregnancy also increases risk for eczema/dermatitis RR 1.28 (95% CI: 1.06 –1.53) and allergic rhinitis RR 1.13 (95% CI: 1.02 – 1.25). One study found an increase in food allergy RR 1.81 (95% CI: 1.11 –2.95).

Conclusions: Maternal antibiotic use during pregnancy is associated with an increased risk for wheeze or asthma development in children, as well as for diseases involved in the atopic march. There was high heterogeneity in the data, and the certainty of the evidence was determined to be low quality, highlighting the need for more high-quality studies on this topic. These results have importance for antibiotics stewardship throughout the prenatal period. This work was supported by the Konrad Adenauer Foundation.

Keywords: Antibiotics, Atopic march, Childhood asthma, Meta-analysis, Pregnancy

Word Count: 3690 Words

Abbreviations:

aIRR – Adjusted incidence risk ratio

CI – Confidence Interval

HR – Hazard Ratio

IRR – Incidence Risk Ratio

NOS - Newcastle-Ottawa Quality Assessment Scale

OR – Odds Ratio

RR – Relative Risk

Introduction:

The atopic march refers to a progression of allergic diseases as they develop over the course of infancy and childhood ¹. Classically, the progression begins with atopic dermatitis, food allergy, or wheeze, then progresses over time to asthma and/or allergic rhinitis ². Underlying atopy, the tendency to become sensitized and produce IgE antibodies after exposure to ordinary proteins ³ is considered a critical link among these diseases ⁴. Though atopy has a poorly understood aetiology, ~~strong~~ evidence indicates that in addition to genetic factors, the environment contributes substantially to disease susceptibility, especially when these exposures occur in prenatally or in early life ^{5,6}. Of particular interest to this study are environmental exposures such as maternal antibiotic use during pregnancy ^{7,8}.

Antibiotics account for 80% of prescribed medications during pregnancy, and it is estimated that 20-25% of pregnant women receive at least one course of an antibiotic during this time period ⁹. Antibiotics alter the maternal gut microbiome ¹⁰, and microbial dysbiosis during

pregnancy can potentially influence the offspring both in utero and immediately following birth. In utero it is hypothesized that the foetus is exposed to bacterial products and metabolites present in the maternal circulation that are generated by the maternal microbiome ¹¹. Additionally, during birth the maternal vaginal and faecal microbiomes are ~~strong~~ determinants of early colonisation of the infant intestinal tract, thus, inheriting dysbiosis can have long lasting implications for childhood health ^{12,13}. The days immediately following birth are an important window of immune system development, during which immune-microbe interactions in the infant intestine are critical for the establishment of immune tolerance ^{14,15}. If prenatal antibiotic exposure influences the infant microbiome and immune system development, this would be advisable to consider when prescribing antibiotics. Therefore, understanding if maternal antibiotic use during the prenatal time period can change the propensity for the atopic march and eventual asthma diagnosis in children is of utmost importance.

Analysing the effects of maternal antibiotic use during pregnancy on the development of asthma in children is important for several reasons. Firstly, there is evidence that prenatal antibiotic exposure can also increase risk for disorders involved in the atopic march such as eczema/dermatitis, food allergy, wheeze and allergic rhinitis, thus having an ~~strong~~ influence on the eventual progression to asthma in children ^{16,17}. Though this connection has been observed however, there are surprisingly few studies that assess these disorders in conjunction with asthma ¹⁸⁻²². Including data on the atopic march in future studies will provide important information regarding the major contributing factors to childhood asthma development. Secondly, though several studies have found that antibiotic use during pregnancy is associated with the development of wheeze/asthma in children, other studies have found no association for wheeze ^{18,23,24} or asthma development ^{21,25}. And finally, still other studies have proposed alternative explanations for the observed risk of antibiotics taken during pregnancy and

progression through the atopic march to asthma. Stockholm et al. (2014) found no temporal association with antibiotic use during pregnancy and asthma development in children, instead suggesting that the association is rather due to a maternal propensity for infection than to antibiotic use during pregnancy ²⁶. Örtkvist et al. (2014) concluded that the association between intrauterine antibiotic exposure and asthma in childhood is confounded by factors shared by siblings ²⁷. This is, however, contradicted by Yoshida et al. (2018) whose study suggested that a weak association still exists, even after adjusting for familial factors ²⁸. Due to the aforementioned factors, a meta-analytic approach is both desirable and timely. To shed light on this important subject, our aim is to conduct a systematic review and meta-analysis to assess the relationship between prenatal antibiotic exposure and subsequent risk of developing along an atopic trajectory culminating in childhood asthma.

Methods

Reporting, Protocol and Registration

A pre-registered review protocol was deposited in the Freie Universität Berlin institutional repository (refubium) on July 1 2020 ²⁹. The protocol and this manuscript are both reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The protocol is registered in the International prospective register of systematic reviews (PROSPERO) ID: CRD42020191940.

Eligibility criteria

Detailed inclusion/exclusion criteria can be found in our published protocol ²⁹. Briefly, studies were included if: (i) they were published in English, German, French, Dutch or Arabic (ii) the intervention was the use of any antibiotic at any time point during pregnancy (iii) the study reported atopic disease incidence in the offspring with a primary outcome of asthma or wheeze.

Study assessments of atopic sensitization, dermatitis/eczema, allergic rhinitis, and/or food allergy in association with wheeze or asthma development were also included. Narrative reviews, preclinical data, and descriptive studies were excluded.

Data sources and searches

Electronic searches were completed on June 9, 2020 using Medline (via Ovid), Proquest Theses & Dissertations (via Proquest), Embase, and the Cochrane central register of controlled trials. No restrictions were placed on the search beyond that of the databases themselves. The specific search strategy was created in MEDLINE (OVID interface, 1946 onwards). Details of the search strategy used for all databases and the number of hits from each database are found in Supplementary Files 1 and 2.

Article selection

Records from searches were uploaded and deduplicated in EndNoteX7.8 (Clairvate Analytics, Philadelphia, USA), exported into DistillerSR (Evidence Partners Inc., Ottawa, Canada), further deduplicated, and then screened in three rounds (title in round one, title and abstract in round two, and full text eligibility in round three) by two independent reviewers. Extracted data consisted of study identification: author, year and journal - study design characteristics: study type, population, measurements of exposure and outcome, sample size, effect size and variance, the type of effect size (Relative Risk-RR/Odds Ratio-OR/Hazard Ratio-HR) and reported p-value - characteristics of the atopic-disease outcome: the type of allergic diagnosis, age at diagnosis, follow-up time, and sex - characteristics of the antibiotic administration during pregnancy - the cause, type, dose and duration of antibiotic treatment, and administration route of the antibiotics. Any conflicts between reviewers were resolved by consensus.

Quality Assessment

The studies were evaluated for risk of bias with the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies. The NOS awards points (stars) in 4 categories: “Selection”, “Comparability”, and “Study outcome”. A study can be awarded a maximum of one star for each numbered item within the “Selection” and “Outcome” categories. A maximum of two stars can be given for “Comparability”. The criteria were used for scoring can be found in Supplementary File 3.

Certainty assessment

The overall certainty of evidence across the studies was evaluated by the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) working group (gradeworkinggroup.org). Subsequently, the quality of the evidence was classified into four categories (high, moderate, low, and very low).³⁰

Statistical analysis

Data preparation. Effect estimates of individual studies included odds ratio (OR), hazard ratio (HR), incidence risk ratio (IRR) and risk ratio (RR). While many meta-analyses assume their numerical equivalence³¹⁻³³, this assumption has repeatedly been shown to be incorrect³⁴⁻³⁷. Hence, we firstly performed a conversion of HR and OR to RR as outlined by Chinn (2000)³⁸ and just recently by Van der Weele (2020)³⁶. One study reporting an adjusted incidence risk ratio (aIRR) could not be included, since there is no adjustment method for this measurement, and the data was not available publicly or via formal request²⁶. Secondly, we also estimated meta-analytic models for each type of effect size and performed pairwise comparisons on the effect estimates. There were no significant differences in the estimated effect sizes, but the model based on the converted estimates showed the highest power for both asthma and wheezing as well as the best model fit for asthma (Supplementary File 4). Consequently, all further analyses were carried out on the original and converted RR data.

Sensitivity Analyses. Firstly, an outlier detection based on Viechtbauer & Cheung's (2010) influential case analysis was performed ³⁹. Secondly, publication bias was evaluated by visual inspection of funnel plots with trim-and-fill method as well as by Egger's parametric and Begg's nonparametric asymmetric distribution tests (Supplementary File 5). Thirdly, subgroup analyses and comparison tests were carried out for the factor of study type (prospective or retrospective).

Model estimation. For the overall effects, inverse-variance weighted random-effects models were estimated. Heterogeneity was assessed by Cochran's Q statistic as well as Higgin's I^2 (values of >40% and >75% indicate moderate and high heterogeneity). Since studies with multiple outcomes did not report correlation matrices, the effects of trimester and antibiotic type were estimated with individual moderated random-effects models with heterogenous τ^2 between the subgroups. Moderation effects were evaluated with an omnibus test as well as Bonferroni-Holm corrected pairwise comparisons.

All analyses were performed in R (R Core Team, 2020) with the *metafor*⁴⁰ and *Evalue*⁴¹.

Results

Study selection, characteristics and methodological quality

The initial search returned a total of 6060 citations, of which 71 assessed the association between antibiotic use during pregnancy with wheeze or asthma as a primary outcome. Of these articles, 44 were excluded due to the following criteria: 1) No follow up during childhood, 2) Antibiotics were given before and after birth, 3) Antibiotics were given to children as well as to mothers, 4) Studies only pertained to animal models, 5) Antibiotics were given with other drugs. 6) Studies did not report asthma or wheeze as primary or secondary outcome, 7)

Duplicates, i.e. conference papers and matched published papers that were not detected and removed at the identification process or theses and papers that reported the same results. Altogether, 27 studies fulfilled the selection criteria (Figure 1).

Of the 27 included studies, 11 were prospective and 16 were retrospective. The publication period for included studies ranged from 2002 to 2020. The studies were conducted in Denmark (4 studies), United States (4), Sweden (3), United Kingdom (2), Italy (2), Canada (2), China (1), Ecuador (1), Finland (1), Japan (1), Poland (1), Norway (1), the Netherlands (1) and Belgium (1). Additionally, there was a pan-Scandinavia study (1) and a large study drawing participants across Austria, Finland, France, Germany, and Switzerland (1). Of the studies investigating prenatal antibiotic exposure and asthma (20) or wheeze (9), the following diseases were also assessed to provide insight into the atopic march: atopic sensitization (1 study), food allergy (1), eczema/dermatitis (5 studies) and allergic rhinitis (3). Exposure to antibiotics during the prenatal period was assessed by either an unsupervised questionnaire (8 studies), an interview by a medical professional (doctor, nurse, or midwife, 4 studies), or obtained from an official medical database (16 studies) (Supplementary File 6). Sensitivity analyses for prospective vs. retrospective studies and for method of data collection for antibiotic exposure were performed and summarized in Supplementary File 7.

The quality of the included studies was assessed according to the Newcastle-Ottawa Quality Assessment Scale for cohort studies, shown in Table 1.

Table 1: Assessment of methodological quality of studies, scored according to the Newcastle-Ottawa Scale for cohort studies. The cohort studies were sorted by year of publication. Stars represent the quality score obtained in each possible category (see Methods for more details on how scoring was determined).

Author	Year	Study ID	Selection /4	Comparability /2	Outcome /3
McKeever	2002	19	****	*	***
Benn	2002	42	***	*	***
Jedrychowski	2006	20	****	*	**
Rusconi	2007	43	***	*	**
Martel	2009	44	****	*	***
Stensballe	2013	45	****	*	***
Dom	2013	18	****	**	**
Kallen	2013	9	****	*	***
Örtqvist	2014	27	****	*	***
Lapin	2015	24	***	*	**
Metsälä	2015	46	****	*	***
Chu	2015	47	****	*	***
Popovic	2015	48	***	*	**
Mulder	2016	49	****	*	***
Wu	2016	50	****	*	***
Zhang	2017	51	***	**	***
Örtqvist	2017	52	****	**	***
Marell Hesla	2017	22	**	*	**
Kashanian	2017	53	***	*	***
Yoshida	2018	28	****	*	***
Fossum	2018	25	****	*	***
Arrieta	2018	54	****	**	***
Loewen	2018	55	****	**	***
Metzler	2019	21	****	**	**
Liu	2019	56	****	*	*
Turi	2020	57	****	*	***
Higgins	2020	23	***	*	**

Atopic Sensitization

Two studies assessed the effect of antibiotic use during pregnancy on atopic sensitization in children; one in association with asthma²¹ and one in association with wheeze¹⁸. Metzler et al. (2019) found a significant association between prenatal antibiotic exposure and atopic sensitization at 4 years of age, defined as positive specific IgE antibodies higher than 0.35 kU/L (RR 1.57, 95% CI 1.11-2.22). When assessing positive specific IgE antibodies higher than 0.7

kU/L, however, there was no longer an association ²¹. In the context of wheezing, Dom et al. (2010) found no association between antibiotic use during pregnancy and atopic sensitization at 1 year of age (RR 1.13, 95% CI 0.93-1.37) ¹⁸.

Food Allergy

Of the 27 studies assessing prenatal antibiotic exposure with relation to asthma risk, only one study included an assessment of food allergy. Metzler et al. (2019) found a significant association between antibiotic use during pregnancy and the development of food allergy, reporting an RR of 1.81 (95% CI: 1.11 –2.95) ²¹.

Dermatitis / Eczema

A total of five studies assessed the risk of developing dermatitis or eczema after prenatal exposure to antibiotics, shown in Figure 2a. The overall RR of 1.28 (95% CI: 1.06 –1.53) indicates that prenatal exposure to antibiotics is a risk factor for the development of dermatitis/eczema. Additional sensitivity analyses indicated that the effect size for studies that used an unsupervised participant questionnaire to collect antibiotic exposure data was significantly smaller than the effect size when a medical professional conducted an interview to collect this information (Supplementary File 7). The studies were quite heterogeneous however, and power analysis indicates that four of the five studies were likely underpowered, shown in Figure 2b.

Allergic rhinitis

Three of the 27 studies assessed the association between prenatal antibiotic exposure and childhood allergic rhinitis (in association with childhood asthma). Risk estimates of the individual studies, shown in Figure 3a, and the overall RR of 1.13 (95% CI: 1.02 –1.25) indicate that antibiotic use during pregnancy is associated with increased risk for allergic rhinitis in

children, another disease in the progression of the atopic march. These results should be interpreted with caution, however, as a sunset plot of these studies indicates that they are likely underpowered, shown in Figure 3b.

Wheeze

Of the nine studies that reported wheeze, eight showed a positive trend in between prenatal antibiotic exposure and the development of childhood wheeze. In half of the studies this relationship was statistically significant. Figure 4a shows the risk estimates of each study with a calculated overall RR of 1.51 (95% CI: 1.17-1.94). Additional sensitivity analyses indicated a moderation effect in a meta regression model with separately estimated residual heterogeneity for how antibiotic exposure data was collected. A pairwise comparison showed a significantly smaller effect size when data was collected using an unsupervised participant questionnaire than when a medical professional conducted an interview to collect this information (Supplementary File 7). There was a slight indication of publication bias after visual inspection of funnel plots (Figure 4b), but this effect was not significant by Egger's parametric or Begg's nonparametric distribution tests. The heterogeneity in the data was substantial (I^2 index = 83.5%, test for heterogeneity $p < 0.001$) and the sunset plot shows that six of nine studies are likely underpowered (Figure 4b).

Asthma

For the overall effects on asthma, meta-analysis of 20 studies showed a strong association between prenatal antibiotic exposure and the risk of developing asthma in childhood (RR 1.28, CI 1.22-1.34) (Figure 5a). In 18 of 20 studies, this relationship was statistically significant. Additional sensitivity analyses indicated a moderation effect in a meta regression model with separately estimated residual heterogeneity for how antibiotic exposure data was collected. A

pairwise comparison showed a significantly smaller effect size when data was collected using an unsupervised participant questionnaire than when a medical professional conducted an interview to collect this information (Supplementary File 7). There was no indication of a significant publication bias, and the sunset plot indicates that the majority of the studies had a high power (Figure 5b), the heterogeneity in the studies was considerable.

Trimester assessment of antibiotic use during pregnancy on wheeze and asthma outcomes in children.

Of the 27 studies included in our study, 11 collected data on the trimester of pregnancy that antibiotics were administered with respect to risk for developing wheeze or asthma during childhood. Our trimester subgroup analysis of three studies assessing wheeze revealed that only antibiotics administered during the third trimester had a significant association with relative risk of childhood wheeze: 1st trimester (RR 1.13, CI 0.95-1.34), 2nd trimester (RR 1.13, CI 0.99-1.29 – only 1 study), 3rd trimester (RR 1.76, CI 1.11-2.79), shown in Figure 6a. Regarding asthma, meta-analysis of eight studies revealed a significant effect of antibiotics taken during any trimester and relative risk of developing childhood asthma: 1st trimester (RR 1.17, CI 1.02-1.35), 2nd trimester (RR 1.22, CI 1.10-1.35), 3rd trimester (RR 1.21, CI 1.10-1.34), shown in Figure 6b. Pair-wise comparisons revealed no differences in asthma development between the three trimester administration periods.

Assessment of antibiotic type during pregnancy on childhood asthma outcomes.

To test if the type of antibiotic used during pregnancy is associated with asthma development in children, we separated the antibiotics into six different classes. From the six studies that collected data on the type of antibiotic administered, subgroup analysis revealed that all antibiotic classes other than Cephalosporins were associated with increased risk for childhood

asthma. Penicillins (RR 1.27, CI 1.19-1.36), Cephalosporins (RR 1.26, CI 0.92-1.74), Macrolides (RR 1.36, CI 1.16-1.59), Sulphonamides / Trimethoprim (RR 1.27, CI 1.11-1.46), Nitrofurantoin (RR 1.17, CI 1.01-1.334), shown in Figure 7, as well as Chloramphenicol (RR of 1.77, CI 1.18-2.65 – one study). Due to the scarcity of studies comparing antibiotic types, however, this analysis should be interpreted with caution.

Grading of evidence

The GRADE protocol was used to evaluate the certainty of the evidence (Table 2) and determined the effect evaluates of antibiotic use during pregnancy on the development of childhood asthma to be of low quality. The effect of antibiotic use during pregnancy on the development of childhood dermatitis, allergic rhinitis, and wheeze were determined to be of very low, very low, and low quality respectively. The reason for the low-quality scoring was due to serious inconsistency (high heterogeneity), and the detection of publication bias. 9 of the 27 studies used for this meta-analysis did not provide population numbers and were therefore excluded from the GRADE analysis^{19,25,42,44,45,47,50,56}. The overall quality of the evidence presented in this systematic review and meta-analysis was regarded as low quality.

Table 2: Grading of Recommendations Assessment, Development, and Evaluation (GRADE) profile for certainty of evidence used in this meta-analysis and systematic review.

						Summary of findings					
Quality Assessment						Number of patients					
no of studies (Design)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	No intervention - No Disease	No intervention - Disease	Intervention - No Disease	Intervention - Disease	Relative Risk (95% CI)	Quality
Childhood dermatitis/ eczema and maternal antibiotic use during pregnancy											
5 (observational)	No serious limitations	serious inconsistency (heterogeneity)	No serious indirectness	No imprecision	Detected	1207	434	278	179	RR 1.28 (1.06, 1.53)	very low
Risk of allergic rhinitis in childhood and maternal antibiotic use during pregnancy											
3 (observational)	No serious limitations	low inconsistency (heterogeneity)	No serious indirectness	No imprecision	Detected	675	73	235	37	RR 1.13 (1.02, 1.25)	very low
Risk of childhood wheeze and maternal antibiotic use during pregnancy											
9 (observational)	No serious limitations	serious inconsistency (heterogeneity)	No serious indirectness	No imprecision	Undetected	48689	4413	10100	1138	RR 1.51 (1.17, 1.94)	low
Risk of childhood asthma and maternal antibiotic use during pregnancy											
20 (observational)	No serious limitations	serious inconsistency (heterogeneity)	No serious indirectness	No imprecision	Undetected	1526198	177668	340683	35755	RR 1.28 (1.22, 1.34)	low

Discussion

Our meta-analysis demonstrates that antibiotic use during pregnancy is significantly associated with the development of asthma in children. Additionally prenatal antibiotic exposure is also associated with disorders present in the atopic march including atopic sensitization, dermatitis/eczema, food allergy, allergic rhinitis and wheeze. Though these disorders are acknowledged as important factors that often precede asthma development in children ^{1,58}, surprisingly few studies assessed them with respect to asthma.

Considering how maternal antibiotic use could influence allergy development in children, the prevailing hypothesis is that the early life microbiome influences immune system development, which subsequently contributes to atopic disease ⁵⁹. Cohort studies demonstrate that antibiotic use during pregnancy has a profound effect on the colonization of the infant gut in early life ^{60,61}. Children with low microbial diversity in the first three months of life had immune cell populations that diverged significantly from healthy children ^{14,62}, and disruptions to the neonatal microbiome are associated with childhood asthma ⁶³. A recent meta-analysis determined that antibiotic use in children also increased risk for asthma development, emphasizing the importance of antibiotic stewardship both during pregnancy and in early life ⁶⁴.

Stokholm et al. (2014), alternatively hypothesized that the observed increase in childhood asthma risk was not due to antibiotic induced alteration of the maternal microbiome, but rather due to an increased propensity for infection as an underlying risk factor for childhood allergy development²⁶. Though there are likely several different mechanisms contributing to this complex transgenerational process, studies in rodents have provided substantial evidence confirming the importance of the maternal microbiome during pregnancy for offspring asthma

susceptibility. Russell et al. demonstrated that mice treated with antibiotics during the perinatal time period showed increased asthma severity ^{65,66}. Additionally, we recently showed that antibiotic treatment of mice during the gestational time period only, resulted in a dysbiotic gut microbiome in mothers that was transferred to the offspring and was associated with increased asthma severity ⁶⁷.

If the infant gut microbiome is a causal link between antibiotic use during pregnancy and increased asthma risk in children, then the timing of antibiotic treatment during pregnancy could influence this association. Our meta-analysis indicates that antibiotic use in the third trimester was associated with wheeze, whereas antibiotic use in any trimester was associated with asthma. Since pair-wise comparison revealed no differences in asthma development according to trimester of antibiotic use, this raises the question, “How long does the maternal gut microbiome remain dysbiotic?” Recent research has demonstrated that particular but bacterial species that were eliminated after antibiotic treatment were still not detectable in humans after 9 months ⁶⁸ and in mice after 6 months ⁶⁹. These studies support the idea that antibiotic use during pregnancy could eliminate specific clades of bacteria that are particularly important for infant development. There are, however, a paucity of data analyzing long term effects of antibiotic use in pregnant women, a topic of utmost importance for future studies.

In addition to trimester of antibiotic treatment, the number of prescriptions during pregnancy and type of antibiotic prescribed could also influence susceptibility to allergic disease in the next generation. Though we could not analyze the number of antibiotic prescriptions, due to differences in categorization of prescription number, the individual studies indicate increased risk for childhood asthma with increasing maternal antibiotic prescriptions. Considering type of antibiotic, our meta-analysis revealed that several different antibiotic types are associated

with increased risk of asthma in children. This indicates that observed increase in childhood asthma risk is likely due to disruption of gut microbial homeostasis rather than due to the elimination of a specific bacterial clade. This highlights an imperative need for maternal and childhood gut microbiome analysis in future studies to assess the effects of antibiotic use during pregnancy on the atopic march with relation to asthma development in early childhood ⁷⁰.

As identified by our GRADE assessment, the certainty of the evidence used was determined to be of low quality, therefore limiting this meta-analysis. Further limitations to our meta-analysis include study populations which were mainly white and European, underpowered studies as well as study protocol inconsistencies in disease diagnosis and adjusting for confounding factors. We also identified that the method by which studies collected antibiotic exposure data was a significant modifier to effect size. Future studies should employ and report more direct and objective measurement methods rather than self-reported questionnaires. The existence of these limitations is justification for why the field needs more high-quality studies on this topic.

Conclusion

Herein, we synthesize data across 27 studies and present a meta-analysis showing a significant association of prenatal antibiotic exposure with increased susceptibility to childhood asthma as well as diseases involved in the atopic march, summary shown in Figure 8. Though there is evidence that antibiotic treatment during pregnancy is a driver of the atopic march, due to a large heterogeneity between studies more research is needed to draw firm conclusions on this matter.

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Author Contributions

Conceptualization: AC, JC, and MC. Article searches, screening, and data extraction: AC, JA, JD, SD, MA, MC. Data analysis: AW. All authors contributed to the writing and editing of the manuscript.

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Conflict of interest statement

The authors declare no conflicts of interest.

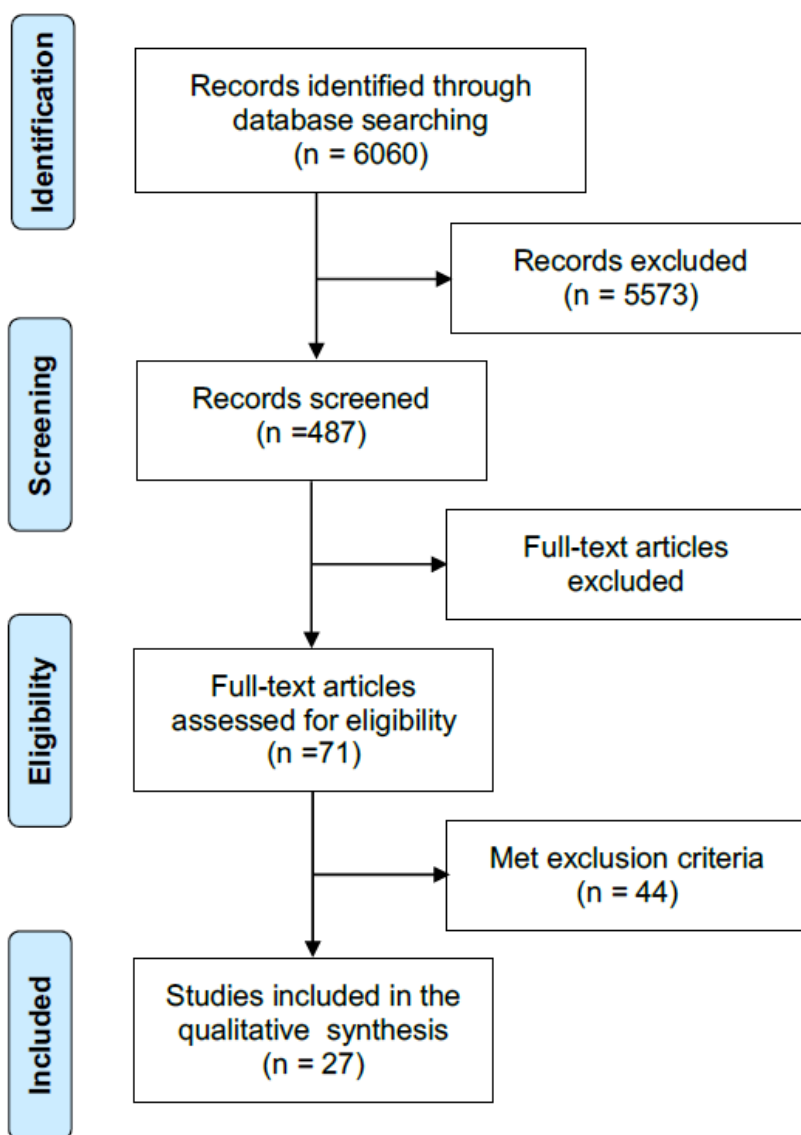


Figure 1: PRISMA flow chart showing the results of the literature search and the selection of studies.

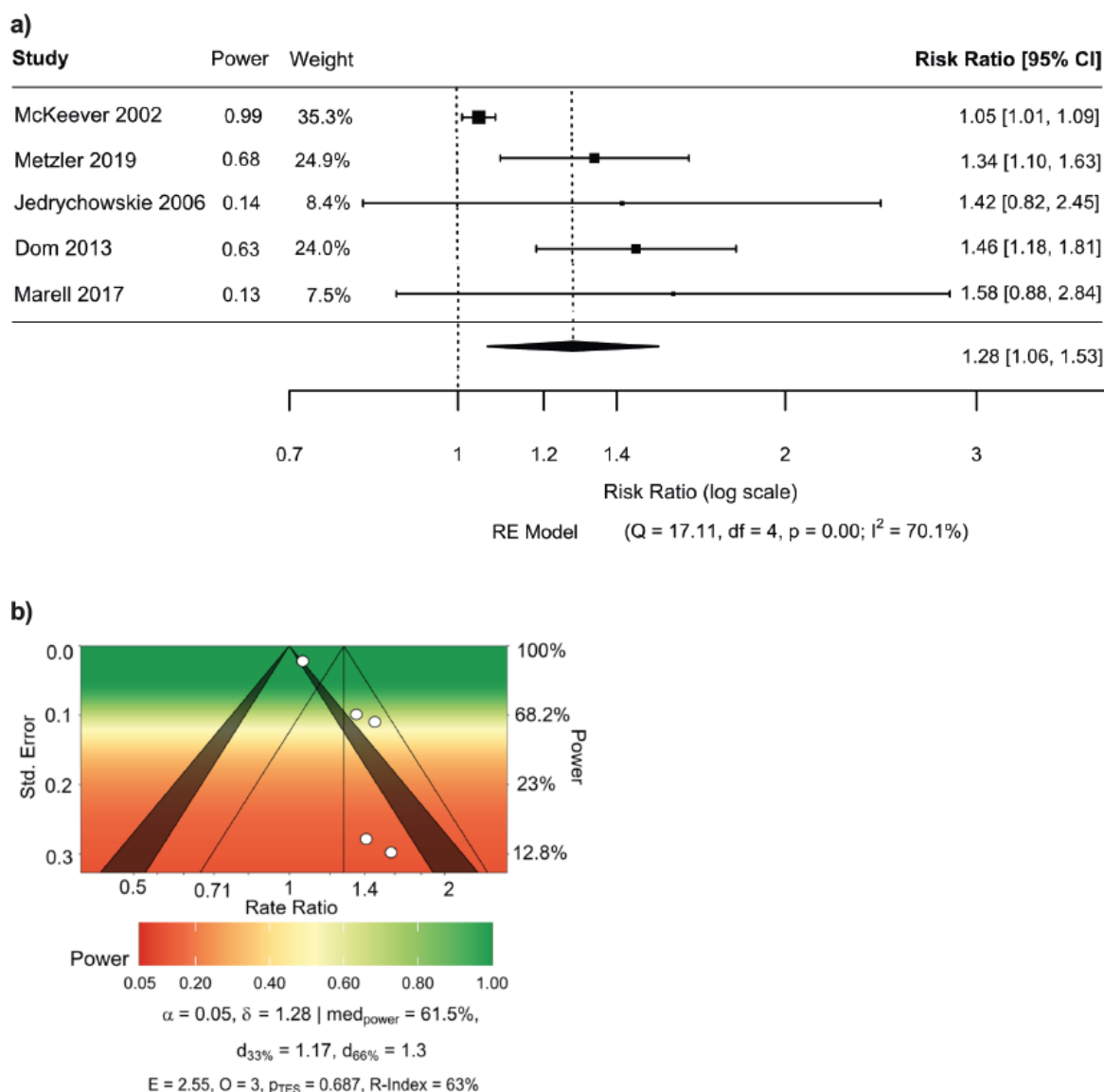


Figure 2 Maternal antibiotic use during pregnancy and relative risk of developing childhood dermatitis/eczema. a) Forest plot for five studies; squares indicate study-specific risk estimates, diamond indicates the overall RR and 95% CI. b) Power enhanced funnel plot (sunset plot). Individual study effect sizes are depicted as open circles.

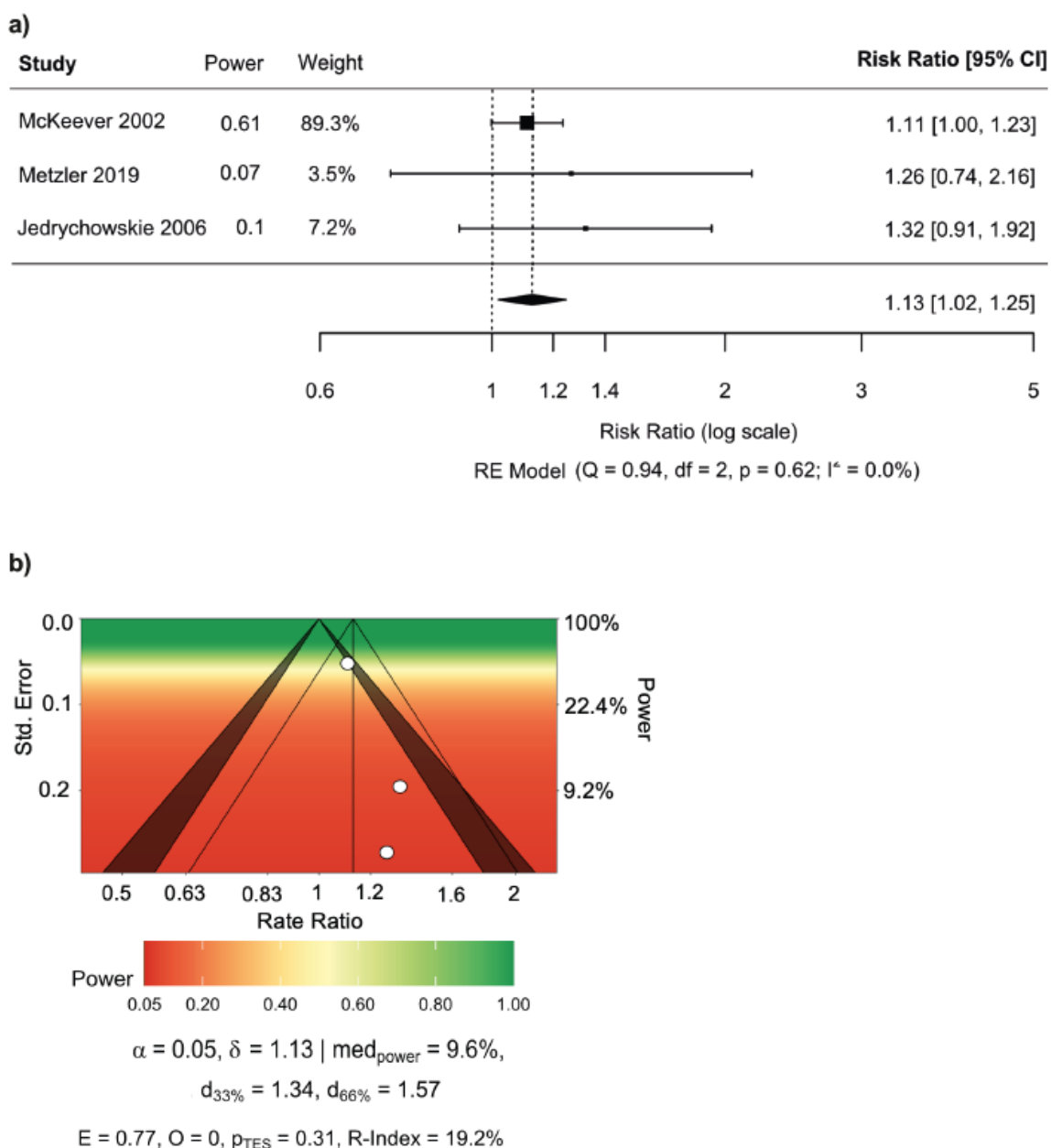


Figure 3: Association between prenatal exposure to antibiotics and risk for allergic rhinitis in childhood. a) Forest plot for three studies; squares indicate study-specific risk estimates, diamond indicates the overall RR and 95% CI. b) Power enhanced funnel plot (sunset plot). Individual study effect sizes are depicted as open circles.

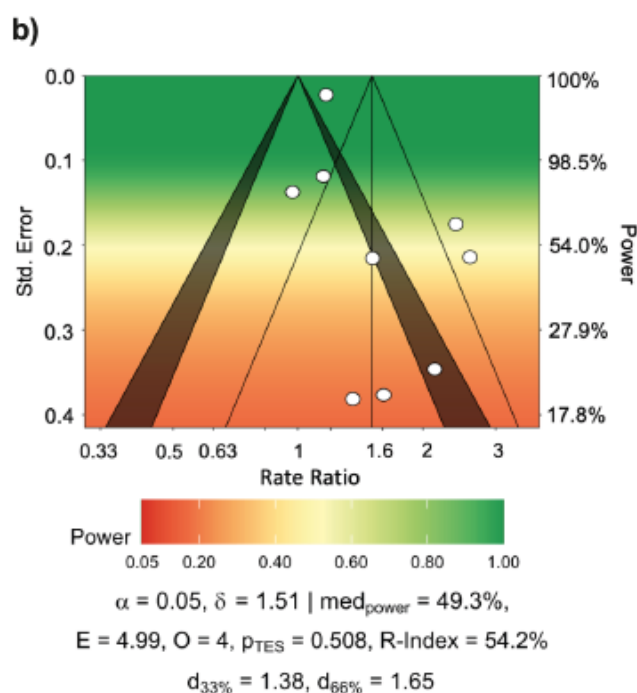
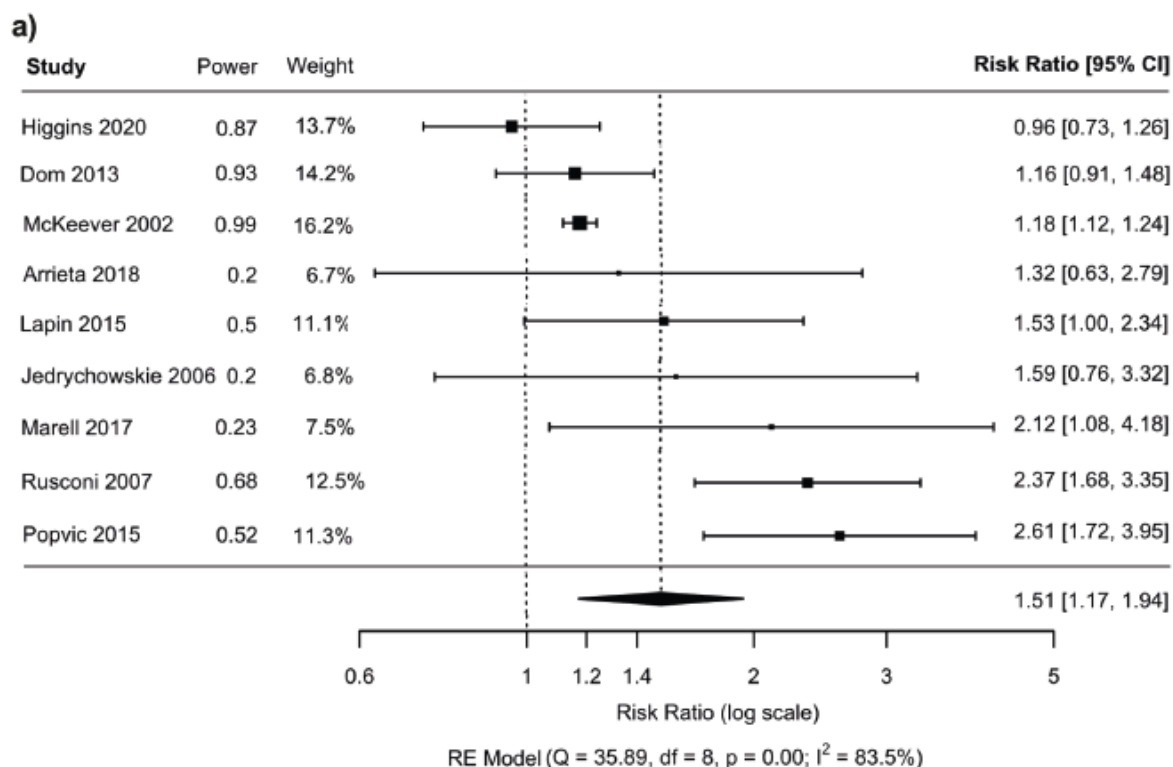
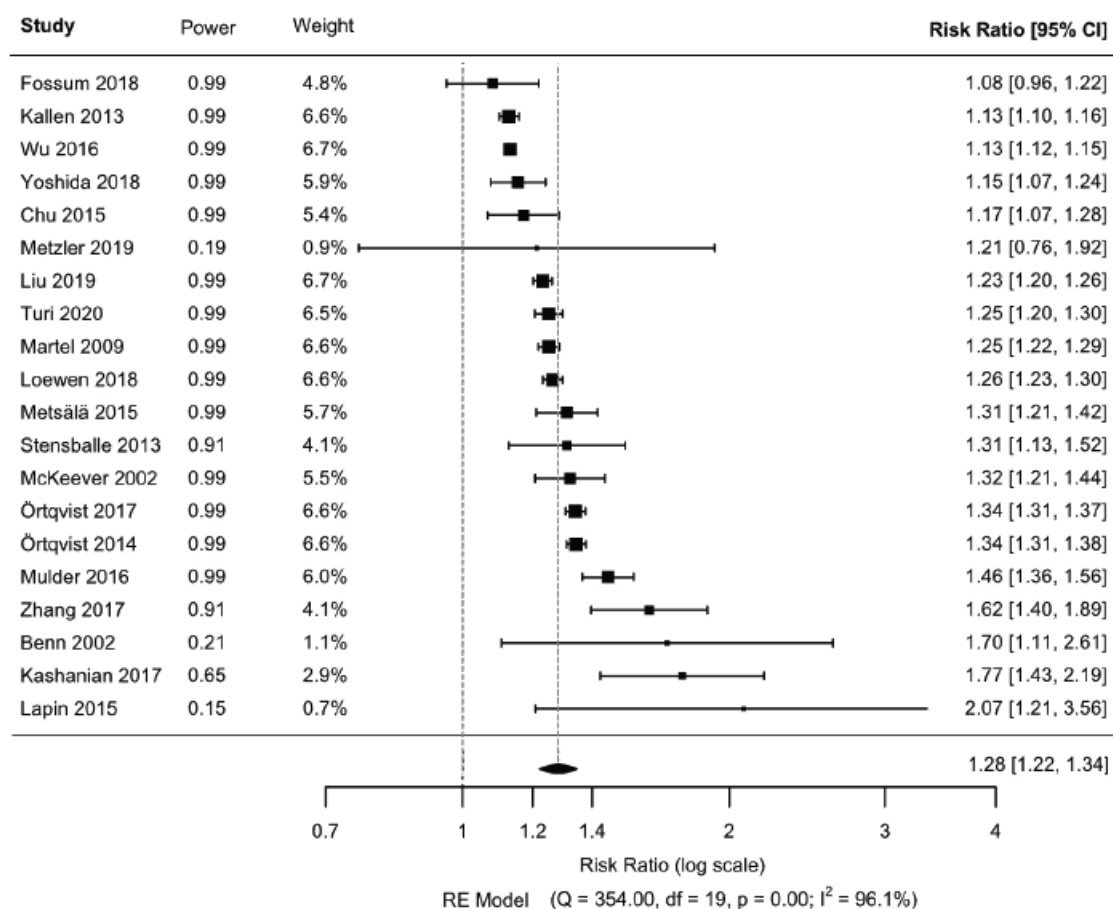


Figure 4: Relative risk of developing childhood wheeze after maternal antibiotic use during pregnancy. a) Forest plot for nine studies, represented as RR and 95% CI. Squares indicate study-specific risk estimates, diamond indicates the overall RR and 95% CI. b) Power enhanced funnel plot (sunset plot). Individual study effect sizes are depicted as open circles.

a)



b)

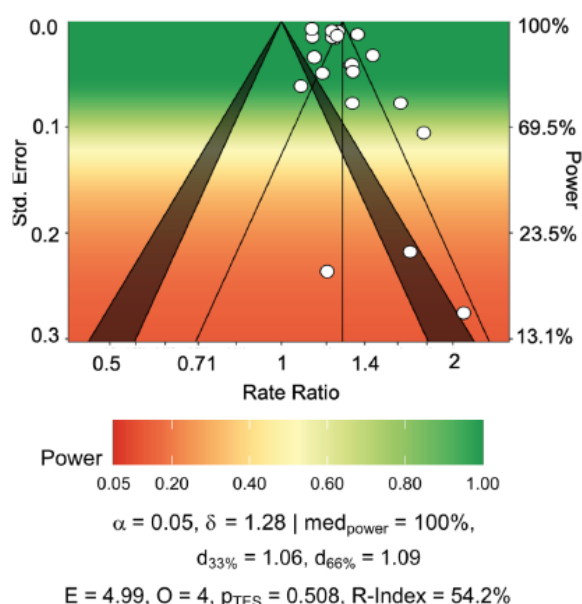
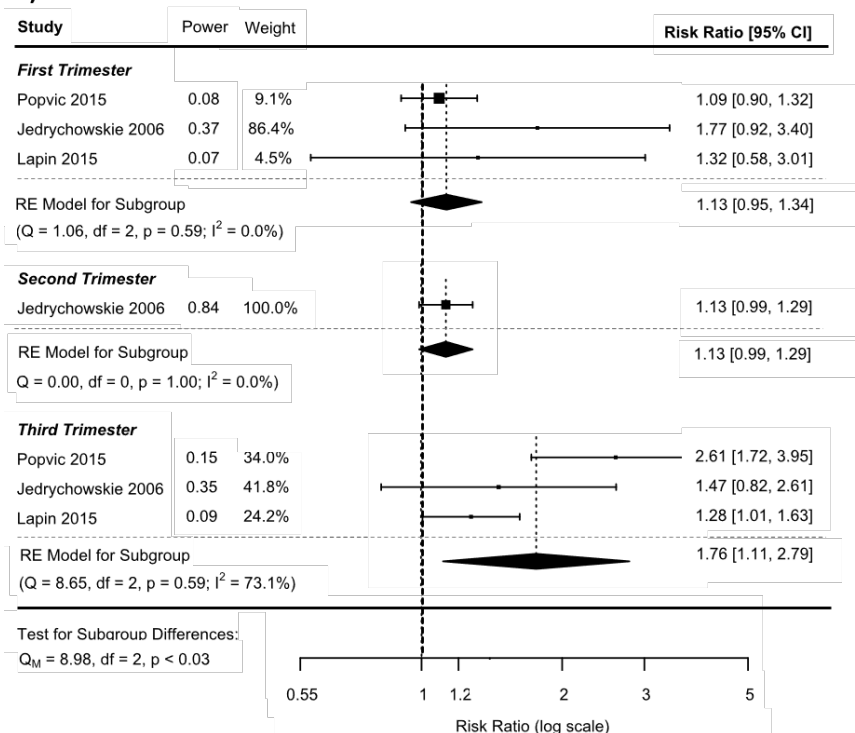


Figure 5 - Prenatal antibiotic exposure and relative risk of developing asthma in childhood. a) Forest plot for 20 studies; squares indicate study-specific risk estimates, diamond indicates the overall RR and 95% CI. b) Power enhanced funnel plot (sunset plot).

a) Wheeze - Trimester



b) Asthma - Trimester

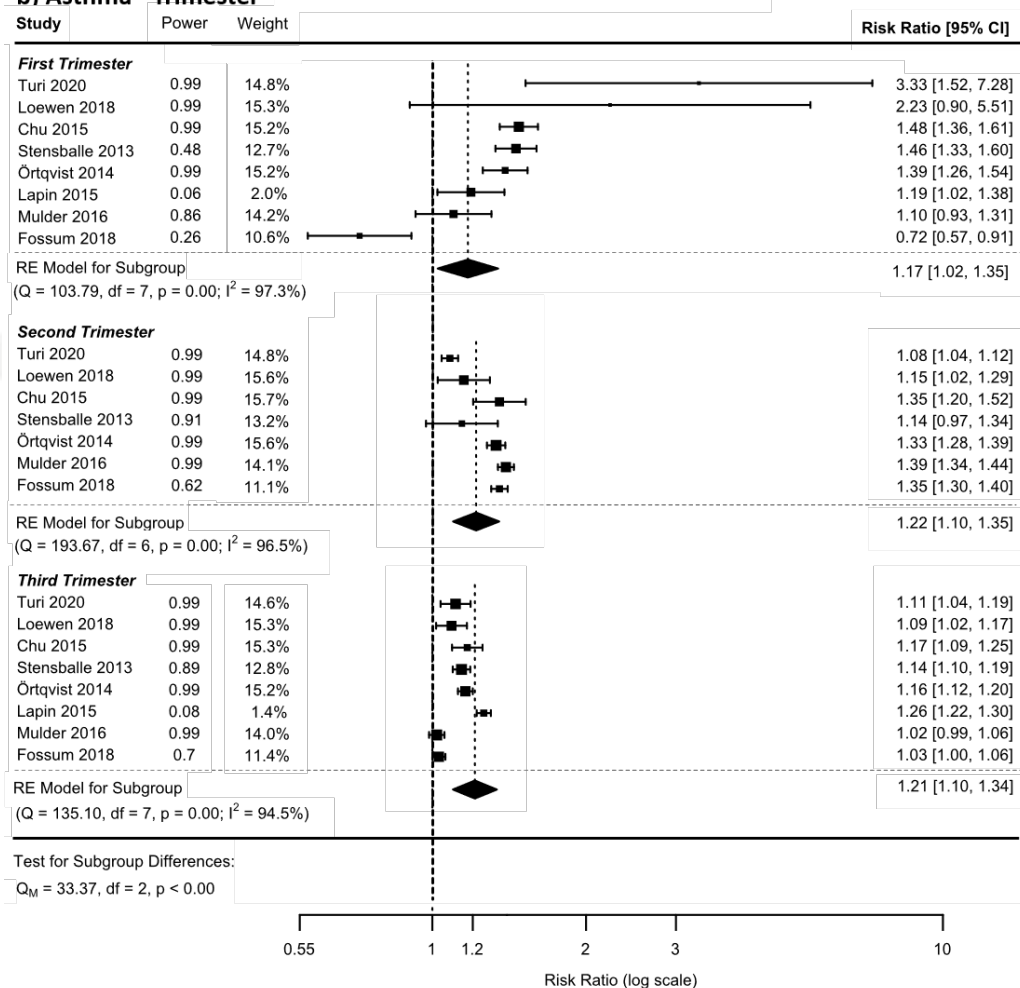


Figure 6: The association between antibiotic use in different trimesters and the development of wheeze or asthma in childhood. a) Forest plot showing subgroup analysis in three studies assessing trimester of prenatal antibiotic administration and relative risk of developing childhood wheeze. b) Forest plot for eight studies assessing trimester of prenatal antibiotic administration and relative risk of developing asthma in childhood. Represented as RR and 95% CI. Squares indicate study-specific risk estimates; diamond indicates overall RR with its corresponding 95% CI.

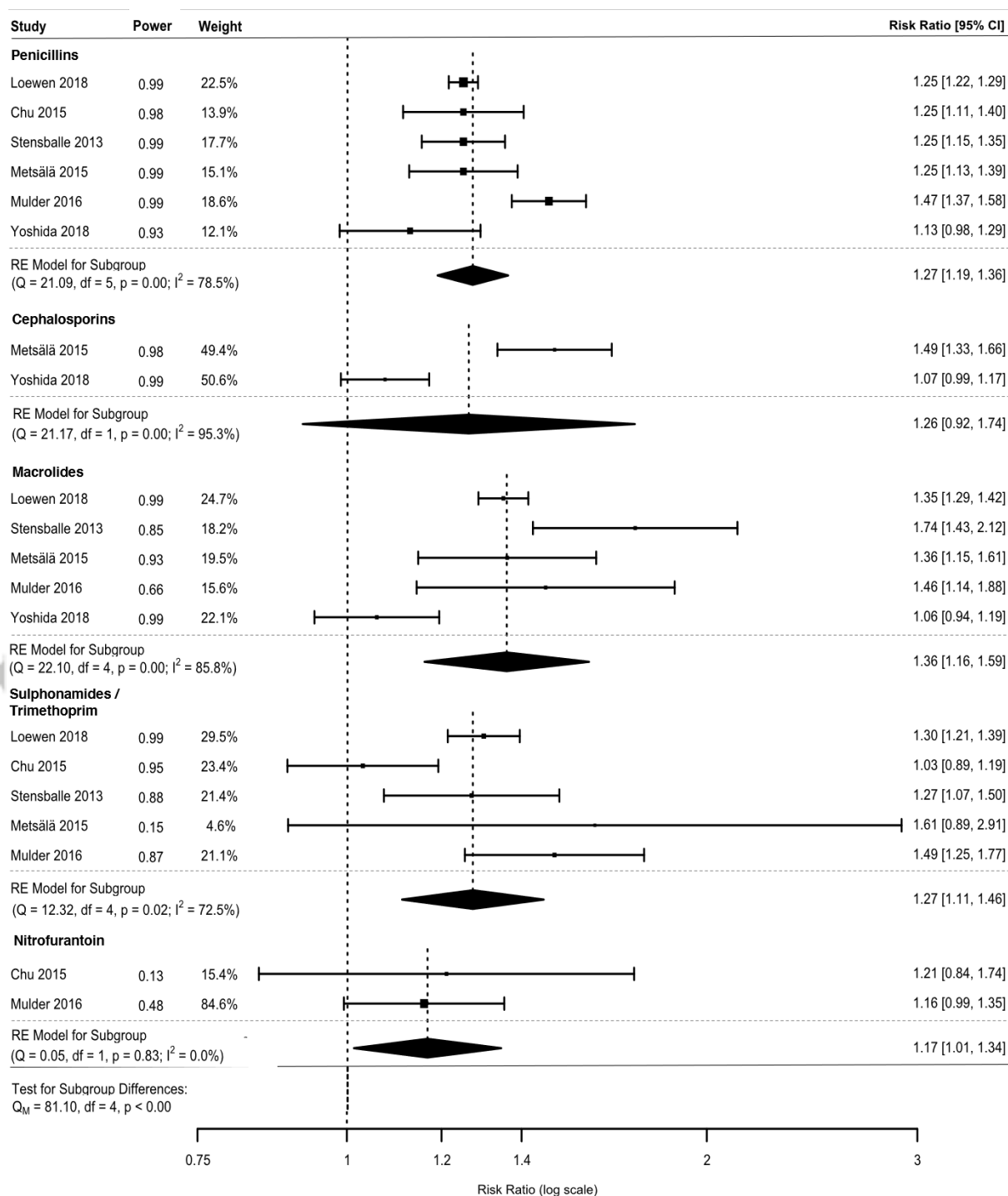


Figure 7: Subgroup analysis of type of antibiotic administered during pregnancy and relative risk of developing childhood asthma. Forest plot showing subgroup analysis of the antibiotic groups: Penicillins (6 studies), Cephalosporins (2 studies), Macrolides (5 studies), Sulphonamides / Trimethoprim (5 studies) and Nitrofurantoin (2 studies). Represented as RR and 95% CI. Squares indicate study-specific risk estimates; diamond indicates the overall RR with its corresponding 95% CI.

Supplementary File 1: Search strategy used to search MEDLINE and EMBASE databases via Ovid.

Maternal Exposure/ or Pregnancy/ or Prenatal Exposure Delayed Effects/ or Prenatal Care/
(maternal or prenatal or gestatio* or pregnan* or perinatal or antenatal).ti,kw,ab.
1 or 2
exp Anti-Bacterial Agents/
(anitbact* or anti\$infecti or anti\$micro or bacteriostat* or bactericid*).ti,kw,ab.
4 or 5
exp Asthma/ or exp Hypersensitivity/
(asthma* or allerg* or respiratory hypersensitivity or hypersensitivity or wheez* or AHR or atopy or atopic dermatitis or eczema or allergic rhinitis or IgE).ti,kw,ab.
7 or 8
3 and 6 and 9

Search strategy used for Proquest database and for Cochrane central register of controlled trials:

TI,KW,AB(maternal or prenatal or gestatio* or pregnan* or perinatal or antenatal)
AND TI,KW,AB(anitbact* or anti\$infecti or anti\$microor bacteriostat* or bactericid*)
AND TI,KW,AB(asthma* or allerg* or respiratory hypersensitivity or hypersensitivity
or wheez* or AHR or atopy or atopic dermatitis or eczema or allergic rhinitis or IgE)

Supplementary File 2: Number of unique hits returned per strategy

Database	No. of unique hits returned
Medline	903
EMBASE	6055
Cochrane central register of controlled trials	44
ProQuest	69
total	7071
total after de-duplication	6060

Supplementary File 3

Selection criteria for evaluated for risk of using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies

- 1) Representativeness of the exposed cohort
 - a) truly representative of the average pregnant woman in the community *
 - b) somewhat representative of the average pregnant woman in the community *
 - c) selected group of users e.g., volunteers
 - d) no description of the derivation of the cohort
- 2) Selection of the non-exposed cohort
 - a) drawn from the same community as the exposed cohort *
 - b) drawn from a different source
 - c) no description of the derivation of the non-exposed cohort
- 3) Ascertainment of exposure
 - a) secure record *
 - b) structured interview *
 - c) written self-report
 - d) no description
- 4) Demonstration that outcome of interest was not present at start of study
 - a) yes *
 - b) no

Comparability

- 1) Comparability of cohorts on the basis of the design or analysis
 - a) study controls for early life antibiotic exposure *
 - b) study controls for any additional factor *

Outcome

- 1) Assessment of outcome
 - a) independent blind assessment *
 - b) record linkage *
 - c) self-report
 - d) no description
- 2) Was follow-up long enough for outcomes to occur (proper diagnosis of asthma or persistent wheeze)
 - a) yes (at least until the age of 5 years) *
 - b) no
- 3) Adequacy of follow up of cohorts
 - a) complete follow up - all subjects accounted for *
 - b) subjects lost to follow up unlikely to introduce bias - small number lost - > 10 % follow up, or description provided of those lost) *
 - c) follow up rate < 10 % and no description of those lost

d) no statement

Supplementary File 4

Results of overall models

	<i>Model results</i>					
	<i>n</i> studies included	Effect Size (95% CI, <i>p</i> -value)	τ^2	I^2	<i>Q</i> -Test	median power
Asthma						
OR	15	1.37 (1.06-1.77, <i>p</i> = .017)	0.231	99.78%	$Q(df = 14) = 2053.78, p < .001$	99.99%
HR	7	1.28 (1.23-1.33, <i>p</i> < .001)	0.002	80.13%	$Q(df = 6) = 35.77, p < .001$	99.99%
RR	20	1.28 (1.23-1.35, <i>p</i> < .001)	0.007	94.84%	$Q(df = 19) = 320.99, p < .001$	99.99%
Wheezing						
OR	6	1.39 (1.06-1.80, <i>p</i> = .015)	0.013	11.11%	$Q(df = 5) = 5.13, p = .400$	16.8%
HR	1	1.27 (1.18-1.37, <i>p</i> < .001)	-	-	-	-
RR	9	1.53 (1.19-1.95, <i>p</i> < .001)	0.096	81.57%	$Q(df = 8) = 31.29, p < .001$	51.3%

Model comparisons

Asthma OR, RR: -0.062 (SE 0.133, p = .642)
Asthma OR, HR: 0.068 (SE 0.133, p = .610)
Asthma HR, RR: 0.006 (SE 0.030, p = .848)
Wheezing, OR, RR: 0.097 (SE 0.184, p = .600)

Model comparisons show no difference in the effect size estimates from models based on different effect size types. Hence, all models should be considered viable for further selection.

Modelfit:

	<i>Asthma Models</i>			<i>Wheezing Models</i>		
	OR	HR	RR	OR	HR	RR
<i>Log-likelihood</i>	-11.58096	9.108083	12.25259	-1.828984	-	-3.321497
<i>dev</i>	23.16191	-18.216165	-24.50518	3.657967	-	6.642994
<i>AiC</i>	27.16191	-14.216165	-20.50518	7.657967	-	10.642994
<i>BiC</i>	28.44003	-14.632646	-18.61631	6.876843	-	10.801878

For asthma, the RR-Model shows the best fit and is therefore selected for further analyses.

For wheezing, the OR-Model shows a better fit, but has lower power.

Hence, the RR-Model is selected for further analyses.

Comparison RR-Model Asthma and Wheezing.

Asthma, Wheezing: 0.170 (SE 0.128, p = .185)

The model comparison shows no significant difference between the overall estimates for wheezing and asthma.

Supplementary File 5

Model Asthma

Sensitivity Analysis

No Outliers

Funnelplot with eggert and beggs test

The tests are not significant, suggesting the absence of publication bias.

```
> regtest(model.re.asthma.rr, model = "lm")
```

Regression Test for Funnel Plot Asymmetry

model: weighted regression with multiplicative dispersion
predictor: standard error

test for funnel plot asymmetry: $t = 1.9875$, $df = 18$, $p = 0.0623$

```
> ##Beggs rank-correlation test  
> ranktest(model.re.asthma.rr)
```

Rank Correlation Test for Funnel Plot Asymmetry

Kendall's tau = -0.0421 , $p = 0.8227$

The funnelplot shows minimal bias with with trim and fill method.

Model Results Asthma

Random-Effects Model (k = 20; tau² estimator: REML)

tau² (estimated amount of total heterogeneity): 0.0089 (SE = 0.0037)
tau (square root of estimated tau² value): 0.0944
I² (total heterogeneity / total variability): 96.12%
H² (total variability / sampling variability): 25.76

Test for Heterogeneity:

Q(df = 19) = 353.9978, p-val < .0001

Model Results:

estimate	se	zval	pval	ci.lb	ci.ub	
0.2470	0.0246	10.0573	<.0001	0.1989	0.2951	***

-> exponentiate to get RR. RR = 1.28

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Asthma Subgroup Analysis Trimester

- Model Results

```
> model.asthma.tri.het
```

```
Multivariate Meta-Analysis Model (k = 23; method: REML)
```

```
Variance Components:
```

```
outer factor: trial (nlvls = 23)
```

```
inner factor: trimester (nlvls = 3)
```

	estim	sqrt	k.lvl	fixed	level
tau^2.1	0.031	0.177	8	no	1
tau^2.2	0.018	0.133	7	no	2
tau^2.3	0.016	0.127	8	no	3

```
Test for Residual Heterogeneity:
```

```
QE(df = 20) = 432.557, p-val < .001
```

```
Test of Moderators (coefficients 1:3):
```

```
QM(df = 3) = 33.374, p-val < .001
```

```
Model Results:
```

	estimate	se	zval	pval	ci.lb	ci.ub		
trimester1	0.160	0.070	2.306	0.021	0.024	0.297	*	-> RR 1.17
trimester2	0.195	0.053	3.686	<.001	0.091	0.299	***	-> RR 1.22
trimester3	0.191	0.050	3.804	<.001	0.093	0.290	***	-> RR 1.21

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- Pairwise Comparisons

```
Hypotheses:
```

```
1: -trimester1 + trimester2 = 0
```

```
2: -trimester1 + trimester3 = 0
```

```
3: -trimester2 + trimester3 = 0
```

```
Results:
```

	estimate	se	zval	pval
1:	0.035	0.087	0.398	0.691
2:	0.031	0.086	0.360	0.719
3:	-0.004	0.073	-0.053	0.957

Asthma Subgroup Analysis Antibiotic Type

- Model Results

Multivariate Meta-Analysis Model (k = 20; method: REML)

Variance Components:

outer factor: trial (nlvls = 20)
inner factor: antibioticcategor (nlvls = 5)

	estim	sqrt	k.lv1	fixed	level
tau^2.1	0.005	0.072	6	no	1
tau^2.2	0.051	0.225	2	no	2
tau^2.3	0.026	0.161	5	no	3
tau^2.4	0.015	0.123	5	no	4
tau^2.5	0.000	0.000	2	no	5

Test for Residual Heterogeneity:
QE(df = 15) = 76.716, p-val < .001

Test of Moderators (coefficients 1:5):
QM(df = 5) = 81.102, p-val < .001

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
antibioticcategor1	0.241	0.035	6.939	<.001	0.173	0.309	***
antibioticcategor2	0.234	0.163	1.431	0.153	-0.086	0.554	
antibioticcategor3	0.307	0.081	3.791	<.001	0.148	0.465	***
antibioticcategor4	0.241	0.070	3.464	<.001	0.105	0.378	***
antibioticcategor5	0.154	0.072	2.128	0.033	0.012	0.296	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

- *Pairwise Comparisons*

Hypotheses:

- 1: $-\text{antibiocategor1} + \text{antibiocategor2} = 0$
- 2: $-\text{antibiocategor1} + \text{antibiocategor3} = 0$
- 3: $-\text{antibiocategor1} + \text{antibiocategor4} = 0$
- 4: $-\text{antibiocategor1} + \text{antibiocategor5} = 0$
- 5: $-\text{antibiocategor2} + \text{antibiocategor3} = 0$
- 6: $-\text{antibiocategor2} + \text{antibiocategor4} = 0$
- 7: $-\text{antibiocategor2} + \text{antibiocategor5} = 0$
- 8: $-\text{antibiocategor3} + \text{antibiocategor4} = 0$
- 9: $-\text{antibiocategor3} + \text{antibiocategor5} = 0$
- 10: $-\text{antibiocategor4} + \text{antibiocategor5} = 0$

Results:

	estimate	se	zval	pval
1:	-0.008	0.167	-0.046	0.963
2:	0.065	0.088	0.742	0.458
3:	0.000	0.078	0.001	0.999
4:	-0.087	0.080	-1.085	0.278
5:	0.073	0.182	0.401	0.688
6:	0.008	0.177	0.044	0.965
7:	-0.079	0.179	-0.445	0.656
8:	-0.065	0.107	-0.611	0.541
9:	-0.153	0.109	-1.405	0.160
10:	-0.087	0.101	-0.868	0.385

Asthma Subgroup Analysis Study Type

```
> model.asthma.pro.het
```

Multivariate Meta-Analysis Model (k = 20; method: REML)

Variance Components:

outer factor: trial (nlvls = 20)

inner factor: Type (nlvls = 2)

	estim	sqrt	k.lvl	fixed	level
tau^2.1	0.000	0.000	5	no	Prospective
tau^2.2	0.010	0.100	15	no	Retrospective

Test for Residual Heterogeneity:

QE(df = 18) = 288.461, p-val < .001

Test of Moderators (coefficients 1:2):

QM(df = 2) = 589.121, p-val < .001

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
TypeProspective	0.293	0.013	22.719	<.001	0.268	0.318	***
TypeRetrospective	0.237	0.028	8.543	<.001	0.182	0.291	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

- Pairwise comparison

Hypothesis:

1: -TypeProspective + TypeRetrospective = 0

Results:

	estimate	se	zval	pval
1:	-0.056	0.031	-1.843	0.065

Test of Hypothesis:

QM(df = 1) = 3.398, p-val = 0.065

Model Wheezing

Sensitivity Analysis

Outliers

No outliers.

Funnel plot with eggers and beggs test

Eggers and beggs test show no asymmetry of the funnelplot, indicating the absence of publication bias.

Regression Test for Funnel Plot Asymmetry

model: weighted regression with multiplicative dispersion
predictor: standard error

test for funnel plot asymmetry: $t = 1.8834$, $df = 7$, $p = 0.1017$

```
>  
> ##Beggs rank-correlation test  
> ranktest(model.re.wheez.rr)
```

Rank Correlation Test for Funnel Plot Asymmetry

Kendall's $\tau = 0.1667$, $p = 0.6122$

The funnel plot with trim and fill supports this assumption.

Model Results Wheezing

Random-Effects Model (k = 9; tau^2 estimator: REML)

tau^2 (estimated amount of total heterogeneity): 0.1016 (SE = 0.0726)

tau (square root of estimated tau^2 value): 0.3188

I^2 (total heterogeneity / total variability): 83.53%

H^2 (total variability / sampling variability): 6.07

Test for Heterogeneity:

Q(df = 8) = 35.8878, p-val < .0001

Model Results:

estimate	se	zval	pval	ci.lb	ci.ub	
0.4119	0.1287	3.2007	0.0014	0.1597	0.6642	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Wheezing Subgroup Analysis Trimester

- Model Results

Multivariate Meta-Analysis Model (k = 6; method: REML)

Variance Components:

outer factor: trial (nlvls = 6)
inner factor: trimester (nlvls = 2)

	estim	sqrt	k.lvl	fixed	level
tau^2.1	0.000	0.000	3	no	1
tau^2.2	0.116	0.341	3	no	3

Test for Residual Heterogeneity:
QE(df = 4) = 9.702, p-val = 0.046

Test of Moderators (coefficients 1:2):
QM(df = 2) = 7.739, p-val = 0.021

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub
trimester1	0.122	0.089	1.367	0.172	-0.053	0.296
trimester3	0.567	0.234	2.423	0.015	0.108	1.026

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

- Pairwise Comparisons

Hypothesis:

1: -trimester1 + trimester3 = 0

Results:

	estimate	se	zval	pval
1:	0.446	0.251	1.779	0.075

Test of Hypothesis:

QM(df = 1) = 3.165, p-val = 0.075

Wheezing Subgroup Analysis Study Type

> model.wheez.pro.het

Multivariate Meta-Analysis Model (k = 9; method: REML)

Variance Components:

outer factor: trial (nlvls = 9)

inner factor: Type (nlvls = 2)

	estim	sqrt	k.lv1	fixed	level
tau^2.1	0.097	0.311	7	no	Prospective
tau^2.2	0.227	0.477	2	no	Retrospective

Test for Residual Heterogeneity:

QE(df = 7) = 34.636, p-val < .001

Test of Moderators (coefficients 1:2):

QM(df = 2) = 8.679, p-val = 0.013

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
TypeProspective	0.387	0.150	2.584	0.010	0.094	0.681	**
TypeRetrospective	0.493	0.348	1.415	0.157	-0.190	1.175	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

- Pairwise comparison

Hypothesis:

1: -TypeProspective + TypeRetrospective = 0

Results:

	estimate	se	zval	pval
1:	0.105	0.379	0.278	0.781

Test of Hypothesis:

QM(df = 1) = 0.077, p-val = 0.781

Model Eczema/Dermatitis

Sensitivity Analysis

Outliers

Cheung and Viechtbauers Outlier Battery show on outlier. The outlier is the single one retrospective study in the sample. It reports a slightly lower effect size, but has a considerably higher power than other studies. Comparing the overall effect size between a model including and excluding the outlier shows no significant difference in the model estimate. Since furthermore the total number of studies is low ($n = 5$) and the outlier had the highest power, we report the model including the outlier.

Model comparison

Fixed-Effects with Moderators Model ($k = 2$)

I^2 (residual heterogeneity / unaccounted variability): 0.00%
 H^2 (unaccounted variability / sampling variability): 1.00

Test for Residual Heterogeneity:
 $QE(df = 0) = 0.000$, $p\text{-val} = 1.000$

Test of Moderators (coefficient 2):
 $QM(df = 1) = 0.704$, $p\text{-val} = 0.401$

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.243	0.092	2.637	0.008	0.063	0.424	**
outlierswithout	0.097	0.116	0.839	0.401	-0.130	0.324	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Funnelplot with egggers and beggs test

Due to small sample size of studies, egggers regression test cannot be interpreted.

```
> ##Beggs rank-correlation test  
> ranktest(model.re.eczderma.rr)
```

Rank Correlation Test for Funnel Plot Asymmetry

Kendall's tau = 0.2000, $p = 0.8167$

Begg's test does not show deviation from funnel plot asymmetry.

However the funnelplot indicates a positive publication bias, so that the meta-analytic effect might be slightly biased towards a bigger effect.

```
Random-effects model:  
> model.re.eczderma.rr
```

```
Random-Effects Model (k = 5; tau^2 estimator: REML)
```

```
tau^2 (estimated amount of total heterogeneity): 0.0238 (SE = 0.0289)  
tau (square root of estimated tau^2 value):      0.1544  
I^2 (total heterogeneity / total variability):    70.09%  
H^2 (total variability / sampling variability):    3.34
```

```
Test for Heterogeneity:  
Q(df = 4) = 17.1139, p-val = 0.0018
```

```
Model Results:
```

estimate	se	zval	pval	ci.lb	ci.ub	
0.2434	0.0923	2.6371	0.0084	0.0625	0.4243	**

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Correctly scaling the model estimate into RR:
```

```
> predict(model.re.eczderma.rr, transf=exp, digits=3)
```

pred	ci.lb	ci.ub	cr.lb	cr.ub
1.276	1.064	1.529	0.897	1.815

Model Hayfever/Rhinitis

Sensitivity Analysis

Outliers

Cheung and Viechtbauers Outlier Battery show one outlier. The outlier is the single one retrospective study in the sample. It reports a slightly lower effect size, but has a considerably higher power than other studies. Comparing the overall effect size between a model including and excluding the outlier shows no significant difference in the model estimate. Since furthermore the total number of studies is low ($n = 3$) and the outlier had the highest power, we report the model including the outlier.

Model comparison

Fixed-Effects with Moderators Model ($k = 2$)

I^2 (residual heterogeneity / unaccounted variability): 0.00%

H^2 (unaccounted variability / sampling variability): 1.00

Test for Residual Heterogeneity:

$QE(df = 0) = 0.000$, $p\text{-val} = 1.000$

Test of Moderators (coefficient 2):

$QM(df = 1) = 0.749$, $p\text{-val} = 0.387$

Model Results:

	estimate	se	zval	pval	ci.lb	ci.ub	
intrcpt	0.120	0.051	2.338	0.019	0.019	0.221	*
outlierswithout	0.143	0.165	0.865	0.387	-0.181	0.466	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Funnelplot with eggers and beggs test

Due to small sample size of studies, eggers regression test cannot be interpreted.

```
> ##Beggs rank-correlation test  
> ranktest(model.re.hayrhini.rr)
```

Rank Correlation Test for Funnel Plot Asymmetry

Kendall's tau = 0.3333, p = 1.0000

Begg's test does not show deviation from funnel plot asymmetry.

However the funnelplot indicates a slight positive publication bias, so that the meta-analytic effect might be very slightly biased towards a bigger effect.

Random-effects model:

```
> model.re.hayrhini.rr
```

Random-Effects Model (k = 3; tau² estimator: REML)

tau² (estimated amount of total heterogeneity): 0 (SE = 0.0236)

tau (square root of estimated tau² value): 0

I² (total heterogeneity / total variability): 0.00%

H² (total variability / sampling variability): 1.00

Test for Heterogeneity:

Q(df = 2) = 0.9447, p-val = 0.6235

Model Results:

estimate	se	zval	pval	ci.lb	ci.ub	
0.1200	0.0513	2.3379	0.0194	0.0194	0.2206	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> predict(model.re.hayrhini.rr, transf=exp, digits=3)
```

pred	ci.lb	ci.ub	cr.lb	cr.ub
1.127	1.020	1.247	1.020	1.247

Supplementary File 6: Breakdown of data used for sensitivity analysis by study.

Author	year	study ID	study type	Antibiotic treatment measurement
Arrieta	2018	54	Prospective	Questionnaire
Benn	2002	42	Prospective	Questionnaire
Chu	2015	47	Retrospective	Interview
Dom	2013	18	Prospective	Interview
Fossum	2018	25	Retrospective	Medical database
Higgins	2020	23	Prospective	Medical database
Jedrychowski	2006	20	Prospective	Interview
Kallen	2013	9	Retrospective	Medical database
Kashanian	2017	53	Retrospective	Medical database
Lapin	2015	24	Prospective	Questionnaire
Liu	2019	56	Retrospective	Medical database
Loewen	2018	55	Retrospective	Medical database
Marell Hesla	2017	22	Prospective	Questionnaire
Martel	2009	44	Retrospective	Medical database
McKeever	2002	19	Retrospective	Questionnaire
Metsälä	2015	46	Retrospective	Questionnaire
Metzler	2019	21	Prospective	Interview
Mulder	2016	49	Retrospective	Medical database
Örtqvist	2014	27	Retrospective	Medical database
Örtqvist	2017	52	Prospective	Medical database
Popovic	2015	48	Prospective	Questionnaire
Rusconi	2007	43	Retrospective	Questionnaire
Stensballe	2013	45	Prospective	Medical database
Turi	2020	57	Retrospective	Medical database
Wu	2016	50	Retrospective	Medical database
Yoshida	2018	28	Retrospective	Medical database
Zhang	2017	51	Retrospective	Medical database

For antibiotic treatment measure: Questionnaire indicates an unsupervised questionnaire completed by participants. Interview indicates an interview performed by a medical professional (doctor, nurse, or midwife). Medical database indicates that data was obtained from official medical records.

Supplemental File 7: Sensitivity analysis for two factors: 1) Comparing prospective and retrospective study designs. 2) Comparing methods of how antibiotic exposure data was collected.

Sensitivity analysis: prospective vs. retrospective studies

Condition		RR prospective	95% CI prospective	RR retrospective	95% CI retrospective		zscore	pval
Atopic Sensitization	too few studies							
Food Allergy	too few studies							
Dermatitis	too few studies							
Allergic Rhinitis	too few studies							
Wheeze		1.47	0.75 - 2.9	1.64	0.51 - 5.21		0.278	0.781 ns
Asthma		1.34	1.31 - 1.38	1.27	1.03 - 1.55		1.843	0.065 ns

Sensitivity analysis: record of antibiotic exposure

Condition		RR interview	95% CI interview	RR questionnaire	95% CI questionnaire	RR database	95% CI database	statistic (z score, QM)	pval
Atopic Sensitization	too few studies								
Food Allergy	too few studies								
Dermatitis		1.58	0.85 - 1.58	1.4	1.21 - 1.61			-3.451	<0.001 ***
Allergic Rhinitis		1.26	0.74 - 2.16	1.16	1.00 - 1.35			-0.6522	0.513 ns
Wheeze		1.2	0.95 - 1.51	1.77	1.33 - 2.36	0.96	0.73 - 1.26	17.98	<0.001 ***
	pairwise, interview vs. questionnaire							2.100	0.036 *
	pairwise, interview vs. database							-1.031	0.302 ns
	pairwise, questionnaire vs database							2.648	0.008 **
Asthma		1.17	1.10 - 1.28	1.33	1.25- 1.41	1.18	1.00 - 1.41	103.891	<0.001 ***
	pairwise, interview vs. questionnaire							2.252	0.024 *
	pairwise, interview vs. database							0.100	0.920 ns
	pairwise, questionnaire vs database							1.242	0.241 ns

* p < 0.05, ** p < 0.01, *** p < 0.001, ns = not statistically significant.

