doi: 10.1111/cea.12756
Clinical & Experimental Allergy, **46**, 1214–1226

ORIGINAL ARTICLE **Epidemiology of Allergic Disease**

Antibiotic use during pregnancy and asthma in preschool children: the influence of confounding

B. Mulder[1], K. B. Pouwels[1], C. C. M. Schuiling-Veninga[1], H. J. Bos[1], T. W. de Vries[2], S. S. Jick[3,4] and E. Hak[1,5]

[1]Department of PharmacoEpidemiology & PharmacoEconomics, University Centre of Pharmacy, University of Groningen, Groningen, [2]Department of Pediatrics, Medical Center Leeuwarden, Leeuwarden, The Netherlands, [3]Department of Epidemiology, Boston University School of Public Health, [4]Boston Collaborative Drug Surveillance Program, Boston, MA, USA and [5]Department of Epidemiology, University Medical Centre Groningen, Groningen, The Netherlands

Clinical & Experimental Allergy

Summary

Background A recent study suggested that early-life intestinal microbiota may play an important role in the development of childhood asthma, indicating that antibiotics taken during early life or in late pregnancy may be associated with childhood asthma.

Objective This study aims to assess the association between prenatal antibiotic use and asthma in preschool children using data from the prescription database IADB.nl. To assess the influence of potential confounding, we conducted both a case–sibling and a case–control study and compared the results.

Methods We conducted a case–sibling study in which 1228 children with asthma were compared to 1228 siblings without asthma, using data from the prescription database IADB.nl. In addition, a case–control study was conducted. Asthma in preschool children was defined as ≥ 3 prescriptions for anti-asthma medication within a year before the fifth birthday. Conditional logistic regression was used to estimate crude and adjusted odds ratios (aORs).

Results In both the case–sibling and case–control analysis, the use of antibiotics in the third trimester of pregnancy was associated with an increased risk of asthma in preschool children (aOR 1.37; 95% CI 1.02–1.83 and aOR 1.40; 95% CI 1.15–1.47). Time-trend analyses showed that results were not influenced by a time trend in antibiotic exposure. A significant association between exposure to antibiotics in any trimester of pregnancy and the development of asthma in preschool children was observed in the case–control analysis only (aOR 1.46; 95% CI 1.34–1.59).

Conclusion Antibiotic use in the third trimester of pregnancy was associated with a small increased risk of asthma in preschool children. This association was robust to time-invariant confounding or exposure time trends, further supporting the important role for early-life intestinal microbiota in the development of childhood asthma.

Keywords antibiotics, asthma, case–sibling study, childhood, maternal–paternal comparison, pregnancy

Submitted 12 November 2015; revised 24 April 2016; accepted 29 April 2016

Correspondence:
B. Mulder, Department of PharmacoEpidemiology & PharmacoEconomics, University Centre of Pharmacy, University of Groningen, P.O. Box XB45, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands.
E-mail: b.mulder@rug.nl
Cite this as: B. Mulder, K. B. Pouwels, C. C. M. Schuiling-Veninga, H. J. Bos, T. W. De Vries, S. S. Jick and E. Hak, *Clinical & Experimental Allergy*, 2016 (46) 1214–1226.

Introduction

The prevalence of atopic diseases, like asthma, has increased dramatically in developed countries [1]. Latest evidence suggests that exposure to environmental factors and drugs during early life or pregnancy may play a role in this sudden increase in atopic disease development [2–12].

Most recent findings showed that a decrease in different bacterial genera may be important in the immune development of young children [13]. Although the immune development in young children is complex, this suggests that early-life microbial dysbiosis may be an important factor in the development of childhood asthma [13]. Antibiotics taken during pregnancy can result in the alteration of vaginal bacterial flora during birth [14, 15]. During birth, the fetus acquires microorganisms from the mother's vaginal flora, and the infants' intestinal flora will be established within the

first years of life [14, 15]. A disturbed maternal microflora has been proposed as a possible explanation for a disturbed infant intestinal flora [16, 17]. However, epidemiological studies that studied the relation between antibiotics during pregnancy show conflicting results. Several studies reported associations between antibiotic use during pregnancy and asthma development in children [4–10]. However, a recent study reported that the association between antibiotic use and childhood asthma was not restricted to the pregnancy period only, suggesting that antibiotic use is a marker of the mother's general propensity for infections [11]. However, they did not account for the correlation between antibiotic use before, during and after pregnancy. In addition, a Swedish case–sibling study stated that previous found associations can be explained by time-invariant confounders shared within families [12].

In the current study, we aimed to assess the association between antibiotic use in pregnancy and the development of asthma in preschool children, by applying a (time-invariant) confounding minimizing case–sibling design in primary analysis. We hypothesized that if an effect would be present, that this would be strongest in the third trimester, as antibiotic use during in late pregnancy likely has the strongest influence on the infants' intestinal flora.

To evaluate the influence of time-invariant confounding, results of the case–sibling analysis were compared with results obtained using a matched case–control design. In addition, we evaluated the influence of potential time trends in exposure frequencies in the case–sibling analysis.

Methods

Setting

Data for this study were collected from the IADB.nl database, a prescription database from the University of Groningen in the Netherlands. The IADB.nl contains prescription records of community pharmacies and covers a population of approximately 600 000 patients, including a mother–infant subset of approximately 40 000 children and their mothers, living in the northern part of the Netherlands. The database is representative of the Netherlands as a whole and is described in detail elsewhere [18, 19]. Over-the-counter medication and medication dispensed during hospitalization were not recorded in the database.

Study designs

In primary analysis, we applied a nested case–sibling study and in secondary analysis a nested matched case–control design to gain an indication about the potential influence of time-invariant confounding. In the case–sibling analysis, preschool children with asthma were compared with their own siblings without asthma, thereby minimizing (un)measured time-invariant confounding (i.e. covariates that have similar statistical distributions in both pregnancies, such as maternal genetic predisposition and other stable familial factors) [20, 21]. In addition, results of the case–sibling analysis were adjusted for potential time trends (see also Appendix A).

In the secondary case–control analysis, cases were not restricted to children with eligible siblings resulting in higher statistical power. Because exposure status was compared between children with asthma and randomly selected children without asthma, this design is per definition more vulnerable to potential confounding.

In another secondary analysis, a maternal–paternal comparison was performed, using the case–sibling subjects. We analysed paternal exposure to systemic antibiotics during pregnancy in the subset of children where paternal data were present as a potential measure for infections in the household, while adjusting for potential confounders [22].

Study population

The study population was restricted to all preschool children present in the database from birth until the age of 5 years. Children who were part of multiple births were excluded from the study population.

Case definitions

Children were defined as cases if they had received at least three prescriptions for asthma medication (Anatomical Therapeutic Chemical (ATC) group R03) within a 12-month period before the fifth birthday according to the primary care drug treatment guidelines [23]. In the case–sibling analysis, a case was eligible for inclusion if there was at least one available control, that is one sibling without any prescriptions from the ATC group R03 before his or her fifth birthday. Sibling controls were matched to cases with a 1 : 1 ratio, thereby preventing the introduction of bias due to exposure dependency between control siblings [24]. Therefore, if multiple sibling controls were available for a case, one eligible sibling control was randomly selected and matched to the case.

In the case–control analyses, cases and controls were selected in the same manner as in the case–sibling analysis. However, in the case–control analysis, cases were eligible for inclusion, irrespective of their sibling status. Controls in the case–control analyses were defined as all children that had not received prescriptions for asthma medication before their fifth birthday. Controls were matched to cases on birth date (± 3 years) with a 6 : 1 ratio. We matched cases to

controls in a 6 : 1 ratio since this was the highest number of controls we could match to one case. We chose the maximum number of controls to pair to each case because of power reasons.

Exposure to antibiotics in and after pregnancy

In the Netherlands, antibiotics are prescription drugs and no over-the-counter sale is allowed. Maternal exposure was defined as at least a 1 day supply of systemic antibiotics (ATC group J01) during pregnancy. Since the actual conception date was unknown, pregnancy was defined as the birth date of the child minus 273 days. Subtypes of antibiotics were stratified into beta-lactam penicillins (ATC group J01C), sulphonamides (ATC group J01E), macrolides (ATC group J01F), nitrofurantoin (ATC group J01XE) and other antibiotics (remaining subgroups of ATC J01). Exposure was further stratified according to trimester of exposure, consisting of 13 weeks each (first 1–91 days; second: 92–189 days; third 190–273 days), thereby adjusting for exposure in the other trimesters. This was done because, based on the hypothesized mechanism [13], an increased risk would be mainly expected during the third trimester. Since only the child's birth date is known, the theoretical conception data were determined as the data of birth minus 273 weeks (39 weeks).

Covariates

Covariates that were considered for inclusion in the regression model were gender of the child and birth order [25–27]. We did not adjust for antibiotic use in the preschool children as early symptoms of undiagnosed asthma are often treated with antibiotics [7, 28]. Adjustment for antibiotic use in preschool children would consequently result in an underestimation of the true effect, if present, due to protopathic bias [7, 29, 30]. Maternal characteristics that are measured in the database and could potentially be confounders or correlated with confounders of the association between maternal antibiotic use and asthma in the offspring are as follows: age at delivery [31], the use of asthma medication (ATC group R03) [31], use of acid-suppressive drugs (ATC group A02B) [20, 21], use of antidepressant drugs (ATC group N06) [32], use of drugs indicated for allergic dermatitis (ATC group D07), use of drugs indicated for allergic rhinitis (ATC group R01AD, R01AC) and use of insulin (ATC group A10A) [31, 32] during pregnancy (defined as having at least a 1 day supply of the drug class).

Statistical analyses

We used conditional logistic regression to obtain the odds ratios (OR) and their corresponding 95% confidence intervals (95% CIs) in both the case–sibling and matched case–control analyses. In multivariate analyses, ORs were adjusted for variables that were significantly associated ($P < 0.05$) with both the outcome (asthma of the child) and the exposure (antibiotic use during pregnancy) in univariate analyses. Each remaining covariate was further assessed for possible confounding by adding it to the multivariate regression to evaluate whether it resulted in a more than 10% change in the effect estimate. The distributions of covariates were measured with paired tests. In addition, ORs for the development of asthma after exposure to antibiotics anytime during pregnancy and during different trimesters of pregnancy were adjusted for potential time trends in the case–sibling analysis (see Appendix A).

Sensitivity analyses

We performed several sensitivity analyses to evaluate the robustness of our results. It should be taken into account that we had limited power to perform some of these sensitivity analyses. First, to further reduce the likelihood that asthma medication had been used for transient wheezing episodes, we restricted the case–sibling pairs of the main case-crossover analysis to the sibling pairs of which the case received at least one prescription between the age of 3 and 5 years. Second, to evaluate whether associations were restricted to the pregnancy period only, we assessed the effect of maternal antibiotic use in the 13 weeks after delivery while adjusting for antibiotic use during pregnancy. Third, although the genetic predisposition for the development of asthma is equal between cases and controls in the case–sibling analysis, the allergic status of the mother can be different [33]. Hence, we also performed a subanalysis on a subgroup of children whose mothers who did not use asthma medication during either pregnancy. Fourth, since data on smoking, respiratory infections and asthma status of the mother during pregnancy were not available in the IADB.nl, both the case–sibling and the case–control design cannot adjust for these potential time-varying confounders. Therefore, we used a simplified sensitivity analysis proposed by VanderWeele et al. [34] to assess the impact of unmeasured confounders on our main analysis of the case–sibling study. A detailed description of this method is available in appendix B. All analyses were conducted using the IBM SPSS Statistics 20 version.

Results

Case–sibling analysis

In the case–sibling analysis, 1228 children with asthma were included as cases and 1228 siblings without asthma

Table 1. Distribution of covariates between cases and controls in case–sibling and case–control analysis

	Case–sibling analysis			Case–control analysis		
	n (%) Cases (*N* = 1228)	*n* (%) Controls (*N* = 1228)	*P*-value	*n* (%) Cases (*N* = 3754)	*n* (%) Controls (*N* = 22 523)	*P*-value
Child characteristics						
Male gender	815 (66.4)	565 (46.0)	< 0.001	2372 (63.2)	10 808 (48.0)	< 0.001
Mean age at index date asthma (years)	1.63			1.65		
Birth order: first born	444 (36.2)	784 (63.8)	< 0.001	NA	NA	
Use of ABs before index date	939 (76.5)	631 (51.4)	< 0.001	2932 (78.1)	9564 (42.5)	< 0.001
Mother characteristics						
Mean age (years)	29.8	29.0	< 0.001	30.3	30.6	< 0.001
Use of medication for atopic diseases during pregnancy	314 (25.6)	320 (26.1)	0.774	1069 (28.5)	4410 (19.6)	< 0.001
Asthma medication	73 (5.9)	82 (6.7)	0.321	301 (8.0)	698 (3.1)	< 0.001
Drugs for atopic dermatitis	123 (10.0)	110 (9.0)	0.362	405 (10.8)	1936 (8.6)	< 0.001
Drugs for rhinitis	171 (13.9)	181 (14.7)	0.535	595 (15.8)	2342 (10.4)	< 0.001
Use of acid-suppressive drugs during pregnancy (ATC A02B)	51 (4.2)	26 (2.1)	0.001	196 (5.2)	627 (2.8)	< 0.001
Use of insulin during pregnancy	10 (0.8)	8 (0.7)	0.687	27 (0.7)	157 (0.7)	0.879
Use of antidepressants during pregnancy	30 (2.4)	27 (2.2)	0.749	101 (2.7)	439 (1.9)	0.003
Father characteristics						
Data present (*N*)	903	835		2544	13 626	
Use of medication for atopic diseases during pregnancy	223 (24.7)	189 (22.6)	0.176	738 (29.0)	2957 (21.7)	< 0.001
Asthma medication	74 (8.2)	49 (5.9)	0.012	251 (9.9)	595 (4.4)	< 0.001
Drugs for atopic dermatitis	99 (11.0)	97 (11.6)	1.000	312 (12.3)	1427 (10.5)	0.025
Drugs for rhinitis	94 (10.4)	80 (9.6)	0.494	349 (13.7)	1438 (10.6)	< 0.001

were included as controls. Analysis of the distribution of covariates between cases and controls showed that case siblings were more often male and were more often born before than after their control siblings. Mothers were older at delivery and used more acid-suppressive drugs during the pregnancy that resulted in a case (Table 1). Children who were exposed to antibiotics during pregnancy were more often born before their sibling. Mothers who used antibiotics during pregnancy also received anti-asthma medication and acid-suppressive drugs more often during that pregnancy (Appendix C).

Of the cases, 24.5% were exposed to antibiotics during pregnancy, while 22.3% were exposed in the control group. The use of antibiotics during pregnancy in general was not associated with a significant increase in risk for the development of asthma in preschool children (aOR 1.06; 95% CI 0.85–1.32). Stratification on trimester of exposure was associated with a small but statistically significant increase in risk for the development of asthma in preschool children (aOR 1.37; 95% CI 1.02–1.83) among those exposed in the third trimester, independent of exposure in the other trimesters. In contrast, the associated risk of asthma development among those with exposure in the first trimester was significantly decreased (aOR 0.70; 95% CI 0.50–0.98). This decreased association explains the difference between the effects in the third trimester compared to the null effect of exposure at any time during pregnancy. Subsequent analyses of first trimester exposure found that the decreased association was present primarily in tetracycline exposure between cases (n = 8; 0.7%) and controls (n = 15; 1.2%)). There were no significant increases in the associated risk for the development of childhood asthma after stratification on the subtypes penicillins, sulphonamides, macrolides and nitrofurantoin (Table 2).

Influence of potential time trends

The case–sibling analysis appeared not to be influenced by a time-trend in exposure frequency, since the time-trend adjusted odds ratio was similar to the original case–sibling odds ratio (1.06; 95% CI 0.81–1.36 vs. 1.06; 95% CI 0.85–1.32) (Table 2). The time-trend adjusted odds ratios for antibiotic use in the different trimesters of pregnancy were also similar to the odds ratios of the original case–sibling analysis (Table 2).

Sensitivity analyses for case–sibling design

Several sensitivity analyses were performed to assess the robustness of the results of the case–sibling analysis. When we restricted cases to children who received anti-asthma treatment at some time after the age of 3, the aOR after exposure in third trimester increased to 1.51 (95% CI 1.07–2.14). When we assessed the effect

Table 2. Unadjusted and adjusted conditional odds ratios for the development of asthma in preschool children after exposure to antibiotic drugs during pregnancy in the case–sibling and case-time-control analysis

	Case–sibling analysis				Time-trend analysis	
	Cases (N = 1228) N (%)	Controls (N = 1228) N (%)	Unadjusted conditional OR (95% CI)	Adjusted conditional OR (95% CI)*	Time-trend OR (95% CI)*	Conditional OR adjusted for potential time trends (95% CI)*
Exposure to any antibiotic during pregnancy	301 (24.5)	274 (22.3)	1.16 (0.94–1.42)	1.06 (0.85–1.32)	1.00 (0.87–1.15)	1.06 (0.81–1.36)
Trimester of exposure						
Trimester 1	89 (7.2)	100 (8.1)	0.88 (0.65–1.19)	0.70 (0.50–0.98)†	1.00 (0.81–1.25)†	0.70 (0.45–1.03)†
Trimester 2	128 (10.4)	112 (9.1)	1.17 (0.89–1.54)	1.25 (0.92–1.69)†	1.01 (0.82–1.24)†	1.24 (0.85–1.79)†
Trimester 3	161 (13.1)	118 (9.6)	1.49 (1.14–1.95)	1.37 (1.02–1.83)†	0.91 (0.75–1.09)†	**1.51 (1.05–2.13)**†
Subgroup of antibiotics‡						
Beta-lactam penicillins	242 (19.7)	208 (16.9)	1.23 (0.99–1.53)	1.13 (0.90–1.43)		
Sulphonamides and trimetoprim	34 (2.8)	33 (2.7)	1.03 (0.63–1.70)	1.06 (0.62–1.82)		
Macrolides	14 (1.1)	14 (1.1)	1.00 (0.45–2.23)	0.96 (0.41–2.28)		
Nitrofurantoin	45 (3.7)	42 (3.4)	0.93 (0.59–1.45)	0.93 (0.57–1.51)		
Other	15 (1.2)	23 (1.9)	0.64 (0.33–1.24)	0.63 (0.31–1.30)		

*Adjusted for gender, birth order, maternal age at delivery and maternal use of acid-suppressive drugs during pregnancy.
†Odds ratios were additionally adjusted for exposure in other trimesters.
‡Women can be exposed to more than one subgroup of antibiotics.

of maternal antibiotic use in the 13 weeks after delivery, the aOR was 1.03 (95% CI 0.80–1.32). When the analysis of the association between antibiotic use in third trimester and the development of asthma in the offspring was restricted to children from mothers who were not using any asthma medication during pregnancy, the aOR was attenuated to 1.22 (95% CI 0.90–1.67). Appendix B shows the effects of a hypothetical unmeasured confounder in the case–sibling analysis. A binary confounder, with a prevalence of 10% among unexposed, that could have biased the third-trimester results from 1.00 to 1.37 should have a strong association with the exposure and/or the outcome (Appendix B), thereby exceeding the effects of any measured confounder. For this scenario, if the prevalence of the binary confounder is two times higher than the prevalence among unexposed women, the OR between this confounder and asthma in the offspring should be 6.9 to bias the effect estimate from 1.00 to 1.37. However, for this scenario, a confounder with an OR of 1.21 could bias the results from statistically non-significant to statistically significant.

Case–control analysis

In the case–control analysis, we included 3754 children with asthma and 22 523 children without asthma (controls). Univariate analysis identified gender, maternal age at delivery, maternal use of acid-suppressive drugs, antidepressant drugs and drugs for atopic disease during pregnancy as potential confounders (Appendix C). 25.4% of cases and 17.8% of controls were exposed to antibiotics during pregnancy. The use of antibiotics during pregnancy in general yielded a small but significant association with childhood asthma (aOR 1.45; 95% CI 1.33–1.58). Associations were even stronger when children were exposed to higher doses (DDDs) during pregnancy (aOR 1.90; 95% CI 1.62–2.22). After adjusting for antibiotic exposure during other trimesters, the association with childhood asthma was strongest in the third trimester, although confidence intervals overlap (Table 3). Stratification on the different subtypes of antibiotics yielded significant associations for the development of childhood asthma only for the penicillin, sulphonamide and macrolide subtypes (Table 3). Exposure to nitrofurantoin was not statistically significantly associated with the development of asthma in preschool children (aOR 1.10; 95% CI 0.91–1.33).

Maternal–paternal comparison

The analysis of paternal exposure to antibiotics during the third trimester of pregnancy as proxy for infections in the household and other factors shared by both parents showed no significant association with an increased risk of asthma (aOR 1.11; 95% CI 0.70–1.74). Effect estimates were almost identical for maternal exposure in the third trimester when restricted to this subset or the main analysis (aOR 1.39 vs. aOR 1.37).

Discussion

Exposure to antibiotics during the second and third trimester of pregnancy was associated with a small

Table 3. Unadjusted and adjusted conditional odds ratios for the development of asthma in preschool children after exposure to antibiotic drugs during pregnancy in the case–control analysis

	Case–control analysis			
	Cases (N = 3754) N (%)	Controls (N = 22 524) N (%)	Unadjusted conditional OR (95% CI)	Adjusted conditional OR (95% CI)*
Exposure to any antibiotic during pregnancy	952 (25.4)	4017 (17.8)	1.57 (1.45–1.71)	1.45 (1.33–1.58)
Trimester of exposure				
Trimester 1	336 (9.0)	1401 (6.2)	1.49 (1.31–1.68)	1.23 (1.08–1.41)†
Trimester 2	404 (10.8)	1603 (7.1)	1.57 (1.40–1.77)	1.30 (1.15–1.47)†
Trimester 3	477 (12.7)	1880 (8.3)	1.61 (1.44–1.79)	1.40 (1.25–1.57)†
Subgroup of antibiotics‡				
Beta-lactam penicillins	773 (20.6)	3159 (14.0)	1.60 (1.46–1.74)	1.48 (1.35–1.62)
Sulphonamides and trimetoprim	105 (2.8)	393 (1.7)	1.63 (1.31–2.03)	1.49 (1.19–1.87)
Macrolides	50 (1.3)	190 (0.8)	1.60 (1.17–2.17)	1.37 (1.00–1.87)
Nitrofurantoin	140 (3.7)	710 (3.2)	1.19 (0.99–1.44)	1.10 (0.91–1.33)
Other	56 (1.5)	250 (1.1)	1.35 (1.01–1.81)	1.20 (0.89–1.61)

*Adjusted for gender, maternal age at delivery, maternal use of acid-suppressive drugs, antidepressant drugs and drugs for atopic diseases during pregnancy.

†Odds ratios were additionally adjusted for exposure in other trimesters.

‡Women can be exposed to more than one subgroup of antibiotics.

increased risk of asthma in preschool children in both our case–sibling and our case–control analyses. The similar estimates and confidence intervals of the case–sibling and case–control study for the second and third trimester, suggest that the influence of potential time-invariant confounding is minimal. Sensitivity analyses showed that results of the case–sibling analysis were restricted to the pregnancy period only and were not influenced by a time trend in antibiotic exposure.

Interpretation and comparison with literature

Exposure to antibiotics during any trimester of pregnancy was associated with a small increased risk of asthma in preschool children in our case–control analysis. In the case–sibling analysis, this association for exposure anytime during the whole pregnancy was considerably reduced, suggesting that confounding by time-invariant factors may have played an important role. However, when stratified by trimester, both designs found an association between exposure to antibiotics in the third trimester and an increased risk of asthma in preschool children. The similar estimates and confidence intervals of the case–sibling and case–control study for the second and third trimester, suggest that the influence of potential time-invariant confounding is minimal. Given these very similar effect estimates, the discrepancy between both designs after exposure anytime during pregnancy is almost solely caused by the significant decreased association found in the first trimester of the case–sibling design. The reason for the discrepant findings between trimesters should be explored. The decreased association in the first trimester of pregnancy may be caused by a biological mechanism. The highest association found in the third trimester is in agreement with the hypothesis that antibiotic drug use during pregnancy alters the vaginal bacterial flora at delivery. Different studies indicate that antibiotic use can have long-term altering effects on the vaginal bacterial flora [5, 16], explaining the slight increased association found after antibiotic drug use in the second trimester. In contrast to the case–control analysis, our case–sibling analysis did find a decreased association for use in the first trimester. Subsequent analyses found that the decreased association in the case–sibling analysis was present primarily in tetracycline exposure between cases (n = 8; 0.7%) and controls (n = 15; 1.2%). Recent studies showed that tetracyclines may lower IgE levels [35–38]. A reduction in cord blood IgE levels – a predictor of allergic disorders in children, especially among children with a family history of allergic diseases – is likely more relevant in mothers with elevated IgE levels and atopic diseases [39]. This may explain the difference between both designs, because all mothers in the case–sibling gave birth to a child with asthma, and most mothers of controls in the case–control did not. Similarly, confounding by allergic status of the mother may explain why another case–control study did not find an association between prenatal exposure to tetracyclines and childhood asthma [10].

Several studies reported associations between antibiotic use during pregnancy and asthma development in children similar to the associations of the current study

[4–10]. However, other studies have proposed that the associations between antibiotic use during pregnancy and asthma development in children can be largely explained by time-invariant confounding or by mother's general propensity of infections. While we did find a difference between trimesters, another case–sibling study did not [12]. This discrepancy may be explained by different analytical choices. Örtqvist et al. included multiple control siblings per case, which may have introduced bias due to exposure dependencies between control siblings [26]. Moreover, in the current study, we took into account that antibiotic exposure in one trimester likely correlates with such exposure in other trimesters. In addition, the odds ratios were adjusted for a different subset of potential time-variant confounders and there may have been different antibiotic prescription patterns between the three studies, which may explain the discrepant results. Alternatively, given the limited sample size for the stratified analyses in comparison with the other study, our results for the different trimester may be chance findings. In addition, a recent study of Stockholm et al. [11] reported that the association between antibiotic use and childhood asthma was not restricted to the pregnancy period only, suggesting that antibiotic use is a marker of the mother's general propensity for infections. However, it was not taken into account that antibiotic exposures before, during and after pregnancy are likely correlated [11]. In a sensitivity analysis, they estimated the effect of exposure before and after pregnancy in women that were not exposed during pregnancy, but results were significantly lower for the period from birth to 40 weeks post-partum (1.42 vs. 1.18). This suggests that this population was not comparable to the population in the main analysis or that the effect post-partum was partly explained by exposure during pregnancy.

Strengths and limitations

A major strength of the present study is the replication of findings in the two study designs. Application of the case–sibling study enabled us to minimize the potential influence of time-invariant confounders that are potential strong risk factors for asthma development. Comparing the results of the case–sibling with the case–control study allowed us to gain insight into the influence of confounding. In addition, this is the first study that evaluated the presence of a time-trend bias in a case–sibling design and corrected for that trend. We also took into account that exposure to antibiotics at different time points is likely correlated within the same mother.

Another strength of our study is that data were obtained from the prescription database IADB.nl [18]. Validation of the identification of mother–infant pairs showed high accuracy; hence, potential information bias was minimal. Since we made use of a prescription database, recall bias with respect to maternal medication use during pregnancy was not present. The study into prenatal medication use and the development of asthma in children is complex. This study merely covers a relatively small piece of this discussion; however, the novel approach on confounders and time trends adds value for future studies. This study also has potential limitations. First, although we minimized potential confounding influences by design, we cannot rule out the possibility of some unmeasured time-variant confounding in the case–sibling analysis, such as smoking, infections and allergic state of the mother [34, 39].

If smoking did confound our estimate, a stronger association would also be expected for exposure to antibiotics in the 3 months after delivery since smoking would increase the risk of infection regardless of pregnancy. It is unlikely that women smoke during the pregnancy and stop immediately after the child is born. However, no increased association was observed after post-natal use of antibiotics, indicating that the results are not confounded by smoking. Moreover, almost identical results were obtained in the case–sibling study of Örtqvist et al. before and after adjustment for maternal smoking, indicating that this is not an important confounder for this association using a sibling design.

Several studies evaluated the possibility of confounding by respiratory infections by investigating whether specific groups of antibiotics were associated with asthma [8, 10, 12]. The only other case–sibling study that investigated the relation between prenatal exposure to antibiotics and asthma development in children showed no differences in the child's asthma risk if antibiotics were used for urinary tract infections or respiratory tract infections [12]. This would indicate that the association between prenatal antibiotic use and the development of asthma is not confounded by respiratory tract infections. Amoxicillin is in those studies often considered as an 'airway antibiotic'; however, at least in the Dutch situation, this would be an inadequate categorization as amoxicillin was during the study period the first-choice treatment for urinary tract infections in pregnant women. In our study, in contrast to, for example, penicillin, exposure to nitrofurantoin showed no association with the child's asthma risk. Given the limited effect of nitrofurantoin on the vaginal flora and *Lactobacillus* colonization [40], this further supports the hypothesis that a reduced microbial exposure during delivery can predispose the child to allergic diseases like asthma. However, since penicillins, such as amoxicillin, are also indicated for respiratory infections, it may also indicate that respiratory infections do confound the association and urinary tract infections do not. Since data on types of infections were not

available, we performed a maternal–paternal comparison with data from the case–sibling analysis. Paternal exposure to antibiotics was used as a proxy for infections in the household and other factors shared by both parents that may change between pregnancies. Paternal exposure did not significantly increase the associated risk of asthma in the child and the point estimate was also lower for paternal than for maternal exposure (aOR 1.11 vs. 1.37). However, since pathogens will not always be transmitted to the mother, a lower effect estimate would also be expected *a priori* for paternal exposure. Thus, we cannot exclude indication bias due to respiratory infections.

The allergic state of the mother, or disease severity, could also change between pregnancies and be associated with the child's asthma risk. When our analysis was restricted to a subgroup of children whose mothers had not used asthma medication during either pregnancy, the associated child's asthma risk was attenuated but antibiotic use during third trimester was still associated with a 22% increase in the associated child's asthma risk.

Since we could not rule out potential confounding by time-varying disease severity and (respiratory) infections, we performed sensitivity analyses to assess how strong such an unmeasured confounder should be to account for the observed association in the third trimester. These analyses showed that potential unmeasured time-variant confounders should have a much stronger association with the outcome and exposure than measured confounders to fully explain our findings. However, given the relatively low power of our case–sibling study, a weaker unmeasured confounder could render our results statistically non-significant.

Since we used the case–sibling design, children that were included in our analysis are per definition not only children. The results from this study only provide information about the association in families with at least two children and results may not be generalized to exposures in single child families.

The IADB.nl contains records of dispensed prescriptions of participating pharmacies and not the actual use of medication. It is possible that some women did not take all of the medication which may have led to small amount of exposure misclassification and an overestimation of actual use. In addition, pregnancy was a standardized at 273 days preceding the date of birth, which also could have led to exposure misclassification. This could have led to over- as well as underestimation of actual use of antibiotic drugs during each trimester of pregnancy.

Since the IADB.nl contains only records of dispensed prescriptions, children that did not receive any prescriptions before the age of 5 years are not included in the database. However, a previously performed validation study showed that approximately 80% of the children aged 2 years had received at least one prescription and could be included in the database. [19] In the current study, a minimal follow up of 5 years was ensured for both cases and controls. Therefore, we do not believe that this could have influenced our results.

In addition, the database lacks information regarding the use of over-the-counter medication. Previous literature has shown that prenatal exposure to paracetamol may be a risk factor for the development of childhood asthma. However, we were not able to control for this. The database lacks information about indications; therefore, case status was determined based on dispensed prescriptions for anti-asthma medications [26]. Using prescription data to identify children with asthma is difficult since asthma medication is also used to treat childhood wheeze that is often transient. To ensure high specificity of case selection, cases were required to receive at least three prescriptions for anti-asthma medication within a 12-month period. Because of this specific inclusion of cases, some children with mild symptoms or untreated asthma may have been missed. However, a recent study of our group in the same source population showed that still 49% of preschool children with an asthma diagnosis could be identified (positive predictive value 0.77) when the same medication proxy as in this study was used [41]. Given these accuracy measures, this would likely not have materially influenced the results of our study. However, it should be noted that no distinction could be made between allergic vs. non-allergic asthma.

Fifth, the database does not include information on the delivery method. Caesarean sections are associated with an increased risk of childhood asthma. Since rates of Caesarean sections are low in the Netherlands (1.0–5.5%), this would not have a substantial influence [26]. Since the actual pregnancy duration is unknown in the IADB.nl database, we were not able to adjust for prematurity. In addition, the database lacks information on the dispensation of in-hospital and thereby intrapartum antibiotic use, including antibiotics prescribed for neonatal group B streptococcal disease during labour [17, 42]. A previous study, however, has shown that the use of intrapartum antibiotic prophylaxis is 5.9% in the hospital setting in the Netherlands. However, this is likely an overestimation, because it is estimated that around 20–30% of births in the Netherlands occur at home. [43].

In conclusion, exposure to antibiotics in the third trimester of pregnancy appeared to be associated with a small increased risk of asthma in preschool children. This association did not appear to be influenced by time-invariant confounders or time trends in antibiotic exposure. More studies are now warranted to focus on whether there is indeed a difference for the different

trimesters and on the potential mechanisms underlying the associations.

Statement of prior postings and presentations

This research article was presented orally at the International Conference of the European Respiratory Society in Munich on 7 September 2014 and awarded 'The best abstract competition award 2014'. In addition, this research was presented orally at the International Conference on Pharmacoepidemiology & Therapeutic Risk management from 24 to 27 October 2014 in Taipei, Taiwan.

Competing interests

All authors have completed the conflict of interest form and declare that they had support from the Department of Pharmacy, University Groningen, for the submitted work; no financial relationships with any organization that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval

Ethical approval for observational studies with anonymized data from IADB.nl is waived in the Netherlands. The University Groningen IADB.nl prescription database and stores data according to the Dutch law on privacy.

Funding

This study was supported by the Department of Pharmacy, University Groningen. The funder had no role in the study design; collection, analysis and interpretation of data; writing of the report; or the decision to submit the paper for publication. The corresponding author had full access to all data and the final responsibility for the decision to submit for publication.

References

1 Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2009; **64**:476–83.

2 Henderson AJ, Warner JO. Fetal origins of asthma. *Semin Fetal Neonatal Med* 2012; **17**:82–91.

3 Prescott SL, Clifton V. Asthma and pregnancy: emerging evidence of epigenetic interactions *in utero*. *Curr Opin Allergy Clin Immunol* 2009; **9**:417–26.

4 Benn CS, Thorsen P, Jensen JS *et al*. Maternal vaginal microflora during pregnancy and the risk of asthma hospitalization and use of antiasthma medication in early childhood. *J Allergy Clin Immunol* 2002; **110**:72–7.

5 McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: a birth cohort study using the West Midlands General Practice Database. *Am J Respir Crit Care Med* 2002; **166**:827–32.

6 Rusconi F, Galassi C, Forastiere F *et al*. Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. *Am J Respir Crit Care Med* 2007; **175**:16–21.

7 Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. *Pediatrics* 2011; **127**:1125–38.

8 Stensballe LG, Simonsen J, Jensen SM, Bonnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr* 2013; **162**:832–8.

9 Källén B, Finnström O, Nygren KG. Otterblad Olausson P. Maternal drug use during pregnancy and asthma risk among children. *Pediatr Allergy Immunol* 2013; **24**:28–32.

10 Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin Exp Allergy* 2015; **45**:137–45.

11 Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med* 2014; **2**:631–7.

12 Örtqvist AK, Lundholm C, Kieler H *et al*. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. *BMJ* 2014; **28**: g6979.

13 Arrieta MC, Stiemsma LT, Dimitriu PA *et al*. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med* 2015; **7**:307ra152.

14 Stokholm J, Schjørring S, Eskildsen CE *et al*. Antibiotic use during pregnancy alters the commensal vaginal microbiota. *Clin Microbiol Infect* 2014; **20**:629–35.

15 Prokopakis E, Vardouniotis A, Kawauchi H *et al*. The pathophysiology of the hygiene hypothesis. *Int J Pediatr Otorhinolaryngol* 2013; **77**:1065–71.

16 Kuo CH, Kuo HF, Huang CH, Yang SN, Lee MS, Hung CH. Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective. 2013 Oct; **46**(5):320–9.

17 Keski-Nisula L, Kyynäräinen HR, Kärkkäinen U, Karhukorpi J, Heinonen S, Pekkanen J. Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth. *Acta Paediatr* 2013; **102**:480–5.

18 Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LT, Postma MJ. The population-based prescription database IADB.nl: its development, usefulness in outcome research and challenges. *Expert Rev Pharmacoecon Outcomes Res* 2013; **13**:285–92.

19 Schirm E, Tobi H, de Jong-van den Berg LT. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn toddlers. *J Clin Epidemiol* 2004; **57**:737–41.

20 Mulder B, Schuiling-Veninga CC, Bos JH, de Vries TW, Hak E. Acid-suppressive drug use in pregnancy and the toddler's asthma risk: a crossover,

case-control study. *J Allergy Clin Immunol* 2013; **132**:1438–40.
21 Hak E, Mulder B, Schuiling-Veninga CC, de Vries TW, Jick SS. Use of acid-suppressive drugs in pregnancy and the risk of childhood asthma: bidirectional crossover study using the general practice research database. *Drug Saf* 2013; **36**:1097–104.
22 Smith GD. Assessing Intrauterine Influences on Offspring Health Outcomes: Can Epidemiological Studies Yield Robust Findings? *Basic Clin Pharmacol Toxicol* 2008; **102**:245–56.
23 NHG (Dutch General Practitioner Guidelines). https://www.nhg.org/standaarden/volledig/nhgstandaard-astma-bij-kinderen. Last accessed 8 August 2013.
24 Vines SK, Farrington CP. Within-subject exposure dependency in case-crossover studies. *Stat Med* 2001; **20**:3039–49.
25 Kawada T. Prevalence of asthma and atopic dermatitis in children with special emphasis on birth order. *Pediatr Allergy Immunol* 2012; **23**:795.
26 Offerhaus PM, de Jonge A, van der Palde Bruin KM, Hukkelhoven CW, Scheepers PL, Lagro-Janssen AL. Change in primary midwife-led care in the Netherlands in 2000–2008: a descriptive study of caesarean sections and other interventions among 789,795 low risk births. *Midwifery* 2014; **30**:560–6.
27 Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. *Clin Exp Allergy* 1993; **23**:941–8.
28 Koster ES, Van der Ent CK, Uiterwaal CS, Verheij TJ, Raaijmakers JA, Maitland-van der Zee AH. Asthma medication use in infancy: determinants related to prescription of drug therapy. *Fam Pract* 2011; **28**:377–84.
29 Kummeling I, Thijs C. Reverse causation and confounding-by-indication: do they or do they not explain the association between childhood antibiotic treatment and subsequent development of respiratory illness? *Clin Exp Allergy* 2008; **38**:1249–51.
30 Semic-Jusufagic A, Belgrave D, Pickles A, *et al*. Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q21: a population-based birth cohort study. *Lancet Respir Med* 2014; **2**: 621–30.
31 Bracken MB, Belanger K, Cookson WO, Triche E, Christiani DC, Leaderer BP. Genetic and perinatal risk factors for asthma onset and severity: a review and theoretical analysis. *Epidemiol Rev* 2002; **24**:176–89.
32 Ter Horst PG, Bos HJ, de Jong-van de Berg LT, Wilffert B. *In utero* exposure to antidepressants and the use of drugs for pulmonary diseases in children. *Eur J Clin Pharmacol* 2013; **69**:541–7.
33 Martel MJ, Rey E, Beauchesne MF *et al*. Control and severity of asthma during pregnancy are associated with asthma incidence in offspring: two-stage case-control study. *Eur Respir J* 2009; **34**:579–87.
34 Vanderweele TJ, Arah OA. Bias formulas for sensitivity analysis of unmeasured confounding for general outcomes, treatments and confounders. *Epidemiology* 2011; **22**:42–52.
35 Dzhindzhikhashvili MS, Joks R, Smith-Norowitz T *et al*. Doxycycline suppresses Chlamydia pneumoniae-mediated increases in ongoing immunoglobulin E and interleukin-4 responses by peripheral blood mononuclear cells of patients with allergic asthma. *J Antimicrob Chemother* 2013; **68**: 2363–8.
36 Joks R, Smith-Norowitz T, Nowakowski M, Bluth MH, Durkin HG. Tetracycline-mediated IgE isotype-specific suppression of ongoing human and murine IgE responses *in vivo* and murine memory IgE responses induced *in vitro*. *Int Immunol* 2010; **22**:281–8.
37 Smith-Norowitz TA, Bluth MH, Drew H *et al*. Effect of minocycline and doxycycline on IgE responses. *Ann Allergy Asthma Immunol* 2002; **89**:172–9.
38 Su W, Wan Q, Han L *et al*. Doxycycline exerts multiple anti-allergy effects to attenuate murine allergic conjunctivitis and systemic anaphylaxis. *Biochem Pharmacol* 2014; **91**:359–68.
39 Scirica CV, Gold DR, Ryan L, *et al*. Predictors of cord blood IgE levels in children at risk for asthma and atopy. *J Allergy Clin Immunol* 2007; **119**:81–8.
40 Raz R, Colodner R, Rohana Y *et al*. Effectiveness of estriol-containing vaginal pessaries and nitrofurantoin macrocrystal therapy in the prevention of recurrent urinary tract infection of postmenopausal women. *Clin Infect Dis* 2003; **36**:1362–8.
41 Mulder B, Groenhof F, Kocabas LI *et al*. Identification of Dutch children diagnosed with atopic diseases using prescription data: a validation study. *Eur J Clin Pharmacol* 2016; **72**:73–82.
42 Trijbels-Smeulders M, de Jonge GA, Pasker-de Jong PC *et al*. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**:F271–6.
43 Christiaens W, Nieuwenhuijze MJ, de Vries R. Trends in the medicalisation of childbirth in Flanders and the Netherlands. *Midwifery* 2013; **29**: e1–8.

Appendix A Adjusting the case–sibling analysis for potential time trends

Potential time trends in the underlying cohort can influence results of the case–sibling study, especially since exposure periods were compared within the same person, that is exposure periods were as per definition always different periods in time [1, 2]. To control for potential time trends in exposure frequencies, we designed a method akin the case-time-control design [1]. In this design, we divided the odds ratio obtained with the case–sibling analysis with a so-called time-trend odds ratio. This time-trend odds ratio was obtained by comparing the frequencies of antibiotic use between two different pregnancies of the same woman that both resulted in a child without asthma (derived from the control group of the case–control analysis), see Fig. 1. In total, 3503 children without asthma and

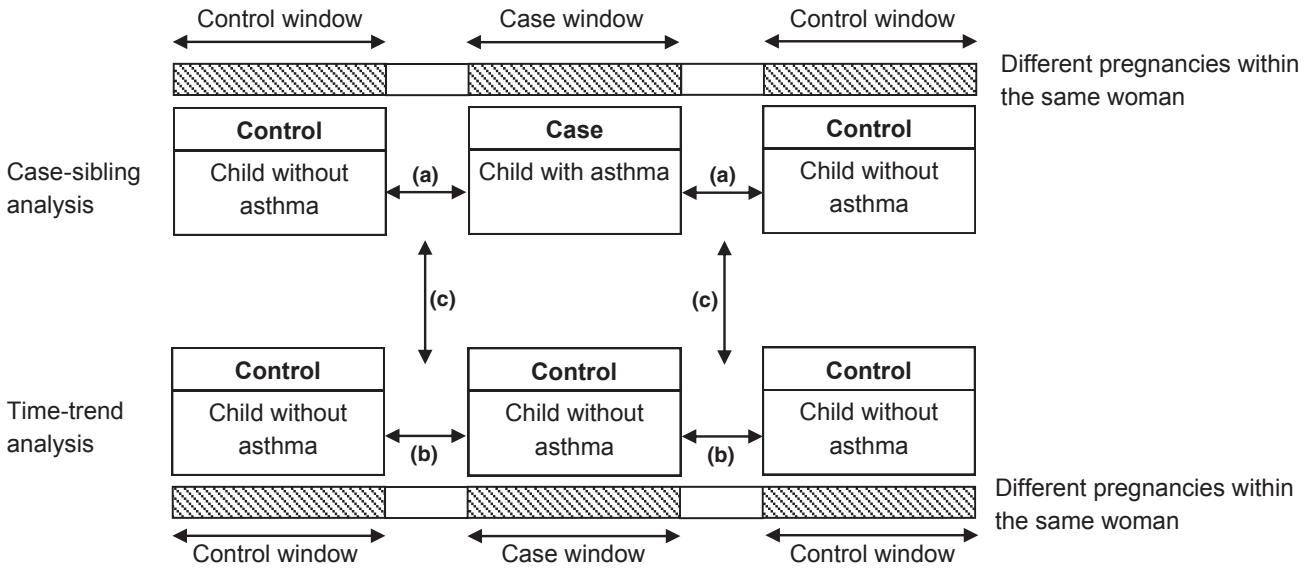

Fig. 1. Schematic overview of the case–sibling analysis and the adjustment for potential time trends. The case–sibling odds ratio after adjustments for potential time trends (c) is the case–sibling odds ratio (a) divided by the time-trend odds ratio (b).

3503 siblings also without asthma could be detected for the time-trend analysis.

We assigned the case and control windows in the time-trend analysis in such a way that this was similar to the distribution of the birth sequence in the case–sibling analysis. Since 66% of the cases in the case–sibling analysis were born after their control sibling (Table 1), 66% of the pregnancies that resembled case windows in the time-trend analysis occurred after the pregnancy that resembled a control window. By comparing exposure frequencies between different pregnancies within mothers that both resulted in children without asthma and by using a similar distribution in birth sequence as the case–sibling analysis, the odds ratio will be an estimate of the exposure frequency in the underlying cohort, a so-called time-trend odds ratio. By dividing the odds ratio obtained with the case–sibling analysis with a 'time-trend' odds ratio, results will be adjusted for potential time trends in the underlying cohort. Confidence intervals for the time-trend adjusted odds ratios were computed by bootstrapping.

References

1 Suissa S. The case-time-control design. *Epidemiology* 1995; **6**:248–53.

2 Hernández-Díaz S, Hernán MA, Meyer K, Werler MM, Mitchell AA. Case-crossover and case-time-control designs in birth defects epidemiology. *Am J Epidemiol* 2003; **158**:385–91.

Appendix B Sensitivity analysis unmeasured confounding

Since data on smoking, infections and IgE levels during pregnancy are not available in the IADB.nl, we used a simplified sensitivity analysis proposed by VanderWeele et al. to assess the impact of a binary unmeasured confounder or several unmeasured confounders combined on the association between third-trimester antibiotic use and childhood asthma in the case–sibling analysis. Assumptions made with this method are (i) that relationships between the outcome and the unmeasured confounder and between the exposure and unmeasured confounder are the same across different levels of measured confounders and (ii) that there is no three-way interaction between exposure, outcome and the unmeasured confounder.

For this study, we assumed that the prevalence of the binary unmeasured confounder ranges from 5% to 15% in the unexposed group and varied the association of this confounder with antibiotic use (OR*xu*) and asthma of the child (OR*yu*).

The following notations will be used in the formulas to obtain the bias term for the unmeasured confounder:

x, Binary treatment status/exposure; y, Binary outcome; u, Unobserved binary confounder; c, Observed confounders; p, Prevalence.

Formula for the prevalence of the unmeasured confounder among the exposed:

$$p(u|x=1) = \frac{\mathrm{OR}xu \times p(u|x=0)}{1 - p(u|x=0) + \mathrm{OR}xu \times p(u|x=0)}$$

Formula for the bias factor according to VanderWeele and Arah's approach

$$\text{bias} = \frac{1 + (\mathrm{OR}yu - 1)p(u|x=1)}{1 + (\mathrm{OR}yu - 1)p(u|x=0)}$$

Table A1 shows the influence of a binary unmeasured confounder that increases the risk of asthma in the offspring two times (OR*yu*). To bias the effect estimate from 1.00 to 1.37, the prevalence of this confounder among exposed pregnant women should be 5.1 times higher than the prevalence among unexposed women (assuming a prevalence of 0.10 in unexposed women). The prevalence of this confounder among exposed women would then be 51% vs. 10% among unexposed women.

If the prevalence of the binary confounder is two times higher than the prevalence among unexposed women (assuming a prevalence of 0.10 in unexposed women), the OR between this confounder and asthma in the offspring (OR*yu*) should be 6.9 to bias the effect estimate from 1.00 to 1.37.

Table A1. Sensitivity analysis (according to the approach of VanderWeele and Arah) of an unmeasured confounder on the child's asthma risk after exposure to antibiotics in third trimester of pregnancy using a OR*yu* of 2

aOR (95% CI)	$p(u\|x=0)$	OR*xu*	Bias	OR$yx \times cu$ (95% CI)
1.37 (1.02–1.83)	0.050	1.25	1.01	1.35 (1.01–1.81)
1.37 (1.02–1.83)	0.050	1.50	1.02	1.34 (1.00–1.79)
1.37 (1.02–1.83)	0.050	1.75	1.03	1.33 (0.99–1.77)
1.37 (1.02–1.83)	0.050	2.00	1.04	1.31 (0.98–1.75)
1.37 (1.02–1.83)	0.050	3.00	1.08	1.27 (0.94–1.69)
1.37 (1.02–1.83)	0.075	1.25	1.02	1.35 (1.00–1.80)
1.37 (1.02–1.83)	0.075	1.50	1.03	1.33 (0.99–1.77)
1.37 (1.02–1.83)	0.075	1.75	1.05	1.31 (0.98–1.75)
1.37 (1.02–1.83)	0.075	2.00	1.06	1.29 (0.96–1.73)
1.37 (1.02–1.83)	0.075	3.00	1.11	1.23 (0.92–1.65)
1.37 (1.02–1.83)	0.100	1.25	1.02	1.34 (1.00–1.79)
1.37 (1.02–1.83)	0.100	1.50	1.04	1.32 (0.98–1.76)
1.37 (1.02–1.83)	0.100	1.75	1.06	1.30 (0.96–1.72)
1.37 (1.02–1.83)	0.100	2.00	1.07	1.28 (0.95–1.70)
1.37 (1.02–1.83)	0.100	3.00	1.14	1.21 (0.90–1.61
1.37 (1.02–1.83)	0.125	1.25	1.02	1.34 (1.00–1.79)
1.37 (1.02–1.83)	0.125	1.50	1.05	1.31 (0.98–1.75)
1.37 (1.02–1.83)	0.125	1.75	1.07	1.28 (0.96–1.72)
1.37 (1.02–1.83)	0.125	2.00	1.09	1.26 (0.94–1.68)
1.37 (1.02–1.83)	0.125	3.00	1.16	1.19 (0.88-1.58)

Table A2. Sensitivity analysis of an unmeasured confounder on the child's asthma risk after exposure to antibiotics in third trimester of pregnancy using a $p(u,x=0)$ of 0,1

aOR (95% CI)	$p(u\|x=1)$*	OR*yu*	Bias	OR$yx \times cu$ (95% CI)
1.37 (1.02–1.83)	0.125	1.5	1.01	1.35 (1.01–1.81)
1.37 (1.02–1.83)	0.125	2	1.02	1.34 (1.00–1.79)
1.37 (1.02–1.83)	0.125	3	1.04	1.32 (0.98–1.76)
1.37 (1.02–1.83)	0.125	4	1.06	1.30 (0.96–1.73)
1.37 (1.02–1.83)	0.150	1.5	1.02	1.34 (1.00–1.79)
1.37 (1.02–1.83)	0.150	2	1.05	1.31 (0.95–1.75)
1.37 (1.02–1.83)	0.150	3	1.08	1.26 (0.94–1.69)
1.37 (1.02–1.83)	0.150	4	1.12	1.23 (0.91–1.64)
1.37 (1.02–1.83)	0.175	1.5	1.04	1.32 (0.98–1.77)
1.37 (1.02–1.83)	0.175	2	1.07	1.28 (0.95–1.71)
1.37 (1.02–1.83)	0.175	3	1.13	1.22 (0.91–1.63)
1.37 (1.02–1.83)	0.175	4	1.17	1.17 (0.87–1.56)
1.37 (1.02–1.83)	0.200	1.5	1.05	1.31 (0.97–1.75)
1.37 (1.02–1.83)	0.200	2	1.09	1.26 (0.94–1.68)
1.37 (1.02–1.83)	0.200	3	1.17	1.17 (0.87–1.57)
1.37 (1.02–1.83)	0.200	4	1.23	1.11 (0.83–1.49)
1.37 (1.02–1.83)	0.250	1.5	1.07	1.28 (0.95–1.71)
1.37 (1.02–1.83)	0.250	2	1.14	1.21 (0.90–1.61)
1.37 (1.02–1.83)	0.250	3	1.25	1.10 (0.82–1.46)
1.37 (1.02–1.83)	0.250	4	1.35	1.02 (0.76–1.36)

*$p(u|x=0) = 0.10$.

Appendix C Distribution of covariates between exposed and unexposed in case–sibling analysis

	Case–sibling analysis			Case–control analysis		
	n (%) Exposed (*N* = 575)	*n* (%) Unexposed (*N* = 1881)	*P*-value	*n* (%) Exposed (*N* = 4969)	*n* (%) Unexposed (*N* = 21 309)	*P*-value
Child characteristics						
Male gender	336 (58.4)	1044 (55.5)	0.215	2533 (51.9)	10 647 (49.8)	0.009
Birth order: first born	263 (45.7)	965 (51.3)	0.020			
Use of ABs before index date	424 (73.7)	1146 (60.9)	< 0.001	2742 (56.1)	9754 (45.6)	< 0.001
Mother characteristics						
Mean age in years	29.9	29.8	0.575	29.8	30.1	< 0.001
Use of medication for atopic diseases during pregnancy	213 (37.0)	421 (22.4)	< 0.001	1566 (31.5)	3913 (18.4)	< 0.001
Asthma medication	59 (10.4)	96 (5.1)	< 0.001	376 (7.6)	623 (2.9)	< 0.001

Appendix C (continued)

	Case–sibling analysis			Case–control analysis		
	n (%) Exposed (*N* = 575)	*n* (%) Unexposed (*N* = 1881)	*P*-value	*n* (%) Exposed (*N* = 4969)	*n* (%) Unexposed (*N* = 21 309)	*P*-value
Drugs for atopic dermatitis	71 (12.3)	162 (8.6)	0.007	592 (11.9)	1749 (8.2)	< 0.001
Drugs for rhinitis	124 (21.6)	228 (12.1)	0.000	882 (17.8)	2055 (9.6)	< 0.001
Use of acid-suppressive drugs during pregnancy (ATC A02B)	34 (5.9)	43 (2.3)	< 0.001	34 (5.9)	43 (2.3)	< 0.001
Use of insulin during pregnancy	4 (0.7)	14 (0.7)	0.905	4 (0.7)	14 (0.7)	0.905
Use of antidepressants during pregnancy	17 (3.0)	40 (2.1)	0.247	17 (3.0)	40 (2.1)	0.247
Father characteristics						
Data present (N)	903	835		3220	12 950	
Use of medication for atopic diseases during pregnancy	115 (27.3)	297 (22.6)	0.045	812 (25.2)	2883 (22.3)	< 0.001
Asthma medication	29 (6.9)	94 (7.1)	0.862	188 (5.8)	658 (5.1)	0.084
Drugs for atopic dermatitis	66 (15.7)	130 (9.9)	0.001	390 (12.1)	1349 (10.4)	0.005
Drugs for rhinitis	44 (10.5)	130 (9.9)	0.730	382 (11.9)	1405 (10.8)	0.101