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# Infant wheezing and prenatal antibiotic exposure and mode of delivery: a prospective birth cohort study

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## ABSTRACT

**Introduction:** Assessments on whether prenatal antibiotic exposure and mode of delivery increase the risk of wheezing in infants and toddlers are inconsistent. Our goal is to evaluate the association between prenatal antibiotic use and Cesarean section with three subtypes of wheezing in infancy.

**Methods:** An ongoing prospective three generations cohort study provides data on prenatal antibiotic use and mode of delivery. Respective questionnaire data was used to distinguish three subtypes of wheezing: any wheezing, infectious wheezing, and noninfectious wheezing. Repeated measurements of wheezing at 3, 6, and 12 months were analyzed using generalized estimation equations. Latent transition analysis assessed patterns of infant wheezing development in the first year of life.

**Results:** The prevalence of any wheezing was highest at 12 months (40.1%). The prevalence of infectious wheezing was higher (3 months 23.8%, 6 months 33.5%, 12 months 38.5%) than of noninfectious wheezing (3 months 13.0%, 6 months 14.0%, 12 months 11.1%). About 11–13% of children had both infectious and noninfectious wheezing at 3, 6, and 12 months (3 months 10.7%, 6 months 13.9%, 12 months 13.1%). Children born via Cesarean section have approximately a 70–80% increase in the risk of any wheezing (RR = 1.83, 95% CI 1.29–2.60) and of infectious wheezing (RR = 1.72, 95% CI 1.18–2.50).

**Conclusions:** Analyses of infectious and noninfectious wheezing subtypes suggests that children born by Cesarean sections may be more susceptible to infectious wheezing, warranting investigations into microbial factors of infectious wheezing. No significant associations were found between prenatal antibiotic exposure and wheezing types.

**Abbreviations:** PFT: Pulmonary function test; GAM: Generalized additive models; LTA: Latent transition analysis; RR: Risk ratio; OR: Odds ratio; 95% CI: 95% confidence interval for the RR

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cohort study

## Introduction

Asthma is one of the most prevalent chronic diseases that affects children and occurs in all countries regardless of the level of development (1). Unfortunately, it is difficult to diagnose asthma in children under the age of five, since pulmonary function tests require full cooperation of the child to sit still and follow breathing directions. Thus, there is a need to identify symptoms in infancy and early childhood that closely resembles asthma. Wheezing, which is a candidate symptom, is classified into two

categories, infectious wheezing and noninfectious wheezing. In the past, infectious wheezing was not clearly distinguished from noninfectious wheezing due to some limitations (2,3).

## Variation in wheezing subtypes

Although previous studies have reported whether wheezing is persistent, recurrent, early, or late (4–10), these studies do not report whether wheezing was related to infections or not. Other studies have also

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reported multiple-trigger wheezing, wheezing between episodes of sickness due to cold air, exercise, allergy, etc (2,11,12), but - with one exception (13) - no wheezing was related to colds or infections, nor has the research focused on wheezing in infancy. Investigating such wheezing phenotypes (infectious vs. noninfectious) may provide more insights into gestational risk factors, in particular, prenatal antibiotic use and mode of delivery.

### **Research regarding the association between prenatal antibiotic use and infant wheezing**

Past reports suggested that prenatal antibiotic use may be associated with offspring wheezing (7,14,15) due to changes in the maternal microbiome. With focus on wheezing information from birth to 10 years of age, we have summarized the results of the past findings related to the association between prenatal antibiotic exposure and infant wheezing in Figure 1 and Appendix Table S1 and found inconsistent results. With some variations, most studies followed children

from birth to early childhood to determine associations between prenatal antibiotic use (4,5,7,8,10) with an increased risk of wheezing. Few studies that explore various definitions of wheezing and antibiotics use (i.e.dose-related, infection-related, continuous or binary exposure, etc.), reported increased risks for prenatal antibiotic exposure and wheezing after birth (4,5,7-9), whereas two studies did not find such associations (6,10). In addition, it has been proposed that prenatal antibiotic use can reduce the amount of 'healthy' bacteria, which the child is exposed to when it comes into contact with maternal vaginal bacteria during normal delivery (4,8,16-20).

### **Research regarding the association between mode of delivery and infant wheezing**

Alteration in bacteria, suggests that mode of delivery is an important risk factor to be taken into account when determining associations between infant wheeze and prenatal antibiotic exposure. Furthermore, non-exposure to the maternal microbiome is considered as

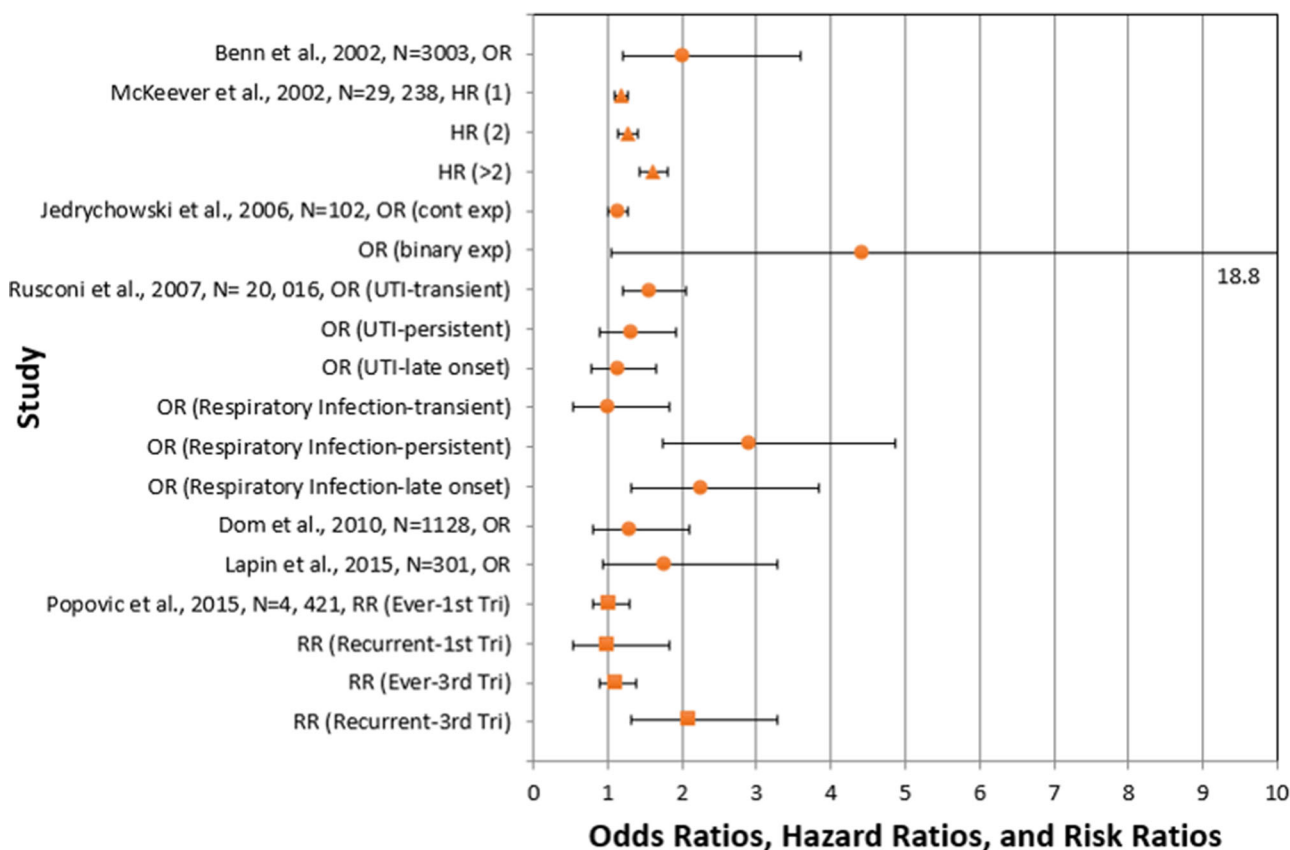


**Figure 1.** Forrest plot of the associations between prenatal antibiotics and wheezing reported in seven studies. (UTI –Urinary Tract Infection, ever – child ever wheezing, recurrent – child had recurring wheezing, transient – or transient early; child has wheezing early in life but not later, persistent – child had repeated episodes of wheezing, late onset – child had wheezing only late in childhood, OR – Odds Ratio, RR – Risk Ratio, HR – Hazard Ratio, Tri – Trimester, cont exp– exposed to antibiotics throughout entire pregnancy).

one factor for the association between Cesarean section and wheezing (4,9). In our overview of past findings (Figure 2), we have applied the same requirements as in our literature review for prenatal antibiotic use. Figure 2 and Appendix Table S2 provide an overview of the past research regarding mode of delivery (4,9,13,21–23) and infant wheezing, showing inconsistent associations. Only one study, Negele et al. (13), found a statistically significant increased risk for wheezing in early life in children delivered through Cesarean section. Three other studies detected non-significant associations between wheezing in children born by Cesarean sections (4,9,22).

## Wheezing

The literature regarding prenatal exposures and wheezing in children revealed varying findings. This could be due to how wheezing was defined in previous studies. Often it was not considered whether the child was wheezing from a cold or infection, or if the child might have early symptoms, like noninfectious wheezing, that can be reflective of asthma (24–26). Using new subtypes of wheezing in this research can be instrumental

to identify associations between prenatal exposures and wheeze. To this end, we used information on infectious and noninfectious wheezing.

## Purpose

Interestingly, the majority of the findings resulted from studies conducted between 1990s and early 2000s (4–10,13,21–23). There is a lack of studies that feature data collected after the year 2000. Hence, new assessments are necessary to update potential associations between prenatal antibiotic use and wheezing and asthma in children. We provided new investigations using the Isle of Wight 3<sup>rd</sup> generation cohort, a three generational cohort including grandmother, mother, and children (F0, F1, and F2). Because of Isle of Wight's ongoing follow-up with deliveries between 2010 and 2016, we were able to contact women early in pregnancy, potentially resulting in a more accurate assessment of exposure and risk factors compared to retrospective ascertainment (27). Our aim was to investigate associations between prenatal antibiotics and mode of delivery for wheezing, focused on infectious and noninfectious wheezing in infants.

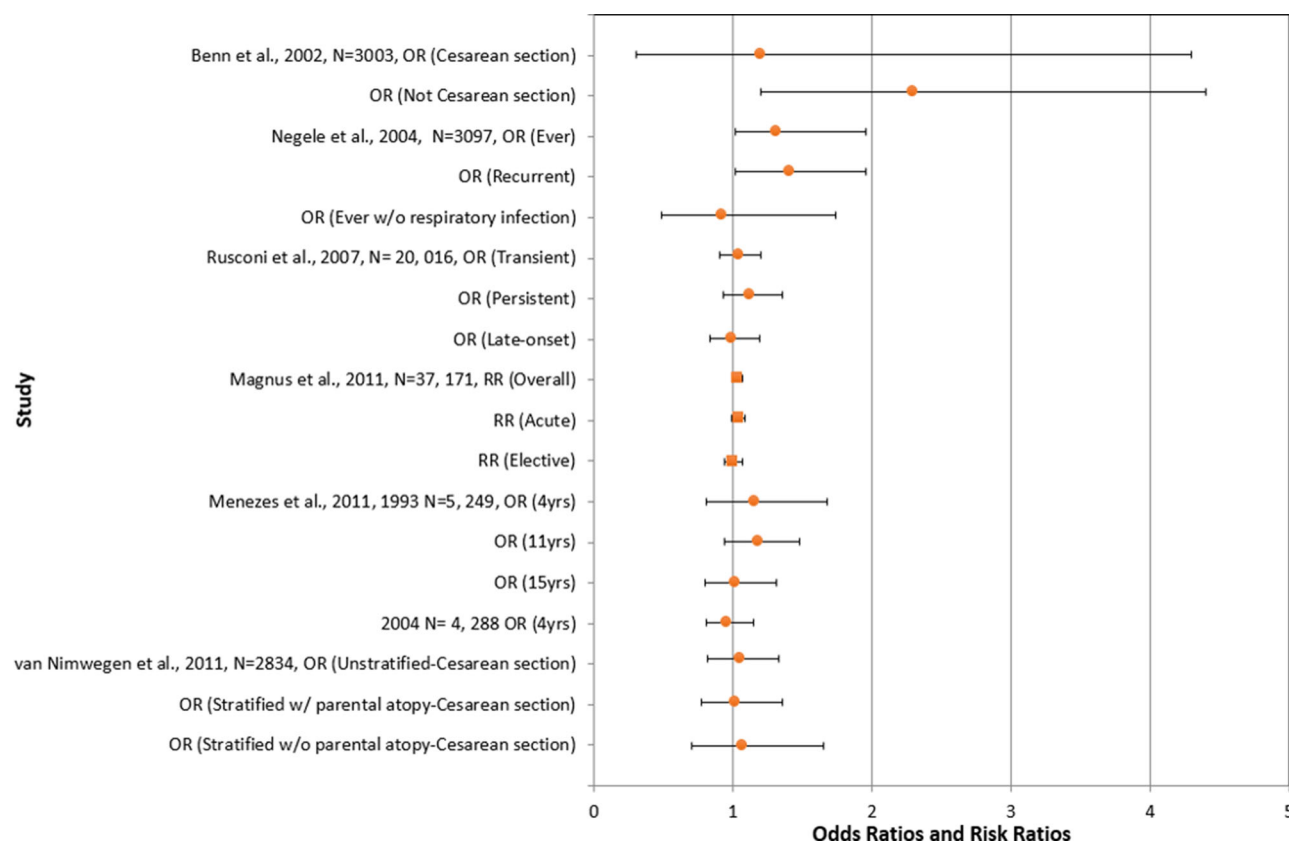


**Figure 2.** Forrest plot of the associations between mode of delivery and wheezing reported in six studies. (ever – child ever wheezing, recurrent – child has recurring wheezing, transient – child has wheezing early in life but not later, persistent – child has repeated episodes of wheezing, late onset – child wheezing only later in childhood, modes of deliveries: Cesarean section- overall, acute, elective, OR – Odds Ratio, RR – Risk Ratio).

## Methods

### Study population

Data from the 3<sup>rd</sup> generation birth cohort (F2) were analyzed. The original birth cohort (F1) was established in 1989 on the Isle of Wight, UK to study natural history and risk factors for allergic disease (28,29). The cohort covers three generations (F0 = grand-parents; F1 = original birth cohort, now parents,  $n = 1456$ , and F2 = grandchildren,  $n = 412$ ) (28). F2 generation is an ongoing cohort with continued recruitment and assessments. Our analysis focuses on the F2 generation, with assessments at 3, 6, and 12 months, with detailed questionnaires. The Isle of Wight is near the British mainland and has a population of 130 000, which is over 99% Caucasian. The investigation was approved by the local ethics committee – National Research Ethics Service, NRES Committee South Central – Hampshire B, U.K. and by the University of Memphis Institutional Review Board in Memphis, U.S (#2423). Written informed consents were obtained from the participants at the time of enrollments and at each follow-up.

### Exposure

Information regarding pre- and perinatal conditions was gathered through questionnaires during early pregnancy (12 and 20 weeks of gestation) and late pregnancy (28 weeks) and hospital and birth records. Questions related to prenatal antibiotic exposure included “Have you taken any medication during pregnancy (e.g. antibiotics, aspirin, paracetamol etc.)? If yes, please specify,” which requested the name of the antibiotics. Children were considered to be exposed to prenatal antibiotic exposure if their mother answered “yes” and specified antibiotic use. Information regarding mode of delivery (Cesarean section or vaginal) was abstracted from birth records.

### Outcome

We focused on the classification of three wheezing subtypes in the first year of life: any wheezing, infectious wheezing, and noninfectious wheezing (asthma). Infants were classified as having any wheezing if the child had “wheezing or whistling in the chest” at any point during the 3, 6, or 12-month follow-up period after birth. If infants had any “wheezing in association with cold or chest infection,” then the infant was classified as having infectious wheezing. To classify infants as having noninfectious wheezing, the infant

must have any wheezing that is not in relation to cold or chest infection, only “wheezing between cold and chest infection.” Each classification was assessed and defined during the 3, 6, and 12 months follow up period via questionnaire. The follow-up data for wheezing were combined separately for each time point to create an overall assessment of each subtype: any wheezing at 3, 6, and 12 months, infectious wheezing at 3, 6, and 12 months, and noninfectious wheezing at 3, 6, and 12 months. The overall assessment outcomes were used for the repeated measurement analysis.

### Covariates

The following risk factors were considered to potentially confound the association between prenatal antibiotics use or mode of delivery and wheezing in offspring and were adjusted for in the analyses: maternal factors (asthma history, age at delivery, smoking, infection) (4–10,13,21–23), gender (4–6,9,10,13,21–23), birth order (6,22), and birth weight (born less than 2500 g (5lbs and 8 oz) (4,6,9,10,13,21–23). Information on birth weight and maternal infection was retrieved from hospital notes. Data on antibiotic use and covariates were acquired via questionnaires.

### Statistical analysis

Frequency counts and percentages are presented for all variables. Chi-square goodness of fit analysis was used to test the statistical significance differences between the analytical sample with the relevant data and the complete F2 birth cohort (status of 2018). We distinguished any wheezing, infectious wheezing, noninfectious wheezing measured at 3, 6, and 12 months in the first year of life. A log-linear model was used to estimate risk ratios of prenatal antibiotic use and mode of delivery for repeated measurement of wheezing using the SAS procedure GENMOD (30). We also assessed first term interactions of prenatal antibiotic exposure and mode of delivery with maternal and infant factors; asthma history, age at delivery, smoking, gender, birth weight, and birth order. Furthermore, we considered that mothers with asthma might have a different use of antibiotics during pregnancy. In addition to assessing interaction on a multiplicative scale using log-linear models, we further evaluated a possible interaction on an additive scale (joint effects) using the SAS procedure GAM (31). This approach allows nonparametric regression modeling and gives flexibility to model predictor-response

relationships in various data types, which may give better insight into biologic interaction (32,33).

Covariates were considered to confound if they changed the risk ratio of the primary exposures (mode of delivery and prenatal antibiotic use) related to the occurrence of wheezing by more than 10%. Backward selection was used to create the most parsimonious model, after investigating interactions and confounding. Risk ratios (RR) and 95% confidence intervals (95% CIs) were estimated. In additional analysis, we investigated the role of missingness to determine whether there was any significant difference due to missing observations between each variable and the observed data. Finally, to assess the development of infant wheezing (infectious and noninfectious wheezing) and co-occurrence of phenotypes in the first year of life, we applied Latent Transition Analysis (LTA) using the SAS procedure LTA (34), to estimate membership for different 'latent wheezing classes'. LTA identified the probability of transition between infectious and noninfectious wheezing at 3 to 6 months and 6 to 12 months and their co-occurrence. In addition, we utilized the log-linear models (GENMOD in SAS) to investigate if there were associations with the two exposure variables with the identified 'latent wheezing classes'. A  $p$  values of  $\leq 0.05$  was used to determine statistical significance. No adjustment for multiple testing was conducted. All data were analyzed using Statistical Analysis System (version 9.4 (SAS Institute, Inc., Cary, NC) software package.

## Results

### Overall prevalence of wheezing

The 3<sup>rd</sup> generation cohort, up to the year 2018, included 412 infants (55% boys). The prevalence of any wheezing in this cohort increased from month 3 to the month 12 (month 3: 29.1%, month 6: 36.6%, months 12: 40.1%, Table 1). The prevalence of infectious wheezing was higher (3 months 23.8%, 6 months 33.5%, 12 months 38.5%) than that of noninfectious wheezing (3 months 13.0%, 6 months 14.0%, 12 months 11.1%).

### Prevalence of wheezing subtypes in infancy

We used LTA to investigate the co-occurrence of infectious and noninfectious wheezing at 3, 6, and 12 months. LTA presented three membership statuses of wheezing subtypes in the first year of life: (infants with no wheezing, infants with infectious wheezing only, and infants with both infectious and noninfectious wheezing). This solution provided the best fit.

**Table 1.** The prevalence of wheezing among infants.

|                        | Month 3<br>(N = 367)<br>n (%) | Month 6<br>(N = 362)<br>n (%) | Month 12<br>(N = 367)<br>n (%) |
|------------------------|-------------------------------|-------------------------------|--------------------------------|
| Any wheezing           | 103 (29.1%)                   | 71 (36.6%)                    | 101 (40.1%)                    |
| Infectious wheezing    | 82 (23.8%)                    | 65 (33.5%)                    | 97 (38.5%)                     |
| Noninfectious wheezing | 46 (13.0%)                    | 27 (14.0%)                    | 28 (11.1%)                     |

The prevalence of the wheezing types is presented in columns and transition probabilities are shown in the arrows (Figure 3). The transition probabilities indicate the proportion of transition in the first year of life between healthy infants, infants with only infectious wheezing, and infants that have both infectious and noninfectious wheezing. The thicker the arrow, the greater the transition probability. At 3, 6 and 12 months the largest latent class is the healthy population (3 months 57.6%, 6 months 53%, 12 months 54.5%), followed by infectious wheezing only (3 months 31.7%, 6 months 33.1%, 12 months 32.5%) and a combination group of infectious and noninfectious wheezing (3 months 10.7%, 6 months 13.9%, 12 months 13.1%), respectively. Interestingly, noninfectious wheezing was not present as a subtype of wheeze of its own. Instead, the LTA identified only a combination group of both infectious and noninfectious wheezing in the first year of life.

### Stability and transition of wheezing subtypes in infancy

The prevalence of healthy infants was stable with a slight decrease from month 3 (57.6%) to month 6 (53%) and a slight increase to month 12 (54.5%); 85.9% of the healthy infants stayed without wheezing from 3 to 6 months and 92.3% remain wheezing-free from 6 to 12 months (Figure 3). Only 7.1% of children transitioned from healthy to having infectious wheezing only and 7.0% to a combination of infectious and noninfectious wheezing at 6 months, while even less, 0.5% and 2.4%, transitioned from healthy to having infectious wheezing or a combination of noninfectious and infectious wheezing at 12 months, respectively. Likewise, the group of infants with only infectious wheezing with about 32% was quite stable from month 3 (89.2%) to month 6 and from month 6 to month 12 (84.8%). Only 10.8% of the infants who had infectious wheezing at month 3 transitioned to have both infectious and noninfectious wheezing at 6 months, while even less, 6.6% transitioned from infectious wheezing at 6 months to a combination of infectious and noninfectious wheezing at 12 months. Eight percent of the infants that had only infectious wheezing at 6 months revert a wheeze-free status at

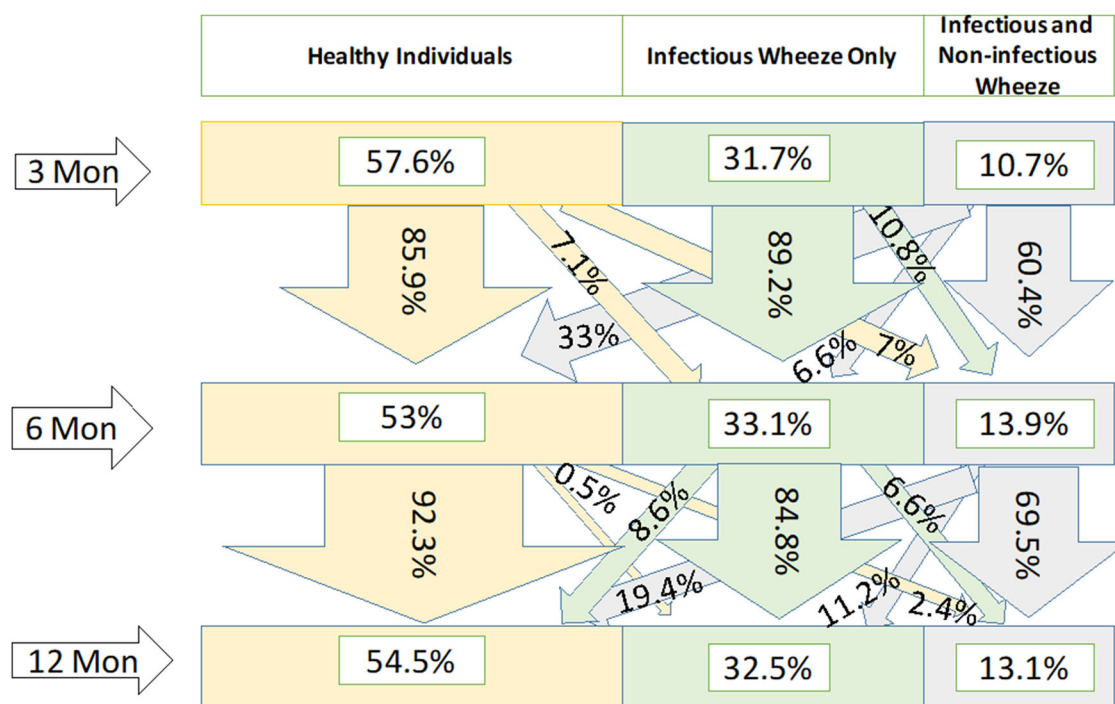


**Figure 3.** Proportion and transition probabilities of wheezing type ( $n=412$ ). Figure shows the change in wheezing subtypes in the first year of life. Healthy individuals are depicted as yellow, infectious wheezing only is depicted as green, and a combination of infectious and noninfectious wheezing is displayed as gray. The groups were based on the probability of the child having the wheezing outcomes (no wheezing, infectious wheezing only, and noninfectious wheezing) at each time point, 3, 6, and 12 months. Latent transition patterns considering 3 time points were most common, though 13% ( $n=54$ ) of infants were grouped considering only 2 time points.

12 months. There was a slight increase in the prevalence of children with a combination of infectious and noninfectious wheezing from 3 months (10.7%) to 6 months (13.9%) and 12 months (13.1%). In addition, the stability of the group of infants with infectious and noninfectious wheezing in the two transition periods is smaller (60.4% and 69.5%). Analyses of these subtypes with regard to antibiotics or mode of delivery did not reveal additional information (data not shown).

### Overall risk factors of the study population

In terms of risk factors, 85 children (20.6%) had prenatal antibiotic exposure and 266 children (64.6%) did not (Table 2). Sixty-one children (14.8%) lacked information related to prenatal antibiotic exposure. Sixty-four children (15.5%) were born by Cesarean Section and 278 (67.4%) by vaginal delivery; 70 children (17%) had missing information on the mode of delivery. Values indicating missingness at different time points are displayed in Table 2 in the rows as “missing”. Maternal smoking, as a time-dependent covariate, showed missing information in 4% (Table 2). Other covariates with missing information are as

follows: maternal asthma ( $n=26$ ; 6%), maternal infection ( $n=138$ ; 33%), birth order ( $n=3$ ; 0.7%), and low-birthweight ( $n=70$ ; 17%). We investigated whether missingness affected the results of our study and found no such effects. Therefore, we concluded that missingness is at random and the missingness in each variable differ from the observed data only by chance.

### Risk of infant wheezing due to prenatal antibiotic exposure or mode of delivery

Prenatal exposure to antibiotics was statistically insignificant for all three subtypes of wheezing (any wheezing, infectious wheezing, and noninfectious wheezing, Table 3). Mode of delivery shows an increased risk for any wheezing among children born by Cesarean section. Children born via Cesarean section had 1.83 times the risk of any wheezing in comparison to children born via vaginal delivery (RR = 1.83, 95% CI 1.29–2.60). Children born via Cesarean section also had 1.72 times the risk of developing infectious wheezing (RR = 1.72, 95% CI 1.18–2.50). There was no significant risk comparing noninfectious wheezing with different mode of delivery.

**Table 2.** Characteristics of study population.

| Characteristic (N = 412)                     |                       | Any Wheezing (N = 367) |     |            | Infectious Wheezing (N = 362) |            |  | Noninfectious Wheezing (N = 367) |           |  |
|----------------------------------------------|-----------------------|------------------------|-----|------------|-------------------------------|------------|--|----------------------------------|-----------|--|
|                                              |                       | N                      | N   | (n) %      | N                             | (n) %      |  | N                                | (n) %     |  |
| Prenatal antibiotic exposure                 | Yes                   | 85                     | 76  | (36) 47.4  | 74                            | (32) 43.2  |  | 76                               | (15) 19.7 |  |
|                                              | No                    | 266                    | 237 | (117) 49.4 | 234                           | (110) 47.0 |  | 237                              | (46) 19.4 |  |
|                                              | Missing               | 61                     | 54  | (22) 40.7  | 54                            | (21) 38.9  |  | 54                               | (9) 16.7  |  |
| Mode of delivery                             | Cesarean              | 64                     | 60  | (35) 58.3  | 59                            | (31) 52.5  |  | 60                               | (13) 21.7 |  |
|                                              | Section               |                        |     |            |                               |            |  |                                  |           |  |
|                                              | Vaginal               | 278                    | 259 | (122) 47.1 | 259                           | (118) 45.6 |  | 259                              | (46) 17.8 |  |
|                                              | Missing               | 70                     | 48  | (18) 37.5  | 44                            | (14) 31.8  |  | 48                               | (11) 22.9 |  |
| Gender                                       | Boy                   | 227                    | 204 | (106) 52.0 | 201                           | (98) 48.8  |  | 204                              | (50) 24.5 |  |
|                                              | Girl                  | 185                    | 163 | (69) 42.3  | 161                           | (65) 40.4  |  | 163                              | (20) 12.3 |  |
| Maternal asthma during pregnancy             | Yes                   | 98                     | 92  | (59) 64.1  | 91                            | (56) 61.5  |  | 92                               | (26) 28.3 |  |
|                                              | No                    | 288                    | 257 | (106) 41.3 | 253                           | (97) 38.3  |  | 257                              | (41) 15.6 |  |
|                                              | Missing               | 26                     | 18  | (10) 55.6  | 18                            | (10) 55.6  |  | 18                               | (3) 16.7  |  |
| Amount of cigarettes smoked during pregnancy | > 10                  | 83                     | 73  | (38) 52.1  | 70                            | (33) 47.1  |  | 73                               | (21) 28.9 |  |
|                                              | 1–10                  | 63                     | 59  | (25) 42.4  | 57                            | (21) 36.8  |  | 59                               | (10) 17.0 |  |
|                                              | 0                     | 249                    | 224 | (104) 46.4 | 224                           | (101) 45.1 |  | 224                              | (36) 16.1 |  |
|                                              | Missing               | 17                     | 11  | (8) 72.7   | 11                            | (8) 72.7   |  | 11                               | (3) 27.3  |  |
| Age of the mother (years)                    | 16–20                 | 89                     | 84  | (39) 46.4  | 83                            | (31) 44.6  |  | 84                               | (12) 14.3 |  |
|                                              | 21–23                 | 129                    | 116 | (72) 62.1  | 116                           | (69) 59.5  |  | 116                              | (34) 29.3 |  |
|                                              | 24–41                 | 194                    | 167 | (64) 38.3  | 163                           | (57) 35.0  |  | 167                              | (24) 14.4 |  |
| Birth order                                  | 3 <sup>rd</sup> child | 49                     | 38  | (23) 60.5  | 37                            | (22) 59.5  |  | 38                               | (13) 34.2 |  |
|                                              | 2 <sup>nd</sup> child | 126                    | 113 | (57) 50.4  | 112                           | (55) 49.1  |  | 113                              | (22) 19.5 |  |
|                                              | 1 <sup>st</sup> child | 234                    | 215 | (95) 44.2  | 212                           | (86) 40.6  |  | 215                              | (35) 16.3 |  |
|                                              | Missing               | 3                      | 1   | 0          | 1                             | 0          |  | 1                                | 0         |  |
| Low birth weight                             | Yes                   | 21                     | 20  | (13) 65.0  | 20                            | (12) 60.0  |  | 20                               | (6) 30.0  |  |
|                                              | No                    | 321                    | 299 | (144) 48.2 | 298                           | (137) 46.0 |  | 299                              | (53) 17.7 |  |
|                                              | Missing               | 70                     | 48  | (18) 37.5  | 44                            | (14) 31.8  |  | 48                               | (11) 22.9 |  |
| Maternal infection                           | Yes                   | 59                     | 57  | (26) 45.6  | 57                            | (25) 43.9  |  | 57                               | (11) 19.3 |  |
|                                              | No                    | 216                    | 197 | (93) 47.2  | 196                           | (87) 44.4  |  | 197                              | (31) 15.7 |  |
|                                              | Missing               | 137                    | 113 | (56) 50    | 109                           | (51) 46.8  |  | 113                              | (28) 24.8 |  |

**Table 3.** Risk ratios for any wheezing, infectious wheezing, noninfectious wheezing, and prenatal antibiotic use in the first year of life using repeated measurements determined at 3, 6, and 12 months.

| Variables               |                       | Any wheezing     |         | Infectious wheezing |         | Noninfectious wheezing |         |
|-------------------------|-----------------------|------------------|---------|---------------------|---------|------------------------|---------|
|                         |                       | RR 95% CI        | P-value | RR 95% CI           | P-value | RR 95% CI              | P-value |
| Prenatal antibiotic use | Yes                   | 1.01 (0.69–1.47) | 0.94    | 0.92 (0.62–1.37)    | 0.69    | 0.88 (0.44–1.78)       | 0.73    |
|                         | No                    | Reference        |         | Reference           |         | Reference              |         |
| Mode of delivery        | Cesarean Section      | 1.83 (1.29–2.60) | 0.0008  | 1.72 (1.18–2.50)    | 0.005   | 1.50 (0.76–2.94)       | 0.24    |
|                         | Vaginal               | Reference        |         | Reference           |         | Reference              |         |
| Gender                  | Boy                   | 1.35 (0.98–1.86) | 0.06    | 1.35 (0.97–1.89)    | 0.07    | 2.83 (1.42–5.69)       | 0.003   |
|                         | Girl                  | Reference        |         | Reference           |         | Reference              |         |
| Age of the mother       | 16–20 years           | 2.16 (1.06–4.41) | 0.03    | 2.32 (1.14–4.76)    | 0.02    | 2.96 (0.61–14.51)      | 0.18    |
|                         | 21–23 years           | 1.50 (1.07–2.11) | 0.02    | 1.42 (1.00–2.04)    | 0.05    | 2.99 (1.52–5.94)       | 0.002   |
|                         | 24–41 years           | Reference        |         | Reference           |         | Reference              |         |
| Birth order             | 3 <sup>rd</sup> child | 1.44 (0.89–2.34) | 0.14    | 1.37 (0.81–2.30)    | 0.24    | 3.86 (1.68–8.86)       | 0.001   |
|                         | 2 <sup>nd</sup> child | 1.65 (1.15–2.36) | 0.006   | 1.75 (1.21–2.54)    | 0.003   | 2.02 (0.96–4.23)       | 0.06    |
|                         | 1 <sup>st</sup> child | Reference        |         | Reference           |         | Reference              |         |
| Low birth weight        | Yes                   | 1.85 (1.03–3.30) | 0.04    | 1.60 (0.85–3.02)    | 0.15    | 4.60 (1.76–12.04)      | 0.002   |
|                         | No                    | Reference        |         | Reference           |         | Reference              |         |

\*Repeated Measures Analysis adjusted for maternal asthma, smoking during pregnancy, maternal infection, and age.

### Interaction by maternal asthma

Additionally, we inspected the relation between wheezing in infants and prenatal maternal use of antibiotics and mode of delivery in asthmatic and non-asthmatic mothers (Table 4). Noninfectious wheezing was more prevalent in children born to mothers who had asthma and used prenatal antibiotics (50% of  $n = 18$ ), but only in 10.5% of 56 children of non-asthmatic mothers who received antibiotics during

pregnancy (Table 4). Thus, there seemed to be a possible joint effect of maternal asthma during pregnancy and prenatal antibiotic use with wheezing in infancy. Although smaller, this type of effect was also seen for the mode of delivery: 38.5% of the 13 offspring born via Cesarean section to asthmatic mothers experienced wheezing in infancy, but only 19.1% of the infants born via Cesarean section in mothers without asthma (Table 4). However, the results using generalized

**Table 4.** Potential joint effects of prenatal antibiotic exposure and mode of delivery with maternal asthma on the occurrence of noninfectious wheezing in the first year of life (infancy) ( $n = 412$ ).

| Noninfectious wheezing     | Maternal asthma      |      |                  |          | No maternal asthma   |      |                  |          |
|----------------------------|----------------------|------|------------------|----------|----------------------|------|------------------|----------|
|                            | Prenatal antibiotics |      | Mode of delivery |          | Prenatal antibiotics |      | Mode of delivery |          |
|                            | No                   | Yes  | Normal           | Cesarean | No                   | Yes  | Normal           | Cesarean |
| Total $n$                  | 59                   | 18   | 66               | 13       | 177                  | 56   | 180              | 42       |
| Noninfectious wheezing (%) | 23.7                 | 50.0 | 24.2             | 38.5     | 18.1                 | 10.7 | 15.0             | 19.1     |

additive models showed no additive interaction of maternal asthma with prenatal antibiotics or Cesarean section (data not shown).

## Discussion

During infancy, most children remain (more than 60%) in a particular symptom class (no wheeze, infectious wheeze, combination of both infectious and noninfectious wheeze) of wheezing in the first year of life. Thus, the subtypes of wheezing in infants were quite stable in infancy. A small percentage (more than 10%) of children showed a combination of infectious and noninfectious wheezing. We did not find significant effects pertaining to prenatal antibiotic use in mothers on wheezing in infants. However, we did find that children born of Cesarean section may have a higher risk of any wheezing and infectious wheezing in the first year of life.

### Wheezing in infancy

The latent transition analysis (LTA) revealed three wheezing outcomes, no wheezing, infectious wheezing only, and a combination of infectious and noninfectious wheezing, in the first year of life. Infectious wheezing was the most prevalent subtype. LTA also illustrated the stability of the wheezing types in infancy. Non-wheezing infants and the infants with infectious wheezing represent more stable groups with majority of participants remaining in the same group in two transitions in the first year of life. LTA did not present noninfectious wheezing only as a stable subtype of its own. This agrees with previous research that has found that children who have noninfectious wheeze (asthma), will often wheeze when they have an infection (35,36). The combined group of infectious and noninfectious wheeze was also found to be more stable than noninfectious wheeze by itself.

### Association between prenatal antibiotic use and wheezing subtypes

For prenatal antibiotic exposure, our results showed no significant associations for noninfectious wheezing

in infancy, but a higher proportion of children with noninfectious wheezing born to mothers that have maternal asthma (Table 4). Thus, there is a possibility that maternal asthma is related to both prenatal antibiotics and noninfectious wheezing in infants and toddlers. However, we found no significant interaction or confounding between prenatal antibiotic use, maternal asthma, and noninfectious wheezing. Nevertheless, maternal asthma should be accounted for in further research with larger sample sizes to achieve more stable results. Furthermore, regarding prenatal antibiotic use, previous studies following children from birth, have found a positive association between wheezing and prenatal antibiotic use (4,5,7). Differences between these studies and our results may be due to variations in the wheezing definition. For example, Dom et al. 2010 (10), analyzing recurrent wheezing used at least two episodes of parent-reported wheezing in a 6 months up to 4 years of age. Jedrychowski et al. 2006 (5), defines persistent wheezing as present at least 9 days after a follow up of wheezing in the first year of life. In our study we have identified wheezing (infectious, noninfectious, and their combinations) in a way no previous study has, that can differentiate the transition of different wheezing phenotypes.

### Association between mode of delivery and wheezing subtypes

Regarding the mode of delivery, log-linear models showed significant increased risk for any and infectious wheezing with Cesarean sections. Children born via Cesarean section have at least a 70% increase in risk of any wheezing ( $RR = 1.83$ , 95% CI 1.29–2.60) and infectious wheezing ( $RR = 1.72$ , 95% CI 1.18–2.50). Our findings are in agreement with one previous study that has researched the association between mode of delivery and wheezing, taking infections into account (13). Negele et al. 2004 (13), found that children born via Cesarean section had 31% increased odds of at least one episode of wheezing ( $OR = 1.31$ , 95% CI 1.02, 1.68), 41% increased odds of recurrent wheezing ( $OR = 1.41$ , 95% CI 1.02, 1.96), and no association between wheezing without

respiratory infection and Cesarean section (OR = 0.92, 95% CI 0.49, 1.74). The similarities in our results and the results found by Negele et al. 2004 (13), may be due to the fact that, for infants and toddlers, viral infection is the most common cause of airway symptoms (13,37). As viral infections are most frequently caused by the respiratory syncytial virus (38), viral infection can be a strong risk factor for infectious wheezing.

An explanation for the observed increased risk between Cesarean section and wheezing, particularly for infectious wheezing, could be a difference in the microbiome for children born via Cesarean section vs. vaginal delivery. A child born through vaginal delivery comes into direct contact with the maternal microbiome. This contact may initiate a balance of the infant immune system, which has a higher proportion of regulatory T cells and a higher T helper-2:T-helper-1 (Th1) ratio (39). Cesarean section may affect the infants' immune system by decreasing the Th1 response (40). The decrease of the Th1 response may increase the risk of infections, which in turn may change offspring's microbiome, as emphasized in the hygiene hypothesis (41,42), and may explain the significant results we see for any wheezing and for infectious wheezing. Furthermore, not establishing a normal intestinal microflora may lead to a different formation of gut microbiota (43–46). Children born via vaginal delivery, they acquire their microbiome from the maternal vaginal canal, which allows strict anaerobes and beneficial gut microbiota, such as *Bacteroides* and *Bifidobacterium* spp. to thrive (43–46). As children born of Cesarean section may have less 'helpful' bacteria that can stimulate immunological responses, the risk to adverse effects, such as wheezing, may increase. Few birth cohorts (4,9), explore this association and found a positive association between Cesarean section and wheezing by 7 years of age. Thus, maternal and infant microbiome are important factors that should be taken account in future wheezing research.

### Limitations

There are some limitations to this study. Our cohort includes a sample size of only about 400 infants, which can limit the power of the analysis. Furthermore, the small sample size could limit the results retained through our analysis, especially for variables with small group sizes or outcomes with low prevalence, such as noninfectious wheezing. For example, when investigating the combined effects of

maternal asthma with antibiotics, the small sample size (Table 4) reduced the ability to detect an effect. In addition to a smaller sample size, this study has been completed in Isle of Wight, UK where the population is 99% Caucasian. Thus, our results may not be generalizable to other races. Also, our definition of wheezing classification is based on questionnaire data. Thus, to better distinguish between outcomes in the future, doctor diagnosed infections, like bronchiolitis, should be used to better separate infectious and non-infectious wheezing. In addition, we did not analyze specific antibiotics. Names of specific antibiotics were provided by 32 (37.6%) of the 85 mothers who used antibiotics. Thus, in our analysis we focused on any antibiotic exposure and not a particular type of antibiotic. The aforementioned variables should be analyzed with a larger sample size in future research to create a more accurate assessment of wheezing in infants and toddlers and confirm inconclusive results.

### Strengths

One strength in our research was the ability to distinguished three new subtypes of wheezing, any wheezing, infectious wheezing (wheezing due to infection), and noninfectious wheezing (asthma). To this end, we were able to demonstrate an increased risk of any and infectious wheezing when exposed to Cesarean section delivery. This suggests that wheezing could be due to a lack of "healthy" or "good" bacteria in the infant's microbiome. Another strength is the longitudinal data and the repeated measurements used to analyze wheezing in the first year of life. Using log-linear models for repeated measurements allowed us to address a clear time order of the risks related to prenatal antibiotic exposure and Cesarean section at different stages of the infant's development.

### Conclusion

Asthma is difficult to diagnose in infants, which makes identification of wheezing central to assessing early life risk factors. It is imperative that we determine the 'type' of wheezing the child has (infectious, noninfectious wheezing, and their overlap). Besides, further explorations of infant exposures such as Cesarean sections and its relation to the fetal microbiome can uncover unknown implications that are crucial in identifying wheezing outcomes. Hence, using detailed wheezing information (infectious, non-infectious, any wheezing) in further research with a large sample size may contribute to increasing

enhancing scientific knowledge of exposures that can increase the risk of asthma in children.

## Declarations of interest

### Ethics approval and consent to participate

The investigation was approved by the local ethics committee –National Research Ethics Service, NRES Committee South Central – Hampshire B, U.K. and by the University of Memphis Institutional Review Board in Memphis, U.S (#2423).

### Consent for publication

Not applicable

### Availability of data and material

Not applicable

### Disclosure of interests

No potential conflict of interest was reported by the author(s).

### Disclaimer

The findings and conclusions in this communication are those of the authors and do not necessarily represent the views or official position of the U.S. Food and Drug Administration (FDA) or the U.S. Department of Health and Human Services (DHHS). The names of vendors or manufacturers are provided as examples of available product sources; inclusion does not imply endorsement of the vendors, manufacturers, or products by the FDA or the DHHS.

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### Notes on contributor

DH analyzed and interpreted data regarding prenatal antibiotic exposure, mode of delivery, and wheezing. DH was

major contributor in writing the manuscript. WK advised the data analysis and interpretation, edited and revised the manuscript. YJ advised the data analysis and interpretation. PB, IS, and HA edited the manuscript. All authors read and approved the final manuscript

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## Appendix:

**Table S1.** Summary of seven studies addressing prenatal exposure to antibiotics and wheezing in children.

| Author                             | Year of Data Col. | Age of Children   | Exposure                                                                 | Outcome                                                                                                                                                                                                                                                                                                                                                          |
|------------------------------------|-------------------|-------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Benn et al., 2002 (Denmark)        | 1992-1994         | 0-3 years         | Maternal colonization of microflora use of antibiotics                   | <b>Infant wheezing</b><br>OR: 2.0 (1.2-3.6)                                                                                                                                                                                                                                                                                                                      |
| McKeever et al., 2002 (UK)         | 1988-1999         | 0-10yrs           | Dose related exposure to any antibiotics                                 | <b>Wheezing</b><br>1 HR:<br>1.17 (1.08-1.26)<br>2 HR:<br>1.26 (1.13-1.41)<br>>2 HR:<br>1.60 (1.42-1.81)                                                                                                                                                                                                                                                          |
| Jedrychowski et al., 2006 (Poland) | 2000-2002         | 0-1 years         | Use of any antibiotics                                                   | <b>Wheezing</b><br><i>Cont. exposure</i><br>OR: 1.14 (1.01–1.27)<br><i>Binary exposure</i><br>OR: 4.42 (1.05– 18.8)                                                                                                                                                                                                                                              |
| Rusconi et al., 2007 (Italy)       | 2002              | 6-7 years         | Maternal health complications during pregnancy that call for antibiotics | <b>Wheezing</b><br><i>Urinary Tract Infection:</i><br><i>Transient:</i><br>OR 1.57 (1.20–2.06)<br><i>Persistent:</i><br>OR 1.31 (0.89-1.91)<br><i>Late-onset:</i><br>OR 1.13 (0.77-1.65)<br><i>Respiratory infection:</i><br><i>Transient:</i><br>OR 0.99(0.54-1.83)<br><i>Persistent:</i><br>OR: 2.91 (1.73–4.86)<br><i>Late-onset:</i><br>OR: 2.25 (1.32–3.83) |
| Dom et al., 2010 (Belgium)         | 1997-2001         | 0-4 years         | Use of any antibiotics                                                   | <b>Wheezing</b><br>OR: 1.29 (0.80–2.09)                                                                                                                                                                                                                                                                                                                          |
| Lapin et al., 2015 (USA)           | 1998-2004         | 4 weeks – 3 years | Use of any antibiotics                                                   | <b>Wheezing</b><br>OR: 1.76: (0.94, 3.28)                                                                                                                                                                                                                                                                                                                        |
| Popovic et al.,                    | 2005              | 0 – 18 months     | Use of any                                                               | <b>Wheezing</b>                                                                                                                                                                                                                                                                                                                                                  |

|                 |           |  |             |                                                                                                                                                                                                                                                                        |
|-----------------|-----------|--|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2015<br>(Italy) | (ongoing) |  | antibiotics | <i>Ever 1<sup>st</sup> Trimester</i><br>RR: 1.02 (0.80–1.30)<br><i>Recurrent 1<sup>st</sup> Trimester</i><br>RR: 0.99 (0.54–1.82)<br><i>Ever 3<sup>rd</sup> Trimester</i><br>RR: 1.12 (0.90–1.39)<br><i>Recurrent 3<sup>rd</sup> Trimester</i><br>RR: 2.09 (1.32–3.29) |
|-----------------|-----------|--|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Table S2.** Summary of six studies researching modes of delivery and wheezing in children.

| <b>Author</b>                       | <b>Year of Data Col.</b> | <b>Age of Children</b> | <b>Exposure</b>                                                 | <b>Outcome</b>                                                                                                                                                                                                                                                                    |
|-------------------------------------|--------------------------|------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Benn et al.,<br>2002<br>(Denmark)   | 1992-1994                | 0-3 years              | Maternal colonization<br>of microflora and<br>Cesarean delivery | <b>Infant wheezing</b><br><i>Cesarean delivery</i><br>OR: 1.2 (0.3-4.3)<br><i>NOT Cesarean delivery</i><br>OR: 2.3 (1.2-4.4)                                                                                                                                                      |
| Negele et al.,<br>2004<br>(Germany) | 1997-1999                | 0-2 years              | Cesarean section                                                | <b>Wheezing</b><br><i>Ever wheeze</i><br>OR: 1.31 (1.02-1.68)<br><i>Recurrent wheeze</i><br>OR: 1.41 (1.02-1.96)<br><i>Ever Wheeze w/o resp.<br/>infection</i><br>OR: 0.92 (0.49-1.74)                                                                                            |
| Rusconi et al.,<br>2007<br>(Italy)  | 2002                     | 6-7 years              | Cesarean delivery                                               | <b>Wheezing</b><br><i>Transient:</i><br>OR 1.04 (0.90–1.20)<br><i>Persistent:</i><br>OR 1.12 (0.93–1.36)<br><i>Late-onset:</i><br>OR 0.99 (0.83–1.19)                                                                                                                             |
| Magnus et al.,<br>2011              | 2001-2007                | 0-3 years              | Cesarean delivery:<br>Overall<br>Acute<br>Elective              | <b>Wheezing</b><br><i>Overall</i><br>OR: 1.03 (0.99, 1.07)<br><i>Acute:</i><br>OR: 1.04 (0.99, 1.09)<br><i>Elective:</i><br>OR: 1.00 (0.94, 1.07)                                                                                                                                 |
| Menezes et<br>al., 2011             | 1993 and<br>2004         | 4, 11, and<br>15 years | Cesarean delivery                                               | <b>Wheezing</b><br><i>Current/Persistent</i><br><i>1993:</i><br><u>4 yr</u><br>OR: 1.16 (0.81-1.68)<br><u>11yr</u><br>OR: 1.18 (0.94-1.48)<br><u>15yr</u><br>OR: 1.02 (0.80-1.31)<br><i>2004: (sample size too<br/>small for 11 and 15)</i><br><u>4yr</u><br>OR: 0.96 (0.81-1.15) |

|                                 |                   |           |                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                          |
|---------------------------------|-------------------|-----------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| van<br>Nimwegen et<br>al., 2011 | 2002<br>(ongoing) | 0-7 years | Parents that have atopy:<br>Cesarean section<br>Vaginal home delivery | <b>Wheezing</b><br><i>Unstratified:</i><br><i>Vaginal home</i><br>OR: 0.88 (0.74-1.04)<br><i>Cesarean section</i><br>OR: 1.05 (0.82-1.33)<br><i>Stratified:</i><br><i>Parental atopy</i><br><i>Vaginal home</i><br>OR: 0.89 (0.73-1.10)<br><i>Cesarean section</i><br>OR: 1.02 (0.77-1.36)<br><i>No Parental atopy</i><br><i>Vaginal home</i><br>OR: 0.85 (0.63-1.15)<br><i>Cesarean section</i><br>OR: 1.07 (0.70-1.65) |
|---------------------------------|-------------------|-----------|-----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|