

ORIGINAL ARTICLE

Pre- and post-natal exposure to antibiotics and the development of eczema, recurrent wheezing and atopic sensitization in children up to the age of 4 years

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Summary

Background Little data are available on the relationship between indirect antibiotic exposure of the child *in utero* or during lactation and allergic diseases. On the other hand, several studies have been conducted on the association with direct post-natal antibiotic exposure, but the results are conflicting.

Objective The aim of this study was to investigate pre- and post-natal antibiotic exposure and the subsequent development of eczema, recurrent wheeze and atopic sensitization in children up to the age of 4 years.

Methods We conducted an aetiological study in 773 children based on a prospective birth cohort project in which environmental and health information were collected using questionnaires. Antibiotic exposure was assessed as maternal antibiotic intake during pregnancy and during lactation and as medication intake of the child. The chronology of exposures and outcomes was taken into account during the data processing. At the age of 1 and 4 years, a blood sample was taken for the quantification of specific IgE.

Results Prenatal antibiotic exposure was significantly positively associated with eczema, whereas no association was found with recurrent wheeze and atopic sensitization. We found a positive, although statistically not significant, association between antibiotic exposure through breastfeeding and recurrent wheeze. Neither eczema nor atopic sensitization was significantly associated with antibiotic exposure through breastfeeding. Finally, we observed a negative association between the use of antibiotics in the first year of life and eczema and atopic sensitization, and also between antibiotic use after the first year of life and recurrent wheeze, eczema and atopic sensitization.

Conclusion Indirect exposure to antibiotics (*in utero* and during lactation) increases the risk for allergic symptoms in children, while direct exposure to antibiotics appears to be protective. The biological mechanisms underlying these findings still need to be elucidated.

Keywords allergy, antibiotics, children, eczema, wheezing

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Introduction

During the last decades, the prevalence of allergic diseases has increased world-wide in adults [1] and especially in children [2]. A possible explanation of this rise in prevalence was postulated by Strachan [3] in 1989 as 'the hygiene hypothesis'. According to this hypothesis, a

decrease of the microbial load in early life increases the risk of atopic immune responses (T-helper cells type 2), and by consequence, allergic disorders.

Together with changes in lifestyle and the health care system, the use of antibiotics has increased during the past three decades [4]. The coincidence of this increase in antibiotic use and the increase in the prevalence of

allergic diseases has led to the hypothesis of a causal relationship. Antibiotics may have an influence on the prevalence of allergic diseases through two pathways. Firstly, several studies have hypothesized that certain bacterial infections protect against atopy [5,6]. Antibiotics may remove this protective effect against allergies by modifying the natural course of these infections. A second possible explanation concerns the influence of antibiotics on the commensal bowel flora. Epidemiologic studies have shown that the composition of the gut flora is altered in atopic subjects, suggesting an important role for this flora in the maturation of the immune system [7,8]. The latter has been confirmed in animal models [9].

Several epidemiologic studies have addressed the association between the use of antibiotics in early life and the prevalence of allergic diseases in children. However, the reported results were conflicting. Some study groups found a significant positive association between early-life antibiotics use and allergic disorders [10–15], whereas others did not find any association [16,17]. Furthermore, only a few studies took the chronology of the exposure and outcome into account, resulting in an uncertainty whether the observed associations are causal or consequential (reverse causation). As far as we know, pre- and post-natal exposures (through medication and breastfeeding) were never included simultaneously in a single study. So far, only two studies have investigated the relationship between prenatal antibiotic exposure and the development of allergic diseases [18,19], and as far as we are aware, only one study has evaluated the exposure to antibiotics through breastfeeding and the subsequent development of allergies [20].

The objective of this study was to investigate the associations between pre- and post-natal exposure to antibiotics (through breastfeeding and through medication), and eczema, recurrent wheeze and atopic sensitization up to the age of 4 years in a prospective birth cohort, taking the chronology of exposures and outcomes into account.

Methods

Study design and population

This is an aetiological study, based on the Prospective Project on the Influence of Perinatal Factors on the Occurrence of Asthma and Allergies (PIPO cohort). In 1997, the PIPO project, a prospective birth cohort, started in the province of Antwerp, Belgium. Between June 1997 and December 2001, 2006 women, who were around 20 weeks pregnant, were recruited via four gynaecologists. Of the women who completed the screening questionnaire concerning their history of asthma and allergies, 1072 agreed to participate (53%), leading to a total study population of 1128 children. All participants resided in

the Antwerp urban or suburban area and had sufficient knowledge of the Dutch language. No other exclusion criteria were applied.

The Ethics Committee of the University of Antwerp granted approval for the assessments. Additionally, written consent was obtained from the parents before each assessment.

Subjects were included in the current study if information on exposure to antibiotics and at least one of the health outcomes was available.

Data collection

Maternal antibiotics use during pregnancy was assessed during home visits at 5 months of pregnancy and 3 months after delivery. During the latter home visit, information on antibiotic use during lactation was obtained. Data on post-natal exposure to antibiotics through medication were collected using postal questionnaires at the age of 1 year and subsequently every 6 months until the age of 4 years. Post-natal antibiotic exposure was divided into three categories according to the timing of the first exposure: not exposed, first exposure during the first year and first exposure after the first year.

Eczema in the children was also assessed by postal questionnaires. At the age of 1 year and subsequently every 6 months until the age of 4 years, the parents were asked the following questions; 'Has your child ever had an itching skin disease in the past 6 months?' and 'If yes, was it eczema?'. The localization of the skin rash was also verified to exclude contact dermatitis. If the questions on eczema were answered affirmatively at least once up to 4 years, the child was classified as having developed eczema in the first 4 years of life. Recurrent wheeze was defined as at least two episodes of parent-reported wheeze in a 6-month period up to the age of 4 years.

Co-variables that might explain or confound the potential associations between the use of antibiotics and the outcomes as described in the literature were taken into account. Parental allergic history was defined as one or more self-reported doctor's diagnoses of asthma, eczema or hayfever of the mother, the father or both, or atopic sensitization of at least one parent. Parental educational level was divided into three categories: (1) both parents who completed secondary school or lower, (2) one parent who had a Bachelors or Masters degree and (3) both parents who had a Bachelors or Masters degree. During two home visits (at 5 months pregnancy and 3 months after birth), information was collected on contact with cats or dogs, active and passive smoking during pregnancy, birth weight in kilogrammes, maternal age at birth, breastfeeding (ever/never) and number of older siblings. In the subsequent semi-annual questionnaires, information was obtained on day care attendance, contact with pets, environmental tobacco smoke exposure (ETS) and

lower respiratory tract infections (LRTI). All questionnaires were based on validated questionnaires of The International Study of Asthma and Allergy in Childhood [21].

Assessment of atopic sensitization

During the visit to the study centre in the University Hospital of Antwerp at the age of 1 year and 4 years, a blood sample was taken for quantification of allergen-specific IgE in the children and their parents. This was carried out with the aid of a fluorescence enzyme immunoassay technique (immunoCAP FEIA systems, Phadia, Brussels, Belgium). In the 1-year-old children, specific IgE against the mite *Dermatophagoides pteronyssinus*, cat, dog, hen's egg and cow's milk were determined in serum. In addition, in the 4-year-old children, specific IgE against birch pollen and timothy grass pollen were also quantified. In the parents, specific IgE against seven common inhalant allergens (*D. pteronyssinus*, cat, dog, birch pollen, timothy grass pollen, mugwort pollen and the fungus *Cladosporium herbarum*) were quantified. Specific IgE levels equal or higher than 0.35 kUa/L were considered as positive. Atopic sensitization was defined as having at least one positive specific IgE at the age of 1 and/or 4 years.

Statistical analyses

For the assessment of the crude and adjusted associations, the chronology of exposures and outcomes was taken into account. The onset of all post-natal exposures (antibiotics, cats or dogs, day care, ETS and LRTI) was evaluated for each outcome separately. To be regarded as exposed-positive, the exposure should be reported in a previous or the same questionnaire as the outcome. As a result, only children who were exposed before or at the onset of the outcome were regarded as exposed. Children were regarded as unexposed when the exposure was only reported after the onset of the outcome.

Because atopic sensitization, recurrent wheeze and eczema are status-type outcomes, prevalence odds ratios (ORs) and 95% confidence intervals (CIs) were computed as a measure of association between the outcome variables and exposure to antibiotics. Crude associations were assessed by contingency tables and evaluated by Pearson's χ^2 -tests. Adjusted associations were studied using multiple logistic regression models with a simultaneous inclusion of both antibiotic variables (pre- and post-natal) and potential confounders (gender, older siblings, parental educational level, allergic diseases of the parents, birth weight, maternal age, breastfeeding, day care attendance, active and passive smoking during pregnancy, ETS, pre- and post-natal exposure to pets and LRTI). The possibility of interaction between prenatal antibiotic exposure and

post-natal antibiotic exposure was tested in the multiple regression models for each outcome separately. Statistical analyses were performed using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

After exclusion of children with missing data on the key variables (pre- and post-natal antibiotic exposure and outcome variables), the study population consisted of 773 children (670 with data on eczema, 667 with data on recurrent wheeze and 450 with data on allergen-specific IgE).

Table 1 presents a description of the study population. The study population consisted of slightly more boys than girls (52.4% vs. 47.6%). In total, 19.9% of the mothers were exposed to antibiotics during pregnancy and 8.4% of the mothers used antibiotics during the first 3 months of lactation. Only 6.9% of the children never received any antibiotics up to the age of 4 years. Most of the children received antibiotics in the first year of life (67.5%). Up to the age of 4 years, 36.3% of the children had eczema, 35.2% had recurrent wheeze and 35.6% had specific IgE against the allergens tested (14.4% house dust mite, 7.1% cat, 3.2% dog, 10.7% milk, 18.2% egg white, 5.6% pollen and 7.5% timothy grass pollen). Children with atopic sensitization were more likely to have eczema and recurrent wheeze than children without atopic sensitization (57.2% vs. 22.8% and 38.9% vs. 28.3%, respectively).

The crude associations between potential confounders and pre- and post-natal antibiotic exposures are summarized in Table 2.

Crude and adjusted associations between prenatal antibiotic exposure, post-natal antibiotic exposure through breastfeeding and post-natal antibiotic exposure through medication and recurrent wheeze up to the age of 4 years are presented in Table 3. Fifteen of the 164 children, who were exposed to antibiotics after the first year of life, already had recurrent wheeze before the first exposure to antibiotics. By consequence, for the analyses with recurrent wheeze as an outcome, these children were reclassified as not exposed. No significant association between recurrent wheeze and prenatal antibiotic exposure or post-natal antibiotic exposure through breastfeeding was found. After adjustment for co-variables, we found a positive trend between post-natal antibiotic exposure through breastfeeding and recurrent wheeze up to the age of 4 years (OR 1.80, 95% CI 0.93–3.46). Antibiotic exposure through medication intake in the first year of life was not significantly associated with recurrent wheeze, whereas exposure to antibiotics through medication after the first year was significantly negatively associated with recurrent wheeze up to the age of 4 years (OR 0.47, 95% CI 0.23–0.94). Confounders responsible for the shift in OR for the association between recurrent wheeze and antibiotic

Table 1. Description of the study population

Individual characteristics	Number (<i>n/d</i>)*	Percentage (%)
Gender: male	405/773	52.4
Siblings	425/773	55.0
Parental education		
Both low	103/675	15.3
One high	169/675	25.0
Both high	403/675	59.7
Parental allergies	519/624	83.2
Breastfeeding ever	622/773	80.5
Prenatal exposure to cats and/or dogs	294/769	38.2
Post-natal exposure to cats and/or dogs	448/662	67.7
Day care attendance	713/733	97.3
Prenatal smoke		
No	566/761	74.4
Passive	127/761	16.7
Active	35/761	4.6
Passive and active	33/761	4.3
Post-natal environmental tobacco smoke	124/618	20.1
Lower respiratory tract infections	253/622	40.7
Eczema ever	243/670	36.3
Recurrent wheeze ever	235/667	35.2
Atopic sensitization	160/450	35.6
Prenatal exposure to antibiotics	154/773	19.9
Exposure to antibiotics through breastfeeding	65/773	8.4
Exposure to antibiotics through medication		
Never	68/773	8.8
Introduction in the first year	522/773	67.5
Introduction after the first year	183/773	23.7
Combinations of antibiotics exposure		
None	53/773	6.9
Prenatal only	9/773	1.2
Through breastfeeding only	5/773	0.6
Through medication only	518/773	67.0
Prenatal and through breastfeeding	1/773	0.1
Prenatal and through medication	128/773	16.6
Through breastfeeding and medication	43/773	5.6
Prenatal and through breastfeeding and medication	16/773	2.1

**n*, Number of children with each characteristic; *d*, the total number of children with information available on this characteristic.

exposure through breastfeeding were post-natal exposure to cat and/or dog and LRTI, and for the association between recurrent wheeze and post-natal antibiotic exposure through medication were LRTI. Furthermore, in the logistic regression model, passive exposure to cigarette smoke during pregnancy, LRTI and male gender were significantly positively associated with recurrent wheeze (OR 2.17, 95% CI 1.32–3.58; OR 1.56, 95% CI 1.02–2.37 and OR 1.99, 95% CI 1.33–2.99, respectively), whereas day care attendance, post-natal exposure to cat or dog and birth weight were negatively associated (OR 0.34, 95% CI 0.15–0.73; OR 0.57, 95% CI 0.35–0.93 and OR 0.63, 95% CI 0.42–0.94, respectively) with this outcome.

Associations between antibiotic exposure and eczema are shown in Table 4. Thirty-eight of the 167 children, who were exposed to antibiotics after the first year of life, already had eczema before the first exposure to antibiotics. By consequence, for the analyses with eczema as an outcome, these children were reclassified as not exposed. After adjustment for potential confounders, children whose mothers used antibiotics during pregnancy were significantly more likely to have eczema up to the age of 4 years (OR 1.82, 95% CI 1.14–2.92). Post-natal exposure to antibiotics through breastfeeding was not associated with eczema up to the age of 4 years, whereas post-natal antibiotic exposure through medication was negatively associated with eczema. This association was statistically significant for exposure after the first year but only borderline significant for exposure in the first year of life (OR 0.11, 95% CI 0.05–0.23 and OR 0.61, 95% CI 0.36–1.01, respectively). Confounders responsible for the shift in OR for the association between eczema and post-natal antibiotic exposure through medication were post-natal environmental tobacco smoke and day care attendance. Furthermore, of the co-variables, parental allergy and prenatal active and passive exposure to cigarette smoke were significantly positively associated with eczema (OR 2.03, 95% CI 1.08–3.80; OR 2.49, 95% CI 1.01–6.11 and OR 2.17, 95% CI 1.28–3.69, respectively), whereas post-natal exposure to cat or dog and day care attendance were significantly negatively associated (OR 0.42, 95% CI 0.25–0.69 and OR 0.23, 95% CI 0.11–0.50, respectively).

Associations between pre- and post-natal exposures to antibiotics and atopic sensitization up to the age of 4 years are summarized in Table 5. Note that the proportions of the children who were assessed for IgE in each of the initial exposure by medication categories for never, first year and after the first year, before adjustment for the timing of exposure and outcome, are 44/68 (64.7%), 301/522 (57.7%) and 105/183 (57.4%), respectively. Twenty-two of the 105 children, who were exposed to antibiotics after the first year of life, already had positive specific IgE at the age of one year. Therefore, for the analyses with atopic sensitization as an outcome these children were reclassified as not exposed. Neither prenatal antibiotics nor antibiotics exposure through breastfeeding were significantly associated with atopic sensitization up to the age of 4 years. However, post-natal antibiotics exposure through medication in the first year as well as after the first year were significantly negatively associated with atopic sensitization in children up to the age of 4 years (crude OR 0.38, 95% CI 0.22–0.65 and crude OR 0.15, 95% CI 0.07–0.32, respectively). After adjustment for potential confounders, these associations remained significant (OR 0.38, 95% CI 0.21–0.71 and OR 0.15, 95% CI 0.07–0.34, respectively). The main confounder responsible for the shift in OR for the association between atopic sensitization and prenatal antibiotic exposure was day care attendance.

Table 2. Associations between the potential confounders and pre- and post-natal antibiotic exposure

Individual characteristics	Prenatal antibiotics		Antibiotics through breastfeeding		Antibiotics through medication in the first year		Antibiotics through medication after the first year	
	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)	%	OR (95% CI)
Gender								
Female	20.7	Ref.	9.0	Ref.	60.9	Ref.	79.1	Ref.
Male	19.3	0.92 (0.64–1.31)	7.9	0.87 (0.52–1.45)	73.8	1.81 (1.33–2.46)	81.4	1.16 (0.80–1.69)
Siblings								
No	15.8	Ref.	6.9	Ref.	67.1	Ref.	85.6	Ref.
Yes	23.3	1.62 (1.12–2.33)	9.6	1.44 (0.85–2.44)	68.2	1.05 (0.77–1.42)	75.8	0.52 (0.36–0.78)
Parental education								
Both low	20.4	Ref.	5.8	Ref.	65.0	Ref.	83.0	Ref.
One high	18.9	0.91 (0.49–1.69)	7.1	1.24 (0.45–3.40)	65.7	1.03 (0.62–1.72)	81.2	0.88 (0.45–1.73)
Both high	19.1	0.92 (0.53–1.58)	9.7	1.73 (0.71–4.21)	67.2	1.10 (0.70–1.74)	78.4	0.75 (0.41–1.35)
Parental allergies								
No	15.2	Ref.	11.4	Ref.	67.6	Ref.	76.8	Ref.
Yes	22.0	1.57 (0.88–2.77)	7.7	0.65 (0.33–1.28)	67.5	1.00 (0.64–1.56)	80.6	1.26 (0.74–2.13)
Breastfeeding								
No	21.9	Ref.	0.0	Ref.	76.0	Ref.	84.8	Ref.
Yes	19.5	0.86 (0.56–1.33)	10.5	–	65.7	0.61 (0.40–0.91)	79.2	0.68 (0.40–1.14)
Prenatal cats and/or dogs								
No	19.6	Ref.	8.4	Ref.	67.0	Ref.	79.7	Ref.
Yes	20.4	1.05 (0.73–1.51)	8.5	1.01 (0.60–1.70)	68.4	1.06 (0.78–1.45)	81.4	1.12 (0.75–1.65)
Post-natal cats and/or dogs								
No	16.8	Ref.	8.4	Ref.	60.3	Ref.	77.0	Ref.
Yes	21.2	1.33 (0.87–2.03)	8.0	0.95 (0.53–1.72)	67.9	1.39 (0.99–1.95)	79.7	1.17 (0.79–1.74)
Day care attendance								
No	10.0	Ref.	10.0	Ref.	45.0	Ref.	75.0	Ref.
Yes	20.1	2.26 (0.52–9.84)	8.7	0.86 (0.19–3.78)	68.1	2.61 (1.07–6.38)	80.4	1.37 (0.49–3.82)
Prenatal smoke								
No	19.3	Ref.	9.0	Ref.	66.9	Ref.	80.7	Ref.
Passive	23.6	1.30 (0.82–2.05)	6.3	0.68 (0.31–1.47)	66.9	1.00 (0.67–1.51)	78.3	0.86 (0.52–1.41)
Active	20.0	1.05 (0.45–2.46)	8.6	0.95 (0.28–3.20)	82.4	2.31 (0.94–5.67)	86.2	1.49 (0.51–4.38)
Passive and active	18.2	0.93 (0.38–2.31)	6.1	0.65 (0.15–2.80)	60.6	0.76 (0.37–1.56)	78.6	0.87 (0.35–2.21)
Post-natal smoke								
No	19.6	Ref.	8.1	Ref.	61.5	Ref.	77.2	Ref.
Yes	19.4	0.98 (0.60–1.61)	8.1	1.00 (0.48–2.05)	70.2	1.47 (0.96–2.26)	79.3	1.13 (0.68–1.87)
Lower respiratory tract infections								
No	16.8	Ref.	8.7	Ref.	61.0	Ref.	67.8	Ref.
Yes	22.9	1.47 (0.99–2.20)	8.7	1.00 (0.57–1.77)	66.7	1.28 (0.92–1.79)	93.5	6.81 (3.92–11.83)

Table 3. The associations between pre- and post-natal antibiotic exposure and recurrent wheeze up to the age of 4 years

Antibiotic exposure*	Recurrent wheeze ever up to 4 years				
	<i>n/N</i> (%)	Crude OR (95% CI)	<i>P</i> -value	Adjusted [†] OR (95% CI)	<i>P</i> -value
Prenatal antibiotics					
No	183/536 (34.1)	1.00 (ref.)		1.00 (ref.)	
Yes	52/131 (39.7)	1.27 (0.86–1.88)	0.22	1.29 (0.80–2.09)	0.30
Post-natal antibiotics					
Through breastfeeding					
No	211/612 (34.5)	1.00 (ref.)		1.00 (ref.)	
Yes	24/55 (43.6)	1.47 (0.84–2.57)	0.17	1.80 (0.93–3.46)	0.08
Through medication					
Not exposed	25/81 [‡] (30.9)	1.00 (ref.)		1.00 (ref.)	
Exposed in the first year	175/437 [‡] (40.0)	1.50 (0.90–2.49)	0.12	0.91 (0.51–1.63)	0.75
Exposed after the first year	35/149 [‡] (23.5)	0.69 (0.38–1.26)	0.23	0.47 (0.23–0.94)	0.03

*Associations of pre- and post-natal (through breastfeeding and medication) exposure were independent from each other because all were included in the logistic regression model simultaneously.

[†]Adjusted for gender, maternal age, birth weight, siblings, breastfeeding, pre- and post-natal exposure to cats or dogs, pre- and post-natal exposure to cigarette smoke, day care attendance, parental education and parental history of allergies, LRTI.

[‡]The discrepancy in numbers of (not) exposed through medication between this table and Table 1 is due to the correction for the timing of exposure and outcome.

LRTI, lower respiratory tract infections.

Table 4. The associations between pre- and post-natal antibiotic exposure and eczema up to the age of 4 years

Antibiotic exposure*	Eczema ever up to 4 years				
	<i>n/N</i> (%)	Crude OR (95% CI)	<i>P</i> -value	Adjusted [†] OR (95% CI)	<i>P</i> -value
Prenatal antibiotics					
No	178/536 (33.2)	1.00 (ref.)		1.00 (ref.)	
Yes	65/134 (48.5)	1.90 (1.29–2.78)	0.001	1.82 (1.14–2.92)	0.01
Post-natal antibiotics					
Through breastfeeding					
No	223/616 (36.2)	1.00 (ref.)		1.00 (ref.)	
Yes	20/54 (37.0)	1.04 (0.58–1.85)	0.90	1.14 (0.56–2.35)	0.90
Through medication					
Not exposed	51/106 [‡] (48.1)	1.00 (ref.)		1.00 (ref.)	
Exposed in the first year	178/435 [‡] (40.9)	0.75 (0.49–1.14)	0.18	0.61 (0.36–1.01)	0.05
Exposed after the first year	14/129 [‡] (10.9)	0.13 (0.07–0.26)	<0.01	0.11 (0.05–0.23)	<0.01

*Associations of pre- and post-natal exposure are independent from each other because all were included in the logistic regression model simultaneously.

[†]Adjusted for gender, maternal age, birth weight, siblings, breastfeeding, pre- and post-natal exposure to cats or dogs, pre- and post-natal exposure to cigarette smoke, day care attendance, parental education and parental history of allergies.

[‡]The discrepancy in numbers of (not) exposed through medication between this table and Table 1 is due to the correction for the timing of exposure and outcome.

Furthermore, in the logistic regression model high parental education was significantly positively associated with atopic sensitization (OR 3.42, 95% CI 1.56–7.37), whereas day care attendance was significantly negatively associated (OR 0.12, 95% CI 0.04–0.31).

For all outcomes, we investigated the possibility of interaction between prenatal exposure and post-natal antibiotic exposure in the multiple regression models. No statistically significant associations were found between the interaction terms and any of the outcome

variables, and were therefore, omitted from the regression models.

Discussion

The results of our study in a prospective birth cohort showed that prenatal antibiotic exposure was significantly positively associated with eczema up to the age of 4 years, whereas no significant association was found with recurrent wheeze and atopic sensitization. Neither

Table 5. The associations between pre- and post-natal antibiotic exposure and atopic sensitization up to the age of 4 years

Antibiotic exposure*	Atopic sensitization ever up to 4 years				
	n/N (%)	Crude OR (95% CI)	P-value	Adjusted [†] OR (95% CI)	P-value
Prenatal antibiotics					
No	125/359 (34.8)	1.00 (ref.)		1.00 (ref.)	
Yes	35/91 (38.5)	1.17 (0.73–1.88)	0.52	1.38 (0.80–2.38)	0.24
Post-natal antibiotics					
Through breastfeeding					
No	144/412 (35.0)	1.00 (ref.)		1.00 (ref.)	
Yes	16/38 (42.1)	1.35 (0.69–2.66)	0.38	1.30 (0.60–2.84)	0.51
Through medication					
Not exposed	39/66 [‡] (59.1)	1.00 (ref.)		1.00 (ref.)	
Exposed in the first year	106/301 [‡] (35.2)	0.38 (0.22–0.65)	<0.01	0.38 (0.21–0.71)	<0.01
Exposed after the first year	15/83 [‡] (18.1)	0.15 (0.07–0.32)	<0.01	0.15 (0.07–0.34)	<0.01

*Associations of pre- and post-natal (through breastfeeding and medication) exposure were independent from each other because all were included in the logistic regression model simultaneously.

[†]Adjusted for gender, maternal age, birth weight, siblings, breastfeeding, pre- and post-natal exposure to cats or dogs, pre- and post-natal exposure to cigarette smoke, day care attendance, parental education and parental history of allergies.

[‡]The discrepancy in numbers of (not) exposed through medication between this table and Table 1 is due to the correction for the timing of exposure and outcome.

eczema nor recurrent wheeze nor atopic sensitization was significantly associated with antibiotic exposure through breastfeeding. Antibiotic intake during the first year was negatively associated with eczema and atopic sensitization. A negative association was also found between antibiotic use after the first year of life and recurrent wheeze, eczema and atopic sensitization up to the age of 4 years.

As far as we know, only two studies have investigated the association between prenatal antibiotic exposure and allergic diseases [18,19]. They both found a positive association between prenatal antibiotic use and wheeze, eczema and hayfever. However, both studies did not correct for post-natal antibiotic exposure in their models. As suggested by Thomas and Price [22], it is important to investigate the association with maternal antibiotic exposure during pregnancy and subsequent child antibiotic exposure independently because different families may show different antibiotic seeking behaviour. The authors concluded that prenatal antibiotic exposure may be a marker for post-natal antibiotic exposure rather than a causal factor for the health outcomes. To our knowledge, our study is the first to include prenatal and post-natal antibiotic exposure simultaneously in a multiple regression model, and also the first to investigate the association between prenatal antibiotics and atopic sensitization. After adjusting for post-natal antibiotics, we found that prenatal antibiotic exposure was independently positively associated with eczema up to the age of 4 years, whereas no significant association was found with recurrent wheeze and atopic sensitization. This finding cannot be explained by the effect of antibiotics on the microbial gut flora because the gut remains sterile *in utero*. Besides

prenatal antibiotics, infections and other complications during pregnancy have also been related to an increased risk of eczema [19]. A possible common explanation for the associations found in this study could be stress during pregnancy. Maternal and fetal stress stimulate the placental secretion of corticotrophin-releasing hormone, which in turn is elevated in the neonatal circulation. This stimulates the fetal hypothalamic–pituitary–adrenocortical axis to secrete glucocorticoids driving the TH1/TH2 balance towards a TH2 cytokine profile [23, 24].

In contrast to prenatal antibiotics, antibiotics used by the mother during breastfeeding can modify the infant's commensal bowel flora and as such affect the function of the immune system [9]. At present, only one other research team has reported on the relationship between antibiotic exposure through breastfeeding and the subsequent development of allergic diseases [20]. This prospective study showed that antibiotic exposure through breastfeeding was associated with a higher risk of recurrent wheeze, whereas no association with eczema and allergic sensitization could be demonstrated. Our findings also did not show a significant association between breastfeeding and eczema and allergic sensitization, whereas a positive trend could be seen for recurrent wheeze.

Several studies have investigated the association between post-natal antibiotic use and the prevalence of allergic diseases, with conflicting results. Some studies found a positive association between early life antibiotic exposure and eczema, hayfever, atopic sensitization, wheeze and asthma [10–12]. Some studies could only find a positive association with wheeze or asthma, but not with allergic conditions like eczema, hayfever or atopic

sensitization [20,25], whereas others did not find any association between post-natal antibiotics and allergic diseases [16,17].

In general, two potential pitfalls may complicate the interpretation of the associations between post-natal exposures and health outcomes. The first one is the correct timing of exposure and outcome. In order to have a genuine causal association, it is a *conditio sine qua non* that the onset of the exposure precedes the onset of the outcome. The second pitfall is 'confounding-by-indication', i.e. that the association between the exposure (antibiotics) and the outcome (wheeze) may be explained by a third factor (respiratory tract infections), which is related to the exposure as well as the outcome. The most commonly used methods to avoid these problems are the exclusion of subjects with respiratory symptoms during the studied antibiotic exposure period [15, 20], exclusion of subjects who received antibiotics for illnesses associated with wheeze [17] and adjustment for LRTI in the multiple regression models [11, 26, 27]. However, these correction methods only tackle one pitfall, namely 'confounding-by-indication'. Owing to the prospective data collection in the PIPO birth cohort, we were not only able to correct for 'confounding-by-indication' by adjustment for LRTI in the regression model for wheeze, but we were also able to take into account the timing of the onset of the outcome and the exposure to antibiotics and confounders (contact with cat or dog, ETS, day care, LRTI). Nevertheless, it should be noted that the nature of wheeze is not always clear, especially in very young children. Consequently, the occurrence of recurrent wheeze does not necessarily imply asthma.

In contrast to previous studies, we found a negative association between exposure to antibiotics through medication in the first year and eczema and atopic sensitization. Furthermore, a negative association was also found between post-natal antibiotic exposure after the first year and recurrent wheeze, eczema and atopic sensitization up to the age of 4 years. The reason for these deviating results is most likely the adjustment of the exposure status in accordance with the timing of exposures and outcomes. After repeating these analyses without this adjustment, post-natal antibiotic exposure appeared to be positively associated with eczema and recurrent wheeze, while no association was found with atopic sensitization (data not shown). The results of these uncorrected analyses were similar to the findings of previous studies [25, 28].

In this study, we found positive associations when the child's exposure to antibiotics was indirect (prenatal or through breastfeeding), whereas negative associations were found with direct exposure to antibiotics through medication. The main difference between these two types of exposures is that exposure to antibiotics through medication is usually preceded by an infection of the child, while indirect exposure is not. Some studies have

hypothesized that infections protect against allergies [5, 29]. It is conceivable that the children's immune system is already sufficiently stimulated when they receive antibiotics, causing the protective effect of the infection to dominate over the effect of the antibiotics.

This study has some methodological limitations. The chance of selection bias has to be considered in the interpretation of the results. For example, higher educated parents are more likely to participate in an epidemiological study than those with a lower education. This may explain the high percentage of highly educated parents in this study. Parents with allergic diseases also tend to participate more in a study on asthma and allergies. Consequently, the prevalence of allergic diseases is likely to be higher than in the general Belgian population. Although bias caused by selective attrition cannot be ruled out because data for bias estimation is not available, preliminary results of a qualitative survey in a small sample of the parents of the PIPO cohort showed that the reasons for discontinuing the study were not related to exposure to antibiotics or outcome variables [30]. Moreover, other studies have shown that loss to follow-up does not necessarily bias associations between early life factors and health outcomes [31, 32].

Furthermore, although we took into account the appropriate order of exposure and outcome, a residual chance of misclassification may still remain. We considered a child as 'exposed' if a question about the exposure was answered affirmatively in a previous or the same questionnaire as the outcome. However, when both questions were answered affirmatively in the same questionnaire, some uncertainty about the appropriate sequence remained. Consequently, some of the children who were classified as 'exposed', may in fact have been 'unexposed'. In order to estimate to what degree our results were influenced by the definition of 'exposed', we also performed the analyses using an alternative, more restricted definition, i.e. the children were considered as 'exposed' only when exposure was reported in a questionnaire before the outcome. When using this alternative definition the associations between post-natal antibiotic exposure and the outcomes were even more negative (data not shown). In the first year, the exposure to antibiotics and the occurrence of symptoms in the first year were assessed using only one questionnaire comprising a 12-month period. Therefore, the chance of misclassification was higher for this period than for the subsequent periods (between the age of 12 and 48 months), which were covered by 6-monthly questionnaires. This may explain the weaker associations that were found for the exposure to antibiotics through medication in the first year of life as compared with antibiotic use after the first year of life.

In conclusion, children with an indirect exposure to antibiotics *in utero* and through breastfeeding were more likely to acquire eczema and recurrent wheeze,

respectively, up to 4 years of age. These results are consistent with the findings of previous studies. However, in contrast to earlier studies, we found that children with direct antibiotic exposure, i.e. through medication, were at less risk of allergic symptoms and sensitization up to the age of 4 years. This contradictory result is most likely due to the fact that the chronology of the exposures and the outcomes was taken into account in our study. Therefore, it is recommended that future studies on post-natal antibiotic exposure also take this temporal sequence into account in their study design. Further research is needed to confirm the associations found in this study.

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