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Gut : 10 January 2018. [Published Online First]

DOI: 10.1136/gutjnl-2017-314352

Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease – a population-based study

Short title: Antibiotics and inflammatory bowel disease

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ABBREVIATIONS

aHR – adjusted Hazard Ratio
CD - Crohn's disease
cHR – crude Hazard Ratio
CI – Confidence Interval
IBD – Inflammatory Bowel Disease
MBR – Medical Birth Register
NPR – National Patient Register

PcV – Phenoxymethylpenicillin

PIN – Personal Identification Number

SPDR – Swedish Prescribed Drug Register

TPR – Total Population Register

UC – Ulcerative Colitis

VEO-IBD – Very Early Onset Inflammatory Bowel Disease

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KEY WORDS: antibiotics; Crohn's disease; Ulcerative colitis; population-based registers; very early onset (VEO) IBD

WORD COUNT: 4309

REFERENCES: 56

TABLES: 5

FIGURES: 2

ABSTRACT

Objective Earlier studies on antibiotics exposure and development of inflammatory bowel disease (IBD, Crohn's disease and ulcerative colitis) may have been biased by familial factors and gastroenteritis. We aimed to estimate the association between antibiotics during pregnancy or infantile age and very early onset (VEO-) IBD.

Design In this cohort study of 827 239 children born in Sweden 2006-2013, we examined the link between exposure to systemic antibiotics and VEO-IBD (diagnosis <6 years of age), using Cox proportional hazard regression models. Information on antibiotics and IBD was retrieved from the nationwide population-based Swedish Prescribed Drug Register and the National Patient Register. We specifically examined potential confounding from parental IBD and gastroenteritis.

Results Children exposed to antibiotics during pregnancy were at increased risk of IBD compared to general population controls (adjusted hazard ratio (aHR) 1.93; 95% confidence interval (CI) 1.06-3.50). Corresponding aHRs were 2.48 (1.01-6.08) for Crohn's disease (CD) and 1.25 (0.47-3.26) for ulcerative colitis (UC) respectively. For antibiotics in infantile age, the aHR for IBD was 1.11 (0.57-2.15); for CD 0.72 (0.27-1.92) and 1.23 (0.45-3.39) for UC. Excluding children with gastroenteritis 12 months prior to the first IBD diagnosis retained similar aHR for antibiotics during pregnancy and CD, while the association no longer remained significant for IBD.

Conclusion We found that exposure to antibiotics during pregnancy, but not in infantile age, is associated with an increased risk of VEO-IBD regardless of gastroenteritis. The risk increase for exposure in pregnancy may be due to changes in the microbiota.

Summary "box"

What is already known about this subject? Very early onset (VEO-) inflammatory bowel disease (IBD) has gradually become more common. One potential risk factor for VEO-IBD is antibiotic exposure during pregnancy and in infantile age.

What are the new findings? In this population-based study of more than 800 000 children, we found a positive association between antibiotics exposure during pregnancy, but not in infantile age, and later VEO-IBD.

How might it impact on clinical practice in the foreseeable future? The risk increase for exposure in pregnancy may be due to changes in the microbiota, which could have an impact on care of pregnant women. However, the absolute risk of disease was very low, and antibiotics during pregnancy should still be used when needed.

INTRODUCTION

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic inflammation of the gastrointestinal tract. Symptoms related to the disease include diarrhoea, rectal bleeding, abdominal pain and weight loss. Although children (diagnosed at <18 years of age) may present with these classical symptoms, non-specific symptoms such as growth failure, anaemia and other extra-intestinal manifestations are also common manifestations of paediatric IBD.[1] Interestingly, patients with very early onset (VEO)-IBD (defined as diagnosed before six years of age) seem to represent a specific entity, possibly more likely to present with rectal bleeding due to a colonic phenotype (in CD)[2] and a family history of IBD.[2-5] This disease entity has gradually become more common and recent data suggest that the increase in IBD incidence is more pronounced in patients with VEO-IBD than among children ≥6 years old, although the overall numbers are still very small compared to young adults.[6]

The pathogenesis is characterized by a complex interaction between genetics, an aberrant mucosal immune response to gut microbiota, disruption of gut barrier, and environmental triggers. While genetic factors clearly play an important role in the etiology of IBD,[7-10] they cannot explain the recent rise in disease incidence or the proband concordance rate of 38-62% in monozygotic twins with CD (even less in UC).[11 12] This has encouraged a search for environmental factors and led to the identification of factors such as smoking, oral contraceptives and appendectomy due to appendicitis (data on appendectomy have however been contradictory)[13] as potential risk factors in adulthood IBD while less is known for childhood IBD.[14-19]

To what extent pathophysiologic mechanism of IBD can be applied to VEO-IBD remains partly unknown. It has been proposed that the influence of genetics might be more pronounced in VEO-IBD, due to the effect of rare variants with a high penetrance for IBD.[20] However, the role of environmental risk factors in VEO-IBD is largely unknown.

One potential risk factor for VEO-IBD is antibiotic exposure during pregnancy and in infantile age. Ungaro *et al*. reported an increased risk of IBD following antibiotic exposure, especially in children, in a recent meta-analysis of eleven observational studies.[21] The association was limited to individuals with newly onset CD and not seen for UC. Some earlier studies have also found a positive dose-response relationship between antibiotics exposure and later IBD, but whether this also applies to VEO-IBD remains unknown.

Therefore, we aimed to estimate the association between antibiotics during pregnancy or infantile age and VEO-IBD (CD and UC), while adjusting for parental IBD, as well as taking gastroenteritis and number of doses into account in a population-based study.

METHOD

Study population and register linkage

This nationwide prospective population-based register study included all children born January 2006 to December 2013, identified together with their mothers from the Swedish Medical Birth Register (MBR). Fathers to the children were identified through the Swedish Multi-generation Register.[22] We used the Swedish Prescribed Drug Register (SPDR)[23] and the National Patient Register (NPR)[24] to retrieve data on exposure and outcome. The SPDR contains complete data on all dispensed drugs from pharmacies since July 2005 while the NPR began in 1964, became nationwide in 1987 (inpatient diagnoses only), and added hospital-based outpatient visit data in 2001. Data from the different registers were linked using the unique personal identity number assigned to all residents in Sweden.[25]

Variables

Antibiotic exposure was defined as filled prescriptions of relevant ATC codes (Anatomical Therapeutic Chemical): J01A-J01X (systemic antibiotics), and were obtained through the SPDR. We categorised antibiotics into two groups: "systemic antibiotics" which included any type of antibiotics; and "PcV" (Phenoxymethylpenicillin), which is by far the most commonly prescribed type of antibiotic in Sweden both during pregnancy and in childhood.[26]

Our outcome IBD was defined as having ≥ 2 diagnoses of either CD or UC according to relevant International Classification of Disease (ICD)-10 codes K50 and K51 respectively recorded in the NPR, with onset before 6 years of age. Crohn's disease was defined as ≥ 2 CD diagnoses but never an ulcerative colitis diagnosis and UC was defined as ≥ 2 UC diagnoses but never a CD diagnosis. These outcome definitions have previously been validated by Jakobsson *et al.* who found a positive predictive value (PPV) of 93% (95% CI: 87–97) for

IBD, 90% (77–97) for UC and 81% (67–91) for CD, when compared to the Copenhagen criteria.[27] While IBD-unclassified is now regarded as a separate entity (and some data suggests that it may represent up to 20% of the total pediatric IBD population),[28] this paper focused on CD and UC.

Co-variates; Through the MBR we retrieved information on the child's date of birth, sex, gestational age (days), mode of delivery (vaginal or caesarean section), maternal smoking at first visit to the antenatal care clinic (yes/no), maternal age at delivery (≤19, 20-24, 25-29, 30-34, or ≥35 years), and parity (child's birth order at current delivery; first-born (1) or not (≥2)). Data from Cnattingius et al. suggest a high quality of data in the MBR with a coverage of >98% of all births in Sweden.[29]

Maternal and paternal IBD was defined from the NPR similarly to that of the children (two records of either: ICD-8: 563.00, 563.10, 569.02; ICD-9: 555, and ICD-10: K50 for CD; and ICD-8: 563.98, 563.99; ICD-9: 556, and ICD-10: K51 for UC). Information on highest level of education of either parent (0-12 vs >12 years) was identified through the Longitudinal integration database for health insurance and labour market studies and parents' country of birth (Sweden or other) was identified from the Total Population Register (TPR).

We defined the beginning of the pregnancy (conception date) as date of birth minus gestational age in days. We furthermore divided pregnancy duration into trimester 1 (day 1-91), 2 (day 92-189), and 3 (day 190+) to examine time-varying effects of fetal antibiotics exposure. To ensure that the full pregnancy would be covered by this study (especially antibiotics exposure in the first trimester in offspring born close to the starting point of the SPDR), we restricted our study population to children with estimated conception date as on or after July 1st, 2005. Information on migration and death was obtained from the TPR to be able to define end of

follow-up. Offspring to women who immigrated to Sweden during pregnancy were excluded from the study (n=14 030).

Statistical analysis

The risk of IBD, and CD and UC separately, in children exposed to antibiotics during pregnancy and in infantile age compared to unexposed children, was examined in Cox proportional hazard models. Attained age was used as the underlying time scale and clustering within families was taken into account by using a sandwich estimator for the standard errors. Follow-up ended with first IBD diagnosis, emigration, death or end of study period (December 31st, 2014 i.e at least one year of follow-up), whichever happened first. The proportional hazards assumption was tested using Schoenfeld's residuals (*p*-values of 0.58 for exposure during pregnancy and 0.84 for exposure in infantile age). Antibiotic exposure during pregnancy was regarded as exposed or non-exposed at the start of follow-up, while exposure after birth was modelled as a time(age)-varying exposure, i.e. a model in which all individuals start as unexposed and then the exposure status changes at the time an individual becomes exposed. However, as there was no sign of non-proportional hazards we did not allow for the effect of the exposure to vary over time.

Potential confounders were identified based on the Directed Acyclic Graphs (DAG) concept,[30] and the final models were adjusted for: mother's and father's history of IBD, parental education, mother's and father's country of birth (in analyses of exposure during pregnancy and in childhood) and mode of delivery (in analysis of exposure in infantile age) as shown in *Figure 1*. Those with missing information on these variables were excluded (n=2848 for exposure in pregnancy and n=3274 for exposure in infantile age) and complete case analyses were performed. Crude and adjusted Hazard Ratios (HR) are presented.

Additional analyses: Sensitivity analyses were performed by excluding all individuals who had been diagnosed with gastroenteritis, either bacterial or viral, according to ICD 10 codes A00-A09, within 12 months prior to onset of their first IBD diagnosis. The risk of IBD with onset after 2 years of age in children exposed to systemic antibiotics in the first year of life, was further tested to investigate potential information bias such as misclassification or reverse causation. This since a possible misclassification of first occurrence of IBD symptoms as gastroenteritis, treated with antibiotics, may induce an association between antibiotics and IBD. A potential dose-relationship between number of antibiotics prescriptions in childhood (1, 2 or ≥3 as a continuous variable) and IBD was tested. Dispensed prescriptions of the same type of antibiotics within 7 days from the prior dispense was counted as one dispense. Too few women had filled more than one prescription of antibiotics during pregnancy to be able to perform dose-response-analyses. The role of timing of systemic antibiotics exposure during the fetal period (1^{st} vs 2^{nd} vs 3^{rd} trimester) and IBD was further explored. We also examined if the risk increase by antibiotic exposure was dependent on parity by adding interaction terms between parity (first-born vs. non-first born) and antibiotic exposure.

STATA statistical software (version 14) was used for all statistical analyses.

The study was approved by the Regional Ethical Review board in Stockholm, Sweden.

RESULTS

The final study population consisted of 827 239 children (*Figure 2*). Some 12 606 children emigrated during follow-up, and another 2428 died.

Overall, 17% (n=140 665) of the children had been exposed to antibiotics during pregnancy, and 5% (n=40 116) had been exposed on two or more occasions. In infantile age, 65% (n=539 809) had been exposed to systemic antibiotics at least once, and 373 802 (70% of those exposed at all) had filled two or more prescriptions (*Table 1*).

222 **Table 1.** Descriptive table of the study population.

	All		IBD		CD		UC	
	N	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Variable	827 239	100.0	51	100.0	20	100.0	24	100.0
Sex								
Males	425 212	51.4	26	51.0	12	60.0	11	45.8
Females	402 027	48.6	25	49.0	8	40.0	13	54.2
Systemic antibiotic exposure during pregnancy	140 665	17.0	15	29.4	7	35.0	5	20.8
Phenoxymethylpenicillin (PcV)	60 701	7.3	8	15.7	4	20.0	3	12.5
Pivmecillinam	34 264	4.1	2	3.9	1	5.0	1	4.2
Nitrofurantoin	30 904	3.7	4	7.8	1	5.0	2	8.3
Other	42 672	5.2	5	9.8	4	20.0	0	0
Number of prescriptions during pregnancy (any)*								
1	100 549	12.2	10	19.6	4	20.0	3	12.5
2	26 357	3.2	3	5.9	2	10.0	1	4.2
≥3	13 759	1.7	2	3.9	1	5.0	1	4.2
Systemic antibiotic exposure in infantile age	539 809	65.3	43	84.3	16	80.0	20	83.3
Phenoxymethylpenicillin (PcV)	460 283	55.6	38	74.5	14	70.0	17	70.8
Amoxicillin	152 052	18.4	15	29.4	7	35.0	5	20.8
Flucloxacillin	80 814	9.8	13	25.5	5	25.0	4	16.7
Other	204 387	24.7	28	54.9	10	50.0	11	45.8
Number of prescriptions in infantile age (any)*								
1	166 007	20.1	3	5.9	1	5.0	2	8.3
2	111 761	13.5	8	15.7	2	10.0	6	25.0
≥3	262 041	31.7	32	62.7	13	65.0	12	50.0
Mothers' diagnoses								
IBD	6 780	0.8	7	13.7	4	20.0	2	8.3
CD	1 943	0.2	2	3.9	2	10.0	0	0.0
UC	3 598	0.4	5	9.8	2	10.0	2	8.3
Fathers' diagnoses								
IBD	7 333	0.9	4	7.8	0	0.0	2	8.3
CD	1 868	0.2	0	0.0	0	0.0	0	0.0
UC	3 972	0.5	1	2.0	0	0.0	0	0.0
Parents' highest achieved education								
0-12 years	337 312	40.8	18	35.3	8	40.0	7	29.2
>12 years	485 926	58.7	33	64.7	12	60.0	17	70.8
Missing	4 001	0.5	0	0.0	0	0.0	0	0.0
Mother's country of birth								
Sweden	646 032	78.1	42	82.4	18	90.0	20	83.3
Other	181 207	21.9	9	17.7	2	10.0	4	16.7
Father's country of birth								

Sweden	630 372	76.2	39	76.5	18	90.0	19	79.2
Other	178 669	21.6	12	23.5	2	10.0	5	20.8
Missing	18 198	2.2	0	0.0	0	0.0	0	0.0
Mode of delivery								
Vaginal	679 693	82.2	43	84.3	16	80.0	20	83.3
Caesarean Section	147 107	17.8	8	15.7	4	20.0	4	16.7
Missing	439	0.1	0	0.0	0	0.0	0	0.0
Maternal smoking at first visit to the antenatal care clinic								
Yes	51 892	6.3	3	5.9	1	5.0	1	4.2
No	741 727	89.7	46	90.2	17	85.0	23	95.8
Missing	33 620	4.1	2	3.9	2	10.0	0	0.0
Maternal age at delivery (years)								
≤19	12 060	1.5	0	0.0	0	0.0	0	0.0
20-24	105 879	12.8	4	7.8	2	10.0	1	4.2
25-29	237 650	28.7	18	35.3	7	35.0	7	29.2
30-34	287 319	34.7	18	35.3	5	25.0	12	50.0
≥35	184 330	22.3	11	21.6	6	30.0	4	16.7
Missing	1	0.0	0	0.0	0	0.0	0	0.0
Parity (at current delivery)								
1	361 332	43.7	26	51.0	11	55.0	10	41.7
≥2	465 907	56.3	25	49.0	9	45.0	14	58.3

Any – Any type of systemic antibiotics (ATC: J01)

* Independent on time in relation to diagnosis (i.e. either before or after onset of diagnosis)

"Other" antibiotics during pregnancy – Tetracyclines (J01A), penicillins with extended spectrum (J01CA) (except pivmecillinam), beta-lactamase sensitive penicillins (J01CE) (except phenoxymethylpenicillin), beta-lactamase resistant penicillins (J01CF), combinations of penicillins, incl. beta-lactamase inhibitors (J01CR), cephalosporins (J01DB-DD), monobactams (J01DF), carbapenems (J01DH), trimethoprim (J01EA), combinations of sulfonamides and trimethoprim (J01EE), macrolides (J01FA), lincosamides (J01FF), other aminoglycosides (J01GB), fluoroquinolones (J01MA), other antibacterials (J01X) (except nitrofurantoin).

"Other" antibiotics during childhood – Tetracyclines (J01A), penicillins with extended spectrum (J01CA) (except amimoxicillin), beta-lactamase resistant penicillins (J01CF) (except flucloxacillin), combinations of penicillins, incl. beta-lactamase inhibitors (J01CR), cephalosporins (J01DB-DE), monobactams (J01DF), carbapenems (J01DH), trimethoprim (J01EA), combinations of sulfonamides and trimethoprim (J01EE), macrolides (J01FA), lincosamides (J01FF), other aminoglycosides (J01GB), fluoroquinolones (J01MA), other antibacterials (J01X).

The median time from conception to exposure to systemic antibiotics was 125 days (interquartile range (IQR) 64-197 days), while the median age at first exposure to systemic antibiotics in childhood was 1.3 years (IQR 0.8-2.1 years).

In total, 51 children with IBD (CD and/or UC), 20 with CD (but never an UC diagnosis) and 24 with UC (but never a CD diagnosis) could be identified through the NPR. Approximately 14% of the children with IBD had a mother with IBD and 8% had a father with IBD, compared to those children without IBD, where the corresponding numbers for parental IBD were less than 1% (0.8% mothers and 0.9% fathers) (*Table 1*). The median age of the first IBD diagnosis was 2.0 years (IQR 0.9-4.1 years).

Antibiotics during pregnancy

Table 2 presents crude and adjusted Hazard Ratios (aHR) and 95% Confidence Intervals (CI) for the association between exposure to antibiotics during pregnancy and IBD, CD and UC respectively. There was a 93% significantly increased risk of IBD in children exposed to systemic antibiotics during pregnancy (aHR1.93, 95% CI 1.06-3.50). An association remained for CD (aHR 2.48, 95% CI 1.01-6.08), but not for UC (aHR 1.25, 95% CI 0.47-3.26). The aHR seemed to remain when restricting systemic antibiotic exposure to PcV (aHR for IBD 2.15, 95% CI 1.02-4.56), although no longer significant for CD (aHR 2.85, 95% CI 0.96-8.45). . Still, no significant association was found between "PcV" and UC.

In total, six children had been diagnosed with gastroenteritis 12 months prior to the first diagnosis of IBD, where five had been diagnosed with a viral or unspecified gastroenteritis and colitis (ICD 10: A09) and one had been diagnosed with a bacterial gastroenteritis with *Clostridium difficile* (ICD 10: A047). In sensitivity analyses, excluding these individuals, similar aHR remained for systemic antibiotics and CD (aHR 2.51, 95% CI 0.96-6.56), although no longer

264 significant, and with lower non-significant risk estimates for systemic antibiotics and IBD (aHR
265 1.68, 95% CI 0.88-3.21) (*Table 2*).

Table 2. Crude and adjusted Hazard ratios and 95% confidence intervals for inflammatory bowel disease (IBD) in relation to exposure to systemic antibiotics and "PcV" during pregnancy, and results from sensitivity analysis excluding those who have been diagnosed with gastroenteritis (GE) 12 months prior to onset of the first IBD diagnosis.

	IBD			Crohn's disease (CD)			Ulcerative colitis (UC)		
Antibiotics	**n**	**cHR (95% CI)**	**aHR (95% CI)**	**n**	**cHR (95% CI)**	**aHR (95% CI)**	**n**	**cHR (95% CI)**	**aHR (95% CI)**
Any	15	1.96 (1.07-3.57)	1.93 (1.06-3.50)	7	2.52 (1.01-6.28)	2.48 (1.01-6.08)	5	1.24 (0.47-3.32)	1.25 (0.47-3.26)
exl.GE	12	1.70 (0.88-3.29)	1.68 (0.88-3.21)	6	2.55 (0.95-6.86)	2.51 (0.96-6.56)	4	1.05 (0.36-3.08)	1.05 (0.36-3.08)
PcV	8	2.19 (1.03-4.66)	2.15 (1.02-4.56)	4	2.92 (0.99-8.67)	2.85 (0.96-8.45)	3	1.71 (0.51-5.71)	1.68 (0.51-5.97)
exl.GE	7	2.17 (0.97-4.84)	2.13 (0.96-4.75)	3	2.50 (0.73-8.62)	2.45 (0.71-8.42)	3	1.87 (0.56-6.34)	1.85 (0.55-6.21)

cHR – Crude Hazard Ratio; 3 945 000 Person-years

aHR – Adjusted Hazard Ratio; 3 874 000 Person-years; analyses adjusted for mother's and father's history of IBD, parental education, mother's and father's country of birth. Those with missing information on these variables are excluded (no cases excluded).

n – cases, *i.e.* those who have been exposed to antibiotics prior to onset of IBD/UC/CD diagnosis

exl.GE – excluding gastroenteritis, either bacterial or viral, according to ICD 10 codes A00-A09 12 months prior to onset of the first IBD diagnosis.

PcV – Phenoxymethylpenicillin

The role of timing of systemic antibiotics exposure during pregnancy and IBD was further explored, where aHR for the first trimester was 1.59 (95% CI 0.64-3.97), second trimester 1.23 (95% CI 0.45-3.40) and with a significant association during the third trimester (aHR 2.57 95% CI 1.10-6.01) (*Table 3*). Too few cases were available for separate analyses of exposure in different trimesters and CD/UC.

Table 3. Crude and adjusted Hazard ratios and 95% confidence intervals for the association between systemic antibiotics and inflammatory bowel disease (IBD) in different trimesters during pregnancy.

	IBD		
Trimester	**n**	**cHR (95% CI)**	**aHR (95% CI)**
First	5	1.59 (0.63-4.01)	1.59 (0.64-3.97)
Second	4	1.27 (0.46-3.51)	1.23 (0.45-3.40)
Third	6	2.60 (1.11-6.10)	2.57 (1.10-6.01)

cHR – Crude Hazard Ratio; 3 945 000 Person-years

aHR – Adjusted Hazard Ratio; 3 874 000 Person-years; analyses adjusted for mother's and father's history of IBD, parental education, mother's and father's country of birth. Those with missing information on these variables are excluded (no cases excluded).

n – cases, *i.e.* those who have been exposed to antibiotics prior to onset of IBD

Antibiotics in infantile age

Table 4 presents crude and adjusted Hazard Ratios (aHR) and 95% Confidence Intervals (CI) for the association between exposure to antibiotics in infantile age and IBD, CD and UC respectively. No significant associations were found for systemic antibiotics and "PcV" for IBD, CD or UC, where aHR varied between 0.72 (95% CI 0.27-1.92) (CD), 1.23 (95% CI 0.45-3.39) (UC) and 1.11 (95% CI 0.57-2.15) (IBD) for systemic antibiotics and 0.87 (95% CI 0.33-2.27) (CD), 1.20 (95% CI 0.51-2.81) (UC) and 1.25 (95% CI 0.70-2.26) (IBD) for "PcV". The associations remained non-significant after excluding individuals with gastroenteritis for both systemic antibiotics and "PcV" and all outcomes (IBD, CD and UC).

Table 4. Crude and adjusted Hazard ratios and 95% confidence intervals for the association between exposure to systemic antibiotics and "PcV" in infantile age and inflammatory bowel disease (IBD), and results from sensitivity analysis excluding those who had been diagnosed with gastroenteritis (GE) 12 months prior to onset of the first IBD diagnosis.

	IBD			Crohn's disease (CD)			Ulcerative colitis (UC)		
Antibiotics	**n**	**cHR (95% CI)**	**aHR (95% CI)**	**n**	**cHR (95% CI)**	**aHR (95% CI)**	**n**	**cHR (95% CI)**	**aHR (95% CI)**
Any	25	1.13 (0.58-2.20)	1.11 (0.57-2.15)	9	0.72 (0.27-1.94)	0.72 (0.27-1.92)	11	1.22 (0.44-3.37)	1.23 (0.45-3.39)
exl.GE	23	1.28 (0.60-2.73)	1.26 (0.60-2.64)	7	0.66 (0.22-1.99)	0.66 (0.23-1.92)	11	1.39 (0.46-4.25)	1.40 (0.46-4.25)
PcV	22	1.27 (0.70-2.31)	1.25 (0.70-2.26)	8	0.88 (0.33-2.31)	0.87 (0.33-2.27)	9	1.19 (0.50-2.80)	1.20 (0.51-2.81)
exl.GE	21	1.55 (0.80-3.00)	1.52 (0.80-2.90)	7	1.07 (0.36-3.21)	1.06 (0.37-3.10)	9	1.29 (0.52-3.23)	1.31 (0.53-3.22)

cHR – Crude Hazard Ratio; 3 945 000 Person-years

aHR – Adjusted Hazard Ratio; 3 874 000 Person-years; analyses adjusted for mother's and father's history of IBD, parental education, mother's and father's country of birth and mode of delivery. Those with missing information on these variables are excluded (no cases excluded).

n – cases, *i.e.* those who have been exposed to antibiotics prior to onset of IBD/UC/CD diagnosis

exl.GE – excluding gastroenteritis, either bacterial or viral, according to ICD 10 codes A00-A09 12 months prior to onset of the first IBD diagnosis.

PcV – Phenoxymethylpenicillin

Exploring the association between individuals who had been exposed to systemic antibiotics during the first year of life and the risk of first IBD diagnosis from 2 years of age, the aHR was 1.49 (95% CI 0.69-3.22). Furthermore, no significant interaction (p=0.48) was found between systemic antibiotics and parity and IBD, where the aHR in first-born was 0.93 (95% CI 0.39-2.22) and 1.38 in non-first-borns (95% CI 0.58-3.27) (data not tabulated).

No dose-response relationship was found between increasing number of prescriptions for systemic antibiotics in infantile age and IBD (*Table 5*).

Table 5. Crude and adjusted Hazard ratios and 95% confidence intervals for inflammatory bowel disease (IBD) in relation to increasing number of antibiotic prescriptions.

	IBD		
Systemic antibiotics, filled prescriptions	**n**	**cHR (95% CI)**	**aHR (95% CI)**
1	6	0.73 (0.28-1.92)	0.73 (0.28-1.89)
2	9	1.87 (0.81-4.34)	1.85 (0.80-4.30)
≥3	10	1.15 (0.48-2.75)	1.12 (0.47-2.65)

cHR – Crude Hazard Ratio; 3 945 000 Person-years

aHR – Adjusted Hazard Ratio; 3 874 000 Person-years; analyses adjusted for mother's and father's history of IBD, parental education, mother's and father's country of birth and mode of delivery. Those with missing information on these variables are excluded (no cases excluded).

n – cases, *i.e.* those who have been exposed to antibiotics prior to onset of IBD diagnosis

DISCUSSION

In this nationwide population-based birth cohort study of more than 800 000 children, we found a positive association between antibiotics exposure during pregnancy and later VEO-IBD and CD, but not UC. The aHR seemed to remain when restricting systemic antibiotic exposure to PcV, although no longer significant for CD. The risk estimates for systemic antibiotics and CD remained similar after exclusion of children with gastroenteritis 12 months prior to their first IBD diagnosis. No association was found between systemic antibiotics or "PcV" in infantile age and later VEO-IBD, CD or UC, independent of exclusion of individuals with gastroenteritis prior to their first IBD diagnosis. This is important as gastroenteritis may have represented undiagnosed IBD, and resulted in antibiotics treatment occurring after IBD rather than preceding it. Furthermore, there was no significant association for children who had been exposed to systemic antibiotics during the first year of life and the risk of first IBD diagnosis from 2 years of age, or between increasing numbers of filled prescriptions for antibiotics and IBD.

Previous literature

While recent research has confirmed that antibiotic use is associated with an increased risk of IBD, including pediatric CD, less attention has been paid to VEO-IBD. In a recent meta-analysis, Ungaro *et al.* examined 11 studies,[31-41] with four focusing on paediatric IBD.[34 37 39 40] The authors regarded only three of these as paediatric,[34 37 39] and when pooling their data the HR was substantially higher than in the overall meta-analysis (HR 2.75, 95% CI 1.72-4.38) for children only).

Antibiotics may influence the risk of acquiring IBD in several ways. Firstly antibiotics may be causally related to IBD, potentially by a mediating effect on the microbiome, causing a reduced diversity and an increased dysbiosis.[42] The microbiome interacts with the host

through production of short-chain fatty acids (including butyrate), induction of the mucosal immune system, stimulation of the local nervous system but also through interaction with the lamina propria by modification of the gut barrier function.[43] A dysbiosis in the gut microbiota, characterized by reduction of beneficial bacteria such as *Faecalibacterium prausnitzii*, and Ruminococcaceae and an increase of pathogens or pathobionts, has consistently been shown in patients with IBD, especially ileal CD.[44] Compared to previous studies we did not find any association between antibiotics treatment in infantile age and IBD, maybe due to that VEO-IBD is partly a different entity from later-onset IBD.[31 39] On the other hand, we found a two-fold increased risk in offspring to mothers receiving antibiotics during pregnancy. We suggest such antibiotics exposure may be detrimental to the risk of VEO-IBD in the offspring. The gut microbiota in pregnant women resembles that of healthy non-pregnant women during the first two trimesters, but undergoes substantial changes during the third trimester.[45] In our study, the highest risk of later VEO-IBD was seen in mothers exposed to antibiotics in the last trimester (aHR 2.57 95% CI 1.10-6.01), i.e. just before birth. Recent animal research indicates that antibiotics administered during pregnancy have substantial effects on the offspring microbiome (reduced bacterial diversity), but may also influence the immune response in the offspring[45] and increase susceptibility to develop colonic inflammation.[46]

While it has long been thought that the intestinal tract is sterile at birth, recent data suggest that the microbial colonization process may be initiated already in utero, [47]a process that may be affected by antibiotics late in pregnancy. Furthermore, it has been suggested in studies of repeated fecal samples from term infants that the use of intrapartum PcV prophylaxis, to prevent early onset group B streptococcal infection in newborns, alters the offspring microbiome,[48 49] although others have found very few differences between antibiotic-exposed and non-antibiotic-

exposed infants.[50]Meanwhile maternal intake of probiotics influence the expression of toll-like receptors in infant meconium,[51] indicating that fetal exposure of antibiotics may play an important role for the development microbiota and the immune system of the child.[48] While a causative association between antibiotics during pregnancy and VEO-IBD thus seems plausible, we cannot exclude possible confounding from an intrauterine infection with fever of the mother and a raised inflammatory reaction, causing epigenetic imprinting in the fetus and subsequent VEO-IBD. Bernstein and colleagues recently suggested that individuals with IBD were no more likely than controls to have been born to mothers with peripartum infections, however they were diagnosed at an earlier age than those whose mothers did not have an infection.[52] Unfortunately, we had no data on the indications of the mother's antibiotic treatment during pregnancy or on either symptoms (or date of symptom onset) or genotype in patients, neither were we able to examine the microbiota per se in children with VEO-IBD

In the meta-analysis by Ungaro *et al*. also fluoroqinolones were highly linked to IBD (pooled OR=1.79, 95% CI 1.03-3.12).[21] During the study period fluoroqinolones were not recommended for younger children in Sweden and only accounted for 0.2% of all antibiotic prescriptions, we therefore chose not to examine fluoroqinolone exposure and IBD separately. The fact that the highest risk estimates for IBD have previously been shown for metronidazole[21] [31] and fluoroqinolones[21] (both used in the treatment of IBD, although not currently recommended[53]) suggest that reverse causation may have been an issue in earlier studies demonstrating an positive association between antibiotics treatment and later IBD.[31 39] We did not find any association for IBD when we restricted our analysis to antibiotics in the first year of life and our outcome to IBD onset beyond 2 years of age. That sub-analysis is similar to the analysis by Ungaro *et al.*[21] limiting their dataset to studies with ≥1 year of exclusion time between

antibiotics exposure and IBD (HR 1.50, 95% CI 1.44-1.57), while we found an aHR of 1.49 with broad confidence intervals (95% CI 0.69-3.22). Reverse causation is obviously not a concern regarding the possible link between antibiotics exposure during pregnancy and risk of VEO-IBD in the offspring.

Strengths and limitations

The main strength of this study is the nationwide cohort, based on prospective information retrieved from high quality population-based register, thereby eliminating recall bias. In addition, we were able to carry out important sub-analyses such as exclusion of individuals with gastroenteritis prior to their first IBD diagnosis, as well as including a time window between exposure and outcome, analyses that allow us to study the potential influence by information bias. Furthermore, we were able to consider familial factors such as parental history of IBD, parents' country of birth and socioeconomic factors including education level.

We acknowledge a number of limitations. Despite our use of a nationwide register-based cohort of more than 800 000 children, we could only identify 51 cases of IBD in total. Thus, the number of individuals in each subgroup (CD, UC) was quite low, which is reflected by the rather wide confidence intervals and may also be of concern in the adjusted analyses, however crude and adjusted estimates were very similar. Furthermore, we acknowledge the potential difficulties to diagnose UC in this age group, why stratification of patients into UC versus CD may not be completely accurate. This means that even a small number of misclassified patients may have affected our conclusions, since some of the results are of borderline significance, specifically in the sensitivity analyses where we excluded individuals whose potential first episode of IBD could have been misclassified as gastroenteritis. Nevertheless, we believe that the strength of our data lies in the results seen in IBD in general, which is not dependent on

stratification into UC versus CD. Misclassification of a child's IBD diagnosis after maternal exposure to antibiotics in pregnancy is most likely non-differential, whereas it could be differential for exposure in infantile age. The misclassification of father's diagnoses related to maternal exposure during pregnancy and to exposure in infantile age is most likely non-differential, whereas misclassification of mother's diagnoses after exposure during pregnancy could be differential, but most likely non-differential for exposure in infantile age. A non-differential misclassification of the outcome will generally bias towards the null, whereas differential misclassification could lead to both higher and lower risk estimates.

Overall, the limited follow-up time of our study means that we were unable to examine the long-term effect of fetal and early life antibiotics on IBD in adulthood, which may also be one possible explanation to the low number of identified cases and lack of association between antibiotic exposure in childhood and later IBD. While a previous validation of IBD, using the same definition (requiring ≥2 diagnoses of IBD) found a positive predictive value (PPV) of 93%,[27] we acknowledge that this PPV was calculated in a population of a much higher median age and we cannot rule out that the PPV for ≥2 IBD records in young age is different. As data from primary out-patient care are not available in the NPR, the sensitivity of our study could be lower than in real life. However, we believe that the sensitivity of IBD in the NPR is high for children as paediatric IBD patients are managed by hospital-based specialists and closely monitored with visits every three to six months.[28] In addition, to minimize the risk of false positive cases and to increase the specificity, we used ≥2 diagnoses for our outcome, even though this could mean that we probably excluded some true cases with a lower sensitivity as result.The small number of cases also limited our possibility to perform sibling analysis, which otherwise would have helped us to control for all factors siblings share (both genetic and environmental).

Unfortunately, no population-based data on exposure to antibiotics in inpatient care are available in Sweden today. We have previously shown that 13% of vaginal deliveries are associated with intrapartum exposure to antibiotics.[54] The corresponding number for elective caesarean section was 14%, and 63% for emergency caesarean section. Thus, while the majority of antibiotics in Sweden (87%) are prescribed in out-patient care[55], we did not capture those exposed to antibiotics during the immediate intrapartum period, but were able to adjust for mode of delivery as a co-variate. Exposure of antibiotics was furthermore defined as having filled a prescription of antibiotics, which is not equivalent to adherence to treatment.[56] Finally, the number of children receiving different subtypes of antibiotics was limited, wherefore we were only able to perform stratified analyses on PcV.

Conclusion and implications

In conclusion, we found an association between antibiotics exposure during pregnancy, specifically during the third trimester, but not in infantile age, and subsequent development of VEO-IBD. Our results may indicate that antibiotic exposure in late pregnancy can lead to changes in the microbiome of the child, however further research is needed to confirm our findings. In addition, the absolute risk of VEO-IBD is very low, and antibiotics during pregnancy should still be used when needed.

ACKNOWLEDGEMENTS

We direct our great appreciation to Christina Norrby and Marcus Boman who contributed with excellent data collection and management.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf. AKÖ, CL, JH, JFL and CA claim no conflict of interest related to the submitted work.

FUNDING

Financial support was provided from the Swedish Research Council through the Swedish Initiative for Research on Microdata in the Social And Medical Sciences (SIMSAM) framework grant no 340-2013-5867, grants provided by the Stockholm County Council (ALF-projects), the Swedish Heart-Lung Foundation and the Swedish Asthma and Allergy Association's Research Foundation.

AUTHOR CONTRIBUTIONS

The study was initiated by JFL and CA, and designed by AKÖ, CL, JFL and CA. AKÖ and CL performed the statistical analysis and wrote the initial draft together with JFL, JH and CA. All authors contributed with invaluable support for data analyses, interpretation of findings and critical revision of the article. CA obtained the financial support. All authors had full access to data, reviewed and approved the final version of the article submitted for publication. AKÖ, CL, JFL, JH and CA are the guarantors for the study and accept full responsibility for the work, had access to the data, and controlled the decision to publish.

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Figure legends and footnotes for figures

Figure 1. A Directed Acyclic Graph (DAG) depicting the included variables in the final models.

Footnote:

The DAG can be applied to various analyses, for example, a study of the association between antibiotic exposure and VEO-IBD. A directed arrow between these two variables indicates that the exposure is associated with the outcome. DAGs can be used to identify all potential confounders that may exist, in order to know which variables that should be adjusted for in the analyses. When the exposure and the outcome are common causes for a third factor (a common effect), this factor is called a collider. Pathways through colliders are closed, unless the collider is adjusted for which will then open the path and potentially cause spurious associations. In this DAG, parental education is a potential collider through parental IBD, however, with adjustment of parental IBD, this backdoor pathway is closed.

* Mode of delivery was only included in analysis of antibiotic exposure in infantile age.

664 **Figure 2.** Flow chart of final study population.

665

666 Footnote:

667 *The first estimated conception date, based on date of birth and gestational age in days, is July 1,

668 2005, and the first child in the cohort was born January 8, 2006. The last estimated conception

669 date was July 7, 2013, and the child was born after 164 days on December 30, 2013.

670 MBR – Medical Birth Register

671 MGR – Multi-generation Register

672 SPDR – Swedish Prescribed Drug Register

673 LISA – Longitudinal integration database for health insurance and labour market studies

674 TPR – Total Population Register

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