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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

26 PcV – Phenoxyethylpenicillin
27 PIN – Personal Identification Number
28 SPDR – Swedish Prescribed Drug Register
29 TPR – Total Population Register
30 UC – Ulcerative Colitis
31 VEO-IBD – Very Early Onset Inflammatory Bowel Disease

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33

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

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48

49

50 **ABSTRACT**

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

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

60 **Results** Children exposed to antibiotics during pregnancy were at increased risk of IBD
61 compared to general population controls (adjusted hazard ratio (aHR) 1.93; 95% confidence
62 interval (CI) 1.06-3.50). Corresponding aHRs were 2.48 (1.01-6.08) for Crohn's disease (CD)
63 and 1.25 (0.47-3.26) for ulcerative colitis (UC) respectively. For antibiotics in infantile age, the
64 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.
65 Excluding children with gastroenteritis 12 months prior to the first IBD diagnosis retained similar
66 aHR for antibiotics during pregnancy and CD, while the association no longer remained
67 significant for IBD.

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

71

72 **Summary “box”**

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

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

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

83

84 INTRODUCTION

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

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

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

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

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

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126 **METHOD**

127 **Study population and register linkage**

128 This nationwide prospective population-based register study included all children born January
129 2006 to December 2013, identified together with their mothers from the Swedish Medical Birth
130 Register (MBR). Fathers to the children were identified through the Swedish Multi-generation
131 Register.²² We used the Swedish Prescribed Drug Register (SPDR)²³ and the National Patient
132 Register (NPR)²⁴ to retrieve data on exposure and outcome. The SPDR contains complete data on
133 all dispensed drugs from pharmacies since July 2005 while the NPR began in 1964, became
134 nationwide in 1987 (inpatient diagnoses only), and added hospital-based outpatient visit data in
135 2001. Data from the different registers were linked using the unique personal identity number
136 assigned to all residents in Sweden.²⁵

137 **Variables**

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

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

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

153 *Co-variables;* Through the MBR we retrieved information on the child’s date of
154 birth, sex, gestational age (days), mode of delivery (vaginal or caesarean section), maternal
155 smoking at first visit to the antenatal care clinic (yes/no), maternal age at delivery (≤ 19 , 20-24,
156 25-29, 30-34, or ≥ 35 years), and parity (child’s birth order at current delivery; first-born (1) or not
157 (≥ 2)). Data from Cnattingius et al. suggest a high quality of data in the MBR with a coverage of
158 $>98\%$ of all births in Sweden.²⁹

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

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

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

174

175 **Statistical analysis**

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

188 Potential confounders were identified based on the Directed Acyclic Graphs (DAG)
189 concept,³⁰ and the final models were adjusted for: mother's and father's history of IBD, parental
190 education, mother's and father's country of birth (in analyses of exposure during pregnancy and
191 in childhood) and mode of delivery (in analysis of exposure in infantile age) as shown in *Figure*
192 *1*. Those with missing information on these variables were excluded (n=2848 for exposure in
193 pregnancy and n=3274 for exposure in infantile age) and complete case analyses were performed.
194 Crude and adjusted Hazard Ratios (HR) are presented.

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

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

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

212
213

214 **RESULTS**

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

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

221

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

Variable	All		IBD		CD		UC	
	N	%	n	%	n	%	n	%
	827 239	100.0	51	100.0	20	100.0	24	100.0
Sex								
<i>Males</i>	425 212	51.4	26	51.0	12	60.0	11	45.8
<i>Females</i>	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
<i>Phenoxymethylpenicillin (PcV)</i>	60 701	7.3	8	15.7	4	20.0	3	12.5
<i>Pivmecillinam</i>	34 264	4.1	2	3.9	1	5.0	1	4.2
<i>Nitrofurantoin</i>	30 904	3.7	4	7.8	1	5.0	2	8.3
<i>Other</i>	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
<i>Phenoxymethylpenicillin (PcV)</i>	460 283	55.6	38	74.5	14	70.0	17	70.8
<i>Amoxicillin</i>	152 052	18.4	15	29.4	7	35.0	5	20.8
<i>Flucloxacillin</i>	80 814	9.8	13	25.5	5	25.0	4	16.7
<i>Other</i>	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								
<i>IBD</i>	6 780	0.8	7	13.7	4	20.0	2	8.3
<i>CD</i>	1 943	0.2	2	3.9	2	10.0	0	0.0
<i>UC</i>	3 598	0.4	5	9.8	2	10.0	2	8.3
Fathers' diagnoses								
<i>IBD</i>	7 333	0.9	4	7.8	0	0.0	2	8.3
<i>CD</i>	1 868	0.2	0	0.0	0	0.0	0	0.0
<i>UC</i>	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								
<i>Sweden</i>	646 032	78.1	42	82.4	18	90.0	20	83.3
<i>Other</i>	181 207	21.9	9	17.7	2	10.0	4	16.7
Father's country of birth								

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

223

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

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

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

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

240

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

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

250 **Antibiotics during pregnancy**

251 *Table 2* presents crude and adjusted Hazard Ratios (aHR) and 95% Confidence Intervals (CI) for
252 the association between exposure to antibiotics during pregnancy and IBD, CD and UC
253 respectively. There was a 93% significantly increased risk of IBD in children exposed to systemic
254 antibiotics during pregnancy (aHR 1.93, 95% CI 1.06-3.50). An association remained for CD
255 (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
256 to remain when restricting systemic antibiotic exposure to PcV (aHR for IBD 2.15, 95% CI 1.02-
257 4.56), although no longer significant for CD (aHR 2.85, 95% CI 0.96-8.45). . Still, no significant
258 association was found between “PcV” and UC.

259 In total, six children had been diagnosed with gastroenteritis 12 months prior to the
260 first diagnosis of IBD, where five had been diagnosed with a viral or unspecified gastroenteritis
261 and colitis (ICD 10: A09) and one had been diagnosed with a bacterial gastroenteritis with
262 *Clostridium difficile* (ICD 10: A047). In sensitivity analyses, excluding these individuals, similar
263 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*).

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

Antibiotics	IBD			Crohn’s disease (CD)			Ulcerative colitis (UC)		
	n	cHR (95% CI)	aHR (95% CI)	n	cHR (95% CI)	aHR (95% CI)	n	cHR (95% CI)	aHR (95% CI)
<i>Any</i>	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)
<i>exl.GE</i>	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)
<i>PcV</i>	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)
<i>exl.GE</i>	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)

269

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

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

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

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

276 *PcV* – Phenoxymethylpenicillin

277 The role of timing of systemic antibiotics exposure during pregnancy and IBD was
 278 further explored, where aHR for the first trimester was 1.59 (95% CI 0.64-3.97), second trimester
 279 1.23 (95% CI 0.45-3.40) and with a significant association during the third trimester (aHR 2.57
 280 95% CI 1.10-6.01) (*Table 3*). Too few cases were available for separate analyses of exposure in
 281 different trimesters and CD/UC.

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

285

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)

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

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

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

294 **Antibiotics in infantile age**

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

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

Antibiotics	IBD			Crohn’s disease (CD)			Ulcerative colitis (UC)		
	n	cHR (95% CI)	aHR (95% CI)	n	cHR (95% CI)	aHR (95% CI)	n	cHR (95% CI)	aHR (95% CI)
<i>Any</i>	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)
<i>exl.GE</i>	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)
<i>PcV</i>	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)
<i>exl.GE</i>	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)

306

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

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

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

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

314 *PcV* – Phenoxymethylpenicillin

315 Exploring the association between individuals who had been exposed to systemic
 316 antibiotics during the first year of life and the risk of first IBD diagnosis from 2 years of age, the
 317 aHR was 1.49 (95% CI 0.69-3.22). Furthermore, no significant interaction ($p=0.48$) was found
 318 between systemic antibiotics and parity and IBD, where the aHR in first-born was 0.93 (95% CI
 319 0.39-2.22) and 1.38 in non-first-borns (95% CI 0.58-3.27) (data not tabulated).

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

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

Systemic antibiotics, filled prescriptions	IBD		
	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 (0.47-2.75)

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

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

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

333

334

335 **DISCUSSION**

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

348 **Previous literature**

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

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

358 through production of short-chain fatty acids (including butyrate), induction of the mucosal
359 immune system, stimulation of the local nervous system but also through interaction with the
360 lamina propria by modification of the gut barrier function.⁴³ A dysbiosis in the gut microbiota,
361 characterized by reduction of beneficial bacteria such as *Faecalibacterium prausnitzii*, and
362 Ruminococcaceae and an increase of pathogens or pathobionts, has consistently been shown in
363 patients with IBD, especially ileal CD.⁴⁴ Compared to previous studies we did not find any
364 association between antibiotics treatment in infantile age and IBD, maybe due to that VEO-IBD
365 is partly a different entity from later-onset IBD.^{31 39} On the other hand, we found a two-fold
366 increased risk in offspring to mothers receiving antibiotics during pregnancy. We suggest such
367 antibiotics exposure may be detrimental to the risk of VEO-IBD in the offspring. The gut
368 microbiota in pregnant women resembles that of healthy non-pregnant women during the first
369 two trimesters, but undergoes substantial changes during the third trimester.⁴⁵ In our study, the
370 highest risk of later VEO-IBD was seen in mothers exposed to antibiotics in the last trimester
371 (aHR 2.57 95% CI 1.10-6.01), i.e. just before birth. Recent animal research indicates that
372 antibiotics administered during pregnancy have substantial effects on the offspring microbiome
373 (reduced bacterial diversity), but may also influence the immune response in the offspring⁴⁵ and
374 increase susceptibility to develop colonic inflammation.⁴⁶

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

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

393 In the meta-analysis by Ungaro *et al.* also fluoroquinolones were highly linked to
394 IBD (pooled OR=1.79, 95% CI 1.03-3.12).²¹ During the study period fluoroquinolones were not
395 recommended for younger children in Sweden and only accounted for 0.2% of all antibiotic
396 prescriptions, we therefore chose not to examine fluoroquinolone exposure and IBD separately.
397 The fact that the highest risk estimates for IBD have previously been shown for metronidazole²¹
398 ³¹ and fluoroquinolones²¹ (both used in the treatment of IBD, although not currently
399 recommended⁵³) suggest that reverse causation may have been an issue in earlier studies
400 demonstrating an positive association between antibiotics treatment and later IBD.^{31 39} We did not
401 find any association for IBD when we restricted our analysis to antibiotics in the first year of life
402 and our outcome to IBD onset beyond 2 years of age. That sub-analysis is similar to the analysis
403 by Ungaro *et al.*²¹ limiting their dataset to studies with ≥ 1 year of exclusion time between

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

408 **Strengths and limitations**

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

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

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

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

450 Unfortunately, no population-based data on exposure to antibiotics in inpatient care
451 are available in Sweden today. We have previously shown that 13% of vaginal deliveries are
452 associated with intrapartum exposure to antibiotics.⁵⁴ The corresponding number for elective
453 caesarean section was 14%, and 63% for emergency caesarean section. Thus, while the majority
454 of antibiotics in Sweden (87%) are prescribed in out-patient care⁵⁵, we did not capture those
455 exposed to antibiotics during the immediate intrapartum period, but were able to adjust for mode
456 of delivery as a co-variate. Exposure of antibiotics was furthermore defined as having filled a
457 prescription of antibiotics, which is not equivalent to adherence to treatment.⁵⁶ Finally, the
458 number of children receiving different subtypes of antibiotics was limited, wherefore we were
459 only able to perform stratified analyses on PcV.

460 **Conclusion and implications**

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

467

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471

472

473 **COMPETING INTERESTS**

474 All authors have completed the ICMJE uniform disclosure form at
475 www.icmje.org/coi_disclosure.pdf. AKÖ, CL, JH, JFL and CA claim no conflict of interest
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479

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486

487 **AUTHOR CONTRIBUTIONS**

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

495

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645 **Figure legends and footnotes for figures**

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

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648

649 Footnote:

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

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

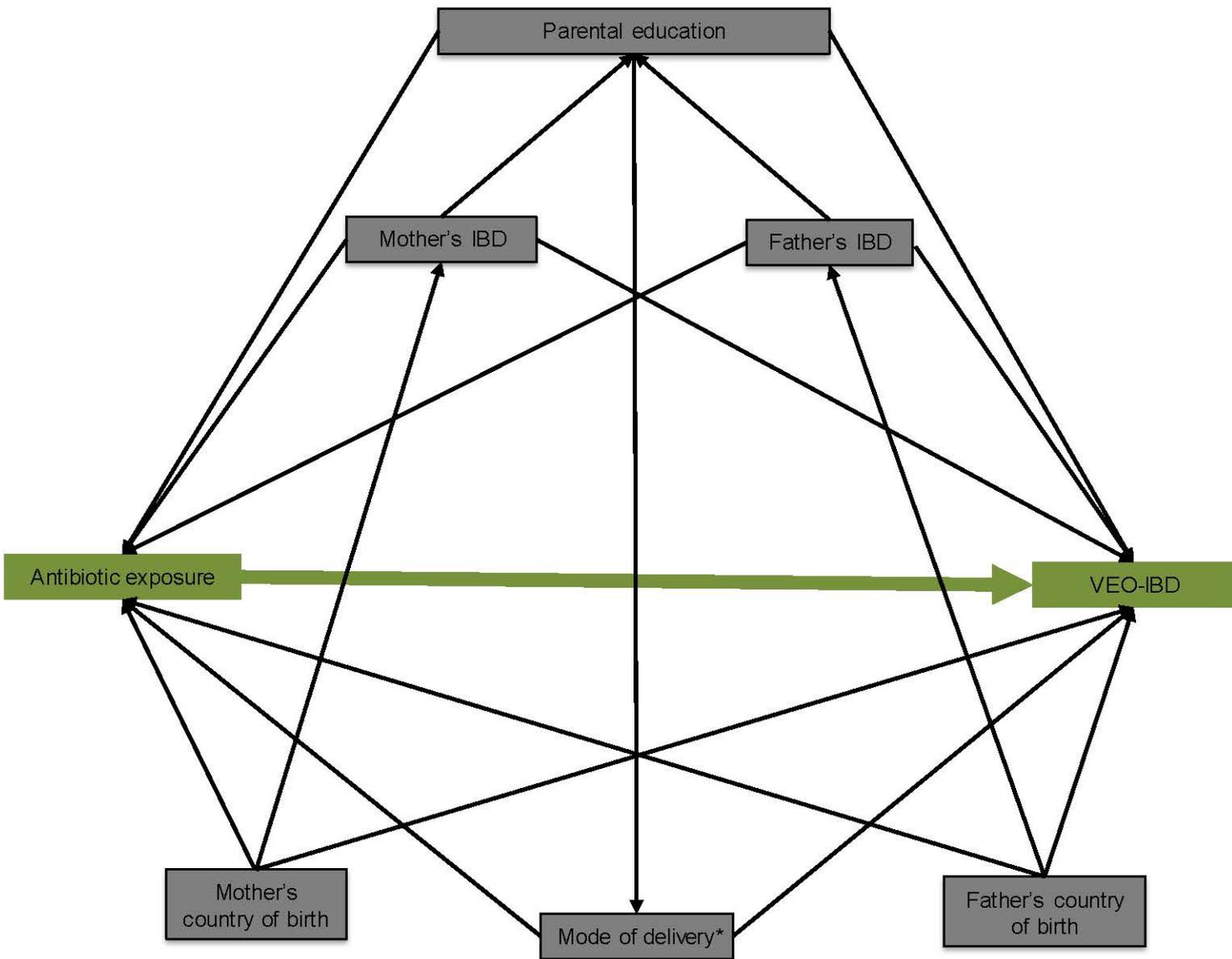
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Figure 1. A Directed Acyclic Graph (DAG) depicting the included variables in the final models.



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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676

Figure 2. Flow chart of final study population

