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Early life antibiotics and childhood gastrointestinal disorders: a systematic review

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Contributors' Statement Page:

K. Kamphorst contributed to the design, the analyses and interpretation of the study, drafting of the initial manuscript, and reviewed and revised the manuscrip*t.*

E. Van Daele contributed to the analysis and interpretation of the study and critically revised the manuscript.

A.M. Vlieger and R.M. van Elburg contributed to the conception of the study, interpretation of the data and critically revised the manuscript.

J.G. Daams conceptualized and performed the systematic search and critically revised the manuscript.

J. Knol contributed to the conception and design of the study and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background: In adults, there is increasing evidence for an association between antibiotic use and gastrointestinal disorders but in children, the evidence is scarce.

Objective: Assess the association between exposure to antibiotics in the first two years of life in term born children and the presence of chronic gastrointestinal disorders later in childhood.

Design: For this systematic review the MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020. Title and abstract screening (n=12,219), full-text screening (n=132) as well as the quality assessment with the Newcastle–Ottawa Scale were independently performed by two researchers.

Main outcome measures: The association between antibiotics and inflammatory bowel disease (n=6), eosinophilic esophagitis (n=5), celiac disease (n=6), infantile colics (n=3), functional constipation (n=2), recurrent abdominal pain, regurgitation, functional diarrhea, and infant dyschezia were examined.

Results: Twenty-two studies were included, 11 cohort and 11 case-control studies. A best evidence synthesis showed strong evidence for an association between antibiotic exposure in the first two years of life and the presence of inflammatory bowel disease, eosinophilic esophagitis, and celiac disease during childhood. Moderate evidence for an absence of an association for functional constipation in the first year of life, and insufficient evidence for the other studied disorders.

Conclusions: The use of antibiotics in early life may increase the risk of gastrointestinal disorders later in life. Further studies are necessary to unravel the underlying mechanisms and determine potential preventive measures. Meanwhile judicious use of antibiotics in early childhood is highly warranted.

Introduction

The incidence of pediatric gastrointestinal disorders (GI-disorders), such as pediatric inflammatory bowel disease (IBD) and celiac disease (CeD), is rising [1, 2]. The increase in pediatric GI-disorders is most likely related to environmental factors and recently the focus has been on the role of the intestinal microbiome. A microbiome that has been disturbed by factors like stress, dietary change, environmental factors or drugs, can result in alterations in the immune system [3]. Several studies have shown that a disturbed microbiome can be a cause or trigger of GI disorders, probably mediated by these immunological changes [4-7].

One of the drugs with the most profound effect on the microbiome are antibiotics [8]. The impact of antibiotics on the microbiome depends on various factors such as type of antibiotic, dosage, and duration of exposure[8]. Furthermore, age at exposure is probably also important. The gut of a newborn infant is almost sterile with a low diversity and matures according to several developmental stages with increasing diversity over time [9]. The microbiome stabilizes around the age of 2 to 3 years [9]. Since this developing gut microbiota plays an important role in the training of both innate and adaptive immune system, it is likely that antibiotics will have their biggest impact when administered in the first two years of life.

In adults, there is increasing evidence for an association between antibiotic use and GI disorders [10], but in children the evidence is scarce [11]. Therefore, the aim of this systematic review was to assess the association between exposure to antibiotics in the first two years of life and the presence of chronic gastrointestinal disorders during childhood.

Method

Study selection

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and registered in PROSPERO CRD42019132631 [12, 13]. MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020 to identify all studies examining the association between antibiotic exposure in the first two years of life and the presence of common chronic (longer than two weeks, in order to exclude viral diarrhea) gastrointestinal disorders during the first 18 years of life. We searched for associations with IBD, eosinophilic esophagitis (EoE), CeD, irritable bowel syndrome (IBS), (functional) abdominal pain, constipation, dyspepsia, aerophagia, infantile colic, gastroesophageal reflux (GERD), regurgitation, dyschezia and chronic diarrhea.

A multi stranded search approach comprised various concept combinations of children aged 0-4 years, prognosis, gastrointestinal disorders and antibiotics. In order to reduce recall noise and enhance search results precision we used VOS-viewer to identify terms for NOTing out irrelevant records from databases searched [14, 15]. See supplementary file 1 for the full search strategies.

In- and exclusion criteria

Studies were included if: 1. Antibiotics were administered between full-term birth and two years of age. 2. Study outcome was diagnosis with a chronic GI-disorder during the first 18 years of life. 3. Antibiotic use was before the diagnosis of the GI-disorder. 4. A control group was included. 5. In case multiple studies were found examining similar outcomes in one cohort, only the study with the largest cohort was included. No restrictions were placed

on the time period of publication. Searches were limited to studies conducted in humans and excluded if the full text was not available in English, Dutch, German or French.

All records found in the search were exported into Rayyan after deduplication [16]. Two researchers (KK and EVD) independently performed title and abstract screening as well as full-text screening. After consensus about the study selection, data were entered into a data extraction form, which included: author, year of publication, country, study design, cases, controls/cohort, population age, sample size exposed to antibiotics, age at exposure, details about classification by type of antibiotics, type of GI disorder, method of diagnosis, confounders for which corrected, and the association between exposure and outcome.

Methodological quality

To assess the risk of bias, two researchers (KK and EVD) independently assessed the methodological quality. Discrepancies were resolved by discussion until consensus was reached. The Newcastle–Ottawa Scale was used, which has been developed to assess the quality of observational studies [17]. The Newcastle–Ottawa Scale includes a different instrument for assessing case-control and cohort studies. Both scales contain a maximum of nine points and assess studies in three core areas: 1. Selection of study participants 2. Comparability of groups 3. Detection of exposure / outcome. One point for comparability of groups was given when the study controlled for the main important confounder and a second point if controlled for a second important confounder, see supplementary file 2. Studies were rated high quality with a score of eight or higher, moderate quality with a score between five and seven and weak quality with a score of four or less [18].

Data analyses

To synthesize the methodological quality of the studies, a commonly used best evidence synthesis was applied per disorder in which the methodological quality was

considered according to the following definitions: 1. strong evidence, provided by generally consistent findings in at least two high-quality studies. 2. moderate evidence, provided by generally consistent results in one high-quality study and at least one moderate- or low-quality study, or generally consistent results in multiple moderate- or low-quality studies. 3. insufficient evidence, when less than two studies were available or inconsistent findings in multiple studies [19-21]. Results were considered consistent when at least 75% of the studies showed results in the same direction.

Results

Search results

Of the 14,731 retrieved records, 12,219 remained after removing duplicates. These records were screened; 132 were assessed as eligible and read in full-text of which 110 were excluded and 22 studies included in this review. Details of the selection procedure are shown in Figure 1.

Study characteristics

The included studies were published between 2010 and 2020: 11 cohort studies [22-32] and 11 case-control studies [33-43]. The studies were performed in Sweden (n=4) [27, 30, 35, 36], the United States of America (USA) (n=5) [33, 34, 37, 41, 42], Italy (n=4) [22, 29, 32, 43], Denmark (n=2) [23, 31], Canada (n=2) [38, 39], and one in the United Kingdom [25], the Netherlands [26], and Finland [40]. There were two international studies, one in Denmark and Norway [28], and another in Finland, Germany, Sweden and the USA [24].

The associations between antibiotics and the following GI-disorders were examined: IBD (n=6, figure 2) [25, 27, 31, 38, 40, 43], EoE (n=5, figure 3) [33, 34, 37, 39, 41], CeD (n=6, figure 4) [22, 24, 28, 35, 36, 42], infantile colics (n=3) [23, 26, 32], functional constipation (n=2) [29, 32], and recurrent abdominal pain (n=1) [30](figure 5). One study examined several functional GI-disorders (FGIDs): infantile colics, functional constipation, functional diarrhea, infant dyschezia, and regurgitation[32](figure 5).

Exposure to antibiotics was studied in the first two years of life (n=4) [24, 30, 35, 42], the first 18 months of life (n=1) [23], the first year of life (n=13) [22, 25, 27-29, 31, 33, 34, 37-40, 43], the first six months of life (n=2) [36, 41], and the first week of life (n=2) [26, 32]. Since only a few studies provided details about type of antibiotics and/or number of antibiotic treatments in the first two years of life, the associations include mostly the overall antibiotic exposure.

Quality assessment

Ten studies were of high quality[22, 26-29, 31, 35, 38, 40, 43], ten studies moderate [23-25, 30, 32, 34, 36, 37, 41, 42], and two weak [33, 39] (Table 1). Frequently observed weaknesses were a high dropout rate in the cohort studies, assessment of antibiotic exposure through parental reports, and no correction for important confounders.

Inflammatory Bowel Disease

Exposure to early life antibiotics was associated with the development of IBD in five out of six studies [25, 31, 38, 40, 43](figure 2), whereas no association was found in one study examining Very Early Onset (VEO) IBD, (before six years of age) [27]. Three studies found a dose-response relation [25, 38, 43] and an increased risk after fluoroquinolone [25], metronidazole [25], and phenoxymethylpenicillin[40] exposure. In two studies IBD was stratified by type and only the odds ratio for Crohn's disease, but not for ulcerative colitis, was significant [38, 40]. Forest plots of the main results are shown in Figure 6a-d.

Eosinophilic esophagitis

In four of the five studies early life antibiotics was associated with EoE[33, 34, 37, 41], whereas in one study [39] the rates of parental reported antibiotic use were similar for cases and controls (figure 3).

Celiac disease

In four studies, of which three had a high quality, a significant association between early life antibiotics and the presence of CeD was found [22, 28, 35, 42], whereas in two moderate quality studies no association was found [24, 36] (figure 4). Three studies showed a dose-response relationship between exposure to antibiotics and the risk of CeD [22, 28, 42].

Furthermore, use of cephalosporin [22] and multiple courses of macrolides [24] showed a positive association with the development of CeD.

Infantile colics

Two studies found a significant association between early life antibiotics and infantile colics [23, 26], while one study found no association [32] (figure 5).

Functional constipation

In both studies, no association was found between early life antibiotics use and functional constipation in the first year of life [29, 32](figure 5).

Recurrent abdominal pain

The only study examining the association between antibiotics use in the first two years of life and the risk of recurrent abdominal pain (AP) at 12 years of age [30] found that only girls, but not boys, who received antibiotics in both the first and second year of life, had an increased risk of AP at 12 years (figure 5).

Regurgitation, dyschezia and functional diarrhea

In one study no association was found between antibiotics in the first week of life and regurgitation, dyschezia and functional diarrhea [32] (figure 5).

Syntheses of individual results

Using the definitions for the best evidence synthesis, described in the method section, it can be concluded that there is strong evidence for an association of antibiotics in early life with IBD, EoE and CeD. There is moderate evidence that there is no association with infantile constipation. The current evidence for an association between antibiotics in early life and the other studied GI-disorders is considered insufficient.

Discussion

This systematic review with best evidence syntheses on the association between antibiotic exposure in the first two years of life and chronic GI disorders during childhood showed strong evidence for this association with inflammatory bowel disease, eosinophilic esophagitis, and celiac disease. For the other studied GI-disorders, only moderate or insufficient evidence was found.

The question remains to what extent the association with IBD, EoE and CeD can be attributed to antibiotic exposure itself or to other factors such as infections and parental health seeking behavior. Infections in early life have been proposed to contribute to the development of chronic GI-disorders [44, 45] and it is difficult to differentiate between the role of infections and antibiotics which are prescribed for (suspected) infections. Furthermore, several GI-disorders like CeD can remain undiagnosed for a long time. Higher parental health seeking behavior can both lead to higher use of antibiotics and a higher change of diagnosing the chronic GI-disorder. Therefore, it remains unknown whether antibiotics are the true causative agent in the observed associations or whether they are intermediates in different mechanistic pathways through microbiome perturbations or changes in immune development after (suspected) infections.

Most studies found a clear association between antibiotics in early life and IBD. The study that focused on very early onset IBD (VEO-IBD), found no association between antibiotics and VEO-IBD. VEO-IBD is considered a different entity from later-onset IBD [44], since genetics play a far more important etiological role than microbial dysbiosis [45]. This may explain the lack of an association with early life antibiotics.

The primary goal of antibiotic administration is to prevent detrimental effects of serious and sometimes even life-threatening infections. However, especially in early life,

antibiotics are overused, since it is often prescribed for viral upper respiratory tract infections [46, 47]. Given its association with the occurrence of IBD, CeD and EoE, it is highly important to prevent antibiotic overuse by strict adherence to guidelines. If antibiotics are necessary, treatment would be adjusted to minimize dysbiosis. Another possible solution is to shorten the time of antibiotic administration. Oosterloo *et al.* found more health issues in the first year of life after seven days compared to two days of antibiotics in the first week of life [26]. Furthermore, whenever possible, small-spectrum antibiotics rather than broad-spectrum should be used, because these specifically reduce the capacity of pathogens to cause disease while leaving commensals unharmed [48]. If adjustment of antibiotic treatment is not possible, interventions that restore or prevent dysbiosis should be considered, such as administration of pre- or probiotics, or fecal transplants [49-52].

Some limitations of this review need to be considered. As no randomized controlled trials were available, only associations but not causality can be examined. Hence, the results must be interpreted with caution. Furthermore, both age at exposure as well as age at diagnosis varied substantially between the studies. In addition, study outcomes were also very heterogeneous, excluding a meta-analysis. Therefore, a best evidence synthesis was applied, taking the quality of the studies into account. Furthermore, the recording of antibiotic exposure was in half of the studies parental reported, which may have led to recall bias. The antibiotics were mostly analyzed as overall use, without distinguishing between types of antibiotics and therefore, it was not possible to determine associations between certain type of antibiotics and GI-disorders. Finally, for several functional gastrointestinal disorders, like IBS or GERD, only few or even no studies were found which prohibits any conclusions on these GI disorders.

One of the strengths of this review is that the search string was built and performed by an information scientist. Besides the published articles, also conference abstracts were

checked for relevant studies. Furthermore, this is the first review studying the association between antibiotics in early life and all chronic GI disorders in childhood, which provides insights in the available evidence but also shows the gap of knowledge for these associations.

For future research, it is recommended to study the association between early life antibiotics and the presence of those GI disorders that currently lack sufficient studies. Furthermore, it is necessary to gain insights in the specific effect of different types of antibiotics on the microbiome in order to optimize therapies that can prevent or counteract the detrimental effects of antibiotics in early life.

Conclusion

This systematic review shows strong evidence for an association between antibiotic exposure in the first two years of life and the presence of IBD, EoE and CeD later in childhood. For the other included GI-disorders, only moderate or insufficient evidence was found. In order to decrease the incidence of IBD, EoE and CeD, antibiotic administration in early life should be critically considered. Moreover, interventions need to be developed to restore the microbiome after unavoidable antibiotic exposure in order to prevent detrimental health consequences later in life.

What is already known

- In adults, there is increasing evidence for an association between antibiotic use and gastrointestinal disorders but in children, the evidence is scarce.
- The incidence of gastrointestinal disorders in childhood is increasing

What this study adds

- Antibiotics in early life may increase the risk of gastrointestinal disorders later in life especially inflammatory bowel disease, eosinophilic esophagitis, and celiac disease.
- Although functional gastrointestinal disorders are the most frequent in childhood, very few studies examined their association with antibiotics in early life.

References

1. Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World journal of gastroenterology. 2018;24(25):2741.
2. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. American Journal of Gastroenterology. 2020;115(4):507-25.
3. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nature Reviews Immunology. 2017;17(4):219-32.
4. Bendtsen KM, Fisker L, Hansen AK, Hansen CH, Nielsen DS. The influence of the young microbiome on inflammatory diseases—lessons from animal studies. Birth Defects Research Part C: Embryo Today: Reviews. 2015;105(4):278-95.
5. Major G, Spiller R. Irritable bowel syndrome, inflammatory bowel disease and the microbiome. Current opinion in endocrinology, diabetes, and obesity. 2014;21(1):15.
6. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? World journal of gastroenterology: WJG. 2014;20(39):14105.
7. Weber TK, Polanco I. Gastrointestinal microbiota and some children diseases: a review. Gastroenterology research and practice. 2012;2012.
8. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016;65(11):1906-15.
9. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Science translational medicine. 2016;8(343):343ra82-ra82.
10. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. Scandinavian journal of gastroenterology. 2018;53(1):1-7.
11. Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho K-L, Kronman MP, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. American Journal of Gastroenterology. 2014;109(11):1728-38.
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336-41.
13. Page MJ, Shamseer L, Tricco AC. Registration of systematic reviews in PROSPERO: 30,000 records and counting. Systematic reviews. 2018;7(1):32.
14. Wilczynski NL, McKibbon KA, Haynes RB, editors. Search filter precision can be improved by NOTing out irrelevant content. AMIA Annual Symposium Proceedings; 2011: American Medical Informatics Association.
15. Van Eck N, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. scientometrics. 2010;84(2):523-38.
16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid AJSr. Rayyan—a web and mobile app for systematic reviews. 2016;5(1):210.
17. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Ottawa Hospital Research Institute; 2019 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
18. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle-Ottawa quality assessment form for cohort studies. pp. E17–E18. 2014.
19. Van Tulder M, Furlan A, Bombardier C, Bouter L, Group EBotCCBR. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine. 2003;28(12):1290-9.

20. Veronese N, Solmi M, Luchini C, Lu R-B, Stubbs B, Zaninotto L, et al. Acetylcholinesterase inhibitors and memantine in bipolar disorder: A systematic review and best evidence synthesis of the efficacy and safety for multiple disease dimensions. Journal of affective disorders. 2016;197:268-80.
21. Slavin RE. Best evidence synthesis: an intelligent alternative to meta-analysis. Journal of clinical epidemiology. 1995;48(1):9-18.
22. Canova C, Zabeo V, Pitter G, Romor P, Baldovin T, Zanotti R, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. American journal of epidemiology. 2014;180(1):76-85.
23. Hestbaek L, Sannes MM, Lous J. Large cohort study finds a statistically significant association between excessive crying in early infancy and subsequent ear symptoms. Acta Paediatrica. 2014;103(5):e206-e11.
24. Kemppainen KM, Vehik K, Lynch KF, Larsson HE, Canepa RJ, Simell V, et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. JAMA pediatrics. 2017;171(12):1217-25.
25. Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics. 2012;130(4):e794-803.
26. Oosterloo BC, van Elburg RM, Rutten NB, Bunkers CM, Crijns CE, Meijssen CB, et al. Wheezing and infantile colic are associated with neonatal antibiotic treatment. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2018;29(2):151-8.
27. Örtqvist AK, Lundholm C, Halfvarson J, Ludvigsson JF, Almqvist C. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study. Gut. 2019;68(2):218-25.
28. Sander SD, Andersen A-MN, Murray JA, Karlstad Ø, Husby S, Størdal K. Association between antibiotics in the first year of life and celiac disease. Gastroenterology. 2019;156(8):2217-29.
29. Turco R, Miele E, Russo M, Mastroianni R, Lavorgna A, Paludetto R, et al. Early-life factors associated with pediatric functional constipation. Journal of pediatric gastroenterology and nutrition. 2014;58(3):307-12.
30. Uusijarvi A, Bergstrom A, Simren M, Ludvigsson JF, Kull I, Wickman M, et al. Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain--a Swedish birth cohort study. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2014;26(6):841-50.
31. Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011;60(1):49-54.
32. Salvatore S, Baldassarre ME, Di Mauro A, Laforgia N, Tafuri S, Bianchi FP, et al. Neonatal Antibiotics and Prematurity Are Associated with an Increased Risk of Functional Gastrointestinal Disorders in the First Year of Life. The Journal of pediatrics. 2019;212:44-51.
33. Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2013;57(1):67-71.
34. Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. Journal of Allergy and Clinical Immunology. 2018;141(1):214-22.

35. Mårild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case–control study. BMC gastroenterology. 2013;13(1):109.
36. Myléus A, Hernell O, Gothefors L, Hammarström M-L, Persson L-Å, Stenlund H, et al. Early infections are associated with increased risk for celiac disease: an incident case-referent study. BMC pediatrics. 2012;12(1):194.
37. Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. The Journal of Allergy and Clinical Immunology: In Practice. 2014;2(4):475-7. e1.
38. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. The American journal of gastroenterology. 2010;105(12):2687-92.
39. Slae M, Persad R, Leung AJ-T, Gabr R, Brocks D, Huynh HQ. Role of environmental factors in the development of pediatric eosinophilic esophagitis. Digestive diseases and sciences. 2015;60(11):3364-72.
40. Virta L, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. American journal of epidemiology. 2012;175(8):775-84.
41. Witmer CP, Susi A, Min SB, Nylund CM. Early infant risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2018;67(5):610-5.
42. Bittker SS, Bell KR. Potential risk factors for celiac disease in childhood: A case-control epidemiological survey. Clinical and experimental gastroenterology. 2019;12:303.
43. Canova C, Ludvigsson JF, Di Domenicantonio R, Zanier L, Barbiellini Amidei C, Zingone F. Perinatal and Antibiotic Exposures and the Risk of Developing Childhood-Onset Inflammatory Bowel Disease: A Nested Case-Control Study Based on a Population-Based Birth Cohort. International Journal of Environmental Research and Public Health. 2020;17(7):2409.
44. Bernstein CN, Burchill C, Targownik LE, Singh H, Roos LL. Events within the first year of life, but not the neonatal period, affect risk for later development of inflammatory bowel diseases. Gastroenterology. 2019;156(8):2190-7. e10.
45. Jiang Hy, Zhang X, Zhou Yy, Jiang Cm, Shi Yd. Infection, antibiotic exposure, and risk of celiac disease: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2020;35(4):557-66.
46. van Houten CB, Naaktgeboren C, Buiteman BJ, van der Lee M, Klein A, Srugo I, et al. Antibiotic overuse in children with respiratory syncytial virus lower respiratory tract infection. The Pediatric infectious disease journal. 2018;37(11):1077-81.
47. Arnolda G, Hibbert P, Ting HP, Molloy C, Wiles L, Warwick M, et al. Assessing the appropriateness of paediatric antibiotic overuse in Australian children: a population-based sample survey. BMC pediatrics. 2020;20:1-8.
48. Melander RJ, Zurawski DV, Melander C. Narrow-spectrum antibacterial agents. Medchemcomm. 2018;9(1):12-21.
49. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. BMJ open. 2014;4(8):e005047.
50. Kumar R, Sood U, Gupta V, Singh M, Scaria J, Lal R. Recent advancements in the development of modern probiotics for restoring human gut microbiome dysbiosis. Indian Journal of Microbiology. 2019:1-14.

51. Chua MC, Ben-Amor K, Lay C, Goh AE, Chiang WC, Rao R, et al. Effect of synbiotic on the gut microbiota of cesarean delivered infants: a randomized, double-blind, multicenter study. Journal of pediatric gastroenterology and nutrition. 2017;65(1):102-6.
52. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome medicine. 2016;8(1):39.

Figure Legends

Figure 1: PRISMA flow diagram of the study selection

Figure 2: Overview of the study characteristics and association with antibiotics for IBD

Figure 3: Overview of the study characteristics and association with antibiotics for EoE

Figure 4: Overview of the study characteristics and association with antibiotics for CeD

Figure 5: Overview of the study characteristics and association with antibiotics for FGID (Infantile colics, FC, recurrent abdominal pain and regurgitation, functional diarrhea and infant dyschezia)

Figure 6: Forest plots per gastrointestinal disorder a. IBD; b. EoE; c. CeD; d. FGID (Infantile colics and functional constipation). CC= case control study, CH = cohort study, (!) Virta 2012 only shows the results of the phenoxymethylpenicillin analyses, overall use of antibiotics was not significant

Table 1 quality assessment

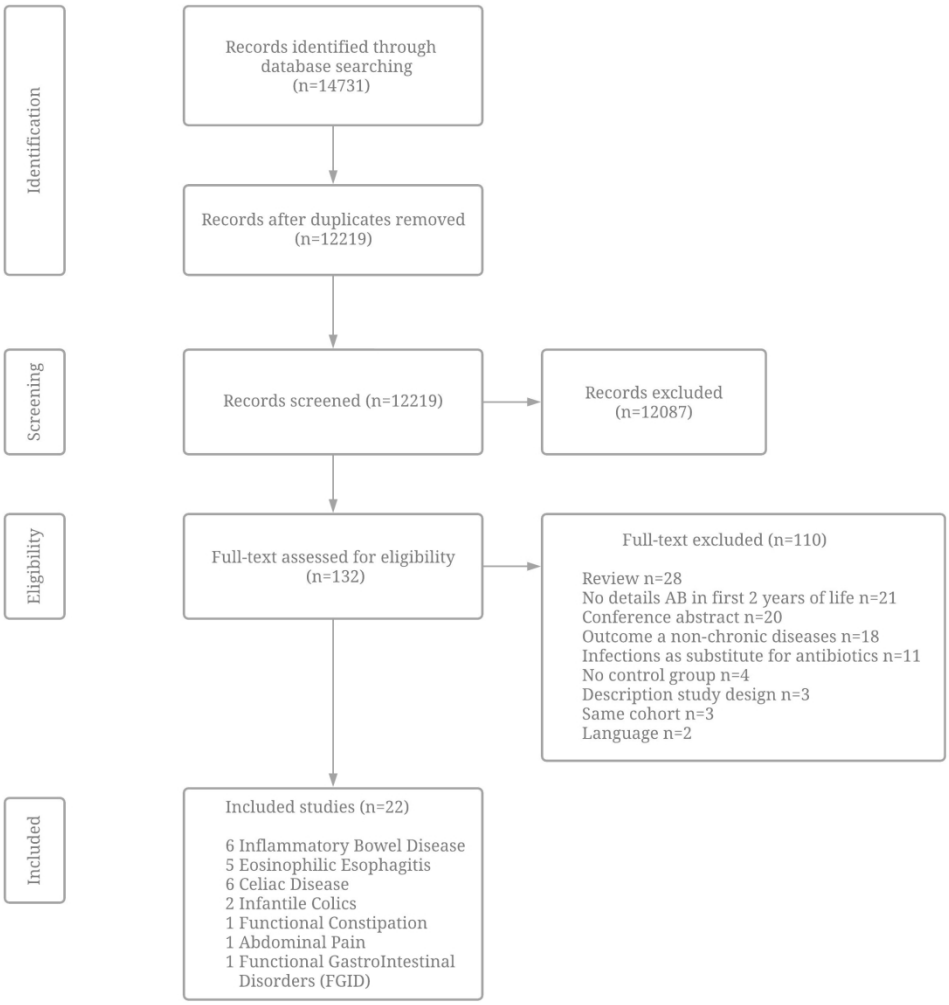
	Selection				Comparability		Outcome / Exposure			Score
	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Cohort studies*	Representativeness	Selection	Exposure	Outcome	Most important	Second important	Assessment	Duration of follow-up	Adequacy follow-up	
Canova [22]	*	*	*	*		*	*	*	*	8/9
Hestbaek [23]	*	*	*	*				*	*	6/9
Hviid [31]	*	*	*	*		*	*	*	*	8/9
Kemppainen [24]		*		*	*	*	*	*		6/9
Kronman [25]		*	*	*	*	*	*	*		7/9
Oosterloo [26]	*	*	*	*	*	*	*	*		8/9
Örtqvist [27]	*	*	*	*	*	*	*		*	8/9
Salvatore [32]	*	*	*	*		*	*	*		7/9
Sander [28]	*	*	*	*	*	*	*	*	*	9/9
Turco [29]	*	*	*	*	*	*		*	*	8/9
Uusijärvi [30]	*	*		*				*	*	5/9
Case-Control studies**	Case definition	Cases	Controls	Definition controls	Most important	Second important	Exposure	Ascertainment	Non-Response rate	Score
Bittker [42]			*	*	*			*	*	5/9
Canova [43]	*	*	*	*		*	*	*	*	8/9
Jensen [33]	*	*		*				*		4/9
Jensen [34]	*	*	*	*				*	*	6/9
Mårild [35]	*	*	*	*		*	*	*	*	8/9
Myleus [36]	*	*	*	*		*		*	*	7/9
Radano [37]	*	*		*	*	*		*	*	7/9
Shaw [38]	*	*	*	*		*	*	*	*	8/9
Slae [39]	*			*				*		3/9
Virta [40]	*	*	*	*		*	*	*	*	8/9
Witmer [41]		*	*	*	*	*		*	*	7/9

*Cohort studies: 1. Representativeness of the exposed cohort, 2. Selection of the non-exposed cohort, 3. Ascertainment of exposure, 4. Demonstration that the outcome of interest was not present at start of the study, 5. Comparability of cohorts on the basis of the design or analysis most important factor, 6. Comparability of cohorts on the basis of the design or analysis second important factor, 7. Assessment of outcome 8. Was follow-up long enough for outcomes to occur and 9. Adequacy of follow up of cohort.

**Case- Control studies: 1. Is the case definition adequate? 2. Representativeness of the cases, 3. Selection of controls, 4. Definition of controls, 5. Comparability of cases and controls on the basis of the design or analysis most important factor, 6. Comparability of cases and controls on the basis of the design or analysis second important factor, 7. Ascertainment of exposure, 8. Same method of ascertainment for cases and controls and 9. Non-Response rate

Comparability: Most important confounder: IBD and CeD: presence of IBD/ CeD in 1ste degree family member, EoE: sex, colics: atopy child and/or family, functional constipation: maternal education/social economic status, abdominal pain: lactose intolerance/ cow's milk allergy.

Comparability: Second important confounder: IBD: race and/or age, EoE: presence of other atopic diseases and/or ethnicity, CeD: sex and/ or season of birth and/or the presence of other autoimmune diseases, colics: presence of GERD and/or type of feeding and/or being a first child, functional constipation: sex and/ or age, abdominal pain: anxiety/ depression/ stress in the child and/or the parents.



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Inflammatory Bowel Disease (IBD)						
Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed Time exposure Recording details	Confounders for which corrected	Significant association	Quality score
Canova, C [43] 2020 Italy Case-control	8.8 yrs[1]	70 / 700	33 (47%) 0-12 months ATC code	Older siblings, season of birth, multiple birth, birth weight, gestation age, Apgar score at 1 minute, mother's age and education at moment of birth.	AB first six months of life childhood onset IBD ✗ Any course aOR = 1.458, 95% CI: 0.81–2.63 ↗ 2-3 courses aOR = 2.29, 95% CI: 1.01–5.24 ↗ >4 courses aOR = 6.25, 95% CI: 1.70–23.05 Ab first 12 months of life childhood onset IBD ✗ Any course aOR = 1.08, 95% CI 0.64–1.80 ↗ >4 courses aOR = 2.92, 95% CI: 1.32–6.46	8/9 High
Hviid, A [31] 2010 Denmark Cohort	3.4 yrs[1]	117 (0.02%) (50 CD and 67 UC) / 577,627	84 (72%) 0-12 months ATC code	Age, calendar period, other types of antibiotics and other times since use	Increased risk of Crohn's disease after: AB use in the last 3 months: ✓ 3-11 months RR = 3.32, 95% CI: 1.15-9.56 ✗ 1 year RR = 1.53, 95% CI: .15-15.46 AB use > 3 months previously before diagnosis: ✓ 0-2 months RR = 4.19, 95% CI: 1.64-10.68	8/9 high
Kronman, M [25] 2012 United Kingdom Cohort	Exposed 4.2 yrs[2]	748 (0.07%) / 1,072,426	436 (58%) 0-12 months Systemic AB prescriptions	Family history of IBD, chronic granulomatous disease and primary sclerosing cholangitis, age at cohort entry, gender, socioeconomic deprivation,	✓ Exposure was associated with a 5.5-fold increased IBD risk (aH = 5.51, 95% CI:1.66–18.28). ↗ Exposure to >2 anti-anaerobic antibiotic courses was more highly associated with IBD development than exposure to 1 to 2 courses (aHR = 4.77, 95% CI: 2.13–10.68) versus (3.33, 95% CI: 1.69–6.58). 💊 Fluoroquinolone (aHR= 2.09, 95% CI: 1.10–3.98) and metronidazole exposure (aHR = 186.25, 95% CI: 10.86–3193.65) was significantly associated with IBD.	7/9 moderate 20
Örtqvist, A [27] 2018 Sweden Cohort	2 yrs[1]	95 (0.01%) 51 IBD (CD and/or UC), 20 CD & 24 UC / 827,239	IBD 43 (84,3%) CD 16 (80%) UC 20 (83.3%) 0-12 months ATC code	Parental history of IBD, parental education, country of birth of parents, and mode of delivery	✗ No significant associations (any and PcV antibiotics) or dose–response relationship were found	8/9 high
Shaw, S [38] 2010 Canada Case-control	8.4 yrs[1]	36 / 360	21 (58%) 0-12 months ATC code	Age, sex, and region of residence	✓ One or more dispensations of antibiotics was associated with 2.9 times the odds (95% CI: 1.2-7.0, P = 0.017) of having IBD. ✓ Stratified by IBD type, only CD was significant (OR = 5.3, 95% CI: 1.6-17.4; P = 0.006). ↗ A dose dependent association was found for 2-4 (OR = 2.9, 95% CI: 1.1-7.8; P = 0.039) and 5+ (OR = 5.0, 95 % CI: 1.3-18.9; P = 0.18) prescriptions.	8/9 high
Virta, L [40] 2012 Finland Case-control	CD: 9.7 yrs[3] UC: 8.5 yrs[3]	595 (233 CD, 362 UC) / 2,380	313 (52,6%) 0-12 months ATC code	Age, gender, place of residence and the presence of additional chronic diseases	✗ Use of AB overall was not significant 💊 Use of phenoxymethylpenicillin was associated with an increased risk of CD. (aOR = 2.54, 95% CI: 1.3-4.98)	8/9 high

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Eosinophilic Esophagitis (EoE)						
Author Year Country Design	Age diagnosis	Cases / Controls	Cases exposed Time exposure Recording details	Confounders for which corrected	Significant association	Quality score
Jensen, E [33] 2013 North Carolina (USA) Case-control	Cases 11 yrs	31 / 52	22 (71%) 0-12 months Motherly reported	None	✔ Antibiotics were associated with EoE (OR= 6, 95% CI: 1.7–20.8)	4/9 weak
Jensen, E [34] 2018 North Carolina (USA) Case-control	Cases 10.6 yrs	127 / 121	91 (72%) 0-12 months Motherly reported	Maternal education and NICU admission	✔ Antibiotics were associated with EoE (aOR = 2.30, 95% CI: 1.21-4.38)	6/9 moderate
Radano, M [37] 2014 Massachusetts (USA) Case-control	Cases 3 yrs	25 / 74	17 (67%) 0-12 months Parental reported	Sex, personal history of atopy, family history of atopy and age	✔ Antibiotics were associated with EoE (OR = 3.61, 95% CI: 1.11-11.74; P = .03)	7/9 moderate
Slae, M [39] 2015 Canada Case-control	Cases 8.6 yrs	102 / 167	60 (59%) 0-12 months Parental reported	Breastfeeding, having older siblings, early day care attendance, exposure to farm animals, fast food consumption	✘ Rates of antibiotic exposure were similar for cases and controls.	3/9 weak
Witmer, C [41] 2018 USA Case-control	4.2 yrs	1410 / 2,820	409 (29%) 0-6 months Pharmaceutical coding	Sex, markers of atopic disease, age, medication exposure, prematurity, caesarean delivery, prolonged rupture/ chorioamnionitis, reflux, feeding problems, infantile colic, oral candidiasis, and erythema toxicum neonatorum	✔ The association with antibiotic exposure was statistically significant (aOR = 1.31, 95% CI: 1.10–1.56).	7/9 moderate

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Celiac Disease (CeD)						
Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed Time exposure Recording details	Confounders for which corrected	Significant association	Quality score
Bittker, S [42] 2019 USA Case-control	6.1 yrs[1]	332 / 241	237 (71%) 0-24 months Parental reported	Child's age and ethnicity, maternal education, and maternal age at birth	✔ Antibiotic exposure is associated with susequent CD (aOR = 1.133, 95% CI: 1.037–1.244; p= 0.007) The ORs on antibiotic use increase with the number of antibiotic courses	5/9 moderate
Canova, C [22] 2014 Italy Cohort	6.4 yrs[1]	1.227 CeD (0.6%) 866 confirmed* and 361 unconfirmed* / 203,557	336 (47%) 0-12 months ATC code	Sex and year of birth. Sensitivity analysis with only pathological confirmed diagnosis of villous atrophy were also corrected for maternal education.	✔ Increased risk of developing CeD after at least 1 AB course (IRR = 1.24, 95% CI: 1.07-1.43), (IRR = 1.31, 95% CI: 1.10-1.56) for histopathologically confirmed CeD The risk increased with increasing number of AB courses (P-trend < 0.01). Cephalosporin use was strongly associated with CeD onset (IRR = 1.42, 95% CI: 1.18-1.73), (IRR = 1.51, 95% CI: 1.21-1.89) for histopathologically confirmed CeD. For first- and second-generation drugs: (IRR = 1.39, 95% CI: 1.11-1.76 and third- and fourth-generation drugs: IRR = 1.49, 95% CI: 1.14-1.95).	8/9 high
Kemppainen, K [24] 2017 Finland, Germany, Sweden and the USA Cohort	21.4 months[1]	783 (11,9%) / 6,558	Unknown 0-24 months Parental reported	First-degree relative with CeD genotype, sex, season of birth, country, caesarean delivery, probiotic use before 90 days of age, breastfeeding status at 90 days of age and maternal AB use during pregnancy.	✘ Exposure to AB was not associated with CeD. Receiving 2 or more doses of macrolides within the first year of life (157 of 6558 [2.4%]) had elevated CeD risk (HR = 1.77, 95% CI: 1.18-2.66; P = .006 before but not after adjustment).	6/9 moderate
Märild, K [35] 2013 Sweden Case-control	0-2 yrs[1]	132 celiac disease / 655 12 inflammation / 60 17 normal mucosa / 85	CeD 51 (39%) Infl* 6 (50%) 0-24 months ATC code	Sex, age, education, number of outpatient visits before biopsy	✔ Exposure to AB was associated with CeD Odds ratios for prior AB use (CeD): cases 51/132 (38.6%) controls 189/655 (28.9%) (OR = 1.58, 95% CI: 1.07-2.34)	8/9 high
Myleus, A [36] 2012 Sweden Case-control	14 months[1]	373 / 581	97 (26%) 0-6 months Parental reported	Sex, age, and family's area of residence	✘ No significantly increased risk for celiac disease (OR = 1.2, 95% CI: 0.87-1.6; P=0.27).	7/9 moderate
Sander, S [28] 2019 Denmark and Norway Cohort	Danish: 11.6 yrs[3] Norwegian: 5.4 yrs[3]	Danish: 1427 (0.12%) / 1,168,656 Norwegian: 1919 (0.36%) / 537,457	Danish: 622 (43.6%) Norwegian: 390 (20.3%) 0-12 months ATC code	Sex, season of birth, parity, maternal educational level, maternal age, associated comorbidity, type 1 diabetes child and/ or mother, hospitalization with infection	✔ Exposure to systemic AB (penicillins and extended spectrum penicillins) was positively associated with diagnosed celiac disease in both cohorts (pooled adjusted OR = 1.26, 95% CI: 1.16–1.36) Dose-dependent relation between number of AB courses and the risk of CeD (pooled adjusted OR for each additional dispensed AB = 1.08, 95% CI: 1.05–1.11).	9/9 high

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Functional GI-disorders (FGIDs)						
Author Year Country Design	Age diagnosis	Cases / Cohort	Cases exposed Time exposure Recording details	Confounders for which corrected	Significant association	Quality score
Infantile colics						
Hestbaek, L [23] 2014 Denmark Cohort	0-6 months	2183 (8,1%) / 26,983	excessive 895 (41%) extreme excessive 355 (50%) 0-6 months Motherly reported	None	✔ At 6-month-olds, statistically significant associations between excessive crying and the use of antibiotics due to ear infections (OR = 1.47, 95% CI: 1.18–1.82) were found.	6/9 moderate
Oosterloo, B [26] 2018 The Netherlands Cohort	0-1 yr	74 (20%) / 362	33 (45%) 0-7 days Broad-spectrum AB intravenous for 2-3 days (AB2) or 7 days (AB7).	Familial history of atopy, duration of breastfeeding, presence of siblings, delivery mode, tobacco exposure, day care attendance and household educational level	✔ Antibiotic treatment was an independent risk factor for infantile colic (aOR = 1.66, 95%CI: 1.00-2.77, P = .05). 🕓 Parent-reported infantile colic was higher in AB7 compared to no antibiotics (AB-) and AB2 (24.8%, 14.4% and 14.3%, P = .048 and P = .015). ✔ Doctors-diagnosed infantile colic was higher in AB+ than in AB− (4.0% vs 0.4%; P = .014).	8/9 high
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	265 (41.9%) / 632	141 (22.3%) 0-7 days Hospital chart and parental report	Feeding pattern at 1 month of life, gestational age, delivery mode, neonatal complications, birth weight and duration of hospitalization at birth	✘ No association was found (OR=1.16; 95%CI: 0.79-1.70, p=0.439)	7/9 moderate
Functional constipation (FC)						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	128 (26.6%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	Feeding pattern at 1 month of life, gestational age, mode of delivery, neonatal complications, birth weight and duration of hospitalization at birth	✘ No association was found (OR=0.77; 95%CI: 0.49-1.20, p=0.242)	7/9 moderate
Turco, R [29] 2014 Italy Cohort	0-1 yr	43 (10.7%) / 465	15 (34.8%) 0-12 months Parental reported	Educational and professional level of the parents, sex, breastfeeding, family history of FGIDs and/or family and/or personal history of atopy, number of siblings, anti-inflammatory drugs or corticosteroids, vitamin and food supplements, weaning, nursery school age, fever episodes before onset, and residence in a community with >3000 citizens.	✘ No statistically significant association was found (26% vs 19%).	8/9 high
Recurrent abdominal pain						
Uusijärvi, A [30] 2014 Sweden Cohort	12 yrs	Monthly: 231 (8,7%) Weekly: 111 (4,2%) / 2,654	Monthly 1900 (71.5%) Weekly 81 (72,9%) 0-24 months Parental reported	Sex, asthma at one year, and/or asthma at 12 years of age	✔ Stratified analyses showed that girls, who received antibiotics during both the first and the second year of life, had an increased risk of AP at 12 years (OR = 1.65, 95% CI: 1.09–2.49).	5/9 moderate
Regurgitation, functional diarrhea and infant dyschezia						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	Regurgitation:236 (37.3%) Functional diarrhea: 24 (3.8%) Infant dyschezia: 199 (31.5%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	Feeding pattern at 1 month of life, gestational age, mode of delivery, neonatal complications, birth weight and duration of hospitalization at birth	✘ No association was found for regurgitation (OR=1.29, 95%CI: 0.88-1.90, p=0.190), functional diarrhea (OR=0.90, 95%CI: 0.33-2.45, p=0.835), or infant dyschezia (OR=1.29, 95%CI: 0.87-1.93, p=0.205).	7/9 moderate

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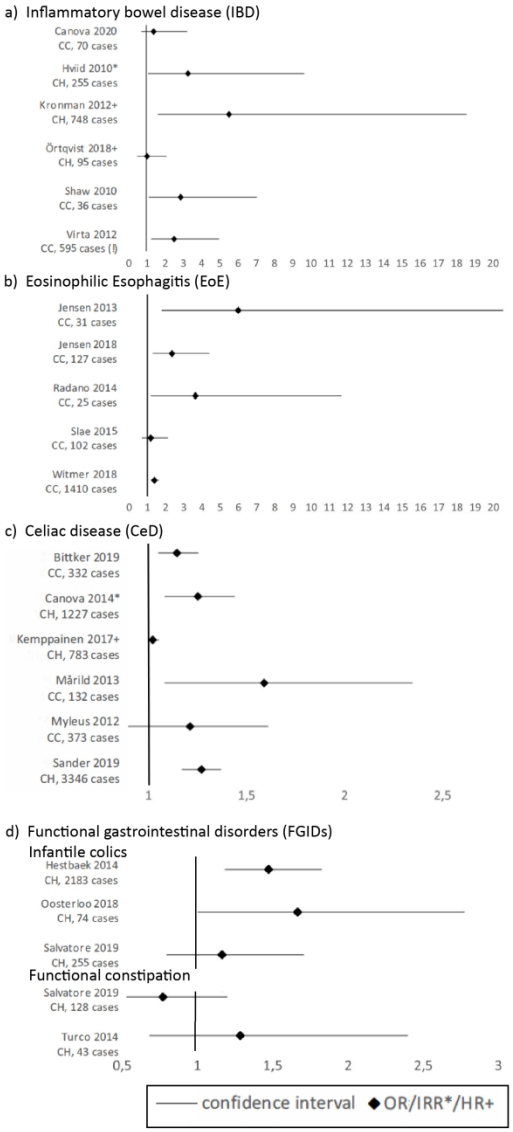

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Supplementary Table 1 search strategy

	Ovid MEDLINE(R) ALL <1946 to 2020 June 08> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp infant death/ or infant/	788526
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kf,ti.	686417
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	655139
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1066665
5	or/1-4 [Ia - children 0-4 yrs]	2564903
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kf,ti. [Ib - children 0-4 yrs]	5357
7	Gentamycins/	18247
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kf,ti.	27205
9	or/7-8 [IIa first week exclusive use]	32706
10	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	399419
11	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kf,ti. [IIc]	48465
12	(sepsis and infant).hw.	9982
13	(sepsis adj2 early).ab,kf,ti.	1919
14	or/12-13 [IId]	11418
15	(childhood disease? and (risk or environmental factor?)).ab,kf,ti. [IIe]	360

16	exp inflammatory bowel disease/ or abdominal pain/ or aerophagy/ or dyspepsia/ or constipation/ or celiac disease/ or appendicitis/ or gastritis/ or enteritis/ or exp diarrhea/ or colic/ or Eosinophilic Esophagitis/ or Gastroesophageal Reflux/ or esophageal stenosis/	266125
17	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kf,ti.	305723
18	Pyloric Stenosis, Hypertrophic/	654
19	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kf,ti.	1513
20	18 or 19	1622
21	limit 20 to yr="2015-current"	184
22	or/16-17,21 [outcomes]	424883
23	follow-up studies/ or longitudinal studies/ or retrospective studies/	1441183
24	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kf,ti.	3775119
25	(case control or cohort study or (risk and review)).mp.	1032965
26	observational study.pt.	80055
27	or/23-26 [study design]	4954421
28	5 or 6 [Ia+b - children 0-4 yrs]	2566952
29	28 and (10 or 11) and 22 and 27	2707
30	and/9,28	4170
31	and/14,22	319
32	or/15,29-31	7477
33	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kf,ti. [NOTing out green]	1105473
34	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kf,ti. [NOTing out blue]	505926
35	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kf,ti. [NOTing out red]	803243

36	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kf,ti. [NOTing out yellow]	1949849
37	or/33-36	4123179
38	32 not 37 [NOTing out]	5238
39	animals/ not humans/	4672110
40	38 not 39	5096
41	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kf,ti.	0
42	40 or 41	5096
	Ovid Embase Classic+Embase <1947 to 2020 June 06> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp *infant/ or *infancy/ or infant.hw.	798854
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kw,ti.	875279
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	1051740
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1708558
5	or/1-4 [Ia - children 0-4 yrs]	3541363
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kw,ti. [Ib - children 0-4 yrs]	7292
7	*Gentamicin/	35017
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kw,ti.	36468

9	"1403-66-3".rn.	104829
10	or/7-9 [IIa first week exclusive use]	113443
11	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	870330
12	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kw,ti. [IIc]	65283
13	(sepsis and infant).hw.	11891
14	(sepsis adj2 early).ab,kw,ti.	2988
15	or/13-14 [IId]	14425
16	(childhood disease? and (risk or environmental factor?)).ab,kw,ti. [IIe]	498
17	exp *inflammatory bowel disease/ or *abdominal pain/ or *aerophagia/ or *dyspepsia/ or exp *constipation/ or *celiac disease/ or *appendicitis/ or *gastritis/ or *enteritis/ or *diarrhea/ or *infantile diarrhea/ or *colic/ or *infantile colic/ or *Eosinophilic Esophagitis/ or *Gastroesophageal Reflux/ or *esophageal stenosis/	267207
18	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kw,ti.	493146
19	*hypertrophic pylorus stenosis/	1263
20	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kw,ti.	1940
21	19 or 20	2152
22	limit 21 to yr="2015-current"	231
23	or/17-18,22 [outcomes]	586712
24	follow up/ or longitudinal study/ or retrospective study/	2412789
25	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kw,ti.	5528713
26	observational study.kw,ti.	27665
27	(case control or cohort study or (risk and review)).mp.	1211338
28	or/24-27 [study design]	6774290
29	5 or 6 [Ia+b - children 0-4 yrs]	3545044
30	29 and (11 or 12) and 23 and 28	5878

31	and/10,28-29	5192
32	and/15,23	576
33	or/16,30-32	11847
34	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kw,ti. [NOTing out green]	1532636
35	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kw,ti. [NOTing out blue]	664053
36	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kw,ti. [NOTing out red]	962439
37	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kw,ti. [NOTing out yellow]	2455192
38	or/34-37	5285640
39	33 not 38 [NOTing out]	9118
40	(animal/ or animal experiment/ or animal model/ or nonhuman/) not human/	6454629
41	39 not 40	8980
42	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kw,ti.	0
43	NTR6681.cn.	0
44	or/41-43	8980
	Web of Science Core Collection: **- SCI-EXPANDED 1975-present** **- SSCI 1975 - present** **-A&HCI 1975 - present** **- ESCI 2015 - present** **Search date: 9 June 2020**	
#	**Searches**	**results**
#1	TS=(early life or infant or infancy or toddler or preschool or (early N4 (childhood or child or children or pediatric)) or minors or baby or babies or kindergarten or newborn)	1085229
#2	AB=(("0" or "1" or "2" or "3" or "4") N1 (age? or yr? or year?))	1805
#3	AB=(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") N1 month?)	1183

#4	TS=((pediatric or infantile or juvenile) N1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic AND Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis))	3
#5	#4 OR #3 OR #2 OR #1	1087634
#6	TS=antibiotic	334292
#7	#6 AND #5	15781
#8	TS=(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3")	25466
#9	#8 OR #7	40687
#10	TS=(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture)	252018
#11	TS=(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis)	1233
#12	#11 OR #10	253145
#13	#12 AND #9	655

Supplementary table 2 Confounders in the quality assessment

Study outcome	Most important	Second important
IBD	Presence of IBD in first degree family members	Ethnicity and/or age
EoE	Sex	Presence of other atopic diseases and/or ethnicity
CeD	Presence of CeD in first degree family member	Sex and/or season of birth and/or the presence of other autoimmune diseases
Colics	Presence of atopy in first degree family members	Presence of GERD and/or type of feeding and/or being a first child
Functional constipation	Maternal education/social economic status	Sex and/or age
Abdominal pain	Lactose intolerance/cow's milk allergy	Anxiety/depression/stress in the child and/or the parents

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Early life antibiotics and childhood gastrointestinal disorders: a systematic review

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Contributors' Statement Page:

K. Kamphorst contributed to the design, the analyses and interpretation of the study, drafting of the initial manuscript, and reviewed and revised the manuscrip*t.*

E. Van Daele contributed to the analysis and interpretation of the study and critically revised the manuscript.

A.M. Vlieger and R.M. van Elburg contributed to the conception of the study, interpretation of the data and critically revised the manuscript.

J.G. Daams conceptualized and performed the systematic search and critically revised the manuscript.

J. Knol contributed to the conception and design of the study and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background: In adults, there is increasing evidence for an association between antibiotic use and gastrointestinal disorders but in children, the evidence is scarce.

Objective: Assess the association between exposure to antibiotics in the first two years of life in term born children and the presence of chronic gastrointestinal disorders later in childhood.

Design: For this systematic review the MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020. Title and abstract screening (n=12,219), full-text screening (n=132) as well as the quality assessment with the Newcastle–Ottawa Scale were independently performed by two researchers.

Main outcome measures: The association between antibiotics and inflammatory bowel disease (n=6), eosinophilic esophagitis (n=5), celiac disease (n=6), infantile colics (n=3), functional constipation (n=2), recurrent abdominal pain, regurgitation, functional diarrhea, and infant dyschezia were examined.

Results: Twenty-two studies were included, 11 cohort and 11 case-control studies. A best evidence synthesis showed strong evidence for an association between antibiotic exposure in the first two years of life and the presence of inflammatory bowel disease, and celiac disease during childhood. Moderate evidence was found for an association with eosinophilic esophagitis and no association with functional constipation in the first year of life. There was insufficient evidence for the other studied disorders.

Conclusions: The use of antibiotics in early life may increase the risk of gastrointestinal disorders later in life. Further studies are necessary to unravel the underlying mechanisms and determine potential preventive measures. Meanwhile judicious use of antibiotics in early childhood is highly warranted.

Introduction

The incidence of pediatric gastrointestinal disorders (GI-disorders), such as pediatric inflammatory bowel disease (IBD) and celiac disease (CeD), is rising [1, 2]. The increase in pediatric GI-disorders is most likely related to environmental factors and recently the focus has been on the role of the intestinal microbiome. A microbiome that has been disturbed by factors like stress, dietary change, environmental factors or drugs, can result in alterations in the immune system [3]. Several studies have shown that a disturbed microbiome can be a cause or trigger of GI disorders, probably mediated by these immunological changes [4-7].

One of the drugs with the most profound effect on the microbiome are antibiotics [8]. The impact of antibiotics on the microbiome depends on various factors such as type of antibiotic, dosage, and duration of exposure[8]. Furthermore, age at exposure is probably also important. The gut of a newborn infant is almost sterile with a low diversity and matures according to several developmental stages with increasing diversity over time [9]. The microbiome stabilizes around the age of 2 to 3 years [9]. Since this developing gut microbiota plays an important role in the training of both innate and adaptive immune system, it is likely that antibiotics will have their biggest impact when administered in the first two years of life.

For the association between antibiotic use and GI disorders, that has been shown in adults, [10], there is only limited evidence in children [11]. Therefore, the aim of this systematic review was to assess the association between exposure to antibiotics in the first two years of life and the presence of chronic gastrointestinal disorders during childhood.

Method

Study selection

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and registered in PROSPERO CRD42019132631 [12, 13]. MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020 to identify all studies examining the association between antibiotic exposure in the first two years of life and the presence of common chronic (longer than two weeks, in order to exclude viral diarrhea) gastrointestinal disorders during the first 18 years of life. We searched for associations with IBD, eosinophilic esophagitis (EoE), CeD, irritable bowel syndrome (IBS), (functional) abdominal pain, constipation, dyspepsia, aerophagia, infantile colic, gastroesophageal reflux (GERD), regurgitation, dyschezia and chronic diarrhea.

A multi stranded search approach comprised various concept combinations of children aged 0-4 years, prognosis, gastrointestinal disorders and antibiotics. In order to reduce recall noise and enhance search results precision we used VOS-viewer to identify terms for NOTing out irrelevant records from databases searched [14, 15]. See supplementary file 1 for the full search strategies.

Patient and Public Involvement statement

As this is a systematic review of the literature, there were no patients involved in the design of the research question nor the study itself. Furthermore, for the same reason no approval for the study was required from an ethical committee.

In- and exclusion criteria

Studies were included if: 1. Antibiotics were administered between full-term birth and two years of age. 2. Study outcome was diagnosis with a chronic GI-disorder during the first

18 years of life. 3. Antibiotic use was before the diagnosis of the GI-disorder. 4. A control group was included. 5. In case multiple studies were found examining similar outcomes in one cohort, only the study with the largest cohort was included. No restrictions were placed on the time period of publication. Searches were limited to studies conducted in humans and excluded if the full text was not available in English, Dutch, German or French.

All records found in the search were exported into Rayyan after deduplication [16]. Two researchers (KK and EVD) independently performed title and abstract screening as well as full-text screening. After consensus about the study selection, data were entered into a data extraction form, which included: author, year of publication, country, study design, cases, controls/cohort, population age, sample size exposed to antibiotics, age at exposure, details about classification by type of antibiotics, type of GI disorder, method of diagnosis, confounders for which corrected, and the association between exposure and outcome.

Methodological quality

To assess the risk of bias, two researchers (KK and EVD) independently assessed the methodological quality. Discrepancies were resolved by discussion until consensus was reached. The Newcastle–Ottawa Scale (NOS) was used, which has been developed to assess the quality of observational studies [17]. The NOS includes different instruments for assessing case-control and cohort studies. Both scales contain a maximum of nine points and assess studies in three core areas: 1. Selection of study participants 2. Comparability of groups 3. Detection of exposure / outcome. One point for comparability of groups was given when the study controlled for the main important confounder and a second point if controlled for a second important confounder, see supplementary file 2. Studies were rated high quality with a score of eight or higher, moderate quality with a score between five and seven and weak quality with a score of four or less [18].

Data analyses

To synthesize the methodological quality of the studies, a commonly used best evidence synthesis was applied per disorder in which the methodological quality was considered according to the following definitions: 1. strong evidence, provided by generally consistent findings in at least two high-quality studies. 2. moderate evidence, provided by generally consistent results in one high-quality study and at least one moderate- or low-quality study, or generally consistent results in multiple moderate- or low-quality studies. 3. insufficient evidence, when less than two studies were available or inconsistent findings in multiple studies [19-21]. Results were considered consistent when at least 75% of the studies showed results in the same direction.

Results

Search results

Of the 14,731 retrieved records, 12,219 remained after removing duplicates. These records were screened; 132 were assessed as eligible and read in full-text of which 110 were excluded and 22 studies included in this review. Details of the selection procedure are shown in Figure 1.

Study characteristics

The included studies were published between 2010 and 2020 (table 1a-d): 11 cohort studies [22-32] and 11 case-control studies [33-43]. The studies were performed in Sweden (n=4) [27, 30, 35, 36], the United States of America (USA) (n=5) [33, 34, 37, 41, 42], Italy (n=4) [22, 29, 32, 43], Denmark (n=2) [23, 31], Canada (n=2) [38, 39], and one in the United Kingdom [25], the Netherlands [26], and Finland [40]. There were two international studies, one in Denmark and Norway [28], and another in Finland, Germany, Sweden and the USA [24].

The associations between antibiotics and the following GI-disorders were examined: IBD (n=6) [25, 27, 31, 38, 40, 43], EoE (n=5) [33, 34, 37, 39, 41], CeD (n=6) [22, 24, 28, 35, 36, 42], infantile colics (n=3) [23, 26, 32], functional constipation (n=2) [29, 32], recurrent abdominal pain (n=1) [30]. One study examined several functional GI-disorders (FGIDs): infantile colics, functional constipation, functional diarrhea, infant dyschezia, and regurgitation[32].

Exposure to antibiotics was studied in the first two years of life (n=4) [24, 30, 35, 42], the first 18 months of life (n=1) [23], the first year of life (n=13) [22, 25, 27-29, 31, 33, 34, 37-40, 43], the first six months of life (n=2) [36, 41], and the first week of life (n=2) [26, 32] (table 1a-d). Since only a few studies provided details about type of antibiotics and/or number of antibiotic treatments in the first two years of life, the associations include mostly the overall antibiotic exposure.

Quality assessment

Ten studies were of high quality[22, 26-29, 31, 35, 38, 40, 43], ten studies moderate [23-25, 30, 32, 34, 36, 37, 41, 42], and two weak [33, 39] (Table 2). Frequently observed weaknesses were a high dropout rate in the cohort studies, assessment of antibiotic exposure through parental reports, and no correction for important confounders.

Inflammatory Bowel Disease

Exposure to early life antibiotics was associated with the development of IBD in five out of six studies [25, 31, 38, 40, 43] (NOS = 7,8,8,8,8), whereas no association was found in one study examining Very Early Onset (VEO) IBD, (before six years of age) [27] (NOS = 8). Three studies found a dose-response relation [25, 38, 43] and an increased risk after fluoroquinolone [25], metronidazole [25], and phenoxymethylpenicillin[40] exposure. In two studies IBD was stratified by type and only the odds ratio for Crohn's disease, but not for ulcerative colitis, was significant [38, 40]. Forest plots of the main results are shown in Figure 2a.

Eosinophilic esophagitis

In four of the five studies early life antibiotics was associated with EoE [33, 34, 37, 41] (NOS = 4,6,7,7), whereas in one study the rates of parental reported antibiotic use were similar for cases and controls [39] (NOS = 3) (figure 2b).

Celiac disease

In four studies, of which three had a high quality, a significant association between early life antibiotics and the presence of CeD was found [22, 28, 35, 42] (NOS = 8,9,8,5), whereas in two moderate quality studies no association was found [24, 36] (NOS = 6,7) (figure 2c). Three studies showed a dose-response relationship between exposure to antibiotics and the

risk of CeD [22, 28, 42]. Furthermore, use of cephalosporin [22] and multiple courses of macrolides [24] showed a positive association with the development of CeD.

Infantile colics

Two studies found a significant association between early life antibiotics and infantile colics [23, 26] (NOS = 6,8), while one study found no association [32] (NOS = 7) (figure 2d).

Functional constipation

In both studies, no association was found between early life antibiotics use and functional constipation in the first year of life [29, 32] (NOS = 8,7).

Recurrent abdominal pain

The only study examining the association between antibiotics use in the first two years of life and the risk of recurrent abdominal pain (AP) at 12 years of age [30] (NOS = 5) found that only girls, but not boys, who received antibiotics in both the first and second year of life, had an increased risk of AP at 12 years.

Regurgitation, dyschezia and functional diarrhea

In one study no association was found between antibiotics in the first week of life and regurgitation, dyschezia and functional diarrhea [32] (NOS = 7).

Syntheses of individual results

Using the definitions for the best evidence synthesis, described in the method section, it can be concluded that there is strong evidence for an association of antibiotics in early life with IBD and CeD. There is moderate evidence for an association with EoE and no association with infantile constipation. The current evidence for an association between antibiotics in early life and the other studied GI-disorders is considered insufficient.

Discussion

This systematic review with best evidence syntheses on the association between antibiotic exposure in the first two years of life and chronic GI disorders during childhood showed strong evidence for this association with inflammatory bowel disease, eosinophilic esophagitis, and celiac disease. For the other studied GI-disorders, only moderate or insufficient evidence was found.

The question remains to what extent the association with IBD, EoE and CeD can be attributed to antibiotic exposure itself or to other factors such as infections and parental health seeking behavior. Infections in early life have been proposed to contribute to the development of chronic GI-disorders [44, 45] and it is difficult to differentiate between the role of infections and antibiotics which are prescribed for (suspected) infections. Furthermore, several GI-disorders like CeD can remain undiagnosed for a long time. Higher parental health seeking behavior can both lead to higher use of antibiotics and a higher chance of diagnosing the chronic GI-disorder. Therefore, it remains unknown whether antibiotics are the true causative agent in the observed associations or whether they are intermediates in different mechanistic pathways through microbiome perturbations or changes in immune development after (suspected) infections.

Most studies found a clear association between antibiotics in early life and IBD. The study that focused on very early onset IBD (VEO-IBD), found no association between antibiotics and VEO-IBD. VEO-IBD is considered a different entity from later-onset IBD [44], since genetics play a far more important etiological role than microbial dysbiosis [45]. This may explain the lack of an association with early life antibiotics.

The primary goal of antibiotic administration is to prevent detrimental effects of serious and sometimes even life-threatening infections. However, especially in early life,

antibiotics are overused, since they are often prescribed for viral upper respiratory tract infections [46, 47]. Given its association with the occurrence of IBD, CeD and EoE, it is highly important to prevent antibiotic overuse by strict adherence to guidelines. If antibiotics are necessary, treatment would be adjusted to minimize dysbiosis. Another possible solution is to shorten the time of antibiotic administration. Oosterloo *et al.* found more health issues in the first year of life after seven days compared to two days of antibiotics in the first week of life [26]. Furthermore, whenever possible, narrow-spectrum antibiotics rather than broad-spectrum should be used, because these specifically reduce the capacity of pathogens to cause disease while leaving commensals unharmed [48]. If adjustment of antibiotic treatment is not possible, interventions that restore or prevent dysbiosis should be considered, such as administration of pre- or probiotics, or fecal transplants [49-52].

Some limitations of this review need to be considered. As no randomized controlled trials were available, only associations but not causality can be examined. Additionally, the studied results were not evaluated for their precision and associations with wide confidence intervals can indicate uncertainty about the magnitude of the association. Hence, the results must be interpreted with caution. Furthermore, both age at exposure as well as age at diagnosis varied substantially between the studies. In addition, study outcomes were also very heterogeneous, excluding a meta-analysis. Therefore, a best evidence synthesis was applied, taking the quality of the studies into account. Furthermore, the recording of antibiotic exposure was in half of the studies parental reported, which may have led to recall bias. The antibiotics were mostly analyzed as overall use, without distinguishing between types of antibiotics and therefore, it was not possible to determine associations between certain type of antibiotics and GI-disorders. Finally, for several functional gastrointestinal disorders, like IBS or GERD, only few or even no studies were found which prohibits any conclusions on these GI disorders.

One of the strengths of this review is that the search string was built and performed by an information scientist. Besides the published articles, also conference abstracts were checked for relevant studies. Furthermore, this review studies the association between antibiotics in early life and all chronic GI disorders in childhood, which provides insights in the available evidence but also shows the gap of knowledge for these associations.

For future research, it is recommended to study the association between early life antibiotics and the presence of those GI disorders that currently lack sufficient studies. Furthermore, it is necessary to gain insights in the specific effect of different types of antibiotics on the microbiome in order to optimize therapies that can prevent or counteract the detrimental effects of antibiotics in early life.

Conclusion

This systematic review shows strong evidence for an association between antibiotic exposure in the first two years of life and the presence of IBD and CeD later in childhood. For the other included GI-disorders, only moderate or insufficient evidence was found. In order to decrease the incidence of IBD and CeD, antibiotic administration in early life should be critically considered. Moreover, interventions need to be developed to restore the microbiome after unavoidable antibiotic exposure in order to prevent detrimental health consequences later in life.

What is already known

- Evidence about the association between antibiotic use and gastrointestinal disorders is increasing for adults, but in children the evidence remains scarce.
- The incidence of gastrointestinal disorders in childhood is increasing

What this study adds

- Antibiotics in early life may increase the risk of gastrointestinal disorders later in life especially inflammatory bowel disease and celiac disease.
- Although functional gastrointestinal disorders are the most frequent in childhood, very few studies examined their association with antibiotics in early life.

References

1. Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World journal of gastroenterology. 2018;24(25):2741.
2. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. American Journal of Gastroenterology. 2020;115(4):507-25.
3. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nature Reviews Immunology. 2017;17(4):219-32.
4. Bendtsen KM, Fisker L, Hansen AK, Hansen CH, Nielsen DS. The influence of the young microbiome on inflammatory diseases—lessons from animal studies. Birth Defects Research Part C: Embryo Today: Reviews. 2015;105(4):278-95.
5. Major G, Spiller R. Irritable bowel syndrome, inflammatory bowel disease and the microbiome. Current opinion in endocrinology, diabetes, and obesity. 2014;21(1):15.
6. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? World journal of gastroenterology: WJG. 2014;20(39):14105.
7. Weber TK, Polanco I. Gastrointestinal microbiota and some children diseases: a review. Gastroenterology research and practice. 2012;2012.
8. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016;65(11):1906-15.
9. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Science translational medicine. 2016;8(343):343ra82-ra82.
10. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. Scandinavian journal of gastroenterology. 2018;53(1):1-7.
11. Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho K-L, Kronman MP, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. American Journal of Gastroenterology. 2014;109(11):1728-38.
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336-41.
13. Page MJ, Shamseer L, Tricco AC. Registration of systematic reviews in PROSPERO: 30,000 records and counting. Systematic reviews. 2018;7(1):32.
14. Wilczynski NL, McKibbon KA, Haynes RB, editors. Search filter precision can be improved by NOTing out irrelevant content. AMIA Annual Symposium Proceedings; 2011: American Medical Informatics Association.
15. Van Eck N, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. scientometrics. 2010;84(2):523-38.
16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid AJSr. Rayyan—a web and mobile app for systematic reviews. 2016;5(1):210.
17. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Ottawa Hospital Research Institute; 2019 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
18. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle-Ottawa quality assessment form for cohort studies. pp. E17–E18. 2014.
19. Van Tulder M, Furlan A, Bombardier C, Bouter L, Group EBotCCBR. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine. 2003;28(12):1290-9.

20. Veronese N, Solmi M, Luchini C, Lu R-B, Stubbs B, Zaninotto L, et al. Acetylcholinesterase inhibitors and memantine in bipolar disorder: A systematic review and best evidence synthesis of the efficacy and safety for multiple disease dimensions. Journal of affective disorders. 2016;197:268-80.
21. Slavin RE. Best evidence synthesis: an intelligent alternative to meta-analysis. Journal of clinical epidemiology. 1995;48(1):9-18.
22. Canova C, Zabeo V, Pitter G, Romor P, Baldovin T, Zanotti R, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. American journal of epidemiology. 2014;180(1):76-85.
23. Hestbaek L, Sannes MM, Lous J. Large cohort study finds a statistically significant association between excessive crying in early infancy and subsequent ear symptoms. Acta Paediatrica. 2014;103(5):e206-e11.
24. Kemppainen KM, Vehik K, Lynch KF, Larsson HE, Canepa RJ, Simell V, et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. JAMA pediatrics. 2017;171(12):1217-25.
25. Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics. 2012;130(4):e794-803.
26. Oosterloo BC, van Elburg RM, Rutten NB, Bunkers CM, Crijns CE, Meijssen CB, et al. Wheezing and infantile colic are associated with neonatal antibiotic treatment. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2018;29(2):151-8.
27. Örtqvist AK, Lundholm C, Halfvarson J, Ludvigsson JF, Almqvist C. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study. Gut. 2019;68(2):218-25.
28. Sander SD, Andersen A-MN, Murray JA, Karlstad Ø, Husby S, Størdal K. Association between antibiotics in the first year of life and celiac disease. Gastroenterology. 2019;156(8):2217-29.
29. Turco R, Miele E, Russo M, Mastroianni R, Lavorgna A, Paludetto R, et al. Early-life factors associated with pediatric functional constipation. Journal of pediatric gastroenterology and nutrition. 2014;58(3):307-12.
30. Uusijarvi A, Bergstrom A, Simren M, Ludvigsson JF, Kull I, Wickman M, et al. Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain--a Swedish birth cohort study. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2014;26(6):841-50.
31. Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011;60(1):49-54.
32. Salvatore S, Baldassarre ME, Di Mauro A, Laforgia N, Tafuri S, Bianchi FP, et al. Neonatal Antibiotics and Prematurity Are Associated with an Increased Risk of Functional Gastrointestinal Disorders in the First Year of Life. The Journal of pediatrics. 2019;212:44-51.
33. Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2013;57(1):67-71.
34. Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. Journal of Allergy and Clinical Immunology. 2018;141(1):214-22.

35. Mårild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case–control study. BMC gastroenterology. 2013;13(1):109.
36. Myléus A, Hernell O, Gothefors L, Hammarström M-L, Persson L-Å, Stenlund H, et al. Early infections are associated with increased risk for celiac disease: an incident case-referent study. BMC pediatrics. 2012;12(1):194.
37. Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. The Journal of Allergy and Clinical Immunology: In Practice. 2014;2(4):475-7. e1.
38. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. The American journal of gastroenterology. 2010;105(12):2687-92.
39. Slae M, Persad R, Leung AJ-T, Gabr R, Brocks D, Huynh HQ. Role of environmental factors in the development of pediatric eosinophilic esophagitis. Digestive diseases and sciences. 2015;60(11):3364-72.
40. Virta L, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. American journal of epidemiology. 2012;175(8):775-84.
41. Witmer CP, Susi A, Min SB, Nylund CM. Early infant risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2018;67(5):610-5.
42. Bittker SS, Bell KR. Potential risk factors for celiac disease in childhood: A case-control epidemiological survey. Clinical and experimental gastroenterology. 2019;12:303.
43. Canova C, Ludvigsson JF, Di Domenicantonio R, Zanier L, Barbiellini Amidei C, Zingone F. Perinatal and Antibiotic Exposures and the Risk of Developing Childhood-Onset Inflammatory Bowel Disease: A Nested Case-Control Study Based on a Population-Based Birth Cohort. International Journal of Environmental Research and Public Health. 2020;17(7):2409.
44. Bernstein CN, Burchill C, Targownik LE, Singh H, Roos LL. Events within the first year of life, but not the neonatal period, affect risk for later development of inflammatory bowel diseases. Gastroenterology. 2019;156(8):2190-7. e10.
45. Jiang Hy, Zhang X, Zhou Yy, Jiang Cm, Shi Yd. Infection, antibiotic exposure, and risk of celiac disease: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2020;35(4):557-66.
46. van Houten CB, Naaktgeboren C, Buiteman BJ, van der Lee M, Klein A, Srugo I, et al. Antibiotic overuse in children with respiratory syncytial virus lower respiratory tract infection. The Pediatric infectious disease journal. 2018;37(11):1077-81.
47. Arnolda G, Hibbert P, Ting HP, Molloy C, Wiles L, Warwick M, et al. Assessing the appropriateness of paediatric antibiotic overuse in Australian children: a population-based sample survey. BMC pediatrics. 2020;20:1-8.
48. Melander RJ, Zurawski DV, Melander C. Narrow-spectrum antibacterial agents. Medchemcomm. 2018;9(1):12-21.
49. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. BMJ open. 2014;4(8):e005047.
50. Kumar R, Sood U, Gupta V, Singh M, Scaria J, Lal R. Recent advancements in the development of modern probiotics for restoring human gut microbiome dysbiosis. Indian Journal of Microbiology. 2019:1-14.

51. Chua MC, Ben-Amor K, Lay C, Goh AE, Chiang WC, Rao R, et al. Effect of synbiotic on the gut microbiota of cesarean delivered infants: a randomized, double-blind, multicenter study. Journal of pediatric gastroenterology and nutrition. 2017;65(1):102-6.
52. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome medicine. 2016;8(1):39.

Figure Legends

Figure 1: PRISMA flow diagram of the study selection

Figure 2: Forest plots per gastrointestinal disorder a. IBD; b. EoE; c. CeD; d. FGID (Infantile colics and functional constipation). CC= case control study, CH = cohort study, (!) Virta 2012 only shows the results of the phenoxymethylpenicillin analyses, overall use of antibiotics was not significant

Table 1a study characteristics and association with antibiotics: Inflammatory Bowel Disease

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score

Canova, C [43]
2020
Italy
Case-control
	8.8 yrs[1]	70 / 700	33 (47%)
			
			0-12 months
			
			ATC code
				• Birth order
				• Age mother (at birth)
				• Apgar score at 1 minute
				• Birth weight
				• Education mother
				• Gestational age
				• Multiple birth
				• Season of birth
					AB first six months of life childhood onset IBD
					• Any course aOR = 1.458, 95% CI: 0.81–2.63
					• **Dose-dependent**
					  ○ 2-3 courses aOR = 2.29, 95% CI: 1.01–5.24
					  ○ >4 courses aOR = 6.25, 95% CI: 1.70–23.05
					Ab first 12 months of life childhood onset IBD
					• Any course aOR = 1.08, 95% CI 0.64–1.80
					• **Dose-dependent**: >4 courses aOR = 2.92, 95% CI: 1.32–6.46
						8/9
						High

Hviid, A [31] 2010 Denmark Cohort	3.4 yrs[1]	117 (0.02%) (50 CD and 67 UC) / 577,627	84 (72%) 0-12 months ATC code	• Age • Calendar period • Other times since use • Other types of antibiotics	Increased risk of Crohn's disease after: AB use in the last 3 months: • 3-11 months RR = 3.32, 95% CI: 1.15-9.56 • 1 year RR = 1.53, 95% CI: .15-15.46 AB use > 3 months previously before diagnosis: • 0-2 months RR = 4.19, 95% CI: 1.64-10.68	8/9 high
Kronman, M [25] 2012 United Kingdom Cohort	Exposed 4.2 yrs[2]	748 (0.07%) / 1,072,426	436 (58%) 0-12 months Systemic AB prescriptions	• Age • Chronic granulomatous disease • IBD family • Primary sclerosing cholangitis • Sex • Socioeconomic deprivation	• Exposure was associated with a 5.5-fold increased IBD risk (aHR = 5.51, 95% CI:1.66–18.28). • **Dose-dependent**: Exposure to >2 anti-anaerobic antibiotic courses was more highly associated with IBD development than exposure to 1 or 2 courses (aHR = 4.77, 95% CI: 2.13–10.68) versus (3.33, 95% CI: 1.69–6.58). • **Type-dependent** Fluoroquinolone (aHR= 2.09, 95% CI: 1.10–3.98) and metronidazole exposure (aHR = 186.25, 95% CI: 10.86–3193.65) was significantly associated with IBD.	7/9 moderate

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Örtqvist, A [27] 2018 Sweden Cohort	2 yrs[1]	95 (0.01%) 51 IBD (CD and/or UC), 20 CD & 24 UC / 827,239	IBD 43 (84,3%) CD 16 (80%) UC 20 (83.3%) 0-12 months ATC code	• Delivery mode • Education parents • Ethnicity parents • IBD parents	No significant associations (any and PcV antibiotics) or dose–response relationship were found	8/9 high
Shaw, S [38] 2010 Canada Case-control	8.4 yrs[1]	36 / 360	21 (58%) 0-12 months ATC code	• Age • Place of residence • Sex	• One or more dispensations of antibiotics was associated with 2.9 times the odds (95% CI: 1.2-7.0, P = 0.017) of having IBD. • Stratified by IBD type, only CD was significant (OR = 5.3, 95% CI: 1.6-17.4; P = 0.006). • **Dose-dependent**: association for 2-4 (OR = 2.9, 95% CI: 1.1-7.8; P = 0.039) and 5+ (OR = 5.0, 95 % CI: 1.3-18.9; P = 0.18) prescriptions.	8/9 high
Virta, L [40] 2012 Finland Case-control	CD: 9.7 yrs[3] UC: 8.5 yrs[3]	595 (233 CD, 362 UC) / 2,380	313 (52,6%) 0-12 months ATC code	• Age • Place of residence • Chronic diseases • Sex	• Use of AB overall was not significant • **Type-dependent:** phenoxymethylpenicillin was associated with an increased risk of CD. (aOR = 2.54, 95% CI: 1.3-4.98)	8/9 high

AB: antibiotic, aHR: adjusted hazard ratio, aOR: adjusted odds ratio, ATC: Anatomical Therapeutic Chemical (ATC) Classification System, CD: Anatomical Therapeutic Chemical (ATC) Classification System, CI: Confidence interval , IBD: Inflammatory bowel disease, IRR: incidence rate ratio, HR: hazard ratio, OR: odds ratio, PcV: Phenoxymethylpenicillin and UC: Ulcerative colitis

Table 1b study characteristics and association with antibiotics: Eosinophilic Esophagitis (EoE)

Author Year Country Design	Age diagnosis [1]	Cases / Controls	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Jensen, E [33] 2013 North Carolina (USA) Case-control	Cases 11 yrs[1]	31 / 52	22 (71%) 0-12 months Motherly reported	None	Antibiotics were associated with EoE (OR= 6, 95% CI: 1.7–20.8)	4/9 weak
Jensen, E [34] 2018 North Carolina (USA) Case-control	Cases 10.6 yrs[1]	127 / 121	91 (72%) 0-12 months Motherly reported	• Education mother • NICU admission	Antibiotics were associated with EoE (aOR = 2.30, 95% CI: 1.21-4.38)	6/9 moderate
Radano, M [37] 2014 Massachusetts (USA) Case-control	Cases 3 yrs[1]	25 / 74	17 (67%) 0-12 months Parental reported	• Age • Atopy • Atopy family • Sex	Antibiotics were associated with EoE (OR = 3.61, 95% CI: 1.11-11.74; P = .03)	7/9 moderate
Slae, M [39] 2015 Canada Case-control	Cases 8.6 yrs[1]	102 / 167	60 (59%) 0-12 months Parental reported	• Breastfeeding • Birth order • Day care attendance (early) • Exposure to farm animals • Fast food consumption	Rates of antibiotic exposure were similar for cases and controls.	3/9 weak
Witmer, C [41] 2018 USA Case-control	4.2 yrs[1]	1410 / 2,820	409 (29%) 0-6 months Pharmaceutical coding	• Age • Atopy (markers) • Delivery mode • Erythema toxicum neonatorum • Feeding problems • Infantile colic • Medication exposure • Oral candidiasis • Prematurity • Prolonged rupture/ chorioamnionitis • Reflux • Sex	The association with antibiotic exposure was statistically significant (aOR = 1.31, 95% CI: 1.10–1.56).	7/9 moderate

Table 1c study characteristics and association with antibiotics: Celiac Disease (CeD)

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Bittker, S [42] 2019 USA Case-control	6.1 yrs[1]	332 / 241	237 (71%) 0-24 months Parental reported	• Age • Age mother (at birth) • Education mother • Ethnicity	• Antibiotic exposure is associated with susequent CeD (aOR = 1.133, 95% CI: 1.037–1.244; p= 0.007) • **Dose-dependent**: ORs increase with number of antibiotic courses	5/9 moderate
Canova, C [22] 2014 Italy Cohort	6.4 yrs[1]	1.227 CeD (0.6%) 866 confirmed* and 361 unconfirmed* / 203,557	336 (47%) 0-12 months ATC code	• Education mother (only in sensitivity analysis with pathological confirmed villous atrophy) • Sex • Year of birth	• Increased risk of developing CeD after at least 1 AB course (IRR = 1.24, 95% CI: 1.07-1.43), (IRR = 1.31, 95% CI: 1.10-1.56) for histopathologically confirmed CeD • **Dose-dependent**: risk increased with more AB courses (P-trend < 0.01). • **Type-dependent:** Cephalosporin use was strongly associated with CeD onset (IRR = 1.42, 95% CI: 1.18-1.73), (IRR = 1.51, 95% CI: 1.21-1.89) for histopathologically confirmed CeD. For first- and second-generation drugs: (IRR = 1.39, 95% CI: 1.11-1.76 and third- and fourth-generation drugs: IRR = 1.49, 95% CI: 1.14-1.95).	8/9 high
Kemppainen, K [24] 2017 Finland, Germany, Sweden and the USA Cohort	21.4 months[1]	783 (11,9%) / 6,558	Unknown 0-24 months Parental reported	• Breastfeeding (at 90 days of age) • CeD genotype with family • Delivery mode • Maternal AB use during pregnancy • Place of residence • Probiotic use before 90 days of age • Season of birth • Sex	• Exposure to AB was not associated with CeD. • **Dose-dependent**: 2 or more doses of macrolides within the first year of life (157 of 6558 [2.4%]) had elevated CeD risk (HR = 1.77, 95% CI: 1.18-2.66; P = .006 before but not after adjustment).	6/9 moderate

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Mårild, K [35] 2013 Sweden Case-control	0-2 yrs[1]	132 celiac disease / 655 12 inflammation / 60 17 normal mucosa / 85	CeD 51 (39%) Inflammation 6 (50%) 0-24 months ATC code	• Age • Education mother • Number of outpatient visits before biopsy • Sex	Exposure to AB was associated with CeD Odds ratios for prior AB use (CeD): cases 51/132 (38.6%) controls 189/655 (28.9%) (OR = 1.58, 95% CI: 1.07-2.34)	8/9 high
Myleus, A [36] 2012 Sweden Case-control	14 months[1]	373 / 581	97 (26%) 0-6 months Parental reported	• Age • Place of residence • Sex	No significantly increased risk for celiac disease (OR = 1.2, 95% CI: 0.87-1.6; P=0.27).	7/9 moderate
Sander, S [28] 2019 Denmark and Norway Cohort	Danish: 11.6 yrs[3] Norwegian: 5.4 yrs[3]	Danish: 1427 (0.12%) / 1,168,656 Norwegian: 1919 (0.36%) / 537,457	Danish: 622 (43.6%) Norwegian: 390 (20.3%) 0-12 months ATC code	• Age mother • Associated comorbidity • Birth order • Education mother • Hospitalization with infection • Season of birth • Sex • Type 1 diabetes child and/ or mother	• Exposure to systemic AB (penicillins and extended spectrum penicillins) was positively associated with diagnosed celiac disease in both cohorts (pooled aOR = 1.26, 95% CI: 1.16–1.36) • **Dose-dependent**: between number of AB courses and risk of CeD (pooled aOR for each additional dispensed AB = 1.08, 95% CI: 1.05–1.11).	9/9 high

Table 1d study characteristics and association with antibiotics: FGIDs: Infantile Colics, Functional constipation (FC), Recurrent abdominal pain (AP) and Regurgitation, functional diarrhea and infant dyschezia

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Infantile colics						
Hestbaek, L [23] 2014 Denmark Cohort	0-6 months	2183 (8,1%) / 26,983	excessive 895 (41%) extreme excessive 355 (50%) 0-6 months Motherly reported	None	At 6-month-olds, statistically significant associations between excessive crying and the use of antibiotics due to ear infections (OR = 1.47, 95% CI: 1.18–1.82) were found.	6/9 moderate
Oosterloo, B [26] 2018 The Netherlands Cohort	0-1 yr	74 (20%) / 362	33 (45%) 0-7 days Broad-spectrum AB intravenous for 2-3 days (AB2) or 7 days (AB7).	• Atopy family • Birth order • Breastfeeding • Day care attendance • Delivery mode • Education parents • Tobacco exposure	• Antibiotic treatment was an independent risk factor for infantile colic (aOR = 1.66, 95%CI: 1.00-2.77, P = .05). • Doctors-diagnosed infantile colic was higher in AB+ than in AB− (4.0% vs 0.4%; P = .014). • **Duration-dependent**: Parent-reported infantile colic was higher in AB7 compared to no antibiotics (AB-) and AB2 (24.8%, 14.4% and 14.3%, P = .048 and P = .015).	8/9 high
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	265 (41.9%) / 632	141 (22.3%) 0-7 days Hospital chart and parental report	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=1.16; 95% CI: 0.79-1.70, p=0.439)	7/9 moderate

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Functional constipation (FC)						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	128 (26.6%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=0.77; 95%CI: 0.49-1.20, p=0.242)	7/9 moderate
Turco, R [29] 2014 Italy Cohort	0-1 yr	43 (10.7%) / 465	15 (34.8%) 0-12 months Parental reported	• Anti-inflammatory drugs or corticosteroids • Atopy & in family • Birth order • Breastfeeding & weaning • Education parents • Fever episodes before onset • FGIDs family • Nursery school age • Place of residence (>3000 citizens) • Sex • Vitamin and food supplements	No statistically significant association was found (26% vs 19%).	8/9 high
Recurrent abdominal pain (AP)						
Uusijärvi, A [30] 2014 Sweden Cohort	12 yrs	Monthly: 231 (8,7%) Weekly: 111 (4,2%) / 2,654	Monthly 1900 (71.5%) Weekly 81 (72,9%) 0-24 months Parental reported	• Asthma at 12 years of age • Asthma at one year • Sex	Stratified analyses showed that girls, who received antibiotics during both the first and the second year of life, had an increased risk of AP at 12 years (OR = 1.65, 95% CI: 1.09–2.49).	5/9 moderate
Regurgitation, functional diarrhea and infant dyschezia						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	Regurgitation:236 (37.3%) Functional diarrhea: 24 (3.8%) Infant dyschezia: 199 (31.5%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found for regurgitation (OR=1.29, 95%CI: 0.88-1.90, p=0.190), functional diarrhea (OR=0.90, 95%CI: 0.33-2.45, p=0.835), or infant dyschezia (OR=1.29, 95%CI: 0.87-1.93, p=0.205).	7/9 moderate

Table 2 quality assessment

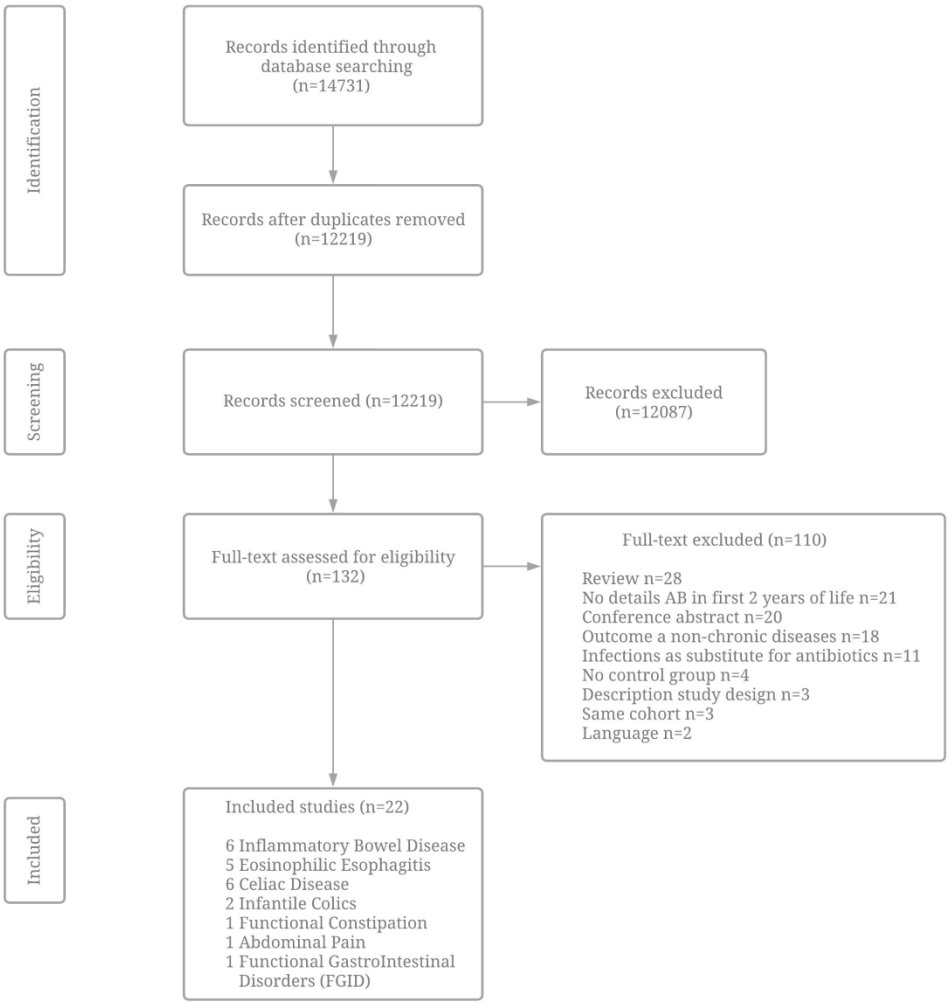
	Selection				Comparability		Outcome / Exposure			Score
	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Cohort studies*	Representativeness	Selection	Exposure	Outcome	Most important	Second important	Assessment	Duration of follow-up	Adequacy follow-up	
Canova [22]	*	*	*	*		*	*	*	*	8/9
Hestbaek [23]	*	*	*	*				*	*	6/9
Hviid [31]	*	*	*	*		*	*	*	*	8/9
Kemppainen [24]		*		*	*	*	*	*		6/9
Kronman [25]		*	*	*	*	*	*	*		7/9
Oosterloo [26]	*	*	*	*	*	*	*	*		8/9
Örtqvist [27]	*	*	*	*	*	*	*		*	8/9
Salvatore [32]	*	*	*	*		*	*	*		7/9
Sander [28]	*	*	*	*	*	*	*	*	*	9/9
Turco [29]	*	*	*	*	*	*		*	*	8/9
Uusijärvi [30]	*	*		*				*	*	5/9
Case-Control studies**	Case definition	Cases	Controls	Definition controls	Most important	Second important	Exposure	Ascertainment	Non-Response rate	Score
Bittker [42]			*	*	*			*	*	5/9
Canova [43]	*	*	*	*		*	*	*	*	8/9
Jensen [33]	*	*		*				*		4/9
Jensen [34]	*	*	*	*				*	*	6/9
Mårild [35]	*	*	*	*		*	*	*	*	8/9
Myleus [36]	*	*	*	*		*		*	*	7/9
Radano [37]	*	*		*	*	*		*	*	7/9
Shaw [38]	*	*	*	*		*	*	*	*	8/9
Slae [39]	*			*				*		3/9
Virta [40]	*	*	*	*		*	*	*	*	8/9
Witmer [41]		*	*	*	*	*		*	*	7/9

*Cohort studies: 1. Representativeness of the exposed cohort, 2. Selection of the non-exposed cohort, 3. Ascertainment of exposure, 4. Demonstration that the outcome of interest was not present at start of the study, 5. Comparability of cohorts on the basis of the design or analysis most important factor, 6. Comparability of cohorts on the basis of the design or analysis second important factor, 7. Assessment of outcome 8. Was follow-up long enough for outcomes to occur and 9. Adequacy of follow up of cohort.

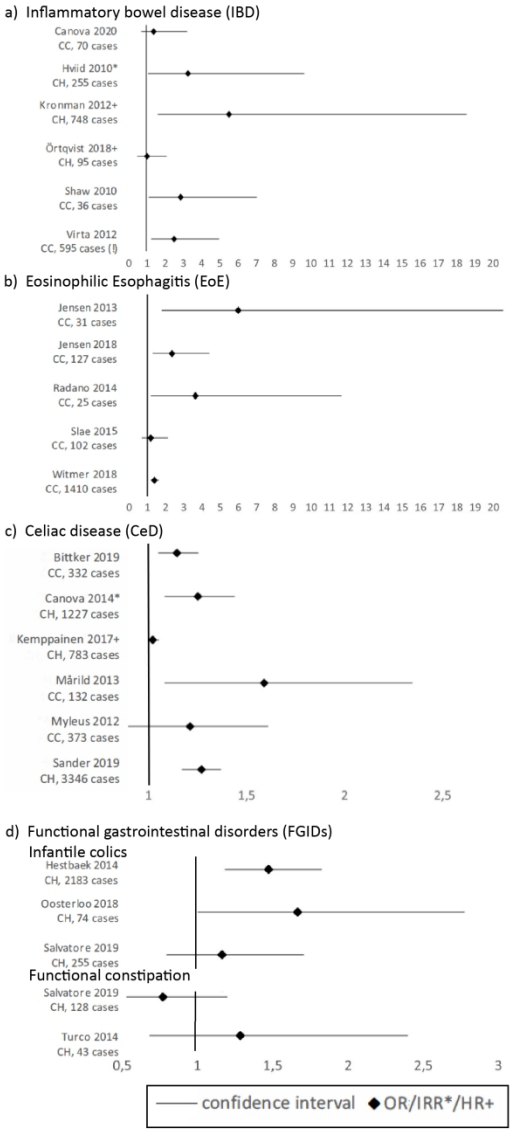
**Case- Control studies: 1. Is the case definition adequate? 2. Representativeness of the cases, 3. Selection of controls, 4. Definition of controls, 5. Comparability of cases and controls on the basis of the design or analysis most important factor, 6. Comparability of cases and controls on the basis of the design or analysis second important factor, 7. Ascertainment of exposure, 8. Same method of ascertainment for cases and controls and 9. Non-Response rate

Comparability: Most important confounder: IBD and CeD: presence of IBD/ CeD in 1ste degree family member, EoE: sex, colics: atopy child and/or family, functional constipation: maternal education/social economic status, abdominal pain: lactose intolerance/ cow's milk allergy.

Comparability: Second important confounder: IBD: ethnicity and/or age, EoE: presence of other atopic diseases and/or ethnicity, CeD: sex and/ or season of birth and/or the presence of other autoimmune diseases, colics: presence of GERD and/or type of feeding and/or being a first child, functional constipation: sex and/ or age, abdominal pain: anxiety/ depression/ stress in the child and/or the parents.



212x223mm (300 x 300 DPI)



210x297mm (300 x 300 DPI)

Supplementary Table 1 search strategy

	Ovid MEDLINE(R) ALL <1946 to 2020 June 08> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp infant death/ or infant/	788526
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kf,ti.	686417
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	655139
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1066665
5	or/1-4 [Ia - children 0-4 yrs]	2564903
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kf,ti. [Ib - children 0-4 yrs]	5357
7	Gentamycins/	18247
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kf,ti.	27205
9	or/7-8 [IIa first week exclusive use]	32706
10	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	399419
11	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kf,ti. [IIc]	48465
12	(sepsis and infant).hw.	9982
13	(sepsis adj2 early).ab,kf,ti.	1919
14	or/12-13 [IId]	11418
15	(childhood disease? and (risk or environmental factor?)).ab,kf,ti. [IIe]	360

16	exp inflammatory bowel disease/ or abdominal pain/ or aerophagy/ or dyspepsia/ or constipation/ or celiac disease/ or appendicitis/ or gastritis/ or enteritis/ or exp diarrhea/ or colic/ or Eosinophilic Esophagitis/ or Gastroesophageal Reflux/ or esophageal stenosis/	266125
17	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kf,ti.	305723
18	Pyloric Stenosis, Hypertrophic/	654
19	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kf,ti.	1513
20	18 or 19	1622
21	limit 20 to yr="2015-current"	184
22	or/16-17,21 [outcomes]	424883
23	follow-up studies/ or longitudinal studies/ or retrospective studies/	1441183
24	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kf,ti.	3775119
25	(case control or cohort study or (risk and review)).mp.	1032965
26	observational study.pt.	80055
27	or/23-26 [study design]	4954421
28	5 or 6 [Ia+b - children 0-4 yrs]	2566952
29	28 and (10 or 11) and 22 and 27	2707
30	and/9,28	4170
31	and/14,22	319
32	or/15,29-31	7477
33	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kf,ti. [NOTing out green]	1105473
34	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kf,ti. [NOTing out blue]	505926
35	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kf,ti. [NOTing out red]	803243

36	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kf,ti. [NOTing out yellow]	1949849
37	or/33-36	4123179
38	32 not 37 [NOTing out]	5238
39	animals/ not humans/	4672110
40	38 not 39	5096
41	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kf,ti.	0
42	40 or 41	5096
	Ovid Embase Classic+Embase <1947 to 2020 June 06> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp *infant/ or *infancy/ or infant.hw.	798854
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kw,ti.	875279
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	1051740
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1708558
5	or/1-4 [Ia - children 0-4 yrs]	3541363
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kw,ti. [Ib - children 0-4 yrs]	7292
7	*Gentamicin/	35017
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kw,ti.	36468

9	"1403-66-3".rn.	104829
10	or/7-9 [IIa first week exclusive use]	113443
11	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	870330
12	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kw,ti. [IIc]	65283
13	(sepsis and infant).hw.	11891
14	(sepsis adj2 early).ab,kw,ti.	2988
15	or/13-14 [IId]	14425
16	(childhood disease? and (risk or environmental factor?)).ab,kw,ti. [IIe]	498
17	exp *inflammatory bowel disease/ or *abdominal pain/ or *aerophagia/ or *dyspepsia/ or exp *constipation/ or *celiac disease/ or *appendicitis/ or *gastritis/ or *enteritis/ or *diarrhea/ or *infantile diarrhea/ or *colic/ or *infantile colic/ or *Eosinophilic Esophagitis/ or *Gastroesophageal Reflux/ or *esophageal stenosis/	267207
18	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kw,ti.	493146
19	*hypertrophic pylorus stenosis/	1263
20	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kw,ti.	1940
21	19 or 20	2152
22	limit 21 to yr="2015-current"	231
23	or/17-18,22 [outcomes]	586712
24	follow up/ or longitudinal study/ or retrospective study/	2412789
25	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kw,ti.	5528713
26	observational study.kw,ti.	27665
27	(case control or cohort study or (risk and review)).mp.	1211338
28	or/24-27 [study design]	6774290
29	5 or 6 [Ia+b - children 0-4 yrs]	3545044
30	29 and (11 or 12) and 23 and 28	5878

31	and/10,28-29	5192
32	and/15,23	576
33	or/16,30-32	11847
34	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kw,ti. [NOTing out green]	1532636
35	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kw,ti. [NOTing out blue]	664053
36	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kw,ti. [NOTing out red]	962439
37	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kw,ti. [NOTing out yellow]	2455192
38	or/34-37	5285640
39	33 not 38 [NOTing out]	9118
40	(animal/ or animal experiment/ or animal model/ or nonhuman/) not human/	6454629
41	39 not 40	8980
42	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kw,ti.	0
43	NTR6681.cn.	0
44	or/41-43	8980
	Web of Science Core Collection: **- SCI-EXPANDED 1975-present** **- SSCI 1975 - present** **-A&HCI 1975 - present** **- ESCI 2015 - present** **Search date: 9 June 2020**	
#	**Searches**	**results**
#1	TS=(early life or infant or infancy or toddler or preschool or (early N4 (childhood or child or children or pediatric)) or minors or baby or babies or kindergarten or newborn)	1085229
#2	AB=(("0" or "1" or "2" or "3" or "4") N1 (age? or yr? or year?))	1805
#3	AB=(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") N1 month?)	1183

#4	TS=((pediatric or infantile or juvenile) N1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic AND Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis))	3
#5	#4 OR #3 OR #2 OR #1	1087634
#6	TS=antibiotic	334292
#7	#6 AND #5	15781
#8	TS=(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3")	25466
#9	#8 OR #7	40687
#10	TS=(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture)	252018
#11	TS=(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis)	1233
#12	#11 OR #10	253145
#13	#12 AND #9	655

Supplementary table 2 Confounders in the quality assessment

Study outcome	Most important	Second important
IBD	Presence of IBD in first degree family members	Ethnicity and/or age
EoE	Sex	Presence of other atopic diseases and/or ethnicity
CeD	Presence of CeD in first degree family member	Sex and/or season of birth and/or the presence of other autoimmune diseases
Colics	Presence of atopy in first degree family members	Presence of GERD and/or type of feeding and/or being a first child
Functional constipation	Maternal education/social economic status	Sex and/or age
Abdominal pain	Lactose intolerance/cow's milk allergy	Anxiety/depression/stress in the child and/or the parents

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Early life antibiotics and childhood gastrointestinal disorders: a systematic review

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Short title early life antibiotics and gastrointestinal disorders

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Contributors' Statement Page:

K. Kamphorst contributed to the design, the analyses and interpretation of the study, drafting of the initial manuscript, and reviewed and revised the manuscrip*t.*

E. Van Daele contributed to the analysis and interpretation of the study and critically revised the manuscript.

A.M. Vlieger and R.M. van Elburg contributed to the conception of the study, interpretation of the data and critically revised the manuscript.

J.G. Daams conceptualized and performed the systematic search and critically revised the manuscript.

J. Knol contributed to the conception and design of the study and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Abstract

Background: In adults, there is increasing evidence for an association between antibiotic use and gastrointestinal disorders but in children, the evidence is scarce.

Objective: Assess the association between exposure to antibiotics in the first two years of life in term born children and the presence of chronic gastrointestinal disorders later in childhood.

Design: For this systematic review the MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020. Title and abstract screening (n=12,219), full-text screening (n=132) as well as the quality assessment with the Newcastle–Ottawa Scale were independently performed by two researchers.

Main outcome measures: The association between antibiotics and inflammatory bowel disease (n=6), eosinophilic esophagitis (n=5), celiac disease (n=6), infantile colics (n=3), functional constipation (n=2), recurrent abdominal pain, regurgitation, functional diarrhea, and infant dyschezia were examined.

Results: Twenty-two studies were included, 11 cohort and 11 case-control studies. A best evidence synthesis showed strong evidence for an association between antibiotic exposure in the first two years of life and the presence of inflammatory bowel disease, and celiac disease during childhood. Moderate evidence was found for an association with eosinophilic esophagitis and no association with functional constipation in the first year of life. There was insufficient evidence for the other studied disorders.

Conclusions: The use of antibiotics in early life may increase the risk of gastrointestinal disorders later in life. Further studies are necessary to unravel the underlying mechanisms and determine potential preventive measures. Meanwhile judicious use of antibiotics in early childhood is highly warranted.

Introduction

The incidence of pediatric gastrointestinal disorders (GI-disorders), such as pediatric inflammatory bowel disease (IBD) and celiac disease (CeD), is rising [1, 2]. The increase in pediatric GI-disorders is most likely related to environmental factors and recently the focus has been on the role of the intestinal microbiome. A microbiome that has been disturbed by factors like stress, dietary change, environmental factors or drugs, can result in alterations in the immune system [3]. Several studies have shown that a disturbed microbiome can be a cause or trigger of GI disorders, probably mediated by these immunological changes [4-7].

One of the drugs with the most profound effect on the microbiome are antibiotics [8]. The impact of antibiotics on the microbiome depends on various factors such as type of antibiotic, dosage, and duration of exposure[8]. Furthermore, age at exposure is probably also important. The gut of a newborn infant is almost sterile with a low diversity and matures according to several developmental stages with increasing diversity over time [9]. The microbiome stabilizes around the age of 2 to 3 years [9]. Since this developing gut microbiota plays an important role in the training of both innate and adaptive immune system, it is likely that antibiotics will have their biggest impact when administered in the first two years of life.

For the association between antibiotic use and GI disorders, that has been shown in adults, [10], there is only limited evidence in children [11]. Therefore, the aim of this systematic review was to assess the association between exposure to antibiotics in the first two years of life and the presence of chronic gastrointestinal disorders during childhood.

Method

Study selection

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and registered in PROSPERO CRD42019132631 [12, 13]. MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020 to identify all studies examining the association between antibiotic exposure in the first two years of life and the presence of common chronic (longer than two weeks, in order to exclude viral diarrhea) gastrointestinal disorders during the first 18 years of life. We searched for associations with IBD, eosinophilic esophagitis (EoE), CeD, irritable bowel syndrome (IBS), (functional) abdominal pain, constipation, dyspepsia, aerophagia, infantile colic, gastroesophageal reflux (GERD), regurgitation, dyschezia and chronic diarrhea.

A multi stranded search approach comprised various concept combinations of children aged 0-4 years, prognosis, gastrointestinal disorders and antibiotics. In order to reduce recall noise and enhance search results precision we used VOS-viewer to identify terms for NOTing out irrelevant records from databases searched [14, 15]. See supplementary file 1 for the full search strategies.

Patient and Public Involvement statement

As this is a systematic review of the literature, there were no patients involved in the design of the research question nor the study itself. Furthermore, for the same reason no approval for the study was required from an ethical committee.

In- and exclusion criteria

Studies were included if: 1. Antibiotics were administered between full-term birth and two years of age. 2. Study outcome was diagnosis with a chronic GI-disorder during the first

18 years of life. 3. Antibiotic use was before the diagnosis of the GI-disorder. 4. A control group was included. 5. In case multiple studies were found examining similar outcomes in one cohort, only the study with the largest cohort was included. No restrictions were placed on the time period of publication. Searches were limited to studies conducted in humans and excluded if the full text was not available in English, Dutch, German or French.

All records found in the search were exported into Rayyan after deduplication [16]. Two researchers (KK and EVD) independently performed title and abstract screening as well as full-text screening. After consensus about the study selection, data were entered into a data extraction form, which included: author, year of publication, country, study design, cases, controls/cohort, population age, sample size exposed to antibiotics, age at exposure, details about classification by type of antibiotics, type of GI disorder, method of diagnosis, confounders for which corrected, and the association between exposure and outcome.

Methodological quality

To assess the risk of bias, two researchers (KK and EVD) independently assessed the methodological quality. Discrepancies were resolved by discussion until consensus was reached. The Newcastle–Ottawa Scale (NOS) was used, which has been developed to assess the quality of observational studies [17]. The NOS includes different instruments for assessing case-control and cohort studies. Both scales contain a maximum of nine points and assess studies in three core areas: 1. Selection of study participants 2. Comparability of groups 3. Detection of exposure / outcome. One point for comparability of groups was given when the study controlled for the main important confounder and a second point if controlled for a second important confounder, see supplementary file 2. Studies were rated high quality with a score of eight or higher, moderate quality with a score between five and seven and weak quality with a score of four or less [18].

Data analyses

To synthesize the methodological quality of the studies, a commonly used best evidence synthesis was applied per disorder in which the methodological quality was considered according to the following definitions: 1. strong evidence, provided by generally consistent findings in at least two high-quality studies. 2. moderate evidence, provided by generally consistent results in one high-quality study and at least one moderate- or low-quality study, or generally consistent results in multiple moderate- or low-quality studies. 3. insufficient evidence, when less than two studies were available or inconsistent findings in multiple studies [19-21]. Results were considered consistent when at least 75% of the studies showed results in the same direction.

Results

Search results

Of the 14,731 retrieved records, 12,219 remained after removing duplicates. These records were screened; 132 were assessed as eligible and read in full-text of which 110 were excluded and 22 studies included in this review. Details of the selection procedure are shown in Figure 1.

Study characteristics

The included studies were published between 2010 and 2020 (table 1a-d): 11 cohort studies [22-32] and 11 case-control studies [33-43]. The studies were performed in Sweden (n=4) [27, 30, 35, 36], the United States of America (USA) (n=5) [33, 34, 37, 41, 42], Italy (n=4) [22, 29, 32, 43], Denmark (n=2) [23, 31], Canada (n=2) [38, 39], and one in the United Kingdom [25], the Netherlands [26], and Finland [40]. There were two international studies, one in Denmark and Norway [28], and another in Finland, Germany, Sweden and the USA [24].

The associations between antibiotics and the following GI-disorders were examined: IBD (n=6) [25, 27, 31, 38, 40, 43], EoE (n=5) [33, 34, 37, 39, 41], CeD (n=6) [22, 24, 28, 35, 36, 42], infantile colics (n=3) [23, 26, 32], functional constipation (n=2) [29, 32], recurrent abdominal pain (n=1) [30]. One study examined several functional GI-disorders (FGIDs): infantile colics, functional constipation, functional diarrhea, infant dyschezia, and regurgitation[32].

Exposure to antibiotics was studied in the first two years of life (n=4) [24, 30, 35, 42], the first 18 months of life (n=1) [23], the first year of life (n=13) [22, 25, 27-29, 31, 33, 34, 37-40, 43], the first six months of life (n=2) [36, 41], and the first week of life (n=2) [26, 32] (table 1a-d). Since only a few studies provided details about type of antibiotics and/or number of antibiotic treatments in the first two years of life, the associations include mostly the overall antibiotic exposure.

Quality assessment

Ten studies were of high quality[22, 26-29, 31, 35, 38, 40, 43], ten studies moderate [23-25, 30, 32, 34, 36, 37, 41, 42], and two weak [33, 39] (Table 2). Frequently observed weaknesses were a high dropout rate in the cohort studies, assessment of antibiotic exposure through parental reports, and no correction for important confounders.

Inflammatory Bowel Disease

Exposure to early life antibiotics was associated with the development of IBD in five out of six studies [25, 31, 38, 40, 43] (NOS = 7,8,8,8,8), whereas no association was found in one study examining Very Early Onset (VEO) IBD, (before six years of age) [27] (NOS = 8). Three studies found a dose-response relation [25, 38, 43] and an increased risk after fluoroquinolone [25], metronidazole [25], and phenoxymethylpenicillin[40] exposure. In two studies IBD was stratified by type and only the odds ratio for Crohn's disease, but not for ulcerative colitis, was significant [38, 40]. Forest plots of the main results are shown in Figure 2a.

Eosinophilic esophagitis

In four of the five studies early life antibiotics was associated with EoE [33, 34, 37, 41] (NOS = 4,6,7,7), whereas in one study the rates of parental reported antibiotic use were similar for cases and controls [39] (NOS = 3) (figure 2b).

Celiac disease

In four studies, of which three had a high quality, a significant association between early life antibiotics and the presence of CeD was found [22, 28, 35, 42] (NOS = 8,9,8,5), whereas in two moderate quality studies no association was found [24, 36] (NOS = 6,7) (figure 2c). Three studies showed a dose-response relationship between exposure to antibiotics and the

risk of CeD [22, 28, 42]. Furthermore, use of cephalosporin [22] and multiple courses of macrolides [24] showed a positive association with the development of CeD.

Infantile colics

Two studies found a significant association between early life antibiotics and infantile colics [23, 26] (NOS = 6,8), while one study found no association [32] (NOS = 7) (figure 2d).

Functional constipation

In both studies, no association was found between early life antibiotics use and functional constipation in the first year of life [29, 32] (NOS = 8,7).

Recurrent abdominal pain

The only study examining the association between antibiotics use in the first two years of life and the risk of recurrent abdominal pain (AP) at 12 years of age [30] (NOS = 5) found that only girls, but not boys, who received antibiotics in both the first and second year of life, had an increased risk of AP at 12 years.

Regurgitation, dyschezia and functional diarrhea

In one study no association was found between antibiotics in the first week of life and regurgitation, dyschezia and functional diarrhea [32] (NOS = 7).

Syntheses of individual results

Using the definitions for the best evidence synthesis, described in the method section, it can be concluded that there is strong evidence for an association of antibiotics in early life with IBD and CeD. There is moderate evidence for an association with EoE and no association with infantile constipation. The current evidence for an association between antibiotics in early life and the other studied GI-disorders is considered insufficient.

Discussion

This systematic review with best evidence syntheses on the association between antibiotic exposure in the first two years of life and chronic GI disorders during childhood showed strong evidence for this association with inflammatory bowel disease and celiac disease, and moderate evidence for this association with eosinophilic esophagitis. For the other studied GI-disorders, insufficient evidence was found.

The question remains to what extent the association with IBD, EoE and CeD can be attributed to antibiotic exposure itself or to other factors such as infections and parental health seeking behavior. Infections in early life have been proposed to contribute to the development of chronic GI-disorders [44, 45] and it is difficult to differentiate between the role of infections and antibiotics which are prescribed for (suspected) infections. Furthermore, several GI-disorders like CeD can remain undiagnosed for a long time. Higher parental health seeking behavior can both lead to higher use of antibiotics and a higher chance of diagnosing the chronic GI-disorder. Therefore, it remains unknown whether antibiotics are the true causative agent in the observed associations or whether they are intermediates in different mechanistic pathways through microbiome perturbations or changes in immune development after (suspected) infections.

Most studies found a clear association between antibiotics in early life and IBD. The study that focused on very early onset IBD (VEO-IBD), found no association between antibiotics and VEO-IBD. VEO-IBD is considered a different entity from later-onset IBD [44], since genetics play a far more important etiological role than microbial dysbiosis [45]. This may explain the lack of an association with early life antibiotics.

The primary goal of antibiotic administration is to prevent detrimental effects of serious and sometimes even life-threatening infections. However, especially in early life,

antibiotics are overused, since they are often prescribed for viral upper respiratory tract infections [46, 47]. Given its association with the occurrence of IBD, CeD and EoE, it is highly important to prevent antibiotic overuse by strict adherence to guidelines. If antibiotics are necessary, treatment would be adjusted to minimize dysbiosis. Another possible solution is to shorten the time of antibiotic administration. Oosterloo *et al.* found more health issues in the first year of life after seven days compared to two days of antibiotics in the first week of life [26]. Furthermore, whenever possible, narrow-spectrum antibiotics rather than broad-spectrum should be used, because these specifically reduce the capacity of pathogens to cause disease while leaving commensals unharmed [48]. If adjustment of antibiotic treatment is not possible, interventions that restore or prevent dysbiosis should be considered, such as administration of pre- or probiotics, or fecal transplants [49-52].

Some limitations of this review need to be considered. As no randomized controlled trials were available, only associations but not causality can be examined. Additionally, the studied results were not evaluated for their precision and associations with wide confidence intervals can indicate uncertainty about the magnitude of the association. Hence, the results must be interpreted with caution. Furthermore, both age at exposure as well as age at diagnosis varied substantially between the studies. In addition, study outcomes were also very heterogeneous, excluding a meta-analysis. Therefore, a best evidence synthesis was applied, taking the quality of the studies into account. Furthermore, the recording of antibiotic exposure was in half of the studies parental reported, which may have led to recall bias. The antibiotics were mostly analyzed as overall use, without distinguishing between types of antibiotics and therefore, it was not possible to determine associations between certain type of antibiotics and GI-disorders. Finally, for several functional gastrointestinal disorders, like IBS or GERD, only few or even no studies were found which prohibits any conclusions on these GI disorders.

One of the strengths of this review is that the search string was built and performed by an information scientist. Besides the published articles, also conference abstracts were checked for relevant studies. Furthermore, this review studies the association between antibiotics in early life and all chronic GI disorders in childhood, which provides insights in the available evidence but also shows the gap of knowledge for these associations.

For future research, it is recommended to study the association between early life antibiotics and the presence of those GI disorders that currently lack sufficient studies. Furthermore, it is necessary to gain insights in the specific effect of different types of antibiotics on the microbiome in order to optimize therapies that can prevent or counteract the detrimental effects of antibiotics in early life.

Conclusion

This systematic review shows strong evidence for an association between antibiotic exposure in the first two years of life and the presence of IBD and CeD later in childhood. For the other included GI-disorders, only moderate or insufficient evidence was found. In order to decrease the incidence of IBD and CeD, antibiotic administration in early life should be critically considered. Moreover, interventions need to be developed to restore the microbiome after unavoidable antibiotic exposure in order to prevent detrimental health consequences later in life.

What is already known

- Evidence about the association between antibiotic use and gastrointestinal disorders is increasing for adults, but in children the evidence remains scarce.
- The incidence of gastrointestinal disorders in childhood is increasing

What this study adds

- Antibiotics in early life may increase the risk of gastrointestinal disorders later in life especially inflammatory bowel disease and celiac disease.
- Although functional gastrointestinal disorders are the most frequent in childhood, very few studies examined their association with antibiotics in early life.

References

1. Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World journal of gastroenterology. 2018;24(25):2741.
2. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. American Journal of Gastroenterology. 2020;115(4):507-25.
3. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nature Reviews Immunology. 2017;17(4):219-32.
4. Bendtsen KM, Fisker L, Hansen AK, Hansen CH, Nielsen DS. The influence of the young microbiome on inflammatory diseases—lessons from animal studies. Birth Defects Research Part C: Embryo Today: Reviews. 2015;105(4):278-95.
5. Major G, Spiller R. Irritable bowel syndrome, inflammatory bowel disease and the microbiome. Current opinion in endocrinology, diabetes, and obesity. 2014;21(1):15.
6. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? World journal of gastroenterology: WJG. 2014;20(39):14105.
7. Weber TK, Polanco I. Gastrointestinal microbiota and some children diseases: a review. Gastroenterology research and practice. 2012;2012.
8. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016;65(11):1906-15.
9. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Science translational medicine. 2016;8(343):343ra82-ra82.
10. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. Scandinavian journal of gastroenterology. 2018;53(1):1-7.
11. Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho K-L, Kronman MP, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. American Journal of Gastroenterology. 2014;109(11):1728-38.
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336-41.
13. Page MJ, Shamseer L, Tricco AC. Registration of systematic reviews in PROSPERO: 30,000 records and counting. Systematic reviews. 2018;7(1):32.
14. Wilczynski NL, McKibbon KA, Haynes RB, editors. Search filter precision can be improved by NOTing out irrelevant content. AMIA Annual Symposium Proceedings; 2011: American Medical Informatics Association.
15. Van Eck N, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. scientometrics. 2010;84(2):523-38.
16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid AJSr. Rayyan—a web and mobile app for systematic reviews. 2016;5(1):210.
17. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Ottawa Hospital Research Institute; 2019 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
18. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle-Ottawa quality assessment form for cohort studies. pp. E17–E18. 2014.
19. Van Tulder M, Furlan A, Bombardier C, Bouter L, Group EBotCCBR. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine. 2003;28(12):1290-9.

20. Veronese N, Solmi M, Luchini C, Lu R-B, Stubbs B, Zaninotto L, et al. Acetylcholinesterase inhibitors and memantine in bipolar disorder: A systematic review and best evidence synthesis of the efficacy and safety for multiple disease dimensions. Journal of affective disorders. 2016;197:268-80.
21. Slavin RE. Best evidence synthesis: an intelligent alternative to meta-analysis. Journal of clinical epidemiology. 1995;48(1):9-18.
22. Canova C, Zabeo V, Pitter G, Romor P, Baldovin T, Zanotti R, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. American journal of epidemiology. 2014;180(1):76-85.
23. Hestbaek L, Sannes MM, Lous J. Large cohort study finds a statistically significant association between excessive crying in early infancy and subsequent ear symptoms. Acta Paediatrica. 2014;103(5):e206-e11.
24. Kemppainen KM, Vehik K, Lynch KF, Larsson HE, Canepa RJ, Simell V, et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. JAMA pediatrics. 2017;171(12):1217-25.
25. Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics. 2012;130(4):e794-803.
26. Oosterloo BC, van Elburg RM, Rutten NB, Bunkers CM, Crijns CE, Meijssen CB, et al. Wheezing and infantile colic are associated with neonatal antibiotic treatment. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2018;29(2):151-8.
27. Örtqvist AK, Lundholm C, Halfvarson J, Ludvigsson JF, Almqvist C. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study. Gut. 2019;68(2):218-25.
28. Sander SD, Andersen A-MN, Murray JA, Karlstad Ø, Husby S, Størdal K. Association between antibiotics in the first year of life and celiac disease. Gastroenterology. 2019;156(8):2217-29.
29. Turco R, Miele E, Russo M, Mastroianni R, Lavorgna A, Paludetto R, et al. Early-life factors associated with pediatric functional constipation. Journal of pediatric gastroenterology and nutrition. 2014;58(3):307-12.
30. Uusijarvi A, Bergstrom A, Simren M, Ludvigsson JF, Kull I, Wickman M, et al. Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain--a Swedish birth cohort study. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2014;26(6):841-50.
31. Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011;60(1):49-54.
32. Salvatore S, Baldassarre ME, Di Mauro A, Laforgia N, Tafuri S, Bianchi FP, et al. Neonatal Antibiotics and Prematurity Are Associated with an Increased Risk of Functional Gastrointestinal Disorders in the First Year of Life. The Journal of pediatrics. 2019;212:44-51.
33. Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2013;57(1):67-71.
34. Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. Journal of Allergy and Clinical Immunology. 2018;141(1):214-22.

35. Mårild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case–control study. BMC gastroenterology. 2013;13(1):109.
36. Myléus A, Hernell O, Gothefors L, Hammarström M-L, Persson L-Å, Stenlund H, et al. Early infections are associated with increased risk for celiac disease: an incident case-referent study. BMC pediatrics. 2012;12(1):194.
37. Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. The Journal of Allergy and Clinical Immunology: In Practice. 2014;2(4):475-7. e1.
38. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. The American journal of gastroenterology. 2010;105(12):2687-92.
39. Slae M, Persad R, Leung AJ-T, Gabr R, Brocks D, Huynh HQ. Role of environmental factors in the development of pediatric eosinophilic esophagitis. Digestive diseases and sciences. 2015;60(11):3364-72.
40. Virta L, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. American journal of epidemiology. 2012;175(8):775-84.
41. Witmer CP, Susi A, Min SB, Nylund CM. Early infant risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2018;67(5):610-5.
42. Bittker SS, Bell KR. Potential risk factors for celiac disease in childhood: A case-control epidemiological survey. Clinical and experimental gastroenterology. 2019;12:303.
43. Canova C, Ludvigsson JF, Di Domenicantonio R, Zanier L, Barbiellini Amidei C, Zingone F. Perinatal and Antibiotic Exposures and the Risk of Developing Childhood-Onset Inflammatory Bowel Disease: A Nested Case-Control Study Based on a Population-Based Birth Cohort. International Journal of Environmental Research and Public Health. 2020;17(7):2409.
44. Bernstein CN, Burchill C, Targownik LE, Singh H, Roos LL. Events within the first year of life, but not the neonatal period, affect risk for later development of inflammatory bowel diseases. Gastroenterology. 2019;156(8):2190-7. e10.
45. Jiang Hy, Zhang X, Zhou Yy, Jiang Cm, Shi Yd. Infection, antibiotic exposure, and risk of celiac disease: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2020;35(4):557-66.
46. van Houten CB, Naaktgeboren C, Buiteman BJ, van der Lee M, Klein A, Srugo I, et al. Antibiotic overuse in children with respiratory syncytial virus lower respiratory tract infection. The Pediatric infectious disease journal. 2018;37(11):1077-81.
47. Arnolda G, Hibbert P, Ting HP, Molloy C, Wiles L, Warwick M, et al. Assessing the appropriateness of paediatric antibiotic overuse in Australian children: a population-based sample survey. BMC pediatrics. 2020;20:1-8.
48. Melander RJ, Zurawski DV, Melander C. Narrow-spectrum antibacterial agents. Medchemcomm. 2018;9(1):12-21.
49. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. BMJ open. 2014;4(8):e005047.
50. Kumar R, Sood U, Gupta V, Singh M, Scaria J, Lal R. Recent advancements in the development of modern probiotics for restoring human gut microbiome dysbiosis. Indian Journal of Microbiology. 2019:1-14.

51. Chua MC, Ben-Amor K, Lay C, Goh AE, Chiang WC, Rao R, et al. Effect of synbiotic on the gut microbiota of cesarean delivered infants: a randomized, double-blind, multicenter study. Journal of pediatric gastroenterology and nutrition. 2017;65(1):102-6.
52. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome medicine. 2016;8(1):39.

Figure Legends

Figure 1: PRISMA flow diagram of the study selection

Figure 2: Forest plots per gastrointestinal disorder a. IBD; b. EoE; c. CeD; d. FGID (Infantile colics and functional constipation). CC= case control study, CH = cohort study, (!) Virta 2012 only shows the results of the phenoxymethylpenicillin analyses, overall use of antibiotics was not significant

Table 1a study characteristics and association with antibiotics: Inflammatory Bowel Disease

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score

Canova, C [43]
2020
Italy
Case-control
	8.8 yrs[1]	70 / 700	33 (47%)
			
			0-12 months
			
			ATC code
				• Birth order
				• Age mother (at birth)
				• Apgar score at 1 minute
				• Birth weight
				• Education mother
				• Gestational age
				• Multiple birth
				• Season of birth
					AB first six months of life childhood onset IBD
					• Any course aOR = 1.458, 95% CI: 0.81–2.63
					• **Dose-dependent**
					  ○ 2-3 courses aOR = 2.29, 95% CI: 1.01–5.24
					  ○ >4 courses aOR = 6.25, 95% CI: 1.70–23.05
					Ab first 12 months of life childhood onset IBD
					• Any course aOR = 1.08, 95% CI 0.64–1.80
					• **Dose-dependent**: >4 courses aOR = 2.92, 95% CI: 1.32–6.46
						8/9
						High

Hviid, A [31] 2010 Denmark Cohort	3.4 yrs[1]	117 (0.02%) (50 CD and 67 UC) / 577,627	84 (72%) 0-12 months ATC code	• Age • Calendar period • Other times since use • Other types of antibiotics	Increased risk of Crohn's disease after: AB use in the last 3 months: • 3-11 months RR = 3.32, 95% CI: 1.15-9.56 • 1 year RR = 1.53, 95% CI: .15-15.46 AB use > 3 months previously before diagnosis: • 0-2 months RR = 4.19, 95% CI: 1.64-10.68	8/9 high
Kronman, M [25] 2012 United Kingdom Cohort	Exposed 4.2 yrs[2]	748 (0.07%) / 1,072,426	436 (58%) 0-12 months Systemic AB prescriptions	• Age • Chronic granulomatous disease • IBD family • Primary sclerosing cholangitis • Sex • Socioeconomic deprivation	• Exposure was associated with a 5.5-fold increased IBD risk (aHR = 5.51, 95% CI:1.66–18.28). • **Dose-dependent**: Exposure to >2 anti-anaerobic antibiotic courses was more highly associated with IBD development than exposure to 1 or 2 courses (aHR = 4.77, 95% CI: 2.13–10.68) versus (3.33, 95% CI: 1.69–6.58). • **Type-dependent** Fluoroquinolone (aHR= 2.09, 95% CI: 1.10–3.98) and metronidazole exposure (aHR = 186.25, 95% CI: 10.86–3193.65) was significantly associated with IBD.	7/9 moderate

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Örtqvist, A [27] 2018 Sweden Cohort	2 yrs[1]	95 (0.01%) 51 IBD (CD and/or UC), 20 CD & 24 UC / 827,239	IBD 43 (84,3%) CD 16 (80%) UC 20 (83.3%) 0-12 months ATC code	• Delivery mode • Education parents • Ethnicity parents • IBD parents	No significant associations (any and PcV antibiotics) or dose–response relationship were found	8/9 high
Shaw, S [38] 2010 Canada Case-control	8.4 yrs[1]	36 / 360	21 (58%) 0-12 months ATC code	• Age • Place of residence • Sex	• One or more dispensations of antibiotics was associated with 2.9 times the odds (95% CI: 1.2-7.0, P = 0.017) of having IBD. • Stratified by IBD type, only CD was significant (OR = 5.3, 95% CI: 1.6-17.4; P = 0.006). • **Dose-dependent**: association for 2-4 (OR = 2.9, 95% CI: 1.1-7.8; P = 0.039) and 5+ (OR = 5.0, 95 % CI: 1.3-18.9; P = 0.18) prescriptions.	8/9 high
Virta, L [40] 2012 Finland Case-control	CD: 9.7 yrs[3] UC: 8.5 yrs[3]	595 (233 CD, 362 UC) / 2,380	313 (52,6%) 0-12 months ATC code	• Age • Place of residence • Chronic diseases • Sex	• Use of AB overall was not significant • **Type-dependent:** phenoxymethylpenicillin was associated with an increased risk of CD. (aOR = 2.54, 95% CI: 1.3-4.98)	8/9 high

AB: antibiotic, aHR: adjusted hazard ratio, aOR: adjusted odds ratio, ATC: Anatomical Therapeutic Chemical (ATC) Classification System, CD: Anatomical Therapeutic Chemical (ATC) Classification System, CI: Confidence interval , IBD: Inflammatory bowel disease, IRR: incidence rate ratio, HR: hazard ratio, OR: odds ratio, PcV: Phenoxymethylpenicillin and UC: Ulcerative colitis

Table 1b study characteristics and association with antibiotics: Eosinophilic Esophagitis (EoE)

Author Year Country Design	Age diagnosis [1]	Cases / Controls	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Jensen, E [33] 2013 North Carolina (USA) Case-control	Cases 11 yrs[1]	31 / 52	22 (71%) 0-12 months Motherly reported	None	Antibiotics were associated with EoE (OR= 6, 95% CI: 1.7–20.8)	4/9 weak
Jensen, E [34] 2018 North Carolina (USA) Case-control	Cases 10.6 yrs[1]	127 / 121	91 (72%) 0-12 months Motherly reported	• Education mother • NICU admission	Antibiotics were associated with EoE (aOR = 2.30, 95% CI: 1.21-4.38)	6/9 moderate
Radano, M [37] 2014 Massachusetts (USA) Case-control	Cases 3 yrs[1]	25 / 74	17 (67%) 0-12 months Parental reported	• Age • Atopy • Atopy family • Sex	Antibiotics were associated with EoE (OR = 3.61, 95% CI: 1.11-11.74; P = .03)	7/9 moderate
Slae, M [39] 2015 Canada Case-control	Cases 8.6 yrs[1]	102 / 167	60 (59%) 0-12 months Parental reported	• Breastfeeding • Birth order • Day care attendance (early) • Exposure to farm animals • Fast food consumption	Rates of antibiotic exposure were similar for cases and controls.	3/9 weak
Witmer, C [41] 2018 USA Case-control	4.2 yrs[1]	1410 / 2,820	409 (29%) 0-6 months Pharmaceutical coding	• Age • Atopy (markers) • Delivery mode • Erythema toxicum neonatorum • Feeding problems • Infantile colic • Medication exposure • Oral candidiasis • Prematurity • Prolonged rupture/ chorioamnionitis • Reflux • Sex	The association with antibiotic exposure was statistically significant (aOR = 1.31, 95% CI: 1.10–1.56).	7/9 moderate

Table 1c study characteristics and association with antibiotics: Celiac Disease (CeD)

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Bittker, S [42] 2019 USA Case-control	6.1 yrs[1]	332 / 241	237 (71%) 0-24 months Parental reported	• Age • Age mother (at birth) • Education mother • Ethnicity	• Antibiotic exposure is associated with susequent CeD (aOR = 1.133, 95% CI: 1.037–1.244; p= 0.007) • **Dose-dependent**: ORs increase with number of antibiotic courses	5/9 moderate
Canova, C [22] 2014 Italy Cohort	6.4 yrs[1]	1.227 CeD (0.6%) 866 confirmed* and 361 unconfirmed* / 203,557	336 (47%) 0-12 months ATC code	• Education mother (only in sensitivity analysis with pathological confirmed villous atrophy) • Sex • Year of birth	• Increased risk of developing CeD after at least 1 AB course (IRR = 1.24, 95% CI: 1.07-1.43), (IRR = 1.31, 95% CI: 1.10-1.56) for histopathologically confirmed CeD • **Dose-dependent**: risk increased with more AB courses (P-trend < 0.01). • **Type-dependent:** Cephalosporin use was strongly associated with CeD onset (IRR = 1.42, 95% CI: 1.18-1.73), (IRR = 1.51, 95% CI: 1.21-1.89) for histopathologically confirmed CeD. For first- and second-generation drugs: (IRR = 1.39, 95% CI: 1.11-1.76 and third- and fourth-generation drugs: IRR = 1.49, 95% CI: 1.14-1.95).	8/9 high
Kemppainen, K [24] 2017 Finland, Germany, Sweden and the USA Cohort	21.4 months[1]	783 (11,9%) / 6,558	Unknown 0-24 months Parental reported	• Breastfeeding (at 90 days of age) • CeD genotype with family • Delivery mode • Maternal AB use during pregnancy • Place of residence • Probiotic use before 90 days of age • Season of birth • Sex	• Exposure to AB was not associated with CeD. • **Dose-dependent**: 2 or more doses of macrolides within the first year of life (157 of 6558 [2.4%]) had elevated CeD risk (HR = 1.77, 95% CI: 1.18-2.66; P = .006 before but not after adjustment).	6/9 moderate

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Mårild, K [35] 2013 Sweden Case-control	0-2 yrs[1]	132 celiac disease / 655 12 inflammation / 60 17 normal mucosa / 85	CeD 51 (39%) Inflammation 6 (50%) 0-24 months ATC code	• Age • Education mother • Number of outpatient visits before biopsy • Sex	Exposure to AB was associated with CeD Odds ratios for prior AB use (CeD): cases 51/132 (38.6%) controls 189/655 (28.9%) (OR = 1.58, 95% CI: 1.07-2.34)	8/9 high
Myleus, A [36] 2012 Sweden Case-control	14 months[1]	373 / 581	97 (26%) 0-6 months Parental reported	• Age • Place of residence • Sex	No significantly increased risk for celiac disease (OR = 1.2, 95% CI: 0.87-1.6; P=0.27).	7/9 moderate
Sander, S [28] 2019 Denmark and Norway Cohort	Danish: 11.6 yrs[3] Norwegian: 5.4 yrs[3]	Danish: 1427 (0.12%) / 1,168,656 Norwegian: 1919 (0.36%) / 537,457	Danish: 622 (43.6%) Norwegian: 390 (20.3%) 0-12 months ATC code	• Age mother • Associated comorbidity • Birth order • Education mother • Hospitalization with infection • Season of birth • Sex • Type 1 diabetes child and/ or mother	• Exposure to systemic AB (penicillins and extended spectrum penicillins) was positively associated with diagnosed celiac disease in both cohorts (pooled aOR = 1.26, 95% CI: 1.16–1.36) • **Dose-dependent**: between number of AB courses and risk of CeD (pooled aOR for each additional dispensed AB = 1.08, 95% CI: 1.05–1.11).	9/9 high

Table 1d study characteristics and association with antibiotics: FGIDs: Infantile Colics, Functional constipation (FC), Recurrent abdominal pain (AP) and Regurgitation, functional diarrhea and infant dyschezia

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Infantile colics						
Hestbaek, L [23] 2014 Denmark Cohort	0-6 months	2183 (8,1%) / 26,983	excessive 895 (41%) extreme excessive 355 (50%) 0-6 months Motherly reported	None	At 6-month-olds, statistically significant associations between excessive crying and the use of antibiotics due to ear infections (OR = 1.47, 95% CI: 1.18–1.82) were found.	6/9 moderate
Oosterloo, B [26] 2018 The Netherlands Cohort	0-1 yr	74 (20%) / 362	33 (45%) 0-7 days Broad-spectrum AB intravenous for 2-3 days (AB2) or 7 days (AB7).	• Atopy family • Birth order • Breastfeeding • Day care attendance • Delivery mode • Education parents • Tobacco exposure	• Antibiotic treatment was an independent risk factor for infantile colic (aOR = 1.66, 95%CI: 1.00-2.77, P = .05). • Doctors-diagnosed infantile colic was higher in AB+ than in AB− (4.0% vs 0.4%; P = .014). • **Duration-dependent**: Parent-reported infantile colic was higher in AB7 compared to no antibiotics (AB-) and AB2 (24.8%, 14.4% and 14.3%, P = .048 and P = .015).	8/9 high
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	265 (41.9%) / 632	141 (22.3%) 0-7 days Hospital chart and parental report	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=1.16; 95% CI: 0.79-1.70, p=0.439)	7/9 moderate

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Functional constipation (FC)						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	128 (26.6%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=0.77; 95%CI: 0.49-1.20, p=0.242)	7/9 moderate
Turco, R [29] 2014 Italy Cohort	0-1 yr	43 (10.7%) / 465	15 (34.8%) 0-12 months Parental reported	• Anti-inflammatory drugs or corticosteroids • Atopy & in family • Birth order • Breastfeeding & weaning • Education parents • Fever episodes before onset • FGIDs family • Nursery school age • Place of residence (>3000 citizens) • Sex • Vitamin and food supplements	No statistically significant association was found (26% vs 19%).	8/9 high
Recurrent abdominal pain (AP)						
Uusijärvi, A [30] 2014 Sweden Cohort	12 yrs	Monthly: 231 (8,7%) Weekly: 111 (4,2%) / 2,654	Monthly 1900 (71.5%) Weekly 81 (72,9%) 0-24 months Parental reported	• Asthma at 12 years of age • Asthma at one year • Sex	Stratified analyses showed that girls, who received antibiotics during both the first and the second year of life, had an increased risk of AP at 12 years (OR = 1.65, 95% CI: 1.09–2.49).	5/9 moderate
Regurgitation, functional diarrhea and infant dyschezia						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	Regurgitation:236 (37.3%) Functional diarrhea: 24 (3.8%) Infant dyschezia: 199 (31.5%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found for regurgitation (OR=1.29, 95%CI: 0.88-1.90, p=0.190), functional diarrhea (OR=0.90, 95%CI: 0.33-2.45, p=0.835), or infant dyschezia (OR=1.29, 95%CI: 0.87-1.93, p=0.205).	7/9 moderate

Table 2 quality assessment

	Selection				Comparability		Outcome / Exposure			Score
	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Cohort studies*	Representativeness	Selection	Exposure	Outcome	Most important	Second important	Assessment	Duration of follow-up	Adequacy follow-up	
Canova [22]	*	*	*	*		*	*	*	*	8/9
Hestbaek [23]	*	*	*	*				*	*	6/9
Hviid [31]	*	*	*	*		*	*	*	*	8/9
Kemppainen [24]		*		*	*	*	*	*		6/9
Kronman [25]		*	*	*	*	*	*	*		7/9
Oosterloo [26]	*	*	*	*	*	*	*	*		8/9
Örtqvist [27]	*	*	*	*	*	*	*		*	8/9
Salvatore [32]	*	*	*	*		*	*	*		7/9
Sander [28]	*	*	*	*	*	*	*	*	*	9/9
Turco [29]	*	*	*	*	*	*		*	*	8/9
Uusijärvi [30]	*	*		*				*	*	5/9
Case-Control studies**	Case definition	Cases	Controls	Definition controls	Most important	Second important	Exposure	Ascertainment	Non-Response rate	Score
Bittker [42]			*	*	*			*	*	5/9
Canova [43]	*	*	*	*		*	*	*	*	8/9
Jensen [33]	*	*		*				*		4/9
Jensen [34]	*	*	*	*				*	*	6/9
Mårild [35]	*	*	*	*		*	*	*	*	8/9
Myleus [36]	*	*	*	*		*		*	*	7/9
Radano [37]	*	*		*	*	*		*	*	7/9
Shaw [38]	*	*	*	*		*	*	*	*	8/9
Slae [39]	*			*				*		3/9
Virta [40]	*	*	*	*		*	*	*	*	8/9
Witmer [41]		*	*	*	*	*		*	*	7/9

*Cohort studies: 1. Representativeness of the exposed cohort, 2. Selection of the non-exposed cohort, 3. Ascertainment of exposure, 4. Demonstration that the outcome of interest was not present at start of the study, 5. Comparability of cohorts on the basis of the design or analysis most important factor, 6. Comparability of cohorts on the basis of the design or analysis second important factor, 7. Assessment of outcome 8. Was follow-up long enough for outcomes to occur and 9. Adequacy of follow up of cohort.

**Case- Control studies: 1. Is the case definition adequate? 2. Representativeness of the cases, 3. Selection of controls, 4. Definition of controls, 5. Comparability of cases and controls on the basis of the design or analysis most important factor, 6. Comparability of cases and controls on the basis of the design or analysis second important factor, 7. Ascertainment of exposure, 8. Same method of ascertainment for cases and controls and 9. Non-Response rate

Comparability: Most important confounder: IBD and CeD: presence of IBD/ CeD in 1ste degree family member, EoE: sex, colics: atopy child and/or family, functional constipation: maternal education/social economic status, abdominal pain: lactose intolerance/ cow's milk allergy.

Comparability: Second important confounder: IBD: ethnicity and/or age, EoE: presence of other atopic diseases and/or ethnicity, CeD: sex and/ or season of birth and/or the presence of other autoimmune diseases, colics: presence of GERD and/or type of feeding and/or being a first child, functional constipation: sex and/ or age, abdominal pain: anxiety/ depression/ stress in the child and/or the parents.

Early life antibiotics and childhood gastrointestinal disorders: a systematic review

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K. Kamphorst contributed to the design, the analyses and interpretation of the study, drafting of the initial manuscript, and reviewed and revised the manuscrip*t.*

E. Van Daele contributed to the analysis and interpretation of the study and critically revised the manuscript.

A.M. Vlieger and R.M. van Elburg contributed to the conception of the study, interpretation of the data and critically revised the manuscript.

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Abstract

Background: In adults, there is increasing evidence for an association between antibiotic use and gastrointestinal disorders but in children, the evidence is scarce.

Objective: Assess the association between exposure to antibiotics in the first two years of life in term born children and the presence of chronic gastrointestinal disorders later in childhood.

Design: For this systematic review the MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020. Title and abstract screening (n=12,219), full-text screening (n=132) as well as the quality assessment with the Newcastle–Ottawa Scale were independently performed by two researchers.

Main outcome measures: The association between antibiotics and inflammatory bowel disease (n=6), eosinophilic esophagitis (n=5), celiac disease (n=6), infantile colics (n=3), functional constipation (n=2), recurrent abdominal pain, regurgitation, functional diarrhea, and infant dyschezia were examined.

Results: Twenty-two studies were included, 11 cohort and 11 case-control studies. A best evidence synthesis showed strong evidence for an association between antibiotic exposure in the first two years of life and the presence of inflammatory bowel disease, and celiac disease during childhood. Moderate evidence was found for an association with eosinophilic esophagitis and no association with functional constipation in the first year of life. There was insufficient evidence for the other studied disorders.

Conclusions: The use of antibiotics in early life may increase the risk of gastrointestinal disorders later in life. Further studies are necessary to unravel the underlying mechanisms and determine potential preventive measures. Meanwhile judicious use of antibiotics in early childhood is highly warranted.

Introduction

The incidence of pediatric gastrointestinal disorders (GI-disorders), such as pediatric inflammatory bowel disease (IBD) and celiac disease (CeD), is rising [1, 2]. The increase in pediatric GI-disorders is most likely related to environmental factors and recently the focus has been on the role of the intestinal microbiome. A microbiome that has been disturbed by factors like stress, dietary change, environmental factors or drugs, can result in alterations in the immune system [3]. Several studies have shown that a disturbed microbiome can be a cause or trigger of GI disorders, probably mediated by these immunological changes [4-7].

One of the drugs with the most profound effect on the microbiome are antibiotics [8]. The impact of antibiotics on the microbiome depends on various factors such as type of antibiotic, dosage, and duration of exposure[8]. Furthermore, age at exposure is probably also important. The gut of a newborn infant is almost sterile with a low diversity and matures according to several developmental stages with increasing diversity over time [9]. The microbiome stabilizes around the age of 2 to 3 years [9]. Since this developing gut microbiota plays an important role in the training of both innate and adaptive immune system, it is likely that antibiotics will have their biggest impact when administered in the first two years of life.

For the association between antibiotic use and GI disorders, that has been shown in adults, [10], there is only limited evidence in children [11]. Therefore, the aim of this systematic review was to assess the association between exposure to antibiotics in the first two years of life and the presence of chronic gastrointestinal disorders during childhood.

Method

Study selection

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and registered in PROSPERO CRD42019132631 [12, 13]. MEDLINE, Embase, WHO trial register, and Web of Science were systematically searched from inception to June 8, 2020 to identify all studies examining the association between antibiotic exposure in the first two years of life and the presence of common chronic (longer than two weeks, in order to exclude viral diarrhea) gastrointestinal disorders during the first 18 years of life. We searched for associations with IBD, eosinophilic esophagitis (EoE), CeD, irritable bowel syndrome (IBS), (functional) abdominal pain, constipation, dyspepsia, aerophagia, infantile colic, gastroesophageal reflux (GERD), regurgitation, dyschezia and chronic diarrhea.

A multi stranded search approach comprised various concept combinations of children aged 0-4 years, prognosis, gastrointestinal disorders and antibiotics. In order to reduce recall noise and enhance search results precision we used VOS-viewer to identify terms for NOTing out irrelevant records from databases searched [14, 15]. See supplementary file 1 for the full search strategies.

Patient and Public Involvement statement

As this is a systematic review of the literature, there were no patients involved in the design of the research question nor the study itself. Furthermore, for the same reason no approval for the study was required from an ethical committee.

In- and exclusion criteria

Studies were included if: 1. Antibiotics were administered between full-term birth and two years of age. 2. Study outcome was diagnosis with a chronic GI-disorder during the first

18 years of life. 3. Antibiotic use was before the diagnosis of the GI-disorder. 4. A control group was included. 5. In case multiple studies were found examining similar outcomes in one cohort, only the study with the largest cohort was included. No restrictions were placed on the time period of publication. Searches were limited to studies conducted in humans and excluded if the full text was not available in English, Dutch, German or French.

All records found in the search were exported into Rayyan after deduplication [16]. Two researchers (KK and EVD) independently performed title and abstract screening as well as full-text screening. After consensus about the study selection, data were entered into a data extraction form, which included: author, year of publication, country, study design, cases, controls/cohort, population age, sample size exposed to antibiotics, age at exposure, details about classification by type of antibiotics, type of GI disorder, method of diagnosis, confounders for which corrected, and the association between exposure and outcome.

Methodological quality

To assess the risk of bias, two researchers (KK and EVD) independently assessed the methodological quality. Discrepancies were resolved by discussion until consensus was reached. The Newcastle–Ottawa Scale (NOS) was used, which has been developed to assess the quality of observational studies [17]. The NOS includes different instruments for assessing case-control and cohort studies. Both scales contain a maximum of nine points and assess studies in three core areas: 1. Selection of study participants 2. Comparability of groups 3. Detection of exposure / outcome. One point for comparability of groups was given when the study controlled for the main important confounder and a second point if controlled for a second important confounder, see supplementary file 2. Studies were rated high quality with a score of eight or higher, moderate quality with a score between five and seven and weak quality with a score of four or less [18].

Data analyses

To synthesize the methodological quality of the studies, a commonly used best evidence synthesis was applied per disorder in which the methodological quality was considered according to the following definitions: 1. strong evidence, provided by generally consistent findings in at least two high-quality studies. 2. moderate evidence, provided by generally consistent results in one high-quality study and at least one moderate- or low-quality study, or generally consistent results in multiple moderate- or low-quality studies. 3. insufficient evidence, when less than two studies were available or inconsistent findings in multiple studies [19-21]. Results were considered consistent when at least 75% of the studies showed results in the same direction.

Results

Search results

Of the 14,731 retrieved records, 12,219 remained after removing duplicates. These records were screened; 132 were assessed as eligible and read in full-text of which 110 were excluded and 22 studies included in this review. Details of the selection procedure are shown in Figure 1.

Study characteristics

The included studies were published between 2010 and 2020 (table 1a-d): 11 cohort studies [22-32] and 11 case-control studies [33-43]. The studies were performed in Sweden (n=4) [27, 30, 35, 36], the United States of America (USA) (n=5) [33, 34, 37, 41, 42], Italy (n=4) [22, 29, 32, 43], Denmark (n=2) [23, 31], Canada (n=2) [38, 39], and one in the United Kingdom [25], the Netherlands [26], and Finland [40]. There were two international studies, one in Denmark and Norway [28], and another in Finland, Germany, Sweden and the USA [24].

The associations between antibiotics and the following GI-disorders were examined: IBD (n=6) [25, 27, 31, 38, 40, 43], EoE (n=5) [33, 34, 37, 39, 41], CeD (n=6) [22, 24, 28, 35, 36, 42], infantile colics (n=3) [23, 26, 32], functional constipation (n=2) [29, 32], recurrent abdominal pain (n=1) [30]. One study examined several functional GI-disorders (FGIDs): infantile colics, functional constipation, functional diarrhea, infant dyschezia, and regurgitation[32].

Exposure to antibiotics was studied in the first two years of life (n=4) [24, 30, 35, 42], the first 18 months of life (n=1) [23], the first year of life (n=13) [22, 25, 27-29, 31, 33, 34, 37-40, 43], the first six months of life (n=2) [36, 41], and the first week of life (n=2) [26, 32] (table 1a-d). Since only a few studies provided details about type of antibiotics and/or number of antibiotic treatments in the first two years of life, the associations include mostly the overall antibiotic exposure.

Quality assessment

Ten studies were of high quality[22, 26-29, 31, 35, 38, 40, 43], ten studies moderate [23-25, 30, 32, 34, 36, 37, 41, 42], and two weak [33, 39] (Table 2). Frequently observed weaknesses were a high dropout rate in the cohort studies, assessment of antibiotic exposure through parental reports, and no correction for important confounders.

Inflammatory Bowel Disease

Exposure to early life antibiotics was associated with the development of IBD in five out of six studies [25, 31, 38, 40, 43] (NOS = 7,8,8,8,8), whereas no association was found in one study examining Very Early Onset (VEO) IBD, (before six years of age) [27] (NOS = 8). Three studies found a dose-response relation [25, 38, 43] and an increased risk after fluoroquinolone [25], metronidazole [25], and phenoxymethylpenicillin[40] exposure. In two studies IBD was stratified by type and only the odds ratio for Crohn's disease, but not for ulcerative colitis, was significant [38, 40]. Forest plots of the main results are shown in Figure 2a.

Eosinophilic esophagitis

In four of the five studies early life antibiotics was associated with EoE [33, 34, 37, 41] (NOS = 4,6,7,7), whereas in one study the rates of parental reported antibiotic use were similar for cases and controls [39] (NOS = 3) (figure 2b).

Celiac disease

In four studies, of which three had a high quality, a significant association between early life antibiotics and the presence of CeD was found [22, 28, 35, 42] (NOS = 8,9,8,5), whereas in two moderate quality studies no association was found [24, 36] (NOS = 6,7) (figure 2c). Three studies showed a dose-response relationship between exposure to antibiotics and the

risk of CeD [22, 28, 42]. Furthermore, use of cephalosporin [22] and multiple courses of macrolides [24] showed a positive association with the development of CeD.

Infantile colics

Two studies found a significant association between early life antibiotics and infantile colics [23, 26] (NOS = 6,8), while one study found no association [32] (NOS = 7) (figure 2d).

Functional constipation

In both studies, no association was found between early life antibiotics use and functional constipation in the first year of life [29, 32] (NOS = 8,7).

Recurrent abdominal pain

The only study examining the association between antibiotics use in the first two years of life and the risk of recurrent abdominal pain (AP) at 12 years of age [30] (NOS = 5) found that only girls, but not boys, who received antibiotics in both the first and second year of life, had an increased risk of AP at 12 years.

Regurgitation, dyschezia and functional diarrhea

In one study no association was found between antibiotics in the first week of life and regurgitation, dyschezia and functional diarrhea [32] (NOS = 7).

Syntheses of individual results

Using the definitions for the best evidence synthesis, described in the method section, it can be concluded that there is strong evidence for an association of antibiotics in early life with IBD and CeD. There is moderate evidence for an association with EoE and no association with infantile constipation. The current evidence for an association between antibiotics in early life and the other studied GI-disorders is considered insufficient.

Discussion

This systematic review with best evidence syntheses on the association between antibiotic exposure in the first two years of life and chronic GI disorders during childhood showed strong evidence for this association with inflammatory bowel disease~~, eosinophilic esophagitis,~~ and celiac disease, and moderate evidence for this association with eosinophilic esophagitis. For the other studied GI-disorders, ~~only moderate or~~ insufficient evidence was found.

The question remains to what extent the association with IBD, EoE and CeD can be attributed to antibiotic exposure itself or to other factors such as infections and parental health seeking behavior. Infections in early life have been proposed to contribute to the development of chronic GI-disorders [44, 45] and it is difficult to differentiate between the role of infections and antibiotics which are prescribed for (suspected) infections. Furthermore, several GI-disorders like CeD can remain undiagnosed for a long time. Higher parental health seeking behavior can both lead to higher use of antibiotics and a higher chance of diagnosing the chronic GI-disorder. Therefore, it remains unknown whether antibiotics are the true causative agent in the observed associations or whether they are intermediates in different mechanistic pathways through microbiome perturbations or changes in immune development after (suspected) infections.

Most studies found a clear association between antibiotics in early life and IBD. The study that focused on very early onset IBD (VEO-IBD), found no association between antibiotics and VEO-IBD. VEO-IBD is considered a different entity from later-onset IBD [44], since genetics play a far more important etiological role than microbial dysbiosis [45]. This may explain the lack of an association with early life antibiotics.

The primary goal of antibiotic administration is to prevent detrimental effects of serious and sometimes even life-threatening infections. However, especially in early life, antibiotics are overused, since they are often prescribed for viral upper respiratory tract infections [46, 47]. Given its association with the occurrence of IBD, CeD and EoE, it is highly important to prevent antibiotic overuse by strict adherence to guidelines. If antibiotics are necessary, treatment would be adjusted to minimize dysbiosis. Another possible solution is to shorten the time of antibiotic administration. Oosterloo *et al.* found more health issues in the first year of life after seven days compared to two days of antibiotics in the first week of life [26]. Furthermore, whenever possible, narrow-spectrum antibiotics rather than broad-spectrum should be used, because these specifically reduce the capacity of pathogens to cause disease while leaving commensals unharmed [48]. If adjustment of antibiotic treatment is not possible, interventions that restore or prevent dysbiosis should be considered, such as administration of pre- or probiotics, or fecal transplants [49-52].

Some limitations of this review need to be considered. As no randomized controlled trials were available, only associations but not causality can be examined. Additionally, the studied results were not evaluated for their precision and associations with wide confidence intervals can indicate uncertainty about the magnitude of the association. Hence, the results must be interpreted with caution. Furthermore, both age at exposure as well as age at diagnosis varied substantially between the studies. In addition, study outcomes were also very heterogeneous, excluding a meta-analysis. Therefore, a best evidence synthesis was applied, taking the quality of the studies into account. Furthermore, the recording of antibiotic exposure was in half of the studies parental reported, which may have led to recall bias. The antibiotics were mostly analyzed as overall use, without distinguishing between types of antibiotics and therefore, it was not possible to determine associations between certain type of antibiotics and GI-disorders. Finally, for several functional gastrointestinal disorders, like IBS

or GERD, only few or even no studies were found which prohibits any conclusions on these GI disorders.

One of the strengths of this review is that the search string was built and performed by an information scientist. Besides the published articles, also conference abstracts were checked for relevant studies. Furthermore, this review studies the association between antibiotics in early life and all chronic GI disorders in childhood, which provides insights in the available evidence but also shows the gap of knowledge for these associations.

For future research, it is recommended to study the association between early life antibiotics and the presence of those GI disorders that currently lack sufficient studies. Furthermore, it is necessary to gain insights in the specific effect of different types of antibiotics on the microbiome in order to optimize therapies that can prevent or counteract the detrimental effects of antibiotics in early life.

Conclusion

This systematic review shows strong evidence for an association between antibiotic exposure in the first two years of life and the presence of IBD and CeD later in childhood. For the other included GI-disorders, only moderate or insufficient evidence was found. In order to decrease the incidence of IBD and CeD, antibiotic administration in early life should be critically considered. Moreover, interventions need to be developed to restore the microbiome after unavoidable antibiotic exposure in order to prevent detrimental health consequences later in life.

What is already known

- Evidence about the association between antibiotic use and gastrointestinal disorders is increasing for adults, but in children the evidence remains scarce.
- The incidence of gastrointestinal disorders in childhood is increasing

What this study adds

- Antibiotics in early life may increase the risk of gastrointestinal disorders later in life especially inflammatory bowel disease and celiac disease.
- Although functional gastrointestinal disorders are the most frequent in childhood, very few studies examined their association with antibiotics in early life.

References

1. Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World journal of gastroenterology. 2018;24(25):2741.
2. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. American Journal of Gastroenterology. 2020;115(4):507-25.
3. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nature Reviews Immunology. 2017;17(4):219-32.
4. Bendtsen KM, Fisker L, Hansen AK, Hansen CH, Nielsen DS. The influence of the young microbiome on inflammatory diseases—lessons from animal studies. Birth Defects Research Part C: Embryo Today: Reviews. 2015;105(4):278-95.
5. Major G, Spiller R. Irritable bowel syndrome, inflammatory bowel disease and the microbiome. Current opinion in endocrinology, diabetes, and obesity. 2014;21(1):15.
6. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? World journal of gastroenterology: WJG. 2014;20(39):14105.
7. Weber TK, Polanco I. Gastrointestinal microbiota and some children diseases: a review. Gastroenterology research and practice. 2012;2012.
8. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut. 2016;65(11):1906-15.
9. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Science translational medicine. 2016;8(343):343ra82-ra82.
10. Theochari NA, Stefanopoulos A, Mylonas KS, Economopoulos KP. Antibiotics exposure and risk of inflammatory bowel disease: a systematic review. Scandinavian journal of gastroenterology. 2018;53(1):1-7.
11. Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho K-L, Kronman MP, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. American Journal of Gastroenterology. 2014;109(11):1728-38.
12. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336-41.
13. Page MJ, Shamseer L, Tricco AC. Registration of systematic reviews in PROSPERO: 30,000 records and counting. Systematic reviews. 2018;7(1):32.
14. Wilczynski NL, McKibbon KA, Haynes RB, editors. Search filter precision can be improved by NOTing out irrelevant content. AMIA Annual Symposium Proceedings; 2011: American Medical Informatics Association.
15. Van Eck N, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. scientometrics. 2010;84(2):523-38.
16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid AJSr. Rayyan—a web and mobile app for systematic reviews. 2016;5(1):210.
17. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P,. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses: Ottawa Hospital Research Institute; 2019 [Available from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
18. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Newcastle-Ottawa quality assessment form for cohort studies. pp. E17–E18. 2014.
19. Van Tulder M, Furlan A, Bombardier C, Bouter L, Group EBotCCBR. Updated method guidelines for systematic reviews in the cochrane collaboration back review group. Spine. 2003;28(12):1290-9.

20. Veronese N, Solmi M, Luchini C, Lu R-B, Stubbs B, Zaninotto L, et al. Acetylcholinesterase inhibitors and memantine in bipolar disorder: A systematic review and best evidence synthesis of the efficacy and safety for multiple disease dimensions. Journal of affective disorders. 2016;197:268-80.
21. Slavin RE. Best evidence synthesis: an intelligent alternative to meta-analysis. Journal of clinical epidemiology. 1995;48(1):9-18.
22. Canova C, Zabeo V, Pitter G, Romor P, Baldovin T, Zanotti R, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. American journal of epidemiology. 2014;180(1):76-85.
23. Hestbaek L, Sannes MM, Lous J. Large cohort study finds a statistically significant association between excessive crying in early infancy and subsequent ear symptoms. Acta Paediatrica. 2014;103(5):e206-e11.
24. Kemppainen KM, Vehik K, Lynch KF, Larsson HE, Canepa RJ, Simell V, et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. JAMA pediatrics. 2017;171(12):1217-25.
25. Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics. 2012;130(4):e794-803.
26. Oosterloo BC, van Elburg RM, Rutten NB, Bunkers CM, Crijns CE, Meijssen CB, et al. Wheezing and infantile colic are associated with neonatal antibiotic treatment. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2018;29(2):151-8.
27. Örtqvist AK, Lundholm C, Halfvarson J, Ludvigsson JF, Almqvist C. Fetal and early life antibiotics exposure and very early onset inflammatory bowel disease: a population-based study. Gut. 2019;68(2):218-25.
28. Sander SD, Andersen A-MN, Murray JA, Karlstad Ø, Husby S, Størdal K. Association between antibiotics in the first year of life and celiac disease. Gastroenterology. 2019;156(8):2217-29.
29. Turco R, Miele E, Russo M, Mastroianni R, Lavorgna A, Paludetto R, et al. Early-life factors associated with pediatric functional constipation. Journal of pediatric gastroenterology and nutrition. 2014;58(3):307-12.
30. Uusijarvi A, Bergstrom A, Simren M, Ludvigsson JF, Kull I, Wickman M, et al. Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain--a Swedish birth cohort study. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2014;26(6):841-50.
31. Hviid A, Svanstrom H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 2011;60(1):49-54.
32. Salvatore S, Baldassarre ME, Di Mauro A, Laforgia N, Tafuri S, Bianchi FP, et al. Neonatal Antibiotics and Prematurity Are Associated with an Increased Risk of Functional Gastrointestinal Disorders in the First Year of Life. The Journal of pediatrics. 2019;212:44-51.
33. Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2013;57(1):67-71.
34. Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. Journal of Allergy and Clinical Immunology. 2018;141(1):214-22.

35. Mårild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case–control study. BMC gastroenterology. 2013;13(1):109.
36. Myléus A, Hernell O, Gothefors L, Hammarström M-L, Persson L-Å, Stenlund H, et al. Early infections are associated with increased risk for celiac disease: an incident case-referent study. BMC pediatrics. 2012;12(1):194.
37. Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. The Journal of Allergy and Clinical Immunology: In Practice. 2014;2(4):475-7. e1.
38. Shaw SY, Blanchard JF, Bernstein CN. Association between the use of antibiotics in the first year of life and pediatric inflammatory bowel disease. The American journal of gastroenterology. 2010;105(12):2687-92.
39. Slae M, Persad R, Leung AJ-T, Gabr R, Brocks D, Huynh HQ. Role of environmental factors in the development of pediatric eosinophilic esophagitis. Digestive diseases and sciences. 2015;60(11):3364-72.
40. Virta L, Auvinen A, Helenius H, Huovinen P, Kolho KL. Association of repeated exposure to antibiotics with the development of pediatric Crohn's disease--a nationwide, register-based finnish case-control study. American journal of epidemiology. 2012;175(8):775-84.
41. Witmer CP, Susi A, Min SB, Nylund CM. Early infant risk factors for pediatric eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2018;67(5):610-5.
42. Bittker SS, Bell KR. Potential risk factors for celiac disease in childhood: A case-control epidemiological survey. Clinical and experimental gastroenterology. 2019;12:303.
43. Canova C, Ludvigsson JF, Di Domenicantonio R, Zanier L, Barbiellini Amidei C, Zingone F. Perinatal and Antibiotic Exposures and the Risk of Developing Childhood-Onset Inflammatory Bowel Disease: A Nested Case-Control Study Based on a Population-Based Birth Cohort. International Journal of Environmental Research and Public Health. 2020;17(7):2409.
44. Bernstein CN, Burchill C, Targownik LE, Singh H, Roos LL. Events within the first year of life, but not the neonatal period, affect risk for later development of inflammatory bowel diseases. Gastroenterology. 2019;156(8):2190-7. e10.
45. Jiang Hy, Zhang X, Zhou Yy, Jiang Cm, Shi Yd. Infection, antibiotic exposure, and risk of celiac disease: A systematic review and meta-analysis. Journal of gastroenterology and hepatology. 2020;35(4):557-66.
46. van Houten CB, Naaktgeboren C, Buiteman BJ, van der Lee M, Klein A, Srugo I, et al. Antibiotic overuse in children with respiratory syncytial virus lower respiratory tract infection. The Pediatric infectious disease journal. 2018;37(11):1077-81.
47. Arnolda G, Hibbert P, Ting HP, Molloy C, Wiles L, Warwick M, et al. Assessing the appropriateness of paediatric antibiotic overuse in Australian children: a population-based sample survey. BMC pediatrics. 2020;20:1-8.
48. Melander RJ, Zurawski DV, Melander C. Narrow-spectrum antibacterial agents. Medchemcomm. 2018;9(1):12-21.
49. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. BMJ open. 2014;4(8):e005047.
50. Kumar R, Sood U, Gupta V, Singh M, Scaria J, Lal R. Recent advancements in the development of modern probiotics for restoring human gut microbiome dysbiosis. Indian Journal of Microbiology. 2019:1-14.

51. Chua MC, Ben-Amor K, Lay C, Goh AE, Chiang WC, Rao R, et al. Effect of synbiotic on the gut microbiota of cesarean delivered infants: a randomized, double-blind, multicenter study. Journal of pediatric gastroenterology and nutrition. 2017;65(1):102-6.
52. Langdon A, Crook N, Dantas G. The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. Genome medicine. 2016;8(1):39.

Figure Legends

Figure 1: PRISMA flow diagram of the study selection

Figure 2: Forest plots per gastrointestinal disorder a. IBD; b. EoE; c. CeD; d. FGID (Infantile colics and functional constipation). CC= case control study, CH = cohort study, (!) Virta 2012 only shows the results of the phenoxymethylpenicillin analyses, overall use of antibiotics was not significant

Table 1a study characteristics and association with antibiotics: Inflammatory Bowel Disease

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score

Canova, C [43]
2020
Italy
Case-control
	8.8 yrs[1]	70 / 700	33 (47%)
			
			0-12 months
			
			ATC code
				• Birth order
				• Age mother (at birth)
				• Apgar score at 1 minute
				• Birth weight
				• Education mother
				• Gestational age
				• Multiple birth
				• Season of birth
					AB first six months of life childhood onset IBD
					• Any course aOR = 1.458, 95% CI: 0.81–2.63
					• **Dose-dependent**
					  ○ 2-3 courses aOR = 2.29, 95% CI: 1.01–5.24
					  ○ >4 courses aOR = 6.25, 95% CI: 1.70–23.05
					Ab first 12 months of life childhood onset IBD
					• Any course aOR = 1.08, 95% CI 0.64–1.80
					• **Dose-dependent**: >4 courses aOR = 2.92, 95% CI: 1.32–6.46
						8/9
						High

Hviid, A [31] 2010 Denmark Cohort	3.4 yrs[1]	117 (0.02%) (50 CD and 67 UC) / 577,627	84 (72%) 0-12 months ATC code	• Age • Calendar period • Other times since use • Other types of antibiotics	Increased risk of Crohn's disease after: AB use in the last 3 months: • 3-11 months RR = 3.32, 95% CI: 1.15-9.56 • 1 year RR = 1.53, 95% CI: .15-15.46 AB use > 3 months previously before diagnosis: • 0-2 months RR = 4.19, 95% CI: 1.64-10.68	8/9 high
Kronman, M [25] 2012 United Kingdom Cohort	Exposed 4.2 yrs[2]	748 (0.07%) / 1,072,426	436 (58%) 0-12 months Systemic AB prescriptions	• Age • Chronic granulomatous disease • IBD family • Primary sclerosing cholangitis • Sex • Socioeconomic deprivation	• Exposure was associated with a 5.5-fold increased IBD risk (aHR = 5.51, 95% CI:1.66–18.28). • **Dose-dependent**: Exposure to >2 anti-anaerobic antibiotic courses was more highly associated with IBD development than exposure to 1 or 2 courses (aHR = 4.77, 95% CI: 2.13–10.68) versus (3.33, 95% CI: 1.69–6.58). • **Type-dependent** Fluoroquinolone (aHR= 2.09, 95% CI: 1.10–3.98) and metronidazole exposure (aHR = 186.25, 95% CI: 10.86–3193.65) was significantly associated with IBD.	7/9 moderate

Author Year Country Design	Age diagnosis[1]/ cohort entry[2]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Örtqvist, A [27] 2018 Sweden Cohort	2 yrs[1]	95 (0.01%) 51 IBD (CD and/or UC), 20 CD & 24 UC / 827,239	IBD 43 (84,3%) CD 16 (80%) UC 20 (83.3%) 0-12 months ATC code	• Delivery mode • Education parents • Ethnicity parents • IBD parents	No significant associations (any and PcV antibiotics) or dose–response relationship were found	8/9 high
Shaw, S [38] 2010 Canada Case-control	8.4 yrs[1]	36 / 360	21 (58%) 0-12 months ATC code	• Age • Place of residence • Sex	• One or more dispensations of antibiotics was associated with 2.9 times the odds (95% CI: 1.2-7.0, P = 0.017) of having IBD. • Stratified by IBD type, only CD was significant (OR = 5.3, 95% CI: 1.6-17.4; P = 0.006). • **Dose-dependent**: association for 2-4 (OR = 2.9, 95% CI: 1.1-7.8; P = 0.039) and 5+ (OR = 5.0, 95 % CI: 1.3-18.9; P = 0.18) prescriptions.	8/9 high
Virta, L [40] 2012 Finland Case-control	CD: 9.7 yrs[3] UC: 8.5 yrs[3]	595 (233 CD, 362 UC) / 2,380	313 (52,6%) 0-12 months ATC code	• Age • Place of residence • Chronic diseases • Sex	• Use of AB overall was not significant • **Type-dependent:** phenoxymethylpenicillin was associated with an increased risk of CD. (aOR = 2.54, 95% CI: 1.3-4.98)	8/9 high

AB: antibiotic, aHR: adjusted hazard ratio, aOR: adjusted odds ratio, ATC: Anatomical Therapeutic Chemical (ATC) Classification System, CD: Anatomical Therapeutic Chemical (ATC) Classification System, CI: Confidence interval , IBD: Inflammatory bowel disease, IRR: incidence rate ratio, HR: hazard ratio, OR: odds ratio, PcV: Phenoxymethylpenicillin and UC: Ulcerative colitis

Table 1b study characteristics and association with antibiotics: Eosinophilic Esophagitis (EoE)

Author Year Country Design	Age diagnosis [1]	Cases / Controls	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Jensen, E [33] 2013 North Carolina (USA) Case-control	Cases 11 yrs[1]	31 / 52	22 (71%) 0-12 months Motherly reported	None	Antibiotics were associated with EoE (OR= 6, 95% CI: 1.7–20.8)	4/9 weak
Jensen, E [34] 2018 North Carolina (USA) Case-control	Cases 10.6 yrs[1]	127 / 121	91 (72%) 0-12 months Motherly reported	• Education mother • NICU admission	Antibiotics were associated with EoE (aOR = 2.30, 95% CI: 1.21-4.38)	6/9 moderate
Radano, M [37] 2014 Massachusetts (USA) Case-control	Cases 3 yrs[1]	25 / 74	17 (67%) 0-12 months Parental reported	• Age • Atopy • Atopy family • Sex	Antibiotics were associated with EoE (OR = 3.61, 95% CI: 1.11-11.74; P = .03)	7/9 moderate
Slae, M [39] 2015 Canada Case-control	Cases 8.6 yrs[1]	102 / 167	60 (59%) 0-12 months Parental reported	• Breastfeeding • Birth order • Day care attendance (early) • Exposure to farm animals • Fast food consumption	Rates of antibiotic exposure were similar for cases and controls.	3/9 weak
Witmer, C [41] 2018 USA Case-control	4.2 yrs[1]	1410 / 2,820	409 (29%) 0-6 months Pharmaceutical coding	• Age • Atopy (markers) • Delivery mode • Erythema toxicum neonatorum • Feeding problems • Infantile colic • Medication exposure • Oral candidiasis • Prematurity • Prolonged rupture/ chorioamnionitis • Reflux • Sex	The association with antibiotic exposure was statistically significant (aOR = 1.31, 95% CI: 1.10–1.56).	7/9 moderate

Table 1c study characteristics and association with antibiotics: Celiac Disease (CeD)

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Bittker, S [42] 2019 USA Case-control	6.1 yrs[1]	332 / 241	237 (71%) 0-24 months Parental reported	• Age • Age mother (at birth) • Education mother • Ethnicity	• Antibiotic exposure is associated with susequent CeD (aOR = 1.133, 95% CI: 1.037–1.244; p= 0.007) • **Dose-dependent**: ORs increase with number of antibiotic courses	5/9 moderate
Canova, C [22] 2014 Italy Cohort	6.4 yrs[1]	1.227 CeD (0.6%) 866 confirmed* and 361 unconfirmed* / 203,557	336 (47%) 0-12 months ATC code	• Education mother (only in sensitivity analysis with pathological confirmed villous atrophy) • Sex • Year of birth	• Increased risk of developing CeD after at least 1 AB course (IRR = 1.24, 95% CI: 1.07-1.43), (IRR = 1.31, 95% CI: 1.10-1.56) for histopathologically confirmed CeD • **Dose-dependent**: risk increased with more AB courses (P-trend < 0.01). • **Type-dependent:** Cephalosporin use was strongly associated with CeD onset (IRR = 1.42, 95% CI: 1.18-1.73), (IRR = 1.51, 95% CI: 1.21-1.89) for histopathologically confirmed CeD. For first- and second-generation drugs: (IRR = 1.39, 95% CI: 1.11-1.76 and third- and fourth-generation drugs: IRR = 1.49, 95% CI: 1.14-1.95).	8/9 high
Kemppainen, K [24] 2017 Finland, Germany, Sweden and the USA Cohort	21.4 months[1]	783 (11,9%) / 6,558	Unknown 0-24 months Parental reported	• Breastfeeding (at 90 days of age) • CeD genotype with family • Delivery mode • Maternal AB use during pregnancy • Place of residence • Probiotic use before 90 days of age • Season of birth • Sex	• Exposure to AB was not associated with CeD. • **Dose-dependent**: 2 or more doses of macrolides within the first year of life (157 of 6558 [2.4%]) had elevated CeD risk (HR = 1.77, 95% CI: 1.18-2.66; P = .006 before but not after adjustment).	6/9 moderate

Author Year Country Design	Age diagnosis[1]/ study endpoint[3]	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Mårild, K [35] 2013 Sweden Case-control	0-2 yrs[1]	132 celiac disease / 655 12 inflammation / 60 17 normal mucosa / 85	CeD 51 (39%) Inflammation 6 (50%) 0-24 months ATC code	• Age • Education mother • Number of outpatient visits before biopsy • Sex	Exposure to AB was associated with CeD Odds ratios for prior AB use (CeD): cases 51/132 (38.6%) controls 189/655 (28.9%) (OR = 1.58, 95% CI: 1.07-2.34)	8/9 high
Myleus, A [36] 2012 Sweden Case-control	14 months[1]	373 / 581	97 (26%) 0-6 months Parental reported	• Age • Place of residence • Sex	No significantly increased risk for celiac disease (OR = 1.2, 95% CI: 0.87-1.6; P=0.27).	7/9 moderate
Sander, S [28] 2019 Denmark and Norway Cohort	Danish: 11.6 yrs[3] Norwegian: 5.4 yrs[3]	Danish: 1427 (0.12%) / 1,168,656 Norwegian: 1919 (0.36%) / 537,457	Danish: 622 (43.6%) Norwegian: 390 (20.3%) 0-12 months ATC code	• Age mother • Associated comorbidity • Birth order • Education mother • Hospitalization with infection • Season of birth • Sex • Type 1 diabetes child and/ or mother	• Exposure to systemic AB (penicillins and extended spectrum penicillins) was positively associated with diagnosed celiac disease in both cohorts (pooled aOR = 1.26, 95% CI: 1.16–1.36) • **Dose-dependent**: between number of AB courses and risk of CeD (pooled aOR for each additional dispensed AB = 1.08, 95% CI: 1.05–1.11).	9/9 high

Table 1d study characteristics and association with antibiotics: FGIDs: Infantile Colics, Functional constipation (FC), Recurrent abdominal pain (AP) and Regurgitation, functional diarrhea and infant dyschezia

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Infantile colics						
Hestbaek, L [23] 2014 Denmark Cohort	0-6 months	2183 (8,1%) / 26,983	excessive 895 (41%) extreme excessive 355 (50%) 0-6 months Motherly reported	None	At 6-month-olds, statistically significant associations between excessive crying and the use of antibiotics due to ear infections (OR = 1.47, 95% CI: 1.18–1.82) were found.	6/9 moderate
Oosterloo, B [26] 2018 The Netherlands Cohort	0-1 yr	74 (20%) / 362	33 (45%) 0-7 days Broad-spectrum AB intravenous for 2-3 days (AB2) or 7 days (AB7).	• Atopy family • Birth order • Breastfeeding • Day care attendance • Delivery mode • Education parents • Tobacco exposure	• Antibiotic treatment was an independent risk factor for infantile colic (aOR = 1.66, 95%CI: 1.00-2.77, P = .05). • Doctors-diagnosed infantile colic was higher in AB+ than in AB− (4.0% vs 0.4%; P = .014). • **Duration-dependent**: Parent-reported infantile colic was higher in AB7 compared to no antibiotics (AB-) and AB2 (24.8%, 14.4% and 14.3%, P = .048 and P = .015).	8/9 high
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	265 (41.9%) / 632	141 (22.3%) 0-7 days Hospital chart and parental report	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=1.16; 95% CI: 0.79-1.70, p=0.439)	7/9 moderate

Author Year Country Design	Age diagnosis	Cases / Controls or Cohort	Cases exposed/ Time exposure/ Recording details	Confounders for which corrected	Significant association	Quality score
Functional constipation (FC)						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	128 (26.6%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found (OR=0.77; 95%CI: 0.49-1.20, p=0.242)	7/9 moderate
Turco, R [29] 2014 Italy Cohort	0-1 yr	43 (10.7%) / 465	15 (34.8%) 0-12 months Parental reported	• Anti-inflammatory drugs or corticosteroids • Atopy & in family • Birth order • Breastfeeding & weaning • Education parents • Fever episodes before onset • FGIDs family • Nursery school age • Place of residence (>3000 citizens) • Sex • Vitamin and food supplements	No statistically significant association was found (26% vs 19%).	8/9 high
Recurrent abdominal pain (AP)						
Uusijärvi, A [30] 2014 Sweden Cohort	12 yrs	Monthly: 231 (8,7%) Weekly: 111 (4,2%) / 2,654	Monthly 1900 (71.5%) Weekly 81 (72,9%) 0-24 months Parental reported	• Asthma at 12 years of age • Asthma at one year • Sex	Stratified analyses showed that girls, who received antibiotics during both the first and the second year of life, had an increased risk of AP at 12 years (OR = 1.65, 95% CI: 1.09–2.49).	5/9 moderate
Regurgitation, functional diarrhea and infant dyschezia						
Salvatore, S [32] 2019 Italy Cohort	0-1 yr	Regurgitation:236 (37.3%) Functional diarrhea: 24 (3.8%) Infant dyschezia: 199 (31.5%) / 632	141 (22.3%) 0-7 days Hospital charts and parental reported	• Birth weight • Breastfeeding (at 1 month of life) • Delivery mode • Duration of hospitalization at birth • Gestational age • Neonatal complications	No association was found for regurgitation (OR=1.29, 95%CI: 0.88-1.90, p=0.190), functional diarrhea (OR=0.90, 95%CI: 0.33-2.45, p=0.835), or infant dyschezia (OR=1.29, 95%CI: 0.87-1.93, p=0.205).	7/9 moderate

Table 2 quality assessment

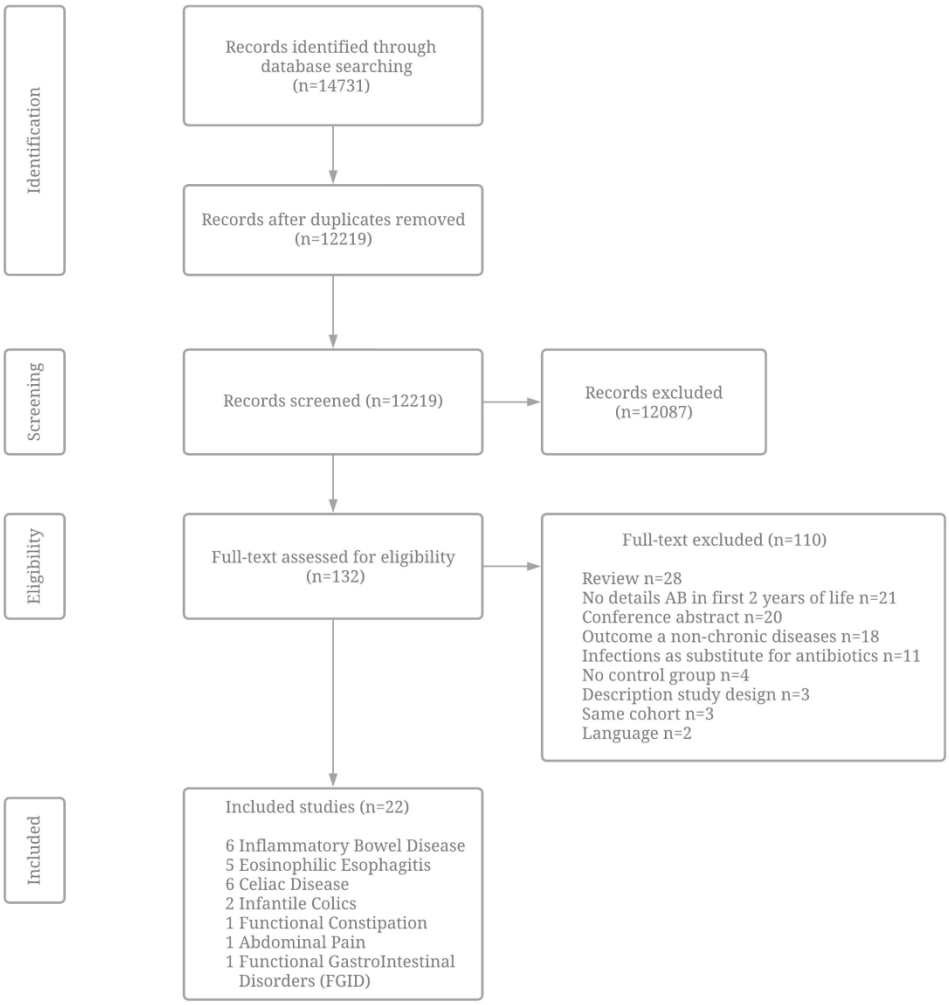
	Selection				Comparability		Outcome / Exposure			Score
	1.	2.	3.	4.	5.	6.	7.	8.	9.	
Cohort studies*	Representativeness	Selection	Exposure	Outcome	Most important	Second important	Assessment	Duration of follow-up	Adequacy follow-up	
Canova [22]	*	*	*	*		*	*	*	*	8/9
Hestbaek [23]	*	*	*	*				*	*	6/9
Hviid [31]	*	*	*	*		*	*	*	*	8/9
Kemppainen [24]		*		*	*	*	*	*		6/9
Kronman [25]		*	*	*	*	*	*	*		7/9
Oosterloo [26]	*	*	*	*	*	*	*	*		8/9
Örtqvist [27]	*	*	*	*	*	*	*		*	8/9
Salvatore [32]	*	*	*	*		*	*	*		7/9
Sander [28]	*	*	*	*	*	*	*	*	*	9/9
Turco [29]	*	*	*	*	*	*		*	*	8/9
Uusijärvi [30]	*	*		*				*	*	5/9
Case-Control studies**	Case definition	Cases	Controls	Definition controls	Most important	Second important	Exposure	Ascertainment	Non-Response rate	Score
Bittker [42]			*	*	*			*	*	5/9
Canova [43]	*	*	*	*		*	*	*	*	8/9
Jensen [33]	*	*		*				*		4/9
Jensen [34]	*	*	*	*				*	*	6/9
Mårild [35]	*	*	*	*		*	*	*	*	8/9
Myleus [36]	*	*	*	*		*		*	*	7/9
Radano [37]	*	*		*	*	*		*	*	7/9
Shaw [38]	*	*	*	*		*	*	*	*	8/9
Slae [39]	*			*				*		3/9
Virta [40]	*	*	*	*		*	*	*	*	8/9
Witmer [41]		*	*	*	*	*		*	*	7/9

*Cohort studies: 1. Representativeness of the exposed cohort, 2. Selection of the non-exposed cohort, 3. Ascertainment of exposure, 4. Demonstration that the outcome of interest was not present at start of the study, 5. Comparability of cohorts on the basis of the design or analysis most important factor, 6. Comparability of cohorts on the basis of the design or analysis second important factor, 7. Assessment of outcome 8. Was follow-up long enough for outcomes to occur and 9. Adequacy of follow up of cohort.

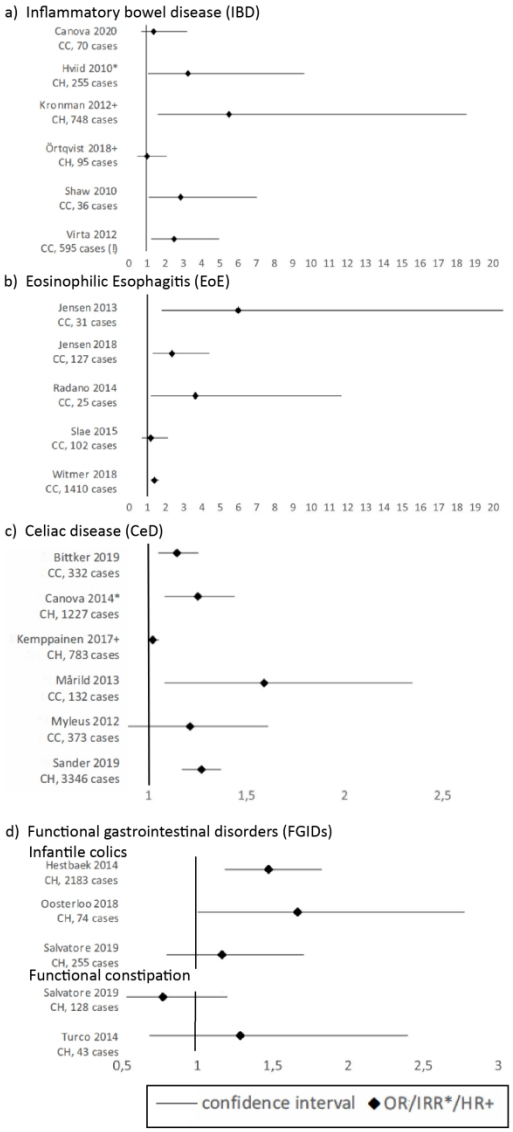
**Case- Control studies: 1. Is the case definition adequate? 2. Representativeness of the cases, 3. Selection of controls, 4. Definition of controls, 5. Comparability of cases and controls on the basis of the design or analysis most important factor, 6. Comparability of cases and controls on the basis of the design or analysis second important factor, 7. Ascertainment of exposure, 8. Same method of ascertainment for cases and controls and 9. Non-Response rate

Comparability: Most important confounder: IBD and CeD: presence of IBD/ CeD in 1ste degree family member, EoE: sex, colics: atopy child and/or family, functional constipation: maternal education/social economic status, abdominal pain: lactose intolerance/ cow's milk allergy.

Comparability: Second important confounder: IBD: ethnicity and/or age, EoE: presence of other atopic diseases and/or ethnicity, CeD: sex and/ or season of birth and/or the presence of other autoimmune diseases, colics: presence of GERD and/or type of feeding and/or being a first child, functional constipation: sex and/ or age, abdominal pain: anxiety/ depression/ stress in the child and/or the parents.



212x223mm (300 x 300 DPI)



210x297mm (300 x 300 DPI)

Supplementary Table 1 search strategy

	Ovid MEDLINE(R) ALL <1946 to 2020 June 08> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp infant death/ or infant/	788526
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kf,ti.	686417
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	655139
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1066665
5	or/1-4 [Ia - children 0-4 yrs]	2564903
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kf,ti. [Ib - children 0-4 yrs]	5357
7	Gentamycins/	18247
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kf,ti.	27205
9	or/7-8 [IIa first week exclusive use]	32706
10	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	399419
11	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kf,ti. [IIc]	48465
12	(sepsis and infant).hw.	9982
13	(sepsis adj2 early).ab,kf,ti.	1919
14	or/12-13 [IId]	11418
15	(childhood disease? and (risk or environmental factor?)).ab,kf,ti. [IIe]	360

16	exp inflammatory bowel disease/ or abdominal pain/ or aerophagy/ or dyspepsia/ or constipation/ or celiac disease/ or appendicitis/ or gastritis/ or enteritis/ or exp diarrhea/ or colic/ or Eosinophilic Esophagitis/ or Gastroesophageal Reflux/ or esophageal stenosis/	266125
17	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kf,ti.	305723
18	Pyloric Stenosis, Hypertrophic/	654
19	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kf,ti.	1513
20	18 or 19	1622
21	limit 20 to yr="2015-current"	184
22	or/16-17,21 [outcomes]	424883
23	follow-up studies/ or longitudinal studies/ or retrospective studies/	1441183
24	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kf,ti.	3775119
25	(case control or cohort study or (risk and review)).mp.	1032965
26	observational study.pt.	80055
27	or/23-26 [study design]	4954421
28	5 or 6 [Ia+b - children 0-4 yrs]	2566952
29	28 and (10 or 11) and 22 and 27	2707
30	and/9,28	4170
31	and/14,22	319
32	or/15,29-31	7477
33	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kf,ti. [NOTing out green]	1105473
34	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kf,ti. [NOTing out blue]	505926
35	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kf,ti. [NOTing out red]	803243

36	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kf,ti. [NOTing out yellow]	1949849
37	or/33-36	4123179
38	32 not 37 [NOTing out]	5238
39	animals/ not humans/	4672110
40	38 not 39	5096
41	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kf,ti.	0
42	40 or 41	5096
	Ovid Embase Classic+Embase <1947 to 2020 June 06> **Search date: 9 June 2020**	
#	**Searches**	**Results**
1	exp *infant/ or *infancy/ or infant.hw.	798854
2	(early life or infant? or infancy or toddler? or preschool* or (early adj5 (childhood or child or children or p?ediatric?)) or minors or baby or babies or kindergarten or newborn?).ab,kw,ti.	875279
3	(("0" or "1" or "2" or "3" or "4") adj1 (age? or yr? or year?)).ab.	1051740
4	(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") adj1 month?).ab.	1708558
5	or/1-4 [Ia - children 0-4 yrs]	3541363
6	((pediatric or infantile or juvenile) adj1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis)).ab,kw,ti. [Ib - children 0-4 yrs]	7292
7	*Gentamicin/	35017
8	(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3").ab,kw,ti.	36468

9	"1403-66-3".rn.	104829
10	or/7-9 [IIa first week exclusive use]	113443
11	(antibiotic? or erythromycin or metoclopramide).mp. [IIb]	870330
12	((meningitis or infection? or inflammat* or pneumonia or pyelonephritis) adj3 (pe?diatric or early or infant* or newborn?)).ab,kw,ti. [IIc]	65283
13	(sepsis and infant).hw.	11891
14	(sepsis adj2 early).ab,kw,ti.	2988
15	or/13-14 [IId]	14425
16	(childhood disease? and (risk or environmental factor?)).ab,kw,ti. [IIe]	498
17	exp *inflammatory bowel disease/ or *abdominal pain/ or *aerophagia/ or *dyspepsia/ or exp *constipation/ or *celiac disease/ or *appendicitis/ or *gastritis/ or *enteritis/ or *diarrhea/ or *infantile diarrhea/ or *colic/ or *infantile colic/ or *Eosinophilic Esophagitis/ or *Gastroesophageal Reflux/ or *esophageal stenosis/	267207
18	(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture).ab,kw,ti.	493146
19	*hypertrophic pylorus stenosis/	1263
20	(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis).ab,kw,ti.	1940
21	19 or 20	2152
22	limit 21 to yr="2015-current"	231
23	or/17-18,22 [outcomes]	586712
24	follow up/ or longitudinal study/ or retrospective study/	2412789
25	(prognos* or predict* or course or follow-up or followup or longitudinal or retrospective or (observational adj2 stud*)).ab,kw,ti.	5528713
26	observational study.kw,ti.	27665
27	(case control or cohort study or (risk and review)).mp.	1211338
28	or/24-27 [study design]	6774290
29	5 or 6 [Ia+b - children 0-4 yrs]	3545044
30	29 and (11 or 12) and 23 and 28	5878

31	and/10,28-29	5192
32	and/15,23	576
33	or/16,30-32	11847
34	(Meniere or Pouchitis or Total hip or Arthroplasty or Whipple or peritoneal dialysis or Bone? or Vertigo? or IUD? or PMMA? or SEM? or debridement or surgical site infection or ssi).ab,kw,ti. [NOTing out green]	1532636
35	(Helicobactor pylori or HIV or Newborn calf or Measle or Varicella or RCT? or Pig).ab,kw,ti. [NOTing out blue]	664053
36	(nalidixic acid? or molecular epidemiology or vitro activity or Strain? or Outbreak?).ab,kw,ti. [NOTing out red]	962439
37	(pharmacokinetic parameter or Rat or premature baby or vlbw or billirubin? or chorioamnionitis? or sisomicin or clearance or therapeutic drug monitoring or serum half life or dosage interval or experiment?).ab,kw,ti. [NOTing out yellow]	2455192
38	or/34-37	5285640
39	33 not 38 [NOTing out]	9118
40	(animal/ or animal experiment/ or animal model/ or nonhuman/) not human/	6454629
41	39 not 40	8980
42	("Twin cohort for the study of (pre)clinical Inflammatory Bowel Disease in the Netherlands" or NTR6681).ab,kw,ti.	0
43	NTR6681.cn.	0
44	or/41-43	8980
	Web of Science Core Collection: **- SCI-EXPANDED 1975-present** **- SSCI 1975 - present** **-A&HCI 1975 - present** **- ESCI 2015 - present** **Search date: 9 June 2020**	
#	**Searches**	**results**
#1	TS=(early life or infant or infancy or toddler or preschool or (early N4 (childhood or child or children or pediatric)) or minors or baby or babies or kindergarten or newborn)	1085229
#2	AB=(("0" or "1" or "2" or "3" or "4") N1 (age? or yr? or year?))	1805
#3	AB=(("0" or "1" or "2" or "3" or "4" or "5" or "6" or "7" or "8" or "9" or "10" or "11" or "12" or "13" or "14" or "15" or "16" or "17" or "18" or "19" or "20" or "21" or "22" or "23" or "24") N1 month?)	1183

#4	TS=((pediatric or infantile or juvenile) N1 (functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator* bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitis or gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or colic or abdominal cramp? or (Eosinophilic AND Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture or Hypertrophic pyloric stenosis))	3
#5	#4 OR #3 OR #2 OR #1	1087634
#6	TS=antibiotic	334292
#7	#6 AND #5	15781
#8	TS=(Alcomicin or Bristagen or G-Mycin or Gentacidin or Gentafair or Gentamar or Jenamicin or Ocu-Mycin or Spectro-Genta or U-gencin or U-Liang or Gentamycin? or Garamycin or Gentacycol or Gentavet or Genticin or G Myticin or GMyticin or Gentamicin or "1403-66-3")	25466
#9	#8 OR #7	40687
#10	TS=(functional gastrointestinal disorders or FIGD? or irritable bowel syndrome or IBS or spastic colon or inflammator bowel or IBD or Colitis ulcerosa or ulcerative colitis or proctocolitis or colitis gravis or crohn or abdominal pain? or aerophag* or dyspepsia or indigestion or constipat* or celiac disease or gluten or appendicitisor gastritis or gastrides or enteritis or diarrh?ea? or loose stool? or liquid stool? or liquid feces or fluid stool? or infantile colic or abdominal cramp? or (Eosinophilic adj2 Esophagitis) or Gastric Acid Reflux or Gastro Esophageal Reflux or Gastroesophageal Reflux or GERD or Esophageal Reflux or Gastro oesophageal Reflux or esophageal stenos* or esophageal stricture)	252018
#11	TS=(Hypertrophic pyloric stenosis or hypertrophic pylorus stenosis)	1233
#12	#11 OR #10	253145
#13	#12 AND #9	655

Supplementary table 2 Confounders in the quality assessment

Study outcome	Most important	Second important
IBD	Presence of IBD in first degree family members	Ethnicity and/or age
EoE	Sex	Presence of other atopic diseases and/or ethnicity
CeD	Presence of CeD in first degree family member	Sex and/or season of birth and/or the presence of other autoimmune diseases
Colics	Presence of atopy in first degree family members	Presence of GERD and/or type of feeding and/or being a first child
Functional constipation	Maternal education/social economic status	Sex and/or age
Abdominal pain	Lactose intolerance/cow's milk allergy	Anxiety/depression/stress in the child and/or the parents