

Enterohepatic *Helicobacter* Species as a Potential Causative Factor in Inflammatory Bowel Disease

A Meta-Analysis

Qiao Yu, MD, Shenghong Zhang, PhD, Li Li, MD, Lishou Xiong, PhD, Kang Chao, PhD, Bihui Zhong, PhD, Yuwen Li, MD, Huiling Wang, MD, and Minhu Chen, PhD

Abstract: The *Helicobacter* species in the gut microbiota comprise *Helicobacter pylori* (*H pylori*) and enterohepatic *Helicobacter* species (EHS), which can colonize the intestinal mucosa. However, it is unclear whether EHS are associated with inflammatory bowel disease (IBD). Therefore, we conducted this meta-analysis to examine the association between EHS and IBD.

PubMed, Scopus, Cochrane Library, and Web of Science databases, as well as abstracts from conference proceedings were searched to identify studies that used polymerase chain reaction to detect *Helicobacter* species in intestinal samples from patients with IBD.

After screening, we carefully reviewed 20 of the 2955 identified studies, and performed a meta-analysis of the findings from 14 studies (11 adult studies and 3 pediatric studies) using STATA v12.0. These studies evaluated 1407 individuals, including 433 patients with Crohn's disease, 306 patients with ulcerative colitis, and 668 controls. The prevalence of *Helicobacter* species was higher among the patients with IBD, compared to that among the controls, which corresponded to a pooled risk ratio (RR) of 1.59 (95% confidence interval [CI]: 1.12–2.27). The RRs for adult and pediatric patients with IBD were 1.61 (95% CI: 1.03–2.52) and 1.76 (95% CI: 1.17–2.64), respectively. Compared to the controls, the patients with IBD tended to have a higher prevalence of EHS in the intestinal mucosa (RR: 2.01, 95% CI: 1.36–2.98), although the prevalence of *H pylori* was not significantly higher (RR: 1.22, 95% CI: 0.77–1.95). Compared to the controls, the RRs for EHS in patients with Crohn's disease and ulcerative colitis were 1.72 (95% CI: 1.20–2.47) and 3.27 (95% CI: 0.93–11.44), respectively.

It appears that EHS was associated with IBD, while intestinal *H pylori* infection was not significantly associated with IBD. Further studies are needed to determine the involvement of EHS in the microbiological etiology of IBD.

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From the Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, P.R. China.

Correspondence: Minhu Chen, Department of Gastroenterology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, P.R. China (e-mail: chenminhu@vip.163.com).

QY and SZ have contributed equally to the study.

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Abbreviations: CD = Crohn's disease, CI = confidence interval, EHS = enterohepatic *Helicobacter* species, *H pylori* = *Helicobacter pylori*, IBD = inflammatory bowel disease, MeSH = Medical Subject Heading, NOS = Newcastle–Ottawa Scale, PCR = polymerase chain reaction, RR = risk ratio, UC = ulcerative colitis.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic idiopathic conditions that are related to gastrointestinal tract inflammation, which primarily involve Crohn's disease (CD) and ulcerative colitis (UC). Unfortunately, IBD is a growing health concern for patients around the world,¹ and its etiology remains unknown. According to existing hypotheses, gastrointestinal microorganisms might trigger a deregulated immune response in genetically predisposed individuals, which might result in chronic inflammation.² A group of spiral or helical microorganisms that are called enterohepatic *Helicobacter* species (EHS) have also recently been implicated as potential pathogens, as they can colonize the mucus layer within the gastrointestinal epithelium.^{3,4} These microorganisms induce an IBD-like colitis in an immunocompromised murine model,^{5–7} and may be involved in the pathogenesis of IBD.⁸

In 2005, 2 Australian scientists were awarded the Nobel Prize in Physiology and Medicine for their discovery that *Helicobacter pylori* (*H pylori*) was the direct causative agent of gastritis and peptic ulcer disease.⁹ A recent study has also reported an inverse association between gastric *H pylori* infection and IBD, which was due to a down-regulation of the pro-inflammatory response.¹⁰ Several meta-analyses have also suggested that *H pylori* infection provides a protective effect against IBD,^{11–13} including among Asian patients with IBD. Nevertheless, those meta-analyses only evaluated serological testing for *H pylori*-specific markers, and their data only reflect the gastric prevalence of *H pylori*. The role of other *Helicobacter* species in IBD, especially that of EHS, has not been sufficiently evaluated.

Thus, we performed the first meta-analysis to investigate the prevalence of intestinal *Helicobacter* species in specimens from patients with IBD. Using that information, we aimed to explore the association between intestinal EHS and IBD, in order to help our understanding of the role of EHS in the etiology of IBD.

METHODS

Search Strategy and Information Sources

This study's design was approved by the Medical Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University. The study was performed in accordance with the

guidelines for meta-analyses of observational studies in epidemiology¹⁴ and the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines.¹⁵ To detect relevant studies, we searched the PubMed, Scopus, Cochrane Library, and Web of Science databases for all relevant articles that were published before October 1, 2014. Additional studies were identified by searching abstracts from the Digestive Disease Week and European Crohn's and Colitis Organization annual meetings. The search strategy employed various Medical Subject Heading (MeSH) terms, as well as free text words to increase the search's sensitivity: "inflammatory bowel disease" (MeSH), "Crohn's disease" (MeSH), "ulcerative colitis" (MeSH), "*Helicobacter*" (MeSH), "gastrospirillum" (free text), "*Helicobacter* species" (MeSH), "*H pylori*" (MeSH), "*Helicobacter mustelae*" (free text), "*Helicobacter hepaticus*" (MeSH), "*Helicobacter heilmannii*" (free text), and "*Helicobacter felis*" (free text) (Supplementary File A). All references within each publication were carefully and manually evaluated to avoid omitting any pertinent study, and any additional studies were verified via email contact with study's investigators.

Eligibility Criteria

Studies were included in our screening if they focused on all *Helicobacter* species and IBD, compromising both intestinal EHS and *H pylori*. The studies were screened using the following criteria: peer reviewed articles that were published in English (to facilitate the review and evaluation of the full text), the study received ethical approval and all participants provided informed consent, the IBD and control groups were matched in terms of age, sex, and study area, *Helicobacter* species were identified via polymerase chain reaction (PCR) using biopsy specimens that were obtained from both the small and large intestine mucosa, and measurable outcomes were fully reported. Animal studies and single-case reports were excluded. Two reviewers (QY and LL) independently assessed the eligibility of each study, and disagreements were resolved via consensus after discussion with the senior investigators (SZ and MC). If the results of a study were reported in multiple publications (with possible overlap), only the most recent and informative publication was included in the analysis.

Quality Assessment

The Newcastle–Ottawa Scale (NOS)¹⁶ was used to assess the quality of the nonrandomized studies (details regarding this evaluation are shown in the Supplementary Material). A score of ≥ 6 stars indicates a high-quality study.¹⁷ Scoring for each study was performed independently by 2 investigators (QY and LL), and kappa statistics were used to assess the inter-rater agreement.¹⁸

Data Extraction and Assessment of the Risk Bias

Four investigators (QY, LL, YL, and HW) independently extracted the data, and agreement was reached via consensus for all items when a discrepancy was found. For each study, we recorded the author, year of publication, geographical location, patient enrollment, sample size, type of IBD, age, sex distribution, disease location, PCR method, and type of *Helicobacter* species (Table 1). Heterogeneity between the studies was assessed using the I^2 test, and an I^2 of $\geq 50\%$ indicated the presence of at least moderate heterogeneity.¹⁹ The presence of

publication bias and related bias were evaluated using funnel plots and Egger's test.²⁰

Statistical Analysis

We constructed 2×2 tables of the numbers of positive cases and total cases for both the IBD and control groups in the included studies. Pooled risk ratios (RRs) and the associated 95% confidence intervals (CIs) were estimated for the binary variables. The priori decision to use a fixed- or random-effects model (DerSimonian and Laird) was guided by the presence or absence of heterogeneity.^{21,22} STATA software (version 12.0; STATA Corporation, College Station, TX) was used for the main and subgroup meta-analyses. Subgroup analyses were performed according to patient age (adults vs children) and disease classification (UC vs CD) for *H pylori* and EHS. A P value of < 0.05 was considered statistically significant.

RESULTS

Eligible Studies

The process for the study selection is shown in Figure 1. After applying the initial search strategy, we identified 2955 citations, and 20 potential studies were subjected to a detailed review. Among these studies, we excluded 3 studies for not using PCR to detect *Helicobacter* species (the culture method was used).^{23–25} Three additional studies were excluded for not reporting useful results to calculate the RR: 1 study included patients with other bowel diseases and did not report the exact number of patients with IBD,²⁶ a 2nd study was excluded because the author did not reply to our enquiry email,²⁷ and a 3rd article focused on T-cell clones instead of *Helicobacter* strains.²⁸ Our meta-analysis included 14 studies that evaluated 1407 individuals. However, 2 studies did not find *H pylori* or EHS in the intestine samples from their patients with IBD or their controls.^{29,30} Therefore, only 12 studies^{31–42} were displayed in the final forest plot for analysis. The NOS quality of the included studies was 3 to 8 stars, and the inter-rater reliability was good ($\kappa = 0.70$, $P = 0.006$).

Study Characteristics

The detailed characteristics of the 14 studies are shown in Table 1. Of 14 studies, 12 used consecutive participant recruitment, the remainders recruited participants randomly. Eleven studies included adult patients, while 3 studies included pediatric patients. Eight studies (8/14) included patients with both CD and UC; 4 studies only evaluated CD; and the other 2 studies only evaluated UC. Patients who were enrolled in the studies were diagnosed using the standard clinical diagnostic criteria for IBD,⁴³ as well as radiography, endoscopy, and pathology findings. Ten studies used non-IBD patients as the control group; 3 studies used normal individuals; 2 studies used patients with culture-negative diarrhea or polyps; and only 1 study used patients with irritable bowel syndrome. The median year of publication was 2005 (range: 2004–2010), and the median sample size was 93 participants (range: 57–143). The median ages at IBD diagnosis for the patients with CD and UC were 38.8 years (range: 20.6–40.5 years) and 49.4 years (range: 22.9–56.8 years), respectively. The median age of the control patients was 49.4 years (range: 22.9–56.8 years). The studies included populations from various countries, including the UK (3 studies), Brazil (2 studies), Germany (2 studies), Sweden, France, Italy, the Netherlands, Canada, Australia, and China. All of the included studies were performed prospectively

TABLE 1. Basic Characteristics of 14 Case–Control Studies That Used PCR to Detect Intestinal Positivity of *Helicobacter* Species in Patients With Inflammatory Bowel Disease and in Controls

| Author | Location | Patient Enrollment | Study Size | Disease | Mean Age, yr | | Female, % | | Specimen | Quality Score* (Newcastle–Ottawa Scale) | Method of Hp Detection | Primers Used for PCR | Organism Identified |
|-------------------------------|-----------------|--------------------|------------|-----------|-----------------|----------|---------------|----------|---|--|------------------------|---|---------------------|
| | | | | | Cases (CD/UC) | Controls | Cases (CD/UC) | Controls | | | | | |
| Tiveljung et al ³¹ | Sweden | Consecutive | 22 | CD | 36 | 50 | 0.27 | 0.36 | Ileal mucosa biopsies | 4 | 16S rDNA PCR | HPV4 and HPV9 | EHS |
| Bell et al ²⁹ | UK | Consecutive | 30 | CD and UC | 38.8/55.2 | 51.3 | 0.33/0.36 | 0.5 | Colonic biopsies | 5 | 16S rRNA PCR | vac3624F/vac3853R, Hp1/Hp2, and HeilF/HeilR | EHS and Hp* |
| Huijsdens et al ³⁰ | The Netherlands | Consecutive | 81 | CD and UC | NR [†] | NR | NR | NR | Colonic biopsies | 3 | 16S rRNA PCR | Only sequence provided | EHS and Hp |
| Bohr et al ³² | Germany | Consecutive | 66 | CD and UC | 41/51.7 | 65.1 | NR | NR | Terminal ileal and colonic biopsies | 5 | 16S rDNA PCR | C97 and H2 | EHS and Hp |
| Grehan et al ³³ | Australia | Consecutive | 70 | CD and UC | 40/49 | 61 | 0.53/0.5 | 0.47 | Ileal, cecal colonic, and rectal biopsies | 5 | 16S rDNA nested PCR | H276f and H676r | Hp |
| Oliveira et al ⁴² | Brazil | Consecutive | 116 | UC | 38.9 | 49.4 | 0.86 | 0.73 | Intestinal mucosa biopsies | 7 | 16S rDNA nested PCR | Outer C70 and C37; inner C97 and C98, HP1 and HP2 | Hp |
| Streutker et al ³⁴ | Canada | Randomized | 85 | CD and UC | 38.8/43.3 | 42 | 0.44/0.42 | 0.52 | Colonic biopsies | 4 | 16S rRNA PCR | Only sequence provided | Hp |
| Oliveira et al ³⁶ | Brazil | Consecutive | 117 | CD | 40.9 | 49.4 | 0.53 | 0.73 | Ileal, colonic, and rectal biopsies | 7 | 16S rRNA PCR | Outer C70 and C37; inner C97 and C98, HP1 and HP2 | Hp |
| Zhang et al ³⁵ | Australia | Consecutive | 16 | CD and UC | 3–15 | 3–15 | 0.24/0.24 | 0.24 | Colonic biopsies | 5 | 16S rDNA PCR | H276f and H676r | EHS |
| Laharie et al ³⁷ | France | Consecutive | 165 | CD | 37.6 | 52.5 | 0.589 | 0.58 | Anastomosis and colonic biopsies | 8 | 16S rRNA PCR | C97–C98 | EHS and Hp |
| Kaakoush et al ³⁸ | Australia | Consecutive | 179 | CD | 11.4 | 9.4 | 0.35 | 0.37 | Intestinal biopsies | 6 | 16S rRNA nested PCR | H276f and H676r | EHS and Hp |
| Thomson et al ³⁹ | UK and USA | Consecutive | 136 | UC | 42 | 63 | 0.54 | 0.41 | Colonic biopsies | 6 | 16S rRNA PCR | C05, C97, and C98 | EHS and Hp |
| Zhang et al ⁴⁰ | China | Randomized | 240 | CD and UC | 29.2/42.3 | 35.1 | 0.30/0.36 | 0.36 | Intestinal biopsies | 7 | 16S rRNA PCR | H276f and H676r, C97 and C98 | Hp |
| Hansen et al ⁴¹ | UK | Consecutive | 100 | CD and UC | 11.9 | 10.6 | 0.31/0.308 | 0.21 | Colonic biopsies | 5 | 16S rRNA PCR | C05/C97 and C98/1067R | EHS |

CD = Crohn's disease, EHS = enterohepatic *Helicobacter* species, Hp = *Helicobacter pylori*, NR = not reported, PCR = polymerase chain reaction, UC = ulcerative colitis.

* Studies with 6 stars or more than 6 stars of 8 in quality score were considered as high-quality; <6 stars of 8 were considered as low-quality.

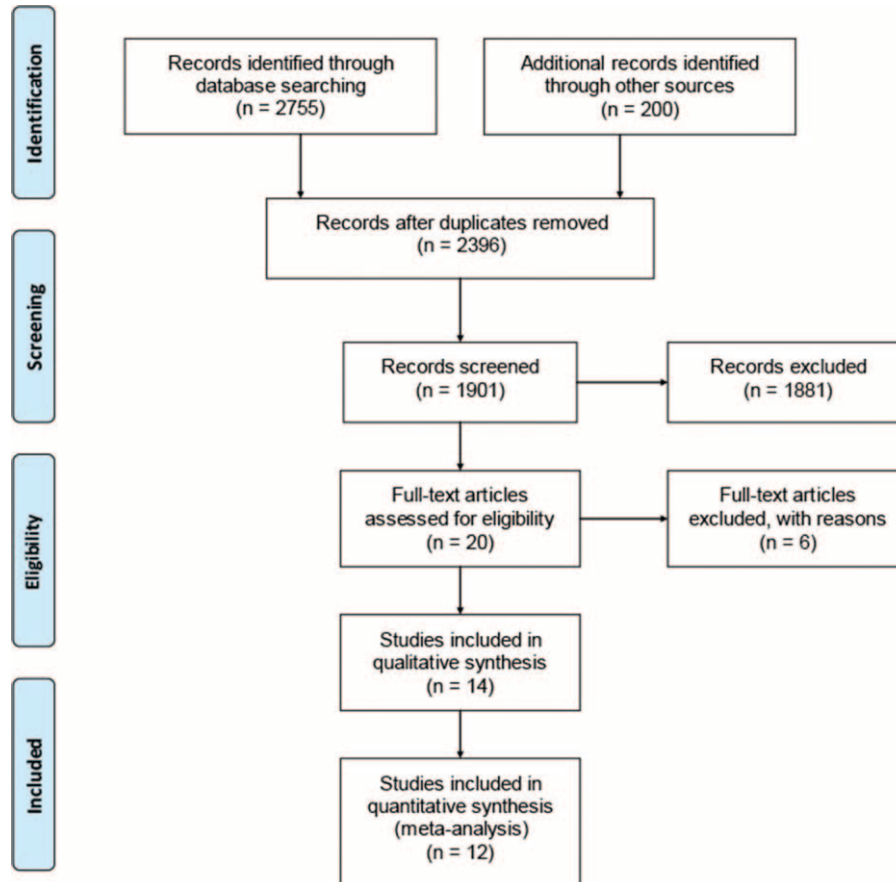


FIGURE 1. Flowchart of the article selection process.

and used PCR to detect 16S rRNA or rDNA from *Helicobacter* species. In 9 studies, PCR products from the intestinal mucosa of patients with IBD were highly similar to *H pylori*, and 7 studies identified EHS via PCR and sequencing.

Analysis of the Association Between All Intestinal *Helicobacter* Species and IBD

Both *H pylori* and EHS were detected in the intestines of the patients with IBD. Given the evidence of moderate heterogeneity among the included studies ($I^2 = 61.1\%$), we used a random-effects model to calculate the RR. The overall prevalence of *Helicobacter* species was higher among the patients with IBD, compared to that among the controls, which corresponded to an overall pooled RR of 1.59 (95% CI: 1.12–2.27) (Fig. 2). We also analyzed the adult and pediatric studies separately, and observed a higher prevalence of *Helicobacter* species among both the adult patients with IBD (9 studies; RR: 1.61, 95% CI: 1.03–2.52) and the pediatric patients with IBD (3 studies; RR: 1.76, 95% CI: 1.17–2.64). There was significant heterogeneity among the studies of adult patients ($I^2 = 66.7\%$), although no heterogeneity was observed for the studies of pediatric patients ($I^2 = 0.0\%$).

The results from the 2 studies that were not included in the forest plot corresponded to 2 zeros in the 2×2 table, which prevented us from calculating an RR. Furthermore, the analysis

of the funnel plot indicated a possible publication bias in these studies (Fig. 3),^{34,36} which fell outside of the funnel plot due to the low number of controls that were positive for *Helicobacter* species (0 and 1 cases, respectively).

Analysis of the Association Between Intestinal *H Pylori* and IBD

Nine studies of 1174 individuals (598 IBD cases) evaluated the prevalence of intestinal *H pylori* in patients with IBD. However, compared to the controls, the RR for intestinal *H pylori* infection was 1.22 (95% CI: 0.77–1.95), and this result was not statistically significant with the presence of moderate heterogeneity ($I^2 = 59.9\%$) (Fig. 4). Figure 4 also shows the subgroup analyses for *H pylori* infection in patients with CD or UC, compared to that in the controls (CD RR: 1.30, 95% CI: 0.74–2.27; UC RR: 1.13; 95% CI: 0.44–2.90). However, significant heterogeneity was also observed in these studies (CD: $I^2 = 57.5\%$; UC: $I^2 = 68.7\%$).

Analysis of the Association Between Intestinal EHS and IBD

As shown in Figure 5, 7 studies of 580 individuals (281 IBD cases) evaluated the prevalence of intestinal EHS. The IBD cases were more likely to have EHS infection, compared to the controls (RR: 2.01, 95% CI: 1.36–2.98), and no significant

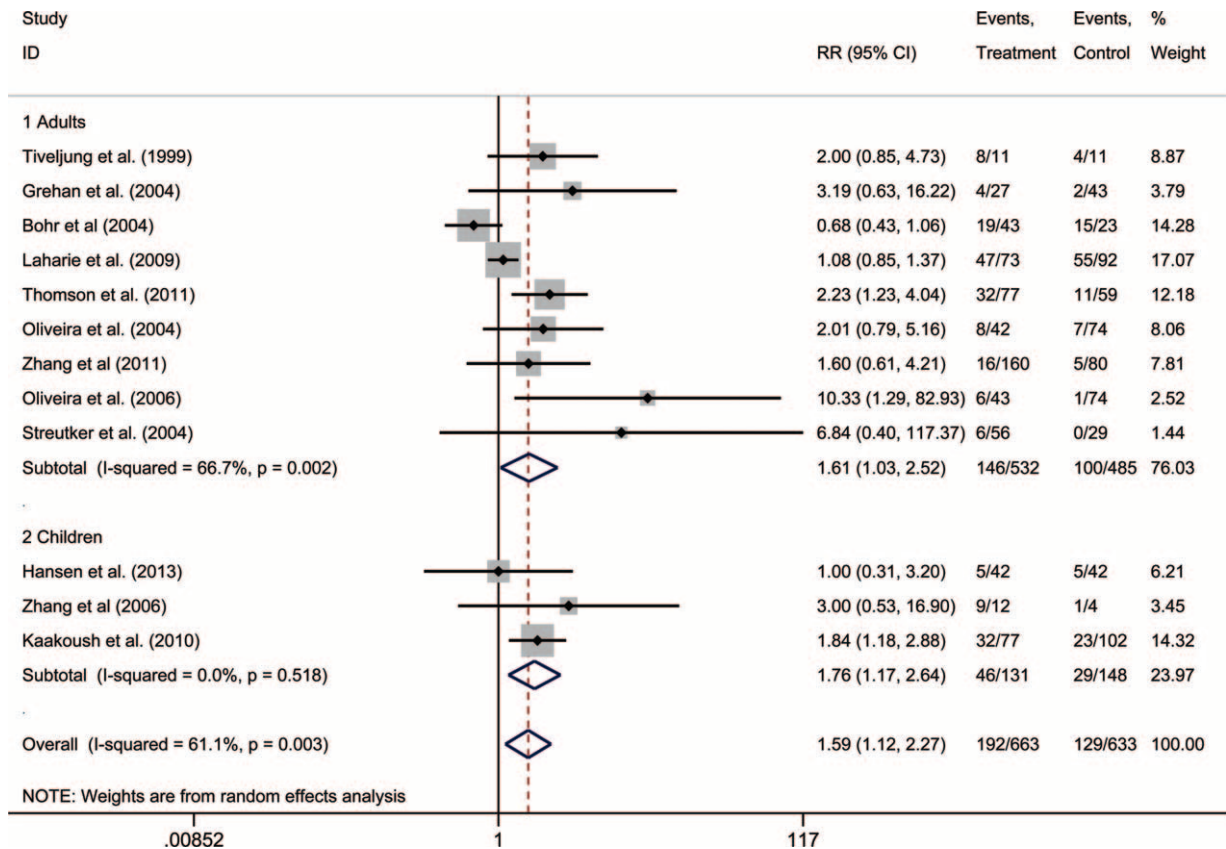


FIGURE 2. Forest plots of adult and pediatric studies that used polymerase chain reaction to detect *Helicobacter* species in patients with inflammatory bowel disease.

heterogeneity was observed ($I^2 = 15.9\%$). Furthermore, the EHS studies exhibited a relatively symmetrical funnel plot, which suggested that publication bias was not present.

We also performed subgroup analyses for the prevalence of EHS in patients with CD and UC. Compared to the controls, the patients with CD had a significantly higher prevalence of EHS (RR: 1.72, 95% CI: 1.20–2.47), and no heterogeneity was observed ($I^2 = 0.0\%$) (Fig. 5). In contrast, patients with UC did not exhibit a significantly higher prevalence of EHS, compared

to the controls (RR: 3.27, 95% CI: 0.93–11.44), and a moderate level of heterogeneity was observed ($I^2 = 51.0\%$).

DISCUSSION

The present meta-analysis included 14 case–control studies from 9 different countries that investigated the association between intestinal *Helicobacter* species and IBD. The majority of the studies (11/14) reported an RR of >1, although the RR was only significant in 3 studies, and 1 study reported a nonsignificant RR of <1. No significant association between intestinal *H pylori* and IBD was found. The results of our meta-analysis suggest that non-*H pylori* EHS infection might be associated with IBD, although publication bias and a moderate level of heterogeneity may confound that interpretation.

There are several potential sources of the heterogeneity for the present meta-analysis. First, different PCR primers were used in various studies. Most included studies (9/14) used the C97–C98 pair of primers,^{32,36,37,39,41,42} as well as 1 excluded study,²⁶ although 4 studies used the H276f–H676r primers.^{33,35,38,40} Furthermore, Tiveljung et al.³¹ used the HPV4–HPV9 pair of primer, which can only identify EHS (not *H pylori*), and reported a higher prevalence of EHS in IBD (72.7%) compared to the controls (36.4%). Second, age may have confounded several studies, especially in the selection of the control groups. Third, we included studies from 9 different countries, with most of the studies being performed in Europe, America, and Australia; only 1 study was performed in Asia (China). However, geographical and population variations may

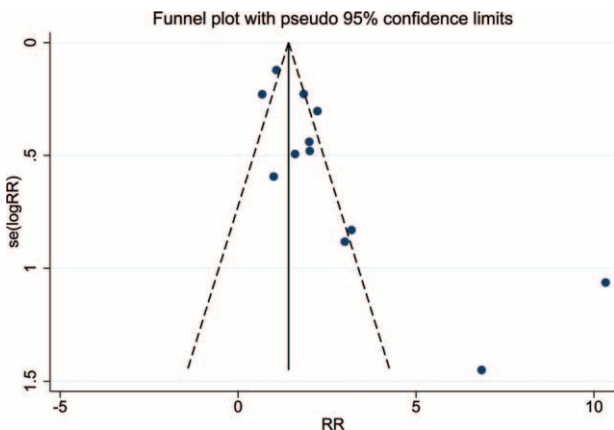


FIGURE 3. Funnel plot showing the publication bias in the included studies. Each solid circle represents one study.

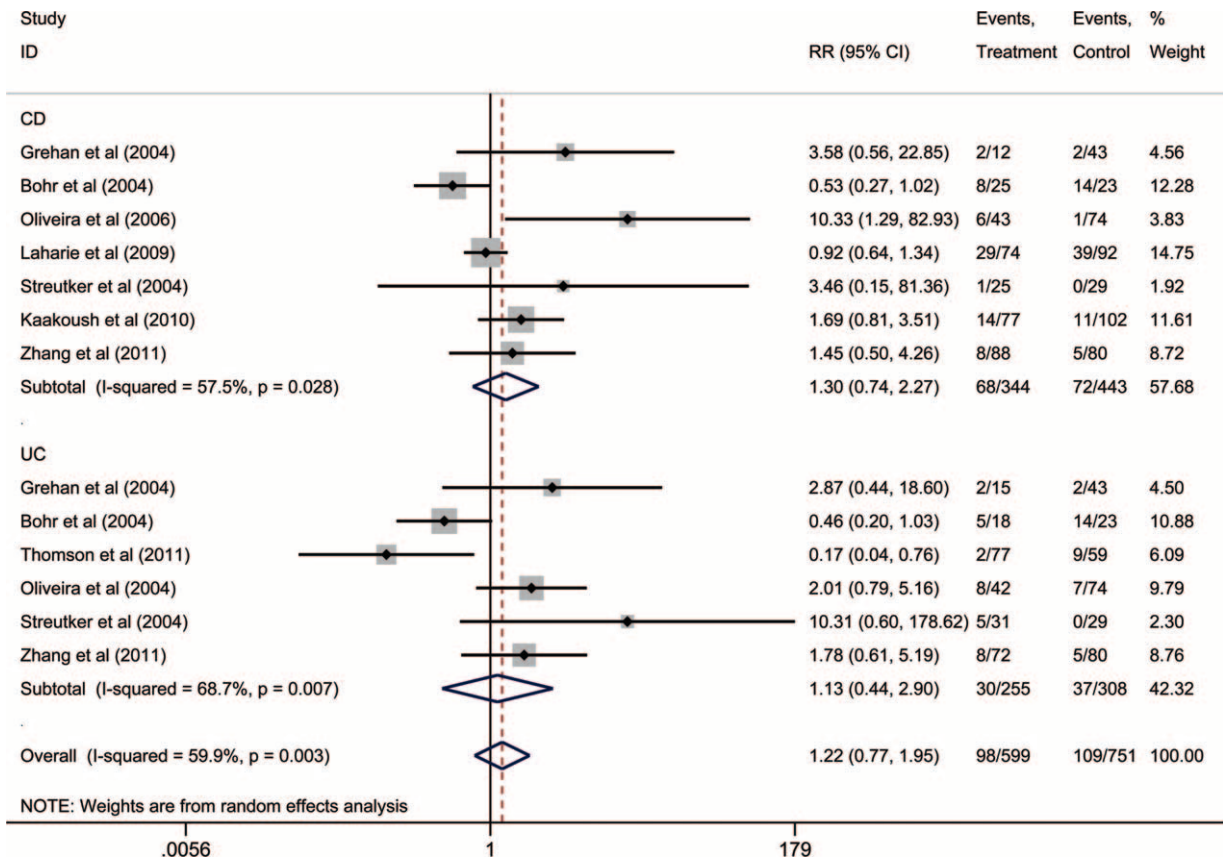


FIGURE 4. Subgroup meta-analysis of *Helicobacter pylori* detection via polymerase chain reaction in patients with Crohn's disease or ulcerative colitis.

affect the detected species of gut microbiota.⁴⁴ Fourth, the sample size of the included studies ranged from 16 to 240 cases, which may have incorporated selection bias into the patient enrollment. These 4 factors may be responsible for the moderate level of heterogeneity in our analysis, and we recommend that additional studies with larger sample sizes should use the same PCR primers to minimize heterogeneity in future studies.

Several meta-analyses have reported that gastric *H pylori* was a protective factor against IBD.¹¹⁻¹³ However, our data did not reveal any significant association between intestinal *H pylori* and IBD. In this context, *H pylori* may be transferred from the mouth to the colon in animals, which may explain why it is detectable in the intestinal tissues.⁴⁵ However, *H pylori* could colonize the intestinal mucosa in a mouse model,⁴⁶ which may partially explain the different prevalence of *H pylori* in the stomach and intestine. The different detection methods may also be responsible for this discrepancy, as gastric *H pylori* infection is usually diagnosed via serology, a urea breath test, or histology. We detected intestinal *H pylori* via PCR, which can more accurately differentiate between intestinal bacteria. Therefore, prospective high-quality controlled studies are needed to confirm our findings.

Unlike *H pylori*, EHS can persistently colonize the lower gastrointestinal tract and hepatobiliary system.⁴⁷ Interestingly, all 7 included studies that evaluated EHS revealed a relationship with IBD,^{31,32,35,37-39,41} although it is not clear whether the presence of EHS is a risk factor or causative factor in the etiology of IBD. One multicenter cross-sectional study detected

EHS in 24.7% of patients with CD, and found that *Helicobacter pullorum* and *Helicobacter canadensis* were the 2 most common species.³⁷ In contrast, a German study found *Helicobacter fennelliae* and *H pullorum* in the colon samples from 12% of patients with CD, compared to in only 4% of the controls.³² These studies unanimously indicate a positive relationship between EHS and IBD. Based on our data, it appears that EHS were most responsible for the dissimilarity between the patients with IBD and the controls, although the role of intestinal *H pylori* in IBD cannot currently be determined. Nevertheless, our data are not sufficient to clearly demonstrate that EHS is a risk factor for IBD, and further studies are needed to address this issue.

In addition, EHS were not detected in the IBD or control groups in 2 studies.^{29,30} The absence of detectable EHS may be related to 3 confounding factors. First, 1 of the studies only evaluated 30 patients, which may preclude an extrapolation to the general patient population (eg, due to sampling error). Second, the gut flora might be contaminated by feces, which could influence the sensitivity of the PCR tests. Third, drug therapies (eg, immunosuppressants or other biologic agents) might inhibit the activation and growth of EHS.

Although we screened for studies that used similar techniques and adhered to strict inclusion criteria, several limitations still existed in our meta-analysis. First, the confounding factors within each study's group increased the heterogeneity, as this type of study is rarely conducted. For example, the baseline patient characteristics varied in terms of age, sex, life experience, and environmental factors.

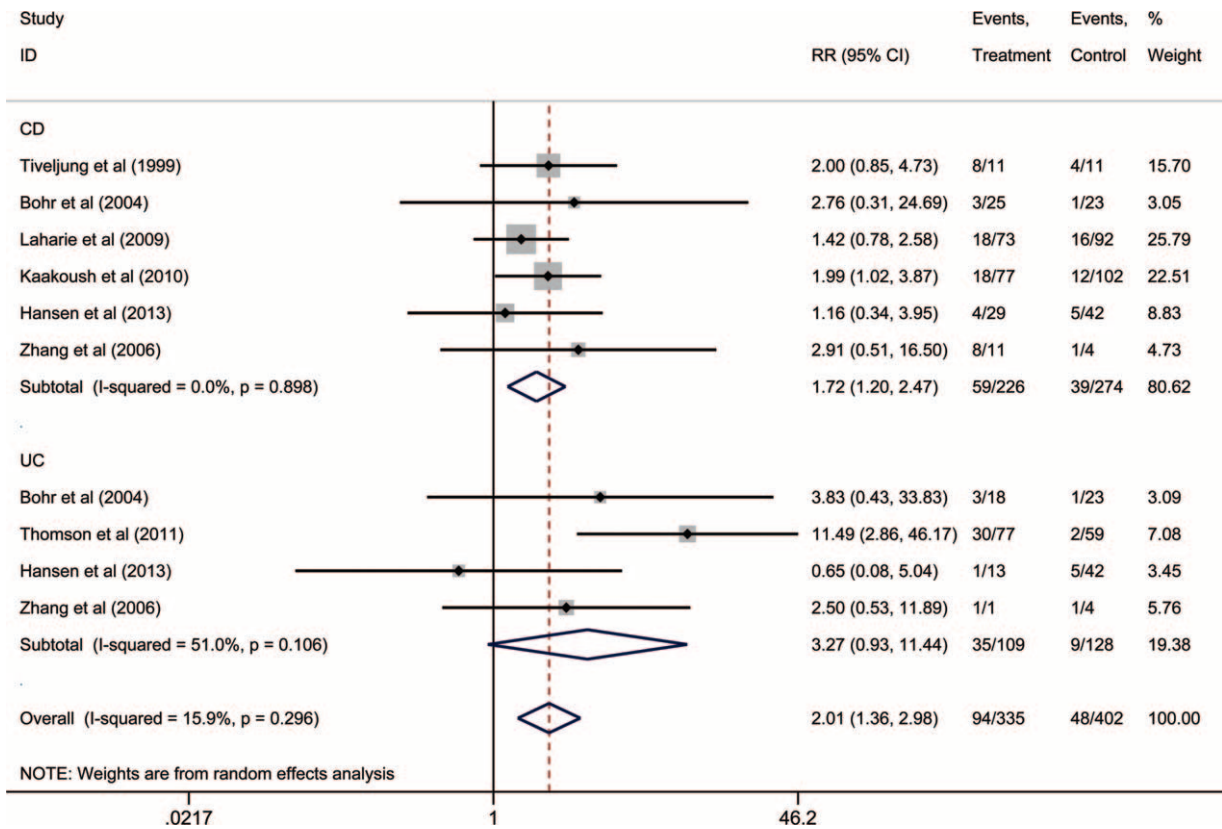


FIGURE 5. Subgroup meta-analysis of enterohepatic *Helicobacter* species detection via polymerase chain reaction in patients with Crohn's disease or ulcerative colitis.

Furthermore, the selection bias for control selection may have also intensified the heterogeneity in this meta-analysis. Second, only PCR was used to identify the infection of *Helicobacter* species. As PCR detection of *Helicobacter* species has good concordance in different intestinal regions for 99.1% of patients,³⁶ CD studies typically involve a biopsy of the ileal tissue and UC studies perform biopsy of the colonic tissue. Therefore, our subgroup analyses of CD and UC might partially reflect the effects of using different specimen locations. However, a single-site detection method has not become routine clinical practice, which may have led to further selection bias. Third, our assessment of study quality via NOS indicated a range of 3 to 8 stars, which suggested that there were discrepancies in the methodological quality of these studies.

Nevertheless, to our knowledge, the current meta-analysis is the first to reveal an association between intestinal *Helicobacter* species and patients with IBD. Future studies should examine this association using other diagnostic methods and sample sources, such as the patients' stool and serum. Furthermore, these studies need to determine whether this association is causative, and what role EHS might play in the pathogenesis of IBD.

CONCLUSION

This meta-analysis revealed a statistically significant association of EHS with IBD using PCR detection of 16S RNA. Furthermore, we found no significant association between intestinal *H pylori* and IBD. However, well-organized

prospective studies are needed to verify our findings and to identify the role of EHS in the immune mechanisms that are involved in the etiology of IBD.

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