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SYSTEMATIC REVIEW

Human intestinal spirochetosis, irritable bowel syndrome, and colonic polyps: A systematic review and meta-analysis

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Abstract

Human colonic spirochetosis (CS) is usually due to*Brachyspira pilosicoli*or *Brachyspira aalborgi*infection. While traditionally considered to be commensal bacteria, there are scattered case reports and case series of gastrointestinal (GI) symptoms in CS and reports of colonic polyps with adherent spirochetes. We performed a systematic review and meta-analysis investigating the association between CS and GI symptoms and conditions including the irritable bowel syndrome (IBS) and colonic polyps. Following PRISMA 2020 guidelines, a systematic search of Medline, CINAHL, EMBASE, and Web of Science was performed using specific keywords for CS and GI disease. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a random-effects model. Of 75 studies identified in the search, 8 case–control studies met the inclusion criteria for meta-analysis and 67 case series studies met the inclusion criteria for pooled prevalence analysis. CS was significantly associated with diarrhea (n = 141/127, cases/controls, OR: 4.19, 95% CI: 1.72–10.21, P = 0.002) and abdominal pain (n = 64/65, OR: 3.66, 95% CI: 1.43–9.35, P = 0.007). CS cases were significantly more likely to have Rome III-diagnosed IBS (n = 79/48, OR: 3.84, 95% CI: 1.44–10.20, P = 0.007), but not colonic polyps (n = 127/843, OR: 8.78, 95% CI: 0.75–103.36, P = 0.084). In conclusion, we found evidence of associations between CS and both diarrhea and IBS, but not colonic polyps. CS is likely underestimated due to suboptimal diagnostic methods and may be an overlooked risk factor for a subset of IBS patients with diarrhea.

Simon Keely and Nicholas J. Talley share equal contribution.

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Introduction

Although human intestinal spirochetosis was identified in 1967,[1] there is still ongoing debate regarding its pathogenic importance in humans.[2] Swine and poultry-infecting intestinal spirochetes can induce severe colitis and diarrhea in those animals,[3] while species colonizing humans (the intestinal spirochetes *Brachyspira pilosicoli*and *Brachyspira aalborgi*) are usually thought to induce few or no symptoms.[4] Our understanding of colonic spirochetosis (CS) has been hampered by the difficulty in working with these species. *Brachyspira* are fastidious, slow-growing, anaerobic bacteria, hard to isolate and to grow in laboratory conditions.[5] As a result, the pathogenesis, transmission pattern, and risk factors of CS remain largely unknown. Studies utilizing transmission electron microscopy (TEM) show that spirochete may adhere to the epithelium, as the "head" of the spirochetes anchors between microvilli structures of the intestinal epithelium while the tail end is directed into the colonic lumen.[6] Although TEM studies using clinical tissues have found spirochetes present in macrophages[7] and close to mast cells,[8] the significance of this finding is unclear as there is little evidence of epithelial cell penetration by spirochetes.[9]

The gold standard diagnosis of CS is based on its characteristic colonization of the epithelial surface, identified by routine histological examination of biopsies taken during colonoscopy.[10] Using hematoxylin and eosin (H&E) staining, colonized spirochetes are stained as light purple, while Warthin–Starry silver staining or specific immunohistochemistry (IHC) staining, using cross-reactive anti-*Treponema pallidum*antibody, better highlight the presence of spirochetes from the background.[10] The limitation of histological diagnosis is that successful detection of spirochetes is largely dependent on the location of biopsy sampling and careful pathology examination, and the basophilic brush line from CS can be easily misinterpreted as a normal brush border structure on H&E staining.[11] While genomic screening of the gut microbiota is advancing rapidly and it has proven to be a powerful tool to investigate microbiota composition,[12] the "conventional" 16S rRNA sequencing approach is unable to detect human colonic spirochetes as the standard 16S rRNA primer sets are incompatible with spirochetes' 16S rRNA region.[13] Consequently, there are currently no non-invasive routine diagnostic methods to diagnose human CS.

Recently, interest in CS has increased with reports of a possible association with diarrhea-predominant irritable bowel syndrome (IBS-D).[14–16] CS has also been observed with colonic polyps, but an association with adenoma formation is uncertain.[17] This systematic review and meta-analysis aimed to determine whether human colonic spirochete infection is associated with specific gastrointestinal (GI) symptoms or GI diseases. We also aimed to identify risk factors associated with the infection and the results of treatment where data were available.

Methods

Search strategy. We followed the PRISMA guidelines for systematic reviews.[18] A protocol was developed before initiation of the systematic review (PROSPERO CRD42019124669). Electronic databases including Medline, CINAHL, EMBASE, and Web of Science were searched on June 31, 2021, with limitations set on human studies published between 1967 and the search date. Each database was searched with the same strategy: [spirochetosis OR spirochaetosis OR spirochete OR spirochaete OR spirochaetose OR *Brachyspira aalborgi*OR *Brachyspira pilosicoli*OR *Serpulina pilosicoli*] AND [intestinal disease OR intestinal].

Study selection. After removal of duplicate studies, two independent reviewers (KF and GLB) screened titles and abstracts for relevance to the review topic. Following this, full texts of all remaining studies were assessed for suitability and relevance based on the review inclusion and exclusion criteria. The inclusion criteria were (i) studies in humans with intestinal (colonic) spirochete infection, (ii) case–control studies, case series, or original research studies of GI symptoms in intestinal (colonic) spirochete infection, and (iii) studies in the English language. Exclusion criteria were (i) reviews, (ii) single case reports, (iii) studies with no mention of patient symptoms, (iv) studies where full text was not available, and (v) studies not in the English language.

Data extraction. Data extraction was performed by two independent reviewers (KF and PMN). Disagreements were resolved by consensus. Data information were extracted where available using a standardized data extraction template and included (a) general: publication year, study type, number of cases, sex, age, travel/work/sex activity, and sexuality; (b) comorbidity: GI comorbidity, non-GI comorbidity, and co-infection; (c) GI symptoms: diarrhea, constipation, diarrhea/constipation mixed, abdominal pain, rectal bleeding, blood in stool, mucus in stool, vomiting, weight loss, fever, nausea, anemia or asymptomatic, and physical examination results when reported; (d) colonoscopy findings: normal colonoscopy, abnormal colonoscopy, and degree and location of the abnormalities; (e) histology findings: presence of spirochetes, location of spirochetes infection, mucosal inflammation, crypt changes, and presence of immune cells (plasma cell, lymphocyte, neutrophil, eosinophil, macrophage, and mast cells); (f) species specificity: *Brachyspira* genus,*B. pilosicoli*, and *B. aalborgi*; (g) diagnostic method: histology, PCR, PCR target, PCR material, culture, culture material, and electron microscopy; and (h) treatment and outcomes: type of treatment, symptom relief, bacterial eradication, pathology recovery, and symptom reoccurrence.

Statistical analysis. For case–control studies, pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the risk correlation of CS and gender, GI symptoms, and GI diseases using a random-effects model.[19] To test the heterogeneity of the studies included for analysis, Cochran's Q statistic was used, with $P < 0.10$ indicating significant effects of heterogeneity. Heterogeneity was assessed using the I^2 statistic with results of 0–25% (low), 25–75% (moderate), and > 75% (high) levels of heterogeneity.[20] Due to the small number of studies included in the meta-analysis, publication bias was not assessed as most methods required at least 10 studies to perform a test.[21]

For case series studies, the prevalence of sex, a range of GI symptoms, colonoscopy findings, diagnostic methods, mucosal inflammation, and treatment effects in reported CS cases were calculated using pooled prevalence rate (event rate [ER]) and 95% CIs

using a random-effects model. Heterogeneity of the studies were evaluated as described above. All analyses were performed with Comprehensive Meta-analysis (version 3.0), Biostat, Englewood, NJ (2014).

Results

Study selection. Of the initial 2157 studies obtained from Medline, EMBASE, Web of Science, and CINAHL, 1079 texts were identified as duplicates. Of the remaining 1078 studies that were screened by title and abstract, 705 were excluded as animal studies, microbiological studies, or incorrect species of bacteria. A total of 373 papers then proceeded to full text screening, and of those, 207 studies were excluded as reviews, lacking patient symptom information, written in non-English language, or had duplicated reports of the same patient cohorts. Ninety-four single case reports were excluded. Three studies were added by hand search. In the end, 75 studies were included for data extraction. Eight case–control studies were included for meta-analysis and 67 case series studies were included for pooled prevalence analysis (Fig. 1).

Meta-analysis of case–control studies

Study characterization. Of the eight case–control studies included (Table 1), in four studies (Walker *et al.*,[14] Goodsall *et al.*,[15] Alsaigh and Fogt,[22] and Higashiyama *et al.*[23]), cases of CS and controls were identified in pathology databases based on histology findings; in Cooper *et al.*,[24] cases were a cohort of homosexual males identified with CS while controls were male patients without CS; for Esteve *et al.*,[25] cases were patients with chronic diarrhea collected prospectively who were later identified with CS, while controls were asymptomatic patients with colonic biopsies available in a pathology database; in Omori *et al.*,[26] cases were patients with sessile serrated adenoma/polyps (SSA/P) while controls were non-SSA/P patients; and in Jabbar *et al.*,[16] cases were patients with IBS and controls were healthy volunteers. In all studies, CS was confirmed by histological examination.

Demographic characterization

Sex. Three studies (Alsaigh and Fogt,[22] Esteve *et al.*,[25] and Jabbar *et al.*[16]) with no sex restrictions for recruitment were included providing $n = 88$ cases and $n = 161$ controls. CS cases were 1.84 times (95% CI = 0.11–29.91, $P = 0.667$) more likely to be male than female, although this was not significant. Heterogeneity of the studies was high ($I^2 = 95.23$, $P < 0.001$) (Fig. 2).

Age. Three studies (Alsaigh and Fogt,[22] Esteve *et al.*,[25] and Jabbar *et al.*[16]) with no age restrictions for recruitment were included providing $n = 88$ cases and $n = 161$ controls. The mean age of the cases and controls was 47.1 and 48.2 years ($P = 0.94$), respectively.

Gastrointestinal symptom in colonic spirochetosis cases

Diarrhea. Four studies assessed diarrhea in association with CS (Alsaigh and Fogt,[22] Walker *et al.*,[14] Goodsall *et al.*,[15] and Jabbar *et al.*[16]). In total, $n = 141$ cases and $n = 127$ controls were included. CS was significantly associated with diarrhea; CS cases were more than three times more likely to have diarrhea compared with controls (OR: 4.19, 95% CI: 1.72–10.21, $P = 0.002$) (Fig. 3a). Heterogeneity of the studies was moderate ($I^2 = 27.32$, $P = 0.25$).

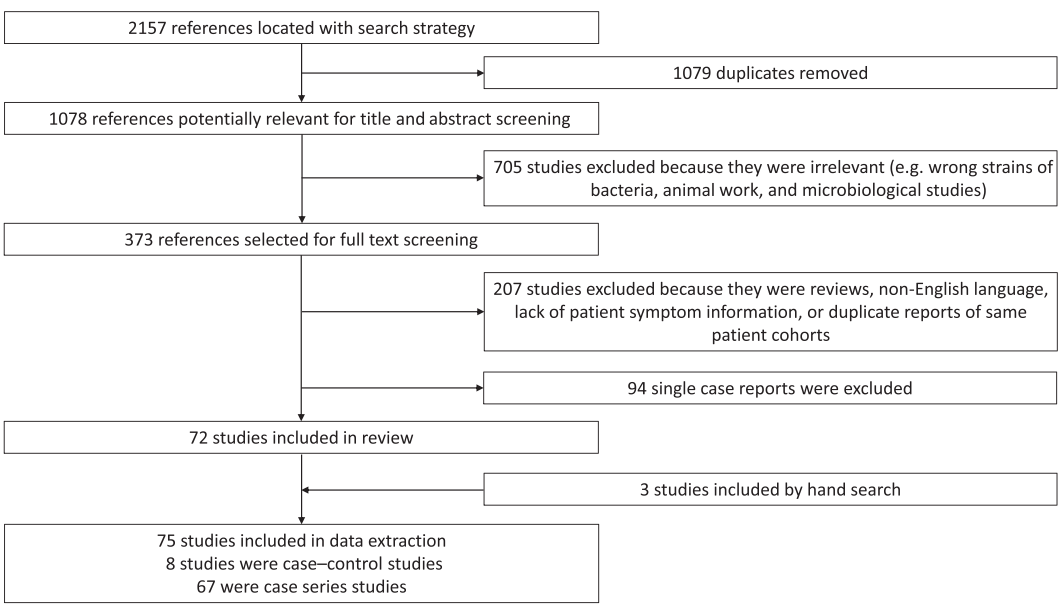

Figure 1 Study selection process. Flow diagram for the identification of studies included in the analysis.

Table 1 Meta-analysis of case–control studies characterization

Paper	Year	Screening cohort selection criteria	CS patient/ screening cohort	Selection criteria for control subjects	CS patient/ control cohort	Data for meta-analysis			
						Gender	Symptom	IBS	Polyps
Cooper *et al.*[24]	1986	Homosexual men with GI symptoms.	5/8	Age matched male patients with available colonic biopsy.	0/5	NA	NA	NA	NA
Alsaigh and Fogt[22]	2002	Pathology database.	15/15	Age and clinical indication matched patients with biopsy.	0/30	Yes	Diarrhea/ rectal bleeding	NA	Yes
Esteve *et al.*[25]	2006	Prospective survey of patients with chronic watery diarrhea and CS patients identified in routine colonoscopy.	11/11	Patients with colonic biopsy taken due to rectal bleeding or polyps histology.	0/100	Yes	NA	NA	NA
Higashiyama *et al.*[23†]	2009	Pathology database from 2005 to 2008.	86/86	Patient with colonic biopsy from 2005 to 2008.	0/702	NA	NA	NA	Yes
Omori *et al.*[26]	2014	Patients with sessile serrated adenoma/polyp identified by histology during 2008–2011.	10/19	Patients with biopsy excluding sessile serrated adenoma/polyp and cancer in 2011.	14/172	NA	NA	NA	Yes
Walker *et al.*[14]	2015	Pathology database.	17/17	Subjects with colonic biopsy from random population.	0/17	NA	Diarrhea/ abdominal pain/rectal bleeding	Yes	Yes
Goodsall *et al.*[15]	2017	Pathology database.	47/47	Subjects with colonic biopsy from random population.	0/48	NA	Diarrhea/ abdominal pain	NA	NA
Jabbar *et al.*[16]	2020	IBS patients diagnosed by Rome III criteria.	19/62	Healthy subjects with colonic biopsy.	0/31	Yes	Diarrhea	Yes	NA

†The Higashiyama *et al.* paper is an abstract.
CS, colonic spirochetosis; GI, gastrointestinal; IBS, irritable bowel syndrome; NA, not applicable.
In all studies, CS was confirmed by histological examination.

Abdominal pain. Two studies assessed abdominal pain in association with CS (Walker *et al.*[14] and Goodsall *et al.*[15]). In total, $n = 64$ cases and $n = 65$ controls were included. CS cases were almost four times more likely to have abdominal pain (OR: 3.66, 95% CI: 1.43–9.35, $P = 0.007$) (Fig. 3b). Heterogeneity of the studies was low ($I^2 = 13.52$, $P = 0.28$).

Rectal bleeding. Two studies examined patients who self-reported symptom of rectal bleeding (Alsaigh and Fogt[22] and Walker *et al.*[14]). In total, $n = 32$ cases and $n = 47$ controls were included. CS cases were twice as likely to experience rectal bleeding (OR: 2.34, 95% CI: 0.36–15.28, $P = 0.374$) (Fig. 3c), although the reason for bleeding was not specified in these studies and the association was not significant. There was no heterogeneity in the studies ($I^2 = 0.00$, $P = 0.44$).

Gastrointestinal diseases in colonic spirochetosis cases

Irritable bowel syndrome. Two studies assessed diagnosis of IBS using Rome III criteria in association with CS (Walker *et al.*[14] and Jabbar *et al.*[16]). In total, $n = 79$ cases and $n = 48$ controls were included. CS was significantly associated with IBS; CS cases are almost four times (OR: 3.84, 95% CI: 1.44–10.20, $P = 0.007$) more likely to have a diagnosis of IBS compared with controls (Fig. 4a). There was no heterogeneity in the studies ($I^2 = 0.00$, $P = 0.71$).

Colonic polyps. Four studies assessed diagnosis of colonic polyps in association with CS (Alsaigh and Fogt,[22] Higashiyama *et al.*,[23] Walker *et al.*,[14] and Omori *et al.*[26]). In Alsaigh and Fogt, Higashiyama *et al.*, and Walker *et al.*, the subtypes of polyps were not discriminated, while Omori *et al.* specifically investigated the correlation of CS and SSA/P in patient cohorts. In total, $n = 127$ cases and $n = 843$ controls were included. CS cases were almost nine times (OR: 8.78, 95% CI: 0.75–103.36, $P = 0.084$) more likely to have colonic polyps compared with controls, but this was not a significant finding (Fig. 4b). Heterogeneity of the studies was high ($I^2 = 89.09$, $P < 0.001$). After the removal of Omori *et al.*, the OR for non-specific polyps dropped to 1.44 (95% CI: 0.33–6.36, $P = 0.632$), with $I^2 = 72.08$, $P = 0.03$ (Fig. 4c).

Colonoscopy findings. Only one study (Alsaigh and Fogt[22]) assessed colonoscopy findings in association with CS, although the definition of normal and abnormal colonoscopy was not specified in the paper. In total, $n = 15$ cases and $n = 30$ controls were

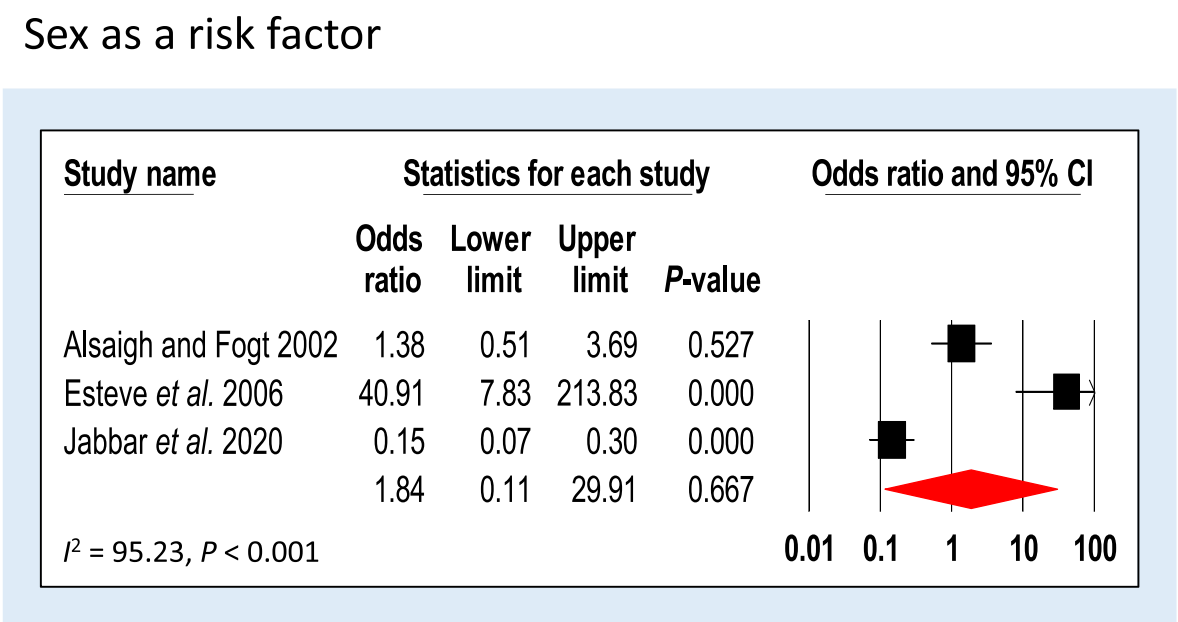

Figure 2 Forest plot of gender risk in colonic spirochetosis cases. Three case–control studies were included for analysis of male sex prevalence. Pooled odds ratios and 95% confidence intervals were calculated using a random-effects model. Heterogeneity of the publications were tested with Cochran's Q statistic and I^2 statistic. Publication bias was tested using the Egger's regression model with the effect of bias assessed using the fail-safe number method. CI, confidence interval.

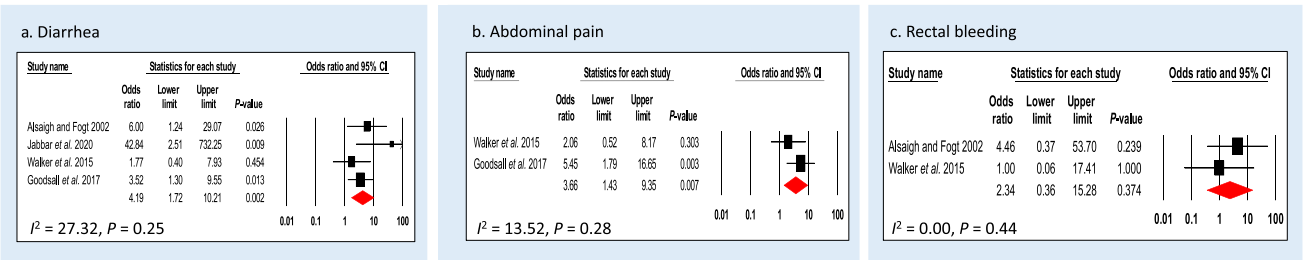

Figure 3 Forest plot of gastrointestinal symptoms risk in colonic spirochetosis cases. (a) Four case–control studies were included for analysis of diarrhea prevalence. (b) Three case–control studies were included for analysis of abdominal pain prevalence. (c) Two case–control studies were included for analysis of rectal bleeding prevalence. Pooled odds ratios and 95% confidence intervals were calculated using a random-effects model. Heterogeneity of the publications were tested with Cochran's Q statistic and I^2 statistic. Publication bias was tested using the Egger's regression model with the effect of bias assessed using the fail-safe number method. For meta-analysis of colonic spirochetosis and rectal bleeding, publication bias could not be tested as the minimal number of study for the Egger's test is 3. CI, confidence interval.

analyzed. The OR of abnormal visible findings on colonoscopy was 0.87 (95% CI: 0.24–3.10, $P = 0.828$).

Case series

Pooled prevalence analysis. Sixty-seven case series studies were included for pooled prevalence analysis. Results are shown in Table 2. In reported CS cases, the most common symptoms were diarrhea (39%) and abdominal pain (34%), followed by symptoms of bloating (29%), undefined rectal bleeding (21%), or a finding of blood in the stool (27%). We also observed that nearly half of the CS cases (48%) were reported to be asymptomatic with CS only identified because biopsies were taken during screening or polyp surveillance.

In CS cases, a range of colonic diseases were assessed. Nearly one third had colonic polyps (29%), one quarter had colon cancer (24%), and one in ten had inflammatory bowel disease (9%) or diverticular disease (12%). In CS cases, the proportion of patients with a normal colonoscopy (47%) *versus* an abnormal colonoscopy (45%) were similar, which was in line with the case–control findings (the remaining 8% were missing data). Among CS cases with abnormal colonoscopy findings, 70% had erosions, 67% had hyperemia, 46% had edema, 33% had erythema, 28% had inflamed mucosa, 25% had pale mucosa, 25% had blood oozing, 23% had ulcers, and 17% had loss of the vascular pattern.

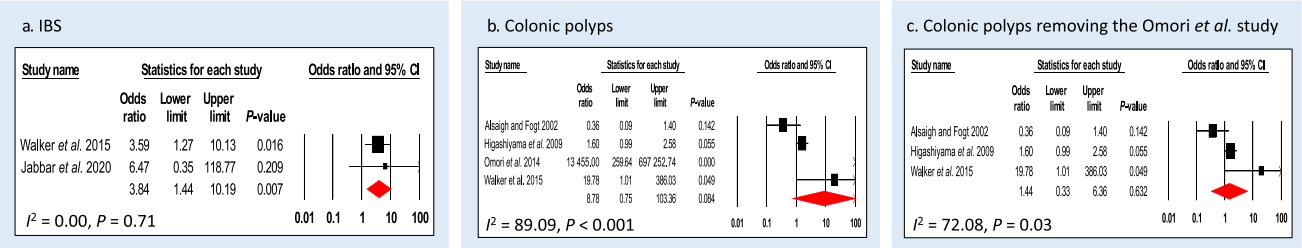

Figure 4 Forest plot of gastrointestinal disease/abnormality risk in colonic spirochetosis cases. (a) Two case–control studies were included for analysis of irritable bowel syndrome risk. (b) Four case–control studies were included for analysis of polyps risk. (c) Sensitivity analysis of polyps risk in colonic spirochetosis cases by removing the Omori *et al.* study from the meta-analysis. Pooled odds ratios and 95% confidence intervals were calculated using a random-effects model. Heterogeneity of the publications were tested with Cochran's Q statistic and I^2 statistic. Publication bias was tested using the Egger's regression model with the effect of bias assessed using the fail-safe number method. CI, confidence interval; IBS, irritable bowel syndrome.

Table 2 Pooled prevalence estimates of colonic spirochetosis-positive patients in case series studies

Event	Pooled study references	Cases	Number of event	Event rate/proportion	Heterogeneity
Demographic characteristics					
Male gender	1, 4, 6, 8, 9, 17, 28, 29, 48, 60–103	2041	1409	0.68 (95% CI 0.63–0.73)	I^2 = 67.24%, $P < 0.001$
Female gender	1, 4, 6, 8, 9, 17, 28, 29, 48, 60–103	2041	632	0.32 (95% CI 0.27–0.37)	I^2 = 67.24%, $P < 0.001$
Travel before symptom onset	29, 61, 66, 70, 81, 83–88, 90, 94, 95	105	28	0.30 (95% CI 0.19–0.45)	I^2 = 29.10%, $P = 0.14$
Childhood sexual abuse	97	8	1	0.13 (95% CI 0.02–0.54)	I^2 = 0.00%, $P = 1$
Frequent sexual activity	85	4	1	0.25 (95% CI 0.03–0.76)	I^2 = 0.00%, $P = 1$
Homosexual	28, 48, 61, 70, 73, 77, 79, 80, 83, 85, 96, 98, 103	530	158	0.44 (95% CI 0.16–0.77)	I^2 = 86.83%, $P < 0.001$
HIV+	4, 28, 48, 61–64, 67, 70, 72, 75, 76, 79, 83, 93, 103, 104	1369	71	0.10 (95% CI 0.04–0.21)	I^2 = 88.64%, $P < 0.001$
HIV−	4, 28, 48, 63, 64, 67, 69–72, 75, 76, 82, 83, 89	654	586	0.89 (95% CI 0.75–0.96)	I^2 = 85.58%, $P < 0.001$
HIV status unknown	1, 4, 6, 8, 17, 27, 29, 61, 62, 65, 66, 68, 74, 77, 81, 84–92, 94–102	429	411	0.90 (95% CI 0.84–0.93)	I^2 = 19.74%, $P = 0.16$
GI abnormality					
Colonic polyps	1, 9, 17, 27, 28, 61–72, 74, 75, 77, 79, 85, 94, 97, 105	1094	370	0.28 (95% CI 0.18–0.40)	I^2 = 89.77%, $P < 0.001$
Diverticular disease	8, 9, 17, 27, 62–64, 66–69, 85, 105, 106	403	39	0.12 (95% CI 0.07–0.19)	I^2 = 47.67%, $P = 0.02$
Inflammatory bowel disease	4, 9, 28, 61–63, 65, 66, 72, 75–77, 79, 91, 92, 106	924	74	0.09 (95% CI 0.06–0.13)	I^2 = 52.59%, $P = 0.007$
Cancer	1, 9, 28, 64–68, 72, 75, 77, 105, 106	532	70	0.24 (95% CI 0.19–0.31)	I^2 = 87.85%, $P < 0.001$
GI symptoms					
Diarrhea	1, 4, 8, 9, 29, 60–73, 75–79, 81–97, 103–105, 107	1770	570	0.39 (95% CI 0.33–0.46)	I^2 = 74.44%, $P < 0.001$
Abdominal pain	1, 4, 6, 8, 9, 29, 60–68, 70–72, 75, 76, 78, 79, 81, 83–88, 90, 92–94, 96, 97, 99, 101, 102, 104, 105	1645	436	0.34 (95% CI 0.26–0.43)	I^2 = 83.92%, $P < 0.001$
Rectal bleeding	1, 4, 8, 29, 61–70, 72, 75, 77, 82, 84–88, 90, 93–95, 97, 99, 105	705	114	0.21 (95% CI 0.15–0.27)	I^2 = 48.94%, $P = 0.001$
Blood in stool	4, 8, 27, 28, 66, 75, 76, 79, 82, 85, 87	576	174	0.27 (95% CI 0.20–0.35)	I^2 = 62.53%, $P = 0.003$

(Continues)

Table 2 (Continued)

Event	Pooled study references	Cases	Number of event	Event rate/proportion	Heterogeneity
Bloating	29, 62, 88	7	2	0.29 (95% CI 0.07–0.68)	I^2 = 0.00%, P = 0.81
Vomiting	60, 79, 81, 84, 96, 99, 101	315	28	0.17 (95% CI 0.08–0.32)	I^2 = 55.47%, P = 0.04
Weight loss	8, 29, 61, 67, 70, 83, 85, 87, 93, 97, 99, 104	527	31	0.17 (95% CI 0.08–0.32)	I^2 = 58.75%, P = 0.005
Anemia	4, 61, 63, 66, 92, 94	112	8	0.10 (95% CI 0.03–0.26)	I^2 = 49.32%, P = 0.08
Mucus in stool	4, 8, 79, 87, 93, 95, 97	232	15	0.12 (95% CI 0.05–0.27)	I^2 = 35.50%, P = 0.17
Asymptomatic	4, 17, 28, 60, 62, 71, 72, 75, 76, 78, 89, 98, 104	1267	648	0.48 (95% CI 0.34–0.63)	I^2 = 90.67%, P < 0.001
Colonoscopy findings					
Normal colonoscopy	4, 8, 17, 29, 61, 63, 65, 69, 75, 82, 84–87, 90–92, 94–97, 101, 102	189	83	0.47 (95% CI 0.34–0.61)	I^2 = 49.19%, P = 0.007
Abnormal colonoscopy	4, 8, 17, 27, 29, 61, 63, 65, 69, 75, 82, 85–88, 90–95, 97, 99, 101, 102	273	109	0.45 (95% CI 0.29–0.61)	I^2 = 69.34%, P < 0.001
Erythema	4, 29, 69, 85, 88, 91	28	17	0.33 (95% CI 0.18–0.54)	I^2 = 34.34%, P = 0.18
Hyperemia	61, 97	21	18	0.67 (95% CI 0.01–1.00)	I^2 = 89.43%, P < 0.001
Loss of vascular pattern	69, 82	14	2	0.17 (95% CI 0.01–0.76)	I^2 = 61.89%, P = 0.11
Pale mucosa	85	3	1	0.25 (95% CI 0.03–0.76)	I^2 = 0.00%, P = 1.00
Edema	91, 97	7	4	0.46 (95% CI 0.02–0.97)	I^2 = 77.36%, P = 0.04
Erosion	61, 69, 92	21	32	0.70 (95% CI 0.06–0.99)	I^2 = 86.00%, P = 0.001
Ulcer	63, 87, 92, 93	26	7	0.23 (95% CI 0.06–0.56)	I^2 = 52.19%, P = 0.10
Mucosal inflammation	27, 69, 75, 86, 87, 97	48	35	0.28 (95% CI 0.09–0.60)	I^2 = 77.80%, P < 0.001
Blood oozing	85	3	1	0.25 (95% CI 0.03–0.76)	I^2 = 0.00%, P = 1.00
Mucosal inflammation					
Inflammation presence	4, 6, 8, 9, 28, 29, 48, 61–64, 66–69, 73, 75, 84, 86–88, 90–97, 99, 100, 102, 105, 106	645	142	0.30 (95% CI 0.21–0.40)	I^2 = 62.76%, P < 0.001
Lymphocyte presence	4, 8, 94, 97	40	11	0.30 (95% CI 0.14–0.53)	I^2 = 25.99%, P = 0.25
Eosinophil presence	62, 84, 86, 87, 97	137	10	0.18 (95% CI 0.03–0.64)	I^2 = 80.79%, P < 0.001
Neutrophil presence	29, 62, 73, 86, 96	160	14	0.18 (95% CI 0.05–0.49)	I^2 = 67.55%, P < 0.001
Mast cell presence	8	2	2	0.83 (95% CI 0.19–0.99)	I^2 = 0.00%, P = 1.00
Macrophage presence	8, 29, 63, 67, 99	51	10	0.45 (95% CI 0.09–0.87)	I^2 = 74.63%, P = 0.003
Crypt involvement	8, 61, 62, 64, 69, 73, 86, 90, 93, 94	217	27	0.20 (95% CI 0.11–0.33)	I^2 = 54.40%, P = 0.02
Diagnosis method					
By histology	1, 4, 6, 8, 9, 17, 27–29, 48, 60–107	2183	1854	0.92 (95% CI 0.85–0.96)	I^2 = 70.44%, P < 0.001
By PCR	1, 4, 6, 8, 9, 17, 27–29, 48, 60–92, 94–107	2104	289	0.15 (95% CI 0.08–0.25)	I^2 = 83.04%, P < 0.001
By culture	1, 4, 6, 8, 9, 17, 27–29, 48, 60–92, 94–107	2104	321	0.08 (95% CI 0.04–0.14)	I^2 = 69.35%, P < 0.001
Species prevalence					
*Brachyspira pilosicoli*presence	28, 29, 61, 63, 64, 67, 70, 75–78, 81, 83, 89–92, 105	504	175	0.20 (95% CI 0.12–0.32)	I^2 = 69.32%, P < 0.001
*Brachyspira aalborgi*presence	28, 29, 61, 63, 64, 67, 70, 75–78, 81, 83, 89–92, 105	504	207	0.58 (95% CI 0.40–0.74)	I^2 = 82.78%, P < 0.001
Metronidazole treatment					
One course of metronidazole/CS patient	4, 8, 61, 63, 69, 79, 82–85, 90, 92–97, 99, 102, 103, 107	358	134	0.49 (95% CI 0.34–0.64)	I^2 = 60.08%, P < 0.001
Symptom relief/metronidazole-treated patient	4, 61, 63, 69, 83–85, 90, 92–94, 97, 102, 103, 107	65	55	0.81 (95% CI 0.68–0.90)	I^2 = 0.00%, P = 0.95
Bacteria eradication/metronidazole-treated patient	61, 63, 79, 85, 92, 94, 97, 103, 107	61	51	0.76 (95% CI 0.62–0.86)	I^2 = 0.00%, P = 0.55
Pathology recovery/metronidazole-treated patient	61, 92, 93	20	19	0.84 (95% CI 0.52–0.96)	I^2 = 60.08%, P < 0.001
Symptom relapse/metronidazole-treated patient	4, 63, 84, 85, 90, 93, 94	20	8	0.39 (95% CI 0.20–0.62)	I^2 = 60.08%, P < 0.001

CI, confidence interval; CS, colonic spirochetosis; GI, gastrointestinal.

The majority of CS cases were diagnosed by histology (92%), while only 15% had specific PCR tests to confirm the species of infection, and only 8% were diagnosed by successful culture. Within those cases where species differentiation was examined, 20% were infected with *B. pilosicoli*, 58% were infected with *B. aalborgi*, and 22% reported undetermined *Brachyspira* genus.

Metronidazole was the most commonly prescribed treatment for CS. Nearly half of the CS patients (49%) received one course of metronidazole treatment (dose and frequency varied between studies). Among these patients, 81% had reported GI symptom relief (symptom assessment varied between studies), while 79% reported successful bacterial eradication in a follow-up colonoscopy examination. Although most patients (84%) with CS-related pathology experienced recovery after treatment, nearly 40% of these patients also reported symptom relapse between a few days to 15 months after treatment.

Anatomical locations of spirochetes were extracted from confirmed CS cases who underwent full colonoscopy and had biopsies taken from each section of the colon, or with colonoscopy that specified the biopsy locations.[11,26–41] Biopsies taken from the ascending colon had the highest success rate (56%) for detecting spirochetes from these CS cases, followed by biopsies taken from the transverse colon (54%), descending colon (48%), sigmoid colon (47%), cecum (40%), and rectum (38%). In total, 964 biopsies were taken by colonoscopy in these confirmed CS cases, with 454 biopsies showing presence of spirochetes, providing a 47% successful detection rate (Fig. 5).

Discussion

To our knowledge, this is the first meta-analysis to investigate human colonic spirochetes infection and GI disease. Although spirochetes are generally considered to be commensals and largely ignored, the results of this review have identified a clear association between CS and diarrhea, abdominal pain, and IBS. However, no association between CS and the presence of polyps was identified. Importantly, we found that CS was strongly associated with IBS, a functional GI disorder that is characterized by abdominal pain and a change of bowel habits.[42] Although the etiology of IBS is still unclear, there is evidence that suggests GI infections may play a role in the initiation and development of IBS.[43] In both studies that directly reported an association of IBS with CS, subtle pathological changes were identified in CS patients, namely, increased eosinophils, mast cells, and lymphoid aggregates in the lamina propria.[14,16] These findings are consistent with low-grade mucosal inflammation that has been observed in other IBS cohorts, although in these studies CS was not evaluated.[44–46] IBS patients usually have normal colonoscopy findings and therefore colonic biopsy is not indicated, which may be the reason that a direct involvement of CS in IBS has not previously been widely reported. Given histology is currently the gold standard for diagnosing CS, standardizing biopsy collection from patients with IBS for careful histological evaluation may reveal the true prevalence of CS in IBS cohorts.

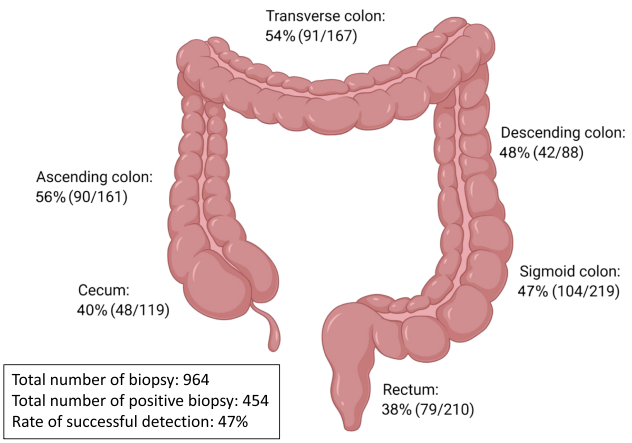

Figure 5 Anatomical location of positive-spirochete biopsy in colonoscopy examination. Anatomical location of spirochetes in patients with colonic spirochetosis was recorded from studies that performed whole colon colonoscopy and had taken biopsies from each section of the colon, or studies that have specified the location which the biopsy had been taken.

In the meta-analysis of case–control studies, we aimed to investigate the association of CS and GI symptoms. Due to the heterogeneity of GI symptoms reported in the available studies, we could only assess diarrhea, abdominal pain, and rectal bleeding by meta-analysis with sufficient sample size. However, the pooled prevalence analysis of case series studies mirrored these findings with diarrhea, followed by abdominal pain, blood in stool, and rectal bleeding, the most commonly reported symptoms with CS. Limitation of the symptom analysis include possible selection bias and reporting bias. CS patients with symptoms may be more likely to seek healthcare and colonoscopy, which could increase the detection of CS compared with asymptomatic patients, and they may also be more likely to be reported and published. Therefore, it is interesting and important that we also assessed and found that 48% of the reported CS cases were asymptomatic and had undergone colonoscopy for polyp surveillance or population screening. It is plausible to assume this number may be higher in general population. Unfortunately, none of the included studies had characterized these cases in detail, and as a result, we were unable to delineate risk factors that may differentiate asymptomatic *versus* symptomatic disease in CS cases. Future studies are needed to comprehensively assess GI symptoms associated with CS with a thorough and standardized symptom evaluation including abdominal pain, diarrhea, rectal bleeding, blood in stool, weight loss, vomiting, bloating, mucus in stool, and anemia. Population studies are urgently needed to determine an accurate infection rate, symptom, and pathology profile of CS.

We also investigated the relationship between sex and CS, as sex differences have been reported in other infectious GI diseases.[47] CS was initially believed to be a sexually transmitted disease, with early work focusing predominantly on homosexual male cohorts.[48] Therefore, CS has largely been regarded as a male dominant disease. In the pooled prevalence analysis of case series, we found that the prevalence of male gender in current reported CS cases was 68%. However, the meta-analysis showed that the OR of a male CS patient is only 1.84 compared with female; the difference was not statistically significant. Randomized population study like Walker *et al.*[14] reported that there was only a slight increase in the likelihood of being male (OR: 1.13) in CS patients. This likely reflects selection bias as past studies have focused on certain patient groups (i.e. male homosexuals). Sex differences in

IBS subtypes have not been extensively studied, but previous meta-analyses suggest that IBS-D is more common in men over women,[49] although whether this is associated with a potentially higher rate of CS is unknown. Alternatively, spirochete infection in males may induce more severe symptoms than in females, as sex differences in infection are known to exist[49] and therefore lead to more male patients seeking care for infection.

There was no significant association between CS and colonic polyps. A sensitivity analysis showed that Omori *et al*.[26] was the source of the increased OR and the major contributor to the high heterogeneity of the analysis. Upon the removal of this study, the association of CS and colonic polyps was reduced from an OR of 8.78 to an OR of 1.44, although neither OR was significant. Interestingly, Omori *et al*. looked specifically at patients with SSA/P, while the remaining studies did not distinguish the polyp pathology subtypes. SSA/P is significantly associated with increased cancer incidence (OR: 1.77 *vs* OR: 1) and mortality (OR: 1.74 *vs* OR: 1) compared with a matched cohort at 10-year follow-up.[50] The case series study by Young *et al*.,[51] which was similar to Omori *et al*., found that 28% (26/93) of patients with SSA/P had CS presence in their GI tract in an Australian population, while 2/4 patients with tubulovillous adenomas and 5/8 patients with colonic resection (reason unspecified) had CS. At the same time, we found that in the pooled prevalence analysis, 28% of patients with CS had colonic polyps, and notably 24% had colorectal cancer. This evidence suggests an association between CS and colonic polyps is possible, notably SSA/P and colorectal cancer. It was not possible to infer any causal relationship of CS and polyps with the available data. Firstly, patients with colon cancer and polyps are much more likely to receive surveillance colonoscopy, so there is a risk of detection bias driving the effect estimates for CS. Furthermore, it is known that colonic polyps and cancers exhibit altered mucosal microbiota,[52–54] and this may include an increase in spirochetes as a consequence of changes to the ecological niche. Given the clinical importance of colonic polyps and cancer, further research to clarify if there is an association between CS with polyps and cancer is warranted.

Only one case–control study assessed colonoscopy findings, indicating that this is a neglected topic and as such no association between CS and abnormal colonoscopy could be made. Given that the current gold standard for diagnosing CS is by histological examination of biopsies taken during colonoscopy, it remains important to characterize macroscopic features of CS infection in order to better inform endoscopists when to take targeted biopsies for CS. A published Digestive Diseases Week abstract[23] reported that red spots or hyperemia and a rough surface with loss of vascular pattern were features of CS at colonoscopy examination. Given that histology is still the gold standard for diagnosing CS, studies for the associations of CS and colonoscopy abnormalities are warranted.

Anatomical locations of spirochetes were assessed in the current case series study analysis. The result showed that the ascending colon and transverse colon had slightly higher positive rates of detecting spirochetes. However, within the confirmed CS cases, the ratio of positive biopsies to the total number of biopsies taken was only 0.47. This suggests that CS infection is patchy and that the current gold standard of diagnosis may be missing more than half of CS patients. 16S rRNA sequencing using stool samples would be an ideal screening method of intestinal microbiota components including spirochetes; however, 16S rRNA sequencing using common primer sets is unable to detect *Brachyspira* genus.[13] Specific PCR primers for *Brachyspira* genus have been developed by some groups, as well as species-specific primers to *B. pilosicoli*and *B. aalborgi*,[55,56] and these approaches can be utilized for screening CS in stool samples. However, this requires a rather complicated protocol of stool sample collection to avoid environmental contamination and DNA extraction, so most clinical facilities would not be able to perform the test. Thus, more sensitive, specific, and non-invasive routine diagnostic methods of CS are urgently needed, for example, a serological test.

One important question that we could not address in our meta-analysis was whether there is a difference in pathogenicity between the two currently isolated species of human intestinal spirochetes, as none of the case–control studies and few case studies distinguished between the two CS species. As many studies used formalin-fixed paraffin-embedded tissue, the quality of DNA isolated from these tissues may be insufficient for subsequent PCR analysis in differentiating between these two species. Furthermore, the presence of yet to be isolated spirochetes species (e.g. *Brachyspira hominis*) may also contribute to this unclassified group of CS infection.[55] Interestingly, we noticed in early studies using culture methods, *B. pilosicoli*was believed to be the main species in human CS as it was easier to isolate, required a shorter incubation period, and was not as nutrient-demanding as *B. aalborgi*. However, subsequent studies refuted this observation, and by PCR, *B. aalborgi*is more commonly reported in literature. Some studies[16,57] have shown that *B. pilosicoli*and *B. aalborgi*live in different niches in the human intestinal tract, with *B. pilosicoli*more "mucus-associated" while *B. aalborgi*was more "membrane-associated"; however, its correlation with symptoms, risk factors, or treatment response are still unclear. Thus, the pathological differences between *B. pilosicoli*and *B. aalborgi*are not fully investigated, and potential bias in their prevalence due to methodological limitations in identifying *Brachyspira* spp. need to be taken into account. More studies are needed to investigate specific factors such as the variation in colonic spirochete species colonization and population diversity, the host immune response, as well as lifestyle and dietary factors that may influence spirochetosis pathology and disease outcome.[58]

Finally, we characterized the efficacy of metronidazole treatment in reducing CS-associated GI symptoms to provide indirect evidence in support of the bacteria playing a pathogenic role and improve clinical guidance for treating CS. Despite being at the early stages of understanding CS, many antibiotics have been explored as a treatment for CS and the current consensus is to use metronidazole as standard.[59] Yet we found a proportion of metronidazole-treated patients would report symptom relapses at follow-up. Whether this is due to re-infection from environmental sources of spirochetes or unsuccessful eradication of the primary infection is still largely unknown. Recently, Jabbar *et al*.[16] found that while the majority of spirochetes were eliminated with metronidazole treatment, some translocated from the colon surface to the colonic crypts and continued to reside within goblet cell granules. This may enable their continued survival despite antibiotic treatment and explain why CS recurs in many patients. A better understanding of spirochete antibiotic sensitivity profiles is therefore required to provide safer and more effective treatment approaches for CS. Eradication of the bacteria and therapeutic effects of

antibiotic treatment should be evaluated through careful pathological assessment over an expanded period of time.

Overall, the limitations of our analysis include a relatively small sample size, and the variability between studies and therefore interpretation of the data, especially related to GI symptoms, must be validated directly. The strengths of this meta-analysis include the comprehensive literature search strategy used to identify studies and the detailed review of each manuscript to obtain complete symptoms, pathology, and treatment data for analysis. In conclusion, patients with CS have a higher risk of a diagnosis of IBS, consistent with the increased risk of experiencing diarrhea and abdominal pain. Importantly, this may occur in the absence of abnormal endoscopy findings. CS may therefore represent a treatable infectious etiology for a proportion of IBS patients, and further study of their role in this condition is warranted.

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