



Original contribution

Colonic spirochetosis is associated with colonic eosinophilia and irritable bowel syndrome in a general population in Sweden^{☆,☆☆}

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Summary Irritable bowel syndrome (IBS) is a functional disorder defined by symptoms in the absence of overt pathology. Colonic spirochetosis (CS), defined by histologic observation of spirochetal strains of *Brachyspira* in colonic biopsies, is uncommon and considered of doubtful significance. We aimed to determine the prevalence of CS in the general population, identify subtle colon pathologies, and evaluate a link with symptoms of IBS. Colonoscopy was performed in 745 subjects (aged 19–70 years, mean age 51 years, 43% male) with biopsies (ileum and 4 colonic sites) from a random population sample, Stockholm, Sweden, who completed a validated questionnaire of gastrointestinal symptoms; IBS was identified by Rome III criteria. CS was identified by histology and immunohistochemistry. In a general population, 17 individuals (2.28%; 95% confidence interval, 1.2%–3.5%) were diagnosed as having CS by histology; 6 (35%) had IBS. CS was always present in the sigmoid colon, but only 14 rectal biopsies. Eosinophils were increased in colon biopsies in CS cases versus controls, in the transverse ($P = .02$), sigmoid colon ($P = .001$), and rectum ($P = .0005$) with subepithelial eosinophil clusters ($P = .053$).

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Lymphoid follicles (at any site) were present in 13 CS ($P = .0003$). There was a 3-fold increased risk of IBS in CS (odds ratio, 3.59; 95% confidence interval, 1.27-10.11; $P = .015$). Polyps and diverticular disease were similar in CS cases and controls. The prevalence of CS in a general population is 2% and associated with nonconstipating IBS. Colonic eosinophilia with lymphoid follicles may signify the presence of CS.

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1. Introduction

Colonic spirochetosis (CS) was first described in 1967, when short spirochaetes adhering to the surface of colonic epithelium were shown by electron microscopy of rectal biopsies [1]. These were also noted at light microscopy to appear as a surface “blue haze or fringe” [1]. Subsequently, CS was also seen in the appendix [2] and a possible association with simulated appendicitis (ie, no histologic evidence of acute appendicitis) was observed in the original reports [2]. Since then, variable and inconsistent gastrointestinal (GI) symptoms have been described in uncontrolled studies of CS. In a Norwegian study in which CS was diagnosed by biopsy, symptoms on referral to the gastroenterologist included diarrhea, abdominal pain, constipation, and blood in the stools [3]. A recent review of 26 patients in North Carolina, United States, with CS presenting at endoscopy over a 5-year period noted that symptoms of diarrhea or alternating bowel habit (46%) and abdominal pain (31%) were most common [4]. However, these were all referral-based studies, and to date, no population-based studies have been performed to document the link, if any, between the infection and symptoms.

The epidemiology of CS is only sporadically described and varies worldwide; in studies based on culture of feces to demonstrate spirochetes there appears to be a lower prevalence in developed countries, United Kingdom (1.5%) [5], whereas in developing countries and rural areas, there is a higher prevalence, such as in the Arab Gulf (11.4%) [6] and remote North West Australia (32.6%) [7]. When detected by histology in biopsy samples, the prevalence of CS ranges from 0.4% in a study from Japan [8], 2.5% in Norway [3] to 16.5% in a Greek population [9]. This infection has a higher prevalence in homosexual men—from a specialist clinic, 30% had infection with a positive association with gonorrhea but not symptoms [10] and CS has also been noted in men with HIV infection [11].

Spirochetosis in the colon is defined by histologic observation of spirochetal strains of *Brachyspira* (*pilosicoli* or *aalborgii*) adherent to colonic epithelium, usually seen in biopsies taken at colonoscopy for investigation of GI symptoms. Microscopically, there are no definitive or consistent mucosal inflammatory lesions reported in association with spirochetosis [2,4,12], although a study from Italy suggested pathogenesis may be due to loss of colonic microvilli when biopsies were examined by transmission electron microscopy [13]. CS has also been suggested to be

tentatively associated with hyperplastic and adenomatous polyps in a small number of cases [14,15]. Histology is said to be normal in most of these cases—however, this is on looking for overt active (neutrophil) infiltration, not subtle changes of innate inflammation (eg, increased eosinophil infiltration) [12]. We have previously described that in functional disorders, pathology is linked to innate immunity [16]. Increased numbers of lymphoid follicles and aggregates in colon mucosal biopsies have also been shown to be associated with persistent diarrhea of unspecified etiology [17].

A study in the general population could clarify any association of CS with histology and symptoms. The PopCol study is a unique epidemiologic study of GI symptoms with colonoscopy and biopsy in randomly selected subjects in Stockholm, Sweden [18,19]. The aim of this study was to investigate the prevalence of CS in a general population by identification through colon biopsy and to ascertain if there were links with subtle pathologies and symptoms in this group.

2. Materials and methods

2.1. Subjects

This article reports on 2 samples. The first consists of all individuals on whom colonoscopy with biopsies was performed ($n = 745$ subjects, aged 19-70 years, mean age 51 years, 49% male) and biopsies taken from the ileum and 4 sites in the colon as part of a population-based questionnaire and colonoscopy study in Stockholm, Sweden, the PopCol (Population-based Colonoscopy) study, which has previously been described in detail [19]. The sample population was sociodemographically similar to the background population, consisting of 49% men (Fig. 1). The 745 who underwent colonoscopy also completed a validated questionnaire of GI symptoms, the abdominal symptom questionnaire [20], and the Rome II questionnaire for functional bowel disorders [21]. The study was approved by the local ethics committee, Forskningskommitté Syd at Karolinska Institutet, Stockholm, Sweden, and all participants gave informed consent.

The second sample consists of the subset of sample 1 who had also histopathology performed and in whom spirochetosis was identified plus 1:1 age (± 2 years) and sex-matched controls who were free of spirochetosis.

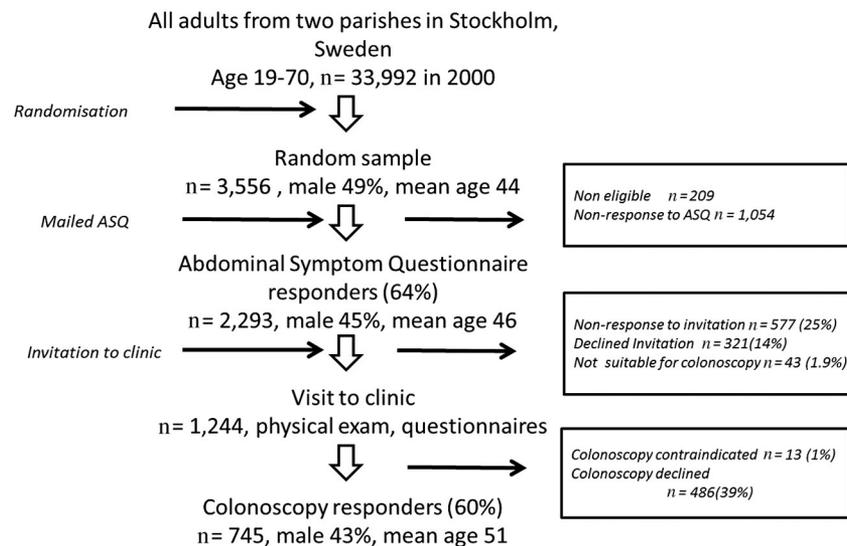


Fig. 1 Study population identification and selection.

2.2. Irritable bowel syndrome

Irritable bowel syndrome (IBS) was defined by applying Rome III criteria as having abdominal pain or discomfort for at least 3 weeks (at least 1 day in each week) in the last 3 months, with at least 2 of 3 of the following: pain or discomfort improved by defecation, onset of pain or discomfort associated with a change in frequency of stool, or onset of pain or discomfort associated with change in stool consistency [22]. IBS-diarrhea (D) was defined as having IBS and reporting loose, mushy, or watery stools. IBS-constipation (C) was defined as having IBS and reporting hard or lumpy stools. IBS-mixed (M) was defined as having IBS and reporting both loose, mushy, or watery stools and hard or lumpy stools. IBS-undefined (U) was defined as IBS but without fulfilling the criteria for IBS-D, IBS-C, or IBS-M. IBS-associated symptoms included abdominal pain, abdominal discomfort, constipation, diarrhea, alternating bowel habit, pain relieved by defecation, pain when defecating, urgency, passing excessive gas, borborygmi, bloating, and passing mucus, assessed using the validated abdominal symptom questionnaire [20].

2.3. Histopathology

At colonoscopy, biopsies were taken from the distal ileum, cecum, transverse colon, sigmoid colon, and rectum. In addition, any overt lesion or polyp was biopsied. Biopsies were assessed by routine histopathology and assessed by 2 pathologists independently (M. M. W. and A. O). CS was identified in biopsies by hematoxylin and eosin (H&E) staining showing a blue fringe effect on the surface of colonocytes and confirmed by immunohistochemistry for *Brachyspira aalborgii* [23]. The numbers of biopsies present on slides were matched for cases and controls. Light microscopy included observation and exclusion of lympho-

cytic colitis and collagenous colitis, granulomata, neutrophil infiltration, eosinophils/mm², lymphoid aggregates/follicles, and intraepithelial lymphocyte counts/100 colonocytes. Lymphoid follicles were assessed by number per biopsy and site of biopsy.

2.4. Statistics

From sample 1, disease prevalence (IBS, polyps, and diverticulosis) has been described overall in the PopCol study as counts of individuals and percentages with 95% confidence intervals (CIs). The associations between spirochetosis and other conditions (Rome III IBS, polyps, and diverticular disease) have been assessed via odds ratios (ORs) reported with 95% CIs, estimated from unconditional logistic regression. ORs greater than 1.0 indicate spirochetosis is associated with higher odds of the other disease, while values less than 1.0 indicate lower odds. From sample 2, numeric scores of cells were compared between CS cases and CS-free controls via the Wilcoxon signed ranks test and, for the yes/no variables, McNemars test for paired proportions.

3. Results

3.1. Demographics

A total of 17 individuals (2.28% of all; 95% CI, 1.2-3.4) were diagnosed as having CS by histology. By light microscopy, CS was present in colonic but not terminal ileal biopsies. There was no significant difference in age (mean 49 years, range 27-69 years) or sex (9 men) in cases of spirochetosis versus controls with no CS. No cases of CS had known HIV infection, but 1 case had hepatitis C. In cases of spirochetosis, 2 cases were taking nonsteroidal anti-

inflammatory drugs (NSAIDs); in controls, 3 subjects were taking NSAIDs. There were no significantly higher eosinophil counts in the cases or controls taking these drugs. No subjects were taking proton pump inhibitors.

3.2. Histopathology

Spirochetosis was diagnosed by light microscopy by the presence of a “blue fringe” adherent to the epithelial surface (H&E staining, Fig. 2). Spirochetes were confirmed by immunostaining (Fig. 2) [23]. No overt pathology (granulomata, neutrophil infiltration, lymphocytic or collagenous colitis) was seen in cases with spirochetosis. No cases had an intraepithelial lymphocyte count above 15/100 colonocytes at any site. No cases of lymphocytic colitis or collagenous colitis were seen in subjects with CS or controls.

Eosinophils were significantly increased in colon biopsies in those with CS in the transverse colon (mean 30/mm² compared with controls, 17/mm²; $P = .015$), sigmoid colon (mean 30/mm² compared with controls, 9/mm²; $P = .0009$), and rectum (mean 17/mm² compared with controls, 4/mm²; $P = .0005$). In non-CS IBS subjects, eosinophils were not significantly increased compared with control subjects (Table 1). We also observed more subepithelial eosinophil clusters in CS present in 50% of CS cases versus 3% of controls in the sigmoid and rectal sites ($P = .053$). Lymphoid follicles at any site were present in 13 cases versus none of the controls ($P = .0003$; Fig. 3). In these cases, CS was always present on immunohistochemistry and H&E in the sigmoid colon, but was only present in 14 of the rectal biopsies.

3.3. Irritable bowel syndrome

For IBS as defined by Rome III, 6 (37%) with full questionnaire data had IBS: 2 IBS-D, 2 IBS-M, and 2 IBS-U [22]. There was more than a 3-fold increased risk of IBS in

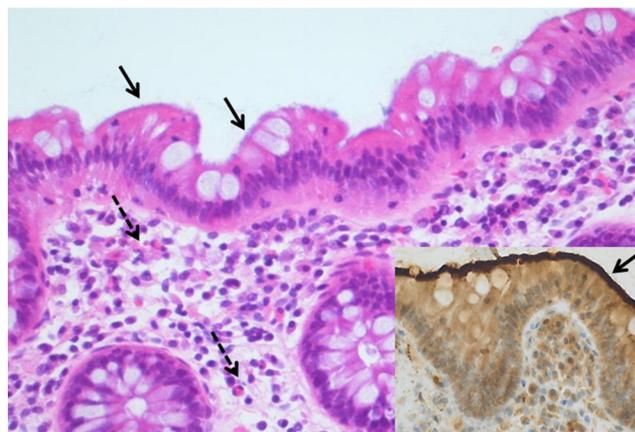


Fig. 2 Spirochetosis on the surface of colonic epithelium (arrows), eosinophils in lamina propria (dashed arrows), and immunocytochemical staining (inset) of *B aalborgii* spirochetes on the surface of colonic epithelium (arrow). H&E.

Table 1 Eosinophilia in colon sites in CS

Eosinophils	Cases with spirochetosis		Controls without spirochetosis		<i>P</i>
	Mean	95% CI	Mean	95% CI	
Ileum	20	13.3-26.3	14	7.0-20.6	.23
Cecum	32	26.3-38.5	33	21.4-45.6	.82
Transverse colon	30	23.2-36.0	17	10.4-22.8	.015
Sigmoid colon	30	23.6-36.4	9	5.4-12.0	.0009
Rectum	17	13.6-19.8	4	2.4-6.0	.0005

those with CS compared with controls with no IBS (OR, 3.59; CI, 1.27-10.11; $P = .01$; Table 2).

3.4. Individual symptoms

There was no significant difference in comparing the individual symptoms of abdominal pain or discomfort, constipation, diarrhea, pain relieved by defecation, abdominal bloating, alternating diarrhea and constipation, urgency, or passing excessive flatus in subjects with and without CS.

3.5. Endoscopy findings

Of 17 cases of CS at colonoscopy, 6 (28%) had colonic polyps. There was no significant difference in the prevalence in polyps in cases versus noninfected subjects in the overall colonoscopy population (27%) [24] and no significant difference in the histologic type of polyp (3 with tubular adenomas, 3 with hyperplastic polyps). Of 17 cases of CS at colonoscopy, 2 (12%) had diverticular disease; there was no significant difference in the prevalence in cases of CS with diverticular disease versus non-infected subjects in the overall colonoscopy population (18%; Table 2).

4. Discussion

This study has shown that the prevalence of histologic CS in a general population in Stockholm, Sweden, is 2%, with

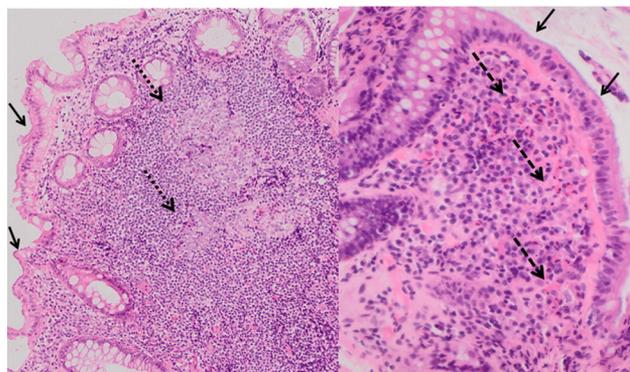


Fig. 3 Lymphoid follicle (dotted arrows) and eosinophil clusters (dashed arrows) in lamina propria with spirochetes on the surface of colonic epithelium (arrows). H&E.

Table 2 Spirochetosis and associated conditions in a general population in Stockholm, Sweden

	Spirochetosis	No spirochetosis	OR (95% CI)	P
No. of subjects (%)	17/745 (2.3%)	728/745 (97.7%)		
Age (y), mean (range)	49 (27-69)	51 (19-70)		.98
% by sex (male)	53	49		.74
Rome III IBS, n (%)	6/17 (35%)	104/728 (14.3%)	3.59 (1.27-10.11)	.015
Polyps	5/17 cases (29%)	206/728 (28.3%)	(25.1-31.8)	.53
Diverticular disease	2/17 (12%)	128/728 (17.7%)	(14.9-20.6)	.53

an equal male-to-female ratio. As these subjects were not health care seeking, this reflects a true prevalence of this condition in this community as a whole. We found spirochetosis to be positively associated with IBS and in particular nonconstipation IBS. Of interest, colon mucosal histopathology shows previously unreported subtle changes in CS with eosinophilia with clusters in the superficial mucosa and increased lymphoid follicles and aggregates, which suggests that pathologists should exercise vigilance for the presence of spirochetes in biopsies when these features are seen at microscopy. CS was always present in the sigmoid, but not in rectal biopsies in all cases.

There are differing views in the literature as to whether spirochetes are responsible for abdominal symptoms. Several studies describe CS as an incidental finding, not associated with symptoms [2,8,10,12]. However, most of these studies were retrospective with imprecise clinical data available. It is notable that in our study, subjects independently completed validated symptom questionnaires which detailed current abdominal symptoms at the time of biopsy.

IBS is a functional bowel disorder defined by chronic symptoms, namely, abdominal pain or discomfort associated with an erratic bowel habit and often bloating in the absence of known overt pathology [22,25]. Previous studies have suggested that CS might be associated with abdominal pain and alternating bowel habits, but these were not definitive and there are no prospective studies on an association with IBS. From the United States, in a study of 26 patients with CS, 31% presented with abdominal pain and 46% with alternating bowel habits [4]. In a study from Norway, the predominant symptoms in 30 cases were diarrhea, abdominal pain, and constipation [3], and in a large study from Germany of 209 patients, 46% presented with abdominal pain, 51% had diarrhea, and 13% had alternating bowel habits [26]. In Australia in 113 cases of CS, diarrhea with and without abdominal pain was seen in 26% of those affected, with 11% having a change in bowel habit [12]. These studies, alongside our observations in the PopCol study, support a role for CS in generation of the conglomeration of symptoms in IBS, and therefore, investigation for CS may be warranted in IBS, especially if diarrhea is present even if mixed with constipation. Importantly, in an uncontrolled study of 84 patients undergoing antibiotic treatment, eradication of spirochetes improved symptoms in 55% of patients, although the specific symptoms which improved were not described

[26]. The role of CS in chronic watery diarrhea is unclear, but 2 studies, one by histologic diagnosis [27] and one by diagnosis from fecal culture [7], suggest a link with this condition and CS. In our study, the symptom of diarrhea alone was not significantly associated with the presence of CS on histology in contrast to the symptom complex of IBS.

The overall prevalence of polyps in this population was 27% [24], and polyps were present in 29% of cases with CS. None of the subjects with CS in this study had positive fecal occult blood test results, but blood in the stools is reported in some studies of CS [4,12,26], and a positive fecal occult blood test result may herald the presence of neoplasia rather than CS [26]. CS has been described colonizing surfaces of polyps (adenomas and hyperplastic polyps, as was seen in our series) [14,15,26]. However, we could not detect an association of CS with colon polyps of any particular type. A study from California, United States, specifically looking at incidental pathology associated with colonic polyps, found 0.5% of cases of polyps harbored CS, but only on nonpolypoid adjacent mucosa [28].

Microscopy in CS shows the typical appearances of a blue fringe on epithelial cells on H&E staining. Confirmation can be made by Warthin and Starry or Giemsa staining, readily available in most histopathology laboratories, by immunostaining for *B aalborgii* [23] or other treponemes [8]. Mild mucosal pathology such as increased intraepithelial lymphocytes [4] or increased chronic inflammation in the lamina propria [8,27,29] has been described, but most studies failed to identify changes by light microscopy in CS [4,9,12]. No cases of lymphocytic or collagenous colitis were seen in this study in accord with a series from Germany where this pathology was not a feature in CS [26]. Mild eosinophilia (mean, 30/mm²), with clusters and the presence of lymphoid follicles and aggregates in the transverse colon, sigmoid colon, and rectal biopsies is a novel and important finding in our population-based study. In a small study of rectal biopsies from 2 cases of spirochetosis, IgE-containing plasma cells were increased, up to 10-fold, compared with controls, which supports a TH2-type response to CS, as seen in our study [30]. Drug use (NSAIDs) had no significant effect on the presence of spirochetosis or eosinophilia. Drug use (NSAIDs) had no significant effect on the presence of spirochetosis or eosinophilia. No subjects were taking proton pump inhibitors. Lymphoid follicles and lymphoid aggregates

have been suggested to be a marker for mucosal damage and described in cases of unresolved diarrhea [17].

We suggest that based on our observations, a mild increase in eosinophils and lymphoid follicles should guide clinicians to seek a cause for IBS including CS, rather than labeling patients as having a purely “functional” disorder.

The pathogenesis of symptomatic CS has been studied at the electron microscope level. The spirochetes are seen to be anchored between colonic microvilli [9]. There is destruction of microvilli in some but not all cases, and it has been speculated that symptomatic CS may be linked to this finding [13,31]. Invasion of the epithelium has been observed by electron microscopy, and a recent review of this phenomenon concluded that this may be associated with symptoms—in 7 cases with invasive spirochetosis as seen by electron microscopy, all had diarrhea at presentation [32].

In a review of spirochetosis from Germany, an analysis of the literature shows that 7.6% of all reported cases have received antibiotics, in which unspecified symptomatic improvement occurred in 51% of patients. It was concluded the treatment of choice for CS is metronidazole. In the largest series to date, of 84 (40.2%) of 209 patients receiving therapy, 52% had resolution of their symptoms with metronidazole, and follow-up showed resolution of spirochetosis at colonoscopy and biopsy in 77% [26].

The strengths of this study include that this is a random population-based study with a large number of participants that represent a wide age range and therefore representative of the population as a whole [19], and thus represented true CS prevalence diagnosed on histology, which is poorly described in the literature. Also, this was a prospective study, with collection of clinical data by validated questionnaires at the time of biopsy, and therefore, symptoms are related to real-time histology and not dependent on recall after this event. The weaknesses of the study are that subjects with CS were not subsequently treated to ascertain if this resolved symptoms, particularly in those with IBS.

In conclusion, we observed that CS is significantly associated with IBS, but not colonic polyps or diverticular disease. Subtle eosinophilia and lymphoid follicles/aggregates were associated with CS on biopsy, and the presence of these features should alert the pathologist to this condition. Although biopsies of the sigmoid colon were always positive for CS, rectal biopsies were not consistently reliable for diagnosis. Previously, CS has been tentatively described in association with polyps, but a link with IBS has not been recognized. This observation should be further explored, and a randomized controlled trial is needed to determine if treatment of CS resolves symptoms of IBS in a subset infected with CS.

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