GASTROENTEROLOGY

Intestinal spirochetosis and chronic watery diarrhea: Clinical and histological response to treatment and long-term follow up

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Abstract

Background: The clinical significance of intestinal spirochetosis is uncertain, therefore the aim of the present paper was to assess the prevalence of histological intestinal spirochetosis in patients with and without chronic watery diarrhea and to evaluate its clinical relevance.

Methods: A prospective diagnostic work-up of intestinal spirochetosis was made on biopsy samples taken from patients with chronic watery diarrhea submitted between 1994 and 2004 (1174 colonoscopies with multiple biopsies). Three other positive cases identified from routine endoscopic biopsies also were reviewed. In addition, samples from 100 asymptomatic control patients and a random sample of another 104 colonic specimens were reviewed for intestinal spirochetosis. The diagnosis was established by light and electron microscopy. Polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA and reduced nicotinamide adenine dinucleotide (NADH) oxidase genes of the intestinal spirochetes Brachyspira aalborgi and B. pilosicoli was performed on tissue biopsies of the 11 positive patients. After diagnosis, treatment with penicillin benzatine (PB) or metronidazole was offered to all symptomatic patients and they were followed for a mean of 45.4 months (range: 37–113 months).

Results: Eight patients with chronic watery diarrhea were positive for intestinal spirochetosis. Intestinal spirochetosis was not diagnosed in the controls. Histological resolution of the infection paralleled clinical recovery in six patients (following metronidazole treatment in three). Most patients showed mild, non-specific colonic inflammation. Invasion by the spirochetes was not demonstrated by electron microscopy. Brachyspira aalborgi and B. pilosicoli each were identified by PCR in two cases.

Conclusions: Histological intestinal spirochetosis appears to be relatively uncommon in Catalonia (Spain) compared to previous reports from other countries, but was identified in patients (0.7%) with chronic watery diarrhea. Sustained clinical recovery after spontaneous or drug-induced spirochetal disappearance in these individuals suggests that intestinal spirochetosis may play a pathogenic role in chronic watery diarrhea. Treatment with metronidazole is advisable in patients with persistent symptoms.

Key words
chronic watery diarrhea, intestinal spirochetosis, metronidazole, microscopic colitis, penicillin.

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Introduction

The clinical relevance of intestinal spirochetosis (IS) is controversial.1–3 The occurrence of spirochetes attached to the luminal surface of the colorectal mucosa has been documented in various case reports and studies of series of patients with intestinal complaints, mainly consisting of watery diarrhea.4–9 In addition, epidemiological studies have shown that chronic diarrhea or wet feces were significantly associated with colonization by intestinal spirochetes in Australian Aboriginal children,10 and in people on the island of Bali.11 There also have been studies in which intestinal spirochete strains isolated from humans have been used to infect and cause
intestinal disease in chickens and pigs. In contrast, in studies where colonic specimens or feces of a large series of patients were assessed, IS was a frequent finding, ranging from 1.9 to 50%, being particularly common in developing communities. In some studies symptoms were not modified by IS eradication, leading to the suggestion that intestinal spirochetes are harmless commensals in humans, and thus specific treatment is not required. This apparent discrepancy in outcomes might be due either to a variable pathogenicity of different spirochete species or strain, or the immunological status of the infected individuals. At least two species of spirochetes, Brachyspira pilosicoli and Brachyspira aalborgi, have been associated with IS in humans, and B. pilosicoli has been isolated from the blood of critically ill patients. In addition, electron microscopic analysis of specimens from some infected individuals has shown spirochetal invasion of colonic epithelial cells, macrophages, goblet cells and Schwann cells.

The aims of the present study were (i) to assess the histological prevalence of IS in adult patients attending a tertiary hospital in Catalonia (Spain); (ii) to evaluate the clinical relevance of IS, mostly diagnosed in the setting of a prospective evaluation of chronic watery diarrhea; and (iii) to determine the spirochete species present in IS cases.

Methods

Patients and controls

Patients with chronic watery diarrhea

A prospective diagnostic work-up was made of biopsies taken from patients with chronic watery diarrhea but a normal appearing mucosa. Procedures followed those established in the Gastroenterology Department of Mutua Terrassa Hospital, Barcelona, which have been previously described in detail. Briefly, total colonoscopy was undertaken and 12 colonic biopsies of five areas of the colon, from the cecum to the rectum, were taken during the course of 1174 colonoscopies (one colonoscopy per patient) performed between 1 January 1994 and 31 December 2004.

Samples from routine biopsies

During the same period, IS had been identified in biopsy samples from three patients without chronic diarrhea, and these cases also were reviewed.

Controls

One hundred patients attending the Endoscopic Unit due to rectal bleeding secondary to anorectal pathology or having a previous history of colonic polyps were included as controls (54 men and 46 women; mean age 48.0 years; range 26–74 years). All these patients had either normal bowel habit or constipation. After informed consent was obtained, multiple biopsies were taken from five areas of the colon, following the same previously described protocol. In addition, a random sample of 104 colonic specimens of patients (67 men and 37 women; mean age 67.28 years; range 4–98 years) operated on within the study period were selected and the slides were reviewed for IS identification. Colonic surgery was performed due to colonic neoplasms (50 cases), diverticular disease (49 cases), irradiation colitis (1 case), Hirschsprung disease (one case) and inflammatory bowel disease (three cases).

Biopsy handling and pathological diagnosis

The biopsy specimens were immediately fixed in 4% phosphate-buffered formaldehyde, pH 7.0 and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE). The diagnosis of IS was established by light microscopy and was confirmed by periodic acid-Schiff stain (PAS) and Steiner silver stains. Because it has been suggested that up to half of IS cases may be overlooked on HE, PAS and Steiner silver stains were performed in all of the control biopsies in spite of a normal appearance on HE.

Biopsy specimens from four patients with IS were examined by electron microscopy before and after treatment. Biopsies were fixed in 2.5% cacodylate-buffered glutaraldehyde, pH 7.4 and embedded in epoxy resin. Two blocks from each case were processed and five ultra-thin sections from each block (10 grids per case) were performed. Three additional colonic samples from IS patients were recovered from formaldehyde-fixed archival tissue, and were deparaffinized and processed for electron microscopic examination. Sections approximately 100 nm thick were cut with a diamond knife and stained with uranyl acetate and lead citrate, and examined under a Philips CM100 transmission electron microscope (Philips, Eindhoven, the Netherlands).

On light microscopy, the following histological findings were recorded: (i) inflammatory infiltrate in the lamina propria; and (ii) epithelial damage: flattening and detachment of the surface epithelium, crypt distortion with polymorphonuclear infiltration and increased number of intraepithelial lymphocytes. The inflammatory infiltrate was subjectively assessed. Care was taken to record the situation when there was an even distribution of cells (lymphocytes, plasma cells and eosinophils) extending into the deeper portion of the lamina propria, as opposed to the usual situation in the normal mucosa, where cells are concentrated in the upper third of the lamina propria.

On electron microscopy, IS was confirmed and the following features were recorded: (i) abnormalities in epithelial cell surface; (ii) intraepithelial spirochetal fragments; and (iii) presence of spirochetal remnants in the phagolysosomes of macrophages.

Identifying spirochete species

DNA from paraffin-embedded tissue (PET) samples from the IS patients was extracted using a modification of a previously described method, with samples from positive and negative control patients included in each analysis. Several sections approximately 10 μm thick were cut from the PET samples. Each sample was dewaxed by adding 400 μL of xylene and placing on a rocker at room temperature until the paraffin dissolved. A total of 400 μL of 100% ethanol was then added, and the samples were centrifuged at 10 000 × g for 10 min, and the supernatant discarded. The samples were dried at 50°C in an oven for at least 30 min. Twenty micrograms of Proteinase K (Boehringer Mannheim, Mannheim, Germany) in 200 μL of 50 mmol/L Tris-HCl, pH 8.3, was added and incubated for 1–2 h at 55°C. The samples...
were then boiled for 8 min, and 2 µL of each resultant extract was used as template DNA for polymerase chain reaction (PCR) analysis.

Specific PCR procedures amplifying portions of the 16S ribosomal RNA (16S rRNA) and reduced nicotinamide adenine dinucleotide (NADH) oxidase (nox) genes of *B. aalborgi* and *B. pilosicoli* were applied to DNA extracted from these tissue biopsies. The PCR products were subjected to electrophoresis in 1.5% (wt/vol) agarose gels in 1× Tris-acetate buffer, stained with ethidium bromide and viewed over ultraviolet (UV) light.

### Clinical assessment of the IS patients and follow up

Following diagnosis, a prospective clinical evaluation, treatment and follow up were offered to all IS patients. Sources of infection were prospectively investigated: sexual habits, employment, travel to endemic areas and possible ingestion of non-potable water. Evaluation of patients at diagnosis included anamnesis, asking about drug consumption (non-steroidal anti-inflammatory drugs, antibiotics, etc.), symptoms (fever, weight loss, abdominal pain, diarrhea, number of bowel movements, duration of diarrhea) and physical examination. In addition, bacterial culture for pathogenic aerobic bacteria and fecal examination for ova and parasites (assessed by stool concentration in samples taken on 3 consecutive days), routine blood analysis and examination for antibody to *B. aalborgi* and *B. pilosicoli* were then boiled for 8 min, and 2 µL of each resultant extract was used as template DNA for polymerase chain reaction (PCR) analysis. Other results were expressed as mean and range.

### Data analysis

The prevalence rates for IS in patients with chronic watery diarrhea and in the controls were compared using Fisher’s exact test. Other results were expressed as mean and range.

### Ethical considerations

The study was performed according to the 1995 Declaration of Helsinki ethical guidelines, and was approved by the research and ethical committees of Mutua Terrassa Hospital, Barcelona.

### Results

#### Controls

Intestinal spirochetosis was not identified in any of the control endoscopic or surgical biopsy specimens. The use of PAS and Steiner silver stain did not identify any additional cases. The control individuals recruited in the endoscopic unit had a normal colonic mucosa on histological examination.

#### Patients with chronic watery diarrhea

Intestinal spirochetosis was identified in biopsies from eight of the 1174 patients with chronic watery diarrhea (0.7%). This prevalence was not statistically different from the prevalence in the control group (*P* = 0.6133). Seven of the IS patients were male. The mean age of the eight patients was 47 years (range 29–72 years). Six of these IS cases (cases 1–5, 7) were diagnosed between January and October 2000. Clinical, endoscopic and histopathological findings and follow-up data for the patients (cases 1–8) are summarized in Table 1. One of these patients had been diagnosed with acquired immunodeficiency syndrome (AIDS) 4 years before IS diagnosis, and was admitted to the hospital due to a reactivation of visceral Leishmaniasis (case 6). Except for this patient, the hematological and biochemical parameters of all patients were normal. The AIDS patient, and one other patient (case 3) were homosexual. The mean number of bowel movements by the patients per day was 4.8 (range 3–8). The mean duration of diarrhea at the time of diagnosis was 12 months (range 1–48 months) and, with the exception of the AIDS patient, it was well-tolerated, and not of major clinical concern.

#### Cases identified in routine biopsy examination

Intestinal spirochetosis was identified in biopsies taken from three other patients with non-specific ulceration of the ileocecal valve, ischemic ulcers, or colonic polyps (cases 9, 10 and 11, respectively: one female and two male; Table 2). Patient 11 worked in a pig and chicken slaughterhouse, where he drank non-potable water.

#### Other potential pathogens

In all 11 IS cases, fecal bacterial cultures for pathogens and examination for ova and parasites were negative.

#### Histopathological findings

Multiple biopsies were available from 10 patients. In six patients a homogeneous distribution of IS was observed throughout the colon. By light microscopy, the presence of a layer of spirochetes attached to the colonic epithelium on HE, PAS and Steiner silver stains was observed in all cases (Fig. 1). All except three patients showed increased inflammatory infiltrate of the lamina propria, which was mild in most cases. Histological findings are detailed in Tables 1 and 2.

Electron microscopy confirmed the presence of a dense band of spirochetes adherent to the colonic surface (Fig. 2). Mild degenerative changes of the epithelium, consisting of stunting and fragmentation of microvilli, were detected in one case. Electron microscopy was also performed on tissue retrieved from the paraffin blocks of the two patients with ulcers in the colonic mucosa. No invasive spirochetes were detected in the enterocytes, goblet cells, macrophages or Schwann cells in any case.

Histopathological follow up was available for eight of the 11 IS patients (four by electron microscopy). After IS disappearance (both spontaneous or therapy-induced), most histopathological abnormalities persisted on light microscopy (Fig. 3). Intestinal spirochetosis was not identified by electron microscopy in any of the specimens obtained during the follow up (Fig. 4).
Table 1  IS patients diagnosed in the prospective diagnostic work-up of chronic diarrhea (cases 1–8)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical features and response to treatment</th>
<th>Colonic distribution of IS</th>
<th>Lamina propria infiltrate</th>
<th>Epithelial lesion</th>
<th>PMN activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (male, 29 years)</td>
<td>Intermittent chronic diarrhea</td>
<td>Right colon sparing</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Persistence of symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After PB treatment</td>
<td>Watery chronic diarrhea</td>
<td>Rectal sparing</td>
<td>Increase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Case 2 (male, 56 years)</td>
<td>Partial improvement</td>
<td>Patchy distribution</td>
<td>Increase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Symptom disappearance</td>
<td>Cure</td>
<td>Increase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>After MZ treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>Increase</td>
<td>Yes</td>
<td>Yes (focal)</td>
</tr>
<tr>
<td>Case 3 (male, 43 years)</td>
<td>Partial improvement</td>
<td>Entire colon</td>
<td>Mild increase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Symptom disappearance</td>
<td>Cure</td>
<td>Mild increase</td>
<td>No</td>
<td>Yes (focal)</td>
</tr>
<tr>
<td>After MZ treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 4 (male, 57 years)</td>
<td>Watery chronic diarrhea</td>
<td>Colon sparing</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Intermittent chronic diarrhea</td>
<td>Right colon sparing</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>After PB treatment</td>
<td>Watery chronic diarrhea</td>
<td>Rectal sparing</td>
<td>Increase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Case 5 (male, 60 years)</td>
<td>Partial improvement</td>
<td>Entire colon</td>
<td>Mild increase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Symptom disappearance</td>
<td>Cure</td>
<td>Mild increase</td>
<td>No</td>
<td>Yes (focal)</td>
</tr>
<tr>
<td>After MZ treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 6 (male, 30 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Improvement</td>
<td>Cure</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>After pentamidine</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>Mild increase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 7 (male, 30 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Watery chronic diarrhea</td>
<td>Rectal sparing</td>
<td>Increase</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>After PB treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>Mild increase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 8 (female, 72 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>After MZ treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 9 (male, 66 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Follow up (not treated)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 10 (female, 87 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>After MZ treatment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Case 11 (male, 41 years)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Follow up 12 months (not treated)</td>
<td>Watery chronic diarrhea</td>
<td>Entire colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

EM, electron microscopy; IS, intestinal spirochetosis; MZ, metronidazole 2 g/day (500 mg q.i.d.) for 10 days; PB, penicillin benzathine 2.4 MU i.m. in a single dose; PMN, polymorphonuclear cells.

1Lymphocytic exocytosis diagnostic of lymphocytic colitis.

Table 2  IS patients diagnosed in routine biopsy examination (cases 9–11)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Colonoscopic findings</th>
<th>Clinical features and response to treatment</th>
<th>Colonic distribution of IS</th>
<th>Lamina propria infiltrate</th>
<th>Epithelial lesion</th>
<th>PMN activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 9 (male, 66 years)</td>
<td>Non-specific ulceration of the ileocecal valve</td>
<td>Malaise and weightloss Melena due to duodenal ulcer</td>
<td>IS in the edge of the cecal ulcer</td>
<td>Mild increase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Normal</td>
<td>Recovery. Cecal and duodenal ulcer healing</td>
<td>Spontaneous cure (multiple biopsies)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Follow up</td>
<td>Extensive areas of inflammation. Ulcers and thickened folds</td>
<td>Abdominal pain</td>
<td>Ischemic ulcers entire colon</td>
<td>Increase</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>(not treated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 10 (female, 87 years)</td>
<td>Normal (adenomatous polyp)</td>
<td>Asymptomatic</td>
<td>IS in the right colon</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Normal</td>
<td>Asymptomatic</td>
<td>Sigmoid and rectal sparing</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>No follow up (death)</td>
<td>Normal</td>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 11 (male, 41 years)</td>
<td>Normal</td>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial assessment</td>
<td>Normal</td>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow up 18 months (not treated)</td>
<td>Normal</td>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IS, intestinal spirochetosis; PMN, polymorphonuclear cells.
Polymerase chain reaction analysis

Spirochete DNA was amplifiable in only four of the 11 cases. For cases 2 and 11, the PCR were positive for *B. pilosicoli*, while the PCR were positive for *B. aalborgi* in cases 3 and 8. *Brachyspira pilosicoli*-specific amplification was also obtained in a sample from case 2 after unsuccessful treatment with penicillin benzathine.

Response to treatment and follow up

All symptomatic patients were followed for a mean of 45.4 months (range: 37–113 months). Four patients were treated with penicillin benzathine. In one patient (case 1) the diarrhea persisted after treatment, but he refused an additional colonoscopy and treatment. At the end of follow up, persistence of intermittent watery diarrhea was recorded. Two patients (cases 2 and 3) showed a partial clinical improvement, but follow-up biopsies showed persistence of IS infection on light microscopy in patchy areas of the colon. In these two cases, treatment with metronidazole resolved the diarrhea and follow-up biopsies showed the disappearance of spirochetes from the colonic surface. The remaining patient (case 4) was first diagnosed with IS in 1994, and treated with penicillin benzathine. A good clinical and histological response was observed after treatment. Six years later he was again re-evaluated due to chronic diarrhea over the previous 8 months. Multiple biopsies of the colon again showed the presence of IS in all the samples. Biopsies taken in the previous IS infection were then recovered from formaldehyde-fixed archival tissue and examined by electron microscopy, confirming the presence of small numbers of spirochetes on the colonic surface, in biopsies taken after penicillin benzathine treatment. The patient was treated with metronidazole, and clinical and histopathological cure occurred.

Two more patients showed self-limited symptoms (cases 5 and 9), and control biopsies showed spontaneous resolution of IS. In the AIDS patient (case 6), gastrointestinal involvement by Leishmania (Fig. 5) was considered to be the main cause of chronic diarrhea, and consequently he was treated with pentamidine 4 mg/kg per day. Colonic biopsies taken 2 months later did not identify either IS or Leishmania. In two patients (cases 7 and 10), clinical and pathological follow up was not available. One patient (case 7) refused to participate in the study and did not receive treatment. At the end of follow up he was contacted by telephone and reported persistence of mild chronic diarrhea. The other patient (case 10) died during hospital admission due to complications related to intestinal ischemia.
Patients 8 and 11 remained asymptomatic in spite of IS persistence for more than 1 year after diagnosis. No specific treatment was administered.

**Discussion**

To our knowledge this is the first study evaluating the prevalence of histological IS in both patients with and without chronic watery diarrhea. In addition, it includes the longest follow up of symptomatic patients. No IS was diagnosed in 204 control subjects, while IS was diagnosed in eight (0.7%) of 1174 patients evaluated for chronic watery diarrhea. The prevalence in the present study is much lower than previously reported in Western countries (1.1–5%), and, perhaps as a result of this, the prevalence rates in the control and chronic diarrhea groups were not statistically different. A much larger control group (>650 individuals) would have been required to obtain a statistically significant difference between the groups. Most previous studies assessing the histological prevalence of IS have been performed using biopsies of the rectosigmoid area or in segmental resection surgical specimens. In the present study multiple biopsies of five areas of the colon were taken from all the patients with chronic diarrhea and from the healthy controls. Thus, taking into account that IS may have a patchy distribution with rectal sparing, differences in prevalence with previous reports could be even higher.

In contrast to previous studies, the routine use of PAS and Steiner silver stains did not increase the number of diagnosed cases in the present study. In fact, the characteristic fuzzy blue

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**Figure 3** Colonic biopsies of the same patient (a) before and (b) after treatment. The bacterial layer has disappeared but surface epithelial damage and inflammation persist (H&E).

**Figure 4** (a) No spiral-shaped microorganisms were detected after treatment (electron microscopy [EM] x4600). (b) Prominent lysosomes are present in the macrophage from the lamina propria (EM x25 000).
been previously described in a number of studies. led a clinical sustained recovery. This is consistent with what has been the infection, whether spontaneous or following treatment, paralysed. The best evidence that IS was a cause of the chronic watery diarrhea biopsies, where IS had been recorded as an incidental finding. The histological sections stained with HE, PAS or Steiner silver stains. archival tissue, while persistent infection was not detected in his biopsies of only four patients. In part, this low identification rate may have been due to the prolonged storage of the samples prior to PCR amplification in 2004, with resultant degradation of the DNA. Another possibility is that the remaining cases may have contained other species of spirochetes that have yet to be characterized. The existence of novel Brachyspira species in human IS has been reported.

No significant differences were found in the occurrence of IS infection between patients with and without chronic diarrhea, while only three cases of IS were obtained from >20,000 routine biopsies, where IS had been recorded as an incidental finding. The best evidence that IS was a cause of the chronic watery diarrhea among the symptomatic patients was the fact that disappearance of the infection, whether spontaneous or following treatment, paralleled a clinical sustained recovery. This is consistent with what has been previously described in a number of studies. It is important not to minimize the importance of IS infection, because invasive forms have been reported. It is not known if IS infection had any influence on the outcome of the patient with intestinal ischemia who subsequently died, but electron microscopy did not show invasion of the colonic mucosa. Thus, IS should be treated in patients with persistent symptoms, and histological follow up is advisable to confirm the cure. In contrast, a wait-and-see policy can be followed in asymptomatic patients with normal colon appearance.

Several therapeutic regimens have been proposed for IS eradication, including penicillin, metronidazole, and neomycin plus bacitracin. Penicillin benzathine has been used previously with success, and this was the first treatment used in the present study because of its simplicity, which ensured therapeutic compliance. However, persistence of infection was documented in all cases. Although a partial therapeutic effect of penicillin was observed, the metronidazole regimen resolved the infection, and thus metronidazole seems to be the drug of choice for treatment of IS.

No consistent risk factors for infection were identified but, in agreement with previous observations, colonization was more commonly found in male patients. Two of the nine male IS patients were homosexual, and homosexuality is a known risk factor for IS. Because there was an aggregation of cases in January–October 2000, an environmental source of infection at that time was suspected, but could not be confirmed. Potential external sources of infection include contaminated water and animals. Two recent reports have demonstrated that colonization with intestinal spirochetes is more frequent in individuals consuming contaminated well water. In addition, B. pilosicoli is a common cause of IS in pigs, dogs and birds, and in some cases B. pilosicoli infection may be zoonotic. It was interesting that one patient (case 11) who was positive for B. pilosicoli worked in a slaughterhouse where he was exposed to both pigs and chickens, and where he also drank non-potable water. It seems likely that he was infected from this environment.

Most IS patients with normal appearing colonic mucosa and chronic watery diarrhea had mild microscopic abnormalities on light and electron microscopy without signs of invasiveness, and these abnormalities remained generally unchanged after IS clearance. This type of abnormality has been referred to as ‘microscopic colitis not otherwise specified (NOS)’ to differentiate it from the well-defined ‘microscopic colitis’ (lymphocytic, collagenous colitis and focal active colitis). In some patients with these histopathological features, intestinal infections or drugs have been recognized as possible etiologic agents. On light microscopy, one patient had a characteristic histopathological pattern of lymphocytic colitis and two other patients had focal active colitis. Persistence of colonic damage in patients with microscopic colitis, in clinical remission, previously has been described. This is probably because the reduced absorptive capacity of the colon in microscopic colitis becomes clinically obvious only in those patients with other concomitant pathological conditions such as celiac disease, bile acid malabsorption or, as in the present study, IS infection.

In conclusion, histological IS is rarely diagnosed in the Catalonia area of Spain, but should be considered as a differential diagnosis in patients with otherwise normal-appearing colonic mucosa and chronic watery diarrhea.

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References


