

This article is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (<http://www.karger.com/Services/OpenAccessLicense>). Usage and distribution for commercial purposes requires written permission.

## Single Case

# Intestinal Bacterial Infection Diagnosed by Histological Examination of Endoscopic Biopsy Specimens

Michiko Yuki Yuko Emoto Katsuhiko Yoshizawa Takashi Yuri  
Airo Tsubura

Department of Pathology II, Kansai Medical University, Hirakata, Japan

## Keywords

Intestinal infection · Bacteria · Biopsy · Immunohistochemistry

## Abstract

Intestinal spirochetosis (IS) in humans is characterized by spirochetal microorganisms attached to the luminal surface of the colonic epithelium. In the present case, attached organisms appeared as 3- to 4  $\mu\text{m}$ -thick (average thickness, 3.4  $\mu\text{m}$ ) basophilic fringes or haze in HE-stained endoscopic biopsy specimens. The basophilic fringes were clearly labeled by *Treponema pallidum* antiserum. Because IS is relatively rare in developed countries, thin basophilic fringes characteristic of IS are readily overlooked. Thus, the recognition of histological characteristics of this disease is important for its diagnosis.

© 2016 The Author(s)  
Published by S. Karger AG, Basel

## Introduction

Intestinal spirochetosis (IS) in humans is a rare disease in which the spirochetes species, coiled Gram-negative bacteria with flagella, infects the gastrointestinal tract. Spirochetes are divided into the Spirochaetaceae, Leptospiraceae, and Brachyspiraceae families. Two members of the Brachyspiraceae family, *Brachyspira aalborgi* and *B. pilosicoli*, are commonly as-

sociated with human IS [1]. *B. pilosicoli* is a pathogen of the large intestine in various animal species including humans, while *B. aalborgi* has only been found in macaques and humans [2]. In the present report, a case of IS was diagnosed by histological examination of endoscopic biopsy specimens.

## Case Report

A 61-year-old man was clinically diagnosed with colitis and underwent endoscopic examination. A colonoscopy revealed scattered varioliform erosions from the ileum end to the rectum. Clinical records indicated that the same colonoscopic findings were seen 4 years ago, and according to the corresponding pathology report, nonspecific colitis was the pathological diagnosis. However, further clinical information regarding the previous colonoscopic findings was unavailable. A differential diagnosis to exclude inflammatory bowel disease and amebic dysentery was required.

Biopsy specimens during endoscopy were taken from the ileum end, ascending colon, and sigmoid colon. In HE-stained sections, all specimens showed marked but nonspecific inflammatory cell infiltration in the mucosal layer. The histology did not support a diagnosis of inflammatory bowel disease, such as Crohn's disease or ulcerative colitis. Moreover, PAS-stained sections did not label trophozoites, so amebic colitis was excluded. However, specimens from the sigmoid colon displayed characteristic morphology, so-called basophilic fringes, haze, or false brush border on the luminal surface and in the crypts of the nonneoplastic epithelium, suggestive of IS (Fig. 1a). Invasion of the microorganism beyond the surface epithelium was not seen in biopsy specimens. The IS infection was confirmed by immunostaining with antiserum against *Treponema pallidum* (rabbit polyclonal antibody; Biocare Medical, Concord, CA, USA), performed according to the manufacturer's instructions (Fig. 1b). This antiserum clearly labels Brachyspiraceae [3]. Therefore, infective colitis associated with IS was the diagnosis.

To measure the thickness of the fringes on the luminal surface, HE-stained and immunostained slides were scanned with a high-resolution digital scanner (NanoZoomer 2.0 Digital Pathology; Hamamatsu Photonics, Hamamatsu, Japan) to prepare digital images. The NDP image files were opened in color mode with NDP.view software (Hamamatsu Photonics). The images were converted to JPEG files at  $\times 400$  magnification in 10 randomly selected areas within each slide. The microorganism thickness was 3–4  $\mu\text{m}$  (average, 3.4  $\mu\text{m}$ ).

## Discussion

Human IS was first described by Harland and Lee in 1967 [4]. IS has been linked to diarrheal illness in swine, poultry, dogs, cats, opossum, guinea pigs, and nonhuman primates [1]. In contrast, IS in humans is typically asymptomatic; if symptoms are present in humans, the most common symptoms are diarrhea and abdominal pain [1, 3, 5]. In the present case, infective colitis associated with IS [6] was the pathological diagnosis. The incidence of IS is 2–7% in Western countries and 11–34% in less-developed countries; the incidence of IS in Japan is 0.4% [5]. However, the incidence of IS is up to 54% in homosexual men and HIV-positive patients [5]. IS should be considered to be an opportunistic infection in immunodeficient patients. Both adult and pediatric cases have been reported [7]. Transmission generally occurs by the fecal-oral route (exposure to contaminated water and/or contact with

infected animals, potential for zoonotic spread), but sexual transmission does occur in homosexual men [7].

The endoscopic appearance of IS is polypoid, erythematous, or normal, but it does not display pathognomonic changes that would aid in its diagnosis [1]. The diagnosis of IS is made exclusively from the histological examination of endoscopic biopsy specimens. IS is characterized by spirochetal microorganisms adherent to the surface of the intestinal mucosa; these appear as basophilic fringes or haze on HE-stained sections. However, due to the rarity of the disease, thin basophilic fringes are readily overlooked. To more clearly visualize the basophilic fringes, Warthin-Starry silver stain, Giemsa stain, and PAS stain are useful. Members of the Brachyspiraceae family are morphologically similar to other spirochetes and cross-react with antitreponemal serum and antileptospiral serum [3, 5, 8]. Immunostaining with these sera is an easy method that yields reproducible and reliable results for the identification and definitive diagnosis of IS. By electron microscopy, the basophilic fringes seen in light microscopy consist of dense spirochetes with flagella between the microvilli that perpendicularly adhere to the intestinal epithelial membrane [1, 9, 10]. In contrast, to detect attached microorganisms in fixed sections, imprint cytology detects floating microorganisms [10].

The causative spirochetes in humans are *B. aalborgi* and *B. pilosicoli*. *B. aalborgi* is the most prevalent species in Western countries and Japan, while *B. pilosicoli* infection is prevalent predominantly in less-developed countries and may be more common in HIV-positive patients [3, 5, 10]. The two species can be differentiated by PCR-based genotyping [2] and a serological agglutination test [11]. The mean length of *B. aalborgi* is 3.8  $\mu\text{m}$  (range, 2–4.5  $\mu\text{m}$ ), which is significantly shorter than that of *B. pilosicoli* (mean, 5.5  $\mu\text{m}$ ; range, 3.2–11  $\mu\text{m}$ ) [3]. Genotyping was not performed in the present study, but the mean length of the microorganisms measured during light microscopy was consistent with that of *B. aalborgi*. Although the patient's immune status is unknown, *B. aalborgi* is commonly found in the nonhomosexual Japanese population [5]. In general, *B. aalborgi* is considered to be nonpathogenic, but the invasion of the microorganism beyond the surface epithelium may be associated with gastrointestinal symptoms [1]. IS was previously found exclusively in nonneoplastic epithelium and hyperplastic epithelium but not in adenomatous or carcinomatous epithelium [5]. However, some adenomas support the growth of IS [12, 13]. Therefore, biopsies for histological diagnosis can be made from both nonneoplastic epithelium and neoplastic epithelium. For IS therapy, metronidazole treatment consisting of 500 mg 3 times a day for 10 days in adults and 15 mg per kg body weight 3 times per day for 5 days in children has been reported to eradicate IS symptoms [7].

In conclusion, as IS is relatively rare in developed countries, the histological recognition of this disease is important for its pathological diagnosis.

#### Statement of Ethics

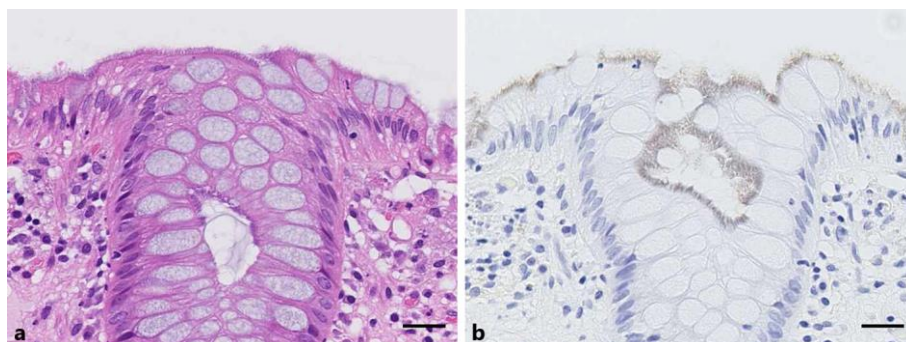
The authors have no ethical conflicts to disclose.

#### Disclosure Statement

The authors declare no conflicts of interest.

## References

- 1 Tsinganou E, Gebbers JO: Human intestinal spirochetosis – a review. *Ger Med Sci* 2010;8:DOC1.
- 2 Mikosza AS, La T, de Boer WB, Hampson DJ: Comparative prevalences of *Brachyspira aalborgi* and *Brachyspira (Serpulina) pilosicoli* as etiologic agents of histologically identified intestinal spirochetosis in Australia. *J Clin Microbiol* 2001;39:347–350.
- 3 Tateishi Y, Takahashi M, Horiguchi S, Funata N, Koizumi K, Okudela K, Hishima T, Ohashi K: Clinicopathologic study of intestinal spirochetosis in Japan with special reference to human immunodeficiency virus infection status and species types: analysis of 5265 consecutive colorectal biopsies. *BMC Infect Dis* 2015;15:13.
- 4 Harland WA, Lee FD: Intestinal spirochaetosis. *Br Med J* 1967;3:718–719.
- 5 Tanahashi J, Daa T, Gamachi A, Kashima K, Kondoh Y, Yada N, Yokoyama S: Human intestinal spirochetosis in Japan; its incidence, clinicopathologic features, and genotypic identification. *Mod Pathol* 2008;21:76–84.
- 6 Calderaro A, Bommezzadri S, Gorrini C, Piccolo G, Peruzzi S, Villanacci V, Zambelli C, Dettori G, Chezzi C: Infective colitis associated with human intestinal spirochetosis. *J Gastroenterol Hepatol* 2007;22:1772–1779.
- 7 Helbling R, Osterheld MC, Vaudaux B, Jatou K, Nydegger A: Intestinal spirochetosis mimicking inflammatory bowel disease in children. *BMC Pediatr* 2012;12:163.
- 8 De Brito T, Sandoval MP, Silva AG, Saad RC, Colaiacovo W: Intestinal spirochetosis: first cases reported in Brazil and the use of immunohistochemistry as an aid in histopathological diagnosis. *Rev Inst Med Trop Sao Paulo* 1996;38:45–52.
- 9 Guccion JG, Benator DA, Zeller J, Termanini B, Saini N: Intestinal spirochetosis and acquired immunodeficiency syndrome: ultrastructural studies of two cases. *Ultrastruct Pathol* 1995;19:15–22.
- 10 Ogata S, Higashiyama M, Adachi Y, Ohara I, Nishiyama J, Okusa Y, Takeo H, Sato K, Nakanishi K, Kawai T: Imprint cytology detects floating *Brachyspira* in human intestinal spirochetosis. *Hum Pathol* 2010;41:249–254.
- 11 Abe Y, Hirane A, Yoshizawa A, Nakajima H, Adachi Y: The specific antibody to *Brachyspira aalborgi* in serum obtained from a patient with intestinal spirochetosis. *J Vet Med Sci* 2006;68:1089–1091.
- 12 Higashiyama M, Ogata S, Adachi Y, Nishiyama J, Ohara I, Okamura M, Matsuzaki K, Okusa Y, Sato K, Hokari R, Miura S: Human intestinal spirochetosis accompanied by human immunodeficiency virus infection: a case report. *Acta Med Okayama* 2009;63:217–221.
- 13 Carr NJ, Mahajan H, Tan KL, Sharma R: The histological features of intestinal spirochetosis in a series of 113 patients. *Int J Surg Pathol* 2010;18:144–148.



**Fig. 1.** Intestinal spirochetosis. **a** Basophilic fringes (average thickness, 3.4  $\mu\text{m}$ ) reflecting a group of spirochetes are seen on the luminal surface of sigmoid colon mucosa by HE staining. **b** Immunostaining with *T. pallidum* antiserum highlights the fringes. Scale bars, 25  $\mu\text{m}$ .