# Human intestinal spirochetosis in Japan; its incidence, clinicopathologic features, and genotypic identification

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**Human intestinal spirochetosis is a common condition in Western countries, but is not well recognized in Japan. To demonstrate the incidence and clinicopathologic findings of human intestinal spirochetosis in Japan, we retrospectively investigated biopsy, and endoscopically or surgically resected specimens of the large intestine. Among a series of 2556 samples, 11 cases of human intestinal spirochetosis were detected (0.4%). Together with additional nine cases sporadically found, 20 cases of human intestinal spirochetosis were subjected to molecular detection of two strains of spirochetes (*Brachyspira aalborgi* and *Brachyspira pilosicoli*) by amplifying species-specific portion of 16S ribosomal RNA and NADH oxydase gene by polymerase chain reaction. *B. aalborgi* was detected in all cases examined, three of which revealed dual infection of both species. Our results suggest that human intestinal spirochetosis infection is relatively rare, and *B. aalborgi* is the most prevalent species in Japan. Most of human intestinal spirochetosis were asymptomatic, although symptomatic in exceptional cases. In addition, we emphasize a usefulness of immunostaining with anti-*Treponema pallidum* and anti-*Mycobacterium bovis* polyclonal antibodies for detecting the spirochetes.**
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Human intestinal spirochetosis, which was first described by Harland and Lee in 1967,[1] is an infestation of spirochetal microorganisms on the surface of the large intestinal mucosa. Causative spirochetes are reported to be either *Brachyspira aalborgi* or *Brachyspira pilosicoli*. The two species may be zoonotic because they have been isolated from the feces of non-human primates and other animals.[2] The incidence in rectal biopsies is reported to be 2–7% in Western countries, 11–34% in less developed countries and up to 54% in homosexual men, and human immunodeficiency virus (HIV)-positive patients.[3] Thus, the incidence of intestinal spirochetosis is considerably varied in geography and immune condition. In Japan, human intestinal spirochetosis has not been well recognized. There have been only two case reports of human intestinal spirochetosis in English language, including the first report by Nakamura *et al* in 1998,[4,5] and has not been a detailed study.

In this paper, we retrospectively reviewed a series of biopsy, endoscopic mucosal resection polypectomy, and surgically resected specimens to search for the incidence of human intestinal spirochetosis in Japan, and performed PCR to identify causative species. In addition, we emphasize a usefulness of immunostaining with anti-*Treponema pallidum* and anti-*Mycobacterium bovis* antibodies for detecting the spirochetes.

## Materials and methods

### Materials

A series of 2985 samples of biopsy (1585) and endoscopically (1004) or surgically (396) resected specimens of the large intestine, which were registered at the department of pathology of Oita City Almeida Memorial Hospital from April 2005 to March 2006, were reviewed by three pathologists. The department is not specialized and handles

many common samples sent from private hospitals or clinics in the city. Intestinal spirochetes have been reported to be present exclusively on non-neoplastic epithelium, including hyperplastic polyp, but not on adenomatous or carcinomatous epithelium.[6] Therefore, we excluded 429 out of 2985 samples, which were biopsy samples almost entirely composed of neoplastic cells and surgically resected samples with ablation of normal surface epithelium due to poor fixation. Nine additional cases of human intestinal spirochetosis, which had been sporadically found at department of pathology of Oita University, were also included in this study (Case 12–20 in Table 2). These 2556 samples were from 1555 men and 1001 women, whose age ranged from 16 to 98 years with mean age of 66 years. Symptoms and endoscopic findings were varied, but most samples were obtained from polypoid lesion. All specimens were stained with hematoxylin and eosin, and were examined at $\times 400$ magnification. Human intestinal spirochetosis was identified by characteristic basophilic fringes on the surface epithelium, and the diagnosis was confirmed by Warthin–Starry staining, which is used to detect spirochetes and *Helicobacter pylori* and immunostaining mentioned below. We used three biopsy specimens of colonic mucosa without histological evidence of human intestinal spirochetosis as negative controls for immunostaining, immunoelectron microscopic study, and PCR.

## Immunostaining

Paraffin-embedded tissues with histologically evident human intestinal spirochetosis were cut at a thickness of $4 \mu m$, deparaffinized in xylene, and rehydrated. Endogenous peroxidase activity was blocked using 3% hydrogen peroxidase for 10 min. Sections were treated with primary antibodies for 30 min. Immunostaining was performed by avidin–biotin–peroxidase complex technique using a Histofine SAB-PO (MULTI) kit (Nichirei Co., Tokyo, Japan) and diaminobenzidine for visualization of binding antibodies. We used two antibodies against the following antigens: *T. pallidum* (polyclonal, dilution 1:20; Biogenesis Ltd., UK) and *M. bovis* (polyclonal, 1:500; Dako Cytomation, Carpinteria, CA, USA). The cross reaction between intestinal spirochetes and *T. pallidum* was first described by De Brito *et al* in 1996.[7] In addition, anti-*M. bovis* antibody has been reported to be a promising screening tool for the detection of microorganism especially in the field of dermatopathology because of its cross reactivity with many bacteria and fungi.[8] Thus, we considered to apply both anti-*T. pallidum* and anti-*M. bovis* immunostaining to the detection of intestinal spirochetes. Specificity of these primary antibodies was examined by using immunoglobulin fraction of non-immunized rabbit serum and anti-*H. pylori* antibodies as primary antibodies.

## Immunoelectron Microscopic Study

Immunoelectron microscopic study was performed in five cases (Case 1, 11–14 in Table 2) according to the method described by Yano *et al.*[9] Briefly, small pieces of the specimens were cut from paraffin block, deparaffinized in xylene, rehydrated in ethanol, immersed in 2% glutaraldehyde, postfixed in 1% $OsO_4$, and embedded in epoxy resin. Ultra thin sections were microwaved in Target Retrieval Solution (pH 10) (Dako Cytomation) and immunostained with anti-*T. pallidum* polyclonal antibody and gold-conjugated secondary antibody.

## DNA Extraction and Amplification of Genes for 16S ribosome RNA and nox by PCR

DNA from paraffin-embedded tissue samples was extracted using DEXPAT™ (Takara, Tokyo, Japan). DEXPAT utilizes ion change resin and surfactants, which is designed to optimize DNA extraction from paraffin-embedded tissue, and PCR-ready DNA is extracted in the supernatant. In brief, $10 \mu m$-thick paraffin-embedded tissues were incubated with $100 \mu l$ of DEXPAT at $100°C$ for 10 min. After centrifugation, supernatant was collected from the tubes and used as template. Samples of DNA from biopsy specimens without histological evidence of human intestinal spirochetosis were used as negative controls. Pairs of primers were designed to detect the genes for 16S ribosome RNA (16S rRNA) and NADH oxidase (*nox*) of the *B. aalborgi* and *B. pilosicoli* according to the method described by Mikosza *et al.*[10] The sequences of these primers and size of the products are listed in Table 1. PCR amplification was performed in $2.5 \mu l$ of the solution of extracted DNA and $22.5 \mu l$ of a reaction mixture that contained $0.4 \mu M$ primers, 0.25 mM dNTPs mix, 1.5 mM $MgCl_2$, PCR buffer source, and 1 U of *Taq* DNA polymerase (AmpliTaq Gold; Applied Biosystems, Foster City, CA, USA). Amplification was performed in a thermal cycler (Gene Amp PCR

**Table 1** Sequences of primers to detect the gene for 16S rRNA and NADH oxidase (*nox*) of *Brachyspira aalborgi* and *Brachyspira pilosicoli*

| Primer | Sequence | Size of products (bp) |
|---|---|---|
| *B. aalborgi* 16S rRNA | F: TACCGCATATACTCTTGAC<br>R: CCTACAATATCCAAGAACC | 472 |
| *B. aalborgi* nox | F: GGTTGACTCAAGCACTAC<br>R: AAACCGTATTTTGTTCCAGG | 334 |
| *B. pilosicoli* 16S rRNA | F: AGAGGAAAGTTTTTTCGCTTC<br>R: GTCGCTCCATCAGACTTT | 196 |
| *B. pilosicoli* nox | F: GTAACTCCTCCTATTGAG<br>R: GCACCATTAGGTAAAGTC | 465 |

F, forward; R, reverse.

system 9700, Applied Biosystems) under the following reaction conditions: one cycle of denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The PCR products were subjected to electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and viewed under ultra violet light.

### DNA Sequencing

To determine the sequences of PCR products, purified products of PCR were subjected to direct sequencing with the forward and reverse primers specific for each products and BigDye™ Terminator Cycle Ready Reaction Mix (Applied Biosystems). Sequencing was performed with an ABI PRISM 310 Genetic Analyser (Applied Biosystems).

## Results

In 11 of 2556 specimens (0.4%), human intestinal spirochetosis was detected (Case 1–11 in Table 2), of which only two cases (Case 1 and 11) were initially diagnosed as human intestinal spirochetosis. Clinico-pathological features and results of PCR of these and nine additional cases previously diagnosed at Oita University (Case 12–20 in Table 2) are summarized in Table 2.

The patients consisted of 17 men and three women, whose age ranged from 35 to 75 years with mean age of 57 years. According to clinical informations, all patients were immunocompetent except for one case with HIV infection (Case 12). With regard to clinical symptoms, nine patients were symptomatic, 10 were asymptomatic (including three cases with fecal occult blood), and no information was available in one case. The commonest symptom was lower abdominal pain, followed by melena, and diarrhea. Affected sites were most frequent in the sigmoid and transverse colon, followed by rectum, cecum, and descending colon. However, it would not be appropriate to refer to a preferential site, because sites other than where biopsy or resection were done were not histologically examined.

In each case, characteristic 2–3 μm-thick blue furry fringes were observed on the luminal surface of the non-neoplastic epithelium, including hyperplastic polyps, and hyperplastic nodule, but not on adenomatous and carcinomatous epithelium (Figures 1a, 2b, 3a and 4b). They were mainly located in surface epithelium, but sometimes also in upper part of crypts, and lumina of hyperplastic polyp (Figure 2b). Inflammatory reaction was slight in most cases, but marked in several cases (Case 5, 7, and 12). The HIV-positive case (Case 12) was accompanied with erosion and marked inflammation (Figure 4a). Histologically, 13 of 20 cases (65%) were associated with other intestinal diseases;

hyperplastic polyp or nodule (Case 2, 13, 16, 19, and 20), tubular adenomas (Case 4, 9, 10, and 14), adenocarcinoma (Case 1), ulcerative colitis (Case 6), inflammatory polyp (Case 7), and amebiasis (Case 5).

To confirm the diagnosis of intestinal spirochetosis, we performed Warthin–Starry staining and immunostaining with anti-*T. pallidum*, and anti-*M. bovis* antibody, by which these furry fringes were highlighted (Figures 1b–d, 3b, and 4c). No positive staining was obtained in negative controls.

Immunoelectron microscopic examination revealed numerous spirochetes positive for anti-*T. pallidum* polyclonal antibody (Figure 5a and b). Each spirochete exhibited spiral or rod-like figure, which measured 3–4 μm in length and 0.2–0.3 μm in width, vertically arranged between microvilli. In severely affected areas, spirochetes themselves looked like long wavy microvilli (Figure 5a-right side, and 5b), whereas in less affected areas, differences between microvilli and spirochetes were evident, that is, microvilli were much smaller than spirochetes (Figure 5a-left side). The surface of the affected cells was slightly depressed to form a pit. Unexpectedly, spirochetes were found within the degenerated cells of unknown origin in the lamina propria, in which cell organelles were hardly discernible (Case 1) (Figure 5c), although they were not detected by light microscopic examination. Microvilli were well preserved in normal mucosa and hyperplastic polyp (Figure 5a-left side, d), although sparse and shortened in adenoma (Figure 5e).

PCR products of the genes for 16S rRNA and *nox* specific for *B. aalborgi* were detected in all cases examined (Case 1–20), while products of the gene for 16S rRNA for *B. pilocicoli* were detected only in three cases (Case 12, 14, and 16). PCR products of the gene for *nox* for *B. pilosicoli* were not obtained in all cases examined. Representative results of electrophoresis of these products are shown in Figure 6, which are consistent with the predicted size shown in Table 1. There was no amplification of DNA in samples from negative controls. Specificity of these products was confirmed by direct sequencing of PCR products. The sequence data were compared with previously reported sequences of 16S rRNA and *nox* genes of the *B. aalborgi*, and *B. pilosicoli* obtained from NCBI database of Gen-Bank (Accession numbers, AY349949, AF060816, and AY514025, respectively). Similarities between these products and reported sequences ranged from 90 to 99% with mean 96.8% (16S rRNA gene of *B. aalborgi*), 83–98% with mean 96.1% (*nox* gene of *B. aalborgi*), and 98% (16S rRNA gene of *B. pilocicoli*).

## Discussion

Our study revealed the incidence of human intestinal spirochetosis in Japan to be 0.4% on the basis of review of a series of specimens of the large intestine

**Table 2** Clinicopathological features and results of PCR amplification

| Case | Age | Sex | Clinical symptoms | Endoscopic diagnosis | Results of PCR | | | | Histopathological features | | |
|------|-----|-----|-------------------|---------------------|------|------|------|------|------|------|------|
| | | | | | Brachyspira aalborgi | | Brachyspira pilosicoli | | Site | Inflammation | Initial diagnosis |
| | | | | | 16S rRNA | nox | 16S rRNA | nox | | | |
| 1 | 59 | M | Abdominal pain | Advanced cancer | + | + | − | − | T | ++ | Adenocarcinoma, human intestinal spirochetosis |
| 2 | 62 | M | No symptom | Polyp | + | + | − | − | T | + | Hyperplastic polyp |
| 3 | 67 | M | No symptom | Polyp | + | + | − | − | T | + | Colitis |
| 4 | 55 | M | No symptom | Polyp | + | + | − | − | C | + | Tubular adenoma |
| 5 | 35 | M | Melena | Amebiasis | + | + | − | − | R | +++ | Amebiasis |
| 6 | 38 | M | Diarrhea, melena | Colitis | + | + | − | − | NR | ++ | Ulcerative colitis |
| 7 | 53 | F | No symptom | Polyp | + | + | − | − | S | +++ | Inflammatory polyp |
| 8 | 52 | F | Fecal occult blood | Proctitis | + | + | − | − | R | ++ | Proctitis |
| 9 | 57 | M | No symptom | Polyp | + | + | − | − | S | ++ | Tubular adenoma |
| 10 | 51 | M | No symptom | Polyp | + | + | − | − | S | + | Tubular adenoma |
| 11 | 58 | M | NR | NR | + | + | − | − | NR | ++ | Human intestinal spirochetosis |
| 12 | 35 | M | Diarrhea, melena | Ulcerative colitis | + | + | + | − | T | +++ | Human intestinal spirochetosis HIV-positive |
| 13 | 62 | M | Fecal occult blood | Polyp | + | + | − | − | S | + | Inverted hyperplastic polyp |
| 14 | 41 | M | Melena | Polyp | + | + | + | − | C | ++ | Tubular adenoma, human intestinal spirochetosis |
| 15 | 61 | M | Lower abdominal pain | Proctitis | + | + | − | − | R | + | Human intestinal spirochetosis |
| 16 | 47 | M | Lower abdominal pain | Polyps | + | + | + | − | D | + | Hyperplastic polyp, human intestinal spirochetosis |
| 17 | 75 | F | No symptom | Polyps | + | + | − | − | A | ++ | Human intestinal spirochetosis |
| 18 | 51 | M | Lower abdominal pain | Polyps | + | + | − | − | S | + | Human intestinal spirochetosis |
| 19 | 54 | M | Lower abdominal pain | Polyps | + | + | − | − | S | + | Hyperplastic polyp, human intestinal spirochetosis |
| 20 | 63 | M | Fecal occult blood | Polyps | + | + | − | − | S | + | Hyperplastic nodule, human intestinal spirochetosis |

F, female; M, male; NR, not recorded; A, ascending colon; C, cecum; D, descending colon; R, rectum; S, sigmoid colon; T, transverse colon; +++, severe; ++, moderate; +, mild.
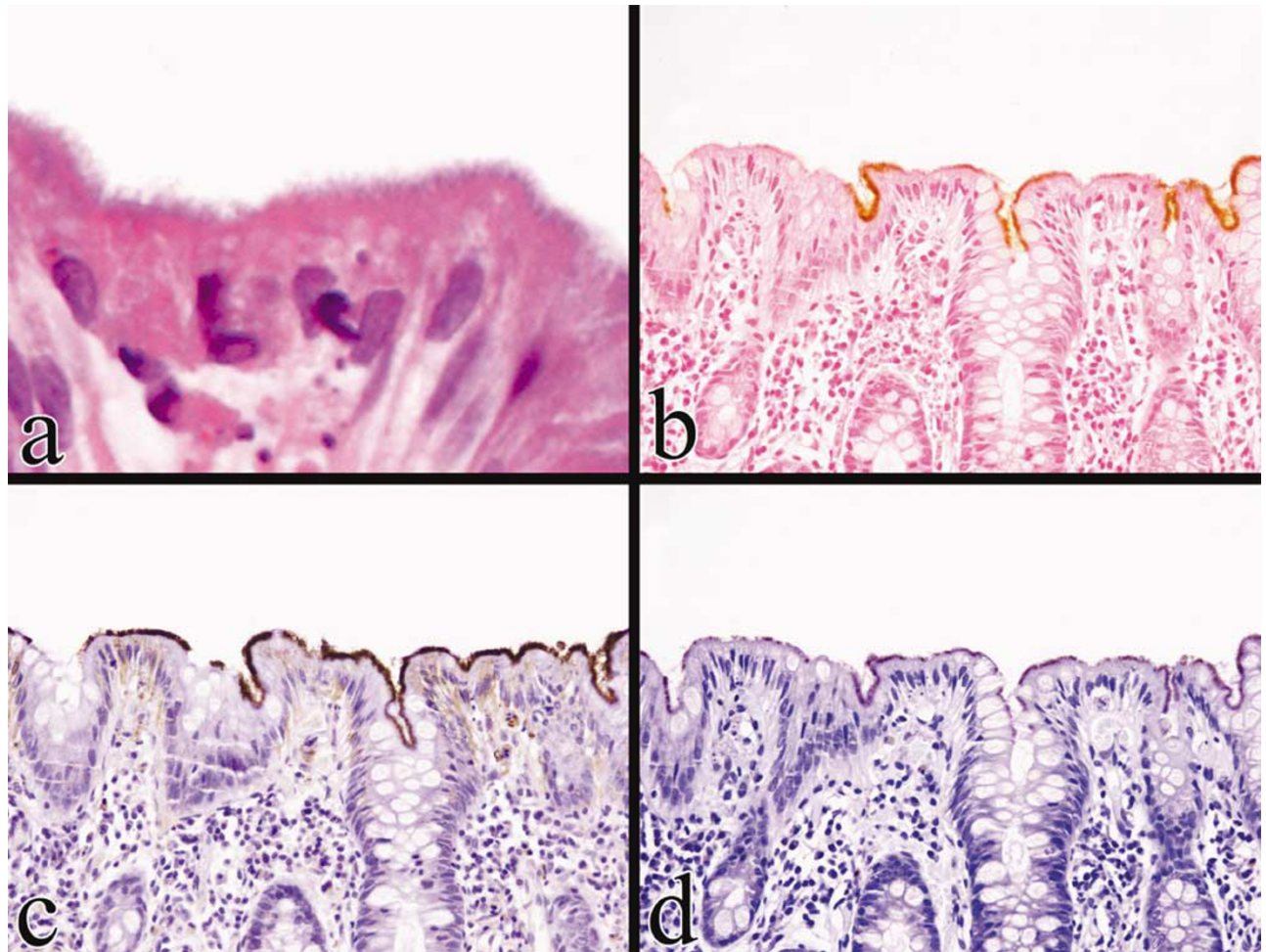
**Figure 1** Typical microscopic appearance of intestinal spirochetosis (Case 2) ((**a**) H&E, (**b**) Warthin–Starry stain, and immunostaining using (**c**) anti-*T. pallidum* and (**d**) anti-*M. bovis* antibodies). (**a**) Approximately 2 $\mu$m-thick basophilic fringes are observed on the luminal surface of large intestinal mucosa. (**b–d**) The fringes are highlighted with Warthin–Starry stain and immunostaining with anti-*T. pallidum* and anti-*M. bovis* antibodies.
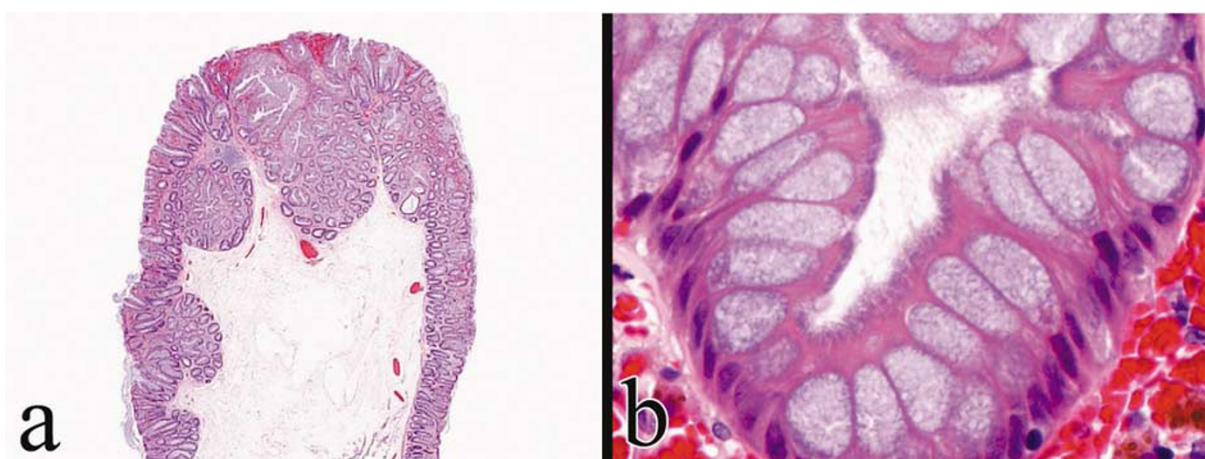


**Figure 2** Microscopic appearance of intestinal spirochetosis in inverted hyperplastic polyp (Case 13) ((**a**) H&E stain—panoramic view and (**b**) H&E stain—high magnification). (**a**) Hyperplastic polyp with endophytic growth. (**b**) The spirochetes are present almost all over the hyperplastic epithelium.

for 1 year in a single institute. This incidence is much lower in comparison with the previous reports from Western and developing countries.[3]

The sex ratio was 17:3 with male predominance, which is consistent with previous reports.[3] Most of intestinal spirochetosis does not exhibit any
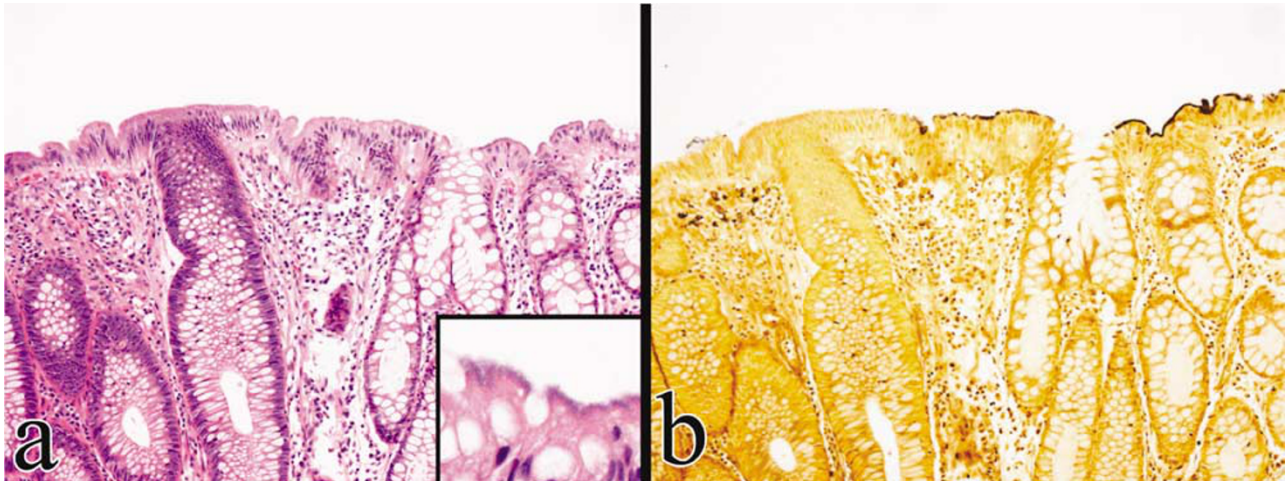
**Figure 3** Microscopic appearance of intestinal spirochetosis in transitional zone between adenoma (left) and normal epithelium (right) (Case 10) ((**a**) H&E stain (inset; high magnification) and (**b**) Warthin–Starry stain). Spirochetes are present exclusively on normal epithelium, but not on adenomatous epithelium.
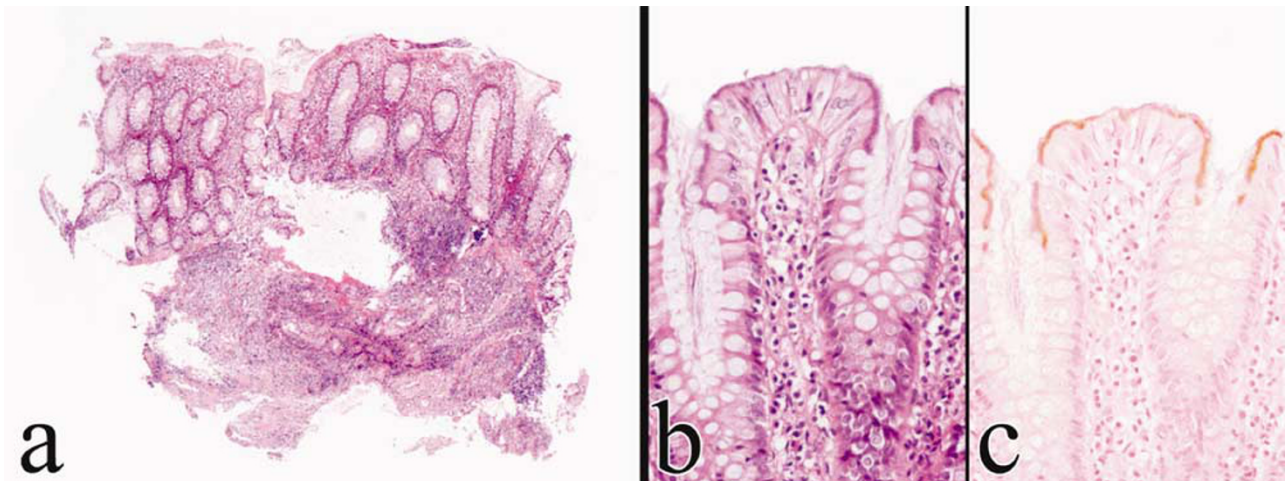


**Figure 4** Microscopic appearance of intestinal spirochetosis in HIV-positive patient (Case 12). (**a**, **b**) H&E stain and (**b**) Warthin–Starry stain) (**a**) Inflammatory reaction is conspicuous with erosion. (**b**, **c**) Spirochetes are present on the surface epithelium and upper part of crypt.

macroscopically or endoscopically recognizable lesions, and is usually diagnosed only histologically. Histologic features of intestinal spirochetosis are characterized by the presence of spirochetal microorganisms adherent to the surface of intestinal mucosa usually without accompanying inflammatory reaction. They were generally reported to be characteristic 'blue furry fringes'. The blue fringes should not be confused with basophilic mucin on the mucosal surface. Although the spirochetes are usually recognizable on hematoxylin–eosin specimen, they are readily overlooked because of their small size and inconspicuous inflammatory reaction. Although the incidence of human intestinal spirochetosis (0.4%) in Japan is low compared to other countries, we may encounter human intestinal spirochetosis with a frequency of 1/250 colorectal

samples. We believe that most cases of human intestinal spirochetosis are overlooked in Japan, where this entity is less recognized. Actually, only two of 11 cases of intestinal spirochetosis were histologically diagnosed initially.

To confirm the diagnosis, special stainings, such as Warthin–Starry stain or Steiner silver stain, are used. However, these stainings require fresh silver solutions and are not always reproducible depending on the performer's skill. Immunostaining with anti-*T. pallidum* antibody is an easy, reproducible procedure to identify spirochetal organisms, which seems to be seldom recognized. In this study, we showed that *T. pallidum* antibody specifically cross reacts with spirochetes by immunoelectron microscopic study, which further confirms a diagnostic usefulness of this procedure. We emphasize the
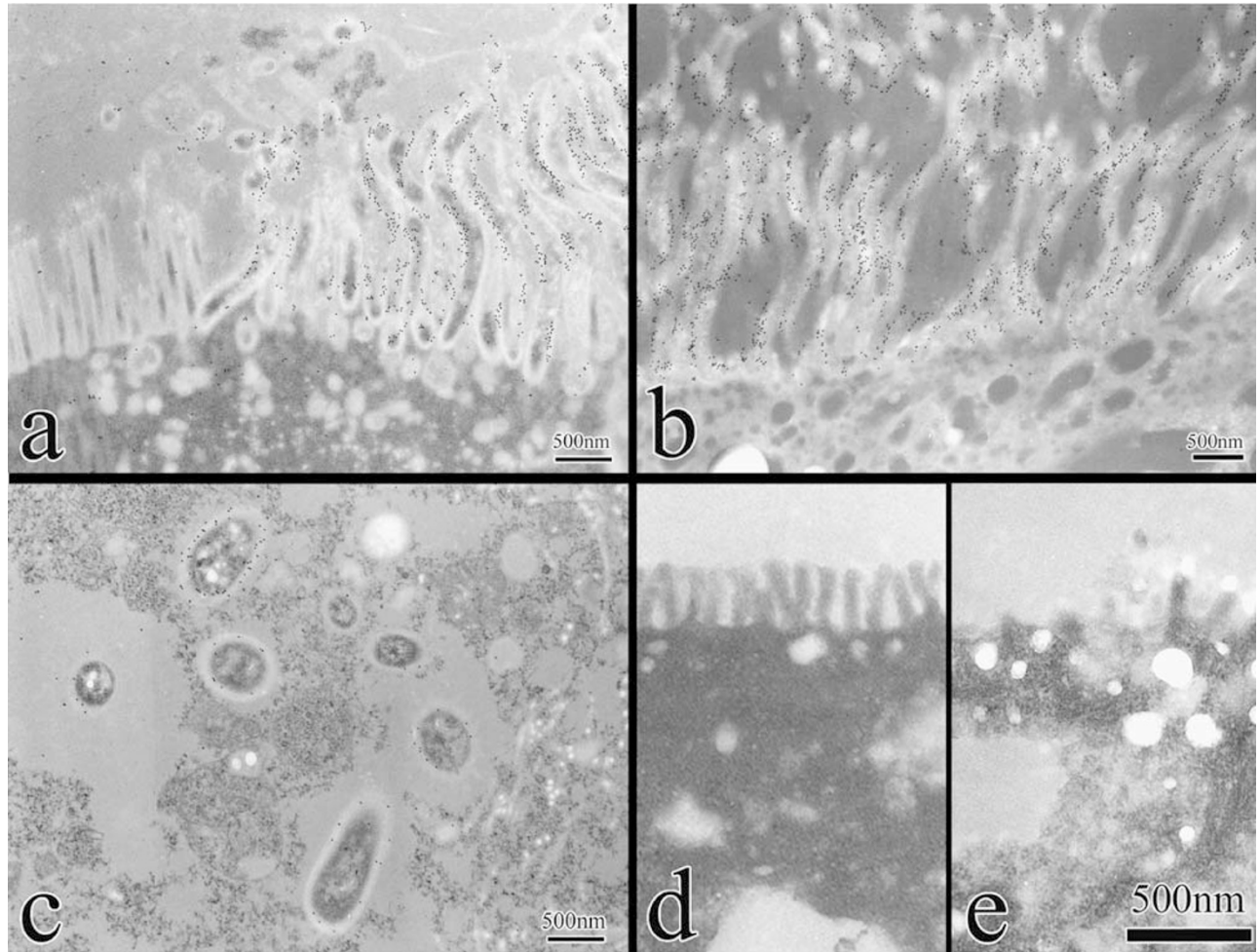
**Figure 5** Electron microscopic findings. (**a**) Numerous spirochetes positive for *T. pallidum* antibody are vertically arranged (right side). Compare them with the much smaller microvilli (left side) (Case 1). (**b**) High magnification of spirochetes positive for *T. pallidum* antibody (Case 13). (**c**) Cross-sectional view of the spirochetes positive for *T. pallidum* antibody are observed within degenerated cell in the lamina propria (Case 1). (**d**) Microvilli in the hyperplastic polyp are well preserved (Case 13). (**e**) Microvilli in adenoma without the spirochetes are very sparse and short.
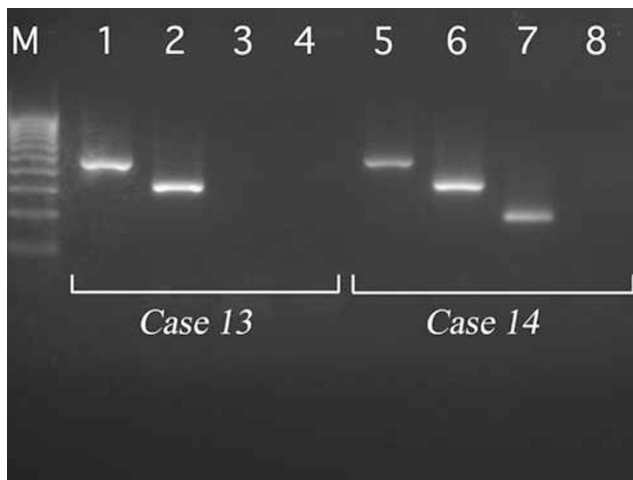


**Figure 6** Representative results of electrophoresis of PCR products in Case 13 (Lane 1–4) and Case 14 (Lane 5–8). M means molecular marker. Lane 1, 5: gene for 16S rRNA of *B. aalborgi.* Lane 2, 6: gene for *nox* of *B. aalborgi.* Lane 3, 7: gene for 16S rRNA of *B. pilocicoli.* Lane 4, 8: gene for nox of *B. pilocicoli.*

usefulness of anti-*M. bovis* antibody, which is more easily available, and provides a clear staining of intestinal spirochetes.

Human intestinal spirochetosis is caused by at least two species of spirochetes, *B. aalborgi* and *B. pilosicoli.* The former is prevalent in the Western people, while the latter is prevalent predominantly in less developed countries and in male homosexuals.[3] However, there have been few reliable studies as to the prevalence of these species, because culture technique was used to detect these organisms in human feces, and *B. aalborgi* is difficult to culture due to its fastidious growth requirements and slow growth. *B. pilosicoli* is easier to culture and therefore is more frequently detected.[11] The fact that *B. pilosicoli* was isolated from only 50% of histologically evident human intestinal spirochetosis specimens suggests that other species including *B. aalborgi* may be involved in culture-negative human intestinal spirochetosis.[11] In this study, we applied PCR amplification to the DNA

extracted from paraffin-embedded tissues for the detection of these two strains of spirochetes, because this method is not biased by culture efficiency. Overall, *B. aalborgi* was the predominant species detected in Japanese patients with histological evidence of human intestinal spirochetosis, whereas *B. pilocicoli* infection was relatively rare with dual infection by both species. This result is consistent with the foregoing investigations as to the prevalence of *B. aalborgi* species in Australia (*B. aalborgi*: 85.7%, *B. pilocicoli*: 14.3%) and Norway (*B. aalborgi*: 100%, *B. pilocicoli*: 0%) by PCR amplification of DNA from biopsy specimens.[12] Two cases of dual infection by both species have also been described by the same authors.[12] We cannot obtain PCR products of the gene for *nox* for *B. pilosicoli*. As *nox* gene is reported to be less conserved than 16S rRNA in intestinal spirochetes[10] and intraspecies *nox* nucleotide variation has not been fully clarified, it is possible that primer pairs designed might not detect all strains of *B. pilosicoli*.

Clinical significance of human intestinal spirochetosis still remains controversial. They have been regarded as non-pathogenic commensals or as a part of normal flora because most cases are asymptomatic. Thus, when the diagnosis of human intestinal spirochetosis is confirmed, the clinicians usually adopt a 'wait and see' policy.[3] Nevertheless, a few cases of human intestinal spirochetosis are associated with clinical symptoms, such as diarrhea, abdominal pain, and rectal bleeding.[13] Some of the recent studies support its association with gastrointestinal symptoms because such symptoms were eliminated by antibiotic therapy.[14] In the present study, nine of these 20 cases were symptomatic; however, in most cases, these symptoms can be explained by coexisting lesions such as advanced colon cancer, colon polyps, ulcerative colitis, and amebiasis, except for three patients (Case 12, 15, and 18). These three cases were accompanied by mild to severe inflammatory reaction (Case 12: severe, Case 15,18: mild). As to correlation with spirochetal species, one (HIV-positive case) of these three patients were infected by both strains of *B. aalborgi* and *B. pilosicoli* spirochetes.

In general, *B. aalborgi* is considered to be non-pathogenic commensal, while *B. pilosicoli* is considered to become opportunistic pathogens. Experimentally, strains of *B. pilocicoli* isolated from human feces have been reported to induce watery, mucoid diarrhea in pigs.[15] Furthermore, *B. pilocicoli* has been isolated from blood of critically ill patients.[16] These evidences support the pathogenic potential of *B. pilocicoli*. On the other hand, Trivett-Moore *et al*[11] claim that there is no apparent association between infection by *B. pilocicoli* species and clinical symptoms. In this study, three cases with *B. pilocicoli* infection (Case 12, 14, and 16 ) were found. Since the number is too small to clarify the clinical significance of *B. pilocicoli* infection, further investigation is required.

In Case 1, by immunoelectrom microscopy, intestinal spirochetes were detected not only on the cell surface, but also in the cytoplasm of degenerated cells. Previously, spirochetes were considered not to penetrate the surface epithelium. However, recent studies have demonstrated invasive spirochetes penetrating into epithelial cells, lamina propria, macrophages, and even Schwann cells.[17–19] Furthermore, marked increase of IgE-producing plasma cells in lamina propria and intraepithelial mast cells has been reported, suggesting close association of immediate-type immune reaction of the host.[20] Körner *et al*[21] postulate that invasive human intestinal spirochetosis with marked inflammatory reaction is related to clinical symptoms. However, clinical significance of 'minimal' invasive human intestinal spirochetosis without prominent inflammation as seen in Case 1 has not been interpreted conclusively. The HIV-positive patient of Case 11 complained of bloody stool, and endoscopic examination revealed multiple ulcers. Histologically, there was prominent inflammatory reaction in the lamina propria, although no apparent invasive spirochetes were ultrastructurally identified. There have been some reports of invasive human intestinal spirochetosis in HIV-positive patients, however, even in such cases, the number of invasive spirochetes was quite small compared with those on the surface epithelium.[18] Thus, in our case, failure to detect invasive spirochetes might be due to inappropriate portion of sectioning and these clinical symptoms might be attributed to spirochetal infection especially by *B. pilocicoli* spirochete, because no other possible enteropathogenic organism was characterized neither by culture nor immunostaining, and these symptoms were eliminated by antibiotic therapy.

As to possible correlation to other intestinal diseases, Delladestima *et al*[22] claimed that human intestinal spirochetosis is frequently associated with various intestinal diseases, such as carcinoma, adenomatous polyp, metaplastic polyp, and ulcerative colitis, and that colonic carcinoma was the most frequent because chronic stasis of intestinal content favors the infestation of the spirochetes.[22] However, the disorders listed above are subject to perform biopsy or resection, leading to more frequent detection of 'concomitant' human intestinal spirochetosis. In our study, we considered the association with intestinal diseases, for example, adenoma, adenocarcinoma, hyperplastic polyp, ulcerative colitis, and amebiasis also to be incidental.

Intestinal spirochetes were detected exclusively on the surface of normal epithelium and hyperplastic epithelium, but not on adenomatous and carcinomatous epithelium. This finding is consistent with previous observations. Coyne *et al*[6] postulated that normal microvilli are essential for spirochetes to attach the surface epithelium, and that the lack of microvilli in neoplastic epithelium prevents their colonization. Electron microscopic study revealed

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shortened and reduced number of microvilli on adenomatous epithelium, whereas well-preserved microvilli on normal and hyperplastic epithelium. A few cases of human intestinal spirochetosis involving adenomatous epithelium have also been reported, in which only areas with well-preserved microvilli were affected.[23] These findings support the hypothesis by Coyne, *et al.*[6]

In conclusion, human intestinal spirochetosis in Japan is relatively rare compared with previous reports from other countries. *B. aalborgi* is the most prevalent species*,* while *B. pilosicoli* is rare. In most cases, there seems to be no apparent correlation between spirochetal infection and clinical symptoms, however, it may become opportunistic pathogen in exceptional cases. To confirm a diagnosis of human intestinal spirochetosis, immunostainings with anti-*T. pallidum* and anti-*M. bovis* antibody is useful.

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