Human intestinal spirochaetosis in northern Japan

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A histological diagnosis of human intestinal spirochaetosis (HIS) was made in 114 patients during the period 1994–2007. All patients lived in three prefectures in the northern part of Honshu, Japan. Most patients were elderly and male. Twenty-nine patients complained of abdominal pain, bloody stools, diarrhoea or bowel symptoms, but most patients showed no direct symptoms of bowel disease, and occult faecal blood detected at medical check-up was the main reason for colonoscopic examination. There were no homosexual patients and no immunosuppressed patients. HIS was evenly distributed throughout the whole colorectum. PCR analysis of *Brachyspira aalborgi* and *Brachyspira pilosicoli* revealed that more patients were infected with *B. aalborgi*. Follow-up PCR studies confirmed that infestation with *B. aalborgi* could be repeatedly detected over a 6 year period. This study, involving over 100 patients, identified the characteristic features of HIS in northern Japan. The results suggest that these spirochaetes may be harmless commensals that cause no obvious pathological alterations in infected individuals.

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INTRODUCTION

Human intestinal spirochaetosis (HIS) is diagnosed on the basis of histopathological examination of tissue sections stained with haematoxylin and eosin (Harland & Lee, 1967). HIS has been reported in both developing and developed countries. The prevalence rates vary, and are higher in developing countries (11.4–64.3%) compared with developed countries (1.1–5%) (Korner & Gebbers, 2003; Mikosza & Hampson, 2001). A high prevalence of HIS has also been found in homosexual men, with or without human immunodeficiency virus infection (Korner & Gebbers, 2003; Trivett-Moore *et al.*, 1998).

Many studies have investigated the clinicopathological characteristics of patients with HIS, including prevalence, male/female ratio, age at diagnosis, clinical symptoms, morphological findings of biopsy specimens and/or bacterial genotypic analysis by PCR (Alsaigh & Fogt, 2002; Calderaro *et al.*, 2007a; Delladetsima *et al.*, 1987; Jensen *et al.*, 2001; Lindboe, 2001; Lindboe *et al.*, 1993; Mikosza *et al.*, 1999, 2001; Nielsen *et al.*, 1983; Tanahashi *et al.*, 2008). These studies identified different clinicopathological characteristics, reflecting the different socioeconomic statuses of the

countries, different races, different immunological backgrounds of the patients and relatively small sample sizes. Local differences in characteristics have been demonstrated, even within a country (Lindboe, 2001; Lindboe *et al.*, 1993).

We reported the first case of a Japanese patient with HIS in 1998 (Nakamura *et al.*, 1998). *Brachyspira aalborgi* was identified in cultures from our second case of HIS, diagnosed by colon biopsy in a Japanese patient with diarrhoea (Tasu *et al.*, 2003).

We diagnosed 114 cases of HIS from 1994 to 2007 in three prefectures located in the northern part of Honshu, Japan. In this study, we examined the clinicopathological, ultrastructural and bacterial genotypic characteristics of HIS in these patients, and compared our results with those from other countries and from the southern part of Japan (Tanahashi *et al.*, 2008). We also discuss the pathogenicity of the bacteria.

METHODS

Patients. One hundred and fourteen patients with intestinal spirochaetosis were registered in the files of the Department of Diagnostic Pathology, Iwate Medical University Hospital, Morioka, Japan, and allied hospitals, and in the Diagnostic Pathology Research Co. Ltd between January 1994 and December 2007. All specimens

Abbreviations: HIS, human intestinal spirochaetosis; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

analysed were from hospitals or clinics in three prefectures (Iwate, Akita and Aomori) located in the northern part of Honshu, Japan.

Clinical records of the patients included information on age, gender, chief complaints, gastrointestinal symptoms, endoscopic findings, anatomical colorectal portions from which biopsies were sampled, and abnormal laboratory data, if present. Histological samples were obtained from 111 patients by colorectal endoscopic biopsy, polypectomy or mucosal resection. In two additional cases, appendices were obtained following appendectomy. One unusual sample was obtained by transrectal prostatic needle biopsy; it inevitably contained small fragments of rectal mucosa, in which intestinal spirochaetosis was detected.

The chief complaints or symptoms prompting referral for colonoscopy and biopsy sampling were analysed in 101 patients. Ten patients were excluded from this analysis because detailed clinical data were not available.

A total of 313 samples were obtained by endoscopy from 111 patients (mean of 2.8 samples per patient). The ratio of the number of samples to the number of samples positive for HIS from each anatomical segment of the colorectum was recorded, and the anatomical prevalence of spirochaete infestation was analysed.

During the period from January to December 2007, one of the authors (S.N.) diagnosed 22 cases of HIS from 2665 patients at a single institute (Diagnostic Pathology Research Co. Ltd). These 2665 patients (1673 male and 992 female) underwent colonoscopy and endoscopic colorectal samples were obtained. However, information on the reasons for colonoscopy in HIS-negative patients was not available. The prevalence of HIS in 2007 was approximately 0.8%.

Histopathology. All specimens were fixed in 10% phosphate-buffered formalin, and dehydrated and embedded in paraffin wax. Thin sections (3–5 µm) were stained with haematoxylin and eosin, and examined under a light microscope. Giemsa and Warthin–Starry stains, and the periodic acid–Schiff reaction, were also used to confirm the presence of the bacteria.

Electron microscopy. The methods and apparatus used for the ultrastructural studies have been described previously (Nakamura *et al.*, 1998). Biopsy specimens from three cases [case 1, species of bacteria not determined; case 2, *B. aalborgi* – this case underwent follow-up study by PCR for 1 year (Table 2); and case 43, *Brachyspira pilosicoli* from a patient with ulcerative colitis in remission] were examined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

PCR for identifying two species of spirochaetes. Eighty-three consecutive cases (numbers 1–83) were analysed using PCR. Paraffin-embedded sections (10 µm thick) were suspended in 200 µl xylene

and rocked for 5 min. The xylene was removed, and the tissue pellet was washed three times with 200 µl 100% ethanol at –20 °C. The ethanol was then aspirated and the pellet was air-dried for 5 min. DNA was extracted from the pellet using phenol/chloroform, with proteinase K digestion, and used as a template for PCR. Four sets of oligonucleotide primers specific for the 16S rRNA gene or NADH oxidase-encoding gene of both *B. aalborgi* and *B. pilosicoli* were used, as described by Mikosza *et al.* (1999). To confirm the suitability of the material for PCR, the human ribosomal protein P0 (36B4)-encoding gene was also examined. All samples were tested in duplicate. To confirm their identity, the DNA sequences of the PCR products were determined using a BigDye terminator v. 1.1 cycle sequencing kit (Applied Biosystems) and an ABI PRISM 310 genetic analyser (Applied Biosystems).

Follow-up study of patients with HIS by PCR. After their initial biopsies, some patients also underwent follow-up colonic endoscopy for neoplasms or inflammation. HIS-positive biopsies from these patients were examined using PCR to confirm the species of spirochaetes and the duration of bacterial infestation.

Ethical considerations. The study was undertaken with the approval of the Ethics Committee of Iwate Medical University School of Medicine, Morioka.

RESULTS AND DISCUSSION

Clinicopathological features of patients

The clinicopathological features of the 114 patients with HIS were as follows: 106 were male and 8 were female, giving a male/female ratio of 13.3/1. All the patients lived in the northern part of Honshu. Two younger patients (24 and 26 years old) were from the Philippines and had come to northern Japan to be married. The patients were relatively elderly, with a mean age of 58.5 years (range 24–83 years). The male patients (mean age 59.2 years, range, 27–83 years) were approximately 10 years older than the female patients (mean age 50 years, range, 24–83 years).

The chief complaints or main reasons for performing colorectal endoscopy in the 101 patients with HIS were as follows: 41 patients had faecal occult blood detected at medical check-up, 13 patients had a screening colonoscopy, 10 patients had endoscopy for detection of the cause of general diseases (including metastatic hepatic or lung

Table 1. Clinical features and results of PCR amplification in 83 patients with intestinal spirochaetosis

PCR result and species		Age range (mean)	M/F	Others (no. of cases)
		(years)		
		No. (%)		
Negative	4	59–75 (65)	4/0	Human 36B4, positive (4)
Positive	79	24–83 (58.6)	72/7	
<i>B. aalborgi</i>	63 (80%)	26–83 (58.2)	58/5	Appendix (2), prostate (1)
<i>B. pilosicoli</i>	11 (14%)	34–83 (65.5)	10/1	Patient with UC in remission
Both species	5 (6%)	24–67 (47.8)	4/1	Filipino patient

F, Female; M, male; UC, ulcerative colitis.

cancer, elevated serum carcinoembryonic antigen or severe anaemia), and 8 patients had detailed colonoscopic examination for colonic polyps. These 72 patients had no abdominal symptoms or abnormal faeces. Only 29 patients (28.7%) complained of abdominal pain or abnormal defecation, such as diarrhoea, bloody stools or constipation. There were no homosexual patients and no patients with immunodeficiencies or human immunodeficiency virus infection in the study group.

The endoscopic findings mainly indicated colorectal tumours, and included hyperplastic polyps, tubular adenomas or carcinomas. No findings specifically suggestive of spirochaete infection were detected endoscopically.

The results of histological diagnosis in the 114 patients were as follows: in addition to spirochaete infestations, 76 patients also had colorectal neoplasms, which were mainly adenomas (72 patients), while 4 patients had adenocarcinomas. Three patients with ulcerative colitis in remission had spirochaete infestations. In one of these three patients, the biopsy specimen taken at the initial diagnosis of ulcerative colitis was reviewed, and no infestation was detected. No colorectal amoebiasis was found in any of the 114 patients with HIS.

The prevalence of spirochaete infestations in different anatomical segments of the colorectum was calculated. Out of a total of 313 specimens, 213 were positive for spirochaetes (68%). The positivity rates for the different segments of the colorectum were: caecum 26/36 (72%), ascending colon 38/55 (69%), transverse colon 45/62 (73%), descending colon 22/33 (67%) sigmoid colon, 46/68 (68%) and rectum, 36/59 (61%).

Weisheit *et al.* (2007) performed a meta-analysis of HIS cases described in the literature. From a total of 442 patients, 78.7% were male and 21.3% were female. Our results also showed a predominance of male patients; however, the reason for this remains unknown.

There were a variety of reasons for performing colonoscopy and for sampling the colonic mucosa in the current study. Only 29 patients (28.7%) showed symptoms such as abdominal pain, bloody stools or diarrhoea, which might have been related to gastrointestinal tract disease. One exceptional case (case 2), in which the patient complained of protracted diarrhoea, showed HIS colonization throughout the large intestine, and eradication of the bacteria with antibiotics improved the persistent diarrhoea. However, no serious diarrhoea or inflammation due to the presence of spirochaetes was found in the other cases, and no additional eradication therapies for spirochaetes were indicated.

Previous studies of the prevalence of HIS in the different segments of the colorectum found a slightly increased frequency in the rectum, though the difference was not significant (Lindboe *et al.*, 1993). The current study found HIS-positive rates for the segments of the large intestine ranging from 61 to 73%, with the lowest rate in the rectum.

Histopathology

The HIS-infested mucosa appeared normal in all 114 cases, with no erosions, ulcerations or dense inflammatory cell infiltration into the lamina propria. Even in the three patients with ulcerative colitis, the disease was in remission. No acute inflammation was found in two cases of HIS of the vermiform appendix. Cases with hyperplastic polyps, and tubular and serrated adenomas with low-grade dysplasia were rarely infested with spirochaetes. However, this study identified two previously unreported manifestations: one case of prostatic needle biopsy with contamination of rectal spirochaetosis, and one case of serrated adenoma with low-grade dysplasia colonized by spirochaetes (Fig. 1). Tubular adenomas with high-grade dysplasia and adenocarcinomas were never infested.

Fig. 1. (a) Serrated adenoma. Spirochaetes were found at the infolded luminal surface (indicated by arrows). (b) Warthin–Starry stain confirmed the presence of the spirochaetes found in (a). Bars, 50 μ m.

Electron microscopy

SEM examination showed numerous bacteria densely infesting the surface of the colonic epithelium, though the crypt openings, crypt borders and surface goblet cells were still traceable (Fig. 2a). Three-dimensional bacterial bodies were long, slender and sigmoidal with smooth surfaces, and tapered at both ends, one of which was inserted into the well-preserved microvilli of the superficial epithelium (Fig. 2b). Bacteria attached to the surface colonic epithelium perpendicularly, with no obvious differences between *B. aalborgi*, *B. pilosicoli* or undetermined species (case 1), in terms of their features observed by SEM.

TEM confirmed that spirochaetes infested the absorptive cells with microvilli, but not the goblet cells, which have no apical microvilli (Fig. 2c). Bacteria inserted one of their tapered ends between the microvilli of the surface epithelium. The bacteria never destroyed the epithelial structures or invaded into the epithelium (Fig. 2d).

PCR for identifying two species of spirochaetes

The results of PCR amplification are shown in Table 1. No PCR products were amplified in four cases, despite clear histological evidence of HIS. In these four cases, the human ribosomal protein P0 (36B4)-encoding gene was amplified. PCR amplification products from the other 79 cases showed that *B. aalborgi* was detected in 63 patients (80%), while *B. pilosicoli* was detected in 11 patients (14%). Five patients (6%) showed PCR amplification products for both *B. aalborgi* and *B. pilosicoli*. There were no apparent relationships between species and clinical symptoms. The bacterial bodies of *B. pilosicoli* seemed to be longer than those of *B. aalborgi* in histological sections, SEM and TEM.

B. aalborgi was first isolated from human biopsy material by Hovind-Hougen *et al.* (1982). *B. aalborgi* only causes spirochaetosis in humans and other primates, and is considered to be a harmless commensal, while *B. pilosicoli* is recognized as a pathogen of various animals, including

Fig. 2. Electron microscopic images. (a) Low-power SEM image (case 1). Crypt openings, shallow boundary lines between neighbouring crypts and small round holes draining the mucous from goblet cells were still visible, despite the dense infestation. (b) High-power SEM image (case 1). Long slender bacteria tapered at both ends, with one end inserted between well-preserved microvilli. (c) Low-power TEM image (case 2). Long slender S-shaped bacteria attached to the surface epithelium and inserted between microvilli at one end. No bacterial infestation was observed in the goblet cell (bottom left). (d) High-power TEM image (case 2). One end of a bacterium was seen between well-preserved microvilli of the surface epithelium.

pigs, dogs and chickens, and its clinical significance in HIS is a matter of controversy (Smith, 2005; Trott *et al.*, 1996a, b).

Despite clear histological evidence of HIS and successful amplification of the control, human ribosomal protein P0 (36B4)-encoding gene, no positive PCR results were obtained in four cases in this study. It is possible that the infestation was due to an unidentified species of spirochaete (Calderaro *et al.*, 2007a; Jensen *et al.*, 2001; Mikosza & Hampson, 2001; Mikosza *et al.*, 1999, 2001, 2004).

Follow-up study of patients with HIS by PCR

After the initial diagnosis of HIS, 20 patients underwent two or more colonoscopic examinations with endoscopic biopsies, for colonic neoplasms or inflammation. HIS-positive biopsies from 10 patients were analysed by PCR to detect the species of spirochaete and the duration of infestation. Nine patients were infested by *B. aalborgi* and one patient was infested by both species. Infestation with *B. aalborgi* could be repeatedly detected over a 6 year period (Table 2). There were no serious symptoms and none of the 10 patients received any treatment to eradicate the spirochaetes.

Chronic diarrhoea is one of the main complaints of patients with HIS (Alsaigh & Fogt, 2002; Calderaro *et al.*, 2007a, b; Esteve *et al.*, 2006; Jensen *et al.*, 2001; Marthinsen *et al.*, 2002; Mikosza & Hampson, 2001; Mikosza *et al.*, 2001; Nielsen *et al.*, 1983; Rodgers *et al.*, 1986; Tanahashi *et al.*, 2008; Weisheit *et al.*, 2007) and this could be interpreted as evidence of direct interference of spirochaetes with the absorptive mechanism of the colorectum. Diarrhoea in HIS has been attributed to reduced height of the microvilli (Hovind-Hougen *et al.*, 1982) or blunting and destruction of microvilli (Esteve *et al.*, 2006; Rodgers *et al.*, 1986). However, no direct destructive action by spirochaetes was observed in our

study, and the bacteria were able to cover the surface of the colonic mucosa without changing the host structures (Fig. 2a, b). Thus, densely packed spirochaetes could reduce absorption and cause diarrhoea by acting as a physical barrier (Gad *et al.*, 1977; Jensen *et al.*, 2001). It is therefore possible that the pathogenic mechanism of the spirochaetes may not involve bacterial toxicity, but may be a feature of the area infested by the bacteria. Limited or segmental infestations could be regarded as harmless (Delladetsima *et al.*, 1987; Nielsen *et al.*, 1983). It is also possible that the spirochaetes could cause diarrhoea by interfering with neural transmission and altering colonic motility (Padmanabhan *et al.*, 1996).

Differences in prevalence and clinicopathological features have been detected even within a country (Lindboe, 2001; Lindboe *et al.*, 1993); 20 cases of HIS reported from Oita, Kyushu, Japan (Tanahashi *et al.*, 2008), showed differences compared with our data. These differences may have been due to differences in the climate, socioeconomic status, social cultures or behaviours between the populations in the two different areas.

In conclusion, HIS in northern Japan is predominant in males and *B. aalborgi* is the prevalent infective agent. Clinical symptoms of colorectal disease are observed in <30 % of patients with HIS, but HIS infection can be detectable over prolonged periods. Spirochaetes may be considered as harmless commensals that cause no obvious histopathological alterations in infected individuals in northern Japan.

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Table 2. Follow-up study using PCR in colonic biopsies from patients with histological diagnoses of HIS

Patient [age (years)/gender]	Species	No. of biopsies	No. of Spi ⁺ PCRs (species)/no. of PCRs	Duration (years)
2 (45/M)	<i>B. aalborgi</i>	3	2(Ba)/3	1
4 (53/M)	<i>B. aalborgi</i>	4	2(Ba)/3	6
8 (58/F)	<i>B. aalborgi</i>	3	3(Ba)/3	3
9 (52/M)	<i>B. aalborgi</i>	4	4(Ba)/4	5
13 (57/M)	<i>B. aalborgi</i>	2	2(Ba)/2	2
14 (58/M)	<i>B. aalborgi</i>	2	2(Ba)/2	1
20 (57/M)	<i>B. aalborgi</i>	2	2(Ba)/2	1
28 (63/M)	<i>B. aalborgi</i>	3	2(Ba)/3	1
33 (57/M)	Both species	2	2(Ba and Bp)/2	1
47 (45/M)	<i>B. aalborgi</i>	3	3(Ba)/3	1

Ba, *B. aalborgi*; Bp, *B. pilosicoli*; F, female; M, male; Spi, spirochaetes.

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