

Human intestinal spirochetosis: *Brachyspira aalborgi* and/or *Brachyspira pilosicoli*?

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Abstract

Intestinal spirochetosis in humans (HIS) is a condition defined by the presence of a layer of spirochetes attached by one cell end to the colorectal epithelium. The pathologic significance of HIS is uncertain, but it has been linked to chronic diarrhea and other abdominal complaints. Two anaerobic intestinal spirochete species have been associated with HIS, namely *Brachyspira pilosicoli* and *Brachyspira aalborgi*. *Brachyspira pilosicoli*, which colonizes many animal species, is common (~30%) in the feces of people from developing countries, including Australian Aborigines, and in HIV+ patients and male homosexuals in Western societies. It is also commonly seen attached to the rectal mucosa of homosexual males. In other groups in Western societies both the presence of *B. pilosicoli* in feces and histologic HIS are uncommon (~1.5%). *Brachyspira aalborgi* is an extremely slow growing and fastidious spirochete, which previously had been isolated from an HIS patient in Denmark. Recent studies using polymerase chain reaction amplification of DNA from intestinal biopsies from a series of cases of HIS in the general Western population demonstrated that *B. aalborgi*, rather than *B. pilosicoli*, was the main spirochete species involved in these patients. This review outlines recent developments in the study of HIS and the two spirochete species, and identifies priorities for future research.

Introduction

Until recently intestinal spirochetosis in humans (HIS) has been regarded as an obscure condition of uncertain significance. First described by Harland and Lee in 1967, diagnosis of HIS has been problematic. Clinical microbiologists have not routinely cultured the associated spirochetes, and consequently this bacterial colonization has been diagnosed almost solely by pathologists – on the basis of histologic findings in biopsies. The pathognomonic histologic feature is a thin carpet-like layer of spirochetes attached by one cell end to the colorectal surface epithelium (Figs 1 and 2). Histologic identification of intestinal spirochetes in biopsies has, with one notable exception (Mathan and Mathan, 1985), been confined to Western populations. This limitation has

undoubtedly resulted in an underestimation of the worldwide prevalence of HIS.

Currently, two species of intestinal spirochetes have been isolated from humans with HIS, *Brachyspira aalborgi* and *Brachyspira (Serpulina) pilosicoli*. *B. aalborgi* grows more slowly than *B. pilosicoli*, but both species require specialized selective media and prolonged anaerobic incubation.

Comparison of HIS with porcine intestinal spirochetosis

Several advances in understanding HIS have come from comparisons with a similar condition of pigs, known as porcine intestinal spirochetosis (PIS). PIS is better defined than HIS, with the causative spirochete having been clearly identified as *B. pilosicoli* (Taylor *et al.*, 1980; Trott *et al.*, 1996a). Although *B. pilosicoli* attaches end-on to the colonic epithelium, this generally is only a feature of the early stages of PIS, and diagnosis is

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Fig. 1. Photomicrograph of paraffin-embedded colonic biopsy tissue from a Norwegian patient (male, 60 years old). Colonoscopy was otherwise normal except for an adenocarcinoma detected elsewhere in the colon. A 'false brush-border' of spirochetes can be seen attached end on to the epithelial surface (arrow). These spirochetes were subsequently shown to be *B. aalborgi* by PCR. Hematoxylin and eosin stain.

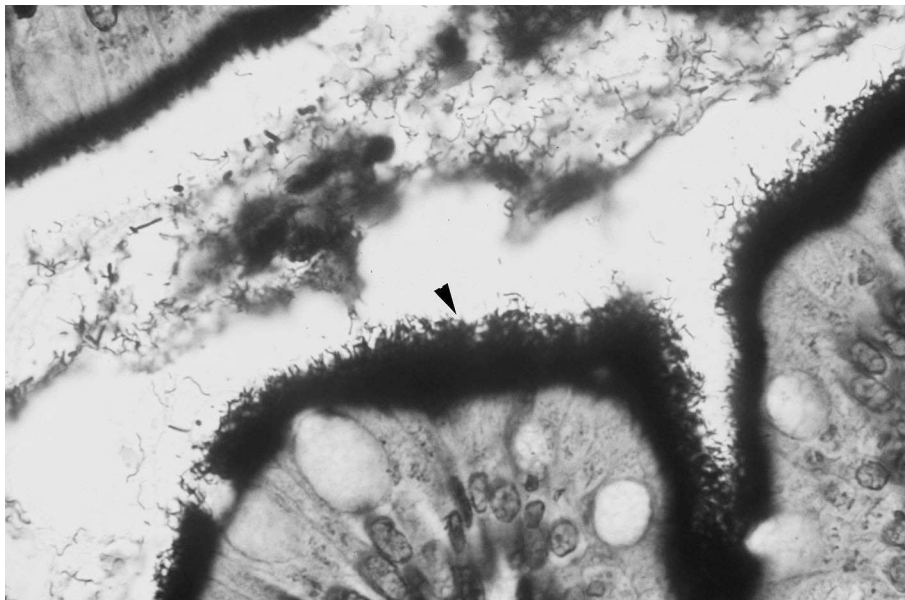


Fig. 2. Photomicrograph of paraffin-embedded colonic biopsy tissue from a French patient (male, 36 years old) with acute diarrhea but otherwise normal colonoscopy. A dense attachment of spirochetes to the epithelial surface can be seen (arrow). These spirochetes were shown subsequently to be *B. pilosicoli* by PCR. Warthin–Starry silver stain.

usually made by fecal culture (Thomson *et al.*, 1997; Hampson and Trott, 1999). *B. pilosicoli* infections in humans could also occur without obvious end-on attachment – resulting in a gross underestimation of the prevalence of infection, since HIS is mainly diagnosed by histology.

PIS usually manifests as a self-limiting watery to 'wet cement'-like diarrhea lasting 2–14 days. Hemorrhagic feces may also be observed. Colonized pigs may appear ill and have poor weight gain. Mortality rates are low (~1%) (Thomson *et al.*, 1998), although *B. pilosicoli* may

concurrently colonize pigs with other, more severe gastrointestinal infections (Girard *et al.*, 1995; Thomson *et al.*, 1998).

Clinical presentations in HIS

Clinically, HIS is an ill-defined condition and many case reports have involved patients who underwent large intestinal biopsies to try to establish a cause for otherwise unexplained symptoms. These patients have varied

from being free from symptoms and with an otherwise normal colonic mucosa (Henrik-Nielsen *et al.*, 1983), to having pseudoappendicitis (Henrik-Nielsen *et al.*, 1985), to having chronic diarrhea and rectal bleeding, lower abdominal cramps and/or colitis (Douglas and Crucoli, 1981; Lo *et al.*, 1994; Guccion *et al.*, 1995; Kostman *et al.*, 1995). In Australian Aboriginal children, diarrhea was the only identified symptom significantly associated with colonization by intestinal spirochetes (Lee and Hampson, 1992). The presenting signs in a series of patients with intestinal spirochetes in their bloodstream varied considerably, but most were critically ill with systemic signs (Lambert and Goursot, 1982; Fournié-amazouz *et al.*, 1995).

The ability of spirochetes to cause clinical signs has been supported by studies where removal of the organisms from the large bowel using penicillin or metronidazole resulted in clinical improvement (Kaplan and Takeuchi, 1979; Douglas and Crucoli, 1981; Cotton *et al.*, 1984; Burns and Hayes, 1985; Cooper *et al.*, 1986; Rodgers *et al.*, 1986; Lo *et al.*, 1994; Peghini *et al.*, 2000). In other studies, removal of the spirochetes did not improve the clinical signs (Henrik-Nielsen *et al.*, 1983), thus suggesting that the spirochetes were not causal.

Prevalence of HIS

HIS has been reported in many parts of the world, including Europe, North and South America, Africa, India, the Gulf States, Australia, Papua New Guinea and Japan (Harland and Lee, 1967; Hovind-Hougen *et al.*, 1982; Sanna *et al.*, 1982; Burns and Hayes, 1985; Mathan and Mathan, 1985; Jones *et al.*, 1986; Surawicz *et al.*, 1987; Barrett, 1990; Lindboe *et al.*, 1993; De Brito *et al.*, 1996; Trott *et al.*, 1997a; Nakamura *et al.*, 1998). HIS also has been reported in virtually all age groups, including children (Henrik-Nielsen *et al.*, 1983; Lee and Hampson, 1992; da Cunha Ferreira *et al.*, 1993; White *et al.*, 1994). Few detailed studies have been made on the prevalence of HIS, and these have used different methodologies, focusing upon specific population groups (Henrik-Nielsen *et al.*, 1983, 1985; Tompkins *et al.*, 1986; Surawicz *et al.*, 1987; Barrett, 1990; Lindboe *et al.*, 1993; Law *et al.*, 1994). Two main types of studies have been conducted – those where feces have been cultured for spirochetes (principally isolating *B. pilosicoli*), and those where intestinal biopsies have been examined for histologic evidence of HIS. In general, the highest prevalence of colonization has been found in three groups: first, in developing communities such as in villagers in Papua New Guinea (22.8% by fecal culture; Trott *et al.*, 1997a) and India (64% by rectal biopsy; Mathan and Mathan, 1985), but also including indigenous populations such as Australian Aboriginals (32.6% by fecal culture; Lee and Hampson, 1992); secondly, in homosexual males in Western societies (21–53.7% by rectal biopsy or fecal

culture; McMillan and Lee, 1981; Tompkins *et al.*, 1981; Law *et al.*, 1994; Trivett-Moore *et al.*, 1998); thirdly, in HIV+ patients in Western countries (44.4% by biopsy; Käsbohrer *et al.*, 1990).

Apart from the high-risk groups, HIS is comparatively rare in Western populations, with a prevalence of around 2.5% in routine diagnostic colorectal biopsies (Lindboe *et al.*, 1993) and 1.5% by specialized fecal culture (Tompkins *et al.*, 1986; Lee and Hampson, 1992).

In a unique study in Papua New Guinea (PNG) multiple isolates of *B. pilosicoli* were isolated from the feces of the same villagers over time. The results indicated that 93.6% of the villagers became infected with *B. pilosicoli* each year, with an average duration of infection of 117 days (Trott *et al.*, 1997a, 1998).

Processes in colonization

By light microscopy of sections stained with hematoxylin and eosin, HIS is identified as a thick, blue fuzzy brush-border on the surface of the colorectal epithelium (Fig. 1). This may be overlooked if the biopsy is not examined at high resolution. Colonization occurs locally or at more than one intestinal site, and can be distributed along the entire length of the large bowel (Lindboe *et al.*, 1993). Colonization densities may reach 1700 organisms/mm² (Takeuchi *et al.*, 1974). The spirochetes invaginate into the enterocyte membrane, forming an electron-lucent pit with a zone of electron-dense material just below the membrane at the point of contact between the cell and the spirochete (Neutra, 1980; Teglbjaerg, 1990). Sometimes there is a loss of microvilli and disruption of terminal web microfilaments in the enterocytes (Cooper *et al.*, 1986; Lindboe *et al.*, 1993; Muniappa *et al.*, 1996).

Spirochetes show differential attachment to neoplastic and non-neoplastic colonic epithelium, suggesting that specific cell receptors may be involved in the attachment process (Lee *et al.*, 1971; Coyne *et al.*, 1995). It is assumed that the spirochetes have specialized attachment mechanisms, but unfortunately there have been relatively few attempts to investigate possible cell surface components on *B. pilosicoli* (Lee and Hampson, 1995, 1999; Tenaya *et al.*, 1998). It is known that *B. pilosicoli* lacks genes homologous to those involved in attachment and invasion processes found in *Yersinia enterocolitica* and enteropathogenic *Escherichia coli* (Hartland *et al.*, 1998). The availability of *in vitro* cell culture systems should help in the future analysis of attachment processes (Muniappa *et al.*, 1999).

Usually little tissue reaction is seen in patients with HIS, although significant changes to the mucosa were noted in rectal biopsies of Indians with HIS (Mathan and Mathan, 1985). In several case reports, invasion of the mucosa has been observed in patients with HIS (Antonakopoulos *et al.*, 1982; Gebbers *et al.*, 1987;

Padmanabhan *et al.*, 1996; Peghini *et al.*, 2000), particularly in individuals with advanced stages of HIV infection (Lafeuillade *et al.*, 1990; Guccion *et al.*, 1995; Kostman *et al.*, 1995). In these studies spirochetes have been seen in colonic enterocytes, goblet cells, macrophages and Schwann cells. In one report, spirochetes morphologically similar to *B. aalborgi* were detected in the liver of a HIV+ patient, although HIS was not detected in the large intestine (Kostman *et al.*, 1995). In another report, spirochetes that were later shown to be *B. pilosicoli* were isolated from the blood of a series of critically ill patients in France and the US (Fournié-amazouz *et al.*, 1995; Trott *et al.*, 1997b).

Taking into account these apparent invasive properties, certain other isolated studies are of interest. Unidentified non-*Borrelia burgdorferi* spirochetes (Gutacker *et al.*, 1998) have been detected in the cerebrospinal fluid and brain tissue of patients with Alzheimer's disease (Miklossy, 1993; Miklossy *et al.*, 1994). In another patient with clinical features of seronegative spondylarthropathy as well as HIS, serology eliminated other possible arthritogenous agents (Pellet *et al.*, 1995). Other case studies involved non-syphilitic spirochetes detected in spontaneously aborted fetuses (Abramowsky, 1991), and a 12-year-old boy with a large perianal condyloma containing non-syphilitic spirochetes that possibly originated from the intestines (Sagerman *et al.*, 1993).

Species of intestinal spirochetes associated with HIS

Although *B. aalborgi* and *B. pilosicoli* are thought to be the main species of intestinal spirochete involved in HIS, it cannot be excluded that other, as yet unidentified, intestinal spirochetes may be associated with certain cases. Intestinal spirochete strain N26, isolated from an Arab from Oman, was originally thought to be one such new spirochete species (Hookey *et al.*, 1994), but further studies using multilocus enzyme electrophoresis (MLEE) have shown that N26 is a strain of *B. pilosicoli* (unpublished data).

Brachyspira aalborgi

B. aalborgi was described in 1982 following its isolation in Denmark from rectal biopsies of five patients with HIS (Hovind-Hougen *et al.*, 1982). A follow-up report concluded that the spirochete was non-pathogenic, as the mucosa of HIS patients appeared normal, and their symptoms remained unchanged after the spirochetes were eliminated (Henrik-Nielsen *et al.*, 1983). Most subsequent case reports, based solely on histology, have referred to *B. aalborgi* as being the etiologic agent of HIS (Rodgers *et al.*, 1986; da Cunha Ferreira *et al.*, 1993; Guccion *et al.*, 1995; Kostman *et al.*, 1995; Padmanabhan *et al.*, 1996).

B. aalborgi remains poorly characterized, and until recently only one strain was available for study. It has not been isolated from animals, although polymerase chain reaction (PCR) amplification of DNA extracted from macaques has indicated that it may colonize this species (Duhamel *et al.*, 1997). *B. aalborgi* fails to colonize experimentally infected chicks, and no experimental animal models are available (Trott and Hampson, 1998). The type strain is 2–6 µm long, with a maximum cell width of about 0.2 µm, and four flagella at each end. The slow growth rate of *B. aalborgi* (at least 2 weeks' anaerobic incubation before colonies were seen on tryptose blood soy agar plates containing 400 µg/ml spectinomycin and 5 µg/ml polymixin) (Hovind-Hougen *et al.*, 1982) has made it difficult to isolate. Recently, strains of *B. aalborgi* have been isolated from biopsy samples taken in Sweden (C. Fellström, personal communication, 1999), Denmark (K. Møller, personal communication, 1999) and Japan (Y. Adachi, personal communication, 1999).

Steps towards defining this organism genetically were made when its allelic profile was determined using MLEE (Lee and Hampson, 1994), and its 16S rRNA gene was sequenced (Stanton *et al.*, 1996). The original isolate of *B. aalborgi* was shown to be genetically distinct from other anaerobic spirochetes previously isolated from human feces (which are now known as *B. pilosicoli*).

Brachyspira (Serpulina) pilosicoli

B. pilosicoli is a weakly hemolytic anaerobic spirochete, the phenotypic properties of which have been described (Trott *et al.*, 1996a, b). Where intestinal spirochetes have been isolated from humans, these have almost exclusively been *B. pilosicoli*. This was the case in studies carried out on indigenous populations in Oman (Barrett, 1990), Western Australia (Lee and Hampson, 1992), and PNG (Trott *et al.*, 1997a), and in homosexual males (Trivett-Moore *et al.*, 1998) and HIV+ patients (Käsbohrer *et al.*, 1990). The role of *B. pilosicoli* as an enteric pathogen in humans was supported by a study in which a volunteer drank a culture containing a strain recovered from an Aboriginal child with diarrhea. The volunteer developed abdominal discomfort and bloating, as well as severe headaches (Oxberry *et al.*, 1998).

Besides humans, *B. pilosicoli* colonizes the large intestine of a variety of animal species, and is recognized as a pathogen of pigs (Trott *et al.*, 1996c; Thomson *et al.*, 1998), chickens (McLaren *et al.*, 1997; Stephens and Hampson, 1999) and dogs (Duhamel *et al.*, 1998). Human strains of *B. pilosicoli* have been used experimentally to infect chickens (Trott *et al.*, 1995; Muniappa *et al.*, 1996), pigs (Trott *et al.*, 1996c) and mice (Sacco *et al.*, 1997), with a proportion of animals

showing end-on attachment of spirochetes, diarrhea and/or reduced growth rates. These studies helped to confirm the pathogenic potential of *B. pilosicoli*, but also suggested that zoonotic transmission may be possible. Although it is assumed that person-to-person transmission of *B. pilosicoli* is direct, via the fecal-oral route, environmental sources of infection may also exist. For example *B. pilosicoli* has been recovered from lake water and wild waterbirds, both of which could be sources of human infection (Oxberry *et al.*, 1998). Under adverse conditions *B. pilosicoli* can assume a resistant cyst-like structure (Gebbers and Marder, 1989; Barber *et al.*, 1995), and this may aid its survival outside the intestinal tract.

Although identified as being associated with disease in pigs in 1980 (Taylor *et al.*, 1980), it was not until 1993 that *B. pilosicoli* was shown, using MLEE analysis, to be a genetically distinct species from other porcine intestinal spirochetes (Lee *et al.*, 1993a). At that time these spirochetes were provisionally called '*Anguillina coli*'. After earlier studies suggesting that human intestinal spirochetes were *B. hyodysenteriae* (the agent of swine dysentery, a severe mucohemorrhagic colitis of pigs), or at least closely related to *B. hyodysenteriae* (Coene *et al.*, 1989; de Wergifosse and Coene, 1989), MLEE analysis and DNA-DNA reassociation studies clearly defined virtually all of the human intestinal spirochetes isolated up to this point as belonging to the same species as the new pig isolates (Lee *et al.*, 1993b; Lee and Hampson, 1994). The unique nature of these spirochetes was confirmed upon further examination of their biochemistry (Fellström and Gunnarsson, 1995) and 16S rRNA gene sequence (Lee and Hampson, 1994; Fellström *et al.*, 1995; Pettersson *et al.*, 1996). A species-specific PCR was first developed for the organisms in 1995 (Park *et al.*, 1995).

The new species was officially named *Serpulina pilosicoli* in 1996, with the original porcine strain P43/6/78^T isolated by Taylor *et al.* (1980) as the type strain (Trott *et al.*, 1996a). Based largely on analysis of 16S rRNA sequences, *S. pilosicoli*, *S. hyodysenteriae* and *S. innocens* were transferred to the genus *Brachyspira* in 1997 (Ochiai *et al.*, 1997).

Strain differentiation

Lack of available strains has prevented development of strain typing techniques for *B. aalborgi*. In contrast, several techniques have been applied for differentiating strains of *B. pilosicoli*, including restriction fragment length (RFLP) analysis (Koopman *et al.*, 1993), MLEE analysis (Lee *et al.*, 1993a; Lee and Hampson, 1994; Trott *et al.*, 1998), pulsed-field gel electrophoresis (PFGE) (Atyeo *et al.*, 1996; Rayment *et al.*, 1997; Trott *et al.*, 1998) and whole-cell protein profiling (Barrett *et al.*, 1996). Porcine isolates of *B. pilosicoli* have also been

typed by amplified fragment length polymorphism (Møller *et al.*, 1999), and this technique has potential for typing human isolates. Of these techniques, MLEE and PFGE have been most widely used. PFGE is more discriminating for strain typing than MLEE, and has been used to confirm that *B. pilosicoli* is genetically diverse. Both techniques proved useful in an epidemiological survey where 164 *B. pilosicoli* isolates from villagers, pigs and dogs in the Eastern Highlands of PNG were analysed (Trott *et al.*, 1998). Both techniques indicated that the isolates were genetically diverse, while a lack of linkage disequilibrium detected by MLEE implied the existence of frequent genetic recombination in the *B. pilosicoli* population. Isolates with the same PFGE types were found in humans and dogs, suggesting the possibility of cross-species transmission. A similar conclusion was drawn in a study in Europe, where strains from dogs and humans had the same RFLP patterns (Koopman *et al.*, 1993). An interesting aspect of the PNG study was the observed genetic variation of isolates obtained from individuals on up to three separate occasions over a period of 3 weeks. Seven individuals were colonized with distinct PFGE types on each occasion. Whether a high rate of reinfection and/or concurrent infection was occurring remains unknown. In another three individuals the first and second isolates differed from each other by only one or two DNA bands, suggesting that recombination may also have occurred. Unfortunately in these studies it was not possible to determine whether individuals had end-on attachment of spirochetes to their colonic mucosa, as biopsies were not taken.

Problems with previous studies of HIS

Problems of interpretation in relation to the etiologic agent have arisen from previous studies of HIS. One difficulty is that most studies have only used either histology or culture, and there have only been two successful attempts at combining these two detection methods in the same study. One involved the only reported isolation of *B. aalborgi* (Hovind-Hougen *et al.*, 1982), while the other isolated *B. pilosicoli* alone from rectal mucosa of a series of Australian homosexual males (Trivett-Moore *et al.*, 1998).

Problems also exist in relation to ease of isolation of the two spirochete species. *B. aalborgi* is slow growing and cultures are very prone to contamination, while *B. pilosicoli* is faster growing, with colonies being visible in approximately 4 days under optimal conditions. It is also uncertain whether the extra antibiotics that have often been added to isolation plates for *B. pilosicoli* (e.g. colistin, vancomycin) may actually inhibit the growth of *B. aalborgi*. These considerations suggest that studies involving bacterial culture may be biased towards the detection of *B. pilosicoli*.

One solution would be to use PCR assays to detect the spirochetes directly. A variety of PCR tests are available for detecting and identifying *B. pilosicoli*, with these amplifying portions of the genes for 16S rRNA (Park *et al.*, 1995), 23S rRNA (Leser *et al.*, 1997) or NADH oxidase (Atyeo *et al.*, 1999), but there are no reports of these tests having been applied to human feces samples. In pigs, direct amplification from feces has been problematic, but improved detection of *B. pilosicoli* has been achieved by amplifying growth harvested from the primary isolation plate (Atyeo *et al.*, 1998). This procedure also identifies the spirochete. To date no PCR tests have been developed to detect *B. aalborgi* in feces, although PCR assays using the 16S rRNA and NADH oxidase gene have been described (Mikosza *et al.*, 1999).

Recent studies designed to determine the etiology of HIS

In recent studies we have attempted to circumvent problems of bias in culture efficiency by applying PCRs directly to DNA extracted from large intestinal biopsies from patients with a histologic diagnosis of HIS (Mikosza *et al.*, 1999, 2001). Biopsies from the large intestine of 45 patients from Australia, Norway, France and the USA were used. All these individuals had had routine biopsies taken for a variety of intestinal problems, and a fringe-like carpet of spirochetes had been observed on the intestinal epithelium. Samples from 15 patients without HIS were included as controls. With the exception of four patients who were HIV+ (two from Australia and both US patients), the patients were categorized as being at low risk for HIS. In most Australian patients, biopsies were taken at multiple sites in the large intestine. PCR primers based upon the 16S rRNA and NADH oxidase (*nox*) genes of *B. aalborgi* or *B. pilosicoli* were developed and tested for specificity in four separate PCR reactions (two target sequences for each species). Total DNA then was extracted from the biopsy samples, and subjected to the four separate PCR reactions.

There was no amplification of DNA from biopsy samples from the control individuals. Biopsies from 10

(22%) HIS patients failed to amplify, biopsies from 33 (73%) patients were positive in both *B. aalborgi* PCRs, and four (9%) were positive in both *B. pilosicoli* PCRs. Two of the above patients (4.5%) were positive for both species in all four PCRs (Table 1). Of the four HIV+ patients, three were colonized with *B. aalborgi* while the other, an Australian, was colonized by *B. pilosicoli*. No correlation could be made between gastrointestinal symptoms and spirochete species, and the two species could not be differentiated by visual examination of the histologic sections (Figs 1 and 2). Much larger studies will be required to dissect out possible differences in distribution or severity of HIS caused by the two species.

These studies showed that *B. aalborgi* is the predominant spirochete species involved in histologically defined HIS involving the colorectal epithelium of patients from the general population in Western countries. *B. pilosicoli* was demonstrated to be involved in only a few individuals, and this low prevalence was consistent with the low rate of fecal carriage of the organism in the general Western population (Tompkins *et al.*, 1986; Lee and Hampson, 1992). In contrast, *B. pilosicoli* has been shown to be the main spirochete species attaching to the rectal mucosa in homosexual males in Western societies (Trivett-Moore *et al.*, 1998), as well as being common in the feces of individuals from developing countries. The only known specific risk factor in the current study was infection with HIV, and only one of the four HIV+ patients was colonized by *B. pilosicoli*. It is important to note that either or both organisms could also have been colonizing the intestinal tract without attaching to the epithelium, and hence would not have been detected in this study. Dual infection with both spirochete species was also found in this study, and a similar situation has been recorded in macaques (Duhamel *et al.*, 1997). The physical disposition of the two spirochete species in the mucosa could be studied further by using fluorescent *in situ* hybridization. This technique has been used to identify *B. pilosicoli* attachment in pigs (Jensen *et al.*, 2000), and could be adapted to the identification and localization of *B. aalborgi* and *B. pilosicoli* in human tissue.

The lack of PCR amplification in 22% of the HIS patients may have been due to technical problems asso-

Table 1. Country of origin of 45 patients diagnosed with HIS, and results of PCR utilizing primers specific for *B. aalborgi* and *B. pilosicoli* 16S rRNA and *nox* genes applied to DNA extracted from biopsy samples from the large bowel

Origin	Number of patients	<i>B. aalborgi</i>	<i>B. pilosicoli</i>	<i>B. aalborgi</i> and <i>B. pilosicoli</i>	Negative
Australia	25	20	1	2	2
Norway	12	7	0	0	5
France	6	2	1	0	3
USA	2	2	0	0	0
Total	45	31	2	2	10

ciated with DNA degradation, but also could have resulted from the HIS being due to other spirochete species that have not been identified. To clarify these possibilities, the DNA could be subjected to PCR using spirochete-specific primers directed at the 16S rRNA gene (Choi *et al.*, 1994). If amplification occurred, sequencing the PCR product could identify the spirochete species present.

Given that fecal carriage of *B. pilosicoli* is common in people in developing countries, and that histologic evidence of HIS has been seen in Indian villagers, it would be useful to apply PCRs to biopsy samples taken from such individuals to determine whether *B. pilosicoli* is the main species involved in HIS in this setting. The comparative distribution of the two spirochete species in different geographic and socio-economic settings could also be studied in sero-epidemiologic surveys, as could possible associations between intestinal spirochetes and more obscure diseases. Development of such serologic tests for the two species is a research priority in our laboratory.

Development of optimal culture techniques for isolation of *B. aalborgi* is another important priority, since, amongst other things, this would allow strain typing to be undertaken. Currently we are attempting to differentiate between strains of *B. aalborgi* in different patients by amplifying and sequencing several genes.

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