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COMMENTARY

Hiding in Plain Sight: Colonic Spirochetosis in Humans

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ABSTRACT In 1967, Harland and Lee made a startling discovery: in some humans, the colonic epithelium is covered with a "forest" of spirochetes (W. A. Harlan, and F. D. Lee, Br Med J 3:718–719, 1967, https://doi.org/10.1136/bmj.3.5567.718). In this issue of *Journal of Bacteriology*, Thorell et al. present a systematic analysis of the prevalence and diversity of the spirochetes *Brachyspira aalborgi* and *Brachyspira pilosicoli* in the human colon. These and prior studies provide avenues toward resolving important questions: what bacterial and host parameters contribute to this extensive colonization, and what impact does it have on human health?

KEYWORDS *Brachyspira*, *Brachyspira aalborgi*, *Brachyspira pilosicoli*, gastrointestinal infection, human infection, intestinal colonization

The association of spirochetes with the gastrointestinal (GI) tracts of humans and other mammals has been known since the time of van Leeuwenhoek, who noted the presence of spiral organisms in human feces. Interest in these spirochetes grew rapidly with the explosion of microbiology during the late 1800s and early 1900s, leading to many publications dating back to 1884 (reviewed in references 1 and 2). However, attempts to culture these organisms during that time were either unsuccessful or not reproducible. Rejuvenation of the study of intestinal spirochetosis during the past 50 years was fueled by two discoveries. Harland and Lee (3) performed electron microscopy of a rectal biopsy specimen from a patient with chronic diarrhea and found that the epithelium was covered with a near-confluent "forest" of spirochetes firmly attached by one end to the surface of the host cells (Fig. 1). Additionally, spirochetes later identified as *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, the latter meaning "short spiral-shaped organisms that make the colon look hairy," were found to be frank pathogens in swine, causing dysentery and wasting in young pigs (4). Currently, nine *Brachyspira* species are recognized (*B. aalborgi*, *B. alvinipulli*, *B. hampsonii*, *B. hyodysenteriae*, *B. innocens*, *B. intermedia*, *B. murdochii*, *B. pilosicoli*, and *B. suanatina*), but to date only *B. aalborgi* and *B. pilosicoli* are known to colonize humans. While the natural host ranges of these organisms have not been studied thoroughly, *B. aalborgi* is reported to colonize humans, nonhuman primates, and opossums, whereas *B. pilosicoli* has been found in humans, swine, dogs, and birds (5, 6). A new species, "*B. catarrhinii*," has been proposed; it encompasses a group of organisms (previously characterized as subset of *B. aalborgi*) that colonize monkeys (7).

A number of studies have been carried out to determine the prevalence of *Brachyspira* colonization in humans and the potential association with disease manifestations. Some of these studies are summarized in Table 1; case reports or studies involving a small number of subjects (<5) are not included. Many of the early studies were published before the *Brachyspira* species were cultured or characterized, and methods such as light and electron microscopy cannot distinguish between different *Brachyspira* species. Therefore, information regarding the relative prevalence of *B. aalborgi* and *B. pilosicoli* was not available for several of the articles. Methods used for detection of colonic spirochetosis range from simple hematoxylin and eosin staining of

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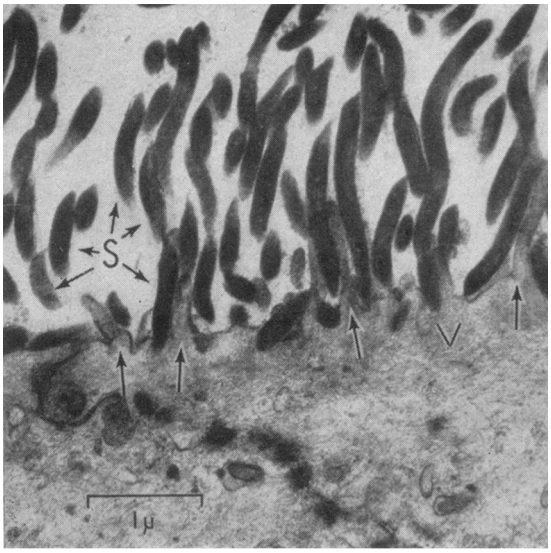

FIG 1 Human colonic spirochetosis. Spirochetes attach end-on to the colonic epithelium, as demonstrated by electron microscopy. This image is from the landmark 1967 publication by Harland and Lee (3) and is reprinted with permission from the publisher. Arrows indicate microvilli. S, spirochetes; V, reactive vacuole. Bar = 1 μm.

tissue sections to culture, PCR, and fluorescent *in situ* hybridization (FISH). Culture from either fresh or frozen fecal samples or biopsy specimens collected during colonoscopy has been effective in isolation of *Brachyspira* organisms, yielding a large number of strains in some studies.

In a comprehensive article featured in this issue of the *Journal of Bacteriology*, Thorell et al. (8) discuss the prevalence and properties of *Brachyspira* species in a group of 745 human subjects in Stockholm, Sweden. This study was part of a larger project called PopCol (9), which investigated the prevalence of endoscopic findings in randomly selected adults in the general population; it thus establishes a firm "baseline" of data useful in assessing the etiology of functional gastrointestinal disorders such as irritable bowel syndrome. Among the strengths of the article is the unbiased sampling of colonoscopy biopsy specimens from a representative cross section of the adult population. Random sampling was made at the terminal ileum (small intestine) and 4 different sites in the colon of each subject. The specimens were screened by light microscopy techniques for the presence of the "false brush border" appearance typical of human colonic spirochetosis (HCS). Seventeen subjects out of the 745 examined (2.3%) were determined to have HCS. Confirmatory studies and culture were performed on the samples from these 17 individuals, and 14 yielded positive cultures; these exhibited a predominance of *B. aalborgi* (13 positive individuals) relative to *B. pilosicoli* (1 positive individual), as reported in other European studies. Perhaps the most valuable information to come out of this study was the nearly complete genomic sequences of 16 *B. aalborgi* strains, including the type strain, 513A; these are the first available *B. aalborgi* genomic sequences. Finally, Thorell et al. determined that the primer sets commonly used for the amplification of 16S rRNA gene sequences for microbiome determinations are ineffective in amplifying *Brachyspira* 16S sequences. They also showed that *Brachyspira* organisms are likely underrepresented in prior human gut microbiome studies.

The article by Thorell et al. (8) thus represents a significant addition to the prior publications (Table 1). To an "outsider" who studies other spirochetes, the accumulating literature on the distribution and characteristics of HCS organisms is very impressive, given the relatively small number of groups contributing to these studies. What are some of the insights that have been derived by this cumulative work, and what questions still remain?

TABLE 1 Selected studies examining the prevalence of human colonic spirochetosis

Study	Reference	Detection method(s)[a]	Location(s)	Study population	Yr(s) of specimen collection
Lee et al. (1971)	2	EM, HE, SS, PAS	Glasgow, Scotland, UK	Patients with diarrhea or suspected cancer	1961
Lee et al. (1971)	2	EM, HE, SS, PAS	Glasgow, Scotland, UK	Appendectomy cases	1963–1966
McMillan and Lee (1981)	11	HE	Glasgow, Scotland, UK	MSM[c]	NI[d]
McMillan and Lee (1981)	11	HE	Glasgow, Scotland, UK	Heterosexual men	NI
Mathan and Mathan (1985)	26	EM	Southern India	Healthy adults	NI
Cooper et al. (1986)	27	EM	Southhampton, Hampshire, England, UK	MSM	NI
Cooper et al. (1986)	27	EM	Southhampton, Hampshire, England, UK	Heterosexual men	NI
Surawicz et al. (1986)	28	HE, AB, SS	Seattle, WA	MSM	NI
Tompkins et al. (1986)	29	C	Great Britain, UK	Healthy adults	NI
Barrett (1990)	30	C	Muskat Region, Oman	Healthy children and adults	1988
Barrett (1990)	30	C	Muskat Region, Oman	Hospitalized patients	1988
Lee and Hampson (1992)	12	C	Western Australia	Aboriginal children and adults	1989–1991
Lee and Hampson (1992)	12	C	Western Australia, Northern Territory	Non-Aboriginal children and adults	1989–1991
De Brito et al. (1996)	32	HE, SS, IHC, EM	Brazil	Patients with GI[f] symptoms	NI
Trivett-Moore et al. (1998)	13	HE, EM, C	Sydney, Australia	MSM attending a sexual health clinic	NI
Brooke et al. (2001)	10	C	Western Australia	Aboriginal rural patients with GI complaints	1998–1999
Brooke et al. (2001)	10	C	Western Australia	Non-Aboriginal rural patients with GI complaints	1998–1999
Brooke et al. (2001)	10	C	Australia	Entering migrants to Australia	1998–1999
Margawani et al. (2004)	33	C	Bali, Indonesia	Adult and child residents	1999
Margawani et al. (2004)	33	C	Bali, Indonesia	Adult and child residents	1999
Esteve et al. (2006)	16	HE, SS, PAS, PCR	Barcelona, Spain	Patients with chronic watery diarrhea and control subjects	1994–2004
Calderaro et al. (2007)	34	PCR, C	Parma, Italy	Patients with suspected gastrointestinal infections	2002–2006
Tanahashi et al. (2008)	35	HE, SS, IHC, immuno-EM, PCR	Oita, Japan	Patients with colonoscopy or surgical resections	2005–2006
Ichimata et al. (2017)	36	HE	Asahi, Matsumoto, Japan	Patients <20 yrs of age with gastrointestinal symptoms	NI
Thorell et al. (2019)	8	HE, IHC, SS, C	Sweden	Adult population	2000–2006
Mikosza et al. (2001)	37	PCR	Australia	HCS subjects (by HE)	NI
Mikosza et al. (2004)	38	PCR	Australia (20); USA (1); France (1); Norway (2)	HCS subjects (by HE)	NI
Westerman et al. (2012)	39	Real-time PCR	The Netherlands	HCS subjects (by HE, IHC)	2001–2011
Rojas et al. (2017)	40	FISH	Germany	HCS subjects (by HE)	NI
Rojas et al. (2017)	40	PCR	Germany	HCS subjects (by HE)	NI

[a]In the studies described in references 37 to 40, specimens were prescreened for colonic spirochetosis. Abbreviations: AB, alcian blue-stained sections; C, culture; EM, transmission electron microscopy; FISH, fluorescent *in situ* hybridization; HE, hematoxylin and eosin-stained sections; IHC, immunohistochemistry; PAS, periodic acid-Schiff-stained sections; SS, silver-stained sections.
[b]—, species not determined.
[c]MSM, men who have sex with men.
[d]NI, not indicated.
[e]FFPE, formalin-fixed, paraffin-embedded tissue.
[f]GI, gastrointestinal.
[g]Includes 36 *B. aalborgi* cluster 1 organisms alone, 6 *B. aalborgi* cluster 2 ("*B. hominis*") organisms alone, 6 *B. pilosicoli* organisms alone, 5 cluster 1 and cluster 2 organisms, 1 *B. pilosicoli* and cluster 2 organisms, and 2 *B. pilosicoli*, cluster 1, and cluster 2 organisms (triple positive).

TABLE 1 (Continued)

Specimen(s)	Total subjects	*B. aalborgi* positive	*B. pilosicoli* positive	Total HCS cases	Percent positive	Comments
Rectal biopsy specimen	144	—[b]	—	10	6.9	Includes the cases from Harland and Lee (3)
Excised appendix	790	—	—	62	7.8	Included acute appendicitis (7/144 [4.4%]), "simulated" appendicitis (15/523 [9.8%]), incidental appendectomy (4/107 [4.4%])
Colorectal biopsy specimen; FFPE[e]	100	—	—	36	36.0	
Colorectal biopsy specimen; FFPE	67	—	—	2	3.0	
Rectal biopsy specimen	14	—	—	9	64.3	
Rectal biopsy specimen	8	—	—	5	62.5	Reduction in microvillus density observed
Rectal biopsy specimen	5	—	—	0	0.0	
Rectal biopsy specimen	100	—	—	28	28.0	
Colorectal biopsy specimen	1,527	—	—	23	1.5	All positive specimens from either MSM or persons of Asian ethnicity
Feces	292	—	—	78	26.7	
Feces	1,000	—	—	114	11.4	
Feces	181	—	—	59	32.6	Isolates were shown subsequently to be *B. pilosicoli* (31)
Feces	695	—	—	8	1.2	
Rectal and sigmoidal colonic biopsy specimens	282	—	—	4	1.4	
Rectal biopsy specimens	41	(0)	(13)	22	53.7	*B. pilosicoli* isolated from biopsy samples positive (11/22) and negative (2/19) for HCS by HE and EM
Feces	151	0	15	15	9.9	High proportion of isolates from subjects aged 2 to 5
Feces	142	0	0	0	0	
Feces	227	0	24	24	10.6	Isolates/subjects for migrants from Asia (2/8), Eastern Europe (3/94), the Middle East (9/65), and Africa (10/50)
Feces (August)	500	0	59	59	11.8	375 subjects were sampled at both time points
Feces (December)	492	0	62	62	12.6	375 subjects were sampled at both time points
Colonic biopsy samples	1,176	(2)	(2)	8	0.7	Of 8 subjects positive for HCS by light microscopy, 2 were positive for *B. pilosicoli* and 2 for *B. aalborgi* by PCR
Feces, colonic biopsy samples, FFPE	234	13	5	16	6.8	Two patients were coinfected with *B. aalborgi* and *B. pilosicoli*
Colonic biopsy samples, FFPE	2,556	20	3	20	0.8	11 cases identified by HE, SS, and IHC. 20 cases positive for *B. aalborgi* by PCR; 3 cases also positive for *B. pilosicoli*
Biopsy specimens, surgical specimens	479	—	—	1	0.2	
Biopsy samples of terminal ileum and colon from cecum to rectum (5 sites)	745	13	1	17	2.3	HCS cases correspond to those described previously by Walker et al. (18); 3 subjects who were positive by HE, IHC, and SS positive were negative by culture
Colon, colorectal, cecum, and appendix biopsy samples; FFPE[b]	28	24	4	26	92.9	2 subjects were positive for both *B. aalborgi* and *B. pilosicoli*; 2 subjects were negative by PCR
Colon, colorectal, cecum, and appendix biopsy samples; PET	24	22	2	24	100	Prescreened for intestinal spirochetosis by histology
Colon biopsy samples; FFPE	56	48	9	56	100	Several genotypes[g]
Intestinal biopsy samples (from ileum to rectum); PET	91	—	—	77	84.6	Prescreened for intestinal spirochetosis by histology; same specimens were analyzed by both FISH and PCR
Intestinal biopsy samples (from ileum to rectum); PET	91	53	23	75	82.4	Prescreened for intestinal spirochetosis by histology; includes one subject with both *B. aalborgi* and *B. pilosicoli*

By all indications, HCS has a global distribution. *Brachyspira* species have been detected in humans on every continent (except Antarctica). However, the prevalence of HCS in healthy populations varies widely in different studies, from 0 to 64.8% (Table 1). Lower prevalences (0.2% to 3.2%) tend to be found among healthy individuals in temperate, highly urbanized areas (such as Western Europe and Japan), whereas higher levels (10.8% to 64.8%) have been observed in Indonesia, Oman, and India and in

Australian Aboriginal populations. This trend does not appear to be associated strictly with rural versus urban environments; for example, Brooke et al. (10) found that Aboriginal and non-Aboriginal individuals with gastrointestinal disorders within the same rural region of Western Australia had HCS prevalences of 9.9% and 0%, respectively. The proportion of individuals with HCS is generally higher in subjects with gastrointestinal complaints. Men who have sex with men (MSM) populations also have relatively high proportions of colonic spirochete colonization, with prevalences ranging from 28 to 62.5% (Table 1). In one study in Scotland, McMillan and Lee (11) found that 36/100 MSM subjects (36%) were positive for colonic spirochetes, whereas a control group of male heterosexual subjects had an HCS prevalence of 2/67 (3%). As stated by Lee and Hampson (12), "there may either be ethnic or environmental influences predisposing to spirochaetal colonization of the intestine."

Another variable aspect of HCS is the proportion of *B. aalborgi* versus *B. pilosicoli* colonization. Early studies utilized only light or electron microscopy to detect spirochete colonization, so distinction between *Brachyspira* species was not possible. As culture and PCR from colonic biopsy or stool specimens came into common use, most studies could utilize the sequences of 16S rRNA and NADH oxidase genes for species and subgroup identification. *B. pilosicoli* tends to be more common in populations with a high incidence of HCS, including Aboriginal Australian, Indonesian, and MSM groups (13). In comparison, *B. aalborgi*, which has not been associated with disease symptoms, is predominant in areas such as Europe, Japan, and urban Australia. The combination of low HCS prevalence (2.3%) and *B. aalborgi* dominance (14 of 16 isolates) is evident in the Swedish population studied by Thorell et al. (8). Genotyping by 16S rRNA gene sequencing further revealed that their *B. aalborgi* isolates fell into cluster 1 of the two major genotype clusters within this species (8).

Brachyspira species exhibit a spectrum of pathogenesis and host ranges, as reviewed previously (5, 14). *B. hyodysenteriae* is a frank pathogen in swine but does not colonize or cause disease in humans. *B. pilosicoli* has a broader host range, with swine, birds, humans, and nonhuman primates being among its known natural hosts. This organism is a cause of diarrheal disease and economic losses in farms raising swine and chickens. In humans, abdominal pain, diarrhea, and perirectal bleeding can be present in some *B. pilosicoli*-positive individuals, whereas many others are asymptomatic; *B. pilosicoli* also has been shown to cause spirochetemia in critically ill patients (15). Thus far, *B. aalborgi* has been detected only in humans, nonhuman primates, and opossums (5); some of the animal isolates may instead be the proposed species "*B. catarrhinii*" (7). Colonization of the colon by *B. aalborgi* is not significantly associated with gastrointestinal symptoms or histopathology, leading some investigators to conclude that it is essentially a commensal organism.

The association between *Brachyspira* colonization and gastrointestinal problems such as chronic diarrhea remains unclear. Thorell et al. (8) and Esteve et al. (16) noted that many individuals were positive for HCS at several regions of the colon yet were asymptomatic, indicating that an extensive "forest" of *Brachyspira* can be present throughout much of the colon without causing GI symptoms. Carr et al. (17) reviewed a series of HCS cases (113 colonic biopsy specimens and 16 appendixes) and concluded that there was a lack of inflammatory changes, except in cases with other causes of inflammation; they further stated that HCS in an inflamed biopsy specimen is "likely to be an incidental finding." However, Walker et al. (18) noted that the occurrence of clusters of eosinophils in the subepithelial tissue of the colon was significantly associated with HCS. Additionally, clearance of HCS spontaneously or through treatment with metronidazole or other antimicrobial agents may result in resolution of symptoms in chronic diarrhea in some cases (6, 16, 19). Perhaps the occurrence of gastrointestinal symptoms may result from the combination of HCS and as yet undefined host factors.

A major contribution of the study by Thorell et al. was the addition of 18 new genomic sequences. The 16 *B. aalborgi* genome sequences revealed that the overall sizes (2.50 to 2.71 Mb) are smaller than those of *B. hyodysenteriae* (2.99 to 3.17 Mb) and

lack the 36-kb plasmid thought to be important in pathogenesis (20). The *B. aalborgi* genomes have the characteristic low G+C content of *Brachyspira* (28.1 to 28.3%) and are otherwise similar to genomes of the 8 other *Brachyspira* species for which sequences are available (20, 21). Intraspecies comparisons of the *B. aalborgi* genomes revealed a heterogeneity greater than expected, although the organisms were clearly separated from other species. Black et al. (20) found that the genomes of *B. hyodysenteriae* strains had undergone significant rearrangements, and the heterogeneity in *B. aalborgi* may also be related in part to rearrangements. Availability of the *B. aalborgi* sequences will extend the comparative genomics possibilities within the *Brachyspira* genus, perhaps helping to reveal key differences important in host interactions, such as pathogenesis or host range (21).

The article by Thorell et al. (8) also revealed that the primer sets commonly used for amplifying 16S rRNA gene sequences for microbiome studies do not amplify *Brachyspira* sequences. As a result, analyses of the human gut microbiome lack representation from *Brachyspira*. An *in silico* reexamination of data from the Human Microbiome Project by Thorell et al. (8) found that only 1 individual out of 179 had *Brachyspira* 16S sequences, and the two samples from this individual contained only 0.03 to 0.04% *Brachyspira* sequences. This paucity of *Brachyspira* sequences is supported by a recent computational analysis of 11,850 human gut microbiomes, which yielded 92,143 metagenome-assembled genomes (MAGs) (22). Of these, only one MAG was identified as a *Brachyspira* species, and this genome assembly turned out to be from a bovine rumen specimen mistakenly included in the collection. Overall, these results are surprising given the extensive forests of *Brachyspira* found in some individuals and the ability to culture *B. pilosicoli* and *B. aalborgi* readily from both colonic biopsy and feces specimens. Perhaps the strong adherence of *Brachyspira* to the colonic epithelium limits the number present in feces, or the amplification of *Brachyspira* sequences in metagenomic studies is relatively inefficient because of its low G+C content.

The study by Thorell et al. and the cumulative work since the 1967 article by Harland and Lee have greatly increased our understanding of human colonic spirochetosis. However, many questions remain, as emphasized in several of the articles cited in this commentary. First and foremost, should *B. pilosicoli* (and perhaps also *B. aalborgi*) be considered a human pathogen? An "*n* of one" *B. pilosicoli* WesB self-inoculation experiment was conducted in the 1990s and resulted in colonization, abdominal discomfort, bloating, and headaches; the infection and symptoms resolved following metronidazole treatment (23). Should a more extensive, blinded, and controlled inoculation study in humans be performed, as has been done with norovirus and other human gastrointestinal pathogens? How is HCS transmitted: through direct human-to-human contact, through animal-to-human transmission (in the case of *B. pilosicoli*), via contaminated water or food supplies (6, 23, 24), or by other means? Do humans vary in susceptibility to colonization or disease manifestations, and to what extent is this aspect related to genetic factors, immune status, hygiene, overall health, sexual activity, socioeconomic influences, climate, and additional parameters? Does the immune response affect colonization or the progression of infection? What determines the host range and pathogenicity of *Brachyspira* species, and do certain strains within each species differ in their infectious and pathogenic properties? Can the expanding genomic and proteomic (25) information be effectively "mined" for this information? Specifically, what genes are involved in these processes, including the characteristic attachment mechanism and cytotoxicity (e.g., the strong and weak hemolysis by *B. hyodysenteriae* and *B. pilosicoli*, respectively)? Is genetic manipulation of *Brachyspira* possible, as has been accomplished to some extent with *Borrelia*, *Treponema*, and *Leptospira* organisms? Some of these questions may be addressed in part by studies of veterinary pathogens, e.g., those affecting the swine and poultry industries. It is hoped that special attention will be paid to the high incidence of *B. pilosicoli* in developing countries and its potential impact on human health.

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