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Commentary:
***Brachyspira* and irritable bowel syndrome with diarrhoea: a Helicobacter pylori moment?**

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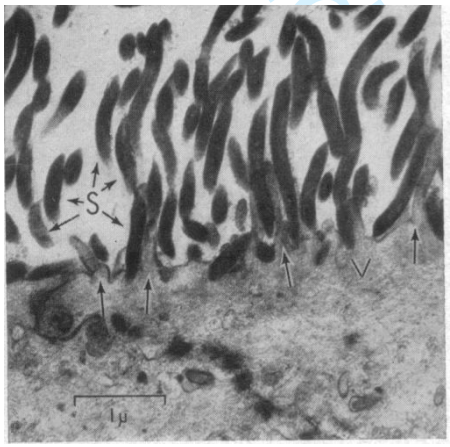

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Abstract
Human intestinal spirochetosis (HIS) has been thought to be largely asymptomatic. The fuzzy brush border seen in HIS is due to an anaerobic spirochaete of the Brachyspira genus aligned perpendicular to the colonic mucosa. Inflammatory submucosal reaction is rare. However several species of Brachyspira found in domestic animals cause diarrhoeal disease and one B. pilosicoli has been documented to transfer freely between dogs, chickens, pigs and humans. This commentary reviews an article which finds evidence of Brachyspira spp. infection in up to 1/3rd of patients with irritable bowel syndrome with diarrhoea. It is unclear whether current treatments are truly effective in the long term. Further work is needed since it remains unclear whether the association is cause or effect.

Human intestinal spirochetosis (HIS) has been recognised since the early days of histopathology but similar to Helicobacter pylori, its presence is inconsistently linked to disease so it is usually dismissed as a harmless commensal(1). The condition is caused by *Brachyspira*, a genus of anaerobic bacteria whose spiral form and periplasmic flagellae allows them to easily glide through colonic mucus which is their normally habitat. They were first reported in 1719 when Van Leeuwenhoek, reported moving spiral "animalcules" in his own stools. HIS can be identified from a blurry red fringe on the intercryptal epithelial surface, seen throughout the colon in sections stained with haematoxylin and eosin. Electron microscopy shows myriads of organisms lined up, perpendicular to the mucosa,

the proximal tip embedded in but not penetrating an invagination of the host cell membrane (Figure). Conventional histology finds HIS in 0.4% in a hospital series in Japan and 2.3% in a population survey in Sweden(2) but 39% in male homosexuals in the UK with a link to ora-anal contact but not HIV status(3). Many cases of HIS appear to be asymptomatic, one large Australian survey of 113 cases of HIS found only 10 cases with obvious inflammation, 6 of whom had other explanations for the inflammatory response(4). Detailed morphological examination in isolated case reports suggests that gastrointestinal symptoms only occur when spirochetes invade beyond the surface epithelium, which appears to be rare and may require additional factors(2).

Veterinary scientists have a rather different perspective since *B. pilosicoli, B. intermedia, B. alvinipulli* and particularly *B. hyodysenteriae*, can all cause diarrhoea and weight loss in domestic animals including dogs, chickens and pigs (5), an problem linked to overcrowding and poor hygiene due to intensive farming practices. *B. pilosicoli* can survive in soil and infect humans particularly in rural communities where the organism appears to move freely between humans and pigs, dogs and chickens(6). Although in this population most infections appear asymptomatic those with watery stools were more likely to harbour *B. pilosicoli*. The two other *Brachyspira species*, *B. aalborgi* and *B. hominis* appear to be primate adapted, being found in humans and subhuman primates(5) where they mostly cause no inflammatory response. An Australian survey found that *B. pilosicoli* infection is largely confined to rural aboriginals (15%), with *B. aalborgi* being less prevalent (5%) but found in both rural and urban living individuals. Interestingly, having *B. aalborgi* increased the risk of having *B. pilosicoli* to 12-fold as did a co-infection with *Blastocystis*(7). Comparative genome sequencing of the various *Brachyspira* species suggests *B. aalborgi* to be evolutionarily the most divergent species of the genus(8). Significantly the primers most commonly used in human microbiome studies fail to detect *Brachyspira* 16S rRNA, which may explain why it has been "hidden in plain sight"(9). The *B. aalborgi* genome is highly heterogeneous with very low GC% and lacks the potentially pathogenic genomic region harboured by the other *Brachyspira spp. (10)*.

The current paper (Jabber, 2020) goes further than previous studies in attempting to separate the effect of different species (*B. aalborgi /hominis /pilosicoli*) and their distribution within the colonic mucus layers (deep/superficial). Mass spectrometry provided proteomic evidence of *Brachyspira* within the deep adherent mucus layer of 3/22 IBS patients but 0/14 healthy controls. Analysis of a further 40 IBS patients and 17 controls showed that overall, 14/50 patients had detectable *Brachyspira spp.* shown by ≥2 methods (PCR or immunofluorescence). Membrane associated spirochaetosis was not found in controls but was found in 9/43 patients with conclusive results from immunofluorescence, seven of whom had IBS-D. When just membrane associated cases were considered, most of whom had *B. aalborgi*, stool frequency was greater, stools looser and transit faster but extracolonic symptoms were less supporting idea that the origin of symptoms was more gut than brain. However, the increase in eosinophils, mast cells and plasma cells in those with *Brachyspira spp.* was seen in both mucus and membrane associated taxa, reducing one's confidence that these are directly linked. There was an increase in many mucosal proteins in those with *Brachyspira spp.,* the most notable being guanylin, a secretagogue which might well explain loose stools.

Only four patients were treated with open label metronidazole. Three of these showed a fall in IBS-SSS of >50 at 1 year but two would still be described as moderate/severely symptomatic. With such a small cohort no comment is really appropriate apart from the fact that a proper randomised placebo-controlled trial is warranted.

Part of the confusion about whether *Brachyspira* causes symptoms could be that studies to date have not distinguished between the significantly different *Brachyspira spp*. and strains, of which only a minority cause disease. It remains also possible that rather than causing IBS, having IBS-D creates an environment where *Brachyspira spp*. can flourish. Given Brachyspira species are ubiquitous, factors which facilitate colonisation like diet (11) and antibiotic treatment (12), may explain differences in colonisation rates. It is possible that the increased risk of being prescribed antibiotics associated with having IBS (13) may contribute to the increased prevalence of *Brachyspira spp.*

Another possible factor allowing *Brachyspira spp.* to cause inflammation might be impaired mucus barrier. IBS patients often report stress which can lead to impaired mucus barrier at least in animals(14). Co-infection with other enteric pathogens exacerbates the inflammatory response in *B. pilosicoli* infection(15) so it is possible that some of the post-infectious IBS cases have *Brachyspira spp*. which causing ongoing symptoms after the original infection has subsided.

Many clinicians reading this will be wondering whether they should be reassessing their IBS-D patients, since if the current cohort is representative, around 1/3 may harbour *Brachyspira spp*.. The current data suggest caution, particularly given that in this study metronidazole may have driven Brachyspira to relocate intracellularly with unknown consequences. Plainly we have a lot to learn. More work on the basic biology in animal models would seem necessary to choose the correct antibiotic for a randomised placebo-controlled trial in humans, which would be an important step forward in this fascinating area.

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Legend to figure

EM from (1) showing surface of colonic epithelial cells with numerous spirochaetes (S) orientated in the long axis of the cells. Arrows indicate microvilli. Reproduced by permission of the publisher