Gut

Leading article

Non-pylori helicobacter species in humans

Introduction

The discovery of *Helicobacter pylori* in 1982 increased interest in the range of other spiral bacteria that had been seen not only in the stomach but also in the lower bowel of many animal species.[1 2] The power of technologies such as the polymerase chain reaction with genus specific primers revealed that many of these bacteria belong to the genus *Helicobacter*. These non-pylori helicobacters are increasingly being found in human clinical specimens. The purpose of this article is to introduce these microorganisms to the clinician, put them in an ecological perspective, and to reflect on their likely importance as human pathogens.

Gastric bacteria

In 1987, Dent *et al* described the presence of a novel bacterium in 3/1300 gastric biopsies.[3] The initial differentiation was based on morphology, the bacterium having a larger tight helical shape compared to the S shape of *H pylori* (fig 1). Subsequent studies have shown that while rarely found in humans it is the dominant gastric organism in a number of animal species including primates, pigs, cats, and dogs.[4] Although first given the name *Gastrospirillum hominis* this gastric bacterium has subsequently been shown to belong to the *Helicobacter* genus and has been given the provisional name of *Helicobacter heilmannii*.[5 6] Another bacterium, *Helicobacter felis*, which is morphologically similar to *H heilmannii* by light microscopy, has also been noted in three cases.[7–9] Its identification is based on the presence of periplasmic fibres which are only visible by electron microscopy. *H felis* has been used extensively in mouse models of *H pylori* infection.[10]

Since the first report in 1987, over 500 cases of human gastric infection with *H heilmannii* have appeared in the literature.[11] The prevalence of this infection is low, ranging from ~ 0.5 % in developed countries[5 7 12–15] to 1.2–6.2% in Eastern European and Asian countries.[16–19]

H heilmannii, like *H pylori*, is associated with a range of upper gastrointestinal symptoms, histologic, and endoscopic findings. The gastritis observed with *H heilmannii* infection tends to be less severe than that due to *H pylori*[20] but infection has been found in association with duodenal ulceration,[20 21] gastric ulceration,[12 20 22 23] gastric carcinoma[17 24] and mucosa associated lymphoid tissue (MALT) lymphoma.[15 20] Indeed, a surprisingly high rate (3.4%) of MALT lymphomas in *H heilmannii* infected patients was noted by Stolte.[20]

The majority of patients are asymptomatic; however, epigastric pain or discomfort, nausea, vomiting, anorexia, weight loss, diarrhoea, and occasionally gastrointestinal bleeding may occur. At gastroscopy, findings range from a

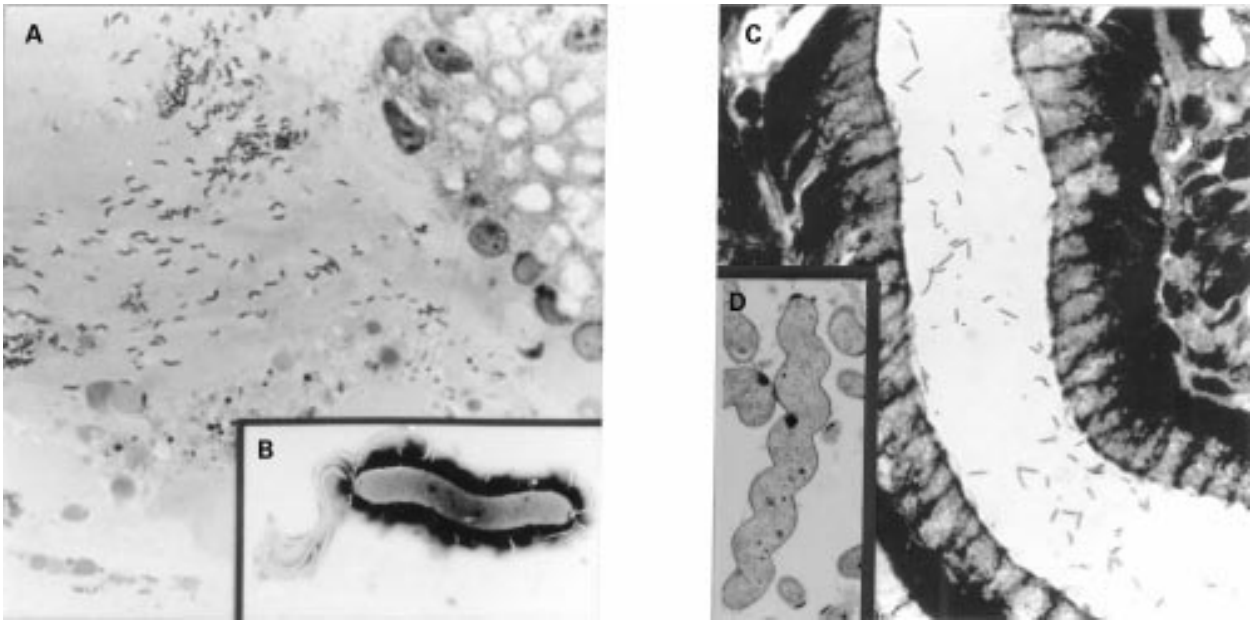

Figure 1 Light micrographs of gastric tissue from humans infected with (A) H pylori and (C) H heilmannii (× 1000). Insets show higher magnifications in which the characteristic S-shape morphology of H pylori (B) can be seen in comparison to the tight helical shape of H heilmannii (D) (× 10 000).

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normal appearance to antral erythema, erosive gastritis, gastric ulceration, duodenal erosions, and ulceration.[8 22 23 25 26] Gastric lymphoid nodules have been reported in cases of *H heilmannii* infection in children as is the case with *H pylori*[23 27–30] (T Bohane and J Mitchell, personal communication). The gastritis is characterised histologically by an infiltrate of polymorphonuclear leukocytes, lymphocytes, and plasma cells in the lamina propria. Acute infection is associated with erosions and a predominantly neutrophilic infiltrate.[7 8 26 31] Of interest, two of the cases of acute infection have been associated with *H felis* infection.[7 8]

The diagnosis of *H heilmannii* infection is often missed unless gastric biopsies are examined for these distinctive helical shaped bacteria. While they can be seen in haematoxylin and eosin stained sections, they are more obvious when a Giemsa or silver stain is used. *H heilmannii* can also be seen by touch cytology of gastric smears.[14] Serology for *H pylori* and rapid urease tests can be relatively insensitive, the latter probably related to the patchy nature of *H heilmannii* colonisation and low numbers of bacteria present when compared with *H pylori*.[22] Large numbers of attempts to culture this bacterium using a variety of media and growth conditions have been unsuccessful. One group has reported on culture of a similar organism from humans, though molecular studies indicate this bacterium is *Helicobacter bizzozeronii*, an organism normally found in canine gastric mucosa.[32] *H heilmannii* can however be readily maintained by in vivo culture techniques in mice.[33 34]

Owing to the relatively small number of reported cases of gastroduodenal disease associated with *H heilmannii*, it is difficult to infer a causal relationship with great certainty. However, treatment with a variety of agents that includes bismuth, amoxicillin, metronidazole, H2 blockers, and proton pump inhibitors, has resulted in complete, or near complete, resolution of symptoms in the majority of patients.[5 13 16 22 25 26 35] In addition, the successful eradication of *H heilmannii* infection with omeprazole and amoxicillin resulted in remission of primary gastric low grade MALT lymphoma in 5 patients.[15]

It is likely that most *H heilmannii* infections represent zoonoses. Meining *et al* showed in a large study of patients infected with *H heilmannii* that, compared with *H pylori* infected individuals, contact with pigs, cats, and dogs was associated with a significantly increased risk of *H heilmannii* infection (odds ratio, 4.99, 1.71, and 1.46 respectively).[36]

In summary, the low frequency of *H heilmannii* infections found in humans means that definite disease association is unlikely to be proven. Imagine if one had to prove multiple disease associations of *H pylori* in less than 1000 reported cases. The reality is that this organism almost certainly does cause gastritis, although this inflammation is generally less aggressive than that seen with *H pylori* infection and may cause proportionately more cases of gastric MALT lymphoma. As it is generally easier to eradicate *H heilmannii* than *H pylori* the best approach would be to offer a standard course of anti *H pylori* therapy to any patient in which a non-*H pylori* gastric helicobacter is detected.

The home of the helicobacters—mucous as a natural niche

Just as the gastric mucosae of most animals studied has been shown to be heavily colonised with spiral shaped bacteria, later shown to be helicobacters, so too had the surfaces of the lower bowel. One of us (AL) spent the 1970s closely examining the intestines of mice as a complex microbial ecosystem in particular the mucosal surfaces and crypts, and found they were packed with a wide range of very distinct bacterial populations. Although varying in size and amplitude, all had in common a spiral shape (fig 2). We reasoned that this morphology gave the bacteria a selective advantage in the viscous mucous environment.[37 38] These bacteria were not limited to mice: when we looked at the lower bowel mucous of cats, dogs, pigs, etc they also showed characteristic populations of spiral bacteria (A Lee, unpublished data). Following the discovery of *H pylori*[1 2] the bacterium's morphology led us to suggest that its natural habitat was the gastric mucous.[39] Many of these spiral bacteria have since been classified as belonging to the *Helicobacter* genus, which is hardly surprising given that gut mucous is their natural habitat. These species include *Helicobacter bilis, Helicobacter hepaticus,* and *Helicobacter muridarum* that are naturally found in small rodents,[40–42] *Helicobacter cinaedi* and *Helicobacter cholecystus* in gerbils and hamsters,[43 44] *Helicobacter pullorum* and *Helicobacter pametensis* in chickens and birds,[45 46] and *Helicobacter canis* in dogs.[47]

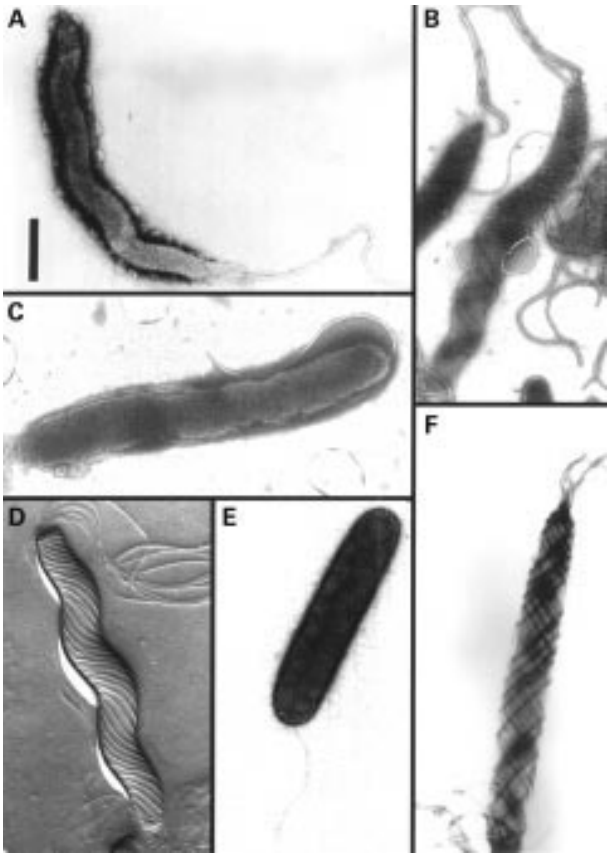

Figure 2 Electron micrographs showing the various morphologies of some of the lower bowel helicobacters. (A) H hepaticus, S-shaped; (B) H bilis, tapered-curved rod with periplasmic fibres; (C) H pullorum, S shaped, (D) H muridarum, S shaped with periplasmic fibres, (E) H cholecystus, curved rod and (F) H rappini, tapered rod with periplasmic fibres. All Helicobacter species possess single or multiple polar flagella. (× 30 000).

Is intestinal mucous a natural niche for helicobacters in humans?

A natural extension of these observations is that humans could also have helicobacters as part of their lower bowel normal flora. However, the evidence to support this hypothesis is surprisingly sparse. In comparison with the detailed mapping studies of the entire gastrointestinal tract in healthy animals, the collection of similar human samples has not been possible and opportunities to obtain suitable mucous samples for culture are limited. Indeed there is only one post mortem study showing spiral shaped bacteria in the gastrointestinal tract of humans. In 1983,

Croucher *et al*, using electron microscopy, observed spiral shaped bacteria in the intestinal mucous of 2 out of 4 individuals who had died suddenly.[48] Mathan also described spirals in the bowels of a group of Indian patients.[49] Despite this sparsity of reports of lower bowel spirals in normal humans, a number of the *Helicobacter* species found in the lower bowels of animals have been found in human clinical specimens.

Intestinal infections

It is possible that *Helicobacter* species are under recognised causes of infective diarrhoea in humans today due to the specialised methods required for their isolation. Culture of helicobacters from faecal samples is best performed with a filter technique that is not widely used in commercial laboratories. These bacteria are often sensitive to the antibiotics used in standard campylobacter selective agars and require incubation for 7 to 10 days in a microaerophilic atmosphere supplemented with hydrogen.[50]

Helicobacters cultured from human diarrhoeal samples include *H cinaedi, H canis, H pullorum*, *Helicobacter fennelliae, Helicobacter canadensis, Helicobacter rappini* and other unclassified but related organisms.[51–55] The mere presence of *Helicobacter* species in a diarrhoeal specimen is not proof of causality. In experiments with rodents we found that induction of diarrhoea with magnesium sulphate resulted in the appearance in the diarrhoeal stools of bacteria we now know were helicobacters whereas they could not be seen in normal rodent faeces.[37 56] Although confirmation is required, the available evidence clearly implicates *H cinaedi* and *H fennelliae* in the causation of human intestinal disease. However, the evidence for a pathogenic role for the other reported isolates is even more limited.

In 1983, a comprehensive study of homosexual males with anorectal and intestinal symptoms involving 119 symptomatic and 75 asymptomatic men was undertaken.[51] These men were sexually active (average 7 exposures per month) with multiple previous sexual partners, recently engaging in anilingus (70%) and receptive anal intercourse (95%). *Campylobacter* or *Shigella* species were isolated from rectal swabs in 11 of 119 symptomatic and 3 of 75 asymptomatic men. A striking feature of this study is that in around twice as many men *Campylobacter* like organisms (CLO) were found—21 of the symptomatic and 6 of the asymptomatic men. The CLOs were further classified into three groups based on phenotypic and genotypic characteristics with two of the groups later classified as new species; *Helicobacter cinaedi* and *Helicobacter fennelliae*.[57 58] The sigmoidoscopic and histopathologic findings in men with CLO infections were similar to those with *Campylobacter jejuni* infection.[59] *C jejuni* or CLOs were not isolated from 150 consecutive asymptomatic heterosexual men and women attending the same clinic.[57 59] It is likely that this cluster of *H cinaedi* and *H fennelliae* cases was first recognised in homosexual men because of sexual behaviours that increase the risk of faecal–oral and mucosa–mucosa transmission. Indeed, local epidemics of bacterial intestinal pathogens are well described in homosexual male communities.[60] As the natural hosts for *H cinaedi* are thought to be gerbils and hamsters, the authors speculate that a zoonosis resulting from contact with these animals may have been the initial source.[43]

Other evidence that intestinal *H cinaedi* and *H fennelliae* are pathogenic and not an incidental finding in stool samples derives from work in infant pig-tailed macaques.[61] Watery or loose stools containing the organisms and bacteraemia developed in the infant monkeys 3 to 7 days after oral inoculation of 10^8–10^9 bacteria. Both *Helicobacter* species could be cultured from stools for 3 or more weeks after inoculation.

In 1993, Burnens *et al* described the isolation of a *spiral* organism from a 5 year old child suffering from gastroenteritis.[52] This organism had previously been found in a large study of faecal samples from healthy and diarrhoeic dogs[62] and was subsequently recognised as a new species, *H canis*.[47]

Helicobacter pullorum originally isolated from chickens[45] has also been cultured from immunocompetent and immunodeficient human patients presenting with acute or chronic diarrhoea.[53 55 63] Recent detailed analysis of four *H pullorum* isolates from Canadian patients presenting with diarrhoea showed they possessed atypical biochemical features and could be differentiated from *H pullorum* on the basis of 16S rRNA sequencing and restriction fragment length polymorphism (RFLP) analysis. Based on this data the name *H canadensis* has been proposed for this group of organisms.[55]

Helicobacter rappini (Flexispira rappini) is another example of a helicobacter whose initial classification was based on morphology, being a tapered rod entwined with periplasmic fibres and multiple bipolar flagella. This bacterium was first described in aborted sheep foetuses.[64] Similar organisms were reported in humans with gastroenteritis in 1988 including a case in which the bacterium was found in both the index case, his 16 year old asymptomatic daughter and a pet puppy.[54 65] Treatment with erythromycin resulted in the resolution of symptoms. *H rappini* was later shown to be part of the faecal flora of rodents, and gastric and faecal flora of dogs.[54 66 67]

Although these less common intestinal helicobacter isolates do not have proven pathogenic potential in the human gut, there is good evidence for disease causation in their animal hosts. When *H rappini* was inoculated into sheep and guinea pigs, it was found the bacteria could cross the placenta of pregnant animals and induce abortion with hepatic lesions seen in the sheep foetuses.[68 69] *H canis* has been seen and cultured from the liver of a puppy with multifocal necrotising hepatitis[70] and *H pullorum* is found in the intestine and liver of diseased birds.[45]

In summary, *H cinaedi* and *H fennelliae* are the helicobacters most frequently isolated from human colonic samples and for which there is clear evidence of pathogenicity. The role of the four other *Helicobacter* species in human disease is unclear. As for the gastric infections, contact with animals is thought to be responsible for initiating clusters of intestinal infections in humans. In contrast to *H heilmannii* gastric infections however, person to person spread of the intestinal bacteria through faecal–oral contact is common. At this stage there is no good evidence that antimicrobial treatment is desirable for immunocompetent hosts with moderate diarrhoeal disease in whom a helicobacter is cultured from stool samples.

Are helicobacters involved in human inflammatory bowel disease?

A substantial body of evidence suggests that bacterial antigens are key contributors to the development of inflammatory bowel disease (IBD) in a genetically or immunologically predisposed host.[71] Recently, murine models have clearly shown that if the normal immune balances are altered then mucosa associated *Helicobacter* species induce a pathology similar to human IBD.[72] This is possibly due to their location in mucous, the microbial niche closest to the susceptible mucosa. Whether an analogous process occurs in humans is unclear. *Helicobacter* species have not consistently been isolated from IBD patients. However, whether an equivalent population of mucous adapted bacteria exists

in the human colon is unknown as this ecological niche has been poorly studied.

Systemic infections

Pathogenic intestinal bacteria such as non-typhi *Salmonella* and *Campylobacter* species can cause bacteraemia and seed to other anatomical locations by translocating from the intestinal lumen. Bacteraemias are up to 20 times more prevalent in human immunodeficiency virus (HIV) infected individuals compared to the general population[73] and enteric bacteria, in particular *Salmonella* species, account for up to 30% of AIDS related bacteraemias.[74] These bacteraemias are often recurrent and occur in the absence of gastrointestinal symptoms or positive stool cultures. All of these observations would lead one to expect human helicobacter associated bacteraemias. The passage of helicobacters into the hepatobiliary system is not well defined or documented. That this is a possibility, at least in primates, was shown in a recent study by Fox *et al* where they found *H cinaedi* in the colon, liver, and mesenteric lymph nodes of a rhesus monkey with colitis and hepatitis. They proposed several mechanisms for hepatic colonisation including direct migration from gut lumen into the bile duct or M cell uptake followed by lymphatic or haematogenous dissemination.[75]

Within a year of the first description of intestinal CLOs in the homosexual population, two cases of bacteraemia were reported in homosexuals with concurrent tuberculosis.[76] Since that time there have been at least 30 reports of bacteraemias associated with *Helicobacter* species involving a total of 65 patients with several reports of recurrent infections.[77–81] The organism most frequently cultured from blood was *H cinaedi*, 51/63 (81%) cases; followed by *H rappini* which was found in 7/63 (11%) cases though bacterial speciation can be difficult and controversial.[82] A majority of cases (55%) occurred in patients infected with HIV and these were predominantly male homosexuals. Other predisposing factors often reported include alcoholism, end stage renal failure, carcinoma, diabetes, and primary immunodeficiencies such as X linked agammaglobulinemia and hypogammaglobulinemia.[78 80 83–87] There have been four reports of *Helicobacter* associated bacteraemias in children[88–90] and 5 cases in healthy immunocompetent adults.[78 81 91]

The secondary sites of infection reported with helicobacter bacteraemia include abdominal abscess, cellulitis, septic arthritis, meningitis, and pneumonia.[76 78 83 87–90 92–94]

Ciprofloxacin has been successfully used in many cases for the treatment of *H cinaedi* bacteraemias[95] however, resistance to this drug may be acquired during infection.[78 83] Other reported antimicrobial regimes include penicillin or ampicillin, tetracycline, or an aminoglycoside such as gentamicin. An infection with *H fennelliae* was successfully treated with ampicillin-sulbactam and ceftazidime[84] and meropenem, imipenem, and gentamicin have been used to treat *H rappini* infections.[80 85] Treatment periods of 2 to 6 weeks are recommended.

In all cases of bacteraemia, helicobacters were detected in automated blood cultures systems with growth apparent 6–10 days after inoculation. Helicobacters are not readily seen by conventional Gram staining methods, and the use of Acridine orange or Giemsa stains, or phase contrast or dark field microscopy is recommended for visualisation. Subculture of the bacteria onto solid agar for further characterisation and antimicrobial sensitivity testing is achieved by the use of enriched media such as Brucella agar supplemented with blood. The agar plates need to be incubated at 37°C under microaerobic conditions, often with added hydrogen, for up to 10 days to detect bacterial growth.

In summary, the frequency of *Helicobacter* bacteraemias may well be underestimated due to their fastidious nature. A lack of suitable blood culture systems in the developing world may mean that many such infections are not being recognised particularly in the third world where HIV is a dramatic problem. Based on case reports, antimicrobial therapy can be successful and is clearly warranted with antimicrobial sensitivity testing performed where possible.

Hepatobilary infections and their significance

Intestinal *Helicobacter* species can enter the bloodstream, particularly in immunocompromised individuals, and thus it would be expected that these organisms could end up in the liver. In murine models, the intestinal helicobacters have been observed to translocate to the liver where viable infection may be associated with inflammation and/or neoplastic change.[41 96] The hypothesis that helicobacters infect the human intestine, liver, or biliary tree and may be responsible for previously unexplained human pathology is certainly very attractive. However, despite detection of helicobacter 16s ribosomal DNA (16s rDNA) by multiple investigators in human hepatic and biliary tissue by polymerase chain reaction (PCR) there have been no published accounts of the culture or consistent ultrastructural identification of helicobacters from these tissues.[97 102] This is in contrast to the relative ease with which helicobacters have been cultured from animal liver tissue.[41 103]

In the absence of the direct isolation of bacteria there are several reasons to be cautious in the interpretation of these PCR based studies. Firstly, the results of these studies have often been conflicting. For example, Fox *et al* detected helicobacter 16s rDNA in 13 of 23 bile samples and 9 of 23 resected gall bladder tissues from Chilean women with chronic cholecystitis.[97] In contrast, Rudi *et al* were not able to detect any helicobacter DNA in bile samples from 73 Germans with biliary disease.[98] Nilsson *et al* reported positive helicobacter PCR in 11 of 12 patients with primary biliary cirrhosis whereas Tanaka *et al* demonstrated helicobacter DNA in only 1 of 29 PBC cases and Harada *et al* in 0 of 5 cases using a PCR cloning methodology.[99 101 104] The main exception to this criticism has been the detection of helicobacter DNA in a majority of primary hepatic and biliary cancers.[100 102] Some of these anomalies may be explained by geographic and clinical differences in the populations studied. In addition, many of these studies do not report the results of PCR for other bacterial species and, when they do so, a plethora of other (possibly contaminating) bacterial species is discovered.[104]

Interestingly, the gene sequence obtained from positive *Helicobacter* species specific 16s rDNA PCRs is usually most analogous to *H pylori*.[99 102] This raises the possibility that the presence of helicobacter DNA in human liver tissue is a reflection of the transport of *H pylori* of gastric origin or its DNA to the liver.[105] At present this hypothesis is speculative. It is important to note that this region of the 16S rRNA gene is not highly variable amongst different *Helicobacter* species[82] and so the evidence to date does not preclude the presence of another human gut *Helicobacter* species. Two studies did however indicate that intestinal helicobacters may be implicated in hepatobiliary disease.[97 101] Accurate species determination was only reported in the study by Fox *et al* where they were able to identify *H bilis*, *H pullorum*, and *H rappini* with no detection of *H pylori*.[97] To make further progress, future studies should include an attempt to culture and directly identify helicobacters ultrastructurally in the hepatobiliary system, in addition to assessing gastric helicobacter status.

Conclusion

The explosion of the genus *Helicobacter* over the past few years poses a challenge for the clinician. Almost monthly, a new species is reported and named. Animal experimentation suggests that several of these bacteria cause disease. The increasing sophistication of culture methods has resulted in the isolation of non-pylori helicobacters from humans. What does this mean? The situation in the stomach is clear. Like *H pylori* in the human, animals have highly specialised populations of helicobacters that have evolved to inhabit the ecological niche of gastric mucous. These animal organisms can transmit to humans and it is likely they cause symptomatic disease.

The lower bowel picture is less clear and requires much more investigation. Most animals also have highly adapted populations of *Helicobacter* species inhabiting their intestinal mucous. In some circumstances, if these bacteria move outside their natural niche they can cause disease—for example, hepatitis in the mouse.[103] If normal homeostasis is altered they can cause severe pathology within their natural niche—for example, inflammatory bowel disease in immunocompromised mice.[72] To date, human adapted lower bowel helicobacters have not been described. Are we looking close enough? Are we looking at the right populations? Yet the intestinal helicobacters of animals have been found in humans where they are able to induce gastrointestinal symptoms and translocate into the bloodstream. The implication is that these bacteria are transmitted to humans from animals. Are they and if so, how? Systematic study of these bacteria using appropriate isolation and identification methods is needed in both health and disease. Only then will we know whether the discovery of these bacteria will influence the management of intestinal and systemic disease in the dramatic way that the discovery of *H pylori* impacted on the management of gastroduodenal disease.

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