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The biliary tract is normally sterile, but bile-tolerant bacteria are frequently isolated from patients with cholecystitis. Since the identification of about 25 *Helicobacter* species, some of which may grow in bile, studies have addressed the role of these organisms in primary biliary cirrhosis, primary sclerosing cholangitis, and cholelithiasis. Most of these bacteria show the presence of *Helicobacter* DNA or antigens in the bile tract and in liver samples. Altogether, data from studies on biliary and hepatic diseases, as well as pancreatic disorders, suggest that bile-tolerant *Helicobacter* species may induce a chronic infection with possible malignant transformation.

Introduction

The biliary tract is normally sterile, but when pigmented and cholestering bile stones are present, various microbes can be cultured from or detected in bile or the gallbladder wall [1,2•] and studied by electron microscopy. Interestingly, Wetter *et al.* [1] suggested that *Escherichia coli* organisms and various other enteric microbes such as enterococci may use the same surface proteins (named adhesins) to adhere and colonize the gut, liver, and biliary tract epithelium. Well-known infectious agents of the liver include several viruses and *Leptospira* species organisms, inducing hepatitis with clinical or subclinical jaundice [3]. Whether these organisms may induce biliary infections has not been elucidated. Today, these infections are rare in Europe and North America. Other biliary infections include acute and chronic cholecystitis induced by *Enterococcus* species, bile-tolerant strains of *Haemophilus influenzae*, *E. coli*, and related enteric microbes [4]. There are serologic indications of chronic infection caused by *Mycobacterium* species in primary biliary cirrhosis (PBC), and multiple examples of retroviral infections preceding PBC, primary sclerosing cholangitis (PSC), and Sjögren's syndrome [5]. Although liver infections caused by parasites are common in tropical and subtropical areas, no biliary infections caused by parasites, except *Entamoeba histolytica*, have been described. Bacterial pathogens, like *Helicobacter*, *Arcobacter*, and *Campylobacter* species organisms, induce inflammation primarily by release of cell-wall associated lipopolysaccharide (LPS) and lipoteichoic acid (LTA) by enterococci and other gram-positive organisms, which are less common causes of liver and biliary tract infection. The extremely low cell toxicity of LPS in *H. pylori* and other *Helicobacter* species organisms (Hynes S, Wadström T, Unpublished observations) suggests a different pathogenesis of these microaerophilic pathogens, lacking the *pho/pho-2* gene structure of *Salmonella typhimurium*, enterohemorrhagic *E. coli*, and other enteric pathogens [6].

More than 20 formally named species of the genus *Helicobacter* have been described [7,8,9•], and at least 13 species colonize the lower gastrointestinal tract of domestic and laboratory animals. Some of these species may have a zoonotic potential, *ie*, *H. pullorum* isolated from the intestine of poultry and humans, *H. cinaedi* from dogs, cats, and humans, and *H. rappini* from dogs, mice, and humans. Most likely, many of these species that naturally colonize the intestinal epithelium and its crypts can also colonize the biliary tract and the liver and induce cholangitis, hepatitis, and malignancies of the bile tract [9•,10]. Interestingly, early reports of *C. jejuni* inducing such infections were confirmed in a study in Japan, demonstrating close adherence of these microbes to the biliary tract epithelium [11]. The pioneer study by Fox *et al.* [12] in Chile showing *H. bilis* and *H. hepaticus* organisms in human cholecystitis-associated infections some years earlier in laboratory mice [8,13,14] has stimulated studies on such enteric *Helicobacter* species as possible causes of biliary tract, liver, and enteric infections in humans (Table 1) [2,15–17,18••].

Enteric *H. cinaedi* (previously *C. cinaedi*) organisms were isolated from stool cultures and blood from homosexual HIV-infected patients as well as from children and adult patients with immunodeficiency syndromes [8]. *H. cinaedi* was recently reported to cause chronic colitis, hepatitis, and mediastinitis in rhesus monkeys, which strongly supports the theory that enteric *Helicobacter* species may translocate from the gut epithelium [19••]. An inflamed colon in animals infected with various enteric *Helicobacter* species, and some *Campylobacter* species, suggests that specific diagnostic methods should be developed to study these infections in animals and humans.

Table 1. *Helicobacter* species as possible causes of hepatobiliary disease in humans

Species	Human disease	Host
H. pylori	Chronic liver disease (?)	Human, monkey
H. hepaticus	Chronic liver disease (?)	Mouse
H. pullorum	Gastroenteritis	Poultry, human
H. bilis	Holecystitis	Mouse, human (?)
H. cinaedi	Septicemia, enteritis	Human, monkey
H. rappini	Cholecystitis	Sheep and others
H. canis	Chronic liver disease	Dog and cat (?)

H. pullorum organisms were first isolated from ceca of normal chicken and from livers and intestines of chicken with hepatitis but rarely from human patients with gastroenteritis [20]. The fastidious nature of *H. pullorum* as well as other enteric *Helicobacter* species demonstrates the need to develop quantitative (real-time) polymerase chain reaction (PCR) and other DNA-based diagnostic methods, such as denaturing gradient gel electrophoresis, to study the possible importance of these pathogens in human intestinal and extraintestinal infections. Interestingly, *H. pullorum* and other enteric species, such as *H. hepaticus* and *H. bilis*, produce toxin(s) like the cytolethal distending toxin (CDT), first characterized in *E. coli*, and later in *C. jejuni* [21]. These toxins may be crucial in intestinal and extragastric infections, and they play a similar role to that of the vacuolating (VAC) toxin of *H. pylori* in establishing infection in the gastric epithelium [22].

Helicobacter-associated Hepatobiliary Infection and Disease

We have recently identified *Helicobacter* species organisms, including *H. pylori*, with genus- and various species-specific primers for PCR in livers from patients with PSC and PBC [17,18••]. Avenaud *et al*. [23••] reported on *H. pylori*-like organism in patients with primary liver carcinoma, and Nilsson *et al*. [24•] reported on similar "liver" *H. pylori*-like organisms (>98% identity with *H. pylori* by DNA sequencing) in patients with primary cholangiocarcinoma. However, more recent studies by Stolzenberg-Solomon *et al*. [25•] and at our institution indicate that such *H. pylori* or *H. pylori*-like organisms may also infect the human pancreas. Similarly, *H. cholecystus* was reported to induce cholangitis and pancreatitis in hamsters [26]. These observations, as well as *H. pylori*-like organisms detected by PCR and immunoblot in human bile samples from patients with gallstones and other biliary tract diseases (Ljungh A, Wadström T, Unpublished), suggest that systematic studies are needed. These studies would employ DNA and immunodiagnostic approaches to reveal how these microbes "hitchhike" from the stomach and/or intestine to reach the liver in humans and various animals, such as dogs, probably often infected by more than one *Helicobacter* species in the liver [27,28]. Furthermore, new experimental models using mice and other laboratory animals, such as hamsters, guinea pigs, Mongolian gerbils, and cotton-top tamarins [29,30], may allow us to understand whether translocation and transport by macrophages and dendritic cells occurs, as it does in hepatogenic *Salmonella* organisms [31], and/or if transport by a direct bloodborne pathway, not associated with professional phagocytes as proposed for some other bacterial liver pathogens, such as *Treponema pallidum* and various *Leptospira* and *Borrelia* species, may be important [32].

Because gastric bile-sensitive *H. pylori* organisms probably translocate the gastric and perhaps the intestinal epithelium after inducing increased epithelial permeability associated with damage of the tight junctions and occlusion of intercellular adhesion molecules (ICAM), extracellular matrix (ECM), and *H. pylori* sialic acid–specific lectin binding, it is tempting to speculate that this process is a major early event in gastric, and possibly intestinal *Helicobacter* infection [33]. Comparative studies in various knockout mouse models may be necessary to identify the various stages of this mucosal invasion process.

Helicobacter hepaticus, Hepatitis, and Hepatic Neoplasia

H. hepaticus was discovered in 1992 to cause hepatitis and hepatic tumors in infected A/JCr mice [13], a strain that normally has a low incidence of hepatic disease. *H. hepaticus* organisms were found in the intestine of all infected mice, and in early colonization of the hepatic bile canaliculi, especially in male mice [34]. Recent studies of hepatic carcinogenesis in this model showed that chemical carcinogens enhance tumor development, which involves a tumor promotion mechanism but no evidence of mutations in the *ras* oncogenes or the *p53* gene. However, the production of superoxide within hepatocytes suggests an important role of reactive oxygen metabolites (ROM) [35,36] in the pathogenesis of chronic *Helicobacter*-induced hepatitis, inflammatory bowel disease [37], and tumor development. This suggests a striking similarity with *H. pylori*-induced stomach neoplasia in chronic gastritis with a strong ROM production by the gastric epithelium and infected professional macrophages and other phagocytes [38]. The possible role of CDT in chronic infection with *H. hepaticus* and other intestinal *Helicobacter* species should be investigated. It is possible that unknown bile adaptation mechanism(s) of *H. pylori* may allow this gastric pathogen to infect the human biliary tract and liver, like other

microbes [1,39,40], and induce ROM-associated inflammation and VAC toxin–induced death of the bile epithelium hepatocytes. These hypotheses will be investigated in murine models of liver disease by selected strains of *H. pylori*, *H. hepaticus*, *H. pullorum*, and other intestinal *Helicobacter* species. Aside from these observations on *H. hepaticus*-infected mice, a few previous reports concerned dogs with liver infected by *H. canis* [27] and other *Helicobacter* species such as *H. bizzozeroni*. Whether these organisms have zoonotic potential is completely unknown today.

Intestinal *Helicobacter* Zoonosis

The first report on *H. hepaticus*– and *H. bilis*–associated chronic cholangitis in humans encouraged us to investigate Swedish patients with various chronic liver diseases for antibodies in serum and bile to this species and other bile-resistant intestinal species such as *H. pullorum* [17,18••,24•]. In brief, infection by possible primarily murine species (*H. hepaticus* and *H. bilis*) seems to be rare in Swedish patients, whereas *H. pullorum* infection seems more common [41•]. We were able to visualize *Helicobacter* species in the portal zone of a patient with PSC [42••]. This observation led us to explore the role of *H. pullorum* and other bile-tolerant intestinal *Helicobacter* species organisms in foodborne intestinal infection and liver disease. Interestingly, patients with *H. heilmannii* infection reported contact with pigs, dogs, and cats, suggesting a zoonotic potential [43]. Analyses are needed of fecal samples, intestinal contents, bile of animal origin, and food samples, with respect to *Helicobacter* species, to elucidate whether these species are examples of zoonosis.

Conclusions

Helicobacter, *Arcobacter*, and *Campylobacter* species probably have a common ancestor in environmental water-associated anaerobic species, such as *Sulfurospirillum* [44]. The early evolution of these microbes in adapting to and colonizing the gut of animals and birds as intestinal and probably late gastric species is very obscure. It seems likely that ancestor *Helicobacter* species first adapted to become bile-resistant and colonize the large bowel of birds and primitive mammals. Much later, bile-sensitive species developed to colonize the stomach, such as *H. heilmannii* and *H. pylori* in humans, *H. suis* in pigs, and so forth. Several of these gastric and intestinal species (like *H. heilmannii* and *H. suis*) are nonculturable, emphasizing the need to develop methods of quantitative detection for infections in the stomach, intestine, and liver as well as for studying possible spread through food and water. The possible role of dormant or coccoidal forms accumulating in old laboratory cultures (*ie*, stationary-phase cells) is controversial [45,46]. Whether these forms may be infective under certain conditions needs further study. It seems most likely that acid as well as bile stress in the gastric environment may select for organisms infecting the biliary tract and liver. The question of whether a bile-sensitive gastric species such as *H. pylori* can infect the gallbladder and liver, or is just a "spillover" of macrophage-associated antigens and DNA, requires study in animal models. However, most recently, for the first time a *Helicobacter* strain was isolated from a woman with cirrhosis and Wilson's disease [47••]. Two strains of *H. pylori*-like organisms were also isolated from two children with "autoimmune" hepatitis at the University Children's Hospital in Warsaw by Dzierzanowska *et al*. (Unpublished). Ribosomal RNA sequence analysis in both studies led to the conclusion that the organisms grown were *H. pylori*. These findings suggest that systematic studies are needed to develop new culture media to grow fastidious organisms from bile and liver biopsies. Addition of charcoal, b-cyclodextrins, and gastric porcine mucin preparations enhances growth of experimentally bile-stressed *H. pylori* strains. This indicates that stress-induced metabolic events must be analyzed in bile-sensitive and bile-resistant *Helicobacter* species and in other microaerophiles, some of which require hydrogen for growth [44].

Finally, the possible role of these and other nonculturable microbes and new hepatotropic viruses should be analyzed, and experimental infection models should be developed. One recent report on a high incidence of *Helicobacter* infection in "healthy" slaughter pigs suggests that diagnostics for transplantation and xenotransplantation should be developed for new hepatotropic microbes [48]. Also, the effect of immunosuppression following liver transplantation should be considered with respect to these pathogens [4].

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