



LUND UNIVERSITY

The role of microorganisms in biliary tract disease.

Ljungh, Åsa; Wadström, Torkel

Published in:
Current Gastroenterology Reports

2002

[Link to publication](#)

Citation for published version (APA):

Ljungh, Å., & Wadström, T. (2002). The role of microorganisms in biliary tract disease. *Current Gastroenterology Reports*, 4(2), 167-71. http://www.current-reports.com/article_frame.cfm?PubID=GR04-2-2-03&Type=Abstract

Total number of authors:

2

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

The Role of Microorganisms in Biliary Tract Disease

Åsa Ljungh, MD, PhD and Torkel Wadström, MD, PhD

Address

Department of Medical Microbiology, Dermatology and Infection,
Lund University, Sölvegatan 23, S-223 62 Lund, Sweden.
E-mail: torkel.wadstrom@mmb.lu.se

Current Gastroenterology Reports 2002, 4:167–171

Current Science Inc. ISSN 1522-8037

Copyright © 2002 by Current Science Inc.

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
<i>H. pylori</i>	Chronic liver disease (?)	Human, monkey
<i>H. hepaticus</i>	Chronic liver disease (?)	Mouse
<i>H. pullorum</i>	Gastroenteritis	Poultry, human
<i>H. bilis</i>	Holecystitis	Mouse, human (?)
<i>H. cinaedi</i>	Septicemia, enteritis	Human, monkey
<i>H. rappini</i>	Cholecystitis	Sheep and others
<i>H. canis</i>	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].

Acknowledgments

The experimental part of this study was supported by the Swedish Medical Research Council (16x-04723) and FORMAS.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Wetter LA, Hamadeh RM, Griffiss JM, *et al.*: Differences in outer membrane characteristics between gallstone-associated bacteria and normal bacterial flora. *Lancet* 1994, 343:444-448.
 2. Monstein H-J, Jonsson Y, Zdolsek J, Svanvik J: Identification of *Helicobacter pylori* DNA in human cholesterol gallstones. *Scand J Gastroenterol* 2002, in press.
- H. pylori* DNA was identified in pigmented gallstones using pyrosequencing, which confirms findings in similar studies using PCR and sequencing.
3. Levett PN: *Leptospirosis*. *Clin Microbiol Rev* 2001, 14:296-326.
 4. Rubin RH: Infectious disease problems. In *Transplantation of the Liver*, edn 3. Edited by Maddrey WC, Schiff ER, Sorrell MF. Philadelphia: Lippincott Williams & Wilkins; 2001:275-296.
 5. Haydon GH, Neuberger J: PBC: an infectious disease? *Gut* 2000, 47:586-588.

6. Van Velkinburgh JC, Gunn JS: PhoP-PhoQ-regulated loci are required for enhanced bile resistance in *Salmonella* spp. *Infect Immun* 1999, 67:1614–1622.
 7. Wadström T, Hänninen M-L: Other *Helicobacters* in the digestive tract. *Curr Opin Gastroenterol* 1999, 1(suppl 15): S53–S66.
 8. Fox JG, Schauer DB, Wadström T: Enterohepatic *Helicobacter* spp. *Curr Opin Gastroenterol* 2001, 17:S28–S31.
 9. O'Rourke J, Grehan M, Lee A: Non-pylori *Helicobacter* species in humans. *Gut* 2001, 49:601–606.
- Discussion of published data on the detection of non-*H. pylori* species in human disease. *H. heilmannii* and *H. felis* are associated with gastric infection and MALT lymphoma, although the former causes a less aggressive disease than *H. pylori*. *H. cinaedi* and *H. fennelliae* are implicated in human intestinal disease, and most likely also *H. canadensis*, *H. rappini*, *H. heilmannii*, and *H. pullorum*. Data conflict regarding detection of *Helicobacter* DNA in biliary disease, but data from patients with primary hepatic and biliary cancers are unequivocal.
10. Bulajic MM, Jovanovic IR, Loehr M: *Helicobacter pylori* infection in patients with bile duct malignancies. *Gut* 2000, 47:A90.
 11. Harada K, Ozaki S, Kono N, et al.: Frequent molecular identification of *Campylobacter* but not *Helicobacter* genus in bile and biliary epithelium in hepatolithiasis. *J Pathol* 2001, 193:218–223.
 12. Fox JG, Dewhirst FE, Shen Z, et al.: Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology* 1998, 114:755–763.
 13. Fox JG, Tully JG, Dewhirst FE: *Helicobacter hepaticus* sp nov, a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *J Clin Microbiol* 1994, 32:1238–1245.
 14. Fox JG, Yan LL, Dewhirst FE, et al.: *Helicobacter bilis* sp. nov., a novel *Helicobacter* species isolated from bile, livers, and intestines of aged, inbred mice. *J Clin Microbiol* 1995, 33:445–454.
 15. Lin T-T, Yeh C-T, Wu C-S, Liaw Y-F: Detection and partial sequence analysis of *Helicobacter pylori* DNA in the bile samples. *Dig Dis Sci* 1995, 40:2214–2219.
 16. Figura N, Cetta F, Angelico M, et al.: Most *Helicobacter pylori*-infected patients have specific antibodies, and some also have *H. pylori* antigens antigens and genomic material in bile. Is it a risk factor for gallstone formation? *Dig Dis Sci* 1998, 43:854–862.
 17. Nilsson H-O, Castedal M, Olsson R, Wadström T: Detection of *Helicobacter* in the liver of patients with chronic cholestatic liver diseases. *J Physiol Pharmacol* 1999, 50:875–881.
 18. Nilsson H-O, Taneera J, Castedal M, et al.: Identification of *Helicobacter pylori* and other *Helicobacter* sp by PCR, hybridization and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *J Clin Microbiol* 2000, 38:1072–1076.
- DNA from *H. pylori*, *H. suis*, and "*Helicobacter* liver" was detected in patients with malignant biliary disease.
19. Fox JG, Handt L, Sheppard BJ, et al.: Isolation of *Helicobacter cinaedi* from the colon, liver, and mesenteric lymph node of a Rhesus monkey with chronic colitis and hepatitis. *J Clin Microbiol* 2001, 39:1580–1585.
- This study suggests translocation of *H. cinaedi* from the intestine to the liver via mesenteric lymph nodes in Rhesus monkeys, with presumable implications for humans.
20. Stanley J, Linton D, Burnens AP, et al.: *Helicobacter pullorum* sp. nov.: genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. *Microbiology* 1994, 140:3441–3449.
 21. Taylor NS, Fox JG, Yan L: In vitro hepatotoxic factor in *Helicobacter hepaticus*, *H. pylori* and other *Helicobacter* species. *J Med Microbiol* 1995, 42:48–52.
 22. Ghiara P, Marchetti M, Blaser MJ, et al.: Role of the *Helicobacter pylori* virulence factors vacuolating cytotoxin, CagA, and urease in a mouse model of disease. *Infect Immun* 1995, 63:4154–4160.
 23. Avenaud P, Marais A, Monteiro L, et al.: Detection of *Helicobacter* species in the liver of patients with and without primary liver carcinoma. *Cancer* 2000, 89:1431–1439.
- DNA from *H. pylori* and "*Helicobacter* liver" with close similarity to *H. pylori* was detected by PCR.
24. Nilsson H-O, Mulchandani R, Tranberg K-G, et al.: *Helicobacter* species identified in human livers from patients with cholangio- and hepatocellular carcinoma. *Gastroenterology* 2001, 120:323–324.
- H. pylori* and "*Helicobacter* liver" were detected in patients with cholangio- and hepatocellular carcinoma, but not in patients with liver metastases from colorectal carcinoma.
25. Stoltzenberg-Solomon RZ, Blaser MJ, Limburg PJ, et al.: *Helicobacter pylori* seropositivity as a risk factor for pancreatic cancer. *J Natl Cancer Inst* 2001, 93:937–941.
- The first serologic evidence of *H. pylori* in pancreatic malignancy is reported here. This finding should be investigated further.
26. Franklin CL, Beckwith CS, Livingston RS, et al.: Isolation of a novel *Helicobacter* species, *Helicobacter cholecystus* sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobar pancreatitis. *J Clin Microbiol* 1996, 34:2952–2958.
 27. Fox JG, Drolet R, Higgins R, et al.: *Helicobacter canis* isolated from a dog liver with multifocal necrotizing hepatitis. *J Clin Microbiol* 1996, 34:2479–2482.
 28. Eaton KA, Dewhirst FE, Paster BJ, et al.: Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. *J Clin Microbiol* 1996, 34:3165–3170.
 29. Hirayama F, Takagi S, Yokoyama Y, et al.: Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 2001, 31:24–28.
 30. Saunders KE, Shen Z, Dewhirst FE, et al.: Novel intestinal *Helicobacter* species isolated from cotton-top tamarins (*Saguinus oedipus*) with chronic colitis. *J Clin Microbiol* 1999, 37:146–151.
 31. Schwan WR, Huang X-Z, Kopecko DJ: Differential bacterial survival, replication, and apoptosis-inducing ability of *Salmonella* serovars within human and murine macrophages. *Infect Immun* 2000, 68:1005–1013.
 32. Marangoni A, Aldini R, Sambri V, et al.: Uptake and killing of *Leptospira interrogans* and *Borrelia burgdorferi*, spirochetes pathogenic to humans, by reticuloendothelial cells in perfused rat liver. *Infect Immun* 2000, 68:5408–5411.
 33. Borén T, Wadström T, Normark S, et al.: Methods for the identification of *Helicobacter pylori* host receptors. In *Methods of Molecular Medicine: Helicobacter pylori*. Edited by Clayton CL, Mobley HLT. Totowa, NJ: Humana Press; 1997:205–224.
 34. Whary MT, Morgan TJ, Dangler CA, et al.: Chronic active hepatitis induced by *Helicobacter hepaticus* in the A/JCr mouse is associated with a Th1 cell-mediated immune response. *Infect Immun* 1998, 66:3142–3148.
 35. Garewal HS: *Antioxidants and Disease Prevention*. Boca Raton: CRC Press; 1997.
 36. Phull PS, Price AB, White KL, et al.: Gastrointestinal mucosal Vitamin C levels in *Helicobacter pylori* infection. *Scand J Gastroenterol* 1999, 34:361–366.
 37. Chin EY, Dangler CA, Fox JG, Schauer DB: *Helicobacter hepaticus* infection triggers inflammatory bowel disease in T cell receptor alpha, beta mutant mice. *Comp Med* 2000, 50:586–594.
 38. Chmiela M, Czkwianianc E, Wadström T, Rudnicka W: Role of *Helicobacter pylori* surface structures in bacterial interaction with macrophages. *Gut* 1997, 40:20–24.
 39. Pope LM, Reed KE, Payne SM: Increased protein secretion and adherence to HeLa cells by *Shigella* spp. following growth in the presence of bile salts. *Infect Immun* 1995, 63:3642–3648.
 40. Erbil Y, Berber E, Özarmagan S, et al.: The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. *Hepatogastroenterology* 1999, 46:2791–2795.

41. • Nilsson I, Lindgren S, Eriksson S, Wadström T: **Serum antibodies to *Helicobacter hepaticus* and *Helicobacter pylori* in patients with chronic liver disease.** *Gut* 2000, 46:410–414.
The authors report on serologic detection of antibodies to *H. hepaticus* and *H. pylori* in patients with cirrhosis and different chronic liver diseases.
42. •• Wadström T, Ljungh Å, Willén R: **Primary biliary cirrhosis and primary sclerosing cholangitis are of infectious origin!** *Gut* 2001, 49:454.
Spiral and coccoid forms of *H. pylori* and other *Helicobacter* species were visualized in portal zones in a patient with PSC.
43. Meining A, Kroher G, Stolte M: **Animal reservoirs in the transmission of *Helicobacter heilmannii*: results of a questionnaire-based study.** *Scand J Gastroenterol* 1998, 33:795–798.
44. On SL: **Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: current status, future prospects and immediate concerns.** *Symp Ser Soc Appl Microbiol* 2001, 90:1–15.
45. Barer MR, Gribbon LT, Harwood CR, Nwoguh CE: **The viable but non-culturable hypothesis and medical bacteriology.** *Rev Med Microbiol* 1993, 4:183–191.
46. Nilsson H-O, Blom J, Al-Soud WA, *et al.*: **Effect of cold starvation, acid stress and nutrient stimuli on metabolic activities and morphology of *Helicobacter pylori*.** *Appl Environ Microbiol* 2002, 68:11–19.
47. • de Magalhaes Queiroz D, Santos A: **Isolation of a *Helicobacter* strain from the human liver.** *Gastroenterology* 2001, 121:1023–1024.
Helicobacter species organisms were isolated from a patient with Wilson's disease (related to copper overload). Further studies are needed to elucidate the possible pathogenetic role of *Helicobacter* in Wilson's disease, which could lead to a change in current treatment schedules.
48. de Groote D, Ducatelle R, van Doorn LJ, *et al.*: **Detection of '*Candidatus Helicobacter suis*' in gastric samples of pigs by PCR: comparison with other invasive diagnostic techniques.** *J Clin Microbiol* 2000, 38:1131–1135.