THE OCCURRENCE AND CLINICAL SIGNIFICANCE OF ENTEROHEPATIC *HELICOBACTER* SPECIES IN LABORATORY RODENTS

Het voorkomen en klinisch belang van enterohepatische Helicobacter-*species bij laboratoriumknaagdieren*

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ABSTRACT

To date, 13 enterohepatic *Helicobacter* species have been detected in laboratory rodents. Some of these species, in particular *Helicobacter hepaticus* and *Helicobacter bilis*, cause disease in immunocompromised and immunocompetent laboratory mice, rats and/or hamsters. In addition, subclinical infections with these bacteria may interfere with results obtained from experimental research in these animals and thus may lead to faulty interpretation of the data. Some laboratory rodent-associated *Helicobacter* species may also be considered zoonotic agents. Apart from *Helicobacter hepaticus*, the pathogenesis of *Helicobacter* infections has not been studied extensively, a fact which means there are substantial gaps in the comprehension of the virulence mechanisms of enterohepatic *Helicobacter* species associated with laboratory rodents. For all these reasons, the potential importance of these bacterial pathogens cannot be overlooked and undoubtedly merits further investigation.

SAMENVATTING

Tot op heden werden 13 enterohepatische *Helicobacter*-species aangetoond bij laboratoriumknaagdieren. Verschillende van deze species, in het bijzonder *Helicobacter hepaticus* en *Helicobacter bilis*, veroorzaken ziekte bij immuundeficiënte en immuuncompetente laboratoriummuizen, -ratten en/of -hamsters. Bovendien kunnen subklinische infecties met deze bacteriën interfereren met de resultaten van experimenteel onderzoek bij deze dieren en dus eventueel leiden tot een foute interpretatie van data. Enkele helicobacters die voorkomen bij laboratoriumknaagdieren kunnen ook de mens infecteren en kunnen dus worden beschouwd als zoönotische agentia. Op *Helicobacter hepaticus* na, is de pathogenese van *Helicobacter*-infecties slechts rudimentair bestudeerd waardoor de kennis van de virulentiemechanismen van enterohepatische *Helicobacter*-species bij laboratoriumknaagdieren schaars is. Omwille van al deze redenen kan het potentieel belang van deze bacteriële agentia niet worden ontkend en is er hiernaar duidelijk meer onderzoek nodig.

INTRODUCTION

The genus *Helicobacter* nowadays includes at least 26 formally named species, with additional novel species in the process of being characterized (Fox, 1997; Whary and Fox, 2004). The genus can roughly be divided into the gastric *Helicobacter* species and the enterohepatic *Helicobacter* species (EHS). All gastric *Helicobacter* species have strong urease activity. They manage to survive gastric acidity by expressing urease at a level higher than that of any other known micro-organism (Dunn *et al*., 1997; Sachs *et al*., 2003). EHS do not normally colonize the gastric mucosa, but do have characteristics of ultrastructure and physiology in common with the gastric *Helicobacter* species. They have been identified in the intestinal tract and/or the liver of humans, mammals and birds (Fox, 1997; Solnick and Schauer, 2001; Inglis *et al*., 2006). EHS infections are associated mostly with intestinal and hepatobiliary disease in a wide range of animals. In laboratory rodents, 13 species of the enterohepatic *Helicobacter* group have been described so far, of which 11

are recognized and two provisionally named. These bacteria may also interfere with results obtained from experimental research in laboratory animals in which they are highly prevalent, and thus may lead to faulty interpretation of the data (Fox *et al*., 1994; Ward *et al*., 1994; Rogers and Fox, 2004; Bohr *et al*., 2006). Finally, members of the enterohepatic *Helicobacter* group may have zoonotic potential for causing gastroenteritis, hepatitis and other disease signs in humans (Solnick and Schauer, 2001; Ljung and Wadstrom, 2002). For all these reasons, the potential importance of these emerging pathogens cannot be overlooked and undoubtedly merits further investigation.

The discovery of EHS has sparked an interest in exploring the pathogenic potential of these organisms in laboratory rodents. Nonetheless, the knowledge of the pathogenesis of EHS infections in these animals still contains large gaps. Additionally, the actual prevalence and clinical significance of most of the EHS remain to be established (Fox, 1997; Solnick and Schauer, 2001). This paper is intended to give a brief overview of the 11 recognized EHS associated with laboratory rodents used in diverse experimental disciplines, with a focus on bacterium-host interaction and the clinical significance for these animals. The methods used for monitoring laboratory rodents for EHS and for the elimination of EHS from laboratory rodent colonies, as well as the zoonotic potential of these bacterial agents, are also concisely considered.

OCCURRENCE, CLINICAL SIGNIFICANCE AND BACTERIUM-HOST INTERACTIONS OF EHS IN LABORATORY RODENTS

The different EHS recognized in laboratory rodents and their rodent hosts are presented in Table 1.

EHS mainly associated with mice and rats

H. hepaticus

H. hepaticus is a helical, motile bacterium that has been identified in mice and more recently in gerbils (Fox *et al.*, 1994; Ward *et al.*, 1994; Euzéby, 2002). Depending on the mouse strain and detection technique, a moderate to large portion of laboratory mice are found to be infected with *H. hepaticus* (Fox *et al.*, 1998a; Goto *et al.*, 2000; Whary *et al.*, 2000; Goto *et al.*, 2004; Nilsson *et al.*, 2004). Spreading by fecal-oral contact between animals is hypothesized. *H. hepaticus* infection may be spread by dirty bedding (Whary *et al.*, 2000). Livingston *et al.* (1997) demonstrated that *H. hepaticus*-free animals can develop antibodies against this EHS within four weeks after contact with dirty cage bedding from *H. hepaticus*-infected mice. Vertical transmission of *H. hepaticus* has been suggested by Li *et al.* (1998), but may depend on the mouse strain involved. Nonetheless, it has been recommended to foster pups within 24 h of birth to keep them free of *H. hepaticus* (Singletary *et al.*, 2003).

In several mouse strains, *H. hepaticus* infection has been associated with chronic active hepatitis and liver tumors. Susceptibility to liver disease seems to be mouse

Table 1. Enterohepatic *Helicobacter* species associated with laboratory rodents.

***Helicobacter* species**	**Rodent host species**	**Type strain**
H. hepaticus	mouse, gerbil	ATCC 51448^T
H. bilis	mouse, rat, gerbil	ATCC 51630^T
H. muridarum	mouse, rat	ATCC 49282^T
H. rodentium	mouse, rat	ATCC 700285^T
H. trogontum	rat	ATCC 700114^T
H. ganmani	mouse	CCUG 43526^T
H. typhlonius	mouse, rat	MU96-1^T
H. cholecystus	hamster	ATCC 700242^T
H. cinaedi	hamster, rat	ATCC 35683^T
H. aurati	hamster	ATCC BBA-1^T
H. mesocricetorum	hamster	MU97-1514^T

strain and sex dependent. While C57BL/6NCr mice appear to be resistant to liver disease caused by *H. hepaticus,* BALB/cNCr, SJL/NCr, A/JCr, SCID/NCr and C3H/HeNCr mice can develop hepatitis which may be worse in males than in females (Fox *et al.*, 1996b; Whary *et al.*, 1998). Table 2 presents the description of lesions and clinical signs which may occur in a number of mouse strains infected with *H. hepaticus*. Finally, Maurer *et al.* (2005) suggested that *H. hepaticus* amongst other EHS might play an important role in the pathophysiology of cholesterol gallstone development in mice and possibly in humans. Forty percent of mice infected with *H. hepaticus* and fed a lithogenic diet developed cholesterol gallstones.

The pathogenesis of *H. hepaticus* infections in mice has been investigated by several research groups with the aim of obtaining a clear view of the relationship of the organism to the host tissues at the molecular level and the development of lesions in the liver and intestines due to *H. hepaticus*. Proteins of *H. hepaticus* have orthologs from both *H. pylori* and *Campylobacter jejuni*, but *H. hepaticus* is deficient in orthologs from most known *H. pylori* virulence factors, including adhesins, VacA cytotoxin, and nearly all *cag* pathogenicity island (PAI) proteins. However, *H. hepaticus* has orthologs of the adhesin PEB1 and the cytolethal distending toxin (CDT) of *C. jejuni*, a 71-kb genomic island (HHGI1) and several genomic islands with a different G+C content than the other sequences of the genome. Interestingly, HHGI1, possessing three basic elements of a type IV secretion system and other virulence protein homologs, constitutes a putative PAI. Within *H. hepaticus*, a large divergence of genome content, including the genomic island HHGI1, is present (Suerbaum *et al.*, 2003). A recent study using male A/JCr mice demonstrated the role of this PAI in the development of hepatitis in these animals (Boutin *et al.*, 2005).

A decade ago, auto-immunity was demonstrated to contribute to hepatocellular damage in *H. hepaticus* infection. *H. hepaticus*-infected mice indeed may build up IgG antibodies against the heat shock protein 70 expressed both by the bacterial agent and the injured liver cells (Ward *et al.*, 1996; Whary *et al.*, 1998).

Additionally, in *H. hepaticus* a toxin activity has been identified that causes vacuole formation in a murine liver cell line, resulting in a granular appearance of the affected cells. The toxin was called granulating cytotoxin (GCT), referring to the induced morphological cell changes (Taylor *et al*., 1995). Despite the innovative and interesting character of this finding, no further research involving this toxin was performed for almost a decade. Only recently, Young *et al.* (2004) alleged that the cytopathic effect induced by GCT could be (CDT)-mediated. Indeed, the same research group previously identified three genes encoding CDT and CDT activity in *H. hepaticus* (Young *et al.*, 2000). This toxic factor has the unique ability to stop the proliferation of various cells that are thus blocked before entering into mitosis. CDT-treated cells are enlarged and the nuclei are distended proportionally. Giant cells and multinucleated cells also can be observed (Ceelen *et al.*, 2006).

Only a few studies on the role of CDT in the pathogenesis of EHS infections have been reported thus far. Ge *et al*. (2005) illustrated that *H. hepaticus* CDT is crucial in the persistent colonization of this bacterial agent in the gut of outbred Swiss Webster Mice, especially in males. Young *et al*. (2004) constructed a CDT-negative *H. hepaticus* mutant and challenged C57/BL6-*Il10*^{tm} mice with this mutant strain. They noticed that, although the isogenic *H. hepaticus* CDT mutant maintained the capacity to colonize C57/BL6-*Il10*^{tm} mice, animals inoculated with the mutant developed markedly less severe disease than mice inoculated with a wild-type *H. hepaticus* strain (Young *et al*., 2004). Furthermore, the results from a recent study of Pratt *et al*. (2006) suggest that CDT has an important immunomodulatory function allowing the persistence of *H. hepaticus* in IL-10$^{-/-}$ mice. In addition, CDT may alter the host immune response, resulting in the development of colitis (Pratt *et al*., 2006).

Finally, *H. hepaticus* expresses high levels of urease activity comparable to half the activity of *H. pylori* (Sachs *et al.*, 2003). It is not clear why these levels of urease activity would be essential in the lower bowel and liver, both non-acidic environments. Possible roles for this enzyme in *H. hepaticus* include the promoting of better endurance during passage through the stomach and the production of ammonia as a source of nitrogen for protein biosynthesis. Urease activity may also be an important factor in the pathology, given that ammonia harms host cells and urease itself provokes phagocyte chemotaxis, stimulates immune cells and induces cytokine production (Beckwith *et al.*, 2001).

H. bilis

This fusiform bacterial species was first identified in aged inbred mice with chronic hepatitis by Fox *et al.* (1995), who recovered the species from liver, bile and intestines of C57BL/6, CBA/CA, BALB/c and DBA/2 mice strains. *H. bilis* infection seems to be widespread in laboratory rodent colonies: 13%-17.1% in mice and 9.7% in rats (Riley *et al*., 1996; Whary *et al*., 2000). Goto *et al.* (2000), however, found a prevalence of only 2.1% in laboratory mice in Japan, and did not detect it at all in

Table 2. Lesions and clinical signs present in *H. hepaticus* infected mouse strains as stated by various authors.

Mouse strain	Lesions and clinical signs			Bacterial exposure timing	Age	Reference
	Generally	males	females			
A/JCr	Liver inflammation and hepatocarcinoma when bacterial exposure at or before 3 weeks of age	Lobular necrogranulomatous and chronic active hepatitis	Intraportal chronic persistent hepatitis	During conception, 10 days after conception, 3 to 12 weeks	3 to 12 months	Roger *et al.*, (2004)
	Typhlitis, IBD, more severe at older age	NA	No significant difference between infected and control animals	3 weeks	Month 1 PI	Myles *et al.*, (2003)
			Mucosal epithelial caecal hyperplasia		Month 3 PI	
	Chronic active hepatitis	Severe perivascular periportal, hepatic parenchymal lesions, lymphohistiocytic and plasmacytic cellular infiltrates	NA	6 to 8 weeks	Month 6 to 12 PI	Whary *et al.*, (1998)
		Transmural typhilitis			Month 12 PI	
	Chronic proliferative hepatitis, liver cancer	Chronic inflammation, cell hyperplasia, hepatomegaly, bile duct proliferation, necrosis, hepatic adenomas, 5-bromr-2'-deoxyuridine proliferation	Identical to males, but less prominent, hepatic adenomas were absent	Newborns and weanlings	Month 3 to 18 PI	Fox *et al.*, (1996a)
A/J	Chronic hepatitis and hepatocarcinoma	Fibrosis, portal inflammation, including lymphoid nodules, more frequent in older mice	NA	3 to 4 weeks	5 to 7 months	Avenaud *et al.*, (2003)
TRC alphabeta mutant	Intestinal epithelial cell hyperplasia and mucosal inflamation	NA	NA	NA	NA	Chin *et al.*, (2000)
	Moderate cecal and colonic lesions	NA	NA	NA	6 months	
SCID/NCr	Hepatitis, proliferate typhilitis, colitis, multifocal to coagulative hepatocyte necrosis	Hepatitis, typhilitis, colitis, colonic epithelial hyperplasia	NA	4 to 6 months	4 to 6 months	Li *et al.* (1998)
		Severe hepatitis, colitis, severe colonic epithelial hyperplasia	NA	9 to 10 months	9 to 10 months	
		NA	Hepatitis, colitis, colonic epithelial hyperplasia	6 to 8 months	6 to 8 months	
Tac: (SW)f	Chronic hepatitis and enterocolitis	NA	Multifocal hepatocytic necrotis, lymphocytic and neutrophilic infiltrates	4 weeks	Week 3 PI	Fox *et al.*, (1996b)
			More severe necrosis		Week 10 to 28 PI	
			Necrosis and multinucleate giant cells present, more prominent inflammation		Week 33 to month 16 PI	
			Disappearance of necrosis, hepatocellular carcinoma in one mouse, mucosal epithelal caecal hyperplasia, prominent Peyer's patches in colon		Month 16 to 24.5 PI	
Genetically altered mouse lines	IBD, rectal prolapse	NA	NA	NA	NA	Foltz *et al.* (1998)

NA: not available, PI: post inoculation

rats. Although *H. bilis* infections primarily occur in mice and rats (Fox *et al.*, 1995), this organism has also been demonstrated in gerbils, hamsters, dogs, cats and pigs (Eaton *et al.,* 1996; De Groote *et al.*, 2000; Roosendaal *et al.*, 2000; Solnick and Schauer, 2001; Euzéby, 2002).

Like other *Helicobacter* species, this micro-organism colonizes the lower part of the intestinal tract in its hosts, generally without inflammation (Fox *et al.*, 1995). *H. bilis* infection, however, has also been associated with IBD, ulcerative typhlitis, typhlocolitis, proctitis, diarrhea and hepatitis in immunodeficient mice and rats (Shomer *et al.*, 1997; Franklin *et al.*, 1998; Shomer *et al.*, 1998; Fox *et al.*, 2004). Several studies (Eaton *et al.*, 1996; Shomer *et al.*, 1997; Burich *et al.*, 2001; Maggio-Price *et al.*, 2002) illustrate that mice and rats may serve as an animal model for IBD and additionally suggest that *Helicobacter* spp. may act as a valuable tool for studying microbially generated IBD.

Furthermore, Maurer *et al.* demonstrated that *H. bilis* can participate in the development of cholesterol gallstones in mice when the animals are fed a lithogenic diet (Maurer *et al.*, 2005). It should be noted that, despite the occurrence of hepatoenteric lesions in *H. bilis*-infected rodents, it is still unproven that this bacterium actually is the primary cause of the disease.

The pathogenesis of *H. bilis* infection has not yet been totally clarified. Ge *et al.* (2001) recognized outer membrane preparation (OMP) proteins in four *H. bilis* strains derived from different host species. These four strains were similar to each other but revealed a different protein profile than *H. pylori* OMP proteins, thus suggesting that *H. bilis* has a conserved, unique OMP profile. The divergence in the OMP structure of these two helicobacters was also illustrated by the absence of cross-antigenicity between the *H. bilis* OMP and a number of *H. pylori* OMP proteins, except for their flagellins. Another finding in this study was the presence of five heat-modifiable proteins (HMP) in the *H. bilis* OMP. Whether these proteins act as porins *in vivo* still needs to be elucidated (Ge *et al.*, 2001). Additionally, *H. bilis* possesses CDT activity, but to a lesser extent than *H. hepaticus* (Chien *et al.*, 2000). The organism also produces strong urease activity, like most other murine helicobacters. The function of this enzyme in *H. bilis* may be similar to that in *H. hepaticus*.

H. muridarum

H. muridarum has a unique cellular ultrastructure among the EHS: it has nine to 11 periplasmic fibers which emerge as concentric helical edges on the surface of the bacterium (Erlandsen and Chase, 1972; Lee *et al.*, 1992; Zenner, 1999). *H. muridarum* colonizes the intestinal mucus in mice and rats. Phillips and Lee (1983) discovered that the species is present in higher numbers in the ileum than in the large intestine of conventional rodents. However, following experimental inoculation of gnotobiotic animals, the colon and mainly the caecum were colonized with *H. muridarum*, suggesting that in the absence of competing bacterial microbiota, these intestinal parts are predisposed to *H. muridarum* colonization. These animals infected with *H. muridarum* in the ileum, caecum and colon did not develop lesions in a study of Queiroz *et al.* (1992). The results of another experiment, however, showed that SCID mice experimentally infected with *H. muridarum* did develop IBD at a higher rate than conventionally reared mice (Jiang *et al.*, 2002). This bacterium apparently is capable of invading epithelial cells and causing degeneration (Queiroz *et al.*, 1992; Solnick and Schauer, 2001). In aging mice, the species is able to reach the stomach, probably due to the lowered parietal cell mass, and consequently play a part in the etiology of chronic gastritis (Fox, 1997; Euzéby, 2002). When mice are enzootically infected with *H. muridarum* in the lower parts of the intestine and are subsequently inoculated with gastric helicobacters, *H. muridarum* is able to colonize the stomach after displacing these gastric *Helicobacter* species. This EHS may hence take advantage of the less acidic environment (Lee *et al.*, 1993). Additionally, Bury-Mone *et al.* (2003) found *H. muridarum* to be the only EHS harboring the *amiE* and *amiF* genes. These genes encode an amidase and a formamidase, respectively, and are only present in helicobacters capable of surviving in the stomach. Their acquisition might be linked to selective pressure in the acid gastric environment.

H. rodentium

H. rodentium is spiral-shaped and possesses inside circular and intraplasmatic structures containing polyphosphate granules (Shen *et al.*, 1997). *H. rodentium* is present in 5% to 17% of several mouse strains, including C57BL/6, BALB/cA and C3H/HeJ (Sher *et al.*, 2000). Goto *et al.* (2000) detected *H. rodentium* in 38.3% and 30% of mice and rats, respectively. In several colonies, mice were infected with both *H. hepaticus* and *H. rodentium*. *H. rodentium* seems to be a normal inhabitant of the intestinal tract of mice and rats. Like *H. hepaticus* and *H. bilis*, this EHS may be transmitted by dirty bedding (Whary *et al.*, 2000). Its possible pathogenicity is hardly documented. SCID mice co-infected with *H. rodentium* and *H. bilis* suffered from severe diarrhea in one study (Shen *et al.*, 1997; Shomer *et al.*, 1998), while the authors of another study suggest that this bacterial agent is not the

cause of hepatitis or enteritis (Myles *et al.*, 2004). The exact role of *H. rodentium* in the development of diarrhea and other diseases in mice and rats requires further investigation (Shen *et al.*, 1997; Solnick and Schauer, 2001).

H. trogontum

Mendes *et al.* (1996) isolated this rod with pointed ends from the colonic mucosa of Wistar and Holtzman rats. *H. trogontum* is genetically most closely related (97%) to *H. hepaticus,* but has the ability to grow at 42°C, in contrast to *H. hepaticus* (Mendes *et al.*, 1996). The prime colonization site in rats probably is the colon. It also has been cultured from the caecum, colon and stomach in experimentally *H. trogontum*-inoculated axenic outbred mice. An organism ultrastructurally indistinguishable from *H. trogontum* was noticed in bile ducts in rats experimentally infected with the liver fluke. However, this bacterium was not cultured and thus not conclusively characterized as *H. trogontum* (Mendes *et al.*, 1996; Solnick and Schauer, 2001). Whether *H. trogontum* induces gastrointestinal lesions in rats has not been studied (Moura *et al.*, 1999). In mice, *H. trogontum* may be associated with gastrointestinal and perhaps hepatic lesions (Moura *et al.*, 1999). Because of the presence of urease activity in this EHS, we can infer that *H. trogontum* has the capacity to colonize the stomach as well, similar to other urease-positive intestinal microbes such as *H. muridarum* in mice (Queiroz *et al.*, 1992; Mendes *et al.*, 1996).

H. ganmani

H. ganmani has been detected in the caecum and liver of both specific pathogen free and conventional mice in Australia (Robertson *et al.*, 2001). A prevalence of 33% and 90% in C3H/HeJ mice and C57BL/6 mice, respectively, has been documented (Nilsson *et al.*, 2004). Remarkably, *H. ganmani* grows anaerobically at 37°C, but not microaerobically. No clear pathogenic significance has yet been attributed to *H. ganmani* (Euzéby, 2002). However, Zhang *et al.* (2005) suggested an association between natural *H. ganmani* infection in *Il10* knock-out mice and the development of IBD.

H. typhlonius

H. typhlonius is a spiral organism that has been isolated from *Il10* knock-out mice suffering from IBD resulting in diarrhea and rectal prolapse and from the intestinal content of BALB/c mice with typhlocolitis. It has also been detected in B6sJl, SCID, BALB/cA, C57BL/6 and C3H/HeJ mouse strains (Fox *et al.*, 1999; Franklin *et al.*, 2001). No conclusive proof of liver colonization by this micro-organism has yet been established. Infected immunodeficient mice only revealed mild portal inflammation (Franklin *et al.*, 1999). Recently, the presence of *H. typhlonius* in sex organs has been reported in three different mouse strains, but no vertical transmission was found (Scavizzi and Raspa, 2006).

Fox *et al.* (1999) demonstrated that *H. typhlonius* was as prevalent (4.88%) as *H. bilis* (4.33%) in laboratory rodents. In a more recent study, *H. typhlonius* was found in not less than 94% of examined SPF-SCID mice by means of pyrosequencing (Nilsson *et al.*, 2004).

EHS mainly associated with hamsters

H. cholecystus

H. cholecystus is the most important EHS in Syrian hamsters. *H. cholecystus* is similar to *H. bilis*, being fusiform in shape. It lacks urease activity (Franklin *et al.*, 1996; Euzéby, 2000; Solnick and Schauer, 2001). The intestinal tract of Syrian hamsters is possibly the normal habitat of *H. cholecystus* and animals older than five weeks seem to be more colonized with this agent than younger ones (Franklin *et al.*, 1996). *H. cholecystus* also may be present in ferrets (Garcia *et al.*, 2002).

In epidemiological studies, isolation of *H. cholecystus* has been strongly associated with cholangiofibrosis and centrilobular pancreatitis in Syrian hamsters. In fact, this micro-organism was first isolated from the gall bladders of hamsters suffering from these diseases (Franklin *et al.*, 1989, 1996). Liver lesions consist of portal neutrophilic and lymphoplasmatic infiltration with hyperplasia of the bile ducts. Formation of lymphoid follicles may also be present. More severe lesions are characterized by massive bile duct hyperplasia and fibrosis with lymphoplasmacytic infiltration. Pancreatic lesions range from mild periductular neutrophilic and lymphoplasmatic infiltration to severe inflammation resulting in interstitial pancreatitis (Brunnert and Altman, 1991; Franklin *et al.*, 1996). Affected hamsters suffering from cholangiofibrosis or centrilobular pancreatitis do not always show overt clinical signs (Franklin *et al.*, 1996). Hamsters have a common duct joining the bile and pancreatic ducts before entering the intestine. Consequently, one may assume that lesions in liver, gall bladder or pancreas may arise from an ascending infection in the common duct (Chen *et al.*, 2003). It is however also possible that *H. cholecystus* is not the primary cause of these lesions, but that already damaged liver, gall bladder or pancreatic tissue simply represents a favorable environment for growth of this micro-organism (Phillips and Lee, 1983; Franklin *et al.*, 1996).

H. cinaedi

The morphology of *H. cinaedi* resembles that of *H. hepaticus.* The agent was first detected in homosexual men, both asymptomatic individuals and men suffering from proctitis, proctocolitis or enteritis. The name 'cinaedi' means homosexual in Latin (Fennel *et al.*, 1984). Since then, many reports regarding individuals infected with *H. cinaedi* have been documented (Cimolai *et al.*, 1987; Ng *et al.*, 1987; Sacks *et al.*, 1991; Orlicek *et al.*, 1993; Burman *et al.*, 1995; Mammen *et al.*, 1995; Van der Ven *et al.* 1996; Sullivan *et al.*, 1997; Peňa *et al.,* 2002; Simons *et al.*, 2004; Uckay *et al.*, 2006).

This micro-organism seems to be a normal inhabitant of the intestinal tract of Syrian hamsters, and it causes neither histological lesions nor clinical signs in these animals. Since 75% of the hamsters harbor this bacterium, these animals may, however, serve as a reservoir of *H. cinaedi* infections for humans (Fennel *et al.*, 1984; Kiehlbauch *et al.*, 1995; Euzéby, 2001; Fox, 2002). Other animals where the species has been found are cats, dogs, foxes, rats, pigtailed macaques and rhesus macaques, in some cases displaying clinical symptoms (Kiehlbauch *et al.*, 1995; Flores *et al.*, 1990; Vandamme *et al.*, 2000; Fernandez *et al.*, 2002).

H. aurati

H. aurati is a fusiform *Helicobacter* species that was first isolated from inflamed stomachs and caeca of Syrian hamsters (Patterson *et al.*, 2000a). The presence of urease in *H. aurati* distinguishes it from the other three helicobacters found in these animals. The preferential colonization site of *H. aurati* in hamsters is the intestinal tract, particularly the caecum. However, subsequent spreading of this bacterial agent to the stomach may occur as well. The micro-organism colonizes the stomach following coprophagia or by retrograde ascending from the intestine in a similar way to *H. muridarum* (Queiroz *et al.*, 1992; Pattserson *et al.*, 2000a). Urease activity allows the bacteria to survive in the acid gastric environment.

The role of *H. aurati* in gastric disease of hamsters is still unknown. Nonetheless, *H. aurati* has been identified in hamsters suffering from gastritis, accompanied by lesions similar to those in *H. muridarum*-infected mice. Hamsters naturally infected with helicobacters suffering from gastritis provide a model for the study of the development of gastric diseases in humans (Patterson *et al.*, 2000a,b; Nambiar *et al.*, 2005, 2006).

H. mesocricetorum

H. mesocricetorum is a urease-negative, spirally curved *Helicobacter* species. It was originally recovered from fecal pellets obtained from clinically healthy hamsters without enteric or hepatic lesions (Simmons *et al.*, 2000). It is phylogenetically most closely related to *H. rodentium*. The habitat and pathogenic potential of *H. mesocricetorum* are not clear. Up to now, the species has not been associated with any lesions or clinical signs in hamsters. *H. mesocricetorum* should possibly be considered a commensal organism of the intestinal tract of hamsters (Simmons *et al.*, 2000; Solnick and Schauer, 2001).

ZOONOTIC POTENTIAL OF EHS ASSOCIATED WITH LABORATORY RODENTS

During the last two decades, *Helicobacter* colonization of the gastrointestinal tract of humans has been the subject of intensive research (Solnick and Schauer, 2001; Fox, 2002; Ljungh and Wadstrom, 2002). The recovery of different helicobacters from both immunocompromised and immunocompetent human patients suffering from enteric and hepatobiliary disease has raised the question about the origin and impact of these infections.

Up to the current time, the causal role of EHS in human hepatoenteric disease has been mostly presumptive (Fox, 1997; Fox *et al*., 1998b; Nilsson *et al*., 1999; 2000a,b; 2001; Solnick and Schauer, 2001; Fox, 2002; Rocha *et al*., 2005).

The rodent EHS *H. hepaticus*, *H. bilis* and *H. cinaedi* are generally considered zoonotic. *H. hepaticus* may play a role in liver carcinogenesis, IBD, irritable bowel syndrome (IBS) and chronic pancreatitis in humans (Nilsson *et al*., 2000b; Apostolov *et al*., 2005; Nilsson *et al*., 2006; Zhang *et al*., 2006). *H. bilis* is the only murine EHS that has actually been isolated from human gallbladder (Andersen, 2001) and its DNA has been demonstrated in patients suffering from chronic cholecystitis (Vorobjova *et al.*, 2006), cholecystolithiasis (Matsukura *et al.*, 2002), choledochocystolithiasis (Murata *et al.*, 2004, Kobayashi *et al*., 2005), biliary tract and gall bladder cancer (Matsukura *et al.*, 2002; Murata *et al.*, 2004; Apostolov *et al.*, 2005; Kobayashi *et al*., 2005).

H. cinaedi was first isolated from homosexual men, both asymptomatic individuals and men suffering from proctitis, proctocolitis and enteritis. Next, many reports regarding individuals infected with *H. cinaedi* have been documented. The agent is mainly found in immunocompromised persons, where it often causes a non-lethal disease with a large possibility for recurrence (Uckay *et al.*,

2006). *H. cinaedi* infection has been associated with septicemia and meningitis in a neonate (Orlicek *et al*., 1993), bacteremia in an afebrile patient with X-linked agammaglobulinemia (Simons *et al.*, 2004), acute diarrhea (Tee *et al*., 1987), bacteremia in immunosuppressed persons due to AIDS or cancer (Cimolai *et al*., 1987; Ng *et al*., 1987; Sacks *et al*., 1991; Mammen *et al.*, 1995; Sullivan *et al*., 1997; Uckay *et al*., 2006) and multifocal cellulitis and monoarticular arthritis (Burman *et al*., 1995; Sullivan *et al*., 1997). Van der Ven *et al*. (1996) reported a case of an HIV-seropositive man who was suffering from a *H. cinaedi* bacteremia with involvement of the soft tissue in the right lower leg causing a localized pain in this area. It was illustrated that endovascular infection was present and could thus be a feature of *H. cinaedi* bacteremia. Despite the association of this species with extragastric infections, Peňa *et al*. (2002) detected *H. cinaedi* DNA in antral gastric biopsies obtained from patients. One patient was diagnosed with erosive gastritis. Another patient had a history of colitis. Very recently, *H. cinaedi* DNA was detected in patients with pancreatic exocrine cancer (Nilsson *et al*., 2006).

At least one other EHS present in rodents that may be transmitted to human beings is *H. ganmani.* This bacterial species has been reported in pediatric patients with liver disorders (Tolia *et al*., 2004).

An association has been seen between *Helicobacter* species DNA in the liver and hepatitis C cirrhosis, with or without hepatocellular carcinoma (HCC) (Rocha *et al.*, 2005). *Helicobacter* species DNA has also been detected in livers from patients with cholangio- (71%) and hepatocellular (75%) carcinoma, but not in patients with hepatic metastases from colorectal carcinoma (Nilsson *et al.*, 2001). Moreover, neither *H. hepaticus* nor *H. bilis* DNA could be demonstrated in liver samples from human patients with primary sclerosing cholangitis (PSC) or primary biliary cirrhosis (PBC) (Nilsson *et al.*, 2000a), or in patients with gallstone formation (Maurer *et al.*, 2005).

Results from a study of Nilsson *et al.* (2001) demonstrated that 39% of patients with chronic liver disease and 20% of patients with PSC showed augmented antibody levels in an *H. hepaticus* enzyme immuno-assay (EIA). Patient serum samples retested by the *H. hepaticus* EIA after absorption with sonicated *H. pylori* cells remained positive in 12 of 37 serum samples. Distinctive antibody reactivity to 55-65 kDa proteins was noticed in *H. hepaticus* immunoblot (IB), after the absorption step, and was supposed to be unique for *H. hepaticus*. These authors suggest that antibodies to *H. hepaticus*, frequently cross reacting with *H. pylori*, occur regularly in persons with chronic liver diseases (Nilsson *et al.*, 2000b; Vorobjova *et al.*, 2006). There may, however, be contradictions between results obtained with molecular techniques, on the one hand, and serology, on the other hand. While Apostolov *et al.* (2005) did not detect *H. bilis* DNA in gallbladder and liver biopsy specimens, 9% of the examined patients did reveal IgG antibodies to *H. bilis* using IB. The same research group also detected anti-*H. hepaticus* antibodies in the sera of patients with chronic cholecystitis using IB. Using the polymerase chain reaction (PCR)-denaturating density gradient gel electrophoresis (DDGE) and DNA sequencing as molecular methods or IHC, no positive results were obtained. The positive serologic reactions might be due to cross-reactivity to antigens of other (not yet identified) helicobacters or antigenically related bacteria. Alternatively, they may also result from an extrahepatic or extra-biliary infection (Apostolov *et al.*, 2005).

Altogether, the data from studies on biliary and hepatic diseases, as well as on pancreatic disorders, suggest that bile-tolerant *Helicobacter* species may induce a chronic infection with possible malignant transformation. Whether they truly participate in the genesis of biliary disease, however, requires additional investigation. At least there is evidence that both gastric and intestinal helicobacters can circulate in human bile (Queiroz *et al.*, 2003; Kobayashi *et al*., 2005).

SCREENING OF LABORATORY RODENTS FOR THE PRENSENCE OF EHS

Since EHS have been associated with gastrointestinal disease in laboratory rodents, it is important to screen accurately for EHS in laboratory facilities. These infections also may interfere with *in vivo* experiments and thus may lead to faulty interpretation of the data (Roger and Fox, 2004; Jacobsen *et al.*, 2005). Rodent EHS can be demonstrated in fecal samples or intestinal, gallbladder and liver tissue by culture, molecular methods, and histologic examination. Alternatively, sera can be examined for the presence of antibodies to these micro-organisms by ELISA (Fox *et al.*, 1994; Shames *et al.*, 1995; Fox *et al.*, 1996a,b; Riley *et al.*, 1996; Livingston *et al.*, 1997).

Columbia, Trypticase Soy and Brucella agar supplemented with 5% sheep or horse blood and occasionally with TVP (trimethoprim, vancomycin, polymyxin) are mainly used as cultivation media. EHS associated with laboratory rodents grow best in a microaerobic environment at 37°C, but not at 25°C. Several species may also grow at 42°C. *H. ganmani* is unusual in that this species grows anaerobically at 37°C, but cannot be cultivated under microaerobic conditions. Isolation can take place using nylon acetate filters with a pore size of 0.45 µm or

0.65 µm, which may reduce contamination by other bacteria. Agar-grown EHS may present as swarming or single pointed colonies. Brucella broth supplemented with 5% fetal calf serum can also be adopted (Fox *et al.*, 1994; Fox *et al.*, 1995; Fox *et al.*, 1996a,b; Franklin *et al.*, 1996; Mendes *et al.*, 1996; Livingston *et al.*, 1997; Foltz *et al.*, 1998; Whary *et al.*, 1998; Chien *et al.*, 2000; Euzéby, 2000; Franklin *et al.*, 2001; Robertson *et al.*, 2001; Euzéby, 2002; Garcia *et al.*, 2002). Satisfactory culture of EHS may be hampered by the fastidious growth requirements of EHS. Additionally, the phenotypic similarity between member species of the genera *Helicobacter* and *Campylobacter* may result in misidentification (On *et al.*, 1996). Moreover, culture may require prolonged periods of time (one to three weeks) (Shames *et al.*, 1995). Because of these shortcomings, different alternative detection methods are appreciated (On *et al.*, 2002).

A frequently applied method is PCR. PCR now makes it possible to detect and identify different and novel species of the *Helicobacter* group. PCR also allows rapid and sensitive detection of the pathogen compared with bacterial culture, electron microscopy, histology and serology (Shames *et al.*, 1995; Livingston *et al.*, 1997). For each EHS in laboratory rodents, specific simplex, nested or reverse transcriptase (RT)-nested PCR methods have been described (Fox *et al.*, 1994; Battles *et al.*, 1995; Foltz *et al.*, 1995; Livingston *et al.*, 1997; Fox *et al.*, 1999; Moura *et al.*, 1999; Goto *et al.*, 2000; Franklin *et al.*, 2001; Nilsson *et al.*, 2004; Jacobsen *et al.*, 2005). However, a PCR assay with fecal samples is often hampered by inhibitory agents. Therefore, Shames *et al.* (1995) developed a PCR method using polyvinylpyropyrollidone and Chelex 100 for demonstrating *H. hepaticus*. A T*th* polymerase supplemented with an enhancer was applied for the DNA amplification. This PCR assay can be employed as a specific, non-invasive way of rapidly screening mice for *H. hepaticus*.

For demonstration of antibodies to *H. hepaticus, H. bilis* and *H. rodentium,* a species-specific ELISA technique can be used (Livingston *et al.*, 1997; Whary *et al.*, 2000; Euzéby, 2002). However, cross-reaction with some unidentifiable *Helicobacter* spp. may be noticed. False positive results can thus occur and confirmation by means of PCR is therefore required (Livingston *et al.*, 1997).

EHS can be visualized in tissue sections using the routinely adopted hematoxylin and eosin (H&E) stain. A Steiner modification of the Warthin-Starry stain, a Silver stain, may be more adequate and specific (Ward *et al.*, 1994a; Zenner, 1999; Rogers *et al.*, 2004).

ELIMINATION OF EHS FROM LABORATORY RODENT COLONIES

Up to the present, only limited information about the effective treatment of EHS infections in laboratory animals has been available. Studies about this subject have mainly been performed in *H. hepaticus*- and *H. bilis*-infected laboratory rodents. Since EHS infections may cause not only gastrointestinal disease in these animals, but may also interfere with *in vivo* experiments, thus leading to the misunderstanding of data, it is of course important to eliminate EHS from laboratory rodent colonies (Rogers and Fox, 2004; Jacobsen *et al.*, 2005).

The best option for obtaining *Helicobacter*-free rodent colonies is probably rederivation by means of embryo transfer. Embryo transfer to rederive infected mouse strains from *H. hepaticus* (and others) has been well established (Van Keuren en Saunders, 2004; Watson *et al.*, 2005). Another possibility for getting rid of *H. hepaticus* infection may be caesarean section (Bergin *et al*., 2005). Since vertical transmission of this bacterium has been implied (Li *et al.*, 1998), caesarean section may not be appropriate. On the contrary, the presence of *H. typhlonius* in sex organs of mice without vertical transmission to their offspring was documented very recently (Scavizzi and Raspa, 2006).

Antibiotic treatment of EHS-infected mice and rats might be an alternative (Bergin *et al*., 2005; Jury *et al.*, 2005). Russell *et al.* (1995) claimed that amoxicillin administered orally for two weeks eliminates or prevents *H. hepaticus* infection in weanlings, but not in older mice with established enteric colonization. A triple therapy of amoxicillin, metronidazole and bismuth administered orally appears to be effective for the eradication of *H. hepaticus*, but not for *H. bilis* or *H. rodentium* infections (Foltz *et al.*, 1995; 1996; Shomer *et al.*, 1998). Recently, an amoxicillin-based triple therapy in combination with cross-fostering onto *Helicobacter*-free foster mothers showed some promise for the eradication of *Helicobacter* infections in several mouse strains. However, a cross-fostering control without amoxicillin triple therapy was not carried out, so no clear-cut conclusions can be drawn about the antibiotic treatment in this study (Kerton and Warden, 2006).

CONCLUSIONS

The number of rodent helicobacters is rapidly growing and will probably continue to increase in the future. Despite the high prevalence of EHS in laboratory animals, little is currently known about the pathogenicity of these bacteria. Only *H. hepaticus* is firmly recognized as

a pathogen in mice, although its pathogenicity is still not totally understood. The pathogenicity of other rodent helicobacters has yet to be established. Some of these may constitute harmless inhabitants of the intestinal tract. In addition, very little information regarding the epidemiology of EHS in rodents is available. The evidence for the zoonotic potential of most EHS and their interference with experimental research is still circumstantial and requires more clarification. This certainly implies the necessity of prospective studies to better understand the pathogenicity of these bacterial organisms.

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REFERENCES

Andersen L.P. (2001). New *Helicobacter* species in humans. *Digestive Diseases 19,* 112-115.

Apostolov E, Al-Soud W, Nilsson I., Kornilovska I., Usenko V., Lyzogubov V., Gaydar Y., Wadstrom T., Ljungh, A. (2005). *Helicobacter pylori* and other *Helicobacter* species in gallbladder and liver of patients with chronic cholecystitis detected by immunological and molecular methods. *Scandinavian Journal of Gastroenterology 40,* 96-102.

Avenaud P., Bail B.L., Mayo K. Marais A., Fawaz R., Bioulac-Sage P., Megraud F. (2003). Natural history of *Helicobacter hepaticus* infection in conventional A/J mice, with special reference to liver involvement. *Infection and Immunity 71*, 3667-3672.

Battles J.K., Williamson J.C., Pike K.M., Gorelick P.L., Ward J.M., Gonda M.A. (1995). Diagnostic assay for *Helicobacter* hepaticus based on nucleotide sequence of its 16S rRNA gene. *Journal of Clinical Microbiology 33,* 1344-1347.

Beckwith C.S., McGee D.J., Mobley H.L., Riley L.K. (2001). Cloning, expression, and catalytic activity of *Helicobacter hepaticus* urease. *Infection and Immunity 69*, 5914-5920.

Bergin I.L., Taylor N.S., Nambiar P.R., Fox J.G. (2005). Eradication of enteric helicobacters in Mongolian gerbils is complicated by the occurrence of *Clostridium difficile* enterotoxemia. *Comparative Medicine 55,* 265-268.

Bohr U.R.M., Selgrad M., Ochmann C., Backert S., König W., Fenke A., Wex T., Malfertheiner P. (2006). Prevalence and spread of enterohepatic *Helicobacter* species in mice reared in a specific-pathogen-free animal facility. *Journal of Clinical Microbiology 44,* 738-742.

Boutin S.R., Shen Z., Rogers A.B., Feng Y., Ge Z., Xu S., Sterzenbach T., Josenhans C., Schauer D.B., Suerbaum S., Fox J.G. (2005). Different *Helicobacter* hepaticus strains with variable genomic content induce various degrees of hepatitis. *Infection and Immunity 73*, 8449-8452.

Brunnert S.R., Altman N.H. (1991). Laboratory assessment of chronic hepatitis in Syrian hamsters. *Laboratory Animal Science 41*, 559-562.

Burman W.J., Cohn D.L., Reves R.R., Wilson M.L. (1995). Multifocal cellulitis and monoarticular arthritis as manifestations of *Helicobacter cinaedi* bacteremia. *Clinical Infectious Diseases 20,* 564-570.

Burich A., Hershberg R., Waggie K. Zeng W., Brabb T., Westrich G., Viney J.L., Maggio-Price L. (2001). *Helicobacter*-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. *American Journal of Physiological Gastrointestinal Liver Physiology 281,* G764-778.

Bury-Mone S., Skouloubris S., Dauga C., Thiberge J.M., Dailidiene D., Berg D.E., Labigne A., De Reuse H. (2003). Presence of active aliphatic amidases in *Helicobacter* species able to colonize the stomach. *Infection and Immunity 71*, 5613-5622.

Ceelen L.M., Decostere A., Ducatelle R., Haesebrouck F. (2006). Cytolethal distending toxin generates cell death by inducing a bottleneck in the cell cycle. *Microbiological Research 161,* 109-120.

Chen W., Li D., Cannan R.J., Stubbs R.S. (2003). Common presence of *Helicobacter* DNA in the gallbladder of patients with gallstone diseases and controls. *Digestive Liver Diseases 35,* 237-243.

Chien C.C., Taylor N.S., Zhongming G.E., Schauer D.B., Young V.B., Fox J.G. (2000). Identification of *cdtB* homologues and cytolethal distending toxin activity in enterohepatic *Helicobacter* spp. *Journal of Medical Microbiology 49*, 525-534.

Chin E.Y., Dangler C.A., Fox J.G., Schauer D.B. (2000). *Helicobacter hepaticus* infection triggers inflammatory bowel disease in T cell receptor alphabeta mutant mice. *Comparative Medicine 50,* 586-94.

Cimolai N., Gill M.J., Jones A., Flores B., Stamm W.E., Laurie W., Madden B., Shahrabadi, M.S. (1987). "*Campylobacter cinaedi*" bacteremia: case report and laboratory findings. *Journal of Clinical Microbiology 25*, 942-943.

De Groote D., Ducatelle R., Haesebrouck F. (2000). Helicobacters of possible zoonotic origin: a review. *Acta Gastroenterologica Belgica 63,* 380-387.

Dunn B.E., Cohen H., Blaser M.J. (1997). *Helicobacter pylori*. *Clinical Microbiology Reviews 10,* 720-741.

Eaton K.A., Dewhirst F.E., Paster B.J., Tzellas N., Coleman B.E., Paola J., Sherding R. (1996). Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. *Journal Clinical Microbiol*ogy *34*, 3165-3170.

Erlandsen S.L., Chase D.G. (1972). Paneth cell function: phagocytosis and intracellular digestion of intestinal microorganisms. II. Spiral microorganism. *Journal of Ultrastructural Research 41,* 319-333.

Euzéby JP (2000). Dictionnaire de Bactériologie Vétérinaire. *Helicobacter cholecystus*. http://www.bacterio.cict.fr/bacdico/hh/cholecystus.html

Euzéby JP (2001). Dictionnaire de Bactériologie Vétérinaire. *Helicobacter cinaedi, Helicobacter fennelliae*. http://www.bacterio.cict.fr/bacdico/hh/cinaedi.html

Euzéby JP (2002). Dictionnaire de Bactériologie Vétérinaire. *Helicobacter bils, Helicobacter ganmani, Helicobacter hepaticus, Helicobacter muridarium, Helicobacter rodentium, Helicobacter typhlonius*. http://www.bacterio.cict.fr/bacdico/hh/helicointestinrongeurs.html

Fennell C.L., Totten C.A., Quinn T.E., Patton D.L., Holmes K.K., Stamm W.E. (1984). Characterization of *Campylobac-*

ter-like organisms isolated from homosexual men. *Journal of Infectious Diseases 149,* 58-66.

Fernandez K.R., Hansen L.M., Vandamme P., Beaman B.L., Solnick J.V. (2002). Captive rhesus monkeys (Macaca mulatta) are commonly infected with *Helicobacter cinaedi*. *Journal of Clinical Microbiology 40*, 1908-1912.

Flores B.M., Fennell C.L., Kuller L., Bronsdon M.A., Morton W.R., Stamm W.E. (1990). Experimental infection of pig-tailed macaques (Macaca nemestrina) with *Campylobacter cinaedi* and *Campylobacter fennelliae*. *Infection and Immunity 58,* 3947-3953.

Foltz C.J., Fox J.G., Yan L., Shames B. (1995). Evaluation of antibiotic therapies for eradication of *Helicobacter hepaticus*. *Antimicrobial Agents Chemotherapy 39,* 1292-1994.

Foltz C.J., Fox J.G., Yan L., Shames B. (1996). Evaluation of various oral antimicrobial formulations for eradication of *Helicobacter hepaticus*. *Laboratory Animal Science 46,* 193-197.

Foltz C.J., Fox J.G., Cahill R., Murphy J.C., Yan L., Shames B., Schauer D.B. (1998). Spontaneous inflammatory bowel disease in multiple mutant mouse lines: association with colonization by *Helicobacter hepaticus*. *Helicobacter 3,* 69-78.

Fox J.G., Dewhirst F.E., Tully J.G., Paster B.J., Yan L., Taylor N.S., Collins Jr. M.J., Gorelick P.L., Ward J.M. (1994). *Helicobacter hepaticus* sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *Journal of Clinical Microbiology 32,* 1238-1245.

Fox J.G., Yan L.L., Dewhirst F.E., Paster B.J., Shames B., Murphy J.C., Hayward A., Belcher J.C., Mendes E.N. (1995). *Helicobacter bilis* sp. nov., a novel *Helicobacter* species isolated from bile, livers, and intestines of aged, inbred mice. *Journal of Clinical Microbiology 33,* 445-454.

Fox J.G., Yan L., Shames B., Campbell J., Murphy J.C., Li X. (1996a). Persistent hepatitis and enterocolitis in germfree mice infected with *Helicobacter hepaticus*. *Infection and Immunity 64,* 3673-3681.

Fox J.G., Li X., Yan L., Cahill R.J., Hurley R., Lewis R., Murphy J.C. (1996b). Chronic proliferative hepatitis in A/JCr mice associated with persistent *Helicobacter hepaticus* infection: a model of helicobacter-induced carcinogenesis. *Infection and Immunity 64,* 1548-1558.

Fox J.G. (1997). The expanding genus of *Helicobacter*: pathogenic and zoonotic potential. *Seminars in Gastrointestinal Disease 8,* 124-141.

Fox L.G., MacGregor J.A., Shen Z., Li X., Lewis R., Dangler C.A. (1998a). Comparison of methods of identifying *Helicobacter hepaticus* in B6C3F1 mice used in a carcinogenesis bioassay. *Journal of Clinical Microbiology 36*, 1382-1387.

Fox J.G., Dewhirst F.E., Shen Z., Feng Y., Taylor N.S., Paster B.J., Ericson R.L., Lau C.N., Correa P., Araya J.C., Roa I. (1998b). Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology 114,* 755-763.

Fox J.G., Gorelick P.L., Kullberg M.C., Ge Z., Dewhirst F.E., Ward J.M. (1999). A novel urease-negative *Helicobacter* species associated with colitis and typhlitis in IL-10-deficient mice. *Infection and Immunity 67,* 1757-1762.

Fox J.G. (2002). The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut 50,* 273-283.

Fox J.G., Rogers A.B., Whary M.T., Taylor, N.S., Xu S., Feng, Y., Keys, S. (2004). *Helicobacter bilis*-associated hepatitis in outbred mice. *Comparative Medicine 54*, 571-577.

Franklin C.L., Gibson S.V., Wagner J.E., Caffrey C.J., Shannon T.R. (1989). Cholangiohepatitis in Syrian hamsters. *Laboratory Animal Science 39,* 469.

Franklin C.L., Beckwith C.S., Livingston R.S., Riley L.K., Gibson S.V., Besch-Williford C.L. Hook R.R. Jr. (1996). Isolation of a novel *Helicobacter* species, *Helicobacter cholecystus* sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobular pancreatitis. *Journal of Clinical Microbiology 34*, 2952-2958.

Franklin C.L., Riley L.K., Livingston R.S., Beckwith C.S., Besch-Williford C.L., Hook R.R. Jr. (1998). Enterohepatic lesions in SCID mice infected with *Helicobacter bilis*. *Laboratory Animal Science 48,* 334-339.

Franlin C.L., Riley L.K., Livingston R.S., Beckwith CS, Hook RR Jr, Besch-Williford CL, Hunziker R, Gorelick PL (1999). Enteric lesions in SCID mice infected with "*Helicobacter typhlonicus*," a novel urease-negative *Helicobacter* species. *Laboratory Animal Science 49,* 496-505.

Franklin C.L., Gorelick P.L., Riley L.K., Dewhirst F.E., Livingston R.S., Ward J.M., Beckwith C.S., Fox J.G. (2001). *Helicobacter typhlonius* sp. nov., a Novel Murine Urease-Negative *Helicobacter* Species. *Journal of Clinical Microbiology 39,* 3920-3926.

García A., Erdman S.E., Xu S., Feng Y., Rogers A.B., Schrenzel M.D., Murphy J.C., Fox J.G. (2002). Hepatobiliary inflammation, neoplasia, and argyrophilic bacteria in a ferret colony. *Veterinary Pathology 39,* 173-179.

Ge Z., White D.A., Whary M.T., Fox J.G. (2001). Fluorogenic PCR-based quantitative detection of a murine pathogen, *Helicobacter hepaticus*. *Journal of Clinical Microbiology 39,* 2598-2602.

Ge Z., Feng Y., Whary M.T., Nambiar P.R., Xu S., Ng V., Taylor N.S., Fox J.G (2005). Cytolethal distending toxin is essential for *Helicobacter hepaticus* colonization in outbred Swiss Webster mice. *Infection and Immunity 73*, 3559- 3567.

Goto K., Ohashi H., Takakura A., Itoh T. (2000). Current status of *Helicobacter* contamination of laboratory mice, rats, gerbils, and house musk shrews in Japan. *Current Microbiology 41,* 161-166.

Goto K., Jiang W., Zheng Q., Oku Y., Kamiya H., Itoh T., Ito M. (2004). Epidemiology of *Helicobacter* infection in wild rodents in the Xinjiang-Uygur autonomous region of China. *Current Microbiology 49,* 221-223.

Inglis D.G., McConville M., de Jong A. (2006). Atypical *Helicobacter* canadensis strains associated with swine. *Applied and Environmental Microbiology 72*, 4464-4471.

Jacobsen K., Mahabir E., Brielmeier M., Wilhelm P., Seidel K.E., Schmidt J. (2005). Monitoring a mouse colony for *Helicobacter bilis* using a *Helicobacter*-genus-specific nested PCR. *Laboratory Animals 39*, 400-412.

Jiang H.Q., Kushnir N., Thurnheer M.C., Bos N.A., Cebra J.J. (2002). Monoassociation of SCID mice with *Helicobacter muridarum*, but not four other enterics, provokes IBD upon receipt of T cells. *Gastroenterology 122,* 1346-1354.

Jury J., Gee L.C., Delaney K.H., Perdue M.H., Bonner R.A. (2005). Eradication of *Heliobacter* spp. from a rat breeding colony. *Contemporary Topics in Laboratory Animal Science 44,* 8-11.

Kerton A., Warden P. (2006). Review of successful treatment for *Helicobacter* species in laboratory mice. *Laboratory Animals 40*, 115-122.

Kiehlbauch J.A., Brenner D.J., Cameron D.N., Steigerwalt A.G., Makowski J.M., Baker C.N., Patton C.M., Wach-

smuth I.K. (1995). Genotypic and phenotypic characterization of *Helicobacter cinaedi* and *Helicobacter fennelliae* strains isolated from humans and animals. *Journal of Clinical Microbiology 33*, 2940-2947.

Kobayashi T., Harada K., Miwa K., Nakanuma Y. (2005). *Helicobacter* genus DNA fragments are commonly detectable in bile from patients with extrahepatic biliary diseases and associated with their pathogenesis. *Digestive Diseases Science 50,* 862-867.

Lee A., Phillips M.W., O'Rourke, Paster B.J., Dewhirst F.E., Fraser G.J., Fox J.G., Sly L.I., Romaniuk P.J., Trust T.J. *et al.* (1992). *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. *International Journal of Systemic Bacteriology 42*, 27-36.

Lee A., Chen M., Coltro N., O'Rourke J., Hazell S., Hu P., Li Y. (1993). Long term infection of the gastric mucosa with *Helicobacter* species does induce atrophic gastritis in an animal model of *Helicobacter pylori* infection. *Zentralblatt für Bakteriologie 280,* 38-50.

Li X., Fox J.G., Whary M.T., Yan L., Shames B., Zhao Z. (1998). SCID/NCr mice naturally infected with *Helicobacter hepaticus* develop progressive hepatitis, proliferative typhlitis, and colitis. *Infection and Immunity 66*, 5477-5484.

Livingston R.S., Riley L.K., Steffen K., Besch-Williford C.L., Hook R.R., Franklin C.L. (1997). Serodiagnosis of *Helicobacter hepaticus* infection in mice by an enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology 35*, 1236-1238.

Ljungh A., Wadstrom T. (2002). The role of microorganisms in biliary tract disease. *Current Gastroenterology Reports 4,* 167-171.

Maggio-Price L., Shows D., Waggie K. Burich A., Zeng W., Escobar S., Morrissey P., Viney J.L. (2002). *Helicobacter bilis* infection accelerates and *H. hepaticus* infection delays the development of colitis in multiple drug resistance-deficient (mdr1a-/-) mice. *American Journal of Pathology 160,* 739-751.

Mammen M.P. Jr., Aronson N.E., Edenfield W.J., Endy T.P. (1995). Recurrent *Helicobacter cinaedi* bacteremia in a patient infected with human immunodeficiency virus: case report. *Clinical Infectious Diseases 21,* 1055.

Matsukura N., Yokomuro S., Yamada S., Tajiri T., Sundo T., Hadama T., Kamiya S., Naito Z., Fox J.G. (2002). Association between *Helicobacter bilis* in bile and biliary tract malignancies: *H. bilis* in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract. *Japanese Journal of Cancer Research 93*, 842-847.

Maurer K.J., Ihrig M.M., Rogers A.B., Bouchard G., Leonard M.R., Carey M.C., Fox J.G. (2005). Identification of cholelithogenic enterohepatic helicobacter species and their role in murine cholesterol gallstone formation. *Gastroenterology 128,* 1023-1033.

Mendes E.N., Queiroz D.M.M., Dewhirst F.E., Paster B.J., Moura S.B., Fox J.G. (1996). *Helicobacter trogontum* sp. nov., Isolated from the rat intestine. *International Journal of Systematic Bacteriology 46,* 916-921.

Moura S.B., Mendes E.N., Queiroz D.M.M., Nicoli J.R., Cabral M.M.D.A., Magalhaes P.P., Rocha G.A., Vieira E.C. (1999). Microbiological and histological study of the gastrointestinal tract of germ-free mice infected with *Helicobacter trogontum*. *Research Microbiology 150,* 205-212.

Murata H., Tsuji S., Tsujii M., Fu H.Y., Tanimura H., Tsujimoto M., Ma N., Kawano S., Hori M. (2004). *Helicobacter bilis* infection in biliary tract cancer. *Alimentary Pharmacological Therapy 20* Suppl 1, 90-94.

Myles M.H., Livingston R.S., Livingston B.A., Criley J.M., Franklin C.L. (2003). Analysis of gene expression in ceca of *Helicobacter hepaticus*-infected A/JCr mice before and after development of typhlitis. *Infection and Immunity 71*, 3885-3893.

Myles M.H., Livingston R.S., Franklin C.L. (2004). Pathogenicity of *Helicobacter rodentium* in A/JCr and SCID mice. *Comparative Medicine 54,* 549-557.

Nambiar R.R., Kirchain S., Fox J.G. (2005). Gastritis-associated adenocarcinoma and intestinal metaplasia in a Syrian hamster naturally infected with *Helicobacter* species. *Veterinary Pathology 42,* 386-930.

Nambiar P.R., Kirchain S.M., Courmier K., Xu S., Taylor N.S., Theve E.J., Patterson M.M., Fox J.G. (2006). Progressive Proliferative and Dysplastic Typhlocolitis in Aging Syrian Hamsters Naturally Infected with *Helicobacter* spp.: A Spontaneous Model of Inflammatory Bowel Disease. *Veterinary Pathology 43,* 2-14.

Ng V.L., Hadley W.K., Fennell C.L., Flores B.M., Stamm W.E. (1987). Successive bacteremias with "*Campylobacter cinaedi*" and "*Campylobacter fennelliae*" in a bisexual male. *Journal of Clinical Microbiology 25,* 2008-2009.

Nilsson H.-O., Castedal M., Olsson R., Wadstrom T. (1999). Detection of *Helicobacter* in the liver of patients with chronic cholestatic liver diseases. *Journal of Physiological Pharmacology 50*, 875-882.

Nilsson H.-O., Taneera J., Castedal M., Glatz E., Olsson R., Wadstrom T. (2000a). Identification of *Helicobacter pylori* and other *Helicobacter* species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *Journal of Clinical Microbiology 38,* 1072-1076.

Nilsson I., Lindgren S., Eriksson S., Wadstrom T. (2000b). Serum antibodies to *Helicobacter hepaticus* and *Helicobacter pylori* in patients with chronic liver disease. *Gut 46,* 410-444.

Nilsson H.-O., Mulchandani R., Tranberg K.G., Stenram U., Wadstrom T. (2001). *Helicobacter* species identified in liver from patients with cholangiocarcinoma and hepatocellular carcinoma. *Gastroenterology 120,* 323-324.

Nilsson H.-O., Ouis I.-S., Stenram U., Ljungh A., Moran A.P., Wadstrom T., Al-Soud W.A. (2004). High prevalence of *Helicobacter* Species detected in laboratory mouse strains by multiplex PCR-denaturing gradient gel electrophoresis and pyrosequencing. *Journal of Clinical Microbiology 42,* 3781-3788.

Nilsson H.-O., Stenram U., Ihse I., Wadstrom T. (2006). *Helicobacter* species ribosomal DNA in the pancreas, stomach and duodenum of pancreatic cancer patients. *World Journal of Gastroenterology 12,* 3038-3043.

On S.L.W., Holmes B., Sackin M.J. (1996). A probability matrix for the identification of campylobacters, helicobacters and allied taxa. *Journal of Applied Bacteriology 81,* 425-432.

On S.L.W., Hynest S., Wadstrom T. (2002). Extragastric *Helicobacter* species. *Helicobacter 7* suppl. 1, 63-67.

Orlicek S.L., Welch D.F., Kuhls T.L. (1993). Septicemia and meningitis caused by *Helicobacter cinaedi* in a neonate. *Journal of Clinical Microbiology 31,* 569-571.

Patterson M.M., Schrenzel M.D., Feng Y., Xu , Dewhirst F.E., Paster B.J., Thibodeau S.A., Versalovic J., Fox J.G. (2000a). *Helicobacter aurati* sp. nov., a urease-positive *Helicobacter* species cultured from gastrointestinal tissues of Syrian hamsters. *Journal of Clinical Microbiology 38,* 3722-3728.

Patterson M.M., Schrenzel M.D., Feng Y., Fox J.G. (2000b). Gastritis and intestinal metaplasia in Syrian hamsters infected with *Helicobacter aurati* and two other microaerobes. *Veterinary Pathology 37,* 589-596.

Peña J.A., McNeil K., Fox J.G., Versalovic J. (2002). Molecular evidence of *Helicobacter cinaedi* organisms in human gastric biopsy specimens. *Journal of Clinical Microbiology 40,* 1511-1513.

Phillips M.W., Lee A. (1983). Isolation and characterization of a spiral bacterium from the crypts of rodent gastrointestinal tracts. *Applied Environmental Microbiology 45,* 675-683.

Pratt J.S., Sachen K.L., Wood H.D., Eaton K.A., Young V.B. (2006). Modulation of host immune responses by the cytolethal distending toxin of *Helicobacter hepaticus*. *Infection and Immunity 74*, 4496-4504.

Queiroz D.M., Contigli C., Coimbra R.S., Nogueira A.M., Mendes E.N., Rocha G.A., Moura S.B. (1992). Spiral bacterium associated with gastric, ileal and caecal mucosa of mice. *Laboratory Animals 26*, 288-294.

Queiroz D.M., Oliveira A.G.M., Guerra J.B., Lima J.C.P., Silva C.P., Rocha G.A. (2003). *Helicobacter* DNA in bile: correlation with hepato-biliary diseases. *Alimentary Pharmacology and Therapeutics 18,* 546-547.

Riley L.K., Franklin C.L., Hook R.R. Jr., Besch-Williford C. (1996). Identification of murine helicobacters by PCR and restriction enzyme analyses. *Journal of Clinical Microbiology 34,* 942-946.

Robertson B.R., O'Rourke J.L., Vandamme P., On S.W.L., Lee A. (2001). *Helicobacter ganmani* sp. nov., a urease-negative anaerobe isolated from the intestines of laboratory mice. *International Journal of Systemic Evolutionary Microbiology 51,* 1881-1889.

Rocha M., Avenaud P., Ménard A., Le Bail B., Balabaud C., Bioulac-Sage P., Queiroz D.M.M., Megraud F. (2005). Association of *Helicobacter* species with hepatitis C cirrhosis with or without hepatocellular carcinoma. *Gut 54*, 396-401.

Rogers A.B., Boutin S.R., Whary M.T., Sundina N., Ge Z., Cormier K., Fox J.G. (2004). Progression of chronic hepatitis and preneoplasia in *Helicobacter hepaticus*-infected A/JCr mice. *Toxicology and Pathology 32,* 668-677.

Rogers A.B., Fox J.G. (2004). Inflammation and Cancer. I. Rodent models of infectious gastrointestinal and liver cancer. *American Journal of Physiological and Gastrointestinal Liver Physiology 286,* G361-366.

Roosendaal R., Vos J.H., Roumen T., Van Vugt R., Cattoli G., Bart A., Klaasen H.L.B.M., Kuipers E.J., Vandenbroucke-Grauls C.M.J.E., Kusters J.G. (2000). Slaughter pigs are commonly infected by closely related but distinct gastric ulcerative lesion-inducing gastrospirilla. *Journal of Clinical Microbiology 38*, 2661-2664.

Russell R.J., Haines D.C., Anver M.R., Battles J.K., Gorelick P.L., Blumen L.L. Gonda M.A., Ward J.M. (1995). Use of antibiotics to prevent hepatitis and typhlitis in male scid mice spontaneously infected with *Helicobacter hepaticus*. *Laboratory Animal Science 45,* 373-378.

Sachs G., Weeks D.L., Melchers K., Scott D.R. (2003). The gastric biology of *Helicobacter pylori*. *Annual Review of physiology 65,* 349-639.

Sacks L.V., Labriola A.M., Gill V.J., Gordin F.M. (1991). Use of ciprofloxacin for successful eradication of bacteremia due to *Campylobacter cinaedi* in a human immunodeficiency virus-infected person. *Reverse Infectious Diseases 13*, 1066-1068.

Scavizzi F., Raspa M. (2006). *Helicobacter typhlonius* was detected in the sex organs of three mouse strains but did not transmit vertically. *Laboratory Animals 40*, 70-79.

Shames B., Fox J.G., Dewhirst F., Yan L., Shen Z., Taylor N.S. (1995). Identification of widespread *Helicobacter hepaticus* infection in feces in commercial mouse colonies by culture and PCR assay. *Journal of Clinical Microbiology 33,* 2968-2972.

Shen Z., Fox J.G., Dewirhst F.E., Paster B.J., Foltz C.J., Yan L., Shames B., Perry L. (1997). *Helicobacter rodentium* sp. nov., a urease-negative *Helicobacter* species isolated from laboratory mice. *International Journal of Systemic Bacteriology 47,* 627-634.

Shen Z., Feng Y., Fox J.G. (2000). Identification of enterohepatic *Helicobacter* species by restriction fragment-lenght polymorphism analysis of the 16S rRNA gene. *Helicobacter 5,* 121-128.

Shomer N.H., Dangler C.A., Schrenzel M.D., Fox J.G. (1997). *Helicobacter bilis*-induced inflammatory bowel disease in scid mice with defined flora. *Infection and Immunity 65,* 4858-4864.

Shomer N.H., Dangler C.A., Marini R.P., Fox J.G. (1998). *Helicobacter bilis*/*Helicobacter rodentium* co-infection associated with diarrhea in a colony of scid mice. *Laboratory Animal Science 48,* 455-459.

Simmons J.H., Riley L.K., Besch-Williford C.L., Franklin C.L. (2000). *Helicobacter mesocricetorum* sp. nov., A novel *Helicobacter* isolated from the feces of Syrian hamsters. *Journal of Clinical Microbiology 38,* 1811-1817.

Simons E., Spacek L.A., Lederman H.M., Winkelstein J.A. (2004). *Helicobacter cinaedi* bacteremia presenting as macules in an afebrile patient with X-linked agammaglobulinemia. *Infection 32,* 367-368.

Singletary K.B., Kloser C.A., Baker D.G. (2003). Optimal age at fostering for derivation of *Helicobacter hepaticus*-free mice. *Comparative Medicine 53,* 259-264.

Solnick J.V., Schauer D.B. (2001). Emergence of Diverse *Helicobacter* Species in the Pathogenesis of Gastric and Enterohepatic Diseases. *Clinical Microbiology Reviews 14,* 59-97.

Suerbaum S., Josenhans C., Sterzenbach T., Drescher B., Brandt P., Bell M., Dröge M., Fartmann B., Fischer H.P., Ge Z., Hörster A., Holland R., Klein K., König J., Macko L., Mendz G.L., Nyakatura G., Schauer D.B., Shen Z., Weber J., Frosch M., Fox J.G. (2003). The complete genome sequence of the carcinogenic bacterium *Helicobacter hepaticus*. Proceedings of the National Academy of Sciences USA 100, 7901-7906.

Sullivan A.K., Nelson M.R., Walsh J., Gazzard B.J. (1997). Recurrent *Helicobacter cinaedi* cellulitis and bacteraemia in a patient with HIV Infection. *Internal Journal STD AIDS 8,* 59-60.

Taylor N.S., Fox J.G., Yan L. (1995). In-vitro hepatotoxic factor in *Helicobacter hepaticus*, *H. pylori* and other *Helicobacter* species. *Journal of Medical Microbiology 42,* 48-52.

Tee W., Anderson B.N., Ross B.C., Dwyer B. (1987). Atypical campylobacters associated with gastroenteritis. *Journal of Clinical Microbiology 25,* 1248-1252.

Tolia V., Nilsson H.-O., Boyer K., Wuerth A., Al-Soud W.A., Rabah R., Wadstrom T. (2004). Detection of *Helicobacter ganmani*-like 16S rDNA in pediatric liver tissue. *Helicobacter 9,* 460-468.

Uckay I., Garbino J., Dietrich P.Y., Ninet B., Rohner P., Jacomo V. (2006). Recurrent bacteremia with *Helicobacter* cinaedi: case report and review of the literature. *BMC Infectious Diseases 6*, 86.

Vandamme P., Harrington C.S., Jalava K., On S.L. (2000). Misidentifying helicobacters: the *Helicobacter cinaedi* example. *Journal of Clinical Microbiology 38,* 2261-2266.

van der Ven A.J., Kullberg B.J., Vandamme P., Meis J.F. (1996). *Helicobacter cinaedi* bacteremia associated with localized pain but not with cellulitis. *Clinical Infectious Diseases 22*, 710-711.

Van Keuren M.L., Saunders T.L. (2004). Rederivation of transgenic and gene-targeted mice by embryo transfer. *Transgenic Research 13*, 363-371.

Vorobjova T., Nilsson S., Terjajev M., Granholm M., Lyyra T., Porkka T., Prükk R., Salupere H.-I., Maaroos T., Wadstrom R., Uibo R. (2006). Serum antibodies to enterohepatic *Helicobacter* spp. in patients with chronic liver diseases and in a population with high prevalence of *H. pylori* infection. *Digestive Liver Diseases 38*, 171-176.

Ward J.M., Fox J.G., Anver M.R., Haines D.C, George C.V., Collins M.J.Jr., Gorelick P.L., Nagashima K., Gonda M.A., Gilden R.V., Tully J.G., Russell R.E., Paster J.B., Dewhirst F.E., Conovan J.C., Anderson L.M., Rice M.J. (1994). Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. *Journal of National Cancer Institute 86,* 1222-1227.

Ward J.M., Benveniste R.E., Fox C.H., Battles J.K., Gonda M.A., Tully J.G. (1996) Autoimmunity in chronic active *Helicobacter* hepatitis of mice. Serum antibodies and expression of heat shock protein 70 in liver. *American Journal of Pathology 148,* 509-517.

Watson J., Thompson K.N., Feldman S.H. (2005). Successful rederivation of contaminated immunocompetent mice using neonatal transfer with iodine immersion. *Comparative Medicine 55,* 465-469.

Whary M.T., Morgan T.J., Dangler C.A., Gaudes K.J., Taylor N.S., Fox J.G. (1998). Chronic active hepatitis induced by *Helicobacter hepaticus* in the A/JCr mouse is associated with a Th1 cell-mediated immune response. *Infection and Immunity 66*, 3142-3148.

Whary M.T., Cline J.H., King A.E., Hewes K.M., Chojnacky D., Salvarrey A., Fox J.G. (2000). Monitoring sentinel mice for *Helicobacter hepaticus*, *H. rodentium*, and *H. bilis* infection by use of polymerase chain reaction analysis and serologic testing. *Comparative Medicine 50,* 436-443.

Whary M.T., Fox J.G. (2004). Natural and experimental *Helicobacter* infections. *Comparative Medicine 54*, 128-158.

Young V.B., Knox K.A., Schauer D.B. (2000). Cytolethal distending toxin sequence and activity in the enterohepatic pathogen *Helicobacter hepaticus*. *Infection and Immunity 68,* 184-191.

Young V.B., Knox K.A., Pratt J.S. Cortez J.S., Mansfield L., Rogers A.B., Fox J.G., Schauer D.B. (2004). *In vitro* and *in vivo* characterization of *Helicobacter hepaticus* cytolethal distending toxin mutants. *Infection and Immunity 72,* 2521-2527.

Zenner L. (1999). Pathology, diagnosis and epidemiology of the rodent *Helicobacter* infection. *Comparative Immunology, Microbiology and Infectious Diseases 22*, 41-61.

Zhang L., Danon S.J., Grehan M., Chan V., Lee A., Mitchell H. (2005). Natural colonization with *Helicobacter* species and the development of inflammatory bowel disease in interleukin-10-deficient mice. *Helicobacter 10,* 223-230.

Zhang L., Day A., McKenzie G., Mitchell H. (2006). Nongastric *Helicobacter* species detected in the intestinal tract of children. *Journal of Clinical Microbiology 44,* 2276-2297.