

Overview

Natural and Experimental *Helicobacter* Infections

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Current information about *Helicobacter* infections in humans and various domestic, wild, and research animal species that have been used or have the potential to be used as animal models of human disease is presented. The *Helicobacter* genus now includes at least 26 formally named species, with additional novel species in the process of being characterized. The natural history, host range with zoonosis potential, pathology, and diagnostic techniques are presented, along with examples of how *Helicobacter* infection has interfered with unrelated in vivo research. Current recommendations for deriving and managing helicobacter-free animal colonies for research are provided.

Helicobacter pylori was first identified in 1982 (163) as an infectious cause of chronic active gastritis and, since then, has been associated with peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma in humans (162). The *Helicobacter* genus now includes at least 26 formally named species, with additional novel species in the process of being characterized (Fig. 1). Beyond the impact of *H. pylori* on human health (13), considerable research has focused on *Helicobacter* spp. isolated from humans and animals and the associated natural diseases that range from subclinical inflammation to cancer in the gastrointestinal tract and liver. Identification and characterization of novel *Helicobacter* spp. associated with clinical disease in their natural host have provided opportunities to investigate idiopathic disease syndromes through experimental *Helicobacter* infection of animal models, particularly rodents. Natural and experimental *Helicobacter* infections reproducibly mimic important features of human disease and are yielding new information on human health issues such as gastritis and its progression to gastric atrophy and cancer; idiopathic hepatitis, including cholecystitis and the development of hepatocellular carcinoma; bacteremia in immunodeficient humans; and inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis (201). This review is an update of a prior publication on the role of *Helicobacter* spp. in newly recognized gastrointestinal tract diseases of animals (85).

The *Helicobacter* Genus

Tropism of the genus *Helicobacter* ranges from the stomach, cecum, and colon to the liver or genital tract of mammals and birds (Table 1). Essentially every species that has been systematically examined to date has one or more helicobacters in its gastrointestinal flora. Some *Helicobacter* species have limited host range (i.e., *H. pylori* in humans and nonhuman primates), whereas others, such as *H. cinaedi*, can infect humans but are found more commonly in various animal reservoirs, including

hamsters, nonhuman primates, dogs, cats, and foxes (85). All known gastric and enterohepatic *Helicobacter* infections appear to persist for the life of the host. Chronic infection is commonly associated with subclinical disease in immunocompetent hosts. Significant innate, humoral and cell-mediated immune responses appear unable to clear infection and, paradoxically, contribute to lesions, particularly in immunologically dysregulated hosts. *Helicobacter* infections in animals and their zoonotic potential remain to be clarified. For example, endemic *H. pylori* infection in a colony of purpose-bred research cats is thought to have originated with a human caretaker (117, 118); otherwise, the risk of *H. pylori* transmission from cats and dogs to humans appears minimal. Helicobacters of the same species of gastric spirals (i.e., '*H. heilmannii*') that have colonized humans (45, 244, 250) have also colonized their dogs or cats; people that work on farms with livestock may also be infected with '*H. heilmannii*.' Other less well-characterized gastric spiral organisms potentially could infect humans. Intestinal *Helicobacter* species (*H. canis*, *H. cinaedi*, *H. pullorum*, *H. canadensis*, and '*H. rappini*') have been isolated from immunosuppressed and immunocompetent patients with septicemia, enteritis, and proctitis, as well as from animal reservoirs (71, 85). *Helicobacter bilis*, isolated from dogs, cats, mice, gerbils, and rats, has been identified in human gallbladder and hepatobiliary cancer by use of molecular techniques (79, 165). Except for *H. pylori*, evidence implicating *Helicobacter* spp. as a cause of human disease is limited but growing. Many investigators have based their study conclusions on molecular techniques because helicobacters are technically difficult to culture. Thus, there may be more frequent causes of *Helicobacter*-associated human disease than is currently appreciated.

The International Committee of Systematic Bacteriology Subcommittee on the Taxonomy of *Campylobacter* and Related Bacteria has established minimum requirements for the formal description of new species of the genus *Helicobacter* (41). Formal naming of a putative new species or subspecies must be based on phenotypic and genotypic examination of at least five strains to characterize the range of potential variation among independent isolates. The *Helicobacter* genus has grown to include 26 formally named species (Fig. 1, Table 1), and includes a diverse group of

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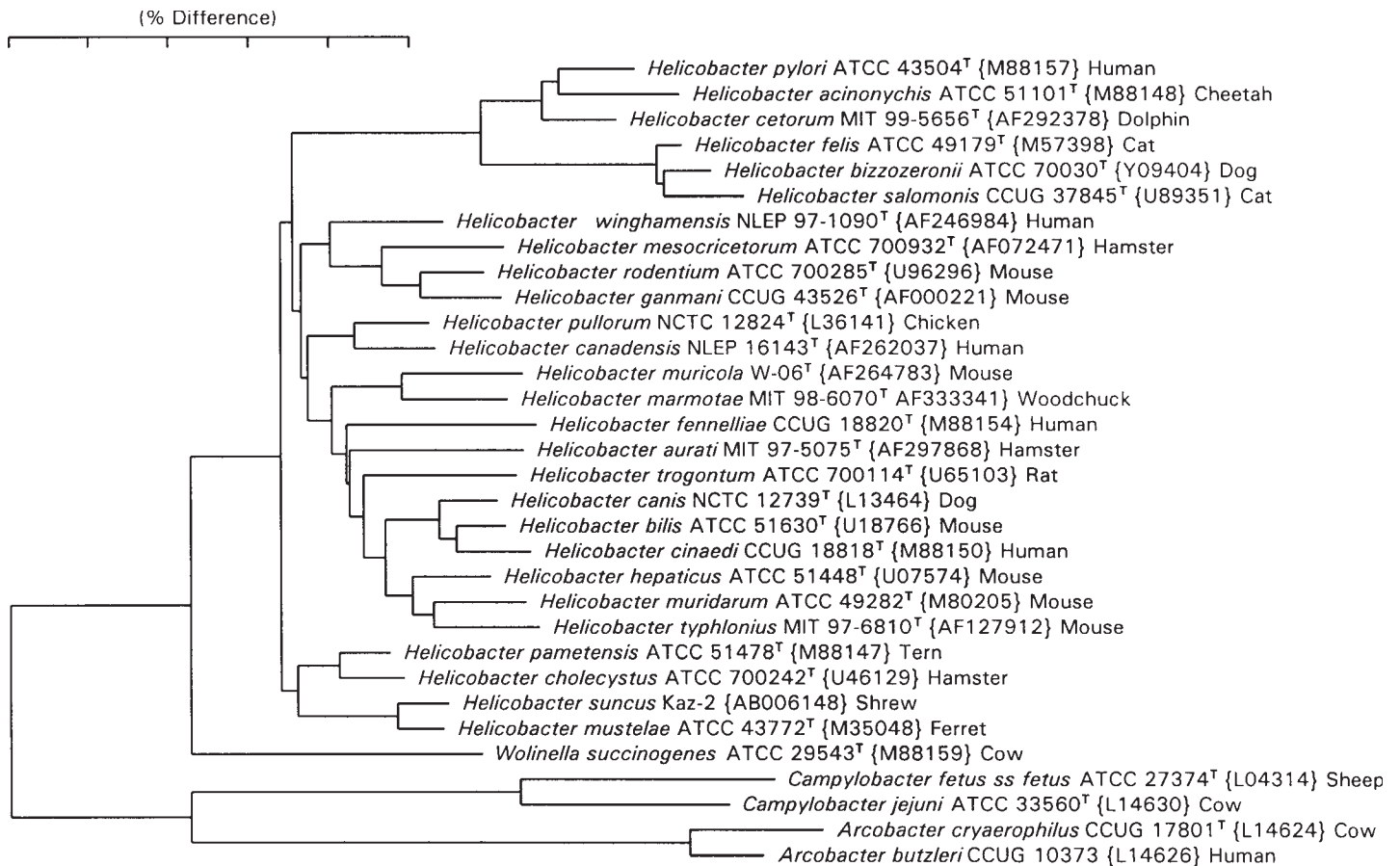


Figure 1. Dendrogram demonstrating genetic relationship among *Helicobacter* species.

potentially emerging pathogens in multiple species—humans, nonhuman primates, cats, dogs, ferrets, swine, sheep, cattle, birds, mice, rats, hamsters, gerbils, and a variety of wildlife, such as exotic cats, foxes, woodchucks, and even dolphins, whales, and seals. *Helicobacters* that have been provisionally named, such as *H. bovis*, are listed as '*H. bovis*' throughout this text.

Helicobacter spp. are gram-negative bacteria that are speciated by morphology (curved to spiral to fusiform in shape, variation in flagella, and presence or absence of periplasmic fibers), growth requirements (microaerobic to anaerobic, optimal growth at 37 and/or 42°C), biochemical profiles (such as oxidase, catalase, and urease production), antibiotic susceptibility, and sequencing of conserved 16S rRNA (Table 2). Many of these characteristic features are shared in common with *Campylobacter* spp., and may explain early misidentification of *H. pylori* as *C. pyloridis* (164). Despite availability of advanced molecular techniques, comparison of near-complete 16S ribosomal DNA sequences does not always provide conclusive evidence for species level identification and can be misleading (249). For example, *Helicobacter nemestrinae* (ATCC 49396^T), believed to be a novel gastric helicobacter isolated from a pigtailed macaque, was later recognized to be a strain of *H. pylori* by use of sequence analysis of 7 housekeeping and 2 flagellin genes that clustered together with sequences from 20 or more *H. pylori* isolates from diverse sources (235). Data also indicated that '*H. westmeadii*' is a strain of *H. cinaedi* and that *Helicobacter* sp. strain Mainz also should be speciated as *H. cinaedi* (249). In addition to 16S rRNA sequencing, hybridization experiments between DNAs from strains of related

taxa of *Campylobacter*, *Wolinella*, and *Helicobacter* spp. and 23S rRNAs from reference strains have been used to revise taxonomic classification of these bacteria (248). Others have recommended that 16S rDNA sequencing is preferable to 23S rDNA analysis (144). Additional identifying information can be gained from whole-cell protein electrophoresis, fatty acid analysis, plasmid profile determination, mass spectrometry, DNA-DNA hybridization, restriction fragment length polymorphism (RFLP) analysis, and random amplified polymorphic DNA (RAPD) fingerprinting analysis (36, 139, 177).

Helicobacter spp. have co-evolved with their hosts and other commensal microbiota of the gastrointestinal tract to reach homeostatic equilibrium that minimizes the risk of clinical disease in most of their natural hosts. Dependent on the host range and tissue tropism of individual *Helicobacter* sp., clinical disease develops not only in immunocompromised humans and animals, but also in a subset of immunocompetent individuals. Risk factors for clinical disease in otherwise clinically normal hosts presumably include environmental factors and host-pathogen genetic polymorphisms. Even within the grouping of gastric helicobacters, the genetic diversity of individual strains ranges from high in *H. pylori* (4, 13) to low in strains of *H. mustelae* (241). This diversity reflects the adaptation of the helicobacter to its natural tissue tropism within a host and potentially to other microbiota, particularly in the case of enterohepatic helicobacters: a topic of great research interest. The importance of genetic and environmental predisposition is well illustrated by the concurrent increase in use of genetically modified mouse

Table 1. Host range and tissue tropism of *Helicobacter* species

<i>Helicobacter</i> taxon	Source(s)	Primary site	Secondary site
<i>H. acinonychis</i>	Cheetah, tiger	Stomach	
<i>H. aurati</i>	Hamster	Stomach, intestine	
<i>H. bilis</i>	Mouse, dog, rat, cat, gerbil, human	Intestine	Liver (mouse), gallbladder (human)
<i>H. bizzozeronii</i> ^a	Human, cat, dog, cheetah, nonhuman primate, wild rat	Stomach	
' <i>H. bovis</i> '	Cattle	Abomasum	
<i>H. canadensis</i>	Human, wild geese	Intestine	
<i>H. canis</i>	Human, cat, dog	Intestine	Liver (dog)
<i>H. cetorum</i>	Dolphin, whale	Stomach	
<i>H. cholecystus</i>	Hamster	Gallbladder	
<i>H. cinaedi</i>	Human, hamster, macaque, dog	Intestine	Blood, brain, joint (human), liver (macaque)
<i>H. felis</i>	Cat, dog, human	Stomach	
<i>H. fennelliae</i>	Human	Intestine	
<i>H. ganmani</i>	Mouse	Intestine	
<i>H. hepaticus</i>	Mouse, gerbil	Intestine	
<i>H. marmotae</i>	Woodchuck, cat	Intestine	Liver (woodchuck)
<i>H. mesocricetorum</i>	Hamster	Intestine	
' <i>H. muricola</i> '	Korean wild mice	Intestine	
<i>H. muridarum</i>	Mouse, rat	Intestine	Stomach (mouse)
<i>H. mustelae</i>	Ferret, mink	Stomach	
<i>H. pametensis</i>	Wild birds, swine	Intestine	
<i>H. pullorum</i>	Human, chicken	Intestine	Liver (chicken)
<i>H. pylori</i>	Human, nonhuman primate, cat	Stomach	
' <i>H. rappini</i> ' ^b	Human, cat, dog, mouse, sheep, swine	Intestine	Placenta/fetus/liver (sheep), blood (humans)
<i>H. rodentium</i>	Mouse, rat	Intestine	
<i>H. salomonis</i>	Dog	Stomach	
' <i>H. suis</i> '	Swine	Stomach	
' <i>H. suncus</i> '	House musk shrew	Stomach	
<i>H. trogonum</i>	Rat	Intestine	
<i>H. typhlonius</i>	Mouse, rat	Intestine	
<i>H. winghamensis</i>	Human	Intestine	
Unnamed novel helicobacters GenBank or MIT accessions	Source	Primary site	Reference
AF333338, AF333339	Rhesus macaque	Intestine	(84)
AF107494	Cotton-top tamarin	Intestine	(207)
MIT 97-6535	Ferret	Liver	(104)
MIT 266-1	Ferret	Intestine	(90)
AF336947	Cat	Intestine	(211)
MIT 01-5926	Sea otter	Intestine	Unpublished (Fox)
MIT 03-5765	Sea lion	Intestine	Unpublished (Fox)
AFAY203898, AFAY203899	Harp seal	Stomach	(124)
AF320621	Mouse	Intestine, liver	(217)
MIT 94-022	Mouse	Intestine	Unpublished (Fox)

^aLikely the same as '*H. heilmannii*.' '*H. heilmannii*' (formerly *Gastrospirillum hominis*) has the same phenotype as listed here for *H. bizzozeronii*. Only a single '*H. heilmannii*' strain has been isolated by use of culture. ^bFormerly regarded as '*Flexispira rappini*'; now subgrouped into ten taxa.

models and associated diseases that have been attributed to natural *Helicobacter* infection.

Rodent *Helicobacter* Infections

Overview. *Helicobacter* spp. colonize the cecum and colon of mice and other rodents and have been associated with liver disease in select strains of mice and rats. Naturally acquired infections are persistent, with long-term shedding of the organism in feces. Endemic infections are common unless specific steps are taken to prevent introduction and dissemination. *Helicobacter*-associated disease is dependent on interaction among host factors of age, sex, genetics, and immune competence, and bacterial virulence factors that either are known or are suspected to influence tissue tropism and host immune responses. Naturally acquired *Helicobacter* infections have been identified in all of the commonly used laboratory animal rodent species (mice, rats, gerbils, hamsters) as well as in wild rodents. *Helicobacter hepaticus*, an enterohepatic helicobacter in mice, was the first murine helicobacter discovered as a confounding factor in long-term carcinogenesis studies (80, 115, 256), and is known to cause typhlocolitis (Table 3) and hepatocellular adenomas and carcinomas in susceptible mouse strains (Table 4). *Helicobacter hepaticus* and the gastric helicobacters that experimentally colo-

nize mice (*H. felis*, *H. pylori*) and gerbils (*H. pylori*), are examples of *Helicobacter*-associated disease models that are strengthening the link between chronic infections, the associated tissue damage from chronic inflammation, and the progression of dysplastic lesions to cancer in the liver and gastrointestinal tract (Table 4).

Natural *Helicobacter* infections in mice. *Helicobacter hepaticus*, a bacterium with a spiral shape and bipolar, single, sheathed flagella, was isolated from the liver of mice with chronic active hepatitis (80). The bacteria also colonized the cecal and colonic mucosae of mice (Fig. 2). The bacterium grows at 37°C under microaerobic and anaerobic conditions, rapidly hydrolyzes urea, is catalase and oxidase positive, reduces nitrate to nitrite, and is resistant to cephalothin and metronidazole (80). *Helicobacter hepaticus* was the first enterohepatic helicobacter identified in mice that was associated with chronic active hepatitis in susceptible mouse strains, including A/JCr, BALB/CAnNCr, SJL/NCr, B6C3F1, SCID/NCr, and C3H/HeNCr mice (80, 256). The A/JCr mouse, an immunocompetent strain, and SCID/NCr, an immunodeficient strain, appear to be among the most hepatitis-prone strains identified to date, with liver lesions that become progressively more severe with age (87, 97, 152). *Helicobacter hepaticus*-associated hepatitis and progression to hepatocellular carcinoma in A/JCr mice is most prevalent after postinfection

Table 2. Biochemical profile, growth characteristics, and flagellar morphology of *Helicobacter* species

Helicobacter taxon	Catalase production	Nitrate reduction	Alkaline phosphatase	Urease	Indoxyl acetate hydrolysis	γ -Glutamyl transferase	Growth		Resistance to ^b :		Flagella
							At 42°C	With 1% glycine	Nalidixic acid ^c	Cephalothin	
<i>H. acinonychis</i>	+	-	+	+	-	+	-	-	R	S	Bipolar
<i>H. aurati</i>	+	-	-	+	+	+	+	-	S	R	Bipolar
<i>H. bilis</i>	+	+	-	+	-	+	+	+	R	R	Bipolar
<i>H. bizzozeronii</i> ^f	+	+	+	+	+	+	+	-	R	S	Bipolar
' <i>H. bovis</i> '	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND
<i>H. canadensis</i>	+	+/-	-	+	+	-	+	+	R	R	Mono/Bipolar
<i>H. canis</i>	-	-	+	-	+	+	+	-	S	I	Bipolar
<i>H. cetorum</i>	+	-	-	+	-	+	+	ND	I	S	Bipolar
<i>H. cholecystus</i>	+	+	+	-	-	-	+	+	I	R	Monopolar
<i>H. cinaedi</i>	+	+	-	-	-	-	-	+	S	I	Bipolar
<i>H. felis</i>	+	+	+	+	-	+	+	-	R	S	Bipolar
<i>H. fennelliae</i>	+	-	+	-	+	-	-	+	S	S	Bipolar
<i>H. ganmani</i>	+	+	-	-	-	ND	-	-	R	S	Bipolar
<i>H. hepaticus</i>	+	+	-	+	+	-	-	+	R	R	Bipolar
<i>H. marmotae</i>	+	-	+	+	-	-	-	+	R	R	Bipolar
<i>H. mesocricetorum</i>	+	+	+	-	ND	-	+	-	S	R	Bipolar
<i>H. muridarum</i>	+	-	+	+	+	+	-	-	R	R	Bipolar
<i>H. mustelae</i>	+	+	+	+	+	+	+	-	S	R	Peritrichous
<i>H. pametensis</i>	+	+	+	-	-	-	+	+	S	S	Bipolar
<i>H. pullorum</i>	+	+	-	-	-	ND	+	-	R	S	Monopolar
<i>H. pylori</i>	+	-	+	+	-	+	-	-	R	S	Monopolar
' <i>H. rappini</i> '	+/-	-	-	+	-	+	+	-	R	R	Bipolar
<i>H. rodentium</i>	+	+	-	-	-	-	+	+	R	R	Bipolar
<i>H. salomonis</i>	+	+	+	+	+	+	-	ND	R	S	Bipolar
' <i>H. suis</i> '	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Bipolar
' <i>H. suncus</i> '	+	+	+	+	ND	ND	-	ND	R	R	Bipolar
<i>H. troglontum</i>	+	+	-	+	-	+	+	ND	R	R	Bipolar
<i>H. winghamensis</i>	-	-	-	-	+	ND	-	+	R	R	Bipolar

ND = not determined, R = resistant, S = susceptible, I = indeterminate.

Table 3. *Helicobacter*-associated typhlocolitis in mice

Genetic status of mice	Type of defect	Pathology
CD45RB ^{high} -reconstituted <i>scids</i>	Reconstitution with naive CD4 ⁺ T cell	Typhlocolitis
TCR $\alpha^{-/-}$, $\beta^{-/-}$ defined flora	Abnormal T cell receptors	Typhlocolitis
Scid ICR defined flora	Lack T and B cells	Typhlocolitis
IL-10 ^{-/-}	Lack IL-10	Typhlocolitis
RAG2 ^{-/-}	Lack T and B cells	Typhlocolitis, colon cancer
IL-7 ^{-/-} /RAG2 ^{-/-}	Lack IL-7, T and B cells	None
A/JCr	Normal	Typhlitis
Swiss Webster gnotobiotic	Normal	Enterocolitis
NF- κ B (p50 ^{-/-} p65 ^{-/-})	Lack NF- κ B	Typhlocolitis
mdr1a ^{-/-}	Lack P-glycoprotein	Typhlocolitis

IL = Interleukin

Table 4. Summary of key rodent models of *Helicobacter* gastrointestinal and liver cancer^a

Rodent	Infective agent/ transgene	Tumor	Comment
C57BL/6 mice	<i>H. felis</i>	Gastric adenocarcinoma	Natural feline pathogen, but lacks <i>vacA</i> and PAI
INS-GAS FVB mice	<i>H. felis</i> and <i>H. pylori</i>	Gastric adenocarcinoma	Constitutive hypergastrinemia promotes tumorigenesis
Mongolian gerbil	<i>H. pylori</i>	Gastric adenocarcinoma	Closely mimics human disease; long time course and few reagents
BALB/c mice	Several <i>Helicobacter</i> spp.	Gastric MALT lymphoma	Usually requires 18-24 months
Genetically engineered mice: IL10 ^{-/-} mice, especially on 129Sv background	"Endogenous microbiota" or <i>H. hepaticus</i>	Large intestinal carcinoma (cecum \pm colon)	Bacteria in endogenous microbiota models not well defined; <i>H. hepaticus</i> reliably induces disease
Lymphocyte-deficient mice: SCID or Rag ^{-/-} ; especially on 129Sv background	<i>H. hepaticus</i>	Large intestinal carcinoma (cecum \pm colon)	Often used for adoptive transfer studies; <i>H. hepaticus</i> induces tumors in untreated Rag2 ^{-/-} mice
A/JCr and other mice	<i>H. hepaticus</i>	Hepatocellular carcinoma	Natural murine pathogen induces chronic active hepatitis and HCC

^aTable adapted from Rogers and Fox (201).

MALT = Mucosa-associated lymphoid tissue; HCC = hepatocellular carcinoma.

month 18, and for unknown reasons, males are predisposed to more severe lesions (87).

Mice of the SCID/NCr strain that were naturally infected with *H. hepaticus* developed hepatitis, proliferative typhlitis, and colitis (152). Lesions in the liver of SCID mice consisted of Kupffer,

Ito, and oval cell hyperplasia, along with multifocal to coalescing coagulative hepatocyte necrosis. Numerous Warthin-Starry-positive bacteria were observed in the parenchyma, and there were minimal to mild accumulations of monocytic cells and neutrophils. Proliferative typhlitis was characterized by moderate to

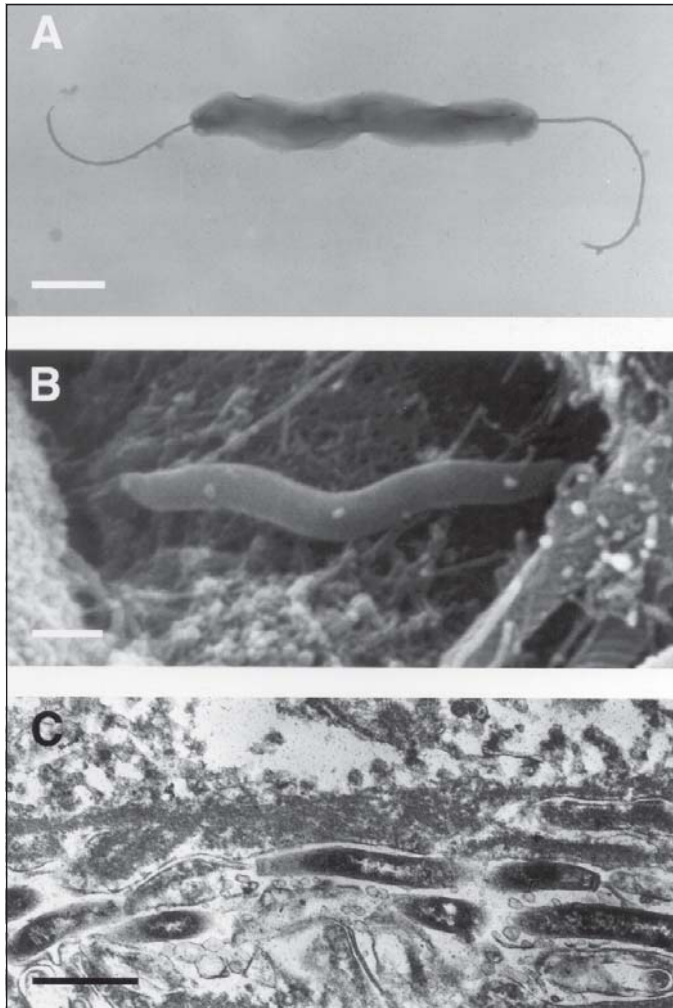


Figure 2. Electron micrograph of *H. hepaticus*. Notice the spiral morphology as well as the single, bipolar flagella (A) in the colon crypt (B) and within the biliary canaliculi of the liver (C). Bars = 0.5 μ m. Reproduced with permission from Dr. Schauer and Dr. Solnick (231).

marked mucosal epithelial cell hyperplasia, with mild monocytic and neutrophilic infiltration. In contrast, infected immunocompetent A/JCr mice, which develop a substantial immune response to *H. hepaticus* associated with prominent multifocal mononuclear cell infiltrates in the liver (262), typically have low numbers of *H. hepaticus* observable at the periphery of foci of inflammation and colonizing the biliary canaliculi (Fig. 3). Therefore, the success rate for recovery of *H. hepaticus* in culture from infected A/JCr mice is high when the cecum and colon are sampled, but is low from liver specimens.

Helicobacter hepaticus infection in A/JCr mice has been characterized on a longitudinal basis through 18 months of age (87). *Helicobacter hepaticus* colonization persisted in the large intestine and liver, and was associated with chronic proliferative hepatitis and liver cancer, and in some mice, chronic typhlitis (87). Infected A/JCr mice developed sustained serum IgG antibody responses to *H. hepaticus* and high serum enzyme activities indicative of hepatocellular injury. To fulfill Koch's postulates, colonization of germfree mice by *H. hepaticus* was induced and the mice went on to develop chronic hepatitis; some mice developed enterocolitis (97).

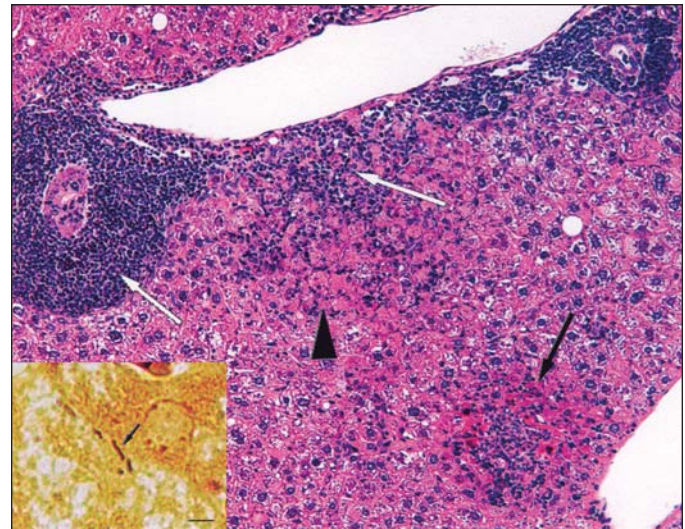


Figure 3. (A) Photomicrograph of a section of the liver from an A/J mouse infected with *H. hepaticus*. Notice characteristic lesions associated with chronic active hepatitis, including cholangiohepatitis (upper left white arrow), interface hepatitis (lower right black arrow), hepatocellular dysplasia (black arrowhead), and lobular inflammation (central upper white arrow). H&E stain; magnification, 20 \times . (B) Inset depicts Warthin-Starry-positive organisms with morphology consistent with *H. hepaticus* in the liver. Magnification, 100 \times .

A clinical syndrome of typhlocolitis, with and without rectal prolapse or concomitant hepatitis, has been described in a variety of inbred strains of immunocompromised mice infected with *H. hepaticus* (Table 3) (66, 254). In immune dysregulated mice, mononuclear inflammatory cells infiltrate the mucosa and submucosa of the cecum and colon. Epithelial cell hyperplasia can be substantial, with frequent villous to papillomatous folds extending into the lumen and a high mitotic index in the crypts. Numbers of goblet cells are variably decreased in the proliferative mucosa. *Helicobacter hepaticus* infection has been associated with development of large intestinal adenocarcinoma in RAG2^{-/-} mice on a 129/SvEv background (59).

Diagnostic Methods for *H. hepaticus*

Culture. Diagnostic specimens (feces, cecal scrapings, tissues) should be stored at -70°C in brain heart infusion or *Brucella* broth containing 30% glycerol. For culture from the gastrointestinal tract, specimens are homogenized in 1 ml of phosphate-buffered saline (PBS) and are filtered through a 0.45- μ m filter to eliminate competitive commensals. For larger helicobacters, such as *H. bilis* or *H. trogontum*, a 0.65- μ m filter is used to aid in isolation. Filtrate from gastrointestinal tract specimens is cultured on blood agar supplemented with 1% trimethoprim, vancomycin, and polymyxin, and specimens from the liver are plated directly onto blood agar. Plates are incubated at 37°C (or 42°C for other *Helicobacter* spp.) under microaerobic conditions in vented jars (90% N_2 , 5% H_2 , and 5% CO_2 ; alternatively, a 80:10:10 mixture of the same gases can be used) for 3 to 7 days. Plates should be kept for 21 days before concluding there is no growth. Growth of *H. hepaticus* occurs as a mucoid film or lawn, but not in distinct colonies, on plates. Bacterial numbers can be expanded by inoculation into *Brucella* broth containing 5% fetal bovine serum and incubation for 24 to 48 h on a rotary shaker. Many *Helicobacter* species require moist agar for efficient growth.

Polymerase chain reaction (PCR) analysis. The PCR methods for *H. hepaticus* and other rodent helicobacters have been described (6, 198, 210). Samples for PCR analysis should be collected aseptically and stored at -20°C prior to DNA extraction. Polymerase chain reaction amplification of bacterial DNA extracted from feces can be performed using the QIAamp Tissue Kit (Qiagen Inc., Valencia, Calif.) and following the kit protocol for the isolation of nucleic acids from blood. The PCR assay for detection of *Helicobacter* DNA in feces permits serial monitoring of individual or groups of laboratory rodents. Cecal scraping specimens obtained at necropsy are extracted for PCR analysis using the Boehringer-Mannheim High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Indianapolis, Ind.) and following the kit protocol for the isolation of nucleic acids from tissue. Primer sequences specific for *H. hepaticus* have been published; 5'-GCA TTT GAA ACT GTT ACT CTG-3' (C68) and 5'-CTG TTT TCA AGC TCC CC-3' (C69) yields an amplification product of 414 basepairs (bp) (210). *Helicobacter hepaticus* has three urease structural genes (*ureA*, *ureB* and *ureC*) that are highly conserved and are homologous to those of the gastric *Helicobacter* species. An RFLP assay, using the nucleotide sequence of the *ureAB* gene sequence, can be used in PCR and RFLP assays for diagnosis of *H. hepaticus* (214).

A RFLP analysis also has been used to differentiate between enterohepatic helicobacters, including *H. hepaticus*, *H. bilis*, and *H. muridarum* (198, 212). A fluorogenic nuclease PCR assay that eliminates post-PCR processing provided sensitive, specific, and high-throughput capacity for detection of *Helicobacter* spp., *H. hepaticus*, *H. bilis*, and *H. typhlonius* in fecal DNA samples from rodents (47). Because *H. hepaticus* does not grow in discrete colonies in vitro, standard limiting dilution methods for bacterial quantification of *H. hepaticus* are difficult to interpret. A real-time PCR assay has been developed to quantify *H. hepaticus* recovered from feces or cecal scraping specimens, using primers for the *H. hepaticus cdtB* gene and mouse 18S rRNA (107).

Serologic testing. To our knowledge, commercial serologic testing for *Helicobacter* infection of rodents is not available. Experimentally, measurement of *Helicobacter*-specific serum IgG or mucosal IgA antibody responses by use of an ELISA can be done using a bacterial sonicate (97), membrane digest preparations (156, 262) or recombinant antigen (62, 138, 155). Presence of infection is readily supported by positive serologic ELISA results for *Helicobacter* antigens and has been documented to be an assay with sensitivity exceeding 90%, although with variable specificity, particularly if mice are infected concurrently with multiple *Helicobacter* spp. (260).

Histologic examination. Warthin-Starry or Steiner silver stains are used to localize *H. hepaticus* in the liver parenchyma and biliary system or gastric helicobacters in the stomach. Silver staining of the large intestine is unrewarding because many other enteric organisms will also be visualized by use of a silver stain. In the liver of infected mice, bacteria appear localized between hepatocytes. Severity of the hepatic lesions appears to be associated with the abundance of bacteria in SCID mice; however, the opposite is true in immunocompetent mice. For example, in infected A/J mice, the organisms can be difficult to find in the liver (Fig. 3). Transmission electron microscopy has revealed spiral bacteria with morphology consistent with *H. hepaticus* within bile canaliculi (Fig. 2). *Helicobacter hepaticus* in tissues can also be localized by use of immunofluorescence with polyclonal rabbit

anti-*H. hepaticus* sera (88).

Genomic analysis and diversity of *H. hepaticus*. Pulsed-field gel electrophoresis (PFGE) was used to establish genomic diversity in 11 strains of *H. hepaticus* obtained from geographically distant locations in the United States and Europe (206). Isolates from three of four independent US sources had similar PFGE patterns, suggesting relative genomic conservation as well as diversity. The balance of 7 isolates from various areas in Europe differed significantly in PFGE patterns from those of the US isolates and from one another. Use of DNA fingerprinting analysis could be helpful in epidemiologic studies of *H. hepaticus*, and this assay highlights the potential strain diversity within *H. hepaticus*.

The genetic relationship among *H. hepaticus*, *C. jejuni*, and *H. pylori* was initially examined by use of comparative sequence analysis of an ordered cosmid library (106). The complete sequence of *H. hepaticus* has since been published (234). The analysis focused on comparison among *H. hepaticus*, *H. pylori*, and *C. jejuni*, providing insights into the diverse mechanisms of tissue tropism and pathogenesis of these three pathogens. *Helicobacter hepaticus* has a genome of 1,799,146 bp, predicted to encode 1,875 proteins; approximately 50% have homologues in *H. pylori*. Importantly, *H. hepaticus* lacks almost all *H. pylori*-specific colonization and virulence factors, including adhesins, vacuolating cytotoxin A (*vacA*), and the cytotoxin-associated gene pathogenicity island (*cagPAI*). *Helicobacter hepaticus* has orthologues of *C. jejuni* adhesin PEB1 and the cytolethal distending toxin (CDT). Experimentally induced infection with an isogenic strain of *H. hepaticus* lacking CDT did not promote typhlocolitis in IL-10^{-/-} mice to the extent caused by wild-type *H. hepaticus*, suggesting a role for CDT in inflammation (266). *Helicobacter hepaticus* has a novel genomic island and putative pathogenicity island encoding the basic components of a type-IV secretion system, as well as other virulence protein homologues. Use of whole-genome microarray analysis indicated that this island is lacking in many isolates of *H. hepaticus* (234). *Helicobacter hepaticus* predictably induces colitis, hepatitis, and hepatocellular carcinoma in susceptible strains of mice. Some investigators have reported lack of expected morbidity and lesion development in *H. hepaticus* infection studies (44). Such differences in outcome may be related to the genetic diversity illustrated by these genomic studies.

***Helicobacter bilis*.** First isolated from bile, liver, and intestine of aged, inbred mice in 1995 (98), *H. bilis* has since been isolated from dogs, rats, and gerbils. As additional evidence that the wide host range of *H. bilis* potentially includes humans, *H. bilis* was detected by use of PCR analysis and was identified by use of 16S rRNA analysis in gallbladder and bile specimens from Chileans with chronic cholecystitis (79), and was associated with biliary tract and gallbladder cancers in two high-risk populations: Japanese and Thai (165). *Helicobacter bilis* is a fusiform bacterium encased in periplasmic fibers, with three to 14 bipolar sheathed flagella. Similar to *H. trogontum* of rats, *H. bilis* grows at 37 and 42°C under microaerobic conditions and is urease, catalase, and oxidase positive. Similar to *H. hepaticus* infection, *H. bilis* infection of mice is associated with moderate to severe proliferative typhlitis and chronic active hepatitis in immunocompromised mice (101). Coinfection with *H. bilis* and *H. rodentium* was associated with diarrhea in a breeding colony of SCID/Trp53^{-/-} mice (215). A third of the colony developed mu-

coid, watery, or severe hemorrhagic diarrhea with mortality. *Helicobacter bilis* and *H. rodentium* were isolated by use of microaerobic culture of feces or cecal specimens from affected mice and their identity was confirmed by use of PCR analysis. Sentinel Swiss mice exposed to bedding from cages containing affected mice acquired the infection, but did not develop clinical signs of disease. Lesions consisted of rectal prolapse and proliferative typhlocolitis, with focal ulcers in the cecum, colon, and rectum. Treatment for 2 weeks with food wafers containing 1.5 mg of amoxicillin, 0.69 mg of metronidazole, and 0.185 mg of bismuth per mouse per day, previously documented to eradicate *H. hepaticus* in immunocompetent mice, resolved the diarrhea but did not eliminate *H. bilis* or *H. rodentium* infection. Experimentally induced infection of 4-week-old defined flora Tac:Icr:Ha(ICR)-scid^{DF} mice with *H. bilis* alone reproducibly caused similar disease (216).

Polymerase chain reaction analysis has been more sensitive than culture or serologic testing at determining *H. bilis* infection status in experimental studies, particularly during the first month after inoculation because seroconversion can take several months (126). Efforts to improve serologic sensitivity and specificity have included identifying an immunodominant *H. bilis*-specific antigen by testing sera from infected mice against a *H. bilis* genomic DNA expression library (62). Immunoreactive clones contained sequences that encoded a predicted 167-kDa protein that identified *H. bilis*- but not *H. hepaticus*-infected mice by use of an ELISA. Immunoblot (western blot) reactivity of sera from *H. bilis*-infected mice against select bands in *H. bilis* lysates was high in comparison with no reaction for immunoblots prepared from *H. hepaticus*, *H. muridarum*, or unrelated bacterial lysates. However, primers for sequences within the coding region of the 167-kDa protein amplified DNA from *H. hepaticus* and *H. muridarum*, indicating that these species may express homologous antigens. Recombinant protein fragments of the P167 protein were compared with *H. bilis* membrane extracts, using an ELISA protocol to determine the seroconversion status of 76 mice naturally infected with *H. bilis* or *H. hepaticus* alone or with an unspiciated *Helicobacter* sp. (138). Assay sensitivity was highest for the membrane extracts, but specificity of the assay was greatly improved using the recombinant P167 fragment proteins.

Characterization of nine immunogenic polypeptide proteins in outer membrane preparations of *H. bilis* indicated that isolates from a variety of sources (mouse, dog, rat, and gerbil) had similar protein profiles that were distinct from those of *H. pylori* (105). Immunoblot cross-reactivity was limited to their flagellins. Terminal sequences of the two membrane proteins did not have homology with protein sequences available in public databases, indicating that *H. bilis* has a conserved, unique outer membrane protein profile that is distinct from that of *H. pylori*.

Helicobacter rodentium. *Helicobacter rodentium* was the first urease-negative *Helicobacter* species isolated from laboratory mice (213). *Helicobacter rodentium* is spiral shaped, with bipolar, single, nonsheathed flagella, which is a structural feature shared with *H. canadensis* (74) and *H. winghamensis* (166) isolated from humans with gastroenteritis, with *H. pullorum* isolated from humans and birds (233), and with *H. ganmani* from mice (199). *Helicobacter rodentium* grows at 37 and 42°C under microaerobic and anaerobic conditions, is urease-negative, and is only weakly positive for catalase and oxidase. In immunocompe-

tent mice, *H. rodentium* appears nonpathogenic as part of commensal intestinal flora, but to our knowledge, pathogenic potential of *H. rodentium* has not been systematically studied. Coinfection with *H. rodentium* and *H. bilis* was implicated in an outbreak of diarrhea and typhlocolitis in a colony of SCID mice (215). Antibiotic therapy helped resolve clinical disease, but failed to eradicate the helicobacters.

Helicobacter ganmani. *Helicobacter ganmani* sp. nov., an urease-negative anaerobe, was isolated from the intestines of laboratory mice at four animal facilities (199). Isolates were unusual in that they only grew anaerobically at 37°C and were incapable of growth under microaerobic conditions. Like *H. rodentium*, isolates had single, bipolar, unsheathed flagella and were urease negative. *Helicobacter ganmani* is positive for oxidase and reduces nitrate to nitrite, but does not hydrolyze hippurate or indoxyl acetate, and can be cultured on charcoal agar, with resistance to cephalothin. Analysis of 16S rDNA sequence indicates that *H. rodentium* is the most closely related species, with 98.2% similarity. Analysis of whole-cell proteins from 9 isolates by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) differentiated *H. ganmani* from *H. rodentium* and other *Helicobacter* spp.

Helicobacter typhlonius. *Helicobacter typhlonius*, an urease-negative helicobacter, was isolated from colonies of laboratory mice independently by personnel at two laboratories (82, 100, 102), and has been detected in rat feces by use of PCR analysis (153). Typhlocolitis in C.B-17 SCID mice was observed, but in contrast to that in mice infected with *H. hepaticus* (152) or *H. bilis* (101), lesions of chronic active hepatitis in C.B-17 SCID mice were not detected in mice inoculated with *H. typhlonius* (102). The IL-10^{-/-} mice naturally infected with a helicobacter later identified as *H. typhlonius*, developed diarrhea, perianal ulceration, intestinal bleeding, and rectal prolapse (82). Helicobacter-free IL-10^{-/-} mice were experimentally challenged with *H. typhlonius*, and typhlocolitis was reproduced (82). *Helicobacter typhlonius* is closely related to *H. hepaticus* and has similar morphology, with spiral shape and bipolar sheathed flagella. A large intervening sequence in its 16S rRNA gene and biochemical differences, including being urease negative, distinguishes *H. typhlonius* from *H. hepaticus*. *Helicobacter typhlonius* infection of research mice appears to be less common than infection with *H. hepaticus* and *H. rodentium*, and its prevalence is similar to the lower prevalence of *H. bilis* in colonies of mice in academic institutions (260).

Helicobacter muridarum. *Helicobacter muridarum* was first isolated from the intestinal mucosa of rats and mice (149, 190). Natural infection of immunocompetent rodents has not been associated with clinical disease. Although the natural niche for *H. muridarum* is the large intestine, *H. muridarum* has been associated with gastritis in mice, some of which had developed gastric atrophy that presumably promoted colonization of *H. muridarum* in the less-acidic stomach (193). In a T-cell transfer model of IBD in C.B17 SCID mice, monoassociation with *H. muridarum* provoked typhlocolitis on receipt of CD45RB^{hi} CD4⁺ T cells from conventionally reared congenic BALB/c mice (134). After cell transfer, C.B17 SCID mice monoassociated with *H. muridarum* developed typhlocolitis within 5 to 6 weeks, compared with 8 to 12 weeks in conventionally housed cohorts.

‘Helicobacter muricola.’ A novel enteric helicobacter was isolated from the cecum and feces of Korean wild mice (*Mus musculus molossinus*) (264). This isolate was provisionally named ‘*H.*

muricola' on the basis of phenotypic characteristics and 16S rRNA gene sequence analysis. '*Helicobacter muricola*' is a microaerophilic helicobacter with a pair of nonsheathed bipolar flagella and is urease, catalase, and oxidase positive, reduces nitrate to nitrite, does not hydrolyze hippurate, and is susceptible to nalidixic acid and resistant to cephalothin.

'*Helicobacter rappini*.' A novel bacterium with fusiform morphology, periplasmic fibers, and bipolar tufts of sheathed flagella was identified in the intestinal mucosae of laboratory mice (208). The isolate grew under microaerobic conditions and was urease positive. On the basis of 16S rRNA gene sequence analysis, the organism was identified as '*Flexispira rappini*,' and is now referred to as '*H. rappini*.'

Experimental *Helicobacter* Infection of Mice

Mouse models of *Helicobacter*-associated hepatitis and hepatic cancer. In experimental studies of *H. hepaticus* infection in A/JCr mice, the development of *Helicobacter*-associated lesions, particularly hepatitis, has been associated with the concomitant development of a significant serum IgG and Th1 cell-mediated immune response to *H. hepaticus* antigens (262), which is consistent with the robust response of IL10^{-/-} mice to *H. hepaticus* infection (145, 146). Significant amounts of mucosal IgA, systemic IgG and T-cell responses develop early after infection, but are ineffective in preventing chronic infection or development of lesions.

The importance of host genetics is indicated by strain susceptibility to liver disease in mice. The predisposition of A/JCr mice to develop *H. hepaticus*-associated hepatitis contrasts with mild or no disease in C57BL/6 mice (129, 258). Colonization by *H. hepaticus* in the cecum of experimentally infected A/JCr and C57BL/6 mice was quantified by use of real-time PCR analysis with primers for the *H. hepaticus cdtB* gene and mouse 18S rRNA (258). Colonization and lesions were evaluated after 6 months of *H. hepaticus* infection. Quantitative PCR analysis of cecal specimens indicated that *H. hepaticus* colonized C57BL/6 mice to a greater extent than it colonized A/JCr mice. Consistent with prior reports, A/JCr mice developed more severe parenchymal necrosis, portal inflammation, and phlebitis in the liver, compared with mild disease observed in infected C57BL/6 mice. Thus, hepatitis in A/JCr mice caused by *H. hepaticus* infection is associated with significantly lower colonization by *H. hepaticus* in the cecum, compared with higher colonization in hepatitis-resistant C57BL/6 mice. Host responses of A/JCr mice that limit cecal colonization with *H. hepaticus* may have important roles in the pathogenesis of hepatic lesions.

To examine the genetic basis for susceptibility to *H. hepaticus*-induced liver disease, disease-susceptible A/JCr mice and disease-resistant C57BL/6 mice were compared, using 9 AXB recombinant inbred (RI) mouse strains (129). After 14 months of infection with *H. hepaticus*, determination of colonization by *H. hepaticus* in the liver and cecum, and morphometric evaluation of the liver lesions were performed to quantify and correlate the severity of inflammation, apoptosis, and proliferation. Significant variation between RI strains in severity of hepatitis suggested a multigenic basis of disease resistance. Quantitative trait analysis, using linear regression, suggested possible linkage to loci on mouse chromosome 19. Hepatocellular and biliary epithelial apoptosis and proliferation indices, including proliferation of oval

cells, were markedly increased and correlated with severity of inflammation. Prevalence of hepatic neoplasia also was increased in susceptible RI strains (129).

Both *H. hepaticus* and *H. bilis* are members of the enterohepatic group of murine helicobacters and are urease positive, a purported virulence factor in common with that of *H. pylori*, that depends on urease activity to survive in the acidic environment of the stomach (24). Urease was found to be critical for colonization of the ferret stomach with *H. mustelae* (3), and in vivo complementation of *ureB* restores the ability of isogenic *H. pylori* urease mutants to colonize mice (53). *Helicobacter pylori* urease has also been documented to stimulate substantial increases in macrophage inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production, implicating it in NO-dependent mucosal damage and carcinogenesis (109). In contrast to gastric helicobacters, all of which are urease-positive, the functional role of urease in enterohepatic helicobacters is not clear because the natural niche of the cecum and colon is not acidic, and many members of the enterohepatic group of helicobacters are urease negative. The urease gene of *H. hepaticus* has been cloned and found to contain a homologue of each gene in the *H. pylori* urease cluster (7). Isogenic urease mutants of *H. hepaticus* were compared with the wild-type parental strain for viability in a low pH environment in vitro and for ability to colonize outbred Swiss Webster mice (22). The urease mutants were more susceptible to acid killing and failed to infect outbred Swiss Webster mice by use of oral gavage or C57BL/6 mice by use of intraperitoneal administration. These results indicate that urease activity is essential for intestinal infection by *H. hepaticus* and that failure to colonize is not attributable to acid susceptibility because parenteral dosing also failed to infect mice.

A urease-negative *Helicobacter* sp. isolated from a wild mouse induced proliferative typhlocolitis in defined flora SCID mice (Tac:ICR:HascidfRFscid) that was accompanied by severe hemorrhagic diarrhea, weight loss, phlebothrombosis, and hepatitis (217). Infected A/J mice did not develop clinical signs of disease, but had mild to moderate proliferative typhlocolitis and moderate to marked cholangiohepatitis at 7 and 24 weeks after infection. This same novel *Helicobacter* isolate was used to examine the ontogeny of a characteristic lesion of *H. hepaticus*-induced hepatitis in the A/J mouse (219). *Helicobacter hepaticus*-associated hepatitis is characterized by random distribution of multifocal accumulation of mononuclear inflammatory cells around small intralobular hepatic venules. These foci of inflammatory infiltrates resemble tertiary lymphoid development that has been observed in other models of chronic autoimmunity or inflammation, and are thought to arise by a process termed lymphoid organ neogenesis. The small high endothelial venules in inflammatory lesions in A/J mice infected with the novel helicobacter were positive by use of immunohistochemical analysis for features typical of lymphoid organ neogenesis that included peripheral node addressin and the mucosal addressin cell adhesion molecule. The chemokines SLC (CCL 21) and BLC (CXCL13) also were present, as were B220⁺ B cells and the naïve T-cell subset that expresses CD45^{lo}CD62L^{hi}. These findings suggest that lymphoid aggregates observed in lesions of *Helicobacter*-induced chronic active hepatitis arise through the process of lymphoid organ neogenesis.

Results of early studies suggested a potential autoimmune-like

mechanism for hepatitis because autoantibodies were detected to hsp70, which was expressed by *H. hepaticus* and diseased hepatocytes (255). The mechanism by which the chronic inflammatory reaction in the liver of *H. hepaticus*-infected mice leads to cancer is unknown. A putative mutagen secreted by *H. hepaticus* has not been detected using the Ames assay (19). Measurement of oxidative damage in liver DNA of infected mice has linked accumulation of 8-oxodeoxyguanosine with disease progression (225). Oxidative stress associated with inflammation can result in lipid peroxidation and the generation of malondialdehyde, which in turn can react with deoxyguanosine in DNA, resulting in the formation of adducts. Adducts may cause mutations that ultimately lead to liver carcinogenesis. Increased amounts of adducts were detected in the liver DNA of *H. hepaticus*-infected A/JCr mice, with highest amounts found at 12 months (223). Although a lobe predisposition for *H. hepaticus*-induced liver tumors has not been reported to our knowledge, adducts were particularly increased in the caudate and median lobes, with the left lobe having the lowest amount of adducts compared with the right and median lobes. Other promutagenic DNA lesions, 7-methylguanine and O6-methylguanine, indicative of nitrosation of endogenous amines by nitric oxide, were not detected. Analysis of carcinomas and adenomas taken from *H. hepaticus*-infected A/JCr mice did not reveal mutations in the *ras* or *p53* oncogenes (226). These results suggest a non-genotoxic tumor promotion mechanism, possibly mediated by reactive oxygen species generated during the inflammatory response (19). Overexpression of epidermal growth factor, transforming growth factor α (TGF- α), cyclin D1, Cdk4, and c-Myc is associated with cellular transformation and were found to be up-regulated in the liver of *H. hepaticus*-infected A/JCr mice, with highest expression in tumors (196).

Early exposure of A/JCr mice to *H. hepaticus* infection promoted the most severe hepatitis and preneoplastic changes in the liver, particularly in male mice (200). Mouse pups were either exposed to *H. hepaticus* by oral infection of the pregnant female, and/or weanlings born to infected and uninfected dams were experimentally re-dosed with *H. hepaticus* at 3 or 12 weeks of age. Effects of gestational exposure to *H. hepaticus* were not significant, but male A/J offspring infected by 3 weeks of age developed the most severe hepatitis, whereas those first infected at 12 weeks of age were hepatitis resistant. Interestingly, compared with females, males were most prone to severe disease as previously reported (87), but males of the current study had a bimodal pattern of susceptibility. The most severe lesions in males included lobular necrogranulomatous and interface (chronic active) hepatitis, whereas females were more prone to develop portal (chronic persistent) hepatitis. Hepatic bacterial load and precursor lesions of hepatocellular carcinoma, including clear and tigroid cell foci of altered hepatocytes, were strongly associated with lobular hepatitis severity (Fig. 3) (200).

Mouse Models of *Helicobacter*-Associated IBD-like Disease

Experimentally induced infection using *H. troglontum*.

National Institutes of Health (NIH) germ-free mice experimentally infected with *H. troglontum* were persistently colonized at various levels of the gastrointestinal tract when necropsied at postinoculation week 6 (173). All infected mice developed microscopically detected inflammation, including gastritis, in at least one region of the bowel. The predominant histologic change was a

moderate diffuse inflammatory infiltrate of mononuclear cells in the lamina propria, often accompanied by mild infiltration of polymorphonuclear neutrophilic leukocytes. Two animals had focal infiltration of inflammatory cells in the liver, although bacteria could not be detected.

The role of the immune response in either limiting colonization or in contributing to the intensity of the inflammatory response to *Helicobacter* infections that can progress to cancer in mouse models has been widely investigated (201). Soon after the discovery of *H. hepaticus* in 1991, case reports associated *H. hepaticus* infection with inflammatory bowel syndromes in nude and SCID mice, manifested as rectal prolapse accompanied by chronic proliferative typhlitis, colitis, and proctitis (254). Infected A/JCr mice produce predominantly IgG2a serum antibodies to *H. hepaticus*, which is consistent with a Th1 immune response, as reported for humans infected with *H. pylori* and mice with *H. felis*. Spleen mononuclear cells from infected A/JCr mice proliferate in vitro in response to *H. hepaticus* antigens and produce more gamma interferon (IFN- γ) than interleukin (IL-) 4 or IL-5, also characteristic of a Th1 immune response (262). A/J mice may develop mild to moderate typhlitis after chronic infection with *H. hepaticus*. To correlate histologic changes with proinflammatory influences from the infection, cecal tissues from A/JCr mice infected with *H. hepaticus* were compared for gene expression profiles to determine potential differences prior to the onset of inflammation (one month) and after chronic inflammation was established at three months (174). One month after infection, 25 genes were up-regulated and three were down-regulated, in contrast to up-regulation of 31 and down-regulation of two genes at the 3-month time point. A subset of proinflammatory genes, including IFN- γ , interferon gamma inducible protein (IP-10), macrophage inflammatory protein 1 α (MIP 1 α), and serum amyloid A1, were among the up-regulated genes.

In murine models, such as IL-10^{-/-} mice, which develop Th1-mediated typhlocolitis, some features of the pathogenesis associated with human IBD are mimicked. Epithelial hyperplasia and progression to dysplasia with neoplastic sequelae have been associated with dysregulated responses to enteric flora, including *H. hepaticus*, in certain strains of mice (8, 59, 261). The most susceptible strains of IL-10^{-/-} mice develop severe spontaneous typhlocolitis when maintained under conventional housing conditions. The importance of genetic background has been highlighted by evidence that severity of typhlocolitis in response to enteric flora varies with mouse strain. Most severe disease developed in IL-10^{-/-} 129/SvEv and IL-10^{-/-} BALB/c strains, intermediate severity was noted in the IL-10^{-/-} 129 \times C57BL/6J outbred crosses, and least-severe disease was observed in IL-10^{-/-} C57BL/6J mice (8). The associated chronic inflammation and epithelial dysplasia progresses to colorectal adenocarcinoma in a high percentage (60%) of IL-10^{-/-} 129/SvEv mice by 6 months of age (8).

The IL-10^{-/-} mice experimentally infected with *H. typhlonius* (82), and most notably *H. hepaticus* (146), develop IBD-like clinical signs of disease, including diarrhea, perianal ulceration, intestinal bleeding, and rectal prolapse (8, 82, 143). The cecocolic junction and distal portion of the colon are most severely affected, with mucosal ulceration and focal transmural inflammation characterized by extensive infiltration of the lamina propria with lymphocytes, plasma cells, macrophages, and scattered neutrophils. The IL-10^{-/-} C57BL/10J mice coinfecting with the murine nematode *Heligmosomoides polygyrus*, which induces a Th2 host

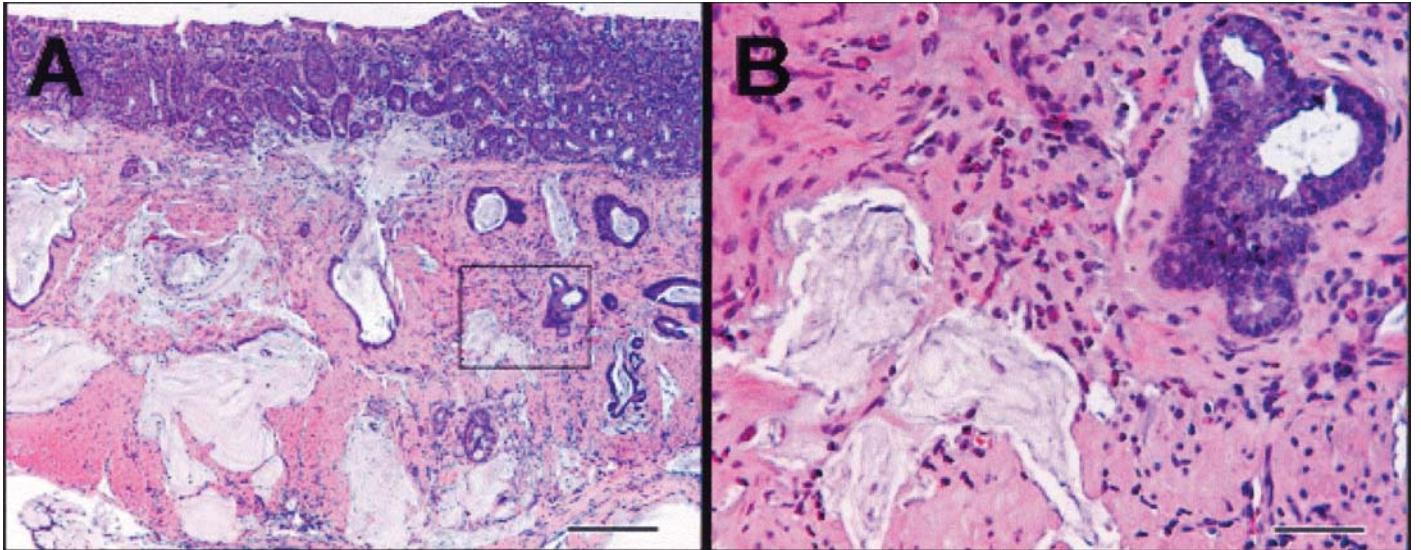


Figure 4. (A) Photomicrograph of a section of the transverse colon of a *H. hepaticus*-infected RAG^{-/-} mouse at 14 months after infection. Notice severely dysplastic glands with atypical epithelium infiltrating the submucosa and muscle and obliterating normal bowel structure. (B) Extracellular mucin was prominent in large pools partially lined by neoplastic epithelium. H&E stain; (A): bar = 250 µm; (B): bar = 100 µm. Figure used with permission from Erdman and co-workers (60).

response, and *H. hepaticus*, which induces a Th1 response, developed less severe typhlocolitis and epithelial hyperplasia than did IL-10^{-/-} mice infected with *H. hepaticus* alone (261). The reduction in mucosal damage in the *H. polygyrus/H. hepaticus*-coinfected IL-10^{-/-} mice was associated with suppressed Th1 responses to *H. hepaticus* despite increased colonization of *H. hepaticus* in the cecum, typical of a Th2 bias toward *Helicobacter* infection. The ameliorating effect of parasitism on the mucosal damage associated with *H. hepaticus* in the IL-10^{-/-} mouse IBD model supports prior findings that enteric parasitism can reduce *Helicobacter*-associated gastric tissue damage (73).

Efforts to define the relevant contributions of innate immunity versus adaptive immunity to IBD have taken advantage of the ability of *H. hepaticus* to reproducibly cause IBD-like disease in immunodeficient mice, such as the SCID C.B-17 strain (18, 160), T cell receptor knockouts (21), NF-kappa B-deficient mice (57), and RAG2^{-/-} mice (60). SCID mice develop typhlocolitis after adoptive transfer of CD4⁺ T cells expressing high levels of CD45RB, a phenotype of naïve T cells. Germfree SCID mice do not develop inflammation after cell transfer, indicating that a response to the intestinal flora is key to the model. When SCID mice were experimentally infected with *H. hepaticus*, development of typhlocolitis was accelerated after reconstitution with CD45RB^{high}CD4⁺ T-cells (18). Transfer of CD45RB^{low}CD4⁺ T cells that contain a regulatory T-cell subset expressing the CD25 IL-2 receptor did not cause inflammation and suppressed the inflammatory response mediated by the CD45RB^{high}CD4⁺ T cells when they were co-transferred. The mechanism(s) for immune suppression by CD4⁺CD25⁺ T-regulatory cells was further studied in *H. hepaticus*-infected RAG^{-/-} mice that lack functional B and T cells. In this model, *H. hepaticus* infection elicited T cell-mediated and T cell-independent intestinal inflammation, both of which were inhibited by adoptively transferred CD4⁺CD25⁺ regulatory T cells. The T cell-independent pathology was accompanied by activation of the innate immune system that was also inhibited by CD4⁺CD25⁺ cells. Suppression of innate immune pathology was dependent on T cell-derived IL-10 and on the production of TGF-β (160).

Because these mice lack functional B and T cells, the inflammatory response to *H. hepaticus* in RAG2^{-/-} mice is mediated by the innate immune system. RAG2^{-/-} mice naturally infected with *H. hepaticus* developed severe colitis, but IL-7^{-/-} RAG2^{-/-} mice colonized by the same flora did not develop spontaneous colitis, suggesting that IL-7 plays a critical role in exacerbating a non-T/non-B cell-mediated chronic inflammatory response. Administration of recombinant (r)IL-10 was able to prevent development of colitis in susceptible mice, suggesting a pivotal role for macrophages (103). *Helicobacter hepaticus*-infected RAG2^{-/-} mice on the 129/SvEv background were reported to rapidly develop colitis and large intestinal carcinoma (Fig. 4) (59). Adoptive transfer with CD4⁺CD45RB^{low}CD25⁺ regulatory T cells in these mice significantly inhibited *H. hepaticus*-induced inflammation and colon cancer. Regulatory cells lacking the anti-inflammatory cytokine IL-10 were unable to inhibit inflammation, dysplasia, or cancer, indicating that IL-10 was required for the protective effects of lymphocytes in these experiments and that the regulatory T cells expressing IL-10 had an anti-inflammatory effect on the innate immune system (60).

NF-kappaB is a transcription factor activated during the inflammatory response, and thus, it was unexpected to discover spontaneous colitis in mice lacking the p50 subunit and having only one allele of the p65 subunit of NF-kappaB (p50^{-/-}p65^{+/-}) (57). These mice were rederived into a helicobacter-free health status, and the phenotype of spontaneous colitis decreased markedly. Experimental infection of NF-kappaB p50^{-/-}p65^{+/-} mice with *H. hepaticus* caused severe colitis to develop within 6 weeks, was milder in control genotypes, such as p50^{-/-}p65^{+/+} and p50^{+/+}p65^{+/-}, and was absent in wild-type mice, suggesting that the p50 and p65 subunits of NF-kappaB have a role in inhibiting the development of colitis, potentially through control of proinflammatory IL-12. It was further documented that NF-kappaB activity is required within the innate immune system to inhibit *H. hepaticus*-induced colitis and that NF-kappaB regulatory activity is mediated, at least in part, through inhibition of the expression of the p40 subunit of IL-12 (246).

Mouse Models of *Helicobacter*-Induced Gastritis

***Helicobacter felis* gastritis.** Isolation of *H. felis* from the cat stomach made it possible to model *Helicobacter* gastritis in mice after it was documented that *H. felis* would colonize germfree mice (147) and gnotobiotic rats (86) and induce gastritis. BALB/c mice infected with *H. felis* for up to 22 months developed B-cell lymphoid follicles in the gastric wall that were reminiscent of MALT lymphoma of humans infected with *H. pylori* (56). These BALB/c mice did not develop gastritis until near the end of the study, which was the first suggestion of strain difference in the host response to *H. felis*. This was further described by Mohammadi and co-workers (172), who observed that the pattern and intensity of gastritis varied from minimal in BALB/c, to moderate in C3H, to severe in C57BL/6 mice. The robust inflammatory response of C57BL/6 mice to *H. felis* was opportune for further model development of *Helicobacter*-induced gastritis because the C57BL/6 background is commonly used for producing transgenic and knockout mice. For example, *H. felis* infection in the IL10^{-/-} mouse led to accelerated severe hyperplastic gastritis with gastric epithelial cell dedifferentiation (9), a feature of *H. pylori* infection in humans that can progress to gastric atrophy and cancer (27). Many studies have followed that documented the roles of innate (130, 131) and adaptive immune responses (171, 204) on impact of colonization levels and inflammatory responses of mice to *H. felis*. Vaccine strategies to prevent infection with *H. felis* (135) or therapeutically ameliorate gastritis (28) are of considerable interest for application to vaccine development in humans. The potential for sex differences in response to *H. felis* infection was reported in female C57BL/6J mice infected with *H. felis* for up to a year. Infected females developed chronic gastric inflammation, epithelial hyperplasia, and oxyntic cell loss earlier than did male mice, with a trend over time for more severe lesions to develop in female mice (29).

Infection with *H. felis* has been used in mice to model gastric cancer. *Helicobacter pylori* infection and adenomatous polyposis coli (*Apc*) gene mutations have been linked to gastric cancer in humans. C57BL/6 and *Apc*1638 heterozygous mice infected with *H. felis* were studied for 7.5 months (78). The infected *Apc*1638 mice had less epithelial proliferation and gastritis, lower serum IgG responses, and higher bacteria and urease scores than did infected C57BL/6 mice. The *Apc*1638 truncating mutation led to gastric dysplasia and polyposis of the antrum and pyloric junction, but *H. felis* infection did not increase the risk of gastric neoplasia. Results of that study indicated that this *Apc* mutation is associated with decreased immune, inflammatory, and gastric hyperplastic responses to *H. felis* infection, suggesting the possibility of a novel role for this tumor suppressor gene in the immune and local tissue responses to gastric bacterial infection. The *p53* heterozygous mice infected with *H. felis* for up to 15 months also had lower risk of *Helicobacter*-associated cancer (94). Antral inflammation and submucosal invasive foci were observed in wild-type C57BL/6 mice accompanied by invasion of adjacent submucosal blood vessels by glandular epithelia in a subset of infected C57BL/6 mice. None of these lesions were observed in *p53*^{+/-} mice, infected or not, and *p53*^{+/-} mice had significantly higher *Helicobacter* colonization consistent with an anti-inflammatory Th2 response. Proinflammatory Th1 responses were significantly higher in C57BL/6, compared with *p53*^{+/-} mice. These results suggested that germline deletion of one *p53* allele results

in a down-regulated Th1 response to gastric *Helicobacter* infection, possibly related to T-cell senescence, which indirectly would be protective against development of gastric cancer and other epithelial-derived neoplasms associated with chronic inflammation. In contrast, infection with *H. felis* in TSG-p53/Big Blue mice (*p53*^{+/-}) resulted in enhanced gastritis and three-fold increase in mutations after 7 months, compared with that in wild-type mice (133). Compared with wild-type mice, TSG-p53/Big Blue mice (*p53*^{+/-}) infected with *H. felis* or *H. pylori* for 6 months developed severe gastritis that was associated with increased iNOS expression and a four-fold and 1.7-fold higher rate, respectively, of mutation (247). After 12 months of infection, gastric hyperplasia was prominent, but the mutagenic effects and iNOS expression decreased in *H. pylori*- and *H. felis*-infected mice. These data suggest a synergistic action between infection with *H. felis* and *p53* deficiency in the accumulation of mutations in gastric tissue.

Studies from our laboratories indicated that Th1-promoted gastric atrophy secondary to *H. felis* infection in mice was ameliorated by the Th2 host response to the murine helminth, *Heligmosomoides polygyrus* (73). Those results suggest that the low incidence of gastric cancer in African populations with a high incidence of *H. pylori* infection is attributable, in part, to high prevalence of concurrent parasitic infection in Africans that tempers the host response to *H. pylori*.

***Helicobacter pylori* gastritis.** The ability to genetically engineer the host response in mice is a powerful advantage to study basic mechanisms of *H. pylori* gastritis and its sequelae. Initial attempts to colonize mice with *H. pylori* were unsuccessful until the Sydney strain of *H. pylori* known as SS1 (*cagA*⁺ and *vacA*⁺) was isolated by dosing mice with a variety of fresh clinical isolates and evaluating long-term colonization (148). High levels of colonization were achieved in C57BL/6, BALB/c, DBA/2, and C3H/He mice, and persistent colonization of mice led to chronic active gastritis with progression to gastric atrophy. The capability of SS1 to colonize mice and cause gastritis has had significant impact on *H. pylori* research. It should be noted that the functional status of *cagA*⁺ in SS1 has been debated because several laboratories have reported that SS1 does not induce IL-8 secretion from human gastric epithelial cells in vitro (31). Thus, although SS1 is probably the best model strain of *H. pylori* to use in mice to study colonization and gastritis, investigators interested in the *cagPAI*, and in particular the significance of IL-8 as a proinflammatory mediator, should use other *H. pylori* strains, and additionally must use other species because mice do not produce IL-8.

Mouse strains vary in their susceptibility to development of gastric atrophy from *Helicobacter*-induced gastritis. The genetic basis for this has been examined for *H. felis* models (239) as well as *H. pylori* models (238). Milder gastritis and lower predisposition to atrophy was identified as a dominant trait associated with increased production of IL-10. In a mouse model of *H. pylori* infection, a high-salt diet linked in humans to increased risk of gastric cancer was shown to exacerbate gastritis, increase epithelial hyperplasia, promote parietal cell loss and enhance *H. pylori* colonization in C57BL/6 mice (77). As for *H. felis*, there has been intense interest in developing vaccine strategies using *H. pylori* in the mouse (237). Therapeutic vaccines have been associated with transient postimmunization gastritis (236).

Epidemiologic evidence in humans and from studies with mice suggests a link between a high-salt diet and *H. pylori* infection and the development of hypergastrinemia and preneoplastic gas-

tronic lesions. *Helicobacter* infection in C57BL/6 mice leads to alterations in gastrin and somatostatin concentrations as the gastritis progresses to atrophy, and is characterized by loss of parietal and chief cells and impairment of gastric acid secretion (43). *Helicobacter felis* infection in insulin-gastrin (INS-GAS) transgenic mice, which develop hypergastrinemia, accelerates development of gastric carcinoma (253). In the INS-GAS/129 mouse model, gastric tissue from males responded more rapidly and aggressively to *H. pylori* infection, high-salt diet, and the combination, compared with tissue from females, a finding that appears consistent with the greater incidence of gastric carcinoma in men. Only male INS-GAS mice infected with *H. pylori* developed in situ and intramucosal carcinoma (92). These results indicated that *H. pylori* can accelerate the development of gastric cancer in the INS-GAS mouse model, and that salt has less of a procarcinogenic effect in the setting of endogenous hypergastrinemia. This same model of INS-GAS mice infected with *H. pylori* or an isogenic *H. pylori* *cagE* mutant of the *cagPAI* developed atrophy, intestinal metaplasia, and dysplasia by 6 weeks and carcinoma by 24 weeks (95). Inactivation of *cagE* delayed the progression to carcinoma, but neoplasia ultimately developed in all males infected with the *H. pylori* mutant. These studies highlight the importance of using both sexes to investigate the pathogenesis of *H. pylori*.

'*Helicobacter suncus*.' A gram-negative, motile bacterium with bipolar sheathed flagella was isolated from the stomach of house musk shrews (*Suncus murinus*) with chronic gastritis and was found on gastric epithelial cells by use of electron microscopy (112). Sequence analysis of 16S rRNA suggested that this bacterium should be classified as a novel *Helicobacter* species with the proposed name '*Helicobacter suncus*.' Gastritis from natural infection was reported, and experimental challenge in shrews, first cleared of naturally acquired '*H. suncus*' by use of antibiotics, resulted in development of gastritis (110).

Natural *Helicobacter* Infections in Rats

Gastric spirals with morphology consistent with that of '*H. heilmannii*' were associated with mild gastritis in wild *Rattus norvegicus* in Italy (108). Spiral organisms with morphology consistent with that of *H. trogontum* were observed by use of microscopy in bile from rats infected with the liver fluke, *Fasciola hepatica* (69). Natural *Helicobacter* infections in research rats appear to be common, with prevalence of 19% on the basis of results of testing rat cecal specimens submitted to a US veterinary diagnostic laboratory and assayed for infection, using genus- and species-specific PCR assays (153, 198). In those two reports, *H. bilis* was detected in 3 to 9% of rat cecal specimens; 10% were positive for *H. typhlonius*, 5 to 8% were positive for *H. hepaticus*, and *H. rodentium* and unspiciated *Helicobacter* spp. each were detected in approximately 2% of the specimens. *Helicobacter rodentium* was detected by use of PCR analysis of fecal specimens collected from 48 of 163 rats (29%) housed in three colonies in Japan (113). Gross lesions were not observed, and histologic examination was not performed. Except for *H. bilis* infection of immunodeficient rats, the potential for *Helicobacter* spp. to cause disease in immunocompetent rats has not been reported to our knowledge.

***Helicobacter bilis*.** Proliferative and ulcerative typhlocolitis and proctitis developed as a clinical problem in nude rats (Cr:NIH-rnu) (116). *Helicobacter bilis* was identified morphologically in the colon crypts using modified Steiner's silver stain, was

recovered by culture, and was further identified using PCR analysis and amplicon sequencing. Typhlocolitis was reproduced in *H. bilis*-free nude rats that were injected intraperitoneally with *H. bilis* recovered from the same colony. *Helicobacter bilis* was detected by use of PCR and RFLP analyses in 9.7% of 113 rat cecal specimens submitted to a veterinary diagnostic laboratory (198). To our knowledge, the potential for *H. bilis* to cause disease in immunocompetent rats has not been reported.

***Helicobacter muridarum*.** Spiral-shaped bacteria with distinctive morphology were first isolated from the intestine of rats and mice using a *Campylobacter*-selective medium and microaerobic incubation (190), and were later named *H. muridarum* (149). To our knowledge, pathology in rats attributable to *H. muridarum* has not been reported.

***Helicobacter trogontum*.** *Helicobacter trogontum* was isolated from the colon of Wistar and Holtzman rats (168). The organism resembles *H. bilis*, with its rod shape entwined with periplasmic fibers. Like many large intestinal helicobacters, *H. trogontum* is catalase and oxidase positive, rapidly hydrolyzes urea, and is susceptible to metronidazole and resistant to cephalothin and nalidixic acid. *Helicobacter trogontum* is microaerophilic and grows at 37 and 42°C, differentiating it from *H. hepaticus* and *H. muridarum*, which grow optimally at 37°C. On the basis of 16S rRNA sequence analysis, *H. trogontum* is most closely related to *H. hepaticus*. Members of *Helicobacter* sp. flexispira 16S rDNA taxa 1, 4, and 5 have been determined to be strains of *H. trogontum* (taxon 6) (121).

Experimental *Helicobacter* Infection of Rats

Rat models of *Helicobacter* gastritis. In comparison with use of mouse models, use of rats for study of *H. pylori* pathogenesis has been limited. Experimentally, *H. felis* and '*H. heilmannii*' colonized the gastric antrum of rats in large numbers and induced mild gastritis (37). Wistar rats, which were documented to be free of naturally acquired gastric spirals, were dosed with '*H. suis*' from swine and the stomach became colonized; mild gastritis had developed when the rats were assessed two weeks later (167). *Helicobacter felis* was reported to colonize the gastric antrum of germfree rats, causing small foci of lymphocytes and eosinophils to form throughout the subglandular region of the antrum by two weeks after infection (86). At 8 weeks after infection, there were increased numbers of lymphocytes and eosinophils in the subglandular areas and some focal aggregates of lymphocytes extending from the submucosa through the muscularis mucosa and lamina propria to the luminal surface. *Helicobacter felis* was detected by use of the Warthin-Starry stain, bacteriologic culture, and a urease assay. Cage-contact control rats were not infected with '*H. suis*' (167) or *H. felis* (86), supporting the lack of fecal-oral spread of gastric helicobacters between rodents housed in the same cage.

'*Helicobacter heilmannii*' infection was used to document that *Helicobacter* infection can delay gastric emptying, possibly related to increased concentration of gastrin and suppressed concentration of somatostatin (15). Short-term infection of rats with mouse-adapted *H. pylori* has been used to indicate that *H. pylori* infection can delay healing of iatrogenic gastric ulcers (151). Other studies in rats have involved examination of interaction between *H. pylori* lipopolysaccharide and administration of nonsteroidal anti-inflammatory drugs (228) and anti-ulcer drugs (191).

Natural *Helicobacter* Infections in Gerbils

***Helicobacter hepaticus*.** In a survey of 50 gerbils housed in four facilities in Japan, shedding of *H. hepaticus* in feces was detected by use of PCR analysis in 3 colonies, but other *Helicobacter* spp. were not detected (113). Gross lesions were not observed, and histologic examination was not performed.

***Helicobacter bilis*.** Natural infection with *H. bilis* in commercially available Mongolian gerbils was recently reported (11). Infection was not associated with lesions or clinical problems except that attempts to eradicate *H. bilis* by use of antibiotics resulted in acute morbidity and mortality from overgrowth of *Clostridium difficile*.

Experimental *Helicobacter* Infection of Gerbils

Gerbil models of *Helicobacter* gastritis. The first successful models of gastric cancer and its precursor lesion, intestinal metaplasia, attributable to chronic *H. pylori* gastritis in a rodent model, were reported using inbred Mongolian gerbils (MGS/Sea) (*Meriones unguiculatus*) infected either with clinical isolates of *H. pylori* or with strain ATCC 43504 (127, 257). The gerbil model for gastric cancer has also combined *H. pylori* infection with the carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine, to establish that chronic *H. pylori* gastritis promoted incidence of chemically induced adenocarcinoma (245). Because risk factors for development of gastric adenocarcinoma in humans include high dietary salt intake and *H. pylori* infection, gastric pathology resulting from *H. pylori* infection and high dietary salt intake were examined as independent variables in outbred Mongolian gerbils (CrI:[MON]) infected with the Sydney strain of *H. pylori* (10). After 37 weeks, 5 of 5 infected gerbils had atrophic gastritis and intestinal metaplasia. Gastric atrophy and intestinal metaplasia without accompanying inflammation also were noted in 6 of 6 uninfected gerbils that had consumed a 2.5% salt diet for 56 weeks. Thus, outbred gerbils provide an excellent animal model for the study of several gastric cancer risk factors.

Only a few *H. pylori* strains have been documented to colonize Mongolian gerbils successfully. A *cagA*+ strain 42GX isolated from a Chinese patient colonized gerbils and induced severe gastritis (252). The gastric mucosa of gerbils infected with the *cagA*+ *H. pylori* SS1 strain for 36 weeks was analyzed for increased expression of pro- and anti-inflammatory cytokines (30). After 36 weeks, female gerbils infected with *H. pylori* had significantly increased expression of transcripts for IFN- γ and IL-12p40, but not TGF- β or IL-10. Significantly reduced IFN- γ and IL-12p40 responses were observed in infected male gerbils, but epithelial proliferative and apoptotic responses were comparable between the sexes. Thus, female gerbil cytokine responses to *H. pylori* appear to have a stronger Th1 profile than do males, and the low expression of TGF- β or IL-10 may account for the more severe gastritis observed with *H. pylori* infection in gerbils than in mice.

The risk for gastric adenocarcinoma from *H. pylori* infection in humans may be related to increased gastric epithelial cell turnover and/or gastrin secretion. Gerbils infected with wild-type *H. pylori* or isogenic mutants of *cagA* and *vacA* developed transient increased rates of antral apoptosis followed by increased but similar rates of gastric epithelial cell turnover (186). Epithelial cell proliferation was significantly related to serum gastrin concentration, whereas antral apoptosis was inversely related to

acute inflammation and formation of lymphoid follicles. Thus, the results from this gerbil model indicate that epithelial cell turnover enhanced by *H. pylori* infection may be mediated by gastrin-dependent mechanisms.

Natural *Helicobacter* Infections in Hamsters

***Helicobacter cinaedi*.** Hamsters are an established reservoir host for *H. cinaedi*, which also is the most commonly reported enterohepatic helicobacter in immunosuppressed humans who frequently have bacteremia or diarrhea as presenting signs (139). Septicemia and meningitis attributed to *H. cinaedi* in a neonate whose mother had contact with pet hamsters during pregnancy was reported (178). *Helicobacter cinaedi* DNA was identified in 8 of 126 urease-negative (i.e., *H. pylori* negative) human gastric biopsy specimens (187). *Helicobacter cinaedi* has also been isolated from a rhesus monkey affected with chronic colitis and hepatitis (83). *Helicobacter cinaedi* produces a bacterial toxin that causes progressive distention and death of Chinese hamster ovary (CHO) cells and HeLa cells, and is termed cytolethal distending toxin (CDT) (242). The CDT causes cell cycle arrest at the G2/M phase, and may be a mechanism for defense against immune clearance. This toxin has been identified in several enteropathogenic bacteria, including *C. jejuni*, *C. coli*, some *Escherichia coli* strains, and *Shigella* spp., as well as *Haemophilus ducreyi* and *Actinobacillus actinomycetemcomitans* (20).

Homologues of the toxin B subunit from five species of enterohepatic helicobacters, including *H. hepaticus*, *H. bilis*, *H. canis*, and a novel *Helicobacter* sp. isolated from mice, and *H. marmotae* from woodchucks, have been identified, and the bacterial lysates from four of these helicobacters have been documented to cause characteristic cytolethal distention of HeLa cells (20).

***Helicobacter mesocricetorum*.** A number of novel helicobacters have been isolated from hamsters obtained from research colonies and commercial vendors. *Helicobacter mesocricetorum* was isolated from the feces of clinically normal Syrian hamsters (220), and appears to be most closely related to *H. rodentium*.

***Helicobacter cholecystus*.** This helicobacter was isolated from the gallbladder of hamsters with cholangiofibrosis and centrilobular pancreatitis (99). Sequence analysis of the 16S rRNA indicated close relationship to *H. pametensis* isolated from swine and birds.

***Helicobacter aurati*.** This helicobacter was isolated from the inflamed stomach and cecum of adult Syrian hamsters (185). It is fusiform, with multiple bipolar sheathed flagella and periplasmic fibers, and is urease and γ -glutamyl transferase positive (Fig. 5). *Helicobacter aurati* represents a distinct taxon, and clusters with *H. muridarum*, *H. hepaticus*, and an unnamed *Helicobacter* isolate (MIT 94-022). Chronic gastritis and intestinal metaplasia associated with naturally acquired colonization by *H. aurati* and two other microaerobic species were reported in Syrian hamsters (184) from research and commercial facilities. *Helicobacter aurati*, a second novel urease-negative *Helicobacter* sp., as well as a smaller, urease-negative *Campylobacter* sp. were cultured from stomach specimens. *Helicobacter* sp. DNA was detected in a total of 35 hamsters by use of Southern blot hybridization; *Campylobacter* sp. DNA was detected in 15 of the 35 hamsters. Argyrophilic bacteria in gastric tissues had morphology consistent with that of isolates recovered by culture. The presence of *Helicobacter*, but



Figure 5. Electron micrograph of *H. aurati* isolated from the stomach of hamsters. Periplasmic fibers and multiple bipolar flagella are typical features of the *Flexispira* taxa. *H. aurati* measures approximately 0.6 by 4.8 μm .

not *Campylobacter* sp., was correlated to gastritis severity and intestinal metaplasia. *Giardia* spp. were observed in the metaplastic gastric pits of the most severe lesions. These findings are consistent with the development of intestinal metaplasia and gastric giardiasis in *H. pylori*-infected humans.

Experimental *H. pylori* Infection of Guinea Pigs

To our knowledge, enterohepatic helicobacters have not been reported in guinea pigs or rabbits, but these species have not been extensively evaluated for *Helicobacter* infection (46). Guinea pigs have been used in *H. pylori* models of gastritis (197, 218) because of the ability of the widely used mouse-adapted SS1 strain of *H. pylori* to colonize them (148). The vitamin C requirement of guinea pigs is a valuable feature of the model because of the role of vitamin C in protection against the formation of carcinogenic nitrosamines in gastric juice. Experimental *H. pylori* infection of guinea pigs induced antral gastritis and gastric MALT in guinea pigs (197, 218). Dunkin-Hartley guinea pigs were infected with the SS1 strain of *H. pylori*, and developed severe antral gastritis over 5 months (227). Natural *Helicobacter* infection was not detected in 5 control animals by use of culture and *Helicobacter* genus-specific PCR analysis of tissues and feces. The persistent

infection, accompanied by severe gastritis and a prominent serum antibody response, and the apparent absence of natural *Helicobacter* infection makes the guinea pig a useful animal model for *H. pylori* research.

Orogastric vaccination of guinea pigs with *H. pylori* sonicate and the adjuvant, cholera toxin, lowered the burden of infection (51). There have been differences in the response of guinea pigs and mice in response to vaccine strategies, which highlights that species differences need to be considered in the eventual application of a vaccine strategy to humans (137).

Interference with Research Attributable to *Helicobacter* Infections in Rodents

The discovery of *H. hepaticus* was the result of an investigation into an abnormally high incidence of hepatic tumors and hepatitis in control A/JCr mice that were part of a long-term carcinogenesis study (80, 256). A review of the impact of *H. hepaticus* infection on 12 National Toxicology Program two-year carcinogenesis studies was published in 1998 (115). Male and female B6C3F1 mice from all 12 studies were naturally infected with *H. hepaticus*, with associated hepatitis in many of the male mice reported from 9 of these studies. The incidences of hepatitis and hepatocellular and hemangiosarcoma of the liver in *H. hepaticus*-infected control male B6C3F1 mice were increased, compared with those in uninfected control males. Typical of the chronic hepatitis attributable to *H. hepaticus*, the liver from infected males had increased hyperplasia and apoptosis. Interestingly, H-ras codon 61 CAA to AAA mutations were less common in liver neoplasms from infected males than those in historical and study control specimens. In inhalation toxicity and carcinogenicity studies of cobalt sulfate, male B6C3F1 mice had liver lesions consistent with *H. hepaticus* infection and increased incidence of hepatic hemangiosarcoma. Because of the confounding infection with *H. hepaticus*, a conclusion could not be reached concerning an association between liver hemangiosarcoma and cobalt sulfate (16).

Helicobacter hepaticus contamination of nonfrozen transplantable human tumors was infective to SCID mice via subcutaneous injection of infected tissue (111). Contaminated but cryopreserved samples were not infective. To our knowledge, this is the first report suggesting that *H. hepaticus* has the ability to spread via biomaterials and that freeze-thawing is able to reduce the numbers of organisms to those that are insufficient for inadvertent infection of immunodeficient mice. *Helicobacter bilis* infection was documented to accelerate and *H. hepaticus* infection to delay development of (otherwise) expected spontaneous colitis that develops with age in multiple drug resistance-deficient (*mdr1a*^{-/-}) mice (159). The cause of spontaneous colitis has been attributed to a lack of P-glycoprotein that contributes to a presumed epithelial cell barrier defect in *mdr1a*^{-/-} mice. Experimental *H. bilis* infection induced diarrhea, weight loss, and typhlocolitis in *mdr1a*^{-/-} mice within 6 to 17 weeks after inoculation and before the expected onset of spontaneous typhlocolitis. In contrast, *H. hepaticus* infection delayed the onset of disease and spontaneous colitis was also less severe. Similar effects were reported in IFN- γ ^{-/-} mice naturally coinfecting with *H. hepaticus* and mouse hepatitis virus (MHV). Clinical disease that included pleuritis, peritonitis, hepatitis, pneumonia, and meningitis developed. Experimental infection with *H. hepaticus* alone did not induce clinical signs of disease, but when mixed with MHV challenge, *H. hepaticus* infection appeared to reduce the severity of MHV-induced lesions dur-

ing the acute phase of infection, and exacerbated hepatitis and meningitis at a later time point (26).

Importantly, subclinical effects of *Helicobacter* infection may confound research. For example, altered gene expression in cecal tissues prior to development of any features of pathology attributable to *H. hepaticus* infection was reported in A/JCr mice (174). One month after infection and prior to any evidence of typhlitis, 25 genes were up-regulated and three were down-regulated in contrast to up-regulation of 31 and down-regulation of two genes at the 3-month time point when inflammation was becoming apparent. A subset of proinflammatory genes, including IFN- γ , IP-10, MIP 1 α , and serum amyloid A1, were among the up-regulated genes.

Colony Management of Helicobacter-Free Animals

Many of the following principles have been applied to maintaining helicobacter-free cats and ferrets for controlled infection studies. Based on management techniques similar to those applied to rodents, larger species, such as swine or cats, can be obtained by cesarean section and hand-raised in a barrier to avoid contact with infected sows/queens or fomites. Helicobacter-free ferrets have been obtained by antibiotic treatment of jills prior to and during nursing, followed by weaning kits in a barrier environment (5). Recrudescence of infection is a risk in the latter approach.

Principles of Eradication of Helicobacter Infections in Mice

Before the impact of *Helicobacter* infection on the colony health of research mice was appreciated, the diagnostic laboratory at the MIT Division of Comparative Medicine frequently identified *Helicobacter*-infected mice from commercial and academic sources (210). Most commercial vendors can now supply mice that are free of *H. hepaticus* and *H. bilis*, and by use of the same production methods (i.e., cesarean section, embryo transfer), it should also be possible to supply mice that are free of other *Helicobacter* spp., such as *H. rodentium*. Natural infection with several murine *Helicobacter* spp. remains common in conventional mouse colonies because of horizontal transmission through fecal-oral contact (154, 260). Because of the endemic nature of the infection, successful eradication strategies consist of restocking with helicobacter-free mice, close adherence to husbandry practices proven to prevent introduction and dissemination of helicobacters, and monitoring colony and sentinel mice to maintain the helicobacter-free health status.

Restocking with helicobacter-free mice. Commercial vendors should be selected on the basis of the availability of helicobacter-free colonies and quality assurance practices that afford reasonable guarantee that the helicobacter-free health status will be maintained and adequately monitored. A percentage of newly received animals should be tested by use of PCR analysis of cecal scrapings or feces, using genus-specific primer sets. The veterinary staff should develop a working relationship with the vendor to emphasize the importance of helicobacter-free mice and to encourage rapid communication should a break in health status be detected. 'Gift mice' received from non-commercial sources can be assumed to be infected with one or more *Helicobacter* spp. until proven otherwise. The best method for maintaining a helicobacter-free barrier is to purchase only helicobacter-free mice and ensure that gift mice be re-derived into the facility via embryo transfer as standard policy. Although PCR screening is highly sensitive

and has reasonable specificity, it requires skill to perform and is subject to false-negative results. Thus, reliance on diagnostic screening of gift animals is subject to error.

Embryo transfer. Embryo transfer is the most efficient method of re-deriving mice into a helicobacter-free (as well as other bacterial, viral and parasitic agents) health status. It requires substantial investment in technical skills and requires support colonies (helicobacter-free recipient females and vasectomized males) that must be monitored closely for health status changes. For large institutions that import substantial numbers of gift mice, an embryo transfer program in a separate quarantine facility can be cost efficient and yields the greatest assurance of maintaining helicobacter-free mice.

Cross fostering. Cesarean section with fostering onto helicobacter-free dams is less ideal than embryo transfer, but has been successful (82, 224). Neonatal mice appear to acquire *Helicobacter* infections soon after birth, and thus, immediate transfer (within 24 h) of pups to clean mothers is imperative. This method is labor intensive and inherently subject to failure from inadvertent contamination. *Helicobacter hepaticus* was isolated from fetal viscera of 2 of 11 pups sampled late in gestation from infected SCID/NCr females (152), suggesting that transplacental infection of *H. hepaticus* is possible in immunodeficient mice. Cross-fostering or cesarean section to derive helicobacter-free mice should be reserved for immunocompetent strains of mice.

Antibiotic therapy. There have not been long-term, large-scale studies that have indicated success in using antibiotics to eradicate helicobacters from a mouse colony. Several oral antimicrobial formulations have been evaluated for eradication of *H. hepaticus* (67, 68). Amoxicillin- or tetracycline-based triple therapy (amoxicillin or tetracycline in combination with metronidazole and bismuth) given by oral gavage three times daily for 2 weeks eradicated *H. hepaticus* in 8- to 10-week-old A/JCr mice (67). In a second study (68), A/JCr male mice received amoxicillin-based triple therapy in drinking water or by oral gavage, or received tetracycline-based triple therapy in the drinking water. Female DBA/2J mice received amoxicillin-based triple therapy in a specially formulated dietary wafer or by oral gavage, or received enrofloxacin in drinking water. All treatments were given for a 2-week period. One month after treatment, *H. hepaticus* was recovered from the liver, cecum, or colon of mice receiving amoxicillin- or tetracycline-based triple therapy in drinking water, but not from those sites in mice receiving amoxicillin-based triple therapy by oral gavage. *Helicobacter hepaticus* was not recovered from DBA/2J mice receiving amoxicillin-based triple therapy in dietary wafers or by oral gavage but was recovered from mice receiving enrofloxacin in water. Although these results indicate that amoxicillin-based triple therapy administered in the diet or by oral gavage could be effective in eradicating *H. hepaticus*, eradication of *H. hepaticus* was not successful in a breeding colony of immunodeficient RAG1^{-/-} mice treated with amoxicillin-based triple combination in dietary wafers or in mice of various genetic backgrounds that had received medicated wafers 4 months after their manufacture. Diarrheic SCID mice infected with *H. rodentium* and *H. bilis* responded clinically to medicated wafers, but infection with both helicobacters persisted (215). Thus, immunocompetence of the mice and stability of antibiotic activity in wafers are necessary considerations (68). Amoxicillin-based therapy also was efficacious in preventing hepatitis and typhlitis in SCID mice infected

with *H. hepaticus*, but eradication data were not reported (205).

Colony management. Fecal-oral spread is the route of natural acquisition of infection with helicobacters in rodents, and *H. hepaticus* (and others) can be transferred on soiled bedding (154, 260). Thus, biocontainment necessary to prevent horizontal transmission of *H. hepaticus* within a mouse colony must include prevention of fecal-oral contact. Use of filter-topped caging, transfer of mice by use of disinfected forceps, and handling uninfected mice before known infected mice or mice of unknown *Helicobacter* status provide a barrier to horizontal transmission (259).

Use of Sentinel Mice to Monitor for *Helicobacter* spp.

Outbred Tac:(SW)fBR sentinel mice have been used to monitor colonies by use of PCR analysis and serologic testing for infection acquired from colony mice shedding *H. hepaticus*, *H. rodentium*, and *H. bilis* (260). The experimental design incorporated an ongoing colony health surveillance program that required systematic transfer of dirty bedding from cages housing colony mice to those housing sentinel mice. Screening colony mice by use of species-specific PCR analysis of cecal scrapings indicated that *H. hepaticus* and *H. rodentium* were most prevalent. Low incidence of *H. bilis* was noted. Concurrence of *Helicobacter* infection detected by use of a PCR-based assay between colony mice and sentinels exposed through dirty bedding was 82% for *H. hepaticus*, 88% for *H. rodentium*, and 94% for *H. bilis*. The anti-helicobacter serum IgG ELISA had high sensitivity, but low specificity in detecting *Helicobacter* infection as early as one month after first exposure to dirty bedding. Low sensitivity may be related to the high antigenic challenge that sentinel mice experience when exposed long term to dirty bedding from a large number of colony mice. Thus, the high sensitivity of the serum ELISA predicts *Helicobacter* infection, but the specificity of the assay was too low for reliable identification of *Helicobacter* species, necessitating the use of culture and PCR analysis for confirmation.

Transmission of *H. hepaticus* infection to sentinel C57BL/6Ncr mice was detected by use of PCR analysis as soon as 2 weeks after exposure to dirty bedding twice a week (154). By 4 weeks after exposure, all sentinels were PCR positive and by 6 weeks, all sentinels had seroconverted to *H. hepaticus*, as measured by use of an ELISA. Both of these protocols for sentinel exposure (154, 260) relied on well-defined conditions consisting of controlled amounts and frequency of dirty bedding exposure, as well as which cages of colony mice were sampled. As for other infective agents, management programs that depend on sentinel exposure to dirty bedding for reliable surveillance of the *Helicobacter* infection status of rodent colonies must standardize their methods and potentially validate them using controlled studies. Increased exposure to dirty bedding or testing an increased number of sentinel mice per time point may improve the sensitivity of surveillance programs that depend on similar protocols.

Helicobacter Infections in Non-Rodent Species

Humans. *Helicobacter pylori* was first identified in 1982 as an infectious cause of chronic active gastritis and peptic ulcer. *H. pylori* has been recognized as one of the most common human infectious diseases, infecting 50% or more humans worldwide (see 13 for review). *Helicobacter pylori* was classified by the World Health Organization as a type-1 gastric carcinogen because of its

association with gastric adenocarcinoma and MALT lymphoma in humans (128). Most *H. pylori*-infected humans develop only subclinical gastritis, but relevant disease with the risk of gastric cancer has an important impact on human health. An enormous amount of investigation has examined the epidemiology, treatment, prevention, and pathogenesis of this important bacterial infection. The literature base covering various aspects of *H. pylori* and associated disease syndromes has grown exponentially and is beyond the scope of this review. Many of the features of *H. pylori* infection of humans are presented here in the context of using animal models to study *H. pylori* gastritis and gastric cancer.

Far less is known about the enterohepatic helicobacters of humans. *Helicobacter winthamensis* was isolated from patients with gastroenteritis, including fever, stomach malaise, and diarrhea (166). It is catalase, urease, alkaline phosphatase, and nitrate negative, but oxidase and indoxyl acetate positive. It grows at 37°C but not at 42°C, and three isolates from two patients were susceptible to nalidixic acid and cephalothin. Sequence analysis of 16S rRNA indicates that the closest relative by phylogenetic analysis is '*Helicobacter* sp. *flexispira*' taxon 1. *Helicobacter winthamensis* has one or two bipolar nonsheathed flagella; however, periplasmic fibers, a characteristic of the known '*Helicobacter* sp. *flexispira*' taxa, are absent. Other human isolates, such as *H. cinaedi*, *H. fennelliae*, *H. canis*, *H. pullorum*, *H. canadensis*, and *H. bilis* will be discussed in other sections.

Natural *Helicobacter* Infections in Nonhuman Primates

Helicobacter pylori. Gastric and large intestinal *Helicobacter* infections have been documented in Old and New World nonhuman primates. To our knowledge, an association between clinical signs of disease and infection of nonhuman primates has not been reported. Nonhuman primates commonly have subclinical but histologic evidence of chronic gastritis and are often coinfecting with *H. pylori* and other large spirals in the stomach such as '*H. heilmannii*' (158). Gastric body and antral biopsy specimens from 23 clinically normal young adult Chinese rhesus macaques were evaluated for *H. pylori* infection by use of PCR analysis, culture, urease testing, histologic evaluation, and serologic testing (119). Results of PCR analysis were positive for 91% of the animals, 52% were culture positive, urease tests were positive in all animals, and *H. pylori* was evident histologically in 48% of the macaques. An ELISA using homologous rhesus *H. pylori* antigen and anti-monkey conjugate was superior in sensitivity (90% of PCR-positive animals) to a commercial ELISA marketed for humans.

Because *H. pylori* is typically acquired by humans early in life, natural acquisition of *H. pylori* infection has been examined in socially housed, newborn rhesus macaques (229). By 3 months of age, 8 of 20 newborns were culture positive for *H. pylori*, with higher recovery of *H. pylori* from newborns from infected dams than from uninfected dams. By 1 year of age, prevalence reached 90%.

Helicobacter cinaedi. This helicobacter was implicated as a cause of enteritis and bacteremia in immunocompromised humans (1), but it also infects children and occasionally immunocompetent adults. *Helicobacter cinaedi* was recently isolated from the colon, liver, and mesenteric lymph nodes of a 2-year-old rhesus macaque with hepatitis and chronic diarrhea (83). Mild to moderate biliary hyperplasia and hypertrophy with periportal inflammation and fibrosis were observed in the liver, and diffuse

chronic typhlocolitis and glandular hyperplasia were noted in the large intestine. *Helicobacter cinaedi* also has been recovered from feces of clinically normal rhesus macaques (5/16), indicating that these captive animals may be a reservoir for human infection with *H. cinaedi* (63) or conversely, like *H. pylori*, they may have acquired the organism in the wild from humans. Unfortunately, colonic biopsy specimens were not taken to ascertain whether colitis was present.

Novel *Helicobacter* spp. Novel *Helicobacter* spp. have been isolated from captive macaques with chronic, moderate to severe typhlocolitis, reactive lymphoid hyperplasia, and multifocal microabscesses (84). Results of microbiological examination for *Salmonella* spp., *Shigella* spp., *Clostridium difficile* A and B toxins, acid-fast bacteria, herpes B virus, simian T-lymphotrophic virus, simian retrovirus, and simian immunodeficiency virus were negative. The helicobacters were recovered by culture of colonic biopsy specimens from 6 clinically affected and 2 clinically normal monkeys. Nine strains were characterized biochemically, and two separate biotypes were identified by use of 16S rRNA analysis. Type-1 strains from animals with subclinical colitis and type-2 strains from animals with diarrhea and colitis differed in catalase activity, ability to reduce nitrate to nitrite, and susceptibility to cephalothin. Complete 16S rRNA analysis of 5 strains indicated that the organisms were newly recognized. Controlled studies are needed to determine whether *Helicobacter* spp. contribute to chronic colitis in macaques. Further microbiological and histologic analyses of clinical cases may prove useful in understanding human IBD.

Cotton-top tamarins (CTTs) can develop clinical signs of disease that include weight loss, chronic diarrhea, and rectal bleeding, with lesions similar to those of human ulcerative colitis. From 25 to 40% of affected animals develop colon cancer within 2 to 5 years. A novel helicobacter with fusiform shape, bipolar flagella, and periplasmic fibers was isolated from feces collected from a colony of CTTs with episodic colitis and colon cancer (207). It grew under microaerobic conditions at 37 and 42°C, was urease negative, and was positive for catalase and oxidase. On the basis of other biochemical testing, antibiotic susceptibilities, and 16S rRNA gene sequence analysis, the organism was classified as a novel helicobacter. The cause of the syndrome in CTTs is unknown, although epidemiologic studies in CTTs incriminate an infectious cause (136). Identification of a novel helicobacter in affected animals warrants further study to determine its potential relationship with chronic colitis and progression to adenocarcinoma.

Experimental *Helicobacter* Infection of Nonhuman Primates

***Helicobacter pylori*.** Studies of *H. pylori* infections in nonhuman primates have been valuable because of the close phylogenetic relationship of macaques to humans, despite the disadvantages of natural infection with *H. pylori* and/or '*H. heilmannii*' (49, 50). Strains of *H. pylori* in humans as well as in rhesus macaques are heterogeneous and are believed to arise from complex adaptive responses of *H. pylori* to its host environment. This was addressed in a study of rhesus macaques first treated to eliminate natural *H. pylori* colonization, then challenged with a mixture of 7 human strains (48). The DNA fingerprinting analysis of isolates recovered from serial samples indicated that multiple strains would co-colonize the stomach for months before one or more strains would predominate. Prior

natural colonization was not protective against *H. pylori* challenge and subsequent infection.

Microarray analysis of *H. pylori* DNA recovered from experimentally challenged macaques indicated that *babA*, one of a family of outer membrane proteins that mediates attachment of *H. pylori* to the Lewis B blood group antigen on gastric epithelium, was deleted and, in some cases, replaced by *babB*, an uncharacterized but related protein (230). In some isolates, the *babA* gene was present but was not expressed because of phase variation in nucleotide repeats in the 5'-coding region. Strains lacking *babA* did not adhere to Lewis B, which is expressed on macaque gastric epithelium. Absence of *babA* and duplication of *babB* were also seen in *H. pylori* isolates derived from human clinical samples, suggesting that this gene conversion event is not unique to experimentally infected rhesus monkeys. These results help support the hypothesis that *H. pylori* regulates outer membrane protein expression in vivo by using antigenic variation and phase variation, potentially to facilitate adherence of *H. pylori* to the gastric epithelium and promote colonization.

Rhesus macaques have also been used in therapeutic vaccine trials. Immunization with recombinant *H. pylori* urease decreased colonization following experimental infection of rhesus macaques, and was correlated with reduced gastritis (150).

***Helicobacter cinaedi* and *H. fennelliae*.** The latter helicobacter has no known animal reservoir, but can be used to experimentally infect nonhuman primates. Experimental challenge of 12- to 25-day-old pig-tailed macaques (*Macaca nemestrina*) with *H. cinaedi* or *H. fennelliae* resulted in watery or loose feces without associated fever or fecal leukocytes within 3 to 7 days after inoculation. Results of culture of fecal specimens remained positive for three weeks after inoculation despite resolution of the clinical illness. All animals became bacteremic, and some had clinical signs of septicemia. Acute inflammation was observed histologically in 1 of 5 rectal biopsy specimens, and hyperplasia of Peyer's patches was noted in one additional animal that was necropsied (64).

Natural *Helicobacter* Infections in Ferrets

***Helicobacter mustelae*.** *Helicobacter mustelae* was the first non-human gastric *Helicobacter* isolate, and shares many biochemical, molecular, and phenotypic characteristics with *H. pylori* (75). It is a gram-negative, curved- to rod-shaped, microaerophilic bacterium that, like *H. pylori*, adheres to the gastric mucosa and is strongly oxidase, catalase, and urease positive. The prevalence of *H. mustelae* infection in adult domestic ferrets (*Mustela putorius furo*) approaches 100%, and the organism has been isolated from mink (241). Similar to that of *H. pylori* infection in humans, persistent colonization of *H. mustelae* is acquired at an early age and is closely associated with development of chronic active gastritis, mucosal ulceration, and experimentally, with promotion of carcinogenesis (96). The ferret is the only domestic animal with *Helicobacter*-induced gastritis associated with duodenal and gastric ulceration, all sequelae of human *H. pylori* infection. Both *H. pylori* and *H. mustelae* produce urease and increase blood gastrin concentration, which alters mucosal pH and increases gastric acid production (189). The immune response of ferrets to *H. mustelae* and of humans to *H. pylori* is incapable of eliminating active infection or preventing reinfection after antibiotic therapy.

Gastric lymphoma resembling MALT lymphoma of humans

infected with *H. pylori* was reported in four ferrets infected with *H. mustelae* (58). Two ferrets developed low-grade, small-cell lymphoma, and two ferrets developed high-grade, large-cell lymphoma at the lesser curvature of the pyloric antrum, which is the site of the most intense *H. mustelae*-induced gastritis. Premalignant gastric lesions associated with neoplasia were described by Fox and co-workers (75) soon after the discovery of *H. mustelae* as the cause of chronic gastritis in ferrets. *Helicobacter mustelae*-induced gastritis results in glandular atrophy of the proximal portion of the antrum, which is associated with progression to gastric adenocarcinoma in ferrets (76) and humans infected with *H. pylori* (27). Thus, the *H. mustelae*-infected ferret can be used to model the progression of *Helicobacter*-associated chronic gastritis to the potential sequela of gastric cancer.

Novel *Helicobacter* spp. Hepatobiliary disease potentially related to *Helicobacter* infection was diagnosed in a single colony of pet ferrets (104). Eight of 34 ferrets, 5 to 8 years old, and sampled over multiple years, developed signs of chronic cholangiohepatitis. There was histologic evidence of hepatocellular hyperplasia progressing to carcinoma in two ferrets. Bacteria with morphology consistent with that of helicobacters were observed histologically in the liver of three ferrets, including the two with carcinoma. Sequence analysis of a 400-bp PCR product derived from fecal bacteria from one of the ferrets revealed 98 and 97% similarity to *H. cholecystus* and a novel strain, *Helicobacter* sp. 266-1 (90), respectively. The prevalence of hepatic disease in this single ferret colony suggests an infectious cause; thus, the role of *Helicobacter* spp. in ferret hepatitis and cancer warrants further study. Novel helicobacters have also been isolated from feces of wild mustelids, California sea otters.

Experimental *Helicobacter*-Associated Gastritis in Ferrets

Experimental infection of the ferret with *H. mustelae* is a model of *H. pylori* infection in humans (75, 89). *Helicobacter mustelae* infection in ferrets has been used to model potential virulence factors that influence colonization of the stomach and the development of gastritis, ulcers, and gastric cancer. Similar to *H. pylori* in humans, *H. mustelae* adheres to ferret gastric tissue via the formation of adhesion pedestals (179). *Helicobacter mustelae* produces unique proteinacious surface ring structures that were investigated for their importance to gastric colonization. *Helicobacter mustelae*-free ferrets were challenged with wild-type or surface ring isogenic mutants of *H. mustelae*, then were evaluated after 18 weeks. Colonization and gastritis in ferrets challenged with mutants was minimal compared with colonization and more robust gastritis in animals infected with wild-type *H. mustelae*. This finding suggests that the novel surface proteins are important for *H. mustelae* colonization of the ferret stomach (183). As with *H. pylori*, flagellar motility of *H. mustelae* is an important virulence factor for colonization and pathogenesis of gastritis in ferrets. Challenge of *H. mustelae*-free ferrets with single or double isogenic mutant strains of the FlaA and FlaB flagellin proteins resulted in no colonization in double knockouts and low colonization in single mutants (2). Colonization with isogenic mutants of flagellar proteins slowly increased over three months, and the degree of gastritis was correlated with the colonization burden of *H. mustelae*. Isogenic flagellum-negative mutants of *H. mustelae* were assessed for their ability to adhere to primary ferret gastric epithelial cells. Compared with

wild-type strains, mutants deficient in production of FlaA, FlaB, or both flagellar proteins did not manifest reduction in adherence to primary ferret gastric epithelial cells (25). These findings suggest that flagella do not play a direct role in promoting adherence of *H. mustelae* to gastric epithelial cells, but genes regulating flagellar biosynthesis may also regulate production of an adhesin.

Helicobacter pylori and *H. mustelae* may partially evade the host immune response through expression of Lewis blood group antigens that mimic those of the host, a mechanism known as molecular mimicry. Cross-reactive antibodies to epitopes expressed by gastric helicobacters and host gastric mucosa may contribute to development of chronic atrophic gastritis through destruction of parietal cells. *Helicobacter mustelae* expresses a blood group-like antigen A as part of its lipopolysaccharide; cross-reactivity of serum from *H. mustelae*-infected ferrets with ferret gastric tissue, but not with duodenal or colonic mucosa, has been documented (32, 33). Absorption of the antiserum with ferret and rabbit gastric tissue removed cross-reactive antibodies (33), but pre-absorption with whole *H. mustelae* cells or ferret erythrocytes failed to reduce antibody binding to ferret gastric mucosa (32). Thus, ferrets naturally infected with *H. mustelae* generate antibodies that react with gastric mucosa, but these antibodies do not appear to be generated through a mechanism involving molecular mimicry (32).

Urease activity was observed to be essential for colonization of the ferret stomach by *H. mustelae* (3). Ferrets that were specific pathogen free (SPF) for *H. mustelae* were orally dosed with wild-type *H. mustelae* or an isogenic urease-negative strain of *H. mustelae*, then were evaluated for 25 weeks. In those dosed with the urease mutant, *H. mustelae* failed to colonize and the animals did not develop gastritis. The urease from *H. pylori* has been used as an antigen combined with the mucosal adjuvant, cholera toxin, to therapeutically immunize ferrets naturally infected with *H. mustelae* (34). By six weeks after oral immunization, *H. mustelae* was eradicated in 30% of all immunized ferrets and gastritis also was reduced.

The *H. mustelae* ferret model has been used to evaluate antibiotic therapies for eradication of gastric helicobacters (5, 161, 180). Triple therapy consisting of amoxicillin (30 mg/kg of body weight), metronidazole (20 mg/kg), and bismuth subsalicylate (17.5 mg/kg) administered orally twice daily for 21 to 28 days cleared *H. mustelae* in 71% (180) and 100% (5) of treated ferrets, respectively.

Ranitidine bismuth citrate, clarithromycin, or a combination of both drugs was administered orally for 14 days to 7-month-old ferrets with *H. mustelae* infection that was documented by gastric biopsy and culture (161). Ferrets were euthanized from 4 to 43 weeks after therapy. Dosages of clarithromycin and ranitidine bismuth citrate that suppressed growth of *H. mustelae* were 12.5 and 24 mg/kg, respectively, given orally every 8 h. Infection was not eradicated in ferrets treated with ranitidine bismuth citrate alone, but was eradicated in all 6 ferrets treated with clarithromycin and ranitidine bismuth citrate and in 4 of 6 treated with clarithromycin alone. A decrease in susceptibility to clarithromycin was detected for *H. mustelae* isolates obtained after treatment. Mild or moderate antral gastritis was observed even in ferrets from which infection was eradicated.

Ferret kits acquire *H. mustelae* from their jill near the time of weaning via fecal-oral exposure (90). Therapeutic vaccination of young ferret kits was attempted to determine whether this natu-

ral route of transmission could be interrupted (263). Weanling kits were orally dosed with a whole-cell sonicate of *H. mustelae* and the adjuvant, muramyl dipeptide. All kits became colonized naturally with *H. mustelae*, and most developed mild to severe gastritis and duodenitis. Unexpectedly, kits that received sonicate and adjuvant developed significantly greater gastritis and duodenitis that led to mucosal ulceration in some animals. In retrospect, the mucosal damage associated with this vaccination regimen potentially enhances the value of the ferret model for studying duodenal ulceration secondary to gastric *Helicobacter* infection.

Natural *Helicobacter* Infections in Cats and Dogs

Helicobacter felis, '*H. heilmannii*' (*bizzoeronii*), '*H. rappini*.' Because many of the helicobacters in the stomach of animals have been isolated only recently, many earlier reports describing these bacteria were based on morphologic criteria. Three morphologic forms of these organisms were reported by Lockard and Boler in dogs (Fig. 6) (157). All three of these morphologically distinct organisms are now known to be helicobacters on the basis of results of 16S rRNA analysis. Lockard type 1 is a bacterium fully entwined with periplasmic fibers and is now known as '*H. rappini*,' a member of the *Flexispira* group also isolated from aborted sheep fetuses (14) and from intestines of normal mice (208). '*Helicobacter rappini*' has been isolated from feces of normal dogs and their owners affected by enteritis (202) and from a bacteremic human (232). Lockard bacterium type 2 also has periplasmic fibers, but they are more sparsely distributed on the organism and can appear singly or in groups of two, three, or four. This organism has been isolated in culture from the stomach of cats and dogs, and has been named *H. felis* (182). The third morphologically distinct organism, type 3, is the bacteria most commonly seen in the animal stomach (dogs, cats, non-human primates, cheetahs, swine) and occasionally in the human stomach. This bacterium, though tightly spiraled, does not have periplasmic fibers and has been given various names—'*Gastrospirillum hominis*' or '*H. heilmannii*'—and most recently, has been cultured from dogs and has been named *H. bizzoeronii* (120). This bacterium measures 0.3 by 5 to 10 μm , and has 10 to 20 sheathed flagella at both ends of the cell.

***Helicobacter pylori*.** Although spiral bacteria were first observed to infect the stomach of dogs and cats more than 100 years ago, there has been renewed interest stimulated by the discovery of *H. pylori* in humans as well as the report of endemic *H. pylori* in one commercial colony of cats bred for research (118). Similar to human *H. pylori* infection, chronic *H. pylori* infection in cats results in chronic diffuse lymphofollicular atrophic gastritis, with areas of mucosal dysplasia in the antrum and predominantly superficial gastritis in the body and cardia (61). Colonization patterns of *H. pylori* in the naturally infected cat, and the cytokine response, such as up-regulation of IL-8 and IL-1 β , are similar to those observations in infected people and nonhuman primates (222). Natural infection with *H. pylori* has not been observed in dogs, except for one recent report of *H. pylori* DNA (notably not recovered by culture) in biopsy specimens from four pet dogs (17). Polymerase chain reaction amplification of 16S rDNA yielded a 400-bp product that was subsequently cloned into *E. coli*, then was re-amplified by use of PCR analysis, and 16S rDNA fragments were analyzed by use of RFLP analysis. Two sequences

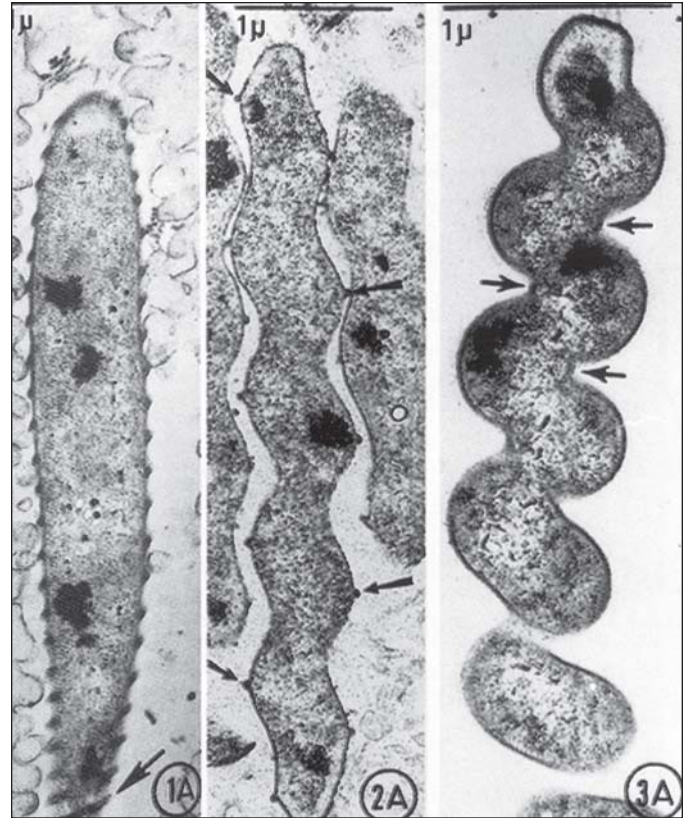


Figure 6. Collage of the original photomicrographs of bacteria in the dog stomach, as seen by electron microscopy, taken from Lockard and Boler (157). Type 1A is '*H. rappini*'-like, Type 2A is *H. felis*-like, and Type 3A is '*H. heilmannii*'-like. Arrows indicate periplasmic fibers in 1A and 2A, and suspected fibers in 3A. Reprinted with permission of *Comparative Medicine*.

obtained from different dogs were identical with the corresponding sequences of *H. pylori* strains. Three sequences had moderate similarity to *H. pylori* (96.6 to 98.0%), and one sequence was similar to *H. salomonis* (97.3%).

***Helicobacter felis*.** This helicobacter was first isolated from cats, and is a large spiral organism with periplasmic fibers (Fig. 7) (182). Infection of domestic cats and dogs with gastric helicobacters other than *H. pylori* are not well characterized. In cats, *H. felis* induces lymphoid follicular hyperplasia, mild gastritis, and seroconversion, but is associated with normal gastric secretory function (221). *Helicobacter felis* has been important to development of *H. pylori* gastritis models, because it persistently colonizes mice and causes robust gastritis that recently was reported to progress to gastric cancer (253). The gastric mucosa of healthy pet cats is commonly colonized with an (until recently) uncultivable '*H. heilmannii*' (a.k.a. *H. bizzoeronii*) that induces moderate or mild gastritis, and has heterogeneity in its 16S rRNA sequence (176). To our knowledge, the relationship between the associated gastritis (inflammation, atrophy of glands, lymphofollicular hyperplasia) and common clinical syndromes in pets (vomiting, anorexia) has not been established (175). The prevalence of gastric *Helicobacter* spp. in dogs and cats is high, irrespective of clinical signs of disease, and, similar to that in humans, mode of transmission is unclear. Urea breath testing and serologic plus urease testing, histologic and cytologic examinations, PCR analysis, and culture of biopsy specimens are useful

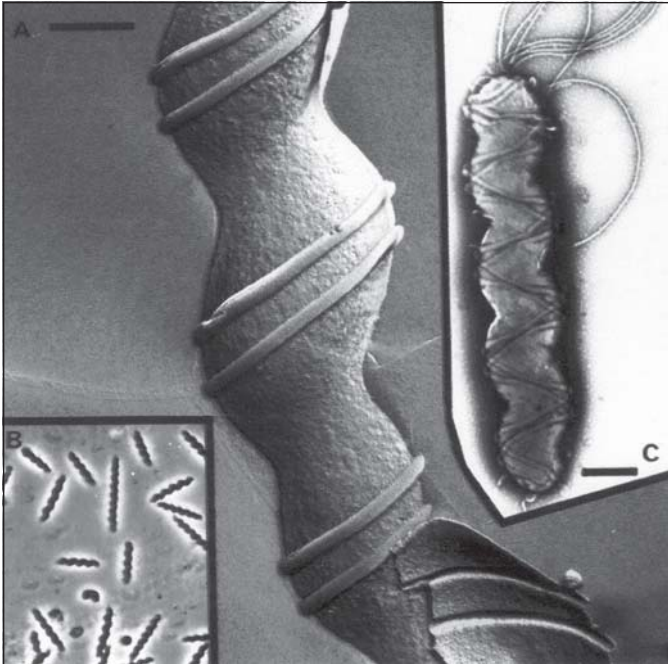


Figure 7. Morphology of *H. felis*, as imaged by electron microscopy (A and C) and phase contrast microscopy (B), demonstrating spiral morphology and periplasmic fibers. Reproduced with permission from Dr. Adrian Lee. Bar = 0.2 μ m.

diagnostically. Therapeutic trials in dogs and cats have not been successful in avoiding recrudescence or reinfection. Gastrin secretion appears unaffected by *Helicobacter* infection (222), and a relationship of infection to gastrointestinal tract ulcers has not been reported.

Miscellaneous helicobacters of cats and dogs. Isolated case reports associating helicobacters with gastroenteritis in young cats and dogs have appeared in the literature. Both *H. cinaedi* and '*H. rappini*,' along with *C. upsaliensis* and two *Anaerobiospirillum* spp., were isolated from a puppy with bloody diarrhea (170). Treatment with amoxicillin was successful, suggesting a bacterial cause for the diarrhea. A spiral-shaped bacilli that ultrastructurally resembled '*H. rappini*' was found to heavily colonize the stomach of a kitten with mucohemorrhagic enteritis; it also was found in the small intestine and cecum of the kitten (140). '*Helicobacter rappini*' is a spiral-shaped helicobacter known to colonize the intestine of dogs and mice. The name is provisional and is given to a taxonomic grouping of gram-negative, microaerophilic, motile, spindle-shaped microorganisms with spiral periplasmic fibers and bipolar tufts of sheathed flagella. Sequencing of 16S rRNA has indicated that this former *Flexispira* is a member of the genus *Helicobacter*, but has not been formally named, probably because '*H. rappini*' strains represent at least 10 *Helicobacter* taxa (40).

Helicobacter canis has been isolated from humans (70) and exotic Bengal cats (65) with diarrhea and from a puppy with signs of gastroenteritis in combination with histologic evidence of Warthin-Starry-positive, spiral to curved bacteria observed in a lesion of necrotizing hepatitis (81). Thus, *H. canis* is associated with inflammation of the colon and potentially the liver of select hosts, particularly those individuals with immune dysregulation. Further studies are needed to determine the importance of *H. canis* as a primary enterohepatic pathogen in dogs and cats and

the possible zoonotic threat to humans.

Helicobacter acinonychis (formerly *H. acinonyx*), a species closely related to *H. pylori*, was isolated from the stomach of cheetahs (52) and from two Sumatran tigers with chronic gastritis (209). *Helicobacter acinonychis* was thought to contribute to an outbreak of vomiting in a group of captive cheetahs (55). Histologic examination revealed chronic gastritis characterized by infiltration of lymphocytes and numerous plasma cells, epithelial erosions, and mucosal hyperplasia. Two groups of *H. acinonychis* strains were identified by use of randomly amplified polymorphic DNA fingerprinting analysis and gene sequencing: one group from cheetahs in a US zoo and two lions in a European circus, and the other group from a tiger and a lion-tiger hybrid in the same circus. Results of PCR analysis and DNA sequencing indicated that each strain lacked the *cag* pathogenicity island and contained a degenerate vacuolating cytotoxin (*vacA*) gene. Mice were dosed with *H. acinonychis* and *H. pylori*, and they became persistently colonized; several variant strains causing a mixed infection were recovered after 2 months (35). The ability of *H. acinonychis* to recombine with *H. pylori* in vivo will allow further studies of virulence mechanisms and genome evolution.

During a survey study of gastric helicobacters in dogs, *H. salomonis* sp. nov., a canine gastric isolate related to *H. felis* and *H. bizzozeronii* was identified (132). *Helicobacter salomonis* was distinguished from *H. felis* and *H. bizzozeronii* by its morphology (slight spiral, 5 to 7 μ m long by 0.8 to 1.2 μ m wide) and unusual slow wavelike motility. Phylogenetic analysis by use of 16S rDNA sequence comparison revealed *H. salomonis* to be a distinct *Helicobacter* species. Reports of associated clinical disease have not been published.

Novel *Helicobacter* spp. Cats have been colonized with mixed infections of enteric *Helicobacter* and *Campylobacter* spp. (211). Feces from commercially bred cats contained isolates identified as *Campylobacter*-like organisms on the basis of biochemical and phenotypic characteristics. Use of PCR analysis with primers specific for *Helicobacter* and *Campylobacter* spp. at the genus level identified mixed infections. Use of species-specific primers identified coinfection with *C. helveticus*, *C. upsaliensis*, and *C. jejuni*. Five RFLP patterns were obtained by digestion of the 1.2-kb PCR products of the 16S rRNA isolated from 19 *Helicobacter* isolates. The RFLP and sequence analyses verified that two isolates clustered with '*Flexispira*' taxon 8, one clustered with *H. bilis*, one with *H. canis*, and the remaining appeared closely related to a novel helicobacter, *H. marmoatae*, isolated from a woodchuck (93). These results illustrate the potential uncertainty in identifying isolates solely on the basis of chemical and phenotypic characteristics. The clinical relevance of enteric *Helicobacter* and *Campylobacter* coinfection in cats is unknown. Importantly, there is the potential for zoonotic transmission. Systematic evaluation of dogs for enterohepatic helicobacters has not been reported to our knowledge.

Experimental *H. pylori* and *H. felis* Infection of Cats and Dogs

Similar to that in dogs, experimental infection of the cat with *H. pylori* results in more severe pathologic changes than does infection with *H. felis*. Cats free of known gastric helicobacters, including *H. pylori*, with normal gastric mucosae were susceptible to colonization by the same *H. pylori* strain, which was negative for the *cagPAI* (cytotoxin gene-associated pathogenicity island),

that was isolated from naturally infected cats from a commercial vendor (72, 118). All cats were persistently colonized over a study period of 7 months and developed multifocal gastritis consisting of lymphoid aggregates plus multiple large lymphoid nodules that were most noticeable in the antrum. Organisms with morphology consistent with that of *H. pylori* were distributed in glandular crypts of the antrum, and two of four cats had high IgG titer to *H. pylori*. The *H. pylori* recovered at the end of the study was confirmed to be the same as the inoculating strain by use of RFLP analysis specific for the *flaA* gene (72). Similar results were obtained after experimental infection of cats with a human cagA+ strain of *H. pylori* (188). Results of experimental studies have indicated that *H. pylori* can be recovered from salivary secretions, gastric juice, and gastric tissue by use of culturing, and the organism has been detected in dental plaque and feces of infected cats by use of PCR analysis (91). Gnotobiotic beagle pups were orally challenged with *H. pylori* at 7 days of age and were persistently colonized for 30 days, but at a lower density than that typically seen in humans. Small (< 1 mm) lymphoid follicles were grossly visible in the stomach, and histologic lesions consisted of focal to diffuse lymphoplasmacytic infiltrates, with follicle formation and mild to moderate infiltration of neutrophils and eosinophils in the gastric lamina propria. A second group of dogs with exposure contact only also became colonized with the *H. pylori* during a 30-day study period (195).

Natural *Helicobacter* Infections in Swine

'*Helicobacter suis*.' Domestic swine were described as a primary host of '*H. heilmannii*' (203), as are cats, dogs (176), and rarely, humans (240). '*Helicobacter heilmannii*' in pigs was renamed '*H. suis*' on the basis of analysis of samples obtained from pig stomach, using 16S rRNA sequencing, results of which identified the isolates as a novel species (23, 38). The potential zoonotic connection between gastric helicobacters of pigs and farmers was highlighted by the observation of high prevalence in humans of '*H. heilmannii*'-associated gastritis in a small, predominantly rural area (240). The pig stomach is also colonized with *Campylobacter* spp., which has complicated the interpretation of studies. Characterization of large gastric spirals that appear to infect most slaughter pigs has been hampered by the inability to successfully recover these organisms by use of culturing (169). Thus genus- and species-specific PCR assays in combination with RFLP and sequence analyses are the most sensitive and reliable diagnostic methods for use in swine studies (121, 203).

'*Helicobacter suis*.' has been linked to a clinical condition in farm pigs, ulceration of the pars esophagea (194), which historically has been attributed to a complex interaction of dietary particle size, gastric fluidity, dietary carbohydrate content, and presence of certain species of commensal gastric organisms capable of fermenting dietary carbohydrates. Unlike that in humans, the relevance of the role of helicobacters in development of porcine gastric ulcers is undefined. The causal relationship between '*H. suis*' and gastritis or ulceration in the pig remains to be clarified. This organism colonizes mainly in the antral and body mucosa, whereas ulceration develops in the esophagus-cardia junction.

***Helicobacter pametensis*.** This helicobacter has been isolated from swine feces, but birds are thought to be the primary host.

'*Helicobacter* sp. *flexispira*' taxa. Potentially, 10 distinct members of the '*Flexispira rappini*' taxa have been described, us-

ing 16S rRNA sequencing (40), and include fusiform-shaped bacteria with helical periplasmic fibrils and bipolar tufts of sheathed flagella. Three '*Flexispira rappini*' members that have been formally renamed as *Helicobacter* spp. include *H. bilis*, *H. trogontum*, and *H. aurati*. Evaluation of gastric organisms from Finnish farm pigs that were characterized by use of phenotypic, biochemical, and molecular techniques revealed close homology to *H. flexispira* taxa 4 and 5 and *H. trogontum* (taxon 6), but a less-close relationship to other members, including taxa 1-3 and 8, *H. bilis*, and *H. aurati* (121).

Experimental Infection of Swine with *Helicobacter pylori*

Germ-free and gnotobiotic pigs (54), SPF farm pigs (192), and miniature pigs (141) have been used to study the pathogenesis of *H. pylori*-induced gastritis. Gnotobiotic piglets have been used to document that the humoral response to *H. pylori* is initiated within the gastric compartment and matures over time to an IgA-dominated mucosal and a systemic IgG-dominated humoral immune response (142). Germ-free neonatal piglets infected with piglet-adapted *H. pylori* strain 26695 were used to document that neither the cagPAI nor the ability to induce IL-8 in vitro is essential for colonization or neutrophilic inflammation in vivo (54).

Natural *Helicobacter* Infections in Sheep

'*Helicobacter rappini*.' Vertical transmission of a helicobacter was first proposed when '*H. rappini*' was suspected to have crossed the placenta and caused hepatic necrosis in fetuses during natural infection in presumably immunocompetent sheep. The same isolate given as an experimental challenge to pregnant guinea pigs resulted in liver necrosis in the fetuses (14). '*Helicobacter rappini*' was reported to cause recurrent bacteremia in an adult human dialysis patient, which suggests that '*H. rappini*' is a pathogen in immunocompromised patients (232). The potential for zoonotic transmission of '*H. rappini*' between humans and dogs has been reviewed (71).

***Helicobacter pylori*.** A higher incidence of *H. pylori* infection in a group of sheep herders, compared with that in the neighboring population, suggested a zoonotic connection with sheep (or at least the occupation), but this remains unresolved (181).

Natural *Helicobacter* Infections in Cattle

'*Helicobacter bovis*.' '*Helicobacter bovis*' is the proposed name for a helicobacter detected in the abomasum of cattle in Europe (39). Although culture of '*H. bovis*' has not been successful, helicobacter-like organisms were detected in abomasal biopsy specimens from adult cattle by use of electron microscopy and biochemical and immunohistochemical methods. Bacterial 16S rDNA was amplified by use of PCR analysis and was sequenced. Phylogenetic analysis placed the organism, corresponding to the reference sequence R2XA, within the genus *Helicobacter*. A diagnostic PCR assay was designed that differentiated all of the bovine 16S rDNA sequences from *Helicobacter* and *Wolinella* species. *Helicobacter bilis* appears to be the most related species, but sequence similarity is low (92.8%), indicating that '*H. bovis*' is a novel *Helicobacter* species. In a separate survey of slaughter cattle, '*H. bovis*' was detected in 85% of the animals (114). In the same survey, the abomasum of goats was tested and the results indicated failure to detect *Helicobacter* infection.

Natural *Helicobacter* Infections in Birds

***Helicobacter pullorum*.** This helicobacter was isolated from the liver, duodenum, and cecum of chickens and from humans with gastroenteritis (233). Supported by relative DNA homology and similar protein electrophoretic patterns, 16S rRNA sequence analysis identified 7 strains as belonging to a single *Helicobacter* species. *Helicobacter pullorum* is biochemically differentiated from other helicobacters, and has typical ultrastructure, except for a non-sheathed flagellum, as seen by use of electron microscopy. Similar to *H. fennelliae* in immunocompromised humans or *H. cinaedi* in a range of different hosts, *H. pullorum* represents a non-gastric, urease-negative *Helicobacter* species that colonizes the large intestine. Because *H. pullorum* has been isolated in culture from feces of diarrheic humans and has been isolated from poultry and poultry products, it may cause an associated zoonosis (12). A species-specific PCR assay for *H. pullorum* is available and will further facilitate identification of the helicobacter and clarification of its epidemiology. A putative virulence factor in *H. pullorum* is CDT (265). Isolates of *H. pullorum* from birds and humans have produced CDT activity in cell culture and are PCR positive for the CDT genes.

***Helicobacter canadensis*.** This helicobacter has been implicated in a human bacteremic patient and was cultured from a series of 11 patients with diarrhea in Canada (74, 243). *Helicobacter canadensis* was previously characterized as *H. pullorum* by determination of its biochemical profile and by use of fatty acid and RFLP analyses. In a reassessment of this classification (74), four isolates differed biochemically from *H. pullorum* by their inability to hydrolyze indoxyl acetate and their resistance to nalidixic acid. Using complete 16S rRNA and RFLP analyses, these four strains clustered near *H. pullorum*, but had a sequence difference of 2%, thereby representing a novel helicobacter renamed as *H. canadensis*. Other investigators re-examined the taxonomic position of *Campylobacter*-like isolates recovered from barnacle geese (*Branta leucopsis*) and Canada geese (*Branta canadensis*) (251). From a collection of 21 isolates, 7 strains were analyzed by use of extensive phenotypic testing, and four strains were characterized by use of 16S rRNA sequence analysis. The results identified the bird isolates as *H. canadensis*; thus, geese are an animal reservoir for this potentially zoonotic helicobacter.

***Helicobacter pametensis*.** An organism in fecal isolates from wild birds and domestic swine was identified as a novel helicobacter with the suggested name of *H. pametensis*. This helicobacter was differentiated from other known *Helicobacter* species by use of full 16S rRNA sequencing and on the basis of unique biochemical and morphologic features (42). The potential for *H. pametensis* to cause disease is unknown. Two additional putative *Helicobacter* species from birds were phenotypically similar to *H. pametensis*, but had a different biochemical profile, including being positive for urease and hydrolysis of indoxyl acetate and resistant to cephalothin. Because only a limited number of isolates were characterized, they were not formally named, but are referred to as *Helicobacter* sp. 'Bird-B' and *Helicobacter* sp. 'Bird-C.'

Natural *Helicobacter* Infections in Woodchucks

***Helicobacter marmotae*.** The association between woodchuck hepatitis virus (WHV) infection of *Marmota monax* and



Figure 8. Morphology, as imaged by electron microscopy, of *H. marmotae* isolated from woodchucks and cats. *H. marmotae* measures approximately 0.2 by 1.5-5 μm .

the high incidence of hepatocellular carcinoma in woodchucks is a model for hepatitis B virus-associated hepatocellular carcinoma of humans. Liver specimens from WHV-infected woodchucks, 50% of which had viral-associated hepatic tumors, and liver specimens from uninfected woodchucks without tumors, were screened for *Helicobacter* infection by use of culture and PCR analysis with *Helicobacter* genus- and species-specific primers (93). Under microaerobic culture, one liver specimen from 10 tumor-positive woodchucks yielded a urease-, catalase-, and oxidase-positive bacterium typical of an enterohepatic helicobacter (Fig. 8). Nine of 10 tumor-bearing liver specimens and 5 of 10 tumor-free specimens were PCR positive, yielding an expected 1,200-bp product, which was further verified to represent a novel *Helicobacter* sp. by use of Southern blot hybridization and sequence analysis. Using 16S rRNA species-specific primers designed for the novel isolate, two additional liver specimens from the nontumor group had PCR-positive amplicons confirmed by use of Southern blot hybridization. By use of 16S rRNA analysis and on the basis of biochemical and phenotypic characteristics, the organism was classified as a novel *Helicobacter* sp., and was named *H. marmotae* (93). Four additional isolates from cat feces were found to be identical to the woodchuck isolate by use of biochemical, phenotypic, and 16S rRNA analyses. Controlled challenge studies are required to ascertain whether *H. marmotae* promotes hepadnavirus-associated tumors in woodchucks or causes enterohepatic disease in cats.

Natural *Helicobacter* Infections in Dolphins and Whales

***Helicobacter cetorum*.** For decades, gastric ulcers have been reported in cetaceans with clinical signs of disease, including

inappetance, lethargy, and anorexia. Ulcers were previously associated with parasitic infections or had undefined causes. Five *Helicobacter* isolates have been obtained from dolphins (122) and whales (125), both wild and captive animals, in habitat ranging from Boston to Hawaii. These isolates are identical and novel, as evidenced by unique RFLP patterns from digestion of a 1.2-kb PCR product, using *Helicobacter* genus-specific primers and further sequencing of 16S rRNA. Positive culture results have been obtained for fecal and biopsy specimens from the gastric mucosa in animals with clinical signs consistent with gastric disease. This novel helicobacter, now formally named *H. cetorum*, potentially causes gastritis and ulcers in marine mammals. Positive fecal culture results indicate potential fecal-oral transmission. Comparison of minimally invasive techniques for detecting the prevalence *H. cetorum* in wild dolphins was undertaken as part of a long-term health study of the animals off the coast of Florida (123). Using serum samples and fecal specimens, results of serologic testing, culture, and PCR analysis, using genus- and *H. cetorum*-specific primers and Southern blot hybridization indicated at least 50% prevalence of *H. cetorum* infection in this group of wild dolphins.

Novel helicobacters isolated from pinnipeds. Novel helicobacters have been isolated from harp seals and California sea lions. Two novel helicobacters were isolated in culture from the gastric mucosa of four harp seals (*Phoca groenlandica*) stranded on the Massachusetts coastline (124). Both isolates were catalase and oxidase positive. One isolate was fusiform shaped, with *Flexispira* morphology, and was urease positive, and the second was spiral shaped and was urease negative. Mild diffuse lymphoplasmacytic gastritis in the superficial mucosa of the pyloric antrum was associated with slender, spiral, and fusiform-shaped bacteria detected by use of the Warthin-Starry stain. On the basis of results of 16S rRNA analysis, the urease-negative isolate clustered with *H. canis* and the urease-positive isolate clustered with other isolates from sea lions and sea otters. These findings suggest that *Helicobacter* spp. play a role in gastrointestinal tract diseases of pinnipeds.

Conclusion

The genus *Helicobacter* has rapidly expanded due to the isolation of new species from a wide range of animals. Many domestic and wild animal species have not been systematically evaluated for *Helicobacter* infections; therefore, more novel isolates can be anticipated with future efforts. The genus now includes 26 formally named species as well as numerous other novel helicobacters currently being characterized. Continued study of the genus *Helicobacter* is yielding new information for varied disciplines within biology and medicine. The zoonotic potential of *Helicobacter* spp. has been established, but is likely under reported. Diagnostic evaluations of human and veterinary patients will increasingly include *Helicobacter*-associated disease in the differential list of potential diagnoses. Advanced molecular techniques have given investigators the ability to study these fastidious organisms when culture has been difficult or appeared unachievable. The advances of evaluating host and bacterial gene expression using microarray technology is in its infancy and will be very applicable to the study of interactions between *Helicobacter* spp. and their hosts. Genomic studies of *Helicobacter* spp. as pathogens and of their hosts will no doubt shed light on critical genes and associated molecular events that could be tar-

gets for preventing or treating infectious diseases in the future. As examples, new insights are emerging concerning the dysregulated host response to enteric flora thought to be the basis for IBD of humans. Particularly noteworthy, mouse models of *Helicobacter*-associated disease are strengthening the link between chronic infections and the associated tissue damage from chronic inflammation and the progression of dysplastic lesions to cancer in the liver and gastrointestinal tract. As more is learned about the helicobacters that naturally infect the species that biomedical research historically has, and will continue to rely on as predictable models of human disease, management of helicobacter-free research colonies will continue to gain in importance.

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