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REVIEW ARTICLE

Gastric and Enterohepatic Helicobacters other than *Helicobacter pylori*

Armelle Ménard,*,† Christelle Péré-Védrenne,*,† Freddy Haesebrouck‡ and Bram Flahou‡

*Laboratoire de Bactériologie, Centre National de Référence des Campylobacters et Hélicobacters, Université de Bordeaux, F33076, Bordeaux, France, †INSERM U853, F33076, Bordeaux, France, ‡Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases, Ghent University, Merelbeke, Belgium

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Reprint requests to: Armelle Ménard, Laboratoire de Bactériologie, INSERM U853, Université de Bordeaux, F33076 Bordeaux, France.
E-mail: armelle.menard@u-bordeaux.fr

Abstract

During the past year, research on non-*Helicobacter pylori* species has intensified. *H. valdiviensis* was isolated from wild birds, and putative novel species have been isolated from Bengal tigers and Australian marsupials. Various genomes have been sequenced: *H. bilis*, *H. canis*, *H. macacae*, *H. fennelliae*, *H. cetorum*, and *H. suis*. Several studies highlighted the virulence of non-*H. pylori* species including *H. cinaedi* in humans and hyperlipidemic mice or *H. macacae* in geriatric rhesus monkeys with intestinal adenocarcinoma. Not surprisingly, increased attention has been paid to the position of *Helicobacter* species in the microbiota of children and animal species (mice, chickens, penguins, and migrating birds). A large number of experimental studies have been performed in animal models of Helicobacter induced typhlocolitis, showing that the gastrointestinal microbial community is involved in modulation of host pathways leading to chronic inflammation. Animal models of *H. suis*, *H. heilmannii,* and *H. felis* infection have been used to study the development of severe inflammation-related pathologies, including gastric MALT lymphoma and adenocarcinoma.

Taxonomy and Phylogeny

In 2014, *Helicobacter valdiviensis* (type strain WBE14T) was described as a novel species [1], isolated from wild bird feces in Southern Chile. The host range of *H. valdiviensis*, its clinical relevance, and zoonotic potential remain to be investigated. Putative novel *Helicobacter* species from Bengal tigers from Thailand were characterized [2]. Gene and protein analysis identified them as novel *H. acinonychis* strains closely related to strains of other big cats. These isolates express homologs of *H. pylori* urease A/B, flagellins, BabA, NapA, HtrA, and γ-glutamyl transpeptidase, but no expression was detected for CagA, VacA, SabA, DupA, or OipA. Novel *Helicobacter* species were detected in the gastrointestinal tract of Australian marsupials [3], and "S"-shaped isolates with bipolar sheathed flagella were cultivated from ringtail possums. No Helicobacters were cultured from the koalas, while Helicobacter DNA was detected in the majority of the animals.

An improved PCR/sequencing of the *atp*A gene was reported for the identification of 14 *Helicobacter* taxa, "*H. winghamensis*," and *Wolinella succinogenes* [4]. A PCR–restriction fragment length polymorphism targeting the 23S rRNA gene was also reported for the differentiation of 27 non-*H. pylori* taxa and *W. succinogenes* [5]. Using two-dimensional gel electrophoresis of the whole proteome of Helicobacter strains, it was possible, based on 66 protein spots, to discriminate between enterohepatic and gastric Helicobacters, despite an extensive heterogeneity [6].

Genomics and Genetics

Genome sequencing was performed for two *H. suis* strains for which no isolates were available in vitro [7]. Genome analysis revealed genes unique to *H. suis*, leading to the development of a new *H. suis*-specific PCR assay based on a homolog of the *car*R gene from *Azospirillum brasilense*, involved in the regulation of carbohydrate catabolism. Two genomes of *H. cetorum* strains, originating from a dolphin and a Beluga whale, were sequenced [8]. The strains were phylogenetically

more closely related to *H. pylori* and *H. acinonychis* than to other *Helicobacter* species. Their genomes are 7–26% larger than *H. pylori* genomes and differ markedly from one another in gene content, sequences, and arrangements of shared genes. They lack the *cag* pathogenicity island (*cag*PAI), but do possess novel alleles of the *vac*A gene. In addition, they reveal an extra triplet of divergent *vac*A genes, metabolic genes distinct from *H. pylori,* and genes encoding an iron and nickel cofactored urease. Although *H. acinonychis* is postulated to descend from the *H. pylori* hpAfrica2 superlineage [9], genome sequences from three South African hpAfrica2 *H. pylori* strains were different from *H. acinonychis* in their gene arrangement and content [10].

H. bilis strain WiWa isolated from the cecum of a mouse (Iowa, USA), *H. canis* strain A805/92 isolated from a boy's stool sample [11], and *H. macacae* type strain MIT 99-5501 isolated from the intestine of a rhesus monkey with chronic idiopathic colitis [12,13] were sequenced (GenBank accession numbers: AQFW0 1000000, AZJJ01000002, and AZJI01000005, respectively). The draft genome sequence [14] of an *H. fennelliae* strain isolated from the blood of a female patient with non-Hodgkin lymphoma [15] is also available (GenBank accession number: BASD00000000). The genome of this strain MRY12-0050 is 2.15 Mb in size, has a G+C content of 37.9%, and contains 2507 genes (2467 protein-coding genes and 40 structural RNAs). No cytolethal distending toxin (CDT) cluster was identified in contrast to its closest neighbors *H. cinaedi* and *H. hepaticus* [15].

Genomic analysis of a metronidazole-resistant human-derived *H. bizzozeronii* strain revealed a frame length extension of a simple sequence cytosine repeat in the 3′ region of the oxygen-insensitive NADPH nitroreductase *rdx*A [16]. This extension was the only mutation, acquired at a high rate, observed in spontaneous *H. bizzozeronii* metronidazole-resistant mutants. The *H. bizzozeronii rdxA* appears to be a contingency gene undergoing phase variation, in contrast to its counterpart in *H. pylori*.

Non-*H. pylori* Helicobacters in humans

Contact with animals is believed to be a risk factor for *H. suis* infection, but consumption of contaminated pork is now also considered to be a possible transmission route [17]. Indeed, viable *H. suis* bacteria were detected in retail pork samples and persisted for days in experimentally contaminated pork. Reports in the literature describe an increased proportional mortality from Parkinson's disease among livestock farmers. In patients (n = 60) with idiopathic parkinsonism, and compared with control patients (n = 256), the relative risk of harboring *H. suis* was 10 times greater than that of having *H. pylori* [18]. This higher frequency was even exaggerated following *H. pylori* eradication therapy.

A 62-year-old Japanese woman, suffering from gastritis and multiple gastric ulcers, was shown to be infected with *H. heilmannii* sensu stricto, which was subsequently eradicated with classic triple therapy [19].

The microaerophilic microbiota was evaluated in colonic biopsies from children presenting for the first time with inflammatory bowel disease (IBD) [20]. The prevalence of *Helicobacter* species (*H. pylori, W. succinogenes, H. brantae,* and *H. hepaticus*), detected by PCR was 11% in 44 patients with treatment naïve de novo IBD vs 12% in 42 children with normal colons, suggesting that Helicobacters may not be associated with IBD in children.

It was proposed that enterohepatic Helicobacters could act as a facilitating agent in the initial infection and progression of *Chlamydia trachomatis*-induced proctitis [21]. A meta-analysis including 10 case–control studies supports the possible association between *Helicobacter* species infection and cholangiocarcinoma [22].

H. hepaticus infection may be involved in the progression of primary hepatocellular carcinoma (HCC) [23]. The anti-*H. hepaticus* IgG detection rate was 50.0% in HCC patients (n = 50), while this rate reached only 7.7 and 6.3% in control groups (patients with benign liver tumor and normal liver tissue, respectively). The *H. hepaticus* 16S rRNA gene was detected in 36% of HCC samples positive by serology of which 44.4% were positive for the *cdt*B gene, while these genes were virtually not detected in control groups.

The fourth clinical case of *H. canis* bacteraemia was reported in a 41-year-old woman, 11 months after kidney transplantation [24]. The patient was fully cured after cefuroxime and ciprofloxacin treatment.

Typing of 46 *H. cinaedi* strains isolated from blood of patients from the same hospital revealed that most isolates exhibited the clonal complex 9 and were mainly isolated from immunocompromised patients in the same ward [14]. Three related *H. fennelliae* isolates were also obtained from the same ward. Antimicrobial susceptibilities of the isolates were similar, although mutations conferring clarithromycin resistance in *H. fennelliae* differed from those in *H. cinaedi*. This study highlights that *H. cinaedi* and *H. fennelliae* must be carefully monitored to prevent nosocomial infection in immunocompromised patients [14]. Among 126 *H. cinaedi*-positive sets of blood cultures isolated from 66 bacteremic patients from two hospitals [25], the time for blood cultures to become positive was ≤5 and >5 days for 55% and 45% of sets, respectively,

confirming that *H. cinaedi* is a fastidious, slow-growing organism, hampering its microbiological diagnosis. All patients except one had an underlying disease. The 30-day mortality rate of *H. cinaedi* bacteremia was 6.3%.

H. cinaedi is rarely encountered in immunocompetent individuals. A case of prosthetic (axillobifemoral bypass) graft infection with *H. cinaedi* was reported in an 85-year-old man [26]. The patient was successfully treated by removal of the infected graft and subsequent antibiotherapy (sulbactam/ampicillin for 2 weeks). A case of *H. cinaedi*-associated meningitis was reported in an immunocompetent 34-year-old woman who had daily contact with a kitten for a month, suggesting that the pet served as a reservoir of transmission [27]. A course of 1 week with ceftriaxone and vancomycin combined antibiotherapy, followed by 2 weeks of meropenem, eliminated the symptoms of *H. cinaedi* meningitis.

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry was shown to be useful for the identification and subtyping of *H. cinaedi* [28]. As for *hsp60* gene-based phylogeny, human isolates formed a single cluster distinct from animal isolates, suggesting that animal strains may not be a major source of infection in humans [28].

Sequencing of an *H. pylori* strain isolated from a patient with gastric cancer in China revealed a new gene sharing 93% identity with a hypothetical protein of *H. cinaedi,* suggesting a possible horizontal gene transfer to *H. pylori* [29].

Natural Infection with Non-*H. pylori* Helicobacters in Animals

Davison et al. [30] described the first isolation of *H. cetorum* from a striped dolphin and they showed that Atlantic white-sided dolphins and short-beaked common dolphins from European waters are also infected with this *Helicobacter* species. In these wild stranded animals, mucosal hemorrhages were present in the pyloric stomach, as well as an ulcerative gastritis resembling previously described gastritis in *H. cetorum*-infected dolphins [31].

H. canis has been associated with digestive diseases in dogs, cats, and humans. Recently, the bacterium was isolated from sheep feces [32], suggesting that sheep could act as *H. canis* reservoirs for zoonotic or foodborne transmission. *H. canis*, *H. bizzozeroni*, *H. bilis*, *H. felis,* and *H. salomonis* were detected by PCR in the crypts of the cecum and colon of healthy and symptomatic stray dogs [33]. Colonization levels of *Helicobacter*-like bacteria correlated with the level of mucosal fibrosis/atrophy and were highest in younger dogs. In another study, gastric mucosal glycosylation profiles were evaluated in *Helicobacter*-free dogs [34]. The canine gastric mucosa was shown to lack expression of type 1 Lewis antigens, while a broad expression of type 2 structures and the A antigen was observed. Lewis X (Le^x) revealed a variable expression in the body, whereas expression was detected in the antrum of all animals. Expression of sialylated Le^x, involved in SabA-mediated adherence of *H. pylori*, was mainly observed in the body. Of known canine non-*H. pylori Helicobacter* species, *H. heilmannii* sensu stricto presented the highest adherence scores to the antral mucosa in canine paraffin-embedded sections.

The relationship between pet ownership or frequent exposure to dogs and infection with different gastric *Helicobacter* species was assessed [35]. A significant correlation was found between human and canine infection for *H. felis* and to a lesser extent for *H. bizzozeronii*.

The poultry gut microbiota was little studied, while chickens are a major meat source worldwide and are considered as important reservoirs for foodborne pathogens. High abundance of *Campylobacter* species *H. pullorum* and *Megamonas* species was found in the cecal microbiome of Ross broiler chickens housed indoors under standard commercial conditions [36]. The gastrointestinal tract microbiota was characterized in king, gentoo, macaroni, and little penguin species [37]. 16S rRNA gene pyrosequencing revealed that Helicobacteriaceae was the third dominant family in king penguins (8%) in contrast to other penguin species. In the Proteobacteria phylum, *Helicobacter* species ranged from 1 to 11% in these four marine seabird species. Of 3889 16S rRNA sequences analyzed from the feces of migrating birds (migratory stopover, Delaware Bay, USA), 6.5% corresponded to Epsilonproteobacteria, that is, *Campylobacter* (82.3%) and *Helicobacter* (17.7%) species. Most *Helicobacter*-like sequences were closely related to *H. pametensis* and *H. anseris*, while the low percentage of sequence identity (92%) with *H. anseris* suggests a different *Helicobacter* species [38]. Helicobacters were detected at low frequence in feces and intestinal tissues of tropical terrestrial wild birds (Venezuela) by molecular methods [39], suggesting that these bacteria may be uncommon in the populations studied.

PCR arrays for commonly reported rodent infectious agents were used in naturally infected index mice and sentinel mice exposed by contact and soiled-bedding transfer [40]. Helicobacters and pinworms were detected in fewer than half of the soiled-bedding sentinels. Of the four *Helicobacter* species identified in index mice, only *H. ganmani* was found in soiled-bedding and contact sentinels.

The prevalence of enterohepatic Helicobacter (EHH) infection was determined in a study on old rhesus

monkeys [41]. Helicobacter infection (PCR, culture) was present in 97% of the monkeys; 13 of 14 monkeys diagnosed with intestinal adenocarcinoma were infected. *H. macacae* and "*Helicobacter* sp. rhesus monkey" taxons 2 and 4 were detected on the epithelial colonic surface. In vitro experiments showed bacterial adherence to epithelia, invasion as well as induction of proinflammatory gene expression, while genes involved in the inflammasome were downregulated. These results suggest that EHH may mediate diarrhea, chronic inflammation, and intestinal cancer in nonhuman primates. Downregulation of inflammasome function by Helicobacter may represent a strategy for long-term persistence in the host.

The impact of *H. pylori* challenge upon the preexisting gastric microbial community members in rhesus macaques was assessed [42]. When comparing non-*Helicobacter* taxa before and after *H. pylori* inoculation, no significant changes in the microflora were observed. Most animals were naturally infected with *H. suis* prior to *H. pylori* inoculation. After *H. pylori* challenge, only one of two gastric *Helicobacter* species was dominant, revealing potential competitive inhibition/exclusion. Interestingly, the proportions of both species were shown to be highly variable in individual animals.

Natural Helicobacter Infection of Mice in Animal Facilities

Helicobacters were shown to be among the dominant organisms in the intestinal tract of mice [43]. *H. ganmani* and an unidentified *Helicobacter* strain (MIT 01-6451) are the predominant *Helicobacter* species infecting specific pathogen-free mice in Japanese animal facilities [44] and lateral gene transfer probably occurs among *Helicobacter* species during coinfection. The prevalence of *Helicobacter* infection in the feces/cecum of laboratory mice in Thailand reached a level of 78–98% [45]. *H. rodentium* (67.0%) and *Helicobacter* strain MIT 01-6451 (15.4%) were the most common *Helicobacter* species, while some species remained unidentified (14.1%).

The beneficial effects of a 4-drug medicated diet, aimed at Helicobacter eradication, were demonstrated in mice with altered adaptive immunity and naturally infected with *H. hepaticus* and *H. typhlonius* [46]. However, mice that were fed a medicated diet developed severe side effects that improved or were resolved after resuming the control diet.

Experimental Infection with non-*H. pylori* Helicobacters

The involvement of the chemokine CXCL-13 in gastric MALT lymphoma development in *H. suis*-infected mice was confirmed by administration of an anti-CXCL-13 antibody, which was able to reduce the formation of lymphoid follicles and germinal centers [47]. Similar results were obtained by administering VEGF receptor antibodies to infected mice [48].

Mongolian gerbils were infected with nine *H. heilmannii* sensu stricto strains [49]. Seven strains caused an antrum-dominant chronic active gastritis after 9 weeks of infection. High colonization levels were observed for four strains, while colonization of four other strains was more restricted and one strain did not colonize the stomach of these animals. A strong IL-1β expression was observed in infected animals, in contrast to IFN-γ expression.

The importance of Th1-mediated immunity in protecting mice against *H. felis* infection was examined [50]. In IL-23p19 KO mice, IL-17 levels remained low but IFN-γ levels were shown to be increased, resulting in colonization levels similar to those in wild-type (WT) mice. In addition, treatment of *H. felis*-infected Balb/c mice with Th1-promoting IL-12 resulted in increased gastric inflammation and even eradication of bacteria in most mice. Infection of mice with *H. felis* was shown to induce expression of the dual oxidase enzyme complex Duox2/Duoxa2 [51]. Higher colonization rates were observed in *Duoxa*$^{-/-}$ mice infected with *H. felis*, compared with WT mice, highlighting the importance of epithelial production of H_2O_2 as a line of defense against *Helicobacter* infection.

Nfkb1$^{-/-}$ mice developed more pronounced gastric atrophy upon *H. felis* infection compared with WT mice, while *nfkb2*$^{-/-}$ mice developed minimal gastric epithelial pathology, and c-Rel-mediated signaling appeared to modulate the risk of lymphomagenesis [52]. Mesenchymal stem cells were shown to promote an accelerated form of *H. felis*-induced gastric cancer [53] and their engraftment in chronic inflammation was shown to be only partially dependent on the CXCR4 receptor. In *H. felis*-infected C57BL/6 mice, gastric metaplasia coincides with the appearance of $CD45^+MHCII^+$ $CD11b^+CD11c^+$ myeloid cells, which were indeed absent in mice suffering from chronic gastritis without concurrent metaplasia [54]. Deletion in mice of *Gli1* inhibited expression of markers of metaplasia, clearly showing that Gli1-dependent myeloid cell differentiation plays a role in the appearance of myeloid cell subtypes required for the development of mucous neck cell metaplasia. In another study, diet-induced obesity in mice was shown to cause an increase in bone marrow-derived immature myeloid cells in blood and gastric tissue of *H. felis*-infected mice, as well as increased expression of IL-17A, GM-CSF, and STAT3 activation [55]. Not only did obesity promote a protumorigenic gastric

microenvironment, but *H. felis*-induced gastric inflammation also augmented obesity-induced adipose inflammation. Besides an increased intake of fat leading to obesity, other dietary factors are increasingly recognized as being important factors in modulating progression of *Helicobacter*-induced gastric pathologies [56]. Partially in contrast to previously published experiments, Yang et al. [57] postulate that bone marrow-derived cells might not be the direct source of gastrointestinal tumor cells induced by *H. felis* infection.

Infection of spontaneously hyperlipidemic mice with *H. cinaedi* was shown to significantly enhance the development of atherosclerosis [58], with increased expression of proinflammatory genes, accumulation of neutrophils, and induction of macrophage-derived foam cell formation in aortic root lesions. Although infection was asymptomatic, detection of CDT RNA of *H. cinaedi* indicated an aortic infection. In vitro, *H. cinaedi* infection altered expression of cholesterol receptors and transporters in macrophages and induced foam cell formation and differentiation of THP-1 monocytes.

$p16^{ink4a}$ and $p19^{arf}$ genes are two distinct tumor suppressors located at the Ink4a/Arf locus. Methylation of $p16^{ink4a}$ and APC genes is increased in colorectal carcinoma after X-ray radiotherapy, and inactivation of $p16^{ink4a}$ and $p19^{arf}$/$p14^{arf}$ by hypermethylation of promoter CpG islands also occurs frequently in various tumors and is implicated in murine carcinogenesis. The small intestine of mice is the most sensitive organ for *ink4a/arf* methylation induced by X-radiation, the chemical carcinogen N-nitrosomethylurea, and *H. felis* infection [59], suggesting that abdomen radiotherapy could be carcinogenic for patients with acute *H. pylori* infection. Young adult mice harbored either conventional intestinal microbiota or intestinal microbiota with a restricted microbial composition. After exposure of mice to irradiation, acute chromosomal DNA lesions were observed in mice with a restricted microbial composition, but not in those with conventional intestinal microbiota [60]. *H. hepaticus* and *Bacteroides stercoris* were more abundant in mice with conventional intestinal microbiota than in those with a restricted intestinal microbiota, suggesting that the intestinal microbiota can influence genotoxic endpoints induced by high-energy protons.

The intestinal microbiota structure was shown to be essential for the development of typhlocolitis in *H. hepaticus*-infected IL-10-deficient mice, and disease can be initiated and progress in the presence of different microbial communities [61]. While the severity of the disease appears to be independent of the microbial community structure, the specific structure of the microbiota may modulate host pathways leading to *H. hepaticus*-induced chronic inflammation. Discrepant results have nevertheless been published. Using the same model, it was shown that mice kept under specific pathogen-free conditions in two different facilities displayed strong differences with respect to their susceptibility to *H. hepaticus*-induced typhlocolitis [62]. This was associated with a different composition of the microbiota. *H. trogontum* infection also induced typhlocolitis in IL-10-deficient mice [63]. Disease is associated with significant intestinal barrier dysfunction characterized by a decreased transepithelial electrical resistance and mRNA expression of tight junction proteins and an increased short-circuit current, myosin light chain kinase mRNA, paracellular permeability, and tumor necrosis factor (TNF)-α and myeloperoxidase plasma levels. Exclusive enteral nutrition, a well-established approach for the management of Crohn's disease, metronidazole treatment or a combination of both, restored barrier function and reversed inflammatory changes along with an *H. trogontum* load reduction, while hydrocortisone treatment did not. These findings provide an explanation as to the observation that patients with Crohn's disease achieve mucosal healing more readily following exclusive enteral nutrition than following corticosteroid treatment.

Dietary vitamin B6 modulates colonic inflammation in IL-10-deficient mice naturally colonized by *H. hepaticus*, suggesting that vitamin B6 supplementation may offer an additional tool for the management of IBD [64].

Patients with IBD are at increased risk for bone loss and fractures. A significant trabecular bone loss was observed in infected IL-10-deficient male mice but not in females [65]. Moreover, *H. hepaticus* infection suppressed osteoblast markers only in male mice. The latter suffered from more severe colitis and presented higher levels of *H. hepaticus* colonization than females.

IL-10 receptor-blocking antibody treatment during chronic *H. hepaticus* infection of mice lacking inducible expression of major histocompatibility complex class II (MHCII) molecules led to colitis associated with increased innate effector cell infiltration and expression of proinflammatory cytokines [66]. Moreover, exacerbated colitis correlated with the inability of intestinal epithelial cells to upregulate MHCII expression.

The Wiskott–Aldrich syndrome protein (WASP) is a hematopoietic cell-specific intracellular signaling molecule that regulates the actin cytoskeleton. WASP deficiency is associated with IBD. *Helicobacter* species were detected in feces of WASP-deficient mice [67]. After *Helicobacter* eradication, these mice did not develop spontaneous colitis, and reinfection with *H. bilis* led to typhlitis and colitis which, in several cases, evolved

toward dysplasia with 10% demonstrating colon carcinoma. In addition, a T-cell transfer model of colitis dependent on WAPS-deficient innate immune cells also required *Helicobacter* colonization.

H. hepaticus-infected Rag2-deficient mice emulate many aspects of human IBD, and infected mice develop severe colitis progressing into colon carcinoma. A translational comparison of protein expression and protein damage products in tissues of *H. hepaticus*-infected Rag2-deficient mice and patients with human IBD assessed the validity of this animal model for human IBD [68]. The study determined some systemic inflammatory markers in serum that were most closely associated with disease activity and were common to human IBD and *H. hepaticus*-associated colitis in Rag2-deficient mice.

Necrotizing enterocolitis (NEC) is the second most common cause of morbidity in premature infants, and dysbiosis is thought to play an important role in disease onset. *H. hepaticus* infection of premature formula-fed rats (model of NEC) induced inflammation, increased levels of TLR4 receptor, altered activation of autophagy, and increased the incidence and severity of NEC in rats exposed to asphyxia and cold stress [69]. These results are consistent with observations in neonates of blooms of proinflammatory microbes just before the onset of NEC and support dysbiosis in the incidence of NEC.

H. hepaticus infection is sufficient to enhance prostate intra-epithelial neoplasia and microinvasive carcinoma in mice with a genetic predilection for dysregulation of wnt signaling ($Apc^{Min/+}$ mutant), in an inflammation-dependent manner [70]. Intraperitoneal injection of mesenteric lymph node cells from *H. hepaticus*-infected mice to noninfected mice is sufficient to transmit early neoplasia to uninfected mice. Transmissibility of neoplasia could be prevented by prior neutralization of inflammation using anti-TNF-α antibody in infected mesenteric lymph node donor mice.

A study evaluating the effects of fish oil on mouse gut microbiota showed that fish oil can suppress the *Helicobacter* growth [71].

Virulence Factors

Similar to *H. pylori*, the gamma glutamyl transpeptidase globulin transferase (GGT) from *H. suis* was shown to impair lymphocyte function [72] and this effect could be modulated by supplementation with glutamine and reduced glutathione, two known GGT substrates. *H. suis* outer membrane vesicles were identified as a possible delivery route of GGT to lymphocytes residing in deeper mucosal layers. GGT is also a virulence factor for *H. bilis* that enhances inflammatory stress response via oxidative stress in colon epithelial cells [73]. IL-8 secretion was upregulated in a GGT-dependent manner, but can be lowered by glutamine supplementation.

The CdtB of *H. pullorum* induces an atypical delocalization of vinculin from focal adhesions to the perinuclear region, formation of cortical actin-rich large lamellipodia with an upregulation of cortactin, and decreased cellular adherence [74].

The CdtB of *H. hepaticus* alone is necessary and sufficient for epithelial cell genotoxicity [75]. As for *H. pylori*, the cholesterol-α-glucosyltransferase of *H. hepaticus* is essential for establishing colonization of the intestine and liver in male A/JCr mice [76]. The PAI of *H. hepaticus* encodes components of a type VI secretion system (T6SS) whose sequence and organization resemble those of the T6SS in *C. coli* and *C. jejuni* [77].

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References

1 Collado L, Jara R, Gonzalez S. Description of *Helicobacter valdiviensis* sp. nov., a novel Epsilonproteobacteria isolated from wild bird faecal samples in Southern Chile. *Int J Syst Evol Microbiol* 2014;64:1913–9.
2 Tegtmeyer N, Rivas Traverso F, Rohde M, Oyarzabal OA, Lehn N, Schneider-Brachert W, Ferrero RL, Fox JG, Berg DE, Backert S. Electron microscopic, genetic and protein expression analyses of *Helicobacter acinonychis* strains from a Bengal tiger. *PLoS One* 2013;8:e71220.
3 Coldham T, Rose K, O'Rourke J, Neilan BA, Dalton H, Lee A, Mitchell H. Detection of *Helicobacter* species in the gastrointestinal tract of ringtail possum and koala: possible influence of diet, on the gut microbiota. *Vet Microbiol* 2013;166:429–37.
4 Miller WG, Yee E, Jolley KA, Chapman MH. Use of an improved *atp*A amplification and sequencing method to identify members of the *Campylobacteraceae* and *Helicobacteraceae*. *Lett Appl Microbiol* 2014;9:e89774.
5 Yadegar A, Alebouyeh M, Lawson AJ, Mirzaei T, Mojarad EN, Zali MR. Differentiation of non-*pylori Helicobacter* species based on PCR-restriction fragment length polymorphism of the 23S rRNA gene. *World J Microbiol Biotechnol* 2014;30:1909–17.
6 Fowsantear W, Argo E, Pattinson C, Cash P. Comparative proteomics of *Helicobacter* species: the discrimination of gastric and enterohepatic *Helicobacter* species. *J Proteomics* 2014;97:245–55.
7 Matsui H, Takahashi T, Murayama SY, et al. Development of new PCR primers by comparative genomics for the detection of *Helicobacter suis* in gastric biopsy specimens. *Helicobacter* 2014; Mar 28. DOI: 10.1111/hel.12127. [Epub ahead of print]
8 Kersulyte D, Rossi M, Berg DE. Sequence divergence and conservation in genomes of *Helicobacter cetorum* strains from a dolphin and a whale. *PLoS One* 2013;8:e83177.

9 Moodley Y, Linz B, Bond RP, et al. Age of the association between *Helicobacter pylori* and man. *PLoS Pathog* 2012;8: e1002693.

10 Duncan SS, Bertoli MT, Kersulyte D, Valk PL, Tamma S, Segal I, McClain MS, Cover TL, Berg DE. Genome Sequences of Three hpAfrica2 Strains of *Helicobacter pylori*. *Genome Announc* 2013;1:e00729–13.

11 Burnens AP, Stanley J, Schaad UB, Nicolet J. Novel *Campylobacter*-like organism resembling *Helicobacter fennelliae* isolated from a boy with gastroenteritis and from dogs. *J Clin Microbiol* 1993;31:1916–7.

12 Fox JG, Handt L, Xu S, Shen Z, Dewhirst FE, Paster BJ, Dangler CA, Lodge K, Motzel S, Klein H. Novel *Helicobacter* species isolated from rhesus monkeys with chronic idiopathic colitis. *J Med Microbiol* 2001;50:421–9.

13 Fox JG, Boutin SR, Handt LK, et al. Isolation and characterization of a novel *helicobacter* species, "*Helicobacter macacae*," from rhesus monkeys with and without chronic idiopathic colitis. *J Clin Microbiol* 2007;45:4061–3.

14 Rimbara E, Matsui M, Mori S, Suzuki S, Suzuki M, Kim H, Sekizuka T, Kuroda M, Shibayama K. Draft Genome Sequence of *Helicobacter fennelliae* Strain MRY12-0050, Isolated from a Bacteremia Patient. *Genome Announc* 2013;1:e00512–13.

15 Rimbara E, Mori S, Kim H, Matsui M, Suzuki S, Takahashi S, Yamamoto S, Mukai M, Shibayama K. *Helicobacter cinaedi* and *Helicobacter fennelliae* transmission in a hospital from 2008 to 2012. *J Clin Microbiol* 2013;51:2439–42.

16 Kondadi PK, Pacini C, Revez J, Hanninen ML, Rossi M. Contingency nature of *Helicobacter bizzozeronii* oxygen-insensitive NAD(P)H-nitroreductase (HBZC1_00960) and its role in metronidazole resistance. *Vet Res* 2013;44:56.

17 De Cooman L, Flahou B, Houf K, Smet A, Ducatelle R, Pasmans F, Haesebrouck F. Survival of *Helicobacter suis* bacteria in retail pig meat. *Int J Food Microbiol* 2013;166:164–7.

18 Blaecher C, Smet A, Flahou B, et al. Significantly higher frequency of *Helicobacter suis* in patients with idiopathic parkinsonism than in control patients. *Aliment Pharmacol Ther* 2013;38:1347–53.

19 Matsumoto T, Kawakubo M, Akamatsu T, Koide N, Ogiwara N, Kubota S, Sugano M, Kawakami Y, Katsuyama T, Ota H. *Helicobacter heilmannii sensu stricto*-related gastric ulcers: a case report. *World J Gastroenterol* 2014;20:3376–82.

20 Hansen R, Berry SH, Mukhopadhya I, et al. The microaerophilic microbiota of *de-novo* paediatric inflammatory bowel disease: the BISCUIT study. *PLoS One* 2013;8:e58825.

21 Mestrovic T, Ljubin-Sternak S, Sviben M. Potential role of enterohepatic *Helicobacter* species as a facilitating factor in the development of *Chlamydia trachomatis* proctitis. *Med Hypotheses* 2013;81:481–3.

22 Xiao M, Gao Y, Wang Y. *Helicobacter* species infection may be associated with cholangiocarcinoma: a meta-analysis. *Int J Clin Pract* 2014;68:262–70.

23 Yang J, Ji S, Zhang Y, Wang J. *Helicobacter hepaticus* infection in primary hepatocellular carcinoma tissue. *Singapore Med J* 2013;54:451–7.

24 van der Vusse ML, van Son WJ, Ott A, Manson W. *Helicobacter canis* bacteremia in a renal transplant patient. *Transpl Infect Dis* 2014;16:125–9.

25 Araoka H, Baba M, Kimura M, Abe M, Inagawa H, Yoneyama A. Clinical characteristics of *Helicobacter cinaedi* bacteremia and time for blood cultures to become positive. *J Clin Microbiol* 2014;52:1519–22.

26 Suematsu Y, Morizumi S, Okamura K, Kawata M. A rare case of axillobifemoral bypass graft infection caused by *Helicobacter cinaedi*. *J Vasc Surg* 2013; S0741-5214,01602-9.

27 Sugiyama A, Mori M, Ishiwada N, Himuro K, Kuwabara S. First adult case of *Helicobacter cinaedi* meningitis. *J Neurol Sci* 2014;336:263–4.

28 Taniguchi T, Sekiya A, Higa M, Saeki Y, Umeki K, Okayama AHayashi T, Misawa N. Rapid identification and subtyping of *Helicobacter cinaedi* strains by intact-cell mass spectrometry profiling with the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2014;52:95–102.

29 You Y, Liu L, Zhang M, Zhu Y, He L, Li D, Zhang J. Genomic characterization of a *Helicobacter pylori* isolate from a patient with gastric cancer in China. *Gut Pathog* 2014;6:5.

30 Davison NJ, Barnett JE, Koylass M, Whatmore AM, Perkins MW, Deaville RC, Jepson PD. *Helicobacter cetorum* infection in striped dolphin (*Stenella Coeruleoalba*), Atlantic white-sided dolphin (*Lagenorhynchus Acutus*), and short-beaked common dolphin (*Delphinus Delphus*) from the Southwest Coast of England. *J Wildl Dis* 2014;50:431–7.

31 Harper CG, Feng Y, Xu S, et al. *Helicobacter cetorum* sp. nov., a urease-positive *Helicobacter* species isolated from dolphins and whales. *J Clin Microbiol* 2002;40:4536–43.

32 Swennes AG, Turk ML, Trowel EM, Cullin C, Shen Z, Pang J, Petersson KH, Dewhirst FE, Fox JG. *Helicobacter canis* colonization in sheep: a Zoonotic link. *Helicobacter* 2014;19:65–8.

33 Abdi FS, Jamshidi S, Moosakhani F, Sasani F. Detection of *Helicobacter* spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigations. *Diagn Pathol* 2014;9:50.

34 Amorim I, Freitas DP, Magalhaes A, Faria F, Lopes C, Faustino AM, Smet A, Haesebrouck F, Reis CA, Gärtner F. A comparison of *Helicobacter pylori* and non-*Helicobacter pylori Helicobacter* spp. binding to canine gastric mucosa with defined gastric glycophenotype. *Helicobacter* 2014; Apr 2. DOI: 10.1111/hel.12125. [Epub ahead of print]

35 Chung TH, Kim HD, Lee YS, Hwang CY. Determination of the prevalence of *Helicobacter heilmannii*-like organisms type 2 (HHLO-2) infection in humans and dogs using non-invasive genus/species-specific PCR in Korea. *J Vet Med Sci* 2014;76:73–9.

36 Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ. Extensive microbial and functional diversity within the chicken cecal microbiome. *PLoS One* 2014;9:e91941.

37 Dewar ML, Arnould JP, Dann P, Trathan P, Groscolas R, Smith S. Interspecific variations in the gastrointestinal microbiota in penguins. *Microbiologyopen* 2013;2:195–204.

38 Ryu H, Grond K, Verheijen B, Elk M, Buehler DM, Santo Domingo JW. Intestinal microbiota and species diversity of *Campylobacter* and *Helicobacter* spp. in migrating shorebirds in Delaware Bay. *Appl Environ Microbiol* 2014;80:1838–47.

39 Garcia-Amado MA, Sanz V, Martinez LM, Contreras M, Lentino M, Michelangeli F. Low occurrence of *Helicobacter* DNA in tropical wild birds, Venezuela. *J Wildl Dis* 2013;49:991–5.

40 Henderson KS, Perkins CL, Havens RB, Kelly MJ, Francis BC, Dole VS, Shek WR. Efficacy of direct detection of pathogens in naturally infected mice by using a high-density PCR array. *J Am Assoc Lab Anim Sci* 2013;52:763–72.

41 Lertpiriyapong K, Handt L, Feng Y, Mitchell TW, Lodge KE, Shen Z, Dewhirst FE, Muthupalani S, Fox JG. Pathogenic properties of enterohepatic *Helicobacter* spp. isolated from rhesus

Macaques with intestinal adenocarcinoma. *J Med Microbiol* 2014;63:1004–16.
42 Martin ME, Bhatnagar S, George MD, Paster BJ, Canfield DR, Eisen JA, Solnick JV. The impact of *Helicobacter pylori* infection on the gastric microbiota of the rhesus macaque. *PLoS One* 2013;8:e76375.
43 Li X, Yang G, Zhang C, Wu D, Tang L, Xin Y. Improvement of intestinal microflora balance by polysaccharide from *Physalis alkekengi* var. *francheti*. *Mol Med Rep* 2014;9:677–82.
44 Yamanaka H, Arita M, Oi R, Ohsawa M, Mizushima M, Takagi T, Kubo N, Yamamoto N, Takemoto T, Ohsawa K. Prevalence of an unidentified *Helicobacter* species in laboratory mice and its distribution in the hepatobiliary system and gastrointestinal tract. *Exp Anim* 2013;62:109–16.
45 Duangchanchot M, Inpunkaew R, Thongsiri P, Hayashimoto N, Gemma N, Nikaido M, et al. Prevalence of *Helicobacter* in laboratory mice in Thailand. *Exp Anim* 2014;63:169–73.
46 Garrett CM, Muth D, Watson J. Effects of medicated diet to eradicate *Helicobacter* spp. on growth, pathology, and infection status in Rag1(−/−) and nude mice. *J Am Assoc Lab Anim Sci* 2014;53:238–45.
47 Yamamoto K, Nishiumi S, Yang L, et al. Anti-CXCL13 antibody can inhibit the formation of gastric lymphoid follicles induced by Helicobacter infection. *Mucosal Immunol* 2014; Mar 19. DOI: 10.1038/mi.2014.14. [Epub ahead of print]
48 Nakamura M, Takahashi T, Matsui H, Takahashi S, Murayama SY, Suzuki H, Tsuchimoto K. New pharmaceutical treatment of gastric MALT lymphoma: anti-angiogenesis treatment using VEGF receptor antibodies and celecoxib. *Curr Pharm Des* 2014;20:1097–103.
49 Joosten M, Blaecher C, Flahou B, Ducatelle R, Haesebrouck F, Smet A. Diversity in bacterium-host interactions within the species *Helicobacter heilmannii sensu stricto*. *Vet Res* 2013;44(1):65.
50 Ding H, Nedrud JG, Blanchard TG, Zagorski BM, Li G, Shiu J, Xu J, Czinn SJ. Th1-mediated immunity against *Helicobacter pylori* can compensate for lack of Th17 cells and can protect mice in the absence of immunization. *PLoS One* 2013;8:e69384.
51 Grasberger H, El-Zaatari M, Dang DT, Merchant JL. Dual oxidases control release of hydrogen peroxide by the gastric epithelium to prevent *Helicobacter felis* infection and inflammation in mice. *Gastroenterology* 2013;145:1045–54.
52 Burkitt MD, Williams JM, Duckworth CA, O'Hara A, Hanedi A, Varro A, Caamaño JH, Pritchard DM. Signaling mediated by the NF-kappaB sub-units NF-kappaB1, NF-kappaB2 and c-Rel differentially regulate *Helicobacter felis*-induced gastric carcinogenesis in C57BL/6 mice. *Oncogene* 2013;32:5563–73.
53 Stoicov C, Li H, Liu JH, Houghton J. Mesenchymal stem cells utilize CXCR4-SDF-1 signaling for acute, but not chronic, trafficking to gastric mucosal inflammation. *Dig Dis Sci* 2013;58:2466–77.
54 El-Zaatari M, Kao JY, Tessier A, Bai L, Hayes MM, Fontaine C, Eaton KA, Merchant JL. Gli1 deletion prevents Helicobacter-induced gastric metaplasia and expansion of myeloid cell subsets. *PLoS One* 2013;8:e58935.
55 Ericksen RE, Rose S, Westphalen CB, et al. Obesity accelerates *Helicobacter felis*-induced gastric carcinogenesis by enhancing immature myeloid cell trafficking and TH17 response. *Gut* 2014;63:385–94.
56 Fox JG, Wang TC. Dietary factors modulate *Helicobacter*-associated gastric cancer in rodent models. *Toxicol Pathol* 2014;42:162–81.
57 Yang C, Gu L, Deng D. Bone marrow-derived cells may not be the original cells for carcinogen-induced mouse gastrointestinal carcinomas. *PLoS One* 2013;8:e79615.
58 Khan S, Rahman HN, Okamoto T, et al. Promotion of atherosclerosis by *Helicobacter cinaedi* infection that involves macrophage-driven proinflammatory responses. *Sci Rep* 2014;4:4680.
59 Yang C, Gu L, Deng D. Distinct susceptibility of induction of methylation of p16 and p19 CpG islands by X-radiation and chemical carcinogen in mice. *Mutat Res, Genet Toxicol Environ Mutagen* 2014; Apr 26, pii: S1383-5718, 00118-1. doi: 10.1016/j.mrgentox.2014.04.012. [Epub ahead of print]
60 Maier I, Berry DM, Schiestl RH. Intestinal microbiota reduces genotoxic endpoints induced by high-energy protons. *Radiat Res* 2014;181:45–53.
61 Nagalingam NA, Robinson CJ, Bergin IL, Eaton KA, Huffnagle GB, Young VB. The effects of intestinal microbial community structure on disease manifestation in IL-10-/- mice infected with *Helicobacter hepaticus*. *Microbiome* 2013;1:15.
62 Yang I, Eibach D, Kops F, et al. Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to *Helicobacter hepaticus*-induced colitis. *PLoS One* 2013;8:e70783.
63 Nahidi L, Leach ST, Mitchell HM, Kaakoush NO, Lemberg DA, Munday JS, Huinao K, Day AS. Inflammatory bowel disease therapies and gut function in a colitis mouse model. *Biomed Res Int* 2013;2013:909613.
64 Selhub J, Byun A, Liu Z, Mason JB, Bronson RT, Crott JW. Dietary vitamin B6 intake modulates colonic inflammation in the IL10-/- model of inflammatory *bowel* disease. *J Nutr Biochem* 2013;24:2138–43.
65 Irwin R, Lee T, Young VB, Parameswaran N, McCabe LR. Colitis-induced bone loss is gender dependent and associated with increased inflammation. *Inflamm Bowel Dis* 2013;19:1586–97.
66 Thelemann C, Eren RO, Coutaz M, et al. Interferon-gamma induces expression of MHC class II on intestinal epithelial cells and protects mice from colitis. *PLoS One* 2014;9:e86844.
67 Nguyen DD, Muthupalani S, Goettel JA, Eston MA, Mobley M, Taylor NS, McCabe A, Marin R, Snapper SB, Fox JG. Colitis and colon cancer in WASP-deficient mice require *Helicobacter* species. *Inflamm Bowel Dis* 2013;19:2041–50.
68 Knutson CG, Mangerich A, Zeng Y, et al. Chemical and cytokine features of innate immunity characterize serum and tissue profiles in inflammatory bowel disease. *Proc Natl Acad Sci USA* 2013 Jun 25;110:E2332–41.
69 Dvorak K, Coursodon-Boyiddle CF, Snarrenberg CL, Kananurak A, Underwood MA, Dvorak B. *Helicobacter hepaticus* increases intestinal injury in a rat model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2013 Oct 15;305:G585–92.
70 Poutahidis T, Cappelle K, Levkovich T, Lee CW, Doulberis M, Ge Z, Fox JG, Horwitz BH, Erdman SE. Pathogenic intestinal bacteria enhance prostate cancer development via systemic activation of immune cells in mice. *PLoS One* 2013;8:e73933.
71 Yu HN, Zhu J, Pan WS, Shen SR, Shan WG, Das UN. Effects of fish oil with high content of n-3 polyunsaturated fatty acids on mouse gut microbiota. *Arch Med Res* 2014;45:195–202.
72 Zhang G, Ducatelle R, Pasmans F, D'Herde K, Huang L, Smet A, Haesebrouck F, Flahou B. Effects of *Helicobacter suis* gamma-glutamyl transpeptidase on lymphocytes: modulation by glutamine and glutathione supplementation and outer membrane vesicles as a putative delivery route of the enzyme. *PLoS One* 2013;8:e77966.
73 Javed S, Mejias-Luque R, Kalali B, Bolz C, Gerhard M. *Helicobacter bilis* gamma-glutamyltranspeptidase enhances inflammatory stress response via oxidative stress in colon epithelial cells. *PLoS One* 2013;8:e73160.

74 Varon C, Mocan I, Mihi B, et al. *Helicobacter pullorum* cytolethal distending toxin targets vinculin and cortactin and triggers formation of lamellipodia in intestinal epithelial cells. *J Infect Dis* 2014;209:588–99.
75 Liyanage NP, Dassanayake RP, Kuszynski CA, Duhamel GE. Contribution of *Helicobacter hepaticus* cytolethal distending toxin subunits to human epithelial cell cycle arrest and apoptotic death *in vitro*. *Helicobacter* 2013;18:433–43.
76 Ge Z, Feng Y, Muthupalani S, Whary MT, Versalovic J, Fox JG. *Helicobacter hepaticus* Cholesterol-alpha-glucosyltransferase is Essential for Establishing Colonization in Male A/JCr Mice. *Helicobacter* 2014; May 23. doi: 10.1111/hel.12135. [Epub ahead of print]
77 Bleumink-Pluym NM, van Alphen LB, Bouwman LI, Wosten MM, van Putten JP. Identification of a functional type VI secretion system in *Campylobacter jejuni* conferring capsule polysaccharide sensitive cytotoxicity. *PLoS Pathog* 2013;9: e1003393.