Helicobacter

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Helicobacter spp. other than *Helicobacter pylori*

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Abstract

Over the last 12 months, new insights into the association of non-*Helicobacter pylori* Helicobacters with a range of human diseases in children and adults, including hepatobiliary disease, Crohn's disease, sepsis, and gastric disease were published. Studies investigating the presence of non-*H. pylori* Helicobacters in domestic animals reinforce previous findings that cats and dogs harbor gastric *Helicobacter* species and thus may be an important source of these organisms in humans. The confounding effect of enterohepatic Helicobacters on the outcome of biomedical research was investigated in several studies and led to recommendations that animals should be screened prior to performing experiments. A number of important and novel investigations regarding pathogenic mechanisms and immune responses to enterohepatic Helicobacters were conducted. Genomic advances in non-*H. pylori* Helicobacters included description of the complete genome of *Helicobacter canadensis*, delineation of two *Helicobacter bilis* genomospecies, and identification of a novel *cis*-regulatory RNA. New insights concerning growth conditions, biochemical characterization, and the effect of certain dietary compounds on *Helicobacter* spp. have also been reported.

Detection of non-*H. pylori Helicobacter* Species in Humans and Disease Association

Hepatobiliary Disease

In a study conducted in 77 children diagnosed with chronic liver disease, Casswall et al. using a *Helicobacter* genus-specific PCR, detected *Helicobacter* spp. DNA in a liver biopsy from 1 child (4.2%) with autoimmune hepatitis (AIH), 3 children (11.1%) with primary sclerosing cholangitis (PSC) and 8.0% of controls. Sequencing of the PCR products from AIH and PSC children showed these to be mostly similar to *Helicobacter hepaticus*, *Helicobacter muridarum*, *Helicobacter canis* and *Helicobacter pylori*, and to *Helicobacter hepaticus*, and *Helicobacter pullorum* in the controls [1]. Culture, nested PCR, and serology were used by Hamada et al. to determine the presence of enterohepatic *Helicobacter* spp. (EHH) in bile samples from patients with cholelithiasis (n = 60), cholecystitis and gastric cancer (n = 28), gall bladder polyps (n = 6), and 32 controls. Based on PCR and serology, *H. hepaticus* DNA was observed in 41% of cholelithiasis patients and 36% of cholecystitis and gastric cancer patients, which was significantly higher ($p = 0.029$) than in the two other groups. The authors concluded that *H. hepaticus* may be associated with diseases of the liver and biliary tract of humans [2]. In a further study, Kosaka et al. used *Helicobacter bilis*-specific primers to determine the presence of *H. bilis* DNA in bile juice and biliary tissue of children (n = 8) and adults (n = 9) with pancreaticobiliary maljunction (PBM). A significantly higher detection rate of *H. bilis* DNA ($p = 0.009$) was observed in patients with PBM [12/17 (70.6%)] when compared to controls [8/27 (29.6%)] suggesting that prolonged biliary colonization with *H. bilis* may contribute to the development of biliary carcinoma in patients with PBM [3]. To determine the incidence of *H. hepaticus* in gallbladder disease associated with gallstones, Pradhan et al. conducted a study in which gallbladder tissue from 30 patients with cholelithiasis was studied by culture and histology. Of 30 samples, 23 (76.7%) showed growth of an oxidase, urease, and catalase-positive Gram-negative bacterium. On histologic analysis, 18/30 samples were positive for an *H. hepaticus*-like bacterium [4]. Further steps to confirm the identity of these isolates would have been advisable.

Case Reports

Yoda et al. and Alon et al. [5,6] reported the isolation of *Helicobacter cinaedi* and *H. canis* from the blood of a febrile 58-year-old man on hemodialysis and a febrile 78-year-old man previously diagnosed with diffuse large B-cell lymphoma, respectively. Three further case reports described the detection of ''*Helicobacter heilmannii*-like organisms'' (HHLO) from gastric biopsies [7–9]. In the first of these, a spiral-shaped HHLO (SH6) was detected in a gastric biopsy from a 70-year-old man. This was shown by 16S rDNA sequence analysis to be most similar (99.4%) to HHLO C4E, however the urease gene sequence had a lower similarity (81.7%), suggesting that SH6 was a novel species [7]. In a further study, Kivisto et al. detected a large spiral bacterium in gastric biopsies from a 45-year-old Finnish dyspeptic woman. Culture of antral and corpus biopsies resulted in the isolation of a large spiral, catalase, and urease positive, Gram-negative bacteria resembling ''*H. heilmannii*''. Based on sequencing of the 16S rRNA and *ure*AB genes as well as a *Helicobacter bizzozeronii* species-specific PCR, the bacterium was shown to be *H. bizzozeronii* [8]. Duquenoy et al. reported the histologic detection of a tightly spiral bacterium similar to ''*H. heilmannii*'' from a gastric biopsy of a 12-year-old boy with an erythematous mucosa. Endoscopy conducted on the boy's two pet dogs found HHLOs to be present in their stomachs. 16S and 23S rDNA sequencing showed these to be identical to that in the boy, suggesting that he was infected by his dogs [9].

Crohn's Disease (CD)

In a multicenter cross-sectional study, Laharie et al. examined intestinal biopsies from 73 CD patients with postoperative recurrence and 92 controls for the presence of EHH using culture, PCR, and genotyping of the Card15/NOD2 mutations, R702W, G908R, and 1007f. EHH DNA was detected in 24.7% of CD patients and 17.4% of controls. In all cases, *H. pullorum* or *Helicobacter canadensis* was identified. Multivariate analysis showed, younger age (OR = 0.89, $p = 0.0001$) and the presence of the Card15/NOD2 1007fs variant to be significantly associated with CD. Following adjustment for age, EHH DNA (OR = 2.58, $p = 0.04$) was significantly associated with CD [10].

Gastric and Enterohepatic non-*H. pylori* *Helicobacter* Species in Animals

Studies on the detection, pathogenicity, and transmission of non-*H. pylori* Helicobacters in animals, including four reviews [11–14], have been published in the past year. The possibility that the oral cavity of stray cats may potentially act as a source for *Helicobacter* spp. transmission was reported by Shojaee Tabrizi et al. who detected *Helicobacter* genus-specific DNA in 93% of oral secretions from 43 clinically healthy cats in Iran. Mixed infections with non-*H. pylori* Helicobacters were also observed in 67.5% of gastric biopsies using PCR, in concordance with rapid urease testing and cytology; however, no correlation between oral and gastric status was found [15]. In addition, a high prevalence (94.6%) of gastric *Helicobacter* species was detected in 56 stray cats from Brazil; however, no correlation was observed between the presence of these gastric bacteria and histopathologic changes [16]. Another study performed in Brazilian pet cats, described a high prevalence (87%) of gastric *Helicobacter* spp. infection based on a *Helicobacter* genus-specific PCR and Warthin–Starry staining, with ''*H. heilmannii*'' being the most frequent species detected. While gastric *Helicobacter* spp. infection was not correlated with gastritis, it was associated with an increased epithelial proliferation and presence of lymphoid follicles [17]. According to the review by Haesebrouck et al. [12], the pathogenic significance of gastric helicobacters in cats and dogs may be related to the species or to differences within strains, although currently little is known about this issue. A wide-ranging culture-independent approach to investigate the spatial distribution of *Helicobacter* spp. in the gastrointestinal tract and hepatobiliary system of dogs was performed by Recordati et al. [18]. In this study, single and nested PCR for the genus *Helicobacter* and for gastric and enterohepatic *Helicobacter* spp., 16S rDNA cloning and sequencing, immunohistochemistry, and fluorescence *in situ* hybridization (FISH) revealed that in addition to the stomach, which was colonized with multiple gastric *Helicobacter* spp. (*H. bizzozeronii, Helicobacter felis* and *Helicobacter salomonis*), the large intestine of dogs was abundantly co-infected with several enterohepatic *Helicobacter* spp. (*H. bilis/*flexispira taxon 8, *H. cinaedi* and *H. canis*) [18]. A review on the significance for human health of gastric helicobacters in domestic animals concluded that in particular pigs, cats, and dogs constitute reservoir hosts for gastric *Helicobacter* species with zoonotic potential, which could cause disease in humans [12]. These authors described the complex and confusing nomenclature used to designate non-*H. pylori* helicobacters and pointed out that ''*H. heilmannii*'' should not be used as a species name according to taxonomic rules [12].

A number of studies have investigated the presence of *Helicobacter* spp. infection in laboratory rodents. A review by Chichlowski and Hale [11] concluded that

natural *Helicobacter* infection of murine models have the potential to influence the outcome and reliability of biomedical research. A major commercial rodent diagnostic laboratory compiled the results of testing a large number of mouse and rat samples from several research institutions to determine the contemporary prevalence of infectious agents and showed *Helicobacter* spp. DNA to be present in 16.1% of fecal pellets from mice and 6.6% from rats [19]. Another study performed in genetically engineered mice reported a 33.9% PCR prevalence of *H. hepaticus* in the cecum of 236 mice representing 46 strains [20]. The authors concluded that cross-fostering as a rederivation method for *H. hepaticus* eradication, was probably not appropriate [20]. Flahou et al. investigated the effect of *Kazachstania heterogenica*, a yeast detected colonizing the gastric antrum of their Mongolian gerbil colony, on the colonization and inflammatory response to *Helicobacter suis*. Gerbils co-infected with *H. suis* and *K. heterogenica* showed a significant increased lymphocytic infiltration when compared with those infected with *H. suis* alone. The authors recommended that Mongolian gerbil stomachs should be screened for *K. heterogenica* [21].

It has been suggested that wild mice might be a potential source of infection to laboratory rodents. Two studies were conducted to assess infectious diseases in wild mice captured in and around rodent facilities. *Helicobacter* spp. DNA was detected in the feces of 7/8 necropsied wild mice (*Peromyscus leucopus*) found in the animal facilities at the University of Michigan, most of which were PCR positive for *Helicobacter rodentium*, representing a potential source of *Helicobacter* infection for laboratory mice [22]. At the University of Pennsylvania (Philadelphia) campus, *Helicobacter* spp. DNA was amplified from fecal pellets of 55/59 (93%) trapped wild mice (*Mus musculus*), with *H. hepaticus* being more prevalent than *Helicobacter typhlonius* and *H. rodentium*. However, histopathologic lesions compatible with *Helicobacter* spp. were not observed in these mice [23]. The authors concluded that wild mice were unlikely to be a source of infection in laboratory animals [23]. An outbreak of *H. pullorum* was reported in mice housed within an isolated barrier unit [24]. Culture of this enterohepatic *Helicobacter* spp. provided an opportunity to study its pathogenesis.

Moyaert et al. [14] reviewed current knowledge on *H. equorum*, a urease-negative species recently described to colonize the lower bowel of horses and reported a high prevalence of *H. equorum* in foals < 6-month-old that decreased with age. Infection was not associated with equine gastrointestinal lesions [14]. A further study related to equine health investigated if bacteria, including *Helicobacter* spp., could be involved in gastric glandular lesions of these animals [25]. Based on urease activity, the presence of bacteria in general and *Helicobacter* spp. in particular, FISH, 16S rDNA amplification, cloning and sequence analysis, gastric Helicobacters were not found in 36 equine gastric lesions. An *Escherichia-like* clone was however found intracellularly, warranting further research into the possible role of this bacterium in equine gastric lesions [25].

Pathogenesis of and Immune Response to non-*H. pylori Helicobacter* Species

To investigate the pathogenic potential of *H. cinaedi* and the role of its cytolethal distending toxin (CDT), Shen et al. infected *Helicobacter*-free C57BL/6 (B6) and IL-$10^{-/-}$ mice on a C57BL/6 background with wild-type (WT) *H. cinaedi* (WT_{HC}) or two *H. cinaedi* CDT mutants (*cdt*B_{HC} or *cdt*B-N_{HC}). Despite similar colonization levels, WT_{HC} induced greater typhlocolitis than the *cdt*B_{HC} and *cdt*B-N_{HC} mutants in IL-$10^{-/-}$ mice. Further, IL-$10^{-/-}$ mice infected with WT_{HC} and *cdt*B_{HC} developed elevated mRNA levels of TNFα, inducible nitric oxide synthase and IFNγ as well as elevated Th1-associated $IgG2a^b$ when compared with B6 mice [26]. To evaluate the role of IL-10 in the signaling pathway used by intestinal microorganisms to regulate inflammation via Toll-like receptor signaling, Matharu et al. assessed parameters of intestinal inflammation in specific pathogen-free $TLR4^{-/-}$, IL-$10^{-/-}$ and $TLR4^{-/-}$ × IL-$10^{-/-}$ mice and in $TLR4^{-/-}$ × IL-$10^{-/-}$ mice following eradication and reintroduction of *H. hepaticus*. To assess regulatory T-cell function, the above-mentioned mice were crossed with transgenic mice that expressed a green fluorescent protein regulated by endogenous regulatory elements of Foxp3. These studies showed that when TLR4 signaling was lacking, pro-inflammatory cells and immunoregulatory cytokines were dysregulated. In $TLR4^{-/-}$ × IL-$10^{-/-}$ mice, Tregs ($Foxp3^+$) secreting IFNγ and IL-17 accumulated in the colonic lamina propria but did not prevent inflammation. The authors concluded that in mice lacking both IL-10 and TLR4-mediated signals, the combination of aberrant regulatory T-cell function and dysregulated control of epithelial homeostasis, leads to an exacerbation of intestinal inflammation [27]. To investigate the effect of gastrin on Helicobacter-associated gastric carcinogenesis, Takaishi et al. infected hypergastrinemic (INS-GAS) mice, gastrin-deficient mice (GAS-KO) on a C57BL/6 background, and C57BL/6 WT mice (B6) orogastrically with *H. felis*.

This study showed that *H. felis* infected INS-GAS and B6 mice progressed to severe corpus dysplasia, while the GAS-KO mice developed severe gastritis with mild gastric atrophy only. While mild to moderate antral dysplasia was observed in GAS-KO and B6 mice, this was absent in INS-GAS mice. Gastrin overexpression or deficiency did not alter *H. felis* colonization or Th1–Th2 polarization. The authors concluded that gastrin is an essential cofactor for gastric corpus carcinogenesis in C57BL/6 mice [28]. In a study to investigate the role of EHH in hepatobiliary cancer, Fox et al. examined the histologic profile of livers from 18–24-month-old Syrian hamsters (Group A) and the presence of *Helicobacter* spp. in paraffin-embedded ceca and liver samples using PCR. Additionally in 6-month-old hamsters (Group B), they investigated whether the presence of *Helicobacter* spp. in the intestine and liver was associated with inflammation, and cultured liver and cecal samples from a subset of Groups A and B. Five cecal isolates from Group A formed a genotypic cluster with the only liver isolate from Group B, and all were closely related to *Helicobacter* sp./flexispira taxon 8 (the *H. bilis/H. cinaedi* group). *Helicobacter*-specific DNA was detected in paraffin-embedded cecal tissue of all Group A and B mice and in the majority of paraffin-embedded liver samples of Group A. Histopathologic analysis showed chronic fibrosing hepatitis in association with Helicobacter infection in the livers of Group A mice. The authors concluded that *H. bilis* and closely related *Helicobacte*r spp. might play a role in hepatobiliary diseases in animals and humans [29]. In a further study, Fox et al. investigated the role of *H. hepaticus* in the promotion of hepatocellular carcinoma in chemical and viral transgenic mouse models in two independent studies. In the first study, *Helicobacter*-free C3H/HeN mice were either inoculated with aflatoxin (AFB1), *H. hepaticus* or AFB + *H. hepaticus or* sham inoculated. In the second study, C57BL/6FL-N/35 mice harboring a full-length hepatitis C virus (HCV) transgene were crossed with C3H/HeN mice and liver cancer rates after 40 weeks compared in mice with and without *H. hepaticus*. These studies showed that in the absence of evident hepatitis, *H. hepaticus* from its niche in the intestine, could promote tumors induced by AFB1 and by HCV. In addition, nuclear factor (NF)–kB was found to be central to signaling networks in both the bowel and the liver [30]. In a study aimed at addressing the role of Th1 immune responses in *Helicobacter*-induced disease, Stoicov et al. infected C57BL/6 and C57BL/6-T-bet knockout (KO) littermates with *H. felis* and followed them for 15 months. While T-bet KO mice and WT mice showed similar colonization levels, a significantly blunted Th1 response (reduced IgG2c/IgG1 ratio) to *H. felis* was observed in T-bet KO when compared with WT mice. Unlike WT mice that progressed over a 15-month period through metaplasia, dysplasia, and carcinoma *in situ*, T-bet KO mice maintained their parietal cell populations and did not develop dysplasia or carcinoma *in situ* [31]. Alam et al. examined the expression of CD39 and CD73 on human T helper (Th) cells, including Tregs, by stimulating Human CD4 + Th cells, gastric T cells, or Treg subsets and assaying for the expression of CD39 and CD73. This showed that CD4 + T cells expressed CD39 and CD73. Activation of CD4 + T cells significantly increased CD73 expression on all Th cells, while inhibition of CD73 enhanced production of interferon-gamma. Investigation of the role of CD73 in regulating *H. felis*-induced gastritis and density in CD73-deficient ($CD73^{-/-}$) and WT mice showed that in *H. felis* infected $CD73^{-/-}$ mice the severity of gastritis and proinflammatory cytokine levels were increased, and *H. felis* colonization levels reduced, when compared with WT mice [32]. FVB/N mice deficient in multidrug resistance gene 1a (mdr1a) gene expression developed spontaneous colitis in 3–4 months. To investigate the role of host genetic background on susceptibility to spontaneous colitis, Staley et al. backcrossed the mdr1a genetic mutation, which results in P-glycoprotein deficiency, onto a C57BL/6J mouse strain; however, these mice did not develop spontaneous colitis. To determine whether they had increased susceptibility to colitis induction following a 2^{nd} insult, B6.mdr1a$^{-/-}$ mice were treated with dextran sulfate sodium (DSS) and *H. bilis.* When compared with B6 mice treated with DSS, treated B6.mdr1a$^{-/-}$ mice had increased histologic inflammation, colonic shortening, fecal blood, and reduced body weight, while *H. bilis* treatment failed to induce colitis [33]. Gulani et al. investigated the effect of *H. hepaticus* colonization on the specific antibody and T-cell-mediated responses to intranasal inoculation with Herpes Simplex Virus (type 1), and on the phenotypic and functional characteristics of dendritic cells (DC) using *H. hepaticus*-free and infected mice. Surface expression of the maturation-associated markers CD40, CD80, CD86, and MHCII and the percentages of IL-12p40 and TNFα-producing DC in the colic lymph nodes of *H. hepaticus*-infected mice were decreased when compared with controls. The authors concluded that Helicobacter-free mice should be used in all immunologic studies [34]. In addition, Hylton et al. [35] reported chronic low levels of *Helicobacter* infection in mice to modulate the response to hemorrhage-induced intestinal damage from a complement-mediated response to a macrophage response.

Genomic and Molecular Biology Studies on Non-*H. pylori* Helicobacters

Loman et al. [36] have suggested that the current taxonomy of *H. canadensis* should be re-evaluated based on their recent sequencing of the complete genome of *H. canadensis* (type strain NCTC13241; accession number CM00776) and on observed phylogenetic discordances. Twenty-nine homopolymeric tract-associated coding regions indicative of phase variation have been identified in the *H. canadensis* genome, including five candidate transcriptional phase variable coding sequences (CDSs), 16 candidate translational phase variable CDSs, and eight candidate *C*-terminal phase variable CDSs that would impact on the function, specificity or antigenicity of the products [37]. Okoli et al. investigated protein expression profiles of *H. hepaticus* grown in bovine bile using two-dimensional gel electrophoresis and tandem mass spectrometry. Fifty-five differentially expressed proteins were identified, which were shown to be involved in a range of biologic functions including cell envelope biosynthesis, cell response to stress, iron homeostasis and transport, motility, primary and secondary metabolism, and virulence [38]. Taxonomic analysis of *H. bilis* strains isolated from dogs and cats showed two different genomic groups to be present with a suggested independent evolution that the authors proposed might be referred as two genomospecies: *H. bilis* sensu stricto and *Helicobacter* sp. 'FL56' [39]. Induction of differential gene expression profiles in the intestinal mucosa due to *H. bilis* colonization was studied using microarray analysis in defined-flora mice experimentally colonized with *H. bilis* (ATCC 51630). Up- or downregulation of genes involved in different functions was suggested to potentially predispose the host to the development of typhlocolitis [40]. Chaouche-Drider et al. conducted *in vitro* coculture studies using a murine cell line (m-IC_{cl2}) and *H. hepaticus*, *H. bilis* or *H. muridarum* and showed that each of these species induced increased gene expression of CxclI and Cxcl2, with *H. bilis* and *H. muridarum* stimulating the highest mRNA levels. Further investigation in HEK293 and AGS cells lines, neither of which expresses functional TLR2 or TLR4, showed that live *H. muridarum* had a dramatic effect on NF-KB reporter activity in HEK293 cells. The possibility that *H. muridarum* may confound studies in colitis mouse models was raised [41]. Finally, based on identification of 104 candidate structured RNAs from genome and metagenome sequences of bacteria and archaea, a newly identified *cis*-regulatory RNA was reported to be implicated in *Helicobacter* gastric infection [42]. The authors suggest that biochemical and genetic investigations are required to validate the biologic functions of the identified structured RNAs.

Effect of Dietary Compounds on *Helicobacter* spp.

In vitro and *in vivo* experiments have demonstrated the bacteriostatic and bactericidal effects of green tea against *H. felis* and *H. pylori*, as well as its ability to prevent gastric mucosal inflammation in mice when consumed prior to Helicobacter exposure [43]. Another study that evaluated the effect of dietary L-glutamine supplementation on the intestinal microbiota and mortality of postweaned rabbits reported a reduced frequency of PCR-RFLP detection of intestinal bacterial species including *Helicobacter* sp. as well as reduced mortality because of epizootic rabbit enteropathy [44].

Diagnostic Methods for *Helicobacter* Species

Based on the International Council for Laboratory Animal Science Animal Quality Network Program, the ''Performance Evaluation Program'' was designed to assist animal diagnostic laboratories in assessing their monitoring methods. The results of the first trial in the developmental phase of this program showed the successful assessment of pathogens including *Helicobacter* spp. [45]. A novel immunoblot analysis was developed to monitor *H. bilis*, *H. hepaticus,* and *Helicobacter ganmani* infections in laboratory rodents, showing promising results after its comparison with PCR-DGGE [46]. Fukuda et al. [47] reported the development of a novel antigen capture ELISA assay for the detection of *H. hepaticus* using a monoclonal antibody HRII-51, which showed 87.0% sensitivity and 97.6% specificity based on specific mouse sera. In a further study, colony sizes and spiral versus coccoid forms of *H. felis* (ATCC 49179) were reported to be influenced by gaseous growth conditions. While a 12% O_2 and 10% CO_2 atmosphere was optimal for colony size, more coccoid than spiral cells were observed [48]. Lastly, Hoosain and Lastovica reported 10 *Helicobacter* spp. (42 strains), tested using the Oxoid Biochemical Identification System Campy test (ID0800M) to be negative for the L-alanine aminopeptidase enzyme. Based on these findings, they suggested that this test may be useful for routine identification of *Campylobacter*, *Arcobacter* and *Helicobacter* species, all Gram-negative, and L-ALA-negative bacteria [49].

Conclusion

Studies published over the last year have added significantly to our understanding of non-*H. pylori* Helicobacters and their potential role in human and animal health.

Conflict of Interest

The authors have no conflicts of interest.

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