

Helicobacters of possible zoonotic origin : a review

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Abstract

Since the isolation of *Helicobacter pylori*, many new *Helicobacter* species have been identified from the gastrointestinal tract in humans and animals. In humans, a spiral organism different from *H. pylori* and provisionally named "*Helicobacter heilmannii*", has been associated with gastritis, gastric ulceration and to a lesser degree, gastric cancer. In addition *Helicobacter cinaedi*, *Helicobacter fennelliae*, *Helicobacter pullorum* and "*Flexispira rappini*" have been isolated from cases of enteric disease, bacteremia and pneumonic illness. In the biliary tract, the presence of *Helicobacter bilis*, *Helicobacter pullorum* and "*Flexispira rappini*" has been demonstrated. Morphological, epidemiological and genotypic data suggest the involvement of animal helicobacters in these infections. In this paper, a review of the literature addressing the current knowledge about epidemiology, diagnosis, pathogenesis and therapy of these infections is given.

Key words : *Helicobacter*, zoonosis, review.

Introduction

The isolation of *Helicobacter pylori* from the human stomach in 1984 (1) and its subsequent identification as a gastric pathogen, ushered a new era both in gastroenterology as in microbiology. Today, *H. pylori* is considered the primary cause of chronic gastritis, peptic ulceration and gastric neoplasia in humans (2-4). The increasing number of *Helicobacter* species identified from a wide variety of animals and from different types of ecological niches along the gastro-intestinal tract, created an awareness within the research community that the impact of these organisms is far wider than that of the human gastric environment (5). To date, the genus *Helicobacter* includes 18 validly named *Helicobacter* species and several more formally unnamed closely related organisms.

In veterinary medicine, the role of *Helicobacter* species as potential pathogens in a diverse range of pathologies is starting to be recognized. Host-specific gastric helicobacters from dogs, cats, cheetahs, ferrets, pigs and non-human primates have been associated with chronic gastritis. In pigs, the involvement of "*Candidatus Helicobacter suis*" in the onset of ulceration of the non-glandular "pars oesophagea" of the stomach is under investigation. Recently, *Helicobacter mustelae* and *Helicobacter felis* have been identified as potential gastric carcinogens. In mice, chronic hepatitis and hepatocellular neoplasia have been linked with the presence of *Helicobacter hepaticus*, while acute outbreaks of diarrhoea infections in immunodeficient

mouse colonies have been attributed to *Helicobacter bilis* and *Helicobacter rodentium*. In sheep, "*Flexispira rappini*", an intestinal helicobacter species, has been isolated from cases of abortion and acute hepatic necrosis in foetuses.

During the last decade, evidence has emerged, suggesting a possible transmission of animal helicobacters to humans. Some of these infections have been associated with different kinds of disease entities in humans. The epidemiological background and the pathogenic pathways by which these organisms act, is still largely unknown. In the following sections, an overview of the literature, addressing the current knowledge about epidemiology, diagnosis, pathogenesis and therapy of these infections, is given.

Gastric pathology

"*Helicobacter heilmannii*"

General

Soon after the discovery of *H. pylori*, gastroenterologists world-wide started to report a second type of spiral-shaped organism in the gastric (6-16) and duodenal mucosa of humans (17,18). These organisms were observed as long, tightly coiled, Gram negative bacteria, 0.4-0.9 µm in diameter, 4-10 µm in length, with 4-8 spiral turns and multiple bipolar flagellae (7,15). In the stomach, they are mostly found in the antral region where they may occur as single organisms or in small groups, located between the mucus layer and the surface cells, or deep within the lumen of the foveolae. In some cases, they have even been observed within the canaliculi of the parietal cells (13,15,19). They are less numerous than *H. pylori* and have a patchy distribution.

Originally, these long tightly coiled bacteria were thought to belong to a genus other than *Helicobacter* and were named "*Gastrospirillum hominis*" (8). However, Solnick and coworkers (20) later identified these organisms as belonging to the genus *Helicobacter*, based on phylogenetic analysis of bacterial 16S rRNA coding genes, cloned from different "*G. hominis*"-infected stomachs. Their data also suggested that at least two different species were involved. As these bacteria

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were yet uncultivated, a formal characterization following the guidelines of the International Committee on Systematic Bacteriology was not possible and as such these organisms were provisionally renamed "*Helicobacter heilmannii*" type 1 and "*Helicobacter heilmannii*" type 2 respectively, in remembrance of Konrad Heilmann, the German histopathologist who described the first large series of "*G. hominis*"-infected patients.

Pathology

Infection of the human stomach with these organisms is almost always accompanied by an active chronic gastritis, generally less severe than in *H. pylori*-infected tissues, and is presented with dyspeptic symptoms such as postprandial discomfort, epigastric pain, vomiting, heartburn and dysphagia (15,21). Glandular atrophy or intestinal metaplasia is not as common as in *H. pylori*-infections (21). Non-*H. pylori*-like spiral organisms have also been identified as a possible cause of acute gastric and duodenal ulceration, independent from NSAID use (22-27). Both types of gastric pathology resolve with clearance of these non-*H. pylori* infections, indicating a possible aetiopathogenic role. A similar finding was observed in a patient suffering from primary gastric low grade lymphoma (stage E1) (28,29), confirming earlier reports linking non-*H. pylori*-like organisms to gastric neoplasia (30-32). Experimental long-term infection of 294 mice with these bacteria, induced a lymphocytic infiltration which in 83.6% of the cases was characterized by the presence of lympho-epithelial lesions. In 13.6% of the animals these lesions progressed to a low grade MALT lymphoma and in 4.1% to a high grade MALT lymphoma (33).

Zoonosis

Based on 16S rRNA comparative data, it was shown that "*H. heilmannii*" type 2 was indistinguishable from *H. felis*, *H. salomonis* and *H. bizzozeroni*, three species isolated from cats and dogs (34,35). The "*H. heilmannii*" type 1 sequence was identical to that of "*Candidatus Helicobacter suis*", a porcine helicobacter (36) and to that of helicobacter-like organisms detected in the stomach of non-human primates (37). In 1996, a "*H. heilmannii*"-like strain was cultured for the first time *in vitro* from an infected human by Andersen and colleagues (38). Very recently, this strain was characterized as *H. bizzozeronii* based on phenotypic analysis, 16S rRNA, DNA-DNA hybridization and whole-cell protein profiling data (39). It was the first study to provide conclusive evidence that the organisms characterised as "*H. heilmannii*" were not a new human-specific *Helicobacter* species but were existing *Helicobacter* species, most probably transmitted through animals.

In a recent epidemiological survey, 802 patients infected with either *H. pylori* or "*H. heilmannii*"-like organisms (HHLO) were questioned about their contact

with animals. Subsequent logistic regression analysis, identified cats, dogs and pigs as reservoirs in the transmission of HHLO (40). For cats and dogs this came as no surprise as many other studies had reported similar findings (7,13,41-47). Other studies demonstrated the presence of *H. felis* in the human stomach either by genotyping (48) or by electronmicroscopic analysis (12).

Additional evidence linking pets directly to human "*H. heilmannii*"-infections was given by Dieterich *et al.* in a comparative study of urease coding gene sequences (*ureB*) determined from HHLO, derived from the stomachs of a "*H. heilmannii*"-infected patient and his two cats (132). Although *Helicobacter* ureases are encoded by conserved genes, intra-species gene heterogeneity has been shown (49). Despite this variability, one cat sequence was found identical to one of the human-derived sequences, while another cat *ureB* gene sequence was a perfect match of that of "*H. heilmannii*" type 2 (50).

Epidemiology

How these feline and canine helicobacters are transmitted is still unclear but a prolonged close contact is indicative. In one case report, intensive licking of a 12-year old girl suffering from chronic active gastritis by her symptomatic dog, was suggested as a possible transmission route.

In addition to pet carnivores, pigs are believed to be another source of non-*H. pylori* infections in humans, based on morphological similarities and a 99.5% 16S rDNA sequence homology between "*Cand. H. suis*" and "*H. heilmannii*" type 1 (36,51-53). Fecal-oral contact probably accounts for these infections, especially since most pigs seem to be colonised by "*Cand. H. suis*" (54).

The prevalence of HHLO-infections in adult humans is rather low when compared to *H. pylori* and ranges between 0.1 and 1.1% with an average of 0.3% (Table 1). However, in one study, a 6.2% prevalence was reported in a series of 257 Thai individuals (55). Children can also be infected by HHLO. Infections have been reported between the age of 1.5 and 19 years with a 0.3-0.4% frequency range (19,41,56-60). This indicates that an age-dependent increase in prevalence as observed in *H. pylori* epidemiology is not present in

Table 1. — "*Helicobacter heilmannii*" prevalence

Author	%	Series
Dent <i>et al.</i> , 1987 (6)	0.2	3/1300
Dye <i>et al.</i> , 1989 (7)	0.5	2/400
Queiroz <i>et al.</i> , 1990 (11)	0.3	1/315
Fisher <i>et al.</i> , 1990 (10)	0.7	4/600
Heilmann <i>et al.</i> , 1991 (15)	0.25	39/15180
Wegmann <i>et al.</i> , 1991 (13)	0.3	5/1551
Mazzucchelli <i>et al.</i> , 1993 (129)	1.1	2/175
Debongnie <i>et al.</i> , 1994 (130)	0.45	17/3800
Monno <i>et al.</i> , 1995 (131)	0.07	2/2781
Hilzenrat <i>et al.</i> , 1995 (62)	0.5	4/1223
Zhang <i>et al.</i> , 1998 (55)	6.2	16/257

HHLO infections. Nevertheless, it has been shown that HHLO are capable to survive for long periods (15). Mixed infections of HHLO and *H. pylori* are uncommon (11,14,15,61,62), suggesting competitive colonization.

Diagnosis

In vitro cultivation of HHLO both in humans as in animals is extremely difficult or even impossible, excluding microbiology as a diagnostic tool. In general, microscopical detection of HHLO either in histological sections or in smears, is the most widely used diagnostic method. However, Fawcett and coworkers described recently an experiment in which *H. pylori* assumed a morphological appearance indistinguishable of that of HHLO, indicating morphology not to be a reliable diagnostic parameter (63). A similar finding was also reported for *H. felis* (35). Recently, an ELISA-test using antigens extracted from the human *H. bizzozeronii* strain was developed and tested with serum samples of 281 Turkish blood donors, determining a prevalence as high as 6%. However, the specificity and sensitivity of this test was not validated (64). 16S rRNA-based PCR methods have recently been developed for the detection of *H. felis*, *H. bizzozeronii* and *H. salomonis* as a group (De Groote D., *et al.* unpublished data) and the specific detection of "*Cand. H. suis*" in gastric biopsy specimens (36). In a preliminary experiment performed in our laboratory, animal helicobacters could be detected in human HHLO-infected tissues with these techniques (De Groote D., *et al.* unpublished data). These studies could help resolve the epidemiological background of HHLO infections.

Pathogenesis

The pathogenic pathway by which HHLO act, is still largely unknown and depends on the species involved. Experimental infection of mice with HHLO-infected human tissues produces a chronic gastritis (65,66). With

the isolation of *H. felis* from cats, a mouse model was developed to study host-pathogen interactions. In a series of studies, it was shown that *Helicobacter felis*-induced gastritis is a cell-mediated, host-dependent process. Experimental infection of specific mouse strains (SJL, CrH/He, DBA/2, C57BL/6) with *H. felis* induced a severe to moderate gastritis in contrast to other mouse lineages (BALB/c, CBA) in which no or only a very mild gastritis was produced (67,68). Recently, evidence was provided, suggesting host T-cell response to be a critical mediator in the onset of *H. felis*-associated gastric pathology (69). In addition, cellular adhesion has been identified as an essential factor in gastric colonization. Mutant *H. felis* strains lacking genes *flaA* and *flaB*, coding for flagellar structures, are not capable to colonize the stomach in a mouse model (70). In another study, infection of rats with either *H. felis* or HHLO did not result in any significant output of gastrin or any inflammatory response. It was concluded that an increase of gastrin levels as seen in *H. pylori* was not mediated through the effects of the urease enzyme but probably is caused by inflammation (71). In *H. pylori*, the *vacA* gene and other genes located on the *cag* pathogenicity island (*cag* PAI) have been identified as important genes regulating and coding for virulence-enhancing processes (72-75). In pigs infected with HHLO resembling "*Cand. H. suis*", the presence of the *vacA* gene was demonstrated by PCR using *H. pylori*-derived primers (76). Further research is needed to detect and characterize these virulence genes in all HHLO-related *Helicobacter* species.

Treatment

Different chemotherapeutic regimens have been used with success to eradicate HHLO-infections in humans using different combinations of antibiotics and acid reducing drugs (Table 2). In an experimental study in which antibiotics were screened in "*H. heilmannii*"-

Table 2. — Therapeutic regimens used for "*Helicobacter heilmannii*" treatment

Author	Therapy	Weeks
Dye <i>et al.</i> , 1989 (7)	1. Bismuth subsalicylate 30 ml, <i>qid</i> (D0-D21) 2. Amoxicillin 500mg, <i>qid</i> (D7-D21) 3. Metronidazole 500 mg <i>tid</i> (D18-D21)	3 w
Morgner <i>et al.</i> , 1999 (28)	1. Omeprazole (40mg <i>tid</i>) 2. Amoxicillin (750mg <i>tid</i>)	2 w
Thomson <i>et al.</i> , (41)	1. Bismuth compound 2. Omeprazole 3. Amoxicillin	6 w
Oliva <i>et al.</i> , 1993 (59)	1. Bismuth compound 2. Amoxicillin 3. Ranitidine	4 w
Oliva <i>et al.</i> , 1993 (59)	1. Bismuth compound 2. Amoxicillin	2 w
Michaud <i>et al.</i> , 1995 (127)	1. Ranitidine 2. Metronidazole 3. Amoxicillin	4 w
Tanaka <i>et al.</i> , 1994 (128)	1. Cimetidine 2. Tetracycline	NM
Alhimyary <i>et al.</i> , 1994 (26)	1. Sucralfate 2. Omeprazole	NM

infected mice, amoxicillin, tetracycline and clindamycin were found effective against HHLO (77). However, as it has become clear that different *Helicobacter* species are involved in these infections, the efficiency of species-specific therapies should be evaluated. In a study by Dick-Hegedus *et al.*, *H. felis*-infected mice were treated orally using either a mono or a triple-based therapy during 4 weeks (133). Only 25% of the mice were cleared with either bismuth subcitrate or erythromycin, 47% with metronidazole, 0% with tetracycline, and 70% with amoxicillin. In contrast, triple therapy with metronidazole, amoxicillin, and bismuth subcitrate resulted in 80% eradication, whereas triple therapy with metronidazole, tetracycline, and bismuth subcitrate eradicated *H. felis* from all the animals (78). Recently, the experimental *H. felis* mouse model has been used by a number of laboratories to investigate the feasibility of immunotherapy to prevent and/or cure *Helicobacter* infection. Oral vaccination with either native or recombinant *Helicobacter pylori* urease (*rUre*) has been shown to confer long-term protection against challenge with *Helicobacter felis* in mice when co-administered with cholera-toxin (CT) or heat labile enterotoxin (LT) of *Escherichia coli* (79,80).

Enteric pathology

During the last decade, an increasing number of helicobacters has been isolated from the lower intestinal tract from a variety of mammals and birds (81-84). Some of these *Helicobacter* species have been associated with enteric disease and sepsis in humans raising questions about the zoonotic significance of these species.

Helicobacter cinaedi and *Helicobacter fennelliae*

Initially, *H. cinaedi* and *H. fennelliae* were isolated from the distal part of the bowel of human immunodeficiency virus (HIV) – infected homosexual men suffering from colitis and proctitis and were first classified as *Campylobacter cinaedi* (CLO-1A) and *Campylobacter fennelliae* (CLO-2) (85,86). Although most additional strains have since been isolated from immunocompromised patients, *H. cinaedi* and *H. fennelliae* were also recovered from immunocompetent men, women and children. In an experimental study using infant pig-tailed macaques, the pathogenic potential of these organisms was confirmed, as a diarrheal illness and bacteremia was consistently observed in infected animals (87). *H. cinaedi*-associated bacteremia and fever, accompanied by leucocytosis and thrombopenia has also been reported in natural cases involving immunocompromised humans (88-90,90-94). Although less frequently, similar cases have also been observed in association with *H. fennelliae* infections (88,89,95,96). Other reports linked *H. cinaedi* with recurrent cellulitis and arthritis (90,97-99). Successful treatment of these infec-

tions has been reported with tetracyclines, aminoglycosides (100) and ampicillin (96).

Beside from humans, *H. cinaedi* and *H. fennelliae* have also been cultured from feces of asymptomatic hamsters, dogs, cats and a macaque in whom they seem to represent normal intestinal flora (101,102).

Recently, scientific interest has focused on the possible zoonotic significance of these animal strains. In a case report, a *H. cinaedi*-strain was recovered from the blood and cerebrospinal fluid of an eight-day old neonate suffering from septicaemia and meningitis (95). During the first two trimesters of her pregnancy, the mother was exposed to hamsters. It was suggested by the authors that the neonate was infected during the birth process by the mother who may have acquired *H. cinaedi* through contact with hamsters as *H. cinaedi* strains were recovered both from the mother and the hamster. No further characterization was performed on these strains. The importance of such typing has recently been demonstrated by Kiehlbauch *et al.* (101). Using ribotyping techniques, human-derived *H. cinaedi* strains could be clearly distinguished from animal isolates. In addition, it was shown that hamster isolates exhibited a ribotype pattern different from that seen with dog and cat strains, establishing the existence of different host-specific biotypes (101). Further studies will be necessary to determine whether these different biotypes also exhibit different virulence properties and if transmission can occur between animals and humans.

Helicobacter pullorum

A group of campylobacter-like organisms (CLO) were isolated from the caeca of asymptomatic poultry and from the livers and intestinal contents of laying hens presented with hepatic lesions performing polyphasic taxonomical analysis, these were identified as a new helicobacter species and were named *Helicobacter pullorum* (81). In the same study, 6 CLO- strains, isolated from different patients suffering from mild to severe diarrhoea, were also characterized as *H. pullorum* strains, indistinguishable from those isolated from poultry, suggesting a possible zoonosis. Both immunocompetent and immunocompromised individuals were involved (103). Recently, a possible transmission route was identified by Atabay *et al.* as they recovered *H. pullorum* from the carcasses of poultry with a prevalence as high as 60% (104). The fact that *Campylobacter jejuni*, a close relative of *H. pullorum*, has been identified as a major food-borne pathogen and more *H. pullorum* associated cases are described (105), raises questions about the importance of *H. pullorum* in human gastrointestinal disease.

“*Flexispira rappini*”

Originally, “*Flexispira rappini*” was isolated from aborted sheep fetuses (106,107). It has been classified as a *Helicobacter* species based on 16S rRNA sequence

analysis (108,109), although a formal description of these organisms has never been published. In humans, it has been isolated from patients suffering from either mild chronic diarrhoea (110), bacteremia (111,112) or pneumonia (113) involving both immunocompetent and immunocompromised individuals. Dogs, cats and rabbits were identified as possible sources for these infections (111,113,114). In one case, "*Flexispira rappini*" was recovered from the puppy of an infected patient (110), while in two other reports the patient's disease coincided with the introduction of a puppy in the household (111,113). Treatment with either erythromycin (113), meropenem (111) or a combination of gentamicin and imipenem (115) resulted in eradication of "*Flexispira rappini*" and subsequent clearance of symptoms.

Recent phylogenetic analysis of 28 "*Flexispira rappini*" strains showed that they represented 9 different species (F. Dewhirst, J. Fox, B. Paster; unpublished observations). This finding illustrates the importance of a well-defined characterization of such strains if we want to learn more about the significance of these organisms.

Hepatic pathology

In recent years, new *Helicobacter* species have been isolated from the livers of a wide variety of animals and have been associated with hepatic disease. *H. hepaticus*, which consistently colonizes the cecum and colon of mice, has been associated with liver tumors in A/JCr mice as well as hepatitis in other susceptible inbred mouse strains (83,116,117). Local urease-induced ammonia (118), the production of cytolethal toxins (119) and the involvement of autoimmune mediated processes (116,120) have been suggested as possible pathogenic pathways.

In addition to *H. hepaticus*, other *Helicobacter* species can colonize the hepatobiliary tract of animals. "*Flexispira rappini*" has been shown to cause hepatic necrosis in fetuses of sheep and guinea pigs (107,114,121). *H. bilis* is originally associated with hepatitis in aged inbred strains of mice (84), but has also been isolated from the gastrointestinal tract of asymptomatic gerbils, dogs and cats (F. Dewhirst, J. Fox, B. Paster; unpublished observations). Other species linked to hepatitis are *H. canis* and *H. pullorum* in dogs and chickens respectively (81,122). In hamsters, cholangiofibrosis and centrilobular pancreatitis are believed to be caused by *H. cholecystus* (123). A common characteristic observed in these species is their ability to grow in the presence of bile in contrast to other non-hepatic helicobacters.

In a recent study by Fox *et al.*, 16S rDNA sequences of three different *Helicobacter* species could be determined from bile and gallbladders of patients suffering from chronic cholecystitis (118). *In vitro* cultivation was unsuccessful. Five sequences represented strains of *H.*

bilis, two of "*Flexispira rappini*" and one strain of *H. pullorum*, suggesting a possible role of animals as reservoirs for these kind of infections. Indirectly, these results were confirmed by two other studies in which DNA of *Helicobacter*-like organisms was sequenced from the biliary tract of humans (124,125). Although these sequences were believed to originate from *H. pylori*, this latter finding was questioned by Fox *et al.* (118).

These results strongly indicate the involvement of *Helicobacter* species in biliary tract related disease. However, the evidence provided is still circumstantial and needs further clarification (126). Further studies are also required to determine the origin of the different *Helicobacter* species observed in the human biliary tract.

Discussion

Review of the literature learns that *H. pylori* is not the only member of the genus *Helicobacter* threatening human health. A growing number of different helicobacters have been identified from the human gastrointestinal tract in association with disease.

In gastric pathology, the involvement of *Helicobacter* species other than *H. pylori* has been clearly demonstrated. The recent isolation of *H. bizzozeronii* from a "*H. heilmannii*" infected patient strongly suggests pet carnivores to be a possible source for these infections but needs further clarification. More studies are equally required to assess the risk of porcine gastric helicobacters as potential food-borne pathogens.

The recent recovery of different *Helicobacter* species from both immunocompromised as immunocompetent patients suffering from enteric and hepatic disease raises questions about the impact and the origin of these infections. Most of the *Helicobacter* species characterized in these cases have also been identified in a variety of animals. In addition, close contact with animals was reported in some cases. However, evidence linking animals directly to these enteric infections has not yet been provided.

Better methods to subtype the different strains isolated from both humans and animals are required to identify the source of these infections. In the same sense, an increased awareness among gastroenterologists and the implication of better and adapted diagnostic methods could help resolve the epidemiological background and identify critical points of entry in animal-to-human relationships.

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