

REVIEW: GASTRIC AND ENTEROHEPATIC HELICOBACTERS OTHER THAN *HELICOBACTER PYLORI* – THE EVOLVING IMPORTANCE OF NON-*HELICOBACTER PYLORI* *HELICOBACTER* SPECIES FOR PUBLIC AND ANIMAL HEALTH

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Abstract – From April 2022 to March 2023, a lot of new evidence was published concerning non-*Helicobacter pylori* *Helicobacter* (NHPH) species and their significance for public and animal health. Five new enterohepatic *Helicobacter* species were described, including *H. anatolicus* and *H. kayseriensis* in urban wild birds, *H. turcicus* in Anatolian ground squirrels, *H. colisuis* in domestic pigs and *H. kumamotonensis* in a human patient. Also, the genomic difference between the human-associated *H. cinaedi* and animal-associated *H. canicola* was established. A potential pathophysiological role for gastric NHPHs was revealed in a Western *H. pylori*-negative cohort study of patients with gastric symptoms, and many case reports were published regarding enterohepatic *Helicobacter* bacteremia in human patients. The public health significance of *H. pullorum* was also an important topic. The significance of gastric and enterohepatic *Helicobacter* infections in animals and the importance of animals as reservoirs were further investigated with cohort studies in pigs and wild boars, dogs, cats, non-human primates, wild mice, and poultry. Experimental infection with NHPHs was still frequently used in several mouse models. In multiple *in vivo* studies, changes in the relative abundance of enterohepatic *Helicobacter* species related to gut microbiota alterations were observed. Novel diagnostic tests were developed, and novel therapeutic strategies were explored in the fight against antibiotic resistance. Finally, new insights into potentially important proteins and virulence mechanisms were obtained.

Keywords: Gastric *Helicobacter* species, Enterohepatic *Helicobacter* species, Non-*Helicobacter pylori* *Helicobacter*, Animal model, Zoonosis, Pathogenesis, Microbiota.



DESCRIPTION OF NEWLY IDENTIFIED SPECIES

In 2022, five novel enterohepatic *Helicobacter* (EHH) species were identified and described. Details on the characteristics of these species are presented in Table 1.

GENOMICS AND GENETICS

Since little was known about the genetic differences between *H. cinaedi* (an established human pathogen) and *H. canicola* (reported to be *H. cinaedi*-like organisms isolated from animals), two distinct EHHs, genome sequence analysis was performed for strains belonging to the *H. cinaedi/canicola/magdeburgensis* complex⁵. *H. cinaedi* sensu stricto (s.s.) was found to represent a distinct clade of a human-adapted lineage within the complex. Furthermore, a method was developed which allows to identify *H. cinaedi* sequences in large metagenome data sets, such as in human fecal metagenome samples.

The draft genome sequence of an unclassified *Helicobacter* strain, designated CaF467b, isolated from a pig manure storage tank in Canada was published⁶. Based on 16S rRNA sequencing, this strain presented the highest similarity to *H. canadensis* (strain MIT 98-5491). On the other hand, average nucleotide identity analysis showed 90.3% similarity of the draft sequence to the reference genome of *H. canadensis* and 98.8% to the draft genome of *Helicobacter* sp. strain 11-8110.

NON-HELICOBACTER PYLORI HELICOBACTER INFECTIONS IN HUMANS AND PUBLIC HEALTH SIGNIFICANCE

Gastric non-*Helicobacter Pylori Helicobacter* Infections

In the past year, the clinical relevance of gastric non-*Helicobacter pylori Helicobacter* (NHPH) species with a zoonotic potential was further explored, and a pathophysiological role for NHPHs in gastric disease was suggested.

In a retrospective cohort of 464 patients presenting with chronic gastritis, peptic ulcer disease or mucosa-associated lymphoid tissue (MALT) lymphoma, which were *a priori* diagnosed as *H. pylori*-negative, gastric NHPHs were detected in 135 (29.1%) of them⁷. In contrast, no gastric NHPHs were detected in a gastric bypass control cohort. The canine/feline gastric NHPHs *H. bizzozeronii* and *H. felis* were the most prevalent species, followed by *H. suis*. Mixed NHPH infections were detected in 17 patients (3.7%). In addition, 65 patients were included prospectively, out of whom 18 (27.7%) presented with gastric NHPH infection. Clinical remission following eradication therapy was reported in 12/17 (70.6%) patients and histological remission in seven out of nine (77.8%). Furthermore, the study confirmed that PCR combined with sequencing in gastric biopsy specimens is the superior diagnostic method for gastric NHPHs.

In Mexico, 59 out of 168 (39%) paediatric patients presenting with dyspeptic symptoms were positive for gastric NHPHs, out of whom 35 presented with co-infections with NHPHs and *H. pylori* or different NHPHs⁸. The canine/feline gastric NHPHs *H. bizzozeronii* and *H. felis* were reported to be the most prevalent species (22% and 18%, respectively), followed by *H. salomonis* and *H. suis*. The same research group further reported on the same topic, but it is, however, not clear whether these patient cohorts overlap⁹. Out of 187 paediatric patients with gastrointestinal symptoms, 47 (25%) were positive for gastric NHPHs, and a positive correlation was found between gastrointestinal bleeding and mucosal erosions in the gastric body. It was further highlighted that neither endoscopic nor clinical findings could distinguish between *H. pylori* and gastric NHPH infection, making molecular analysis for gastric NHPH detection crucial.

Three case reports added further evidence for the pathophysiological role of gastric NHPHs in gastric patients. Key findings from these cases are summarized in Table 2.

A literature review regarding *H. heilmannii* s.s.-associated gastritis in patients with dyspepsia further demonstrated a worldwide prevalence of 1.9%, with a higher prevalence in Asian compared to Western countries (3.1% vs. 1.2%, respectively)¹³. Typically, *H. heilmannii* s.s. infection was associated with focal, chronic gastritis which was less active compared to *H. pylori*.

TABLE 1. CHARACTERISTICS OF NEWLY IDENTIFIED NON-*HELICOBACTER PYLORI* *HELICOBACTER* SPECIES.

Species name	Isolated from	Urease	Catalase	Closest relative	Type strain	References
<i>H. anatolicus</i>	Faeces of urban wild birds (crow, magpie and sparrow)	+	+	<i>H. mustelae</i> (98.5-98.6% 16S rRNA sequence similarity)	Faydin-H8 ^T (= LMG 32237 ^T = DSM 112312 ^T)	Aydin et al ¹
<i>H. kayseriensis</i>	Faeces of urban wild birds (magpie)	-	+	<i>H. pamatensis</i> (98.5% 16S rRNA sequence similarity)	Faydin-H23 ^T (= LMG 32236 ^T = CECT 30508 ^T)	Aydin et al ¹
<i>H. turcicus</i>	Faeces of Anatolian ground squirrels	-	-	<i>H. ganmani</i> (97.0-97.1% 16S rRNA sequence similarity)	Faydin- H70 ^T (= DSM 112556 ^T = LMG 32335 ^T)	Aydin et al ²
<i>H. colisuis</i>	Caecal contents of domestic pigs	-	+	<i>H. canadensis</i> (≥ 99.8% 16S rRNA sequence similarity)	11154-15 ^T (= DSM 113688 ^T = CCUG 76053 ^T)	Gruntar et al ³
<i>H. kumamotonensis</i>	Blood of a human patient suffering from diffuse large B-cell lymphoma	-	+	<i>H. equorum</i> (99.1% 16S rRNA sequence similarity)	PAGU 1991 ^T (= GTC 16810 ^T = CCUG 75774 ^T)	Kawamura et al ⁴

+ = Positive, - = Negative.

TABLE 2. CASE REPORTS ON NATURAL GASTRIC NON-*HELICOBACTER PYLORI* *HELICOBACTER* INFECTIONS IN HUMANS.

<i>Helicobacter</i> species	Patient characteristics	Associated disease	Main findings	References
<i>H. suis</i>	45-year-old, <i>H. pylori</i> -negative, Japanese male	Gastritis	Endoscopic features indistinguishable from <i>H. pylori</i> -associated atrophic gastritis	Iwamuro et al ¹⁰
<i>H. suis</i>	68-year-old male with elevated CA 19-9 levels	Chronic gastritis & dyspeptic symptoms	Along with eradication of <i>H. suis</i> , CA 19-9 levels completely normalized	Capirchio et al ¹¹
<i>H. ailurogastricus</i>	61-year-old, Japanese male	Multiple refractory gastric ulcers	- Isolated strain resistant to levofloxacin + carried mutation conferring resistance to fluoroquinolones - Infection possibly transmitted through patient's cats	Sano et al ¹²

CA = carbohydrate antigen.

Another review of the public health significance of dog-, cat- and pig-associated gastric NPHs concluded that a lot remains to be elucidated, including global prevalence rates, exact modes of transmission and mechanisms of action underlying disease development and progression¹⁴.

Enterohepatic *Helicobacter* Infections

The public health significance of several EHHs was also further investigated. Noteworthy, several case reports were published concerning severe EHH bacteraemia (Table 3) and awareness for these EHH infections was raised since they are often overlooked. This was highlighted in a study in which, *H. cinaedi* was detected as the causative bacterium in four of ten patients with infected aortic aneurysms¹⁵. All four patients showed good clinical courses. Median blood culture growth time was 90.6 hours, and levofloxacin-resistance was established.

A laboratory in France also took a closer look at blood cultures that were considered false positive based on a positive evaluation by the automated diagnostic system, but a negative evaluation by microscopy and subculture¹⁶. These false positives were mainly due to hyperleukocytosis. In one false positive case, *H. cinaedi* bacteraemia was finally diagnosed through *16S rRNA* and nanopore sequencing in a patient with digestive problems, fever and rash.

In an Egyptian study, *H. pullorum* was detected in 14 out of 300 chicken meat samples (4.67%), applying PCR techniques to DNA from pure cultures, but not in swabs from chicken processing surfaces²¹. Phylogenetic analysis of two *H. pullorum* isolates showed a 100% similarity with an isolate from a human patient with gastroenteritis and 99.8% similarity with an isolate from the cirrhotic liver of a hepatitis C patient, suggesting its potential as a food-borne zoonotic pathogen. *H. pullorum* further presented high resistance towards erythromycin and ciprofloxacin.

The public health significance of *H. pullorum* was also highlighted in a literature review, particularly in developing countries. In Iran, prevalence rates of *H. pullorum* in chicken meat and in the human population ranged from 0 to 49% (depending on the type of meat samples and the applied methods (PCR and/or culture)) and from 0% (using PCR methods only) to 12% (using both culture and PCR methods), respectively. The overall occurrence rate in such samples appeared higher compared to the global occurrence rate, considering, for instance, figures obtained in Portugal, Spain and Belgium²².

TABLE 3. CASE REPORTS ON NATURAL ENTEROHEPATIC *HELICOBACTER* BACTERAEMIA IN HUMANS.

<i>Helicobacter</i> species	Patient characteristics	Associated symptoms and disease	Main findings	References
<i>H. canis</i>	49-year-old immunocompromised female	2-week history of pain, swelling and erythema of the left ankle and calf, malaise and nausea associated with cellulitis	- Cellulitis under control via an antibiotic combination regimen - Infection possibly transmitted through patient's pet dog	van Wezel et al ¹⁷
<i>H. troglontum</i>	22-year-old male with XLA	Fever, malaise and skin lesions	Several intravenous and oral antibiotic regimens administered for 10 months, each resulting in (para)clinical remission, although relapse occurred multiple times	Fjordside et al ¹⁸
<i>H. cinaedi</i>	23-year-old male with XLA	Erythematous scaly eruption adjacent to the chest port for antibiotic therapy + history of non-responsive cellulitis	2 skin lesion-free years after 6-month therapy with oral doxycycline, after which the patient again presented with a 2-day fever and newly appearing skin lesions	Lenskaya et al ¹⁹
<i>H. cinaedi</i>	61-year-old male treated with ibrutinib in the context of chronic lymphocytic leukaemia	Recurrent multifocal cellulitis	- <i>H. cinaedi</i> infection identified through blood culture incubation and 16S rRNA sequencing from single bacterial colonies - Clinical remission following discontinuation of ibrutinib and 6 weeks of amoxicillin/clavulanate, without relapse after 6 months	Roupie et al ²⁰

XLA = X-linked agammaglobulinemia.

Natural non-*Helicobacter pylori* *Helicobacter* Infections in Animals

As a first point of note, a study showed that Portuguese veterinarians are in general badly informed about the animal and public health significance of gastric NHPHs such as *H. suis*²³.

Pigs and Wild Boars

A study in domestic pigs with the majority suffering from gastritis, found *Helicobacter* species in 56 out of 71 (78.9%) pig stomachs, including *H. suis* (57.7%), *H. pylori*-like organisms (50.7%) and sporadically *H. salomonis* and *H. felis* (2.8% each)²⁴. Species described as *H. pylori*-like organisms were mainly detected in the *pars oesophagea* and were positive in a *ureAB* based *H. pylori*-specific PCR, but not in a *glmM* based *H. pylori*-specific PCR, and therefore deserve further investigation. Also in this study, *H. bizzozeronii*, *H. salomonis*, *H. pylori*-like organisms and *H. suis* were detected in four, four, two and one out of 14 wild boars, respectively.

Dogs

In a cohort of 84 pet dogs presenting with gastrointestinal symptoms, *Helicobacter* species using PCR in gastric biopsy specimens were detected in 71.4%²⁵. Here, *H. heilmannii* s.s. was most

prevalent. Confirming findings of previous studies, there was no association between gastric NHPH infection and the occurrence of gastritis. However, mono-infections showed a higher risk for more severe inflammation compared to mixed infections (possibly due to the lack of competitive inhibition) and gastric NHPH infections were associated with lymphoid follicular hyperplasia. Although species-specific PCR was confirmed to be the preferred diagnostic method, cytology and the rapid urease test were also found to be possibly reliable and suitable methods.

A case report described *Helicobacter* gastritis, based on histopathological findings, in an eight-year-old female neutered golden retriever²⁶. The dog presented with chronic vomiting and multiple raised mass-like lesions in the fundus that were evident upon ultrasonography and endoscopy. Following eradication therapy, clinical and histological remission was obtained.

In Taiwan, faecal samples from 95 dogs were collected for *Helicobacter* analysis²⁷. EHHs were detected in 50 (including *H. canis*, *H. canicola*, *H. bilis*, *H. typhlonius*, *H. winghamensis*, *H. canadensis*, *H. cinaedi* and two unclassified species) and gastric *Helicobacters* in 20 (including *H. pylori* and *H. heilmannii* s.s.) dogs, using PCR and sequencing methods. Being a stray dog and mixed-breed were identified as risk factors for *Helicobacter* infections. Given the zoonotic potential of the species identified, awareness for a public health risk was raised.

A study was conducted in dogs diagnosed with EHH infection in which the association between expression of the pro-inflammatory response regulator IL-1R8 in the large intestine as a potential biomarker, and chronic enteropathy was assessed²⁸. IL-1R8 was significantly downregulated in acute and chronic cases compared to healthy controls and was decreased in case of higher *Helicobacter* colonization levels as measured by qPCR. Also, expression of IL-1R8 negatively correlated with the severity of macroscopic lesions and hyperplasia.

Cats

A study in 71 cats, including 17 without and 54 with histopathological alterations of the gastric mucosa representative of gastritis, further added to the evidence for the absence of a pathophysiological involvement of gastric NHPHs in gastritis in cats²⁹. Gastric NHPHs detected in 53 of these cats (74.6%), through histological evaluation and/or PCR on DNA extracts, included *H. salomonis*, *H. heilmannii* s.s., *H. felis* and *H. bizzozeronii*. These species were therefore suggested to be able to highly adapt to the feline gastric microenvironment or even to possibly belong to the gastric microbiome.

Another study investigated the immunoexpression of ezrin in parietal cells of domestic cats diagnosed with *Helicobacter* infection and chronic gastritis³⁰. Ezrin is a membrane cytoskeletal crosslinker protein involved in stimulation dependent processes of membrane remodelling in epithelial cells and suggested to play a role in tumour progression and metastasis³¹. Although there was no association between *Helicobacter* infection and ezrin expression, ezrin expression was associated with the intensity of the inflammatory infiltrate in the gastric mucosa and was, therefore, higher in chronic gastritis.

Non-Human Primates

In faecal samples of a captive colony of olive baboons, the presence of *Helicobacter* species most closely related to *H. fennelliae* was reported based on PCR and sequencing³². *H. fennelliae* is a human-associated EHH that had not been reported in non-human primates before. The inspection for *Helicobacter* species in this colony was prompted due to a case of regurgitation and hematemesis during anaesthesia. In this case, eradication of this *Helicobacter* species resulted in clinical remission, however, eradication in eight other baboons was unsuccessful.

Wild Mice

Since the house mouse is a prominent model for microbiota-host interactions, a study was performed to investigate the role of gastrointestinal microbiota in mouse speciation³³. Using standard 16S rRNA microbiota profiling, a clear difference was observed between the gut microbiomes of *Mus musculus* and *Mus musculus domesticus*. Indeed, this subspecies-specific difference was

mainly driven by abundances of several *Helicobacter* species (including *H. ganmani*, *H. typhlo-nius* and *H. hepaticus*), which could be traced at species and strain level. Two *H. ganmani* strains showed a signal of co-divergence within the subspecies.

Next-generation sequencing in caecal samples of striped field mice from Korea revealed that *H. rodentium* was present in all 48 cases, *H. aurati* in 38 and *H. fennelliae* in one, with *H. rodentium* being relatively less abundant during spring compared to fall³⁴.

Poultry

Microbiota analyses of caecal contents of free-range chickens showed the presence of *H. pullorum* in 16 out of 18 cases, with a significantly higher relative abundance in indoor (2.46%) compared to outdoor (0.52%) chickens³⁵. *H. pullorum* was negatively correlated with 17 bacterial species of which several are associated with a healthy gut, suggesting that outdoor access may play an important role in gut health.

Varriale et al³⁶ reported similar findings in caecal samples of free range grown chickens, of which the outdoor group showed a significant decrease in *H. pullorum* colonization compared to the indoor group. In addition to the gut health benefit of outdoor access, it was suggested that the lower abundance of *H. pullorum* at slaughter age reduces the risk of spreading this food-borne zoonotic pathogen. Furthermore, mutual exclusion analysis pointed towards a role for *Bacteroides barnesiae*, an uncultured organism of the genus *Synergistes* and *Bacteroides gallinaceum* in reducing the relative abundance of *H. pullorum*. Competitive exclusion strategies may thus be of interest.

H. pullorum was also detected in gastric samples of two out of 40 broiler chickens and one out of ten resident wild birds (domestic pigeons) through PCR and sequencing, indicating that these animals may be important reservoirs³⁷.

EXPERIMENTAL INFECTION WITH NON-HELICOBACTER PYLORI HELICOBACTER SPECIES

In different mouse models, experimentally induced NHPH infection was performed to further investigate the pathogenic mechanisms associated with *Helicobacter* infection. On the other hand, experimental *H. hepaticus* infection was conducted to generate different colitis mouse models for inflammatory bowel disease (IBD) research. Studies in which experimental NHPH infection was performed are summarized in Table 4.

As reviewed by Hand et al⁴⁶, *H. hepaticus* infection in immunocompetent mice reduces susceptibility to colitis and intestinal tumour formation. This was suggested to be related to a complex relationship between bacterial colonization, taking into account the genomic, locational and functional characteristics of *H. hepaticus*, and the local host immune response.

Another literature review described the innate and adaptive immune responses elicited by *H. bilis*, which is a known opportunistic pathogen in humans, and its related mechanisms of action in rodent models where the bacterium triggers IBD⁴⁷.

The role of non-*Helicobacter pylori* *Helicobacter* Species in gut Microbiota Alterations in In Vivo Research

Several *in vivo* studies reported shifts in the relative abundance of EHHs as part of the gut microbiome (Table 5). These included *H. hepaticus* and *H. rodentium*, naturally colonizing the gut of mice, although they were never the only bacteria showing relative abundance alterations. The specific roles of these shifts in EHH abundance remain to be further investigated.

NOVEL DIAGNOSTIC TECHNIQUES AND THERAPEUTIC STRATEGIES

Diagnostics

A novel primer set for a 16S rRNA based *Helicobacter* genus-specific PCR assay was developed and its sensitivity and specificity turned out to be superior compared to nine other, existing assays⁵³.

TABLE 4. EXPERIMENTAL NON-*HELICOBACTER PYLORI* *HELICOBACTER* INFECTIONS IN MOUSE MODELS.

<i>Helicobacter</i> species	Conditions	Research outcome	Main findings	References
Experimentally induced NHPH infection for <i>Helicobacter</i> research				
<i>H. suis</i>	WT	Effect of acid suppressants on the viability of NHPHs within parietal cells	Vonoprazan induced NHPH damage through a pH increase	Nakamura et al ³⁸
<i>H. felis</i>	WT and Tlr9 ^{-/-}	Role of TLR9 in the initiation of gastric inflammation and hyperplasia in <i>H. pylori</i> gastric tumorigenesis	- WT: Tlr9 expression ↑ & TLR9-dependent NF-κB and ERK1/2 MAPK signalling pathway suggested to contribute to gastric pathogenesis - KO: Significantly protected against <i>H. felis</i> -induced gastric inflammation and hyperplasia, despite elevated <i>H. felis</i> colonization	Tang et al ³⁹
<i>H. felis</i>	WT and Aim2 ^{-/-}	Role of the AIM2 inflammasome in cancerous and pre-cancerous gastric disease related to GH infection	- WT: Upregulated <i>Aim2</i> expression - KO: Less severe gastric inflammation and hyperplasia & ↓ levels of inflammasome activity and the mature inflammasome effector cytokine IL-1β	Dawson et al ⁴⁰
<i>H. hepaticus</i>	Rotenone-treated human α-syn gene transgenic PD mouse model	Role of <i>H. hepaticus</i> (abundantly present in PD faecal microbiome) in the gut-brain-axis	Induction of α-syn pathology, gut and brain inflammation and motor dysfunctions in an asparagine endopeptidase-dependent way	Ahn et al ⁴¹
Experimentally induced <i>H. hepaticus</i> infection for IBD research				
<i>H. hepaticus</i>	IL-10 ^{-/-}	Development of a colitis mouse model facilitating research in <i>C. difficile</i> infection in IBD	Infection triggered gut inflammation which drove alterations of the microbiota composition, making the mice susceptible to <i>C. difficile</i> infection without antibiotic pre-treatment	Barron et al ⁴²
<i>H. hepaticus</i>	IL-10 ^{-/-}	Impact of raw broccoli sprouts in an IBD mouse model	Sprout-sourced sulforaphane positively impacted enterocolitis related symptoms and gut microbiota composition	Holcomb et al ⁴³
<i>H. hepaticus</i>	IL-10 ^{-/-} and IL-17a ^{-/-}	Role of IL-17A in IBD and in the development of a colitis mouse model	- Infection in the IL-17a ^{-/-} model triggered moderate colitis through impaired intestinal barrier integrity and upregulated gut inflammation - IL-17A may play a role in epithelial repair and crypt development, thereby suppressing colitis following <i>H. hepaticus</i> infection	Zhu et al ⁴⁴
<i>H. hepaticus</i>	Stat1 ^{-/-}	Role of IL-10R/IFN-γR/STAT1 pathway in controlling inflammatory macrophage accumulation in IBD	Impaired colonic macrophage accumulation in the infected model	Patik et al ⁴⁵

NHPH = non-*Helicobacter pylori Helicobacter*, WT = wild type, TLR = toll-like receptor, KO = knockout, AIM = Absent In Melanoma, GH = gastric *Helicobacter*, α-syn = α-synuclein, PD = Parkinson's Disease, IBD = inflammatory bowel disease, IL = interleukin, STAT = Signal transducer and activator of transcription, IFN = interferon.

TABLE 5. NON-HELICOBACTER PYLORI HELICOBACTER RELATED GUT MICROBIOTA ALTERATIONS IN RODENT MODELS.

Rodent model	NHPH related microbiota alteration	Main findings	References
Intestinal goblet cell <i>Piezo1</i> ^{-/-} mouse model	Abundance <i>H. hepaticus</i> ↑	Disturbed intestinal mucus layer and gut microbiota balance	Liu et al ⁴⁸
Mouse model simulating a microgravity environment	Abundance <i>H. hepaticus</i> ↓ (among 10 other bacteria)	Impact of these gut microbiota composition alterations for astronauts remains to be investigated	Khan et al ⁴⁹
Mice treated with fluconazole for gut fungi depletion (used as a research model for drug-induced liver injury)	Abundance <i>H. rodentium</i> ↑	Significance of this enteric bacteria-fungi interaction needs further investigation	He et al ⁵⁰
Ovariectomized osteoporotic rats	Abundance <i>H. rodentium</i> ↑	Gut microbiota shifts reflected those associated with estrogen deficiency induced osteoporosis	Wang et al ⁵¹
AD rat model administered Jiedu-Yizhi formula, a Chinese herbal prescription for AD treatment	Abundance of <i>Helicobacter</i> species, and particularly of <i>H. rodentium</i> ↓	These gut microbiota shifts may contribute to the neuroprotective effect of the formula	Wang et al ⁵²

AD = Alzheimer's Disease.

Butt et al⁵⁴ developed and successfully validated a multiplex PCR able to detect *H. hepaticus*, *H. bilis*, *H. typhlonius*, *H. pylori*, *H. muridarum*, *H. pullorum*, *H. cinaedi*, *H. heilmannii* s.s. and *C. jejuni* in a high-throughput manner, enhancing routine health monitoring in laboratory mice.

A non-invasive diagnostic test was developed to simultaneously detect antibodies against *H. suis* and *H. pylori* in a reliable manner⁵⁵. This is an ELISA test which was also able to measure the decline in anti-*H. suis* antibodies in the serum following eradication therapy. A limitation of the test were the low positive predictive values (76.9% for *H. suis* and 65.2% for *H. pylori*).

Therapeutics

The antibacterial activity of newly synthesized *Paraclostridium benzoelyticum* bacterium-mediated zinc oxide nanoparticles (ZnO-NPs) was investigated against *H. suis*, *H. bizzozeronii*, *H. felis* and *H. salomonis*⁵⁶. These ZnO-NPs generate reactive oxygen species (ROS) resulting in microbe cell wall damage, causing membrane permeability, DNA damage and mitochondrial dysfunction, which leads to cell death. All *Helicobacter* species tested presented good inhibitory zones when introduced to five mg/mL of the ZnO-NPs, which is promising in the fight against antibiotic resistance.

Also, in light of combatting antibiotic resistance, a promising multi-epitopes vaccine candidate was proposed to prevent dissemination of *H. cinaedi*, using *in silico* techniques⁵⁷. To this end, pan-genome analysis of published *H. cinaedi* strains was performed, revealing TonB dependent receptor, flagellar hook protein FlgE, Hcp family type VI secretion system effector and flagellar motor protein MotB as potential vaccine candidates. Subsequently, epitopes were mapped and prioritized, physicochemical properties were determined, interaction with the host immune system was simulated, and molecular docking and molecular dynamic simulations were performed, among other computational analyses.

Functional and Evolutionary Analysis of Proteins and Virulence Mechanisms

Since virulence mechanisms of NHPHs have not been fully unraveled yet, several studies aimed to elucidate the roles of specific proteins, genes, other potential virulence factors and the evolution thereof within the genus *Helicobacter*. Such research is increasingly being done via *in silico* techniques. Table 6 presents a summary of these research findings achieved over the past year.

TABLE 6. RESEARCH RELATED TO FUNCTIONAL AND EVOLUTIONARY ANALYSIS OF PROTEINS/GENES AND VIRULENCE MECHANISMS IN NON-*HELICOBACTER PYLORI* *HELICOBACTER* SPECIES.

Investigated protein (family)/genes/virulence	Associated <i>Helicobacter</i> species	Applied methods	Main findings	References
HcaA (a novel autotransporter protein)	<i>H. cinaedi</i>	<i>In vitro</i> and <i>in vivo</i> experiments using HcaA KO and recombinant strains	HcaA is an important factor in host cell adhesion	Aoki et al ⁵⁹
Hemolysin co-regulated protein (Hcp)	T6SS positive <i>H. pullorum</i>	In silico techniques and <i>in vitro</i> adhesion and invasion assays using HepG2 cells	- Hcp could be both a structural and secretory protein according to molecular structure - Hcp possibly involved in bacterial internalization into host hepatocytes, thereby promoting persistence of infection	Javed et al ⁶⁰
Virulence mechanisms in cholangiocytes	<i>H. bilis</i>	Coculture experiments with MMNK-1 cells	IL-6 and IL-8 secretion ↑ Cell proliferation ↑ ROS without DNA damage → Since chronic inflammation and oxidative stress are risk factors for cholangiocarcinoma, further investigation was suggested	Yamashita et al ⁶¹
Evolution of histidine-rich proteins (HRPs) involved in nickel storage	GHs and EHHs	Bioinformatics, proteomics, and phylogenetics of 18 GHs, 11 EHHs and 10 other Epsilonproteobacteria	In contrast to EHHs, GHs were found to be enriched in HRPs, particularly HRPs with C-terminal histidine-rich domains, possibly linked to the presence of the urease nickel enzyme	Fischer et al ⁶²
Genes for the chemotaxis methylation adaptation enzymes CheB and CheR (absent in <i>H. pylori</i>)	GHs and EHHs	In silico techniques	- Genes for CheB and CheR absent in gastric NHPHs, but EHHs have retained them - CheB/CheR chemoreceptor target methylation sites less conserved in GHs compared to EHHs	Liu et al ⁶³
Gain and loss events in virulence factor genes	GHs and EHHs	Computational analysis of the complete genomes of 22 <i>Helicobacter</i> species	- Gene-specific gains and losses support a process of molecular adaptation from EHHs (ancestral species) to GHs - Many genetic changes related to host adaptation and pathogenic phenotype	Prada et al ⁶⁴

KO = knockout, T6SS = Type six secretory system, IL = interleukin, ROS = reactive oxygen species, GH = gastric *Helicobacter*, EHH = enterohepatic *Helicobacter*, NHPH = non-*Helicobacter pylori Helicobacter*.

The motility of different gastric *Helicobacter* species and strains was reviewed as well, including respective swimming characteristics according to their different flagellar architectures and numbers, and cell shapes⁵⁸.

CONCLUSIONS

In summary, NHPH research has again provided many new insights in the past year, especially regarding the potential public health significance of several gastric NHPHs and EHHs. Zoonotic infections with these *Helicobacter* species bring together veterinary and human health research. Policymakers should provide a venue for integrated and global approaches to zoonoses and public health. Continued efforts to further elucidate the pathogenicity of NHPHs is proving to be of great importance for obtaining that goal.

Conflict of Interest

None of the authors have any conflicts of interest to disclose.

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Informed Consent

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Authors' Contribution

ET performed the literature search, wrote the original draft manuscript and reviewed and edited the manuscript. CVS and FH supervised the project and reviewed and edited the manuscript. All authors approved the final version of the article.

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