Gastric Helicobacters in Domestic Animals and Nonhuman Primates: the Agents and their Significance for Human Health

Running title: Gastric helicobacters in domestic animals and primates

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INTRODUCTION

It was first reported in 1984 that gastric ulcer disease in humans is caused by a bacterial infection (141). The causative agent, *Helicobacter (H.) pylori*, has also been associated with gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (132, 174, 215). This bacterium is very successful in the way that it colonizes the human stomach, since in developing countries, more than 80% of the population is infected with *H. pylori,* even at young age. In developed countries the prevalence rate of *H. pylori* generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (132, 181).

Various tests have been developed for the diagnosis of *H. pylori* infections (reviewed in 132). For routine diagnostic purposes, histology or culture of biopsies from patients who have undergone endoscopy and urea breath testing are most often used. On histology, *H. pylori* bacteria are identified on the basis of their typical localization and their characteristic, slightly curve-shaped morphology. In 0.2 to 6% (depending on the literature source and the geographical region) of these biopsies, however, bacteria with a different, typically long spiral-shaped morphology are found. These spiral-shaped non-*H. pylori* helicobacters were first described in 1987 (46). They were originally referred to as "*Gastrospirillum hominis*" (143). Analysis of the 16S rRNA gene of these uncultivated organisms resulted in their classification in the genus *Helicobacter*. They were provisionally named "*H. heilmannii*" after the German pathologist Konrad Heilmann, who first studied the pathology associated with these microorganisms (101). "*H. heilmannii*" has also been associated with gastritis (40), gastric ulcers (41) and gastric MALT lymphoma (153), but not with gastric adenocarcinoma. Further research on "*H. heilmannii"* has been seriously hampered by the very fastidious nature of these microorganisms. Even today, to our knowledge, only two "*H. heilmannii"* strains have been cultured from human tissue (2, 127).

63 Long spiral-shaped helicobacters have also been demonstrated in the stomach of different
64 animal species. A summary of these helicobacters is given in Table 1.
65 This article aims to provide an overview of *Helicobacter* species naturally colonizing the
66 stomach of food producing animals, pet animals and nonhuman primates. First, the very
67 complex and confusing nomenclature used to designate non-*H. pylori Helicobacter* species
68 colonizing the human stomach is considered. Thereafter, an overview of helicobacters
69 colonizing the stomach of domestic animals and nonhuman primates is presented and their
70 possible pathogenic significance for their animal hosts is discussed. The main aim of this
71 article, however, is to have a closer look at the significance of these microorganisms for
72 human health: should they be considered as zoonotic agents, what are the disease signs in
73 infected humans, how are they transmitted and what is known about their virulence factors?
74 The article ends with some thoughts on what the study of gastric *Helicobacter* infections in
75 animals might teach us about these infections in humans.
76

77 **GASTRIC NON-*H. PYLORI HELICOBACTER* NOMENCLATURE: THE NEED FOR**
78 **CLARIFICATION**

79 Since the description of *H. pylori,* the number of species in the genus *Helicobacter* has
80 rapidly expanded. Today, a large number of non-*H. pylori Helicobacter* species have been
81 described in a wide variety of animals and humans and the genus *Helicobacter* contains at
82 least 32 species with validly published names
83 (http://www.bacterio.cict.fr/h/helicobacter.html). The frequent changes in nomenclature of
84 non-*H. pylori* helicobacters colonizing the stomach of humans have caused quite a lot of
85 confusion, not only among clinicians, but also among bacteriologists. Today, there is a serious
86 problem in trying to reach international agreement on this complex and expanding group of

87 microorganisms, all of which have in common their tightly coiled morphology and their
88 difficulty to culture *in vitro*.
89 Subsequent to the renaming of "*Gastrospirillum hominis*" as "*H. heilmannii*", further
90 genetic analysis of the 16S rRNA gene revealed two types that differed by more than 3% in
91 their nucleotide sequence, which prompted the subclassification of the non-*H. pylori*
92 helicobacters into "*H. heilmannii"* type 1 and "*H. heilmannii"* type 2. Sequencing of the 23S
93 ribosomal RNA encoding genes also makes it possible to distinguish between the two types
94 (48).
95 "*H. heilmannii*" type 1 is both morphologically and genetically identical to a bacterium
96 colonizing the stomach of pigs (43, 166) that was first designated "*Gastrospirillum suis*"
97 (146, 184). Almost ten years later, sequencing of the 16S rRNA gene, fluorescent in-situ
98 hybridization (FISH) and electron microscopy showed that these organisms belong to the
99 genus *Helicobacter* and are sufficiently different from all existing species to constitute a new
100 taxon. Because at that time this species could not be thoroughly characterized due to the lack
101 of pure *in vitro* isolates, the organism was described as "*Candidatus* Helicobacter suis*"* (43).
102 Only recently have *in vitro* cultures been obtained, resulting in the description of *H. suis* as a
103 species (14).
104 The situation with regard to "*H. heilmannii*" type 2 is even more complex. This type does
105 not represent a single *Helicobacter* species but rather a group of species, including three
106 helicobacters that have been isolated from the stomachs of cats and dogs, namely *H. felis, H.*
107 *bizzozeronii* and *H. salomonis.* To add to the confusion, one uncultivable species detected in
108 the stomachs of humans, wild felids, dogs and cats was named "*Candidatus* Helicobacter
109 heilmannii" (166). Two other closely related species, one of which was isolated from a dog
110 and the other from a cat, have been described as *H. cynogastricus* and *H. baculiformis,*

111 respectively (15, 233). However, up till now no information is available about the presence of
112 these bacteria in humans.
113 Differences in morphology between different gastric non-*H. pylori Helicobacter* species
114 have been described (Table 2), but this is not an accurate method for species identification. It
115 has been stated that periplasmic fibrils wrapped around the cell body are a typical feature of
116 *H. felis* (134). However, *H. cynogastricus* also possesses a periplasmic fibril running along
117 the external side of the helix and both species are tightly coiled organisms (233). *H.*
118 *salomonis* is less tightly coiled and does not have periplasmic fibrils (115). *H. baculiformis* is
119 a large, slender to slightly spiral rod with periplasmic fibrils (15). "*Candidatus* H.
120 heilmannii", *H. bizzozeronii* and *H. suis* are morphologically very similar. These
121 microorganisms do not possess periplasmic fibrils and show very tight coils (14, 92, 166).
122 Sequencing of the 16S and 23S ribosomal RNA encoding genes allows differentiation of
123 *H. suis* from the other gastric non-*H. pylori Helicobacter* species mentioned above, but it can
124 not distinguish between *H. felis*, *H. bizzozeronii*, *H. salomonis, H. cynogastricus, H.*
125 *baculiformis* and "*Candidatus* H. heilmannii" (15, 48, 233). For differentiation between these
126 species sequencing of the *hsp60* gene (149), the urease A and B genes (161, 166) and *gyrB*
127 gene (95) is useful, as well as whole cell protein profiling (228) if pure *in vitro* cultures are
128 available.
129 Phylogenetic trees for the gastric helicobacters discussed in this review are shown in
130 Figures 1 and 2. Fig. 1 is based on 16S rRNA gene sequence similarity data, and Fig. 2 on the
131 partial *ureA* and *ureB* gene sequences. The sequences that have been detected in human
132 stomachs are also indicated.
133 In literature, gastric infections with spiral-shaped bacteria in humans are often referred to
134 as "*H. heilmanni"* or "*H. heilmannii*-like organism" infections. However, at present, the name
135 "*H. heilmannii*" can not be used as a species name, according to taxonomical rules. To avoid

confusion, we propose to use the term "gastric non-*H. pylori* helicobacters" to designate these spiral-shaped bacteria when only results of histopathology or crude taxonomic data are available and to reserve true species designations for those situations in which the species is defined.

To non-bacteriologists, the changes in "*H. heilmannii"* nomenclature may appear unwieldy and unnecessary. However, it should be kept in mind that several important traits, including pathogenicity and antimicrobial susceptibility, may vary, depending on the bacterial species. At the present time it is not known whether certain non-*H. pylori Helicobacter* species are more often associated with a certain disease outcome in humans than others.

GASTRIC HELICOBACTERS IN DOMESTIC ANIMALS AND NONHUMAN PRIMATES: AN OVERVIEW

A summary of gastric lesions described in domestic animals and nonhuman primates naturally or experimentally infected with helicobacters is presented in Table 3. Below, infections with gastric helicobacters in pigs, dogs, cats, rabbits, ferrets, hamsters, ruminants, horses and nonhuman primates are considered. Guinea pigs and Mongolian gerbils are also often kept as pets, but natural infections with gastric helicobacters have not been described in these animal species.

Gastric helicobacters associated with pigs

The main *Helicobacter* species colonizing the stomach of pigs is *H. suis.* Its prevalence at slaughter age in most reports is 60% or more. *H. suis* causes gastritis in experimentally and naturally infected pigs (87, 104, 147, 173, 185). It has also been associated with ulcers of the non-glandular part of the stomach (18, 28, 185, 190), although the exact role of *H. suis* in

porcine gastric pathology remains to be elucidated. Indeed, Grasso et al. (87), Melnichouk et al. (145), Park et al. (173) and Szeredi et al. (222) did not find this association. These discrepancies might be due to differences in laboratory techniques for demonstration of *Helicobacter,* different sampling practices or differences in virulence between different *H. suis* strains. In any case, in a recent study carried out by our research group, gastric ulcers were induced in pigs experimentally infected with *H. suis* (T. Meyns, R. Ducatelle, B. Flahou, K. Chiers, F. Pasmans and F. Haesebrouck, submitted for publication). In this study, 6-week-old piglets that were free of *H. suis* were used. Nine piglets were intragastrically inoculated with a pure culture of *H. suis,* while 5 sham-inoculated piglets were used as controls. All piglets were fed a finely ground diet. Hyperkeratosis and ulcer formation were clearly present in the gastric non-glandular mucosa of all *H. suis* inoculated pigs, while none of the sham-inoculated piglets developed gastric lesions.

Hyperkeratosis and ulceration of the non-glandular part of the stomach have been reported in many countries. Up to 80% of the market pigs in Australia (189) and 60% of the sows (106) in the Netherlands showed gastric lesions. Hessing et al. (106) found gastric ulcers in 10-15% of the sows.

The stomach mucosa of pigs can be divided into a glandular part (cardiac gland zone, fundic gland zone and antrum with pyloric glands) and a non-glandular part, the latter being a small rectangular area around the esophageal opening. It is also called the pars esophagea of the stomach and is covered by a stratified squamous epithelium (Fig. 3). After experimental infection, *H. suis* mainly colonizes the antrum and the fundic gland zone and, to a lesser extent, the cardiac gland zone (103). *H. suis* DNA was also detected in the pars esophagea by PCR (190), but bacteria were not detected in the non-glandular part of the stomach by microscopic examination (103). Ulceration of the porcine gastric non-glandular mucosa may result in decreased feed intake, a decrease in daily weight gain, and even sudden death (10),

186 thus leading to significant economic losses. There is little doubt that this disease can cause
187 pain and discomfort.
188 The non-glandular region and the cardiac gland zone, together representing almost 50% of
189 the stomach, have a pH range between 5 and 7 due to the presence of saliva and cardiac gland
190 bicarbonate secretions (109). The distal compartment, composed of the fundic and pyloric
191 glands, ensures postprandial pepsin digestive enzymatic activity through acid secretion.
192 Pepsin activity is only possible at the low pH of the distal compartment. It has been suggested
193 that no mixing of luminal content is taking place between the proximal and the distal stomach
194 compartments, and that the porcine stomach normally maintains these two compartments with
195 distinct pH and enzymatic conditions (58). Anything contributing to a breakdown in the
196 segregation of the proximal and distal compartments may allow the stratified squamous
197 epithelium of the non-glandular region to come into contact with the luminal content of the
198 distal part with acid, bile (refluxed from the duodenum) and pepsin. Chronic insult of the non-
199 glandular region will eventually lead to ulceration (Fig. 4).
200 Ulceration in the non-glandular stomach of pigs is a disease of complex etiology in which
201 multiple factors are involved, including dietary and stress factors. Small particle size of feed,
202 interruption of feed intake and presence of highly fermentable carbohydrates in the diet
203 promote ulcera (10). In general, all conditions increasing the fluidity of the stomach contents
204 may cause a breakdown of the pH gradient between the proximal and the distal parts of the
205 stomach and may play a role in ulcer development (77). An infection with *H. suis* may result
206 in secretion of excessive amounts of gastric acid, leading to increased contact of the non-
207 glandular part of the stomach with hydrochloric acid. In the fundic gland region of pigs
208 experimentally or naturally infected with *H. suis,* these micro-organisms were found in close
209 contact with parietal cells, which might indicate that the bacterium may have an impact on
210 these hydrochloric acid-producing cells (103). An *H. suis* infection results in gastritis, which

211 is mainly localized in the antrum (103, 147). In *H. pylori* infections in humans, increased acid
212 production has been associated with antral predominant gastritis (132). In a recent study,
213 Sapierzynski et al. (192) demonstrated that an *H. suis* infection in pigs results in an increased
214 number of gastrin producing cells and a decreased number of somatostatin producing cells.
215 Since gastrin stimulates and somatostatin inhibits the secretion of hydrochloric acid by
216 parietal cells, this may also result in excessive acid production. However, Silva et al. (202)
217 did not find increased postprandial serum gastrin concentrations in pigs with ulceration of the
218 pars esophagea.
219 Krakowka et al. (129) isolated a curve-shaped *Helicobacter* species from naturally
220 infected young piglets different from the tightly coiled *H. suis*. This microorganism is
221 morphologically similar to but antigenically different from *H. pylori.* As far as we know, no
222 genomic data of this *H. pylori*-like bacterium have been published yet. Gnotobiotic piglets
223 experimentally inoculated with this microorganism developed ulcers of the pars esophagea
224 (130).
225 Hänninen et al. (93, 94) demonstrated that spindle-shaped microorganisms that had been
226 isolated from the stomach and feces of pigs and that had tufts of sheated flagella at both ends
227 and external fibrils outside the cell, belonged to the species *H. bilis* and *H. trogontum.* These
228 are enterohepatic helicobacters that were originally isolated from mice and rats, respectively,
229 and were provisionally called “flexispira”. The pathogenicity of these microorganisms for
230 pigs is unknown and their main site of colonization is most probably the lower intestinal tract.
231 They are urease-positive, which may help them to survive during passage through the
232 stomach. It remains to be determined whether they are able to colonize the porcine stomach,
233 as has been shown for some other urease-positive enterohepatic helicobacters in other
234 animals, such as *H. aurati* in Syrian hamsters (177) and *H. muridarum* in mice (183).
235

Gastric helicobacters associated with dogs and cats

The majority of *Helicobacter* infections of the canine and feline gastric mucosa are mixed infections of various *Helicobacter* species, including *H. felis*, *H. bizzozeronii*, *H. salomonis* and "*Candidatus* H. heilmannii". Recently, one additional species was isolated from the stomach of a dog, namely *H. cynogastricus* (233), and one additional species was isolated from the stomach of a cat, namely *H. baculiformis* (15).

In dogs, spiral shaped bacteria are commonly found in the stomach. They are present in 67 to 86% of clinically healthy dogs and in 61 to 100% of dogs presenting chronic vomiting (105, 112). In cats, spiral-shaped organisms have been detected in 41 to 100% of the animals investigated, with a slightly higher rate in animals presenting chronic vomiting (62, 80, 105, 112, 161, 171, 172, 237, 243). Bridgeford et al. (23) hypothesized that gastric *Helicobacter* species may be a cause of feline gastric lymphoma.

The pathogenic significance of gastric *Helicobacter* species in dogs and cats remains enigmatic and may be *Helicobacter* species- or even strain-dependent.

Cats experimentally infected with *H. felis* presented a pangastric mononuclear infiltration throughout the gastric mucosa, which was equivalent to the inflammatory response in uninfected animals. However, follicular organization of the inflammatory cells was restricted to the infected animals (193, 204).

The only spiral organism which has been identified in dogs with chronic active gastritis, and not in dogs with a normal gastric histology, is *H. felis* (51). Also, young gnotobiotic dogs experimentally infected with *H. felis* presented marked lymphoid hyperplasia in the fundus and the body of the stomach (137). These observations suggest a cytopathogenic effect in the canine stomach for at least *H. felis*, which may be enhanced due to a possible synergistic effect with *H. bizzozeronii*. However, Simpson and others (204) found a similar degree of inflammation both in mature SPF dogs experimentally infected with *H. felis* and in uninfected

control dogs. These conflicting observations may be due to differences in virulence between different *H. felis* isolates, as has also been described for *H. pylori* (59, 132). Very little is known, however, about differences in pathogenicity between different strains within the same species of non-*H. pylori* helicobacters. De Bock et al. (36) reported significant differences in inflammation scores in the gastric mucosa of SJL mice at 3 weeks following experimental infection with two different *H. felis* strains, but it is unclear whether this difference in inflammation score persists in time. Inflammation is considered only one aspect of pathogenicity and it is not known whether these strains differ in other aspects of pathogenicity as well, or whether this observation also holds in other animal species.

Gastric and duodenal ulcers are reported infrequently in dogs and cats, and no clear association has been made with *Helicobacter* infections (25).

Several research groups concluded on the basis of a species-specific *ureB* PCR (13, 161) that *H. bizzozeronii* is the predominant *Helicobacter* species in the canine stomach (182, 230, 241). Using a multiplex PCR, it was found that more than 50% of the Belgian dogs and cats investigated were harboring *H. felis* (230). The prevalence of "*Candidatus* H. heilmannii" (designated "*H. heilmannii*", or "HLO135" at that time) was found to vary from between 20 and 100% in both cats and dogs (112, 161, 218, 230, 241). According to Van den Bulck et al. (230), *H. felis* and "*Candidatus* H. heilmannii" (designated "HLO135") are the predominant *Helicobacter* species in cats. *H. salomonis* has only sporadically been detected in both dogs and cats. The prevalence of *H. cynogastricus* and *H. baculiformis* in these animal species is presently unknown.

Gastric helicobacters associated with rabbits

To the authors' knowledge, only two reports describe the detection of *Helicobacter* DNA in the stomach of rabbits (231, 234). This concerned *H. felis* and *H. salomonis.* No attempts

were made to cultivate these organisms from rabbits, nor is there anything known about their pathogenicity towards this animal species. Further research is recommended, especially since rabbits are gaining importance as pet animals, often living in intimate contact with their adoptive family.

Gastric helicobacters associated with ferrets

Shortly after the discovery of *H. pylori* in humans, spiral organisms were isolated from a gastric ulcer of a ferret and from the gastric mucosa of two healthy ferrets (66). These organisms were first described as *Campylobacter (C.) pylori* subsp. *mustelae* (68). Later on, they were designated *C. mustelae* (69) and finally *H. mustelae* (85). Only a minority of ferrets younger than 6 weeks are colonized by this bacterium, in contrast to approximately 100 % of the adult ferrets (67). This indicates that widespread colonization occurs after weaning and it seems to persist throughout the adult life of the ferret (71).

In ferrets naturally infected with *H. mustelae*, often only a superficial gastritis is present in the corpus region, where these bacteria colonize the mucosal surface (140). In the antrum, however, a diffuse mononuclear gastritis is observed with inflammatory cells often occupying the full thickness of the mucosa (72). In this stomach region, *H. mustelae* colonizes the surface, gastric pits and the superficial portion of the glands (72). A retrospective study revealed that persistent colonization with *H. mustelae* over time increases the severity of gastric disease (71).

Gastric and, to a lesser extent, duodenal ulcers have been reported in ferrets infected with *H. mustelae* (66, 70) and the incidence of gastric ulceration in this animal species varies between 1.4 and 35% (3, 4). However, since the prevalence of *H. mustelae* is very high in adult ferrets, long-term observations of experimentally infected pathogen-free ferrets are

needed to elucidate the exact role of *H. mustelae* infection in the development of peptic ulcer disease (211).

Fox and coworkers (75) reported on the presence of *H. mustelae* in the pyloric mucosa of two ferrets suffering from pyloric adenocarcinoma. In both cases, the invasion of neoplastic tubules into the deep submucosa is described. An increased epithelial cell proliferation has also been detected in the gastric mucosa of ferrets infected with *H. mustelae.* This may play a role in the development of gastric tumours (247). Gastric MALT lymphoma has also been described in ferrets infected with *H. mustelae* (63). Replacement of normal epithelium by uniform populations of lymphoid cells was seen, with invasion and destruction of the gastric glands. These lymphomas arose in the antrum, where *H. mustelae*-induced gastritis is most severe. However, for both types of gastric malignancy, evidence remains circumstantial and the role of *H. mustelae* in the development of gastric tumours needs to be confirmed (211).

Gastric helicobacters associated with hamsters

H. aurati has been isolated from the stomachs of hamsters. Several not further characterized *Helicobacter* spp. have also been reported to be present in the stomach of these animals (159, 176) but no further information is available on these species.

H. aurati has been isolated from the inflamed stomachs and caeca of adult Syrian hamsters. Various features, such as the fusiform shape and the presence of periplasmic fibrils, allow morphologic discrimination between *H. aurati* and the three other helicobacters that have thus far been identified in hamsters, namely *H. cholecystus* (76), *H. mesocricetorum* (203) and *H. cinaedi* (26, 236). The presence of urease activity also distinguishes it from these three enterohepatic *Helicobacter* species. The preferential colonisation site of *H. aurati* in hamsters is probably the intestinal tract, particularly the caecum with subsequent spreading of this bacterial agent to the stomach in selected animals. The coprophagic behaviour of

hamsters may play a role in this gastric colonisation by *H. aurati*. At present, the exact role of *H. aurati* in gastric disease of hamsters has not yet been fully clarified, although the organism has been identified in hamsters suffering from chronic gastric inflammation and intestinal metaplasia (176, 177). The same authors reported the presence of another helical, urease-negative *Helicobacter* species, as well as a smaller, urease-negative *Campylobacter* sp. in the stomachs of these hamsters. Likewise, Nambiar et al. (159) reported a case of gastritis-associated adenocarcinoma and intestinal metaplasia in a Syrian hamster naturally infected with different *Helicobacter* species, including *H. aurati*. They suggested that chronic *Helicobacter*-associated gastritis in hamsters may develop into an infiltrative gastric adenocarcinoma, similar to what has been described in chronic *H. pylori* infections in humans. There are no indications that *H. aurati* is of zoonotic significance.

Gastric helicobacters associated with ruminants

Gastric ulcers regularly occur in calves and adult cattle with an incidence varying between 2 and 87% (52, 96, 117, 163, 206, 240). "*Candidatus* Helicobacter bovis" has been demonstrated in the pyloric part of the abomasum of calves and adult cattle but has not yet been cultivated *in vitro* (44). Although it is highly prevalent in bovines (88, non-published results), its involvement in gastric disease in cattle is presently unknown. In contrast, *Helicobacter* DNA was not detected in the abomasum of 70 goats, using a genus specific PCR (88).

Gastric helicobacters associated with horses

Gastric ulcers are common in horses and their incidence in racehorses in active training may exceed 90% (20, 156). Various stress factors, diet, management and training practices are

359 regarded as potential risk factors (31, 156). The proximal half of a horse's stomach is entirely
360 lined with a stratified squamous epithelium resembling the pars esophagea of the porcine
361 stomach. The more distal portion is the glandular part. Ulcers are most frequently seen close
362 to the junction between the non-glandular and the glandular part of the stomach (31). In some
363 studies, *Helicobacter*-like organisms or their DNA have been detected in the stomach of
364 horses but their role in development of gastric ulcers remains speculative. Contreras *et al.* (31)
365 detected *Helicobacter*-like DNA in the gastric mucosa of 11 thoroughbred racehorses.
366 Sequencing of the 16S rRNA gene revealed 99% similarity with *H. pylori*, but all samples
367 were negative when tested with *H. pylori*-specific PCR assays targeting the *cagA* and *glmM*
368 genes which might indicate that the DNA was from a *Helicobacter* species different from *H.*
369 *pylori*. It remains to be determined whether horses may indeed be infected with a gastric
370 *Helicobacter* species specifically associated with this animal host. Attempts should be made
371 to try and isolate these microorganisms from the horse's stomach, both *in vitro* and *in vivo* by
372 intragastric inoculation of specific pathogen free mice (166).
373

374 **Gastric helicobacters associated with nonhuman primates**

375 In several studies, nonhuman primates were used as models for human *H. pylori*
376 infections (111, 128, 209, 210, 212). Captive rhesus monkeys (*Macaca mulatta*) are
377 commonly infected with *H. pylori* (54). The rhesus monkey model, therefore, provides an
378 opportunity to examine natural acquisition of *H. pylori* using an experimental set up that
379 closely resembles human infection. Socially housed rhesus monkeys rapidly acquire *H. pylori*
380 infection. Newborns from infected dams are more commonly infected than those from
381 uninfected dams, particularly during the peripartum period, suggesting that close contact
382 during this time facilitates oral-oral transmission (213, 214). Once acquired, infection is
383 associated with chronic gastritis that resembles that seen in humans.

384 The number of reports dealing with natural infections with gastric non-*H. pylori*
385 helicobacters in nonhuman primates is limited and at present, a gastric *helicobacter* species
386 specifically associated with these animals has not yet been described. Bronsdon et al. (24)
387 isolated and described *H. nemestrinae* from the stomach of a pigtailed macaque (*Macaca*
388 *nemestrina*). This microorganism is able to grow at 42°C and possesses bipolar flagella,
389 which is different from *H. pylori* strains isolated from humans. Based on the sequencing of 7
390 housekeeping genes and 2 flagellin genes, *H. nemestrinae* was later shown to be an atypical
391 *H. pylori* strain (219). *H. nemestrinae* should therefore be considered a later heterotypic
392 synonym of *H. pylori.*
393 In the stomach of rhesus monkeys (*Macaca mulatta*), gastric non-*H. pylori* helicobacters
394 which were not identified to the species level, have been observed in the mucus covering the
395 surface epithelial cells, in the lumina of the gastric glands and in close contact with parietal
396 cells (57). These micro-organisms were able to invade and on occasion to damage parietal
397 cells, while apparently causing hyperchlorhydria. This is in contrast to *H. pylori*, which
398 caused gastritis in these animals without modifying the acid output (57). Long, spiral-shaped
399 bacteria have also been reported in the stomach of baboons (*Popio hamadryas*). This was
400 associated with gastritis by Mackie and O'Rourcke (138), but not by others (34, 35). Non-*H.*
401 *pylori* helicobacters, without clarification about the species, have been described to be
402 naturally present in the stomach of up to 100% of cynomolgus monkeys from many different
403 geographic regions (55, 187). These micro-organisms were found in the superficial portions
404 of the gastric epithelium, most frequently in the fundic region. The bacteria were located in
405 the gastric pits, superficial glands or on the surface epithelium. However, no correlation was
406 observed between the presence of these bacteria and the infiltration of lymphoplasmacytic
407 cells and inflammatory lesions in these gastric tissues (55).

H. suis has been demonstrated in the stomach of two mandrill monkeys (*Papio Sphinx*), two cynomolgus monkeys (*Macaca fasicularis*) and one Rhesus macaque (*Macaca mulatta*) from a zoo (167). One isolate, first described as "*H. heilmannii"* (114) and later identified as "C*andidatus* H. heilmannii" (158), was obtained by intragastric inoculation of mice with gastric tissue from a cynomolgus monkey (*Macaca fascicularis)*. No information on the pathogenic significance of these *Helicobacter* species for nonhuman primates is available and the source of infection remains to be determined.

Although it is clear that nonhuman primates may be infected with different types of gastral helicobacters, little information on these bacteria and their interactions with these hosts is available. Since these animals are closely related to humans, further research is wanted. Monkeys might serve as a possible reservoir for human infections.

NON-*H. PYLORI HELICOBACTER*-ASSOCIATED GASTRIC DISEASES IN HUMANS: ZOONOSES?

Although some data in the literature indicate that animals, including cats, dogs and sheep, occasionally may be infected with *H. pylori* (53, 74, 226), it is unlikely that animals play an important role in the transmission of this microorganism to humans. Moreover, it cannot be excluded that in some of these cases *H. pylori*-like organisms – but not *H. pylori* itself – were involved. *H. pylori* has been demonstrated by culture and PCR methods in the gastric mucosa of SPF laboratory cats in one study (90). This observation may concern an anecdotic anthroponosis, especially since these bacteria have not been identified in stray cats (62).

Human infections with non-*H. pylori Helicobacter* organisms, however, most likely originate from animals, although the fingerprinting of *Helicobacter* species present in the human and animal gastric mucosa should be considered in order to fully understand the zoonotic hazard originating from different animal species.

O'Rourke et al. (166) demonstrated that 16S rRNA gene sequences and partial *ureA* and *ureB* gene sequences from 3 human and 4 porcine non-*H. pylori Helicobacter* strains isolated *in vivo* by inoculation in specific pathogen free mice showed a very high degree of homology, $\geq$ 99.3%. This led to the conclusion that they represent the same species, later described as *H. suis* (14, 166).

Another human non-*H. pylori Helicobacter* strain, also isolated *in vivo* in specific pathogen free mice, could be easily differentiated from *H. suis.* Urease gene sequence analysis demonstrated that it clustered with helicobacters from domestic and exotic feline species. These microorganisms were designated "*Candidatus* H. heilmannii" (166).

Two urease-based PCRs, one developed by O'Rourke et al. (166) and another one by Neiger et al. (161), detected only "*Candidatus* H. heilmannii" DNA and not DNA from pure *in vitro* cultures of *H. suis, H. felis, H. salomonis, H. bizzozeronii, H. baculiformis* and *H. cynogastricus* strains (non-published results), and can therefore be considered species-specific. Using the test described by Neiger et al. (161), Chisholm and Owen (27) demonstrated the presence of *Candidatus* H. heilmannii DNA in one of 113 gastric biopsies from human patients with dyspeptic symptoms.

Trebesius et al. (227) used fluorescent in situ hybridization (FISH) and partial 16S ribosomal sequencing for analyzing 89 gastric biopsy samples from humans in Germany with histological evidence of non-*H. pylori* helicobacters. Five short 16S rRNA directed probes of about 20 nucleotides were used in FISH. In total, 71 (80%) of these samples hybridized with a probe designated Hhe-1, which recognizes fragments of the 16S rRNA gene of *H. suis.* The 16S ribosomal gene sequences of the former "*H. heilmannii*" type 2 are highly related, and results obtained with the other probes are therefore more difficult to interpret. Two probes (Hhe2 and Hhe4) are identical to fragments of the 16S rRNA gene of "*Candidatus* H. heilmannii", leading to the conclusion that DNA of this helicobacter was detected in 17 (19%)

458 of the samples. Probe Hhe5 recognizes the 16S rRNA gene of *H. felis, H. bizzozeronii* and *H.*
459 *salomonis.* Five samples (6%) hybridized with this probe. Finally, one sample hybridized with
460 probe Hhe3. It is possible that this probe recognizes a not yet described *Helicobacter* species,
461 since the sequence obtained from HHLO-3 (Genbank accession N° AY014859) shows less
462 than 97% similarity with any of the known *Helicobacter* species. More research is needed to
463 confirm this, however.

464 De Groote et al. (45) used three PCR assays targeting the 16S rRNA gene for screening of
465 parrafin-embedded gastric biopsy specimens of 101 patients with chronic active gastritis and
466 histological evidence of a non-*H. pylori Helicobacter* infection. Fourteen samples tested
467 positive in a PCR assay that specifically detected *H. suis* DNA. DNA of *Candidatus* H. bovis
468 was detected in one sample. Samples of 49 patients tested positive in a third assay that
469 simultaneously detected *H. felis, H. bizzozeronii, H. salomonis, Candidatus* H. heilmannii, *H.*
470 *bacculiformis* and *H. cynogastricus.*

471 Van den Bulck et al. (230) studied the presence of *Helicobacter* species in 123 gastric
472 biopsies of humans from Belgium and Germany with histological evidence of a non-*H. pylori*
473 *Helicobacter* infection, using a multiplex PCR based on the tRNA intergenic spacers, the
474 urease gene and the 16S rRNA gene (13). In 37% of the samples, *H. suis* was detected. In
475 descending order, *H. salomonis* (21%), *H. felis* (15%), *Candidatus* H. heilmannii (8%,
476 designated HLO135 by the authors) and *H. bizzozeronii* (4%) were found.

477 The data presented above show that *H. suis* is the most prevalent gastric non-*H. pylori*
478 *Helicobacter* species in humans and there are strong indications that pigs may be a source of
479 infection for humans. Apart from the stomachs of pigs and humans, *H. suis* has also been
480 detected in the stomachs of macaques and mandrill monkeys, as has been demonstrated by
481 16S rRNA gene and urease gene sequencing (167), and in one cat (230).

Dogs, cats and perhaps also pet rabbits may serve as a source of infection in humans with *H. felis*, *Candidatus* H. heilmannii and *H. bizzozeronii.* Anecdotal reports of the presence of the same *Helicobacter* species in the stomach of a person and his favorite pet animal further point in the same direction. Several reports indeed suggest the transmission of gastric non-*H. pylori* spiral bacteria from dogs to humans (39, 116, 226) or from cats to humans (50, 107, 133, 217, 235). In 1999, Andersen et al. (2) succeeded in the isolation of a non-*H. pylori* helicobacter strain from human gastric mucosa. Later, phenotypic analysis, sequencing of the 16S rRNA gene, DNA-DNA hybridization analysis and whole-cell protein profiling revealed that this isolate belongs to the species *H. bizzozeronii* (116). Recently, another *in vitro* isolate was obtained from a human gastric non-*H. pylori Helicobacter* infection. Despite the low prevalence of *H. bizzozeronii* in human biopsies, polyphasic identification analysis revealed that this isolate also belonged to this species (127). In the study of Van den Bulck et al. (230), *H. salomonis* represented 21% of the human samples. The frequent identification of *H. salomonis* in human gastric biopsies, however, is in contrast to its rare identification in pet carnivore samples, thus inclining us to suspect additional sources of infection. In none of the studies described above were tests included that specifically detect *H. baculiformis* and *H. cynogastricus,* and it is presently not known whether these recently described species are able to colonize the human stomach.

GASTRIC DISEASE IN HUMANS INFECTED WITH NON-*H. PYLORI HELICOBACTER* SPECIES

Studying the effects of non-*H. pylori* helicobacters in humans is complicated by the fact that these infections are uncommon and it seems likely that there may be variation among non-*H. pylori* helicobacters in their ability to cause inflammation or disease in humans. In addition, the presence of *H. pylori* must be excluded in order to assess the effects of the

507 species under consideration. De Groote et al. (45) detected *H. pylori* DNA in 7 of 64 and Van
508 den Bulck et al. (230) in 6 of 89 human gastric biopsy specimens that were positive for non-
509 *H. pylori* helicobacters. Human *H. pylori* and non-*H. pylori Helicobacter* co-infections have
510 also been identified by histology (113), but the possible significance of this in terms of
511 disease development has not been determined.

512 Non-*H. pylori Helicobacter* infections of the human stomach are consistently
513 accompanied by active chronic gastritis. The lesions, however, appear less severe than those
514 associated with *H. pylori* (216). Acute gastritis is also occasionally observed (246). There
515 may be glandular atrophy or intestinal metaplasia of the fundic mucosa, but these lesions are
516 less common with non-*H. pylori Helicobacter* than with *H. pylori* infections (216). Gastric
517 erosions mainly located in the antrum (22, 41, 50, 199, 221, 246) and duodenal ulcers (21, 81,
518 118, 194) have also been reported in association with non-*H. pylori Helicobacter* infections.
519 Furthermore, these infections have been associated with low grade MALT lymphoma of the
520 stomach, and the risk of developing MALT lymphoma is higher with non-*H. pylori*
521 helicobacters than with *H. pylori* (153, 154). Both the gastritis and the MALT lymphomas
522 have been reported to resolve after clearance of the non-*H. pylori Helicobacter* infections,
523 further underlining the causal relationship (154).

524 Clinical symptoms associated with non-*H. pylori* helicobacters in man can be
525 characterized by atypical complaints such as acute or chronic epigastric pain and nausea.
526 Other aspecific symptoms include hematemesis, recurrent dyspepsia, irregular defecation
527 frequency and consistency, vomiting, heartburn and dysphagia, often accompanied by a
528 decreased appetite (50, 81, 101, 123, 148, 164, 194, 199, 221, 235, 239, 245, 246). Some
529 people infected with non-*H. pylori* helicobacters do not present obvious clinical signs (142).

530 Inspection of the gastric mucosa of people infected with long spiral bacteria through
531 endoscopy reveals a variety of lesions, ranging from a normal to slightly hyperemic mucosa,

to mucosal edema, and to multiple erosions and ulcerations in the antrum or in the duodenum (50, 81, 194, 199, 221, 235, 244, 246).

Histologically, the inflammation induced by non-*H. pylori* helicobacters in the gastric tissue is generally characterized by lymphocytic exudation into gastric foveolae, sometimes admixed with plasma cells. In some cases, lymphocytes are organized into lymphoid aggregates. Epithelial mucus is occasionally depleted (64, 113, 123, 154, 164, 194).

In human patients presenting severe pathology and clinical symptoms associated with the presence of non-*H. pylori* helicobacters, treatment is indicated, although the efficacy of such treatment is not always easy to determine due to the lack of randomized trials. Such trials are difficult to organize in view of the low frequency of these infections in humans. In practice, identical treatment regimens as used for *H. pylori* have been prescribed. Triple therapy using combinations of a proton pump inhibitor and two antimicrobial agents selected from clarithomycin, metronidazole, amoxicillin and tetracycline may be effective (39, 81, 123, 221, 235).

Because of the low number of *in vitro* isolates available, very little data exists on the antimicrobial susceptibility and acquired resistance of gastric non-*H. pylori Helicobacter* species. Determination of minimal inhibitory concentrations of various antimicrobials against *in vitro* isolates indicated that acquired resistance to metronidazole may occur in *H. bizzozeronii* and *H. felis* strains of animal origin (232). Experimental *H. felis* infections in mice showed that several therapies using only one antimicrobial were only effective in 25 to 70% of the animals tested, while triple therapy using metronidazole, tetracycline and bismuth subcitrate eradicated *H. felis* from all the animals (49). In a BALB/c mouse model it was shown that treatment with ampicillin/omeprazole results in the suppression of *H. suis*. Differences in sensitivity were seen between different *H. suis* isolates, which might indicate acquired antimicrobial resistance (102).

TRANSMISSION OF GASTRIC NON-*H. PYLORI HELICOBACTER* SPECIES

Little data is available on how non-*H. pylori Helicobacter* species are transmitted among their hosts.

Hellemans et al. (104) screened stomach samples of pigs of different ages from different herds for the presence of *H. suis*. The prevalence of the infection was very low in suckling piglets, increased rapidly after weaning and reached 90 per cent in the adult boars and sows. The low degree of infection before weaning despite the high prevalence in adult pigs and thus most probably in the dams of the suckling piglets, may indicate maternal protection through antibodies or other antibacterial factors present in sow milk (89), which disappears at weaning. The regrouping of animals at the time of weaning most probably favors the spread of the microorganism from the few piglets infected before weaning to non-infected animals. Another factor that may enhance *H. suis* colonization in weaned pigs is that after weaning the lactobacilli disappear temporarily from the stomach. An antagonistic effect of lactobacilli against *H. pylori* (1, 119, 200) and *H. felis* (30) infection has been demonstrated in a murine model. The fact that the *H. suis* infection persisted in the adult boars and sows indicates that any natural immune response against this microorganism did not lead to its clearance. Despite numerous attempts, we were not able to detect *H. suis* in feces of infected pigs, which may indicate that fecal-oral spread between pigs and from pigs to humans is limited. Transmission of *H. suis* may be oral-oral via saliva, or gastric-oral via vomitus, but this remains to be investigated. Persistent stomach colonization with *H. suis* can be achieved in laboratory mice (102), and it may be worthwhile to determine whether wild mice can act as vectors or even as reservoirs of the infection.

Hardly any data on the transmission of non-*H. pylori Helicobacter* infections in dogs and cats is available in the literature. Hänninen et al. (91) described the transmission of *H.*

582 *salomonis* from a dam to her puppies, as well as between infected and non-infected pups,
583 which was proved by the similar pattern found for all cultured isolates using pulsed field gel
584 electrophoresis. Transmission is suspected to be through oral-oral or gastric-oral contact, as
585 nursing dogs have very intimate contact with their offspring and puppies eat material vomited
586 by the dam. *Helicobacter* DNA was detected in the oral cavity of dogs (186). Lee et al. (135)
587 found that gastric mucus derived from *Helicobacter*-infected cats was highly infectious for
588 mice, while rectal contents were not. Moreover, the isolation procedure starting from fecal
589 material was unsuccessful (91). These observations may indicate that fecal-oral transmission
590 is less important.

591 Fecal-oral transmission has, however, been suggested for *H. mustelae* (73). In a group of
592 36 ferrets, *H. mustelae* was isolated from the feces of 11 animals. A correlation was found
593 with periods of transient hypochlorhydria, also seen in experimentally infected animals (72),
594 which may allow larger numbers of *H. mustelae* to exit the stomach. Keeping in mind the ease
595 by which ferrets vomit, oral-oral and gastric-oral contact may also play a role in transmission
596 of this bacterium (71).

597 It is not exactly known how gastric helicobacters are transmitted from animals to humans,
598 but most likely it occurs through direct contact. Living in close proximity to dogs, cats and
599 especially swine has indeed been identified as a significant risk factor for these infections
600 (108, 144, 220). The intensity of contact with animals is thought to be important as well, since
601 a higher incidence of these infections has been noted in pig farmers, the staff of pig
602 slaughterhouses and people having intensive contact with pet animals (217, 221, 235, 246). It
603 is remarkable that *H. suis* is the most prevalent gastric non-*H. pylori Helicobacter* species in
604 humans (27, 227, 230). This might indicate that the infectivity in humans of cat or dog related
605 strains is less than that of *H. suis.*

606 It is not known whether, besides direct contact with animals, other routes of transmission
607 of non-*H. pylori* helicobacters are of importance. Recently, it was shown that gastric
608 helicobacters can survive in water for more than 4 days, a fact which may suggest a possible
609 role for water in the transmission of *Helicobacter* species between hosts (11). There is no data
610 available on the survival of *H. suis* on carcasses of slaughtered pigs, and it remains to be
611 determined whether raw or undercooked pork meat might be a source of infection for humans.
612 It is also not known how frequently transmission of non-*H. pylori* helicobacters from
613 animals to humans occurs. Only in a low percentage of human patients with severe gastric
614 complaints long spiral shaped bacteria are found at microscopic examination of gastric
615 biopsies. However, it is possible that this represents only the tip of the iceberg and it can not
616 be excluded that infections with these bacteria often pass inapparent or result in mild disease
617 signs which are not further examined (142).
618
619 **VIRULENCE FACTORS OF GASTRIC NON-*H. PYLORI HELICOBACTER* SPECIES**
620 Most of the research concerning *Helicobacter* virulence factors and the evoked host
621 response has been done with *H. pylori* and much less information is available about the
622 virulence mechanisms of non-*H. pylori* helicobacters. Although some virulence factors of
623 these bacteria may indeed be similar to those described for *H. pylori,* there may also be
624 differences.
625 *H. pylori* is a diverse pathogen and several bacterial virulence factors are considered to
626 play a role in pathogenesis of infections with this agent. The key enzymes and proteins found
627 in *H. pylori* that are important for colonization include the urease system, alpha carbonic
628 anhydrase, sheathed flagella, pH taxis *tlpB* gene, arginase and several adhesins. Key virulence
629 factors also include the *cag* pathogenicity island (*cag* PAI) and the vacuolating toxin VacA.

630 Describing these virulence factors in detail goes beyond the scope of this article and readers
631 are referred to recent reviews dealing with this subject (100, 132, 152, 160, 179, 196).
632 All gastric *Helicobacter* species require a family of genes involved in the production of
633 urease. This enzyme consists of two subunits, UreA and UreB. It hydrolizes urea to ammonia
634 and carbon dioxide and is an important mechanism of survival required to colonize the
635 stomach. The ammonia produced neutralizes the hydrochloric acid of the stomach, creating a
636 neutral microenvironment around the bacterium. Urease is mainly localised in the cytoplasm
637 but also becomes associated with the surface of the viable bacteria after autolysis of
638 surrounding bacteria (131, 139, 178). *In vitro*, this autolysis occurs at the culture stage when
639 the growth of *H. pylori* ceases (78). Although urease has been associated with the outer
640 membrane (16), the concentration of surface-bound urease is probably too low to contribute to
641 acid resistance (197). The proton-gated urea channel UreI regulates the rate of the urea entry
642 into the cytoplasm and is required for acid survival and gastric infection (151, 205, 238).
643 When UreI is activated by an acidic pH of the medium, urease moves from the inner portion
644 to the outer portion of *H. pylori*, closer to the source of urea. So presumably, ammonia
645 production occurs at or near the inner membrane (110). In *H. felis,* a second urease system,
646 ureA2B2, has been detected (180), but its function and regulation is currently unknown. One
647 study showed that an *H. pylori* strain unable to produce functional urease was able to colonize
648 and damage the gastric mucosa of Mongolian gerbils (150). This seems to contrast with
649 results from many other studies stating that urease is essential for colonization of the stomach
650 of several animals, including gnotobiotic piglets (61). An isogenic urease-negative mutant of
651 *H. mustelae* (208) produced no detectable urease and failed to colonize the ferret stomach (5).
652 In any case, the role of urease seems not to be limited to colonization. Ammonia is probably
653 also used as a nitrogen source (242) and is thought to assist in damaging the mucosal barrier,
654 thereby releasing nutrients for the bacterium and maintaining the inflammation process (180,

655 207). *In vitro* experiments have shown that *H. pylori* urease is capable of activating peripheral
656 blood mononuclear leukocytes and mucosal macrophages, resulting in production of
657 proinflammatory cytokines (98, 99). Moreover, the *H. pylori* urease B subunit is able to
658 induce NF-κB activation and IL-8 production (19). Urease may also play a role in chemotaxis
659 (157).

660 Motility is essential for stomach colonization by helicobacters, allowing them to move
661 towards the gastric mucosa (122, 170), which has a neutral pH. Gastric helicobacters possess
662 monopolar, bipolar or peritrichous bundles of 2-23 flagella. The flagella consist of a body,
663 hook and flagellar filament. The latter is composed of two flagellin subunits namely the
664 predominant FlaA and the minor FlaB. It works as a propeller and is covered by a sheath
665 which is suspected to play a role in acid protection, masking of antigens and maybe adhesion
666 (120). The basal body of the flagella is embedded in the bacterial cell wall and contains
667 proteins required for rotation and chemotaxis. The hook links the body and the filament. *H.*
668 *mustelae* mutants defective in hook production are nonmotile and devoid of flagellar
669 filaments (169). Double mutants of *H. mustelae* in *flaA* and *flaB* genes are completely
670 nonmotile and unable to colonize the ferret whereas single-gene *flaA* and *flaB* mutants have
671 decreased motility (6, 121). These single-gene mutant strains were still able to initially
672 colonize the ferret's stomach at a low level and establish persistent infection with increasing
673 numbers of organisms over time (6).

674 Using a microscope slide-based pH gradient assay, it has been shown that *H. pylori*
675 displays pH-tactic behavior. In response to hydrochloric acid, the microorganism moves away
676 from the strong acid. Chemotaxis receptor TlpB is required for this pH taxis and *tlpB* mutants
677 are defective for mouse colonization (33). Homologous genes have not yet been described in
678 gastric non-*H. pylori* helicobacters, although it seems likely that similar mechanisms exist,
679 allowing them to escape from the higly acidic stomach lumen.

Once highly motile *H. pylori* bacteria have escaped pH stress, motility decreases or ceases. The ability of *H. pylori* to stop swimming in response to a neutral pH environment might optimize attachment to gastric epithelial cells (33). While the bacteria can persist deep in the mucus layer, they also attach tightly to gastric epithelial cells via a number of adhesins (162). Nothing is known about the adhesins of non-*H. pylori* helicobacters and information about specific localizations in the stomach of these microorganisms is limited. Gerbils have been experimentally infected with *H. felis* and *H. bizzozeronii* as a model for the study of pathogenesis and virulence mechanisms. Transmission electron microscopy revealed *H. felis* bacteria often in close proximity of parietal cells in contrast to what was found for *H. bizzozeronii* (38). A close apposition between the microvilli membrane of parietal cells and the outer membrane of the bacteria at the level of the periplasmic fibrils was regularly seen. Bacteria were also found surrounded by necrotic debris of parietal cells. *H. suis* cells were found in close proximity of mucus-producing epithelial cells and parietal cells of the stomach of experimentally infected pigs. This has also been seen in naturally infected pigs and in these animals the bacteria were also found inside the canaliculi of the parietal cells (103). *H. mustelae* adheres firmly to the gastric epithelium and only few bacteria are seen lying in the mucus (165). The exact mechanisms promoting its adhesion remain unknown. Most strains of *H. mustelae* agglutinate red blood cells from various hosts (223). Probably, more than one receptor is involved. In *H. mustelae* infected ferrets, the gastral mucosal hydrophobicity is reduced, which is correlated with the degree of mucosal inflammation (84). This may promote the attachment of *H. mustelae*, which is thought to be mainly hydrophilic. *H. mustelae* binds to the same receptor lipids as *H. pylori*, particularly phosphatidylethanolamine (82). Adhesion to eukaryotic cells *in vitro* correlates with the amount of phosphatidylethanolamine present (83). Clyne et al. (29) showed that flagella do not play a direct role in promoting adherence of *H. mustelae* to gastric epithelial cells.

Several virulence factors and genes are thought to be important in the pathogenesis of *H. pylori* infections once contact with the host cell epithelium is established, including VacA and the *cag* PAI (17, 100, 132, 160, 179, 196, 229). The *cag* PAI encodes a type IV secretion system, which forms a syringe-like structure capable of penetrating gastric epithelial cells and delivering CagA into the host cells. Once delivered inside the cell, CagA becomes phosphorylated on tyrosine residues, which are present in EPIYA motifs, resulting in morphological epithelial cell changes (162, 198). *H. pylori* strains that deliver CagA with more phosphorylation motifs are able to induce more severe cytoskeletal changes and are most often associated with gastric cancer (7, 12). The vacuolating toxin VacA plays a role in the development of vacuoles in epithelial cells and in the induction of apoptosis of these cells (32, 132). It is involved in the osmotic swelling of endosomes into vacuoles (79) and is encoded by the *vacA* gene, possessing several polymorphic sites, namely the signal region, the midregion and the intermediate region. For the first two regions, *H. pylori* strains with *vacA* type s1/m1 have been shown to be associated with duodenal and gastric ulceration and gastric adenocarcinoma, whereas the intermediate region is an important marker for *H. pylori* strains associated with gastric adenocarcinoma (188). Recombinant strains with altered toxicity can emerge during human infection, both for *vacA* and *cag* PAI (9, 124). Moreover, these two genes are thought to downregulate each other's effects on epithelial cells, raising the possibility of avoiding excessive cellular damage (8). Although homologous genes have not been found in gastric non-*H. pylori* helicobacters, *H. felis,* in particular, induces extensive apoptosis and necrosis of parietal cells in experimentally infected gerbils (37). *H. felis* and *H. suis* induce apoptosis in the murine gastric epithelial cell line GSM06 and in the human gastric adenocarcinoma cell line AGS (non-published results). Gamma-glutamyl-transpeptidase which has been associated with the induction of apoptosis in gastric epithelial

729 cells and the inhibition of T-cell proliferation by *H. pylori* (125, 195, 201), is also produced
730 by gastral non-*H. pylori Helicobacter* species (Table 2).
731 *H. mustelae* produces an array of surface rings which have not yet been described in other
732 *Helicobacter* species. These rings are composed of the *Helicobacter* surface ring (Hsr)
733 protein, comprising approximately 25% of the total envelope protein of *H. mustelae* (168). An
734 Hsr-deficient mutant strain was able to colonize the ferret stomach, but cultures from mutant-
735 dosed ferrets showed reduced levels of bacteria (175). Moreover, animals inoculated with the
736 Hsr-negative strain showed reduced gastric inflammation compared to ferrets infected with
737 the wild-type strain. This underlines the impact of these surface rings on the long-term
738 survival of *H. mustelae* in the ferret stomach.
739
740 **STUDYING GASTRIC *HELICOBACTER* INFECTIONS IN DOMESTIC ANIMALS:**
741 **WHAT MIGHT IT TEACH US ABOUT THESE INFECTIONS IN HUMANS?**
742 One of the main reasons for the lack of knowledge of the main characteristics and
743 bacterium-host interactions for non-*H. pylori* helicobacters colonizing the human stomach is
744 that these bacteria are infrequently cultured *in vitro*, if at all, from gastric biopsies taken from
745 infected humans. Hence, there are two major drawbacks to this approach: the difficulty of
746 obtaining fresh human non-*H. pylori* spiral bacteria positive stomach biopsies, and the small
747 size of these samples. Tackling this problem from a veterinary perspective may be helpful.
748 Indeed, all known gastric non-*H. pylori Helicobacter* species infecting humans, except
749 "*Candidatus* Helicobacter heilmannii" and "*Candidatus* Helicobacter bovis", have now been
750 isolated from the stomach of animals and can be cultivated *in vitro* (14, 92, 115, 134). This
751 will facilitate the study of bacterium-host interactions and will make it possible to determine
752 the antimicrobial susceptibility of these microorganisms. Whole genome sequencing of non-
753 *H. pylori* helicobacters may be used for detecting genes homologous to *H. pylori* virulence

754 genes. The *H. mustelae* and *H. suis* genome have recently been completed but results have not
755 yet been published. Studying the genetic diversity of different isolates belonging to a single
756 species may make it possible to determine whether certain genotypes are more often
757 associated with disease in animals and humans than other genotypes. The availability of pure
758 isolates should also enable the development of typing methods, such as multilocus sequence
759 typing, which can be directly applied on gastric tissue. These techniques can then be used to
760 determine whether animal and human strains are clonally related. It can indeed not be
761 excluded at this time that gastric non-*H. pylori Helicobacter* strains infecting humans may
762 possibly be somewhat different from those found in animals.
763
764 **CONCLUSIONS**
765 There are clear indications that gastric helicobacters other than *H. pylori* can cause disease
766 in humans. These tightly coiled microorganisms comprise at least five different *Helicobacter*
767 species. Diagnostic methods enabling the identification of these bacteria to the species level
768 are needed to help clarify the epidemiology and pathology of these infections in humans.
769 Evidence is accumulating that especially pigs, dogs and cats constitute reservoir hosts for
770 gastric *Helicobacter* species with zoonotic potential. The recent successes with *in vitro*
771 isolation of these fastidious microorganisms from domestic animals open new perspectives
772 for developing typing techniques that can be directly applied on gastric biopsies from humans.
773 These techniques should make it possible to determine whether animal and human strains
774 belonging to the same *Helicobacter* species are clonally related. The availability of *in vitro*
775 isolates also opens new perspectives for better understanding the pathogenesis of non-*H.*
776 *pylori Helicobacter* associated gastric pathology and for developing treatment and prevention
777 measures.

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Frank Pasmans obtained his degree in veterinary medicine in 1998 and a PhD in veterinary sciences in 2002 at Ghent University, Belgium. After three years of post-doc positions at the department of Pathology, Bacteriology and Avian Diseases, Ghent University, he was appointed professor at the same department in the disciplines of veterinary bacteriology and mycology and diseases of reptiles and amphibians. His research focuses on host–pathogen interactions of bacteria and fungi with animals. In this respect, gastric non *Helicobacter pylori Helicobacter* infections in animals are of special interest because they provide an exciting new area in which nearly all aspects of the pathogenesis await discovery. Recent findings confirm the suspected role of *H. suis* in gastric disease in swine, rendering this research highly relevant for both animal and human health.

Bram Flahou is a veterinarian who graduated at the Faculty of Veterinary Medicine, Ghent University, Belgium. In 2005 he started his PhD studies on animal-associated gastric

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Koen Chiers is a veterinarian and PhD from Ghent University, Belgium. In 1995, he became assistant and later assistant-professor at the Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University. Currently, he is employed as a professor in veterinary pathology at the same department. In 2006, he became diplomate of the European College of Veterinary Pathologists (ECVP) and the European College of Porcine Health Management (ECPHM). Since 2001, he has been involved in several studies dealing with animal-associated helicobacters.

Margo Baele studied Bioscience Engineering at Ghent University and graduated in 1997. After her studies, she joined the Department of Pathology, Bacteriology and Avian Diseases at the Faculty of Veterinary Medicine of the same university and obtained her PhD in 2001. During this period, she performed taxonomical research, developed PCR-based methods for the identification and typing of bacteria and described several new bacterial species. After obtaining her PhD degree, she joined the *Helicobacter* team of the same department and studied the taxonomy of *Helicobacter* species from dogs, cats and pigs. This research resulted in the description of several new *Helicobacter* species and the first cultivation of and taxonomical studies on *Helicobacter suis*. Since 2008, Margo Baele has joined the Department of Research Affairs of Ghent University, advising researchers in applying for and managing European Research projects in the European Framework Programme.

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Annemie Decostere received her education at the Faculty of Veterinary Medicine, Ghent University, Belgium. She obtained a Master of Science degree in Aquatic Veterinary Studies at the Institute of Aquaculture, Stirling University, Scotland. Up until December 2006, she was full-time employed as a professor in veterinary bacteriology and mycology. Since the beginning of 2007, she is working at pharma.be (the Belgian association of the pharmaceutical industry) as Advisor in Public Health and Regulatory Affairs Manager. She is currently still involved as a visiting professor in teaching and supervising scientific research at the above Faculty. Her active involvement in research on helicobacters in domestic animals started in 2001. This resulted in her being promoter of various defended doctoral dissertations on helicobacters in domestic animals mainly focussing on taxonomy and clinical significance both in animals and human beings.

Richard Ducatelle is a veterinarian and PhD from Ghent University, Belgium. After a few years working for a Belgian research granting organization as a research grant evaluator, he joined Ghent University where he became professor in avian pathology, general and special animal pathology and animal hygiene. Currently, he is full professor in general and special animal pathology and animal hygiene. Main focus of his research is on interactions between bacteria and the animal host. For many years he has been interested in the problem of gastric ulcers in domestic animals. A major breakthrough in this field was the recent isolation of *Helicobacter suis* which, together with a number of other animal helicobacters, appears to play a role in human gastric pathology as well.

Figure legends.

Fig. 1. Phylogenetic tree based on the near-complete 16S rRNA gene sequences from gastric *Helicobacter* species and other closely related bacteria. The sequences were aligned using the CLUSTAL W program (225) and a phylogenetic tree was constructed using the neighbour-joining method (191) via the PHYLIP package (65). DNADIST was used for distance analysis (126). Bootstrap values (for branches present in more than 50 out of 100 resamplings of the data) are indicated at the nodes. Original names found at the Entrez Nucleotide database (NCBI) are shown between brackets. Sequences marked with an * are derived from bacteria demonstrated in the stomach of humans.

Fig. 2. Phylogenetic tree based on the partial *ureA* and *ureB* gene sequences from gastric *Helicobacter* species and other closely related bacteria. The sequences were aligned using the CLUSTAL W program (225) and a phylogenetic tree was constructed using the neighbour-joining method (191) via the PHYLIP package (65). DNADIST was used for distance analysis (126). Bootstrap values (for branches present in more than 50 out of 100 resamplings of the data) are indicated at the nodes. Original names found at the Entrez Nucleotide database (NCBI) are shown between brackets. Sequences marked with an * are derived from bacteria demonstrated in the stomach of humans.

Fig. 3. In the normal porcine stomach there is a small rectangular area around the cardia which is covered by a slightly keratinized squamous epithelium presenting as a white, slightly irregular surface on visual inspection. This area is named the pars esophagea, since the epithelium is similar to that of the esophagus.

Figure 4. As opposed to most other animal species and to humans, pigs do not usually develop stomach ulcers in the pyloric antrum, but rather in the pars esophagea. These lesions are characterized by hyperkeratosis, which typically presents as a bile-stained thickening of the mucosa (small arrow). This can evolve to clefts, erosions and ulcerations (large arrow).

ABSTRACT

Helicobacters other than *Helicobacter (H.) pylori* have been associated with gastritis, gastric ulcers and gastric MALT lymphoma in humans. These very fastidious microorganisms with typical large spiral shaped morphology were provisionally designated "*H. heilmannii",* but in fact they comprise at least five different *Helicobacter* species, all of them known to colonize the gastric mucosa of animals. *H. suis,* which has been isolated from the stomach of pigs, is the most prevalent gastric non-*H. pylori Helicobacter* species in humans. Other gastric non-*H. pylori* helicobacters colonizing the human stomach are *H. felis, H. salomonis, H. bizzozeronii* and the until now uncultivable *Candidatus* H. heilmannii. These microorganisms are often detected in the stomach of dogs and cats. *Candidatus* H. bovis is highly prevalent in the abomasum of cattle, but has only occasionally been detected in the stomach of humans. There are clear indications that gastric non-*H. pylori Helicobacter* infections in humans originate from animals, and it is likely that transmission to humans occurs through direct contact. Little is known about the virulence factors of these microorganisms. The recent successes with *in vitro* isolation of non-*H. pylori* helicobacters from domestic animals open new perspectives for studying these microorganisms and their interactions with the host.

Table 1. *Helicobacter* species naturally colonizing the stomach of animals and their pathogenic significance for humans

Helicobacter species	Natural host (prevalence)	Associated with gastric disease in humans	References
H. suis	Pig (60 - > 80% in slaughter pigs), macaque (NA**), mandrill monkey (NA)	Yes	14, 42, 45, 167, 227, 230
H. felis	Dog (47%), cat (63%), rabbit (2-9%), cheetah (NA***)	Yes	45, 134, 166, 227, 230, 231, 234
H. bizzozeronii	Dog (70%), cat (35%)	Yes	45, 92, 227, 230
H. salomonis	Dog (9%), cat (2%), rabbit (0-4%)	Yes	45, 115, 227, 230
Cand. H. heilmannii	Dog (20-100%), cat (20-100%), wild felidae (NA***), nonhuman primates (66%)	Yes	166, 227, 230
H. baculiformis	Cat (NA)	No	15
H. cynogastricus	Dog (NA)	No	233
Cand. H. bovis	Cattle (NA)	Yes	44, 45
H. mustelae	Ferret (0-100%)	No	66, 67, 68, 69, 70, 71, 85
H. aurati	Syrian hamster (50-100%)	No	177
*H. nemestrinae**	Macaque (NA)	No	24
H. acinonychis	Cheetah (low), tiger (NA)	No	60, 224
H. cetorum	Whales (NA), dolphins (NA)	No	97
H. muridarum	Mice (0-62%)	No	86, 136, 183

* Later heterotypic synonym of *H. pylori* (219)
** Not available
*** Terio et al. (224) and Mörner et al. (155) found the gastric mucosa of 75% of cheetahs and 68% of free-ranging lynx to be colonized with "pet carnivore associated" helicobacters. These studies do not allow differentiation between *H. felis, H. bizzozeronii, H. salomonis, H. bacculiformis, H. cynogastricus* and *Cand.* H. heilmannii.

Table 2. Differential characteristics of gastric *Helicobacter* species associated with domestic animals and nonhuman primates. Data were obtained from Baele *et al.* (14, 15), De Groote *et al.* (44), Dewhirst *et al.* (47), Fox *et al.* (68), Hänninen *et al.* (92, 93), Jalava *et al.* (115), Lee *et al.* (134), O'Rourke *et al.* (166), Patterson *et al.* (177) and Van den Bulck *et al.* (233). All taxa are positive for catalase production and possess sheathed flagella.

Characteristic	*H. baculiformis*	*H. cynogastricus*	*H. bizzozeronii*	*H. felis*	*H. salomonis*	*H. pylori*	*H. suis*	*Cand.* H. heilmannii	*H. mustelae*	*H. nemestrinae**	*Cand.* H. bovis	*H. aurati*
length (µm)	10	10–18	5–10	5–7.5	5–7	2.5–5	2.3–6.7	5–10	2	ND	1.5–2.5	4–8
Cell width (µm)	1	0.8–1.0	0.3	0.4	0.8–1.2	0.5–1.0	0.9-1.2	0.5–0.6	0.5	ND	0.3	0.6
Nitrate reduction	+**	+	+	+	+	–	–	ND	+	–	ND	–
Urease	+	+	(+)	(+)	+	+	+	+	+	+	+	+
Alkaline phosphate hydrolysis	+	+	V	V	V	+	+	ND	+	+	ND	–
-Glutamyl transpeptidase	+	+	+	+	+	+	+	ND	+	ND	ND	+
Indoxyl acetate hydrolysis	–	–	(–)	(–)	(–)	(–)	–	ND	+	–	ND	+
Growth at 42 °C	–	–	V	V	–	(–)	–	ND	V	+	ND	+
Growth on 1 % glycine	–	–	(–)	–	–	–	–	ND	–	–	ND	–
Periplasmic fibril	+	+	–	+	–	–	–	–	–	–	–	+
No. of flagella/cell	11	6–12	10–20	14–20	10–23	4–8	4–10	10–20	4–8	4–8	≥4	7–10
Distribution of flagella	BP***	BP	BP	BP	BP	MP	BP	BP	LP	BP	ND	BP

* Later heterotypic synonym of *H. pylori* (219)
** +, 100% of strains positive; –, 0% strains positive; (+), 80–94% strains positive; (–), 7–33% strains positive; v, 42–66% strains positive; ND, not determined
*** BP, bipolar; MP, monopolar; LP, lateral polar.

Table 3. Summary of gastric lesions in different animal species naturally or experimentally infected with helicobacters

Animal species	**Helico-bacter**	**Infection**	**Gastric gross lesions**	**Gastric histological lesions**	**Localization of *Helicobacter spp***	**Ref.**
Pig	*H. suis*	Natural	Surface redness	ND	Antrum, fundus	87
			none	ND	Antrum, fundus	104
			Mucosa redness and edema, occasional erosions and hemorrhage	Antrum, fundus: diffuse mononuclear cell infiltration with occasional neutrophilic infiltrate and lymphoid follicles	Antrum, fundus: in mucus, in lumen of the pits, in mucosal surface; positive correlation between presence of bacteria and pyloric gastritis	147
			Fundus: proliferation of gastric folds with occasional necrosis, severe mucosal congestion Pars esophagea: hyperkeratosis	Diffuse lymphocytic infiltration and lymphoid follicles in lamina propria	Antrum: in mucus, gastric pits and lumen of gastric glands	173
			Pars esophagea: hyperkeratosis with yellow discoloration or chronic ulcers	Pars esophagea: increased thickness of epithelium, elongation of papillae, parakeratosis, balloon cells or chronic peptic ulcers (layers of necrosis, numerous inflammatory cells, and granulation tissue and fibrosis) Antrum: mild diffuse mononuclear cell infiltration in lamina propria with multiple lymphocytic aggregates or lymphoid follicles	Antrum, fundus, cardia; positive correlation between presence of bacteria and lesions in pars esophagea	185
			Pars esophagea ulcerative gastritis	Pars esophagea: erosion of surface epithelium, necrosis, mixed inflammatory infiltrate and granulation tissue	Antrum, fundus: in mucous layer and foveolae, occasionally in lumen of gastric glands; positive correlation between presence of bacteria and lesions in pars esophagea	18
			Pars esophagea ulcerative gastritis	ND	Pars esophagea: positive correlation between presence of bacteria and lesions in pars esophagea	28

			Pars esophagea ulcerative gastritis	Diffuse mononuclear cell infiltration in propria mucosa	Antrum: positive correlation between presence of bacteria and lesions in pars esophagea	190
		Experi-mental	Pars esophagea: no, pre-ulcerative and ulcerative lesions	Antrum: mild diffuse mononuclear infiltration in lamina propria with multiple lymphocytic aggregates or lymphoid follicles	Antrum: in mucus overlying the surface epithelium and in the surface foveola Fundus: in glandular foveola extending half way down the gastric pits, and often in close association with mucus-producing cells and parietal cells	103
	Curve-shaped bacteria morpholo-gically similar to *H. pylori*	Natural	None	Antrum, fundus, cardia: diffuse mononuclear cell infiltration in lamina propria with multiple lymphoid follicles, and occasional neutrophilic infiltrates and exudation into the glandular lumens	Antrum, fundus, cardia; in close apposition to the gastric epithelia and in the gastric mucus	129
		Experi-mental	Gastroesophageal ulceration, glandular mucosal ulcers, lymphoid follicles, excess luminal mucus, and mucosal edema	Pars esophagea: peptic ulceration Antrum, cardia, fundus: diffuse mononuclear cell infiltration in lamina propria with multiple lymphoid follicles	Cardia, antrum: extracellularly	130
Dog	*H. felis*	Natural	ND	Chronic active gastritis: diffuse lymphoplasmic infiltration, lymphocytic aggregates and occasional neutrophilic infiltration	Fundus, corpus, antrum: in mucus adjacent to surface epithelium, glandular lamina adjacent to parietal cells and gastric pits	51
		Experi-mental	No lesions	Fundus, antrum: diffuse mononuclear cell infiltration in lamina propria with multiple variable-sized lymphoid follicles	Fundus, corpus, antrum: in mucous layer on mucosal surface within gastric pits and glandular lumen, occasional intracellular in glandular epithelial cells	137
			No lesions	Antrum: mild diffuse lymphoplasmacytic inflammation of lamina propria	Fundus, corpus, antrum: in superficial gastric mucous layer, in gastric glands and parietal cells	204
	NHPH	Natural	Mucosal redening, edema, erosions and ulcerations	Fundus: glandular degeneration with accumulation of lymphocytes and neutrophilic granulocytes, edema, fibrosis, diffuse lymphoplasmacytic infiltrates and lymphoid follicles in lamina propria	Fundus: in mucus covering the surface epithelium, the gastric pits, the glandular lumina and the parietal cells; presence of very high numbers of bacteria was directly related to the number of lymphoid follicles	105

Cat	NHPH	Natural	Mucosal redening, edema, erosions and ulcerations	Fundus: glandular degeneration with accumulation of lymphocytes and neutrophilic granulocytes, edema, fibrosis, diffuse lymphoplasmacytic infiltrates and lymphoid follicles in lamina propria	Fundus: in mucus covering the surface epithelium, the gastric pits, the glandular lumina and the parietal cells; increased bacterial colonization was directly related to the number of lymphoid follicles, fibrosis, lesions of surface epithelium and glandular degeneration	105
			ND	Antrum: diffuse, mixed subglandular leukocytic infiltrates and multiple lymphoid nodules in the lamina propria	Antrum: in the canaliculi or cytoplasm of viable parietal cells	171
	NHPH	Natural	ND	Antrum: moderate lymphoid follicles in lamina propria Antrum and fundus: moderate mononuclear infiltrates in lamina propria	Antrum, fundus: at the mucosal surface, in the lumina of gastric glands, and in cytoplasm of parietal cells of the fundus	193
	H. felis	Experi-mental	ND	Antrum: mild diffuse lymfoplasmacytic and eosinophilic infiltration, lymphoid follicular hyperplasia and mild fibrosis of lamina propria	Antrum, fundus: at the mucosal surface, in the lumina of gastric glands, and in cytoplasm of parietal cells of the fundus	193
	H. pylori	Experi-mental	ND	Antrum: mild diffuse lymfoplasmacytic and granulocytic infiltration, severe lymphoid follicular hyperplasia and mild fibrosis of lamina propria	Antrum, fundus: at the mucosal surface, in the lumina of gastric glands, and in cytoplasm of parietal cells of the fundus	193
Ferret	*H. mustelae*	Natural	Gastritis, peptic ulcer	Proximal antrum: mononuclear cell infiltrates in superficial layer of lamina propria, mucus depletion and occasional neutrophilic infiltrate, gland necrosis and regeneration Distal antrum: diffuse mononuclear cell infiltration and mucus depletion Fundus: mononuclear cell infiltrates in superficial layer of lamina propria, mucus depletion and occasional neutrophilic infiltrate	Proximal antrum: at surface and lumen of foveola and occasionally in deep glandular lumen Distal antrum: at surface and lumen of foveola and occasionally in superficial glandular lumen Fundus: at surface and lumen of foveola adjacent to the inflammation	70, 140
			Pyloric adenocarcinoma	Antrum: multifocal segmental glandular proliferation and surface erosion, multifocal mucosal lymphoid aggregates, mixed inflammatory cell infiltration, and multifocal fibrosis	Antrum: in the lumen of gastric pits, adherent to apical surface of the mucous epithelium	75

			Gastric lymphoma	High and low grade B-cel lymphoma	Antrum, fundus: within the mucosal glands	63
		Experi-mental	ND	Antrum: focal minimal lymphocytic infiltrates with moderate numbers of eosinophils and neutrophils Fundus: superficial gastritis consisting of lymphocytes and occasional neutrophils	Antrum, fundus: on the surface of the gastric epithelium within the mucous layer and within gastric pits	72
	H. mustelae + *MMNPG*	Experi-mental	Pyloric adenocarcinoma	ND	Antrum: epithelial surfaces of the neck glands	75
Ham-ster	*H. aurati*	Natural	ND	Distal antrum: diffuse lymphoplasmacytic inflammation, scattered heterophils and eosinophils, and goblet cell hyperplasia	Antrum: within gastric pits or glands	176
			Pyloric adenocarcinoma	Distal antrum: locally extensive chronic gastritis with intestinal metaplasia, and occasional well-differentiated and moderately pleomorphic tubular to tortuous gastric glands	Antrum: within gastric glands	159
Cattle	*Cand.* Helicobac-ter bovis	Natural	ND	ND	Distal antrum: in mucus layer and proximal gastric crypts	44
Horse	NHPH	Natural	No lesions, gastritis or gastric ulcers	Gastric mucosa: loss of continuity with submucosa exposure and edema; parakeratotic hyperkeratosis; lymphoplasmocytic mononuclear infiltrate	Glandular and non-glandular stomach near *margo plicatus*	31
Non-human primates	*H. pylori*	Natural	No lesions, superficial gastritis	Distal fundus, antrum: superficial erosions, marked mononuclear and polynuclear infiltration	Antrum: proximity to the mucosal epithelial cells or in the lumen of the gastric pits	57
			None	No lesions or diffuse lymphoplasmacytic infiltration of the lamina propria, occasional neutrophilic infiltrates and lymphoid follicles	Antrum: superficial mucosa	55
			ND	Antrum: diffuse lymfoplasmacytic infiltraton in lamina propria, prominent lymphoid follicles, and occasional glandular epithelial hyperplasia, patchy necrosis, attenuation of glandular epithelium, and neutrophilic infiltrates	Antrum: gastric pits and the upper portions of gastric glands, often in intimate association with the epithelial cell surface	138

			Localized to multifocal reddening of mucosa	Antrum, cardia: lymphoplasmacytic infiltrates, gastric gland epithelial hyperplasia, reduction in mucin content of surface and gland epithelia, increased lymphoid follicles, minor infiltrates of neutrophils in superficial lamina propria and gastric glands, and occasional erosions	Antrum, cardia: mucosal regions of inflammation; located in gastric pits and upper portions of gastric glands, often associated with epithelial cell surface	187
		Experi-mental	ND	Antrum: chronic-active gastritis, marked atrophy of the mucosae, microerosions and loss of mucus from superficial epithelial cells	Antrum	56
	NHPH	Natural	No lesions, superficial gastritis	No lesions to occasional mononuclear and polynuclear infiltration	Fundus: mucus covering the surface of epithelial cells, in the lumina of the gastric glands, and overlying parietal cells	57
	NHPH	Natural	None	No lesions or diffuse lymphoplasmacytic infiltration of the lamina propria, occasional neutrophilic infiltrates and lymphoid follicles	Fundus: gastric pits, superficial glands or on the surface epithelium,	55
			ND	Fundus: minor scattered aggregates of lymphocytes, low numbers of plasma cells and occasional lymphoid follicles	Fundus: in the gland lumens, sometimes attached to the epithelial cell surface, and within the cytoplasm of parietal cells	138
			Localized to multifocal reddening of mucosa	Fundus: no lesions	Fundus: in gland lumens, parietal cells, and surface mucus	187

ND: not described; NHPH: Non-*H. pylori Helicobacter* species; MNNG: N-methyl-N-nitro-N'-nitrosoguanidine