博士論文（要約）

Studies on the epidemiology and pathogenicity of canine and feline gastric *Helicobacter* spp. in Japan

（国内の犬・猫の胃に感染する *Helicobacter* 属菌の疫学調査および病原性の検討）

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CONTENTS

General introduction

The genus *Helicobacter* is gram-negative, microaerophilic spiral bacteria that contains at least 40 species (Baele et al., 2008a), and it inhabits throughout the digestive systems of humans and several animals including dogs and cats (Haesebrouck et al., 2009a). These species are divided into gastric and enterohepatic *Helicobacter* spp. based on their preferential site of colonization and phylogenetic analysis of 16S and 23S rRNA genes (Fox, 2002; Dewhirst et al., 2005).

Helicobacter pylori (*H. pylori*) is the most common gastric *Helicobacter* species in humans, and it is associated with various human gastric diseases including gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (Parsonnet et al., 1991; Wotherspoon et al., 1993; Kusters et al., 2006). *H. pylori* infection is also associated with extragastric diseases such as idiopathic thrombocytopenic purpura and iron deficiency anemia in humans (Wu et al., 2008).

In dogs and cats, *H. pylori* infection has been reported be rare (Handt et al., 1994; Buczolits et al., 2003; Canejo-Teixeira et al., 2014; Sasani et al., 2014), and non-*H. pylori* helicobacters (NHPH) are more common gastric *Helicobacter* spp. NHPH had generally been referred to as *H. heilmannii*, a designation that include *H. suis*, which colonizes the stomachs of pigs, and several species, includeing *H. felis, H. bizzozeronii, H. salomonis, H. cynogastricus, H. baculiformis*, and *H. heilmannii* sp. nov., that colonize the gastric mucosa of dogs and cats (Haesebrouck et al., 2011). To avoid nomenclatural confusion, the term *H. heilmannii* sensu lato (s.l.) was proposed in 2011 to refer to NHPH detected in the stomachs of humans or animals when only histopathological, electron microscopy, or crude taxonomic data were available, whereas *H. heilmannii* sensu stricto (s.s.) or other species names would

be used if NHPH were definitively identified at the species level (Haesebrouck et al., 2011). According to previous reports, the prevalence of NHPH ranges between 61% and 100% in dogs, and between 41% and 100% in cats (Haesebrouck et al., 2009a). The predominant gastric NHPH that are found in dogs and cats have been reported to be *H. bizzozeronii*, *H. felis*, and *H. heilmannii* s.s. (Haesebrouck et al., 2009a). In addition, *H. salomonis* is sometimes detected in both dogs and cats. *H. cynogastricus* and *H. baculiformis* were recently isolated from the stomach of a dog and a cat, respectively (Van den Bulck et al., 2006; Baele et al., 2008b), and their prevalence have not yet been determined.

Recently, NHPH have also been observed in human stomach, and most NHPH including *H. bizzozeronii*, *H. felis*, *H. heilmannii* s.s., and *H. salomonis* are associated with human gastric diseases such as active chronic gastritis, acute gastritis, gastric ulcer, and gastric low-grade MALT lymphoma (Stolte et al., 1997; Debongnie et al., 1998; Morgner et al., 2000; Yoshimura et al., 2002), which is attracting growing concern as zoonosis. In contrast, there are many conflicting reports regarding the pathogenicity of each NHPH in dogs and cats, and therefore, the pathogenic significance of these gastric NHPH in dogs and cats is controversial at present. The conflicting results of the pathogenicity of each NHPH in dogs and cats may occur due to the differences in virulence between different isolates, as has also been described for *H. pylori* (Dunn et al., 1997; Kusters et al., 2006). Despite their potential importance, there is insufficient epidemiological data to estimate the prevalence of *Helicobacter* spp. in the stomach of dogs and cats in Japan. Further studies of the relationship between infection with the various NHPH species, as identified by their specific sequences, and canine and feline gastric lesions from larger sample sizes in Japan are needed.

Therefore, the purposes of the present thesis were to determine the prevalence of various *Helicobacter* spp. in the stomachs of dogs and cats in Japan, and to evaluate their pathogenic significance in dogs and cats. In Chapter 1, I compared several methods for detecting gastric *Helicobacter* spp. infection in dogs as a preliminary step toward the investigation of the infection status of gastric *Helicobacter* spp. in dogs. In Chapter 2-1, the prevalence of various *Helicobacter* spp. in the canine stomachs in Japan was investigated at the strain level, and the first suggestion that *H. pylori* could be transmitted between humans and dogs was reported in Chapter 2-2. In Chapter 3, the pathogenicity of NHPH in dogs was investigated using various methods, including the UBT, for clinical cases. In Chapter 4, the prevalence of various *Helicobacter* spp. in the feline stomachs in Japan was investigated at the strain level, as a preliminary step toward evaluating the pathogenic significance of *Helicobacter* spp. in cats. In Chapter 5, the pathogenicity of NHPH in cats was investigated using various methods for clinical cases.

Chapter 1

Value of the ^{13}C-urea breath test for detection of gastric *Helicobacter* spp. infection in dogs undergoing an endoscopic examination

Helicobacter spp. colonize the stomach and intestine of humans and several animal species (Haesebrouck et al., 2009a). The *Helicobacter* genus currently includes about 40 formally named members with numerous other putative species under investigation (Baele et al., 2008a; Harbour and Sutton, 2008). In humans, *Helicobacter pylori* (*H. pylori*) is known to be the major agent of chronic diffuse superficial gastritis, plays a causative role in peptic ulcers and is considered a co-factor in the development of gastric malignancies (Parsonnet et al., 1991; Stolte and Eidt, 1993; Kusters et al., 2006). *H. pylori* infection in dogs is reported to be rare (Haesebrouck et al., 2009a), but a variety of gastric non-*H. pylori Helicobacter* species (NHPH), including *H. felis*, *H. bizzozeronii*, *H. salomonis, H. heilmannii* sensu stricto (s.s.), and *H. cynogastricus*, can colonize the stomachs of dogs. The reported prevalence of *Helicobacter* spp. in dogs is between 61 and 100%; it is found in 61―82% of dogs with recurrent vomiting (Geyer et al., 1993a; Hermanns et al., 1995b; Yamasaki et al., 1998a), 67―86% of clinically healthy pet dogs (Eaton et al., 1996a; Yamasaki et al., 1998a) and almost 100% of laboratory beagles and shelter dogs (Henry et al., 1987b; Eaton et al., 1996a), but insufficient epidemiological data are available to estimate the prevalence in Japan. In addition, although *H. felis*, *H. bizzozeronii*, *H. salomonis,* and *H. heilmannii* s.s. are also found in the human stomach (Trebesius et al., 2001; De Groote et al., 2005; Van den Bulck et al., 2005) and are suggested to be associated with gastric diseases in humans (Morgner et al., 1995; Stolte et al., 1997; Morgner et al., 2000; Yoshimura et al., 2002), their pathogenic significance in dogs is controversial (Happonen et al., 1998b; Peyrol et al., 1998b; Simpson et al., 1999b; Sapierzynski et al., 2003b; Sapierzynski and Malicka, 2004a; Sapierzyniski et al., 2006; Leib et al., 2007b; Shabestari et al., 2008b).

Diagnostic tests for gastric *Helicobacter* spp. in dogs and cats include polymerase chain reaction (PCR) testing, rapid urease test, histology and cytology; all of these require anesthesia and gastric biopsy (Happonen et al., 1996b; Neiger et al., 1998; Neiger et al., 1999). The ^{13}C-urea breath test (UBT) is a non-invasive test with high sensitivity and specificity that is widely used in human medicine (Graham et al., 1987; Slomianski et al., 1995; Kato et al., 1998; Ohara et al., 1998a). In a multicenter trial in Japan, UBT using an infrared spectral analyzer showed 98.1% sensitivity and 97.9% specificity in human when a cut-off value of 2.5‰ was used to distinguish between patients with and without *Helicobacter* infections (Ohara et al., 1998a). In veterinary medicine, UBT using an isotope ratio mass spectrometer has been used only experimentally in dogs and cats (Cornetta et al., 1998a; Neiger et al., 1998; Neiger et al., 1999), and UBT using an infrared spectral analyzer, despite being much more rapid and economical than gas isotope ratio mass spectrometric analysis (Ohara et al., 1998b), has not yet been applied in the clinical setting.

The main purposes of this study were to determine the reference range of UBT using an infrared spectrograph in dogs and to evaluate its validity in clinical cases.

Materials and Methods

Animals

Six healthy laboratory beagles (one male and five females) kept in the animal facility of the Veterinary Medical Center of the University of Tokyo (VMC-UT) were used for the determination of the reference range of the UBT. The dogs' ages ranged from 2.8 to 6.7 years and their body weights from 7.8 to 14.2 kg. No dog showed any gastrointestinal clinical sign or other abnormality upon physical examination. To evaluate the validity of the reference range of the UBT, dogs that visited VMC-UT and underwent upper gastrointestinal endoscopy for various reasons between March 2011 and August 2012 were enrolled in this study. Dogs were excluded from the study when collection of breath samples failed or endoscopic samples could not be obtained because of incidents during anesthesia. The experiments and animal care procedures were approved by the Animal Use and Care Committee of the University of Tokyo (approval number: P11-527).

Experimental design

For the determination of the reference range of the UBT, six laboratory beagles underwent UBT and upper gastrointestinal endoscopy in that order. Dogs were judged to be *Helicobacter* spp.-negative when all of the gastric biopsy samples from the gastric fundus, corpus and antrum were negative by all of the tests performed (PCR and histology/cytology). For *Helicobacter* spp.-positive beagles, amoxicillin (250 mg/dog, PO, q 12 h), metronidazole (250 mg/dog, PO, q 12 h) and

omeprazole (10 mg/dog, PO, q 24 h) were administered for 14 days (Cornetta et al., 1998a; Papich, 2007), and dogs that became *Helicobacter* spp.-negative were used to determine the reference range.

In the canine patients, the UBT and upper gastrointestinal endoscopy were performed in that order, and the sensitivity and specificity of the UBT based on the results of PCR as the gold standard for the detection of *Helicobacter* spp. were calculated.

UBT

After overnight fasting, a breath sample was collected using a close-fitting anesthesia mask and a breath sampling bag (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). A breath sample was collected until the sampling bag was inflated adequately without disconnecting the mask from a mouth. The laboratory beagles were given ^{13}C-urea UBIT® tablets (Otsuka Pharmaceutical Co., Ltd.) orally at a dose of 50 mg/dog based on a previous report (Cornetta et al., 1998a). The canine patients were dosed with ^{13}C-urea UBIT® tablets as follows: 25 mg/dog for dogs weighing less than 6 kg, 50 mg/dog for dogs weighing 6 kg or more but less than 15 kg, and 100 mg/dog for dogs weighting 15 kg or more (Cornetta et al., 1998a). A second breath sample was obtained 30 min after administration. These paired breath samples were analyzed using a POCone device (Otsuka Pharmaceutical Co., Ltd.), an infrared spectral analyzer that measures the change in the carbon isotope ratio ($^{13}CO_2/^{12}CO_2$) in the exhaled air. The difference between the ratios before and after ^{13}C-urea administration was expressed as $\Delta^{13}CO_2$.

Endoscopic biopsy

After the UBT, an intravenous catheter was placed, and anesthesia was induced with propofol and maintained with isoflurane by endotracheal intubation. Dogs were restrained in left recumbency and subjected to gastroscopy. Endoscopic biopsy samples of the stomach were obtained from the antrum, corpus and fundus in this order using an endoscope designed for animals, the OLYMPUS VQ-8143B (Olympus Medical Systems Corp., Tokyo, Japan), and a biopsy forceps (FB-54Q-1, Olympus Medical Systems Corp.). The biopsy forceps was intensively washed with water and 70% ethanol every time the targeted gastric regions were changed. The samples obtained were subjected to PCR, and histology/cytology.

PCR

Gastric biopsy samples were frozen at -20°C until DNA extraction. DNA was extracted from the specimens using a QIAamp DNA Blood Mini Kit (Qiagen, Santa Clarita, CA, U.S.A.). PCR was performed with primers specific to *Helicobacter* 16S rRNA (forward primer: 5′-GCTATGACGGGTATCC-3′, reverse primer: 3′-ACTTCACCCCAGTCGCTG-5′) that generate a 1200-bp amplicon (Fox et al., 1998). All PCR reactions consisted of 1 μL of extracted DNA, 12.5 μL of HotStar Taq Master Mix (Qiagen) and 0.5 μM of each primer in a final volume of 25μL and were run using a TaKaRa PCR Thermal Cycler Dice® Standard (Takara Bio Inc., Otsu, Japan). PCR amplification was carried out according to the following protocol: 94°C for 10 min, followed by 30 cycles of denaturing at 94°C for 1 min, primer annealing at 58°C for 1 min and 30 sec, and extension

at 72°C for 2 min, followed by a final extension at 72°C for 10 min. A 10-μL volume of each amplification product was analyzed by electrophoresis on a 1% agarose gel in Tris-acetate EDTA buffer. The gel was stained with ethidium bromide, and the DNA was visualized using a UV transilluminator.

Histology/cytology

For histology, the gastric mucosal samples were fixed with neutral-buffered formalin, processed routinely and embedded in paraffin. Sections were cut, stained with hematoxylin and eosin and evaluated by light microscopy by two veterinary pathologists (K. U. and Y. M.). Impression smears of the gastric biopsy samples were stained with Wright-Giemsa stain and evaluated by light microscopy. The specimens were considered to be *Helicobacter* spp.-positive by histology/cytology when spiral-shaped bacteria were observed in at least 1 histologic section and/or cytology smear and negative when none was seen in all sections and smears.

Statistical Analysis

The Mann—Whitney *U*-test was used to compare the numerical results of the UBT between the *Helicobacte*r spp.-positive and -negative groups of canine patients. The analysis was performed using JMP version 5.0.1 (SAS Institute, Cary, NC, U.S.A.). The level of significance was set at $P = 0.05$.

Results

Determination of the reference range of the UBT in dogs

All of the gastric tissue samples obtained by endoscopy from the 6 laboratory beagles were positive for *Helicobacter* spp. by all tests (PCR and histology/cytology; Table 1-1). In two dogs, PCR detected *Helicobacter* spp. in the fundus and corpus of the stomach, but not in the antrum. After eradication using antimicrobial agents, all dogs were negative in all regions by all examinations (Table 1-1).

The mean ± 2 SD value of UBT was 22.2 ± 18.2‰ before the eradication of *Helicobacter* spp. and decreased markedly after eradication (to 0.6 ± 1.8‰). As all dogs were negative by all examinations after the eradication of *Helicobacter* spp., the reference range of the UBT in dogs was determined to be less than 2.5‰, which is equivalent to the cut-off value for humans (2.5‰).

Validity of the reference range of the UBT in canine patients

The UBT was tried to be performed for 32 dogs, and succeeded in 27 dogs. Of these, 17 were female (13 spayed) and 10 male (six castrated). The dogs' ages ranged from 1.2 to 13.1 years and their body weights from 1.8 to 25.4 kg. The 19 pure breeds represented were Miniature Dachshund (n = 5), Yorkshire Terrier (n = 3), Chihuahua and Shih Tzu (n = 2 each), and Pug, French Bulldog, Japanese Shiba Inu, German Shepherd Dog, Toy Poodle, Toy Manchester Terrier, Boston Terrier, West Highland White Terrier, Jack Russell Terrier, Cairn Terrier, Miniature Schnauzer, Siberian Husky,

Pekingese, Kaninchen Dachshund and Pembroke Welsh Corgi (n = 1 each).

By PCR, 18 dogs were positive and 9 negative for *Helicobacter* spp. (Table 1-2). *Helicobacter* spp. was detected by PCR in the fundus region specimens of all 18 positive dogs, but not in the antrum specimens of 10 or the corpus specimens of 2 of these dogs (Table 1-2). The median value of the UBT in the positive group (n = 18) was 21.1‰ (range: 0.8—157.3‰), which was significantly higher than that of the negative group (n = 9, median: 0.6‰, range: 0.2—6.4‰; Fig. 0-1, $P < 0.01$). On the basis of the reference range of 0—2.4‰ determined in the first part of the present study, 17 dogs were judged to be positive and 10 to be negative for *Helicobacter* spp. by the UBT. Of the 18 dogs that were *Helicobacter*-positive by PCR, 16 were also positive by the UBT, while among the 9 dogs that were *Helicobacter*-negative by PCR, 8 were also negative by the UBT (Fig. 1-1). Therefore, UBT exhibited 89% (16/18) sensitivity and 89% (8/9) specificity.

Discussion

Helicobacter spp. has been reported to be widespread in dogs regardless of their clinical signs and/or disease states (Geyer et al., 1993b; Hermanns et al., 1995a; Eaton et al., 1996b; Yamasaki et al., 1998b), and the prevalence is also reportedly high in laboratory and shelter dogs (Henry et al., 1987a; Eaton et al., 1996b). In the present study, all laboratory beagles (n = 6) were initially judged to be *Helicobacter* spp.-positive by all examinations (PCR, histology, and cytology), indicating that *Helicobacter* spp. was prevalent in the kennel housing the laboratory beagles used in the present study.

Helicobacter spp. was eradicated from the stomachs of the infected dogs in the present study by administration of amoxicillin (250 mg/dog, PO, q 12 h), metronidazole (250 mg/dog, PO, q 12 h) and omeprazole (10 mg/dog, PO, q 24 h) for 14 days; this protocol was based on previous reports (Papich; Cornetta et al., 1998b). As all dogs were *Helicobacter* spp.-negative by all examinations after eradication, this protocol seemed to be quite efficacious for the eradication of *Helicobacter* spp. from the canine stomach.

On the basis of the post-eradication results of the UBT in the six laboratory beagles, the reference range of the UBT for dogs was calculated to be less than 2.5‰, which is equivalent to the cut off value for humans. Next, the validity of this reference range was evaluated in 27 canine patients that underwent upper gastrointestinal endoscopy by determining the sensitivity and specificity of the UBT using PCR as the gold standard for the detection of *Helicobacte*r spp. The UBT proved to be highly sensitive (89%, 16/18) and specific (89%, 8/9), indicating that the UBT could be quite helpful

for detecting *Helicobacter* spp. in the canine stomach.

In the present study, histology, and cytology were also performed on the laboratory beagles before and after eradication, and the results of all of these agreed with the results of the UBT and PCR, indicating that these examinations are also useful when gastric biopsy samples are available.

While *H. pylori* has been most frequently observed in the gastric antrum region in humans (Leodolter and Megraud, 2001), several studies in dogs have reported that *Helicobacter* spp. has been identified at higher frequency in the fundus and corpus than in the antrum (Happonen et al., 1996a; Happonen et al., 1998a; Yamasaki et al., 1998b; Simpson et al., 1999a). In the present study, *Helicobacter* spp. was most frequently detected by PCR in the fundus in both laboratory beagles and canine patients (Tables 1-1 and 1-2). These observations indicated that gastric endoscopic biopsy samples should be collected from at least the gastric fundus for the detection of *Helicobacter* spp. in dogs. Though the possibility of the contamination between the gastric regions through the biopsy forceps cannot be completely denied, we believed it was minimized by intensively washing of the biopsy forceps with water and 70% ethanol every time the targeted gastric regions were changed.

In the *Helicobacter* spp.-positive patients, the UBT values of three dogs seemed to be relatively higher than those of the rests. The number of *Helicobacter* spp. in the stomach of each patient wasn't counted in the present study, but there is no report which shows a positive correlation between the UBT value and the number of *Helicobacter* spp. in the stomach at present. Considering that the urease activities of *Helicobacter* spp. are different according to their species and strains (Haesebrouck et al., 2009b), the identification of *Helicobacter* spp. by their specific sequences may

be helpful for the investigation into the cause of the high values.

In the present study, one canine patient showed false-positive and two false-negative UBT results. The false-positive result may in fact have reflected the local presence of *Helicobacter* spp. in the stomach and the collection of endoscopic biopsy samples for PCR from areas in which there was no *Helicobacter* spp. In addition, oral or gastric urease-producing bacteria other than *Helicobacter* spp., such as *Enterobacter cloacae*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, may affect the results of the UBT, as has been reported in humans (Ohara et al., 1996; Osaki et al., 2008). The false-negative results may have occurred, because the number of *Helicobacter* spp. organisms was too small to detect by the UBT or because the ^{13}C-urea did not sufficiently contact the organisms due to gastric hyperactivity.

During the study of canine patients, we failed to collect breath samples in five snub-nosed or small/large dogs: Pug (n = 2), and Pomeranian, German Shepherd Dog and Belgian Shepherd Dog (n = 1 each). Therefore, the UBT may require preparing a mask of appropriate shape and size for each dog. Furthermore, the UBT may be impossible to perform on very aggressive dogs.

The pathogenicity of *Helicobacter* spp. in the canine stomach is controversial at present. Some authors have denied the pathogenicity of *Helicobacter* spp. in dogs (Happonen et al., 1998a; Simpson et al., 1999a; Shabestari et al., 2008a), but other reports have supported the relationship between *Helicobacter* infection and gastric disease (Peyrol et al., 1998a; Sapierzynski et al., 2003a; Sapierzynski and Malicka, 2004b; Sapierzynski et al., 2006; Leib et al., 2007a). Therefore, treating *Helicobacter* infection in dogs is controversial, and whether treatment is needed in all cases or not is

unclear at this point. Further studies of the relationships between infection with each NHPH species (as identified by their specific sequences) and canine gastric lesions with larger sample sizes are needed. The present study showed that the status of gastric infection with *Helicobacter* spp. in canine patients can be determined by the UBT with high sensitivity and specificity and without anesthesia and invasive procedure. UBT using an infrared spectrograph would thus be a useful screening test for domestic epidemiological studies of *Helicobacter* spp. in the canine stomach, and would make the study of the pathogenicity of *Helicobacter* spp. in the canine stomach more efficient. In addition, as the UBT values decreased after eradication of *Helicobacter* spp., UBT could be useful for monitoring the gastric infection status in dogs treated with antibiotics for suspicious NHPH-related gastritis.

Table 1-1. Results of PCR, and histology or cytology for *Helicobacter* spp. in gastric biopsy samples from six laboratory beagles

	Before eradication[a)]		After eradication	
Dog no.	PCR	Histology/Cytology[b)]	PCR	Histology/Cytology
1	+ (+/+/+) [c)]	+ (+/+/+)	–	–
2	+ (–/+/+)	+ (+/+/+)	–	–
3	+ (+/+/+)	+ (+/+/+)	–	–
4	+ (+/+/+)	+ (+/+/+)	–	–
5	+ (–/+/+)	+ (+/+/+)	–	–
6	+ (+/+/+)	+ (+/+/+)	–	–

[a)] *Helicobacter* spp. was eradicated by amoxicillin (250 mg/dog, PO, q 12 h), metronidazole (250 mg/dog, PO, q 12 h) and omeprazole (10 mg/dog, PO, q 24 h) for 14 days

[b)] Positive when spiral-shaped bacteria were observed in at least one histologic section and/or cytology smear and negative when none was seen in all sections and smears.

[c)] Total result and partial results (antrum/corpus/fundus); total result was positive when at least one region had positive results, and negative when all the three regions had negative results.

Partial results are arranged in order of the obtention of endoscopic biopsy samples

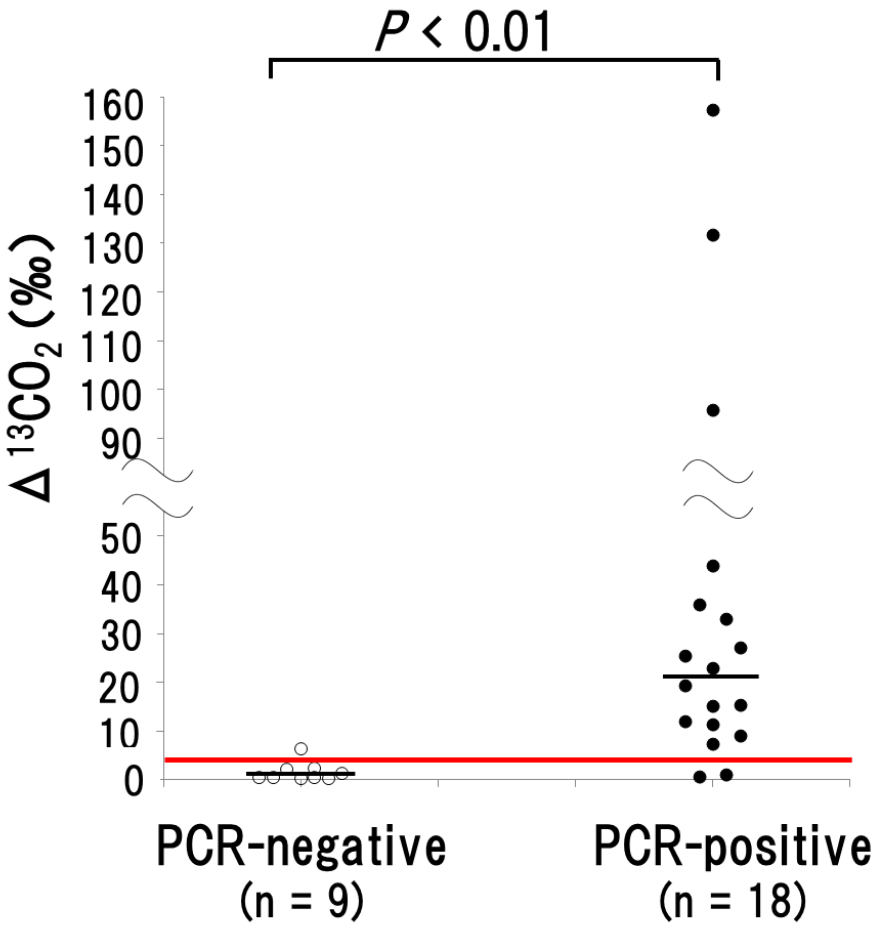
Table 1-2. Results of PCR for *Helicobacter* spp. in the stomachs of canine patients (n=27)

Dog no.	Total [a] (antrum/corpus/fundus) [b]
1	– (–/–/–)
2	**+ (+/+/+)**
3	**+ (+/+/+)**
4	– (–/–/–)
5	– (–/–/–)
6	**+ (–/–/+)**
7	**+ (+/+/+)**
8	**+ (–/+/+)**
9	**+ (–/+/+)**
10	**+ (–/+/+)**
11	– (–/–/–)
12	– (–/–/–)
13	– (–/–/–)
14	**+ (–/+/+)**
15	**+ (–/+/+)**
16	**+ (–/+/+)**
17	– (–/–/–)
18	**+ (–/+/+)**
19	– (–/–/–)
20	**+ (–/+/+)**
21	**+ (–/+/+)**
22	**+ (+/+/+)**
23	**+ (–/–/+)**
24	– (–/–/–)
25	**+ (+/–/+)**
26	**+ (+/+/+)**
27	**+ (+/+/+)**

[a] Total result was considered to be positive when at least one region had positive results, and negative when all the three regions had negative results

[b] Partial results are arranged in order of the obtention of endoscopic biopsy samples

Fig. 1-1



$\Delta^{13}CO_2$ values from the urea breath test (UBT) for *Helicobacter* spp.-negative and -positive canine patients. Each point represents one dog. The reference range for the UBT is under the red solid horizontal line. The black solid horizontal lines represent the median values of each.

Chapter 2

Infection status of gastric *Helicobacter* spp. in dogs that underwent an endoscopic examination in Japan

Chapter 2-1

Infection status of gastric *Helicobacter* spp. in dogs that underwent an endoscopic examination in Japan: diversity of *H. heilmannii* s.s.

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Chapter 2-2

First report of transmission of *Helicobacter pylori* between human and dogs

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Chapter 3

Relationship between gastric *Helicobacter* spp. infection and clinical, clinicopathological, and pathological findings in dogs that underwent an endoscopic examination

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Chapter 4

Infection status of gastric *Helicobacter* spp. in cats that underwent an endoscopic examination in Japan

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Chapter 5

Relationship between gastric *Helicobacter* spp. infection and clinical, clinicopathological, and pathological findings in cats that underwent an endoscopic examination

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Conclusion

Non-*H. pylori Helicobacter* species (NHPH) including *H. felis*, *H. bizzozeronii*, and *H. heilmannii* s.s. are common gastric *Helicobacter* spp. in dogs and cats (Haesebrouck et al., 2011). These NHPH are thought to be transmitted from dogs and cats to humans, and cause some human gastric diseases (Stolte et al., 1997; Debongnie et al., 1998; Morgner et al., 2000; Yoshimura et al., 2002). Therefore, the zoonoses have been attracting growing concern in human medicine. On the other hand, their pathogenic significance in dogs and cats is controversial, and, despite their potential importance, there is no sufficient epidemiological data on *Helicobacter* spp. in the stomachs of dogs and cats in Japan. In Chapter 1, I showed that the ^{13}C-urea breath test (UBT) could non-invasively determine the status of gastric *Helicobacter* spp. infection in canine patients with high sensitivity and specificity, and that the UBT would be helpful in studying the epidemiology and pathogenicity of gastric *Helicobacter* spp. in dogs. As a next step, a series of studies were conducted to determine the prevalence and evaluate the pathogenic significance of *Helicobacter* spp. in the stomachs of dogs and cats in Japan.

In Chapter 1, I performed a comparative review of several methods for detecting gastric *Helicobacter* spp. infection in dogs as a preliminary step toward the investigation of the infection status of gastric *Helicobacter* spp. in dogs. The present study showed that the status of gastric infection with *Helicobacter* spp. in canine patients can be determined by the UBT with high sensitivity and specificity and without anesthesia and invasive procedure. Moreover, UBT could be useful for monitoring the gastric infection status in dogs treated with antimicrobial agents.

In Chapter 2-1, the prevalence of *Helicobacter* spp. in the stomachs of dogs in Japan was

found to be 35%. In a sequence analysis of the partial *ureAB* gene, most of the sequences obtained clustered with those of known *H. heilmannii* s.s. strains, with sequence identities ranging from 89% to 99%. The low prevalence of *Helicobacter* spp. in the stomachs of canine patients in Japan compared to those in Europe or Korea is thought to be attributed to regional differences. The situation is similar to *H. pylori* infection in humans, which has a range of prevalence in different countries (Pounder and Ng, 1995; Kusters et al., 2006). Although a history of antibiotic use may have affected the *Helicobacter* spp. infection rate in the present study, the influence is thought to be small because cases were excluded from this study if the canine patients had already received the effective eradication protocols for gastric *Helicobacter* spp. for dogs (Cornetta et al., 1998a; Fox, 1998; Hall, 2000). The genetic diversity of the partial *ureAB* gene of *H. heilmannii* s.s. suggests the possibility that *H. heilmannii* s.s. in Japan can be divided into several subspecies.

In Chapter 2-2, I introduced the first report that suggests *H. pylori* could be a zoonosis between humans and dogs. *H. pylori* was found in the stomach of a dog (Chapter 2-1), and the infection was also found in the dog's owner and another dog within the same household (Chapter 2-2). The sequences of the partial *ureAB* gene from the dogs and owner were identical. The most closely related organism was *H. pylori* oki 128, which was isolated from a human patient in Okinawa, far from the area where the dogs and owner in the current study live. These results suggest that the infection of *H. pylori* among the study's patients was caused by transmission between human and dogs. Taken together with the fact that NHPH are widely known to be transferable between humans and animals, including dogs and cats, strong cooperation between human and veterinary medicine is needed to

investigate the infection route of *Helicobacter* spp. between humans and animals to prevent the spread of these zoonoses.

In Chapter 4, PCR results showed that the prevalence of *Helicobacter* spp. in the stomachs of cats in Japan was 50%, and the majority of the partial *ureAB* gene sequences were most closely related to *H. heilmannii* s.s. with sequence similarities of 91–99%. The prevalence of *Helicobacter* spp. in this study falls within the range previously reported in cats outside of Japan (41–100%) (Haesebrouck et al., 2009a). Of the partial *ureAB* gene sequences most closely related to *H. heilmannii* s.s., some strains showed high similarities (99%) with those of *H. heilmannii* s.s. strains obtained from human patients with gastric diseases. Others were most closely related to known *H. heilmannii* s.s. with relatively low similarities of about 90%. These results suggest possible zoonotic transmission of some *H. heilmannii* s.s. strains between humans and cats, and that there are novel *H. heilmannii* s.s. strains in the stomachs of cats in Japan.

The relationship between gastric *Helicobacter* spp. infections and gastrointestinal symptoms, clinicopathological findings, gastric endoscopic findings, and gastric histopathological changes were investigated in Chapter 3. The dogs infected with *Helicobacter* spp. most similar to *H. heilmannii* s.s. showed a higher incidence of moderate to severe gastritis than *Helicobacter*-negative dogs. Furthermore, the clinical response to eradicating gastric *Helicobacter* spp. could be determined in the cases of four patients, three of which showed an improvement in vomiting frequency. Taken together with these results, the predominant *Helicobacter* species detected in the stomachs of dogs in Japan were most closely related to *H. heilmannii* s.s. with substantial genetic diversity, and infection with

this *Helicobacter* may have increased the severity of gastritis in dogs from Japan. Eradicating *Helicobacter* spp. from the canine patients' stomachs ameliorated the vomiting frequency for some dogs in the present study, but I could not perform a statistical analysis of the results because there were too few test subjects. To confirm the statistically significance of difference in the treatment result in dogs, it would be necessary to perform an additional study using a greater number of dogs.

There were no significant differences, however, between *Helicobacter* spp.-positive and -negative cats in the severity of chronic gastritis, as shown in Chapter 5. The clinical response of eradicating gastric *Helicobacter* spp. could be evaluated in the cases of six feline patients, five of which experienced a reduction in vomiting frequency after eradicating *Helicobacter* spp. Histopathological observations indicate that *Helicobacter* spp. distributed in the stomachs of cats in Japan seem to have low pathogenicity, but also that they may be related to some upper gastrointestinal symptoms in cats. Taken together with the results described in Chapters 3 and 5, the predominant gastric *Helicobacter* species were most closely related to *H. heilmannii* s.s. in both dogs and cats in Japan, but the pathogenicity, as observed in histopathological studies, seemed to differ between dogs and cats in the present study. This may be attributed to the differences in the host factor, the *H. heilmannii* s.s. strains, or the numbers used in the analysis. Regarding the relationship between NHPH infection and gastric lymphoma in cats as reported before (Bridgeford et al., 2008), the prevalence of gastric lymphoma was slightly higher in the *Helicobacter* spp.-positive group (25%) than the -negative group (11%) in the present study. However, it is unclear if this difference is significant because I could not perform the statistical analysis owing to an insufficient number of patients with gastric lymphoma

in this study (n = 10). To more properly investigate the effectiveness of eradicating gastric NHPH to reduce vomiting frequency in cats and the relationship between NHPH infection and gastric lymphoma in cats, it is necessary to perform an additional study using a greater number of cats.

Regarding the relationship between *Helicobacter* spp. infection and idiopathic thrombocytopenic purpura and iron deficiency anemia, *H. pylori* infection has been associated with these diseases in humans (Wu et al., 2008), but the relationship between NHPH infection and these diseases in dogs and cats could not be proved in the present study. For a more proper investigation, it would be necessary to perform studies using dogs and cats with idiopathic thrombocytopenic purpura and iron deficiency anemia. These studies should involve eradication of gastric NHPH species to see whether the hematological and clinical responses would change after the treatment, as the case of *H. pylori* infection in human. UBT will be helpful for this time-dependent investigation of the relationship between the extragastric diseases and gastric NHPH infection in dogs and cats because it is a non-invasive test.

The pathogenicity of each NHPH infected in the stomachs of dogs and cats in Japan remains uncertain in the present study because *H. heilmannii* s.s. was detected in almost all the *Helicobacter*-positive cases in both dogs and cats, as determined by partial *ureAB* gene sequencing. Therefore, *in vitro* cultivation of each NHPH and experimental infection of the gastric cell lines of dogs or cats would be necessary to better understand the pathogenicity of respective NHPH. In fact, I tried to isolate *Helicobacter* spp. from seven dogs and two cats that were infected with *Helicobacter* spp. using the same procedure used for the isolation of *H. pylori* from the human stomachs as described in Chapter

2-2, but all the attempts ended in failure. Therefore, it is necessary to develop an appropriate method to isolate NHPH from canine and feline stomachs for future studies.

In conclusion, the most prevalent *Helicobacter* sp. distributed in the stomachs of dogs and cats in Japan is *H. heilmannii* s.s. with putative subspecies. *H. heilmannii* s.s. infection may be associated with the severity of gastritis in dogs and some upper gastrointestinal symptoms in dogs and cats. To investigate the pathogenicity of NHPH in greater detail, a larger number of dogs and cats, not only with gastric diseases but also extragastric diseases such as liver and blood diseases, should be examined making full use of UBT in some cases. Furthermore, it will be necessary to investigate the relationship between NHPH infection and histopathological changes by performing *in vitro* cultivation of each *Helicobacter* sp. for experimental infection of canine or feline gastric cell lines.

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References

Adu-Aryee, N.A., Aabakken, L., Dedey, F., Nsaful, J., Kudzi, W., 2016. Comparison of endoscopic based diagnosis with *Helicobacter* urease test for *Helicobacter pylori* infection. BMC Res. Notes 9, 421.

Amorim, I., Smet, A., Alves, O., Teixeira, S., Saraiva, A.L., Taulescu, M., Reis, C., Haesebrouck, F., Gartner, F., 2015. Presence and significance of *Helicobacter* spp. in the gastric mucosa of Portuguese dogs. Gut Pathog. 7, 12–19.

Baele, M., Decostere, A., Vandamme, P., Ceelen, L., Hellemans, A., Mast, J., Chiers, K., Ducatelle, R., Haesebrouck, F., 2008a. Isolation and characterization of *Helicobacter suis* sp. nov. from pig stomachs. Int. J. Syst. Evol. Microbiol. 58, 1350–1358.

Baele, M., Decostere, A., Vandamme, P., Van den Bulck, K., Gruntar, I., Mehle, J., Mast, J., Ducatelle, R., Haesebrouck, F., 2008b. *Helicobacter baculiformis* sp. nov., isolated from feline stomach mucosa. Int. J. Syst. Evol. Microbiol. 58, 357-364.

Boyanova, L., Koumanova, R., Lazarova, E., Jelev, C., 2003. *Helicobacter pylori* and *Helicobacter heilmannii* in children. A Bulgarian study. Diagn. Microbiol. Infect. Dis. 46, 249–252.

Bridgeford, E.C., Marini, R.P., Feng, Y., Parry, N.M., Rickman, B., Fox, J.G., 2008. Gastric *Helicobacter* species as a cause of feline gastric lymphoma: a viable hypothesis. Vet. Immunol. Immunopathol. 123, 106–113.

Buczolits, S., Hirt, R., Rosengarten, R., Busse, H.-J., 2003. PCR-based genetic evidence for occurrence of *Helicobacter pylori* and novel *Helicobacter* species in the canine gastric mucosa. Vet. Microbiol. 95, 259–270.

Canejo-Teixeira, R., Oliveira, M., Pissarra, H., Niza, M., Vilela, C., 2014. A mixed population of *Helicobacter pylori*, *Helicobacter bizzozeronii* and "*Helicobacter heilmannii*" in the gastric mucosa of a domestic cat. Ir. Vet. J. 67, 25.

Cornetta, A.M., Simpson, K.W., Strauss-Ayali, D., McDonough, P.L., Gleed, R.D., 1998a. Use of a ^{13}C urea breath test for detection of gastric infection with *Helicobacter* spp in dogs. Am. J. Vet. Res. 59, 1364–1369.

Cornetta, A.M., Simpson, K.W., Strauss-Ayali, D., McDonough, P.L., Gleed, R.D., 1998b. Use of a [13C]urea breath test for detection of gastric infection with *Helicobacter* spp in dogs. Am. J. Vet. Res. 59, 1364-1369.

Covacci, A., Telford, J.L., Del Giudice, G., Parsonnet, J., Rappuoli, R., 1999. *Helicobacter pylori* virulence and genetic geography. Science 284, 1328–1333.

Craven, M., Recordati, C., Gualdi, V., Pengo, G., Luini, M., Scanziani, E., Simpson, K.W., 2011. Evaluation of the Helicobacteraceae in the oral cavity of dogs. Am. J. Vet. Res. 72, 1476–1481.

Day, M.J., Bilzer, T., Mansell, J., Wilcock, B., Hall, E.J., Jergens, A., Minami, T., Willard, M., Washabau, R., World Small Animal Veterinary Association Gastrointestinal Standardization, G., 2008. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. J. Comp. Pathol. 138 Suppl 1, S1–43.

De Groote, D., Van Doorn, L.J., Van den Bulck, K., Vandamme, P., Vieth, M., Stolte, M., Debongnie, J.C., Burette, A., Haesebrouck, F., Ducatelle, R., 2005. Detection of non-*pylori Helicobacter* species in "*Helicobacter heilmannii*"-infected humans. Helicobacter 10, 398–406.

Debongnie, J.C., Donnay, M., Mairesse, J., Lamy, V., Dekoninck, X., Ramdani, B., 1998. Gastric ulcers and *Helicobacter heilmannii*. Eur. J. Gastroenterol. Hepatol. 10, 251–254.

Dewhirst, F.E., Shen, Z., Scimeca, M.S., Stokes, L.N., Boumenna, T., Chen, T., Paster, B.J., Fox, J.G., 2005. Discordant 16S and 23S rRNA gene phylogenies for the genus *Helicobacter*: implications for phylogenetic inference and systematics. J. Bacteriol. 187, 6106-6118.

Dieterich, C., Wiesel, P., Neiger, R., Blum, A., Corthesy-Theulaz, I., 1998. Presence of multiple "*Helicobacter heilmannii*" strains in an individual suffering from ulcers and in his two cats. J. Clin. Microbiol. 36, 1366–1370.

Diker, K.S., Haziroglu, R., Akan, M., Celik, S., Kabakci, N., 2002. The prevalence, colonization sites

and pathological effects of gastric helicobacters in dogs. Turk. J. Vet. Anim. Sci. 26, 345–351.

Dunn, B.E., Cohen, H., Blaser, M.J., 1997. *Helicobacter pylori*. Clin. Microbiol. Rev. 10, 720-741.

Eaton, K.A., Dewhirst, F.E., Paster, B.J., Tzellas, N., Coleman, B.E., Paola, J., Sherding, R., 1996a. Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. J. Clin. Microbiol. 34, 3165–3170.

Eaton, K.A., Dewhirst, F.E., Paster, B.J., Tzellas, N., Coleman, B.E., Paola, J., Sherding, R., 1996b. Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. J. Clin. Microbiol. 34, 3165-3170.

Ekman, E., Fredriksson, M., Trowald-Wigh, G., 2013. *Helicobacter* spp. in the saliva, stomach, duodenum and faeces of colony dogs. Vet. J. 195, 127–129.

Fox, J.G., 1998. Gastric helicobacters, In: Infectious Diseases of the Dog and Cat. W.B. Saunders Co, Philadelphia, PA, USA, pp. 229–233.

Fox, J.G., 2002. The non-*H pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. Gut 50, 273-283.

Fox, J.G., Dewhirst, F.E., Shen, Z., Feng, Y., Taylor, N.S., Paster, B.J., Ericson, R.L., Lau, C.N., Correa, P., Araya, J.C., et al., 1998. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 114, 755–763.

Geyer, C., Colbatzky, F., Lechner, J., Hermanns, W., 1993a. Occurrence of spiral-shaped bacteria in gastric biopsies of dogs and cats. Vet. Rec. 133, 18–19.

Geyer, C., Colbatzky, F., Lechner, J., Hermanns, W., 1993b. Occurrence of spiral-shaped bacteria in gastric biopsies of dogs and cats. Vet. Rec. 133, 18-19.

Graham, D.Y., Klein, P.D., Evans, D.J., Jr., Evans, D.G., Alpert, L.C., Opekun, A.R., Boutton, T.W., 1987. *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet 1, 1174–1177.

Haesebrouck, F., Pasmans, F., Flahou, B., Chiers, K., Baele, M., Meyns, T., Decostere, A., Ducatelle, R., 2009a. Gastric helicobacters in domestic animals and nonhuman primates and their

significance for human health. Clin. Microbiol. Rev. 22, 202–223.

Haesebrouck, F., Pasmans, F., Flahou, B., Chiers, K., Baele, M., Meyns, T., Decostere, A., Ducatelle, R., 2009b. Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. Clin. Microbiol. Rev. 22, 202-223, Table of Contents.

Haesebrouck, F., Pasmans, F., Flahou, B., Smet, A., Vandamme, P., Ducatelle, R., 2011. Non-*Helicobacter pylori Helicobacter* species in the human gastric mucosa: a proposal to introduce the terms *H. heilmannii* sensu lato and sensu stricto. Helicobacter 16, 339–340.

Hall, J.A., 2000. Diseases of the stomach, In: Textbook of Veterinary Internal Medicine, 5th ed ed. W.B. Saunders Co, Philadelphia, PA, USA, pp. 1154–1181.

Handt, L.K., Fox, J.G., Dewhirst, F.E., Fraser, G.J., Paster, B.J., Yan, L.L., Rozmiarek, H., Rufo, R., Stalis, I.H., 1994. *Helicobacter pylori* isolated from the domestic cat: public health implications. Infect. Immun. 62, 2367–2374.

Happonen, I., Linden, J., Saari, S., Karjalainen, M., Hanninen, M.L., Jalava, K., Westermarck, E., 1998a. Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. J. Am. Vet. Med. Assoc. 213, 1767-1774.

Happonen, I., Linden, J., Saari, S., Karjalainen, M., Hanninen, M.L., Jalava, K., Westermarck, E., 1998b. Detection and effects of helicobacters in healthy dogs and dogs with signs of gastritis. J. Am. Vet. Med. Assoc. 213, 1767–1774.

Happonen, I., Linden, J., Westermarck, E., 2000. Effect of triple therapy on eradication of canine gastric helicobacters and gastric disease. J. Small Anim. Pract. 41, 1–6.

Happonen, I., Saari, S., Castren, L., Tyni, O., Hanninen, M.L., Westermarck, E., 1996a. Comparison of diagnostic methods for detecting gastric *Helicobacter*-like organisms in dogs and cats. J. Comp. Pathol. 115, 117-127.

Happonen, I., Saari, S., Castren, L., Tyni, O., Hanninen, M.L., Westermarck, E., 1996b. Comparison of diagnostic methods for detecting gastric *Helicobacter*-like organisms in dogs and cats. J. Comp. Pathol. 115, 117–127.

Harbour, S., Sutton, P., 2008. Immunogenicity and pathogenicity of *Helicobacter* infections of veterinary animals. Vet. Immunol. Immunopathol. 122, 191–203.

Henry, G.A., Long, P.H., Burns, J.L., Charbonneau, D.L., 1987a. Gastric spirillosis in beagles. Am. J. Vet. Res. 48, 831-836.

Henry, G.A., Long, P.H., Burns, J.L., Charbonneau, D.L., 1987b. Gastric spirillosis in beagles. Am. J. Vet. Res. 48, 831–836.

Hermanns, W., Kregel, K., Breuer, W., Lechner, J., 1995a. *Helicobacter*-like organisms: histopathological examination of gastric biopsies from dogs and cats. J. Comp. Pathol. 112, 307-318.

Hermanns, W., Kregel, K., Breuer, W., Lechner, J., 1995b. *Helicobacter*-like organisms: histopathological examination of gastric biopsies from dogs and cats. J. Comp. Pathol. 112, 307–318.

Hill, K.K., Hill, D.B., Humphries, L.L., Maloney, M.J., McClain, C.J., 1999. A role for *Helicobacter pylori* in the gastrointestinal complaints of eating disorder patients? Int. J. Eat. Disord. 25, 109–112.

Hwang, C.Y., Han, H.R., Youn, H.Y., 2002. Prevalence and clinical characterization of gastric *Helicobacter* species infection of dogs and cats in Korea. Journal of Veternary Science 3, 123–133.

Kato, M., Asaka, M., Ohara, S., Toyota, T., 1998. Clinical studies of ^{13}C-urea breath test in Japan. J. Gastroenterol. 33 Suppl 10, 36–39.

Kubota, S., Ohno, K., Tsukamoto, A., Maeda, S., Murata, Y., Nakashima, K., Fukushima, K., Uchida, K., Fujino, Y., Tsujimoto, H., 2013. Value of the ^{13}C-urea breath test for detection of gastric *Helicobacter* spp. infection in dogs undergoing endoscopic examination. J. Vet. Med. Sci. 75, 1049–1054.

Kuipers, E.J., Klinkenberg-Knol, E.C., Vandenbroucke-Grauls, C.M., Appelmelk, B.J., Schenk, B.E., Meuwissen, S.G., 1997. Role of *Helicobacter pylori* in the pathogenesis of atrophic gastritis.

Scand. J. Gastroenterol. Suppl. 223, 28–34.

Kusters, J.G., van Vliet, A.H., Kuipers, E.J., 2006. Pathogenesis of *Helicobacter pylori* infection. Clin. Microbiol. Rev. 19, 449–490.

Lee, A., Krakowka, S., Fox, J.G., Otto, G., Eaton, K.A., Murphy, J.C., 1992. Role of *Helicobacter felis* in chronic canine gastritis. Vet. Pathol. 29, 487–494.

Lee, S.Y., 2016. Endoscopic gastritis, serum pepsinogen assay, and *Helicobacter pylori* infection. Korean. J. Intern. Med. 31, 835–844.

Leib, M.S., Duncan, R.B., Ward, D.L., 2007a. Triple antimicrobial therapy and acid suppression in dogs with chronic vomiting and gastric *Helicobacter* spp. J. Vet. Intern. Med. 21, 1185-1192.

Leib, M.S., Duncan, R.B., Ward, D.L., 2007b. Triple antimicrobial therapy and acid suppression in dogs with chronic vomiting and gastric *Helicobacter* spp. J. Vet. Intern. Med. 21, 1185–1192.

Leodolter, A., Megraud, F., 2001. Doagnosis of *Helicobacter pylori* infection. Curr. Opin. Gastroenterol. 17 (suppl 1), S19-S23.

Matsumoto, T., Kawakubo, M., Akamatsu, T., Koide, N., Ogiwara, N., Kubota, S., Sugano, M., Kawakami, Y., Katsuyama, T., Ota, H., 2014. *Helicobacter heilmannii* sensu stricto-related gastric ulcers: a case report. World J. Gastroenterol. 20, 3376–3382.

Matsumoto, T., Kawakubo, M., Shiohara, M., Kumagai, T., Hidaka, E., Yamauchi, K., Oana, K., Matsuzawa, K., Ota, H., Kawakami, Y., 2009. Phylogeny of a novel "*Helicobacter heilmannii*" organism from a Japanese patient with chronic gastritis based on DNA sequence analysis of 16S rRNA and urease genes. J. Microbiol. 47, 201–207.

Mikkonen, T.P., 2004. Phylogenetic analysis of gastric and enterohepatic *Helicobacter* species based on partial HSP60 gene sequences. Int. J. Syst. Evol. Microbiol. 54, 753-758.

Morgner, A., Bayerdorffer, E., Meining, A., Stolte, M., Kroher, G., 1995. *Helicobacter heilmannii* and gastric cancer. Lancet 346, 511–512.

Morgner, A., Lehn, N., Andersen, L.P., Thiede, C., Bennedsen, M., Trebesius, K., Neubauer, B., Neubauer, A., Stolte, M., Bayerdorffer, E., 2000. *Helicobacter heilmannii*-associated primary

gastric low-grade MALT lymphoma: complete remission after curing the infection. Gastroenterology 118, 821–828.

Neiger, R., Dieterich, C., Burnens, A., Waldvogel, A., Corthesy-Theulaz, I., Halter, F., Lauterburg, B., Schmassmann, A., 1998. Detection and prevalence of *Helicobacter* infection in pet cats. J. Clin. Microbiol. 36, 634–637.

Neiger, R., Seiler, G., Schmassmann, A., 1999. Use of a urea breath test to evaluate short-term treatments for cats naturally infected with *Helicobacter heilmannii*. Am. J. Vet. Res. 60, 880–883.

O'Rourke, J.L., Solnick, J.V., Neilan, B.A., Seidel, K., Hayter, R., Hansen, L.M., Lee, A., 2004. Description of '*Candidatus* Helicobacter heilmannii' based on DNA sequence analysis of 16S rRNA and urease genes. Int. J. Syst. Evol. Microbiol. 54, 2203–2211.

Ohara, S., Kato, M., Asaka, M., Toyota, T., 1998a. Studies of ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* infection in Japan. J. Gastroenterol. 33, 6–13.

Ohara, S., Kato, M., Asaka, M., Toyota, T., 1998b. The UBiT-100 $^{13}CO2$ infrared analyzer: comparison between infrared spectrometric analysis and mass spectrometric analysis. Helicobacter 3, 49–53.

Ohara, S., Sekine, H., Moriyama, S., Sugiyama, K., Arikawa, T., Iijima, K., Honda, K., Asaki, S., Toyota, T., Sato, A., 1996. Methodological study of 13C-urea breath test for detection of *Helicobacter pylori* infection. pp.530-536. *In*: Nihon Shokakibyo Gakkai Zasshi, 3rd Department of Internal Medicine, Tohoku University School of Medicine, Miyagi (in Japanese).

Osaki, T., Mabe, K., Hanawa, T., Kamiya, S., 2008. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J. Med. Microbiol. 57, 814-819.

Osaki, T., Okuda, M., Ueda, J., Konno, M., Yonezawa, H., Hojo, F., Yagyu, K., Lin, Y., Fukuda, Y., Kikuchi, S., Kamiya, S., 2013. Multilocus sequence typing of DNA from faecal specimens

for the analysis of intra-familial transmission of *Helicobacter pylori*. J. Med. Microbiol. 62, 761-765.

Papich, M., 2007. pp. 476-478. *In*: Saunders Handbook of Veterinary Drugs, 2nd ed. Elsevier, St. Louis.

Papich, M., 2007. pp. 476-478. *In*: Saunders Handbook of Veterinary Drugs, 2nd ed. Elsevier, St Louis.

Parsonnet, J., Friedman, G.D., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentreich, N., Sibley, R.K., 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. The New England Journal of Medicine 325, 1127–1131.

Peyrol, S., Lecoindre, P., Berger, I., Deleforge, J., Chevallier, M., 1998a. Differential pathogenic effect of two *Helicobacter*-like organisms in dog gastric mucosa. J. Submicrosc. Cytol. Pathol. 30, 425-433.

Peyrol, S., Lecoindre, P., Berger, I., Deleforge, J., Chevallier, M., 1998b. Differential pathogenic effect of two *Helicobacter*-like organisms in dog gastric mucosa. J. Submicrosc. Cytol. Pathol. 30, 425–433.

Pounder, R.E., Ng, D., 1995. The prevalence of *Helicobacter pylori* infection in different countries. Aliment. Pharmacol. Ther. 9 Suppl 2, 33–39.

Priestnall, S.L., Wiinberg, B., Spohr, A., Neuhaus, B., Kuffer, M., Wiedmann, M., Simpson, K.W., 2004. Evaluation of "*Helicobacter heilmannii*" subtypes in the gastric mucosas of cats and dogs. J. Clin. Microbiol. 42, 2144–2151.

Sapierzyniski, R., Malicka, E., Zmudzka, M., Cywinska, A., 2006. The diagnosis of gastritis and *helicobacter*-like organisms infection in endoscopic biopsies of the canine gastric mucosa. Pol. J. Vet. Sci. 9, 17–21.

Sapierzynski, R., Malicka, E., 2004a. Effect of gastric *Helicobacter*-like organisms on gastric epithelial cell proliferation rate in dogs. Pol. J. Vet. Sci. 7, 275–281.

Sapierzynski, R., Malicka, E., 2004b. Effect of gastric *Helicobacter*-like organisms on gastric epithelial cell proliferation rate in dogs. Pol. J. Vet. Sci. 7, 275-281.

Sapierzynski, R., Malicka, E., Bielecki, W., Sendecka, H., 2003a. The presence of *Helicobacter*-like

microorganisms in the gastric mucosa in dogs. Pol. J. Vet. Sci. 6, 247-252.

Sapierzynski, R., Malicka, E., Bielecki, W., Sendecka, H., 2003b. The presence of *Helicobacter*-like microorganisms in the gastric mucosa in dogs. Pol. J. Vet. Sci. 6, 247–252.

Sapierzynski, R., Malicka, E., Zmudzka, M., Cywinska, A., 2006. The diagnosis of gastritis and *helicobacter*-like organisms infection in endoscopic biopsies of the canine gastric mucosa. Pol. J. Vet. Sci. 9, 17-21.

Sasani, F., Javanbakht, J., Kabir, F.R., Agha Mohammad Hassan, M., Pashaei, A.R., 2014. Evaluation of gastric lesions based on *Helicobacter pylori* and *Helicobacter*-like organisms (HLOs) in cats; a histopathological and bacteriological study. Jundishapur. J. Microbiol. 7, e9129.

Satou, K., Shiroma, A., Teruya, K., Shimoji, M., Nakano, K., Juan, A., Tamotsu, H., Terabayashi, Y., Aoyama, M., Teruya, M., Suzuki, R., Matsuda, M., Sekine, A., Kinjo, N., Kinjo, F., Yamaoka, Y., Hirano, T., 2014. Complete genome sequences of eight *Helicobacter pylori* strains with different virulence factor genotypes and methylation profiles, isolated from patients with diverse gastrointestinal diseases on Okinawa Island, Japan, determined using PacBio Single-Molecule Real-Time Technology. Genome Announc. 2.

Scanziani, E., Simpson, K.W., Monestiroli, S., Soldati, S., Strauss-Ayali, D., Del Piero, F., 2001. Histological and immunohistochemical detection of different *Helicobacter* species in the gastric mucosa of cats. J. Vet. Diagn. Invest. 13, 3–12.

Seo, W.J., Park, C.S., Cho, Y.J., Cha, K.W., Lee, S.W., Lim, S.T., Sung, Y.H., Baek, A.R., 2003. A case of gastric ulcer induced by *Helicobacter heilmannii*-like organism. Korean. J. Gastroenterol. 42, 63–66.

Shabestari, A.S., Mohammadi, M., Jamshidi, S., Sasani, F., Bahadori, A., Oghalaie, A., 2008a. Assessment of chronic gastritis in pet dogs and its relation with *helicobacter*-like organisms. Pak. J. Biol. Sci. 11, 1443-1448.

Shabestari, A.S., Mohammadi, M., Jamshidi, S., Sasani, F., Bahadori, A., Oghalaie, A., 2008b. Assessment of chronic gastritis in pet dogs and its relation with *helicobacter*-like organisms.

Pak. J. Biol. Sci. 11, 1443–1448.

Silva, C.P., Pereira-Lima, J.C., Oliveira, A.G., Guerra, J.B., Marques, D.L., Sarmanho, L., Cabral, M.M., Queiroz, D.M., 2003. Association of the presence of *Helicobacter* in gallbladder tissue with cholelithiasis and cholecystitis. J. Clin. Microbiol. 41, 5615–5618.

Simpson, K.W., McDonough, P.L., Strauss-Ayali, D., Chang, Y.F., Harpending, P., Valentine, B.A., 1999a. *Helicobacter felis* infection in dogs: effect on gastric structure and function. Vet. Pathol. 36, 237-248.

Simpson, K.W., McDonough, P.L., Strauss-Ayali, D., Chang, Y.F., Harpending, P., Valentine, B.A., 1999b. *Helicobacter felis* infection in dogs: effect on gastric structure and function. Vet. Pathol. 36, 237–248.

Slomianski, A., Schubert, T., Cutler, A.F., 1995. ^{13}C urea breath test to confirm eradication of *Helicobacter pylori*. Am. J. Gastroenterol. 90, 224–226.

Smet, A., Flahou, B., D'Herde, K., Vandamme, P., Cleenwerck, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., 2012. *Helicobacter heilmannii* sp. nov., isolated from feline gastric mucosa. Int. J. Syst. Evol. Microbiol. 62, 299–306.

Solnick, J.V., O'Rourke, J., Lee, A., Tompkins, L.S., 1994. Molecular analysis of urease genes from a newly identified uncultured species of *Helicobacter*. Infect. Immun. 62, 1631–1638.

Stolte, M., Eidt, S., 1993. Healing gastric MALT lymphomas by eradicating *H. pylori*? Lancet 342, 568.

Stolte, M., Kroher, G., Meining, A., Morgner, A., Bayerdorffer, E., Bethke, B., 1997. A comparison of *Helicobacter pylori* and *H. heilmannii* gastritis. A matched control study involving 404 patients. Scand. J. Gastroenterol. 32, 28–33.

Suzuki, M., Kiga, K., Kersulyte, D., Cok, J., Hooper, C.C., Mimuro, H., Sanada, T., Suzuki, S., Oyama, M., Kozuka-Hata, H., Kamiya, S., Zou, Q.M., Gilman, R.H., Berg, D.E., Sasakawa, C., 2011. Attenuated CagA oncoprotein in *Helicobacter pylori* from Amerindians in Peruvian Amazon. J. Biol. Chem. 286, 29964–29972.

Sykora, J., Hejda, V., Varvarovska, J., Stozicky, F., Gottrand, F., Siala, K., 2003. *Helicobacter heilmannii* related gastric ulcer in childhood. J. Pediatr. Gastroenterol. Nutr. 36, 410–413.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Trebesius, K., Adler, K., Vieth, M., Stolte, M., Haas, R., 2001. Specific detection and prevalence of *Helicobacter heilmannii*-like organisms in the human gastric mucosa by fluorescent in situ hybridization and partial 16S ribosomal DNA sequencing. J. Clin. Microbiol. 39, 1510–1516.

Van den Bulck, K., Decostere, A., Baele, M., Driessen, A., Debongnie, J.C., Burette, A., Stolte, M., Ducatelle, R., Haesebrouck, F., 2005. Identification of non-*Helicobacter pylori* spiral organisms in gastric samples from humans, dogs, and cats. J. Clin. Microbiol. 43, 2256–2260.

Van den Bulck, K., Decostere, A., Baele, M., Vandamme, P., Mast, J., Ducatelle, R., Haesebrouck, F., 2006. *Helicobacter cynogastricus* sp. nov., isolated from the canine gastric mucosa. Int. J. Syst. Evol. Microbiol. 56, 1559–1564.

Wiinberg, B., Spohr, A., Dietz, H.H., Egelund, T., Greiter-Wilke, A., McDonough, S.P., Olsen, J., Priestnall, S., Chang, Y.F., Simpson, K.W., 2005. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to *Helicobacter* spp. infection. J. Vet. Intern. Med. 19, 4–14.

Wotherspoon, A.C., Doglioni, C., Diss, T.C., Pan, L., Moschini, A., de Boni, M., Isaacson, P.G., 1993. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 342, 575–577.

Wu, M.S., Chow, L.P., Lin, J.T., Chiou, S.H., 2008. Proteomic identification of biomarkers related to *Helicobacter pylori*-associated gastroduodenal disease: challenges and opportunities. J. Gastroenterol. Hepatol. 23, 1657–1661.

Yamasaki, K., Suematsu, H., Takahashi, T., 1998a. Comparison of gastric lesions in dogs and cats with and without gastric spiral organisms. J. Am. Vet. Med. Assoc. 212, 529–533.

Yamasaki, K., Suematsu, H., Takahashi, T., 1998b. Comparison of gastric lesions in dogs and cats with

and without gastric spiral organisms. J. Am. Vet. Med. Assoc. 212, 529-533.

Yoshimura, M., Isomoto, H., Shikuwa, S., Osabe, M., Matsunaga, K., Omagari, K., Mizuta, Y., Murase, K., Murata, I., Kohno, S., 2002. A case of acute gastric mucosal lesions associated with *Helicobacter heilmannii* infection. Helicobacter 7, 322–326.