J. Vet. Sci. (2002), 3(2), 123-133

JOURNAL OF
Veterinary
Science

Prevalence and Clinical Characterization of Gastric *Helicobacter* Species Infection of Dogs and Cats in Korea

Cheol-Yong Hwang, Hong-Ryul Han* and Hwa-Young Youn

Department of Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

Received Dec. 16, 2001 / Accepted May 16, 2002

ABSTRACT

This study was carried out to evaluate the prevalence and clinical characterizations of gastric *Helicobacter* spp. infection of dogs and cats in Korea. The prevalence of *Helicobacter* spp. infection of dogs and cats determined by urease test was 78.4% and 64%, respectively, although *Helicobacter* genus-specific PCR assay showed that it was 82.3% and 84%. Urease mapping results based on urease test showed that total positive rate of tested tissues from clinically abnormal dogs was significantly higher than that from clinically normal dogs (p=0.0018; Odds ratio = 6.118; 95% Confidence Interval = 1.96~19.103). These findings were consistent with the results of *Helicobacter* genus-specific PCR assay which showed that positive rate of the fundus (100%) and the antrum (100%) of clinically abnormal dogs was significantly higher than that of same gastric regions of clinically normal dogs (77.5 and 67.5% respectively). In comparison of gastric regions between clinically normal dogs and abnormal dogs, positive rate of urease test for the fundus (100%) and body (90.9%) in clinically abnormal dogs was significantly higher than that of abnormal dogs (72.5% and 57.5% respectively; p<0.05). The results of urease mapping in dogs and cats also indicated that *Helicobacter* colonization in the fundus was more dense compared with the density in the body and antrum. In *Helicobacter* species-specific PCR assay for dogs, 32 of 42 fundic tissues (76.2%) were positive for *H. heilmannii* and two (4.8%) were positive for *H. felis*. In cats, 18 of 21 fundic tissues (85.7%) were positive for *H. heilmannii* and 2 (9.5%) were positive for *H. felis*. Gastritis scores of fundic tissues from clinically abnormal infected dogs were similar to that from noninfected dogs and evidence of upregulation of IL-1β, IL-8, and TNF-α mRNA was not detected in gastric fundic tissues from clinically abnormal infected dogs. This study suggested that *Helicobacter* spp. infection in domestic dogs including private owned pet dogs and cats is highly prevalent usually with no clinical sign but high density of colonization can be related to gastrointestinal signs

Key words: *Helicobacter* spp., Prevalence, PCR, Dog, Cat

Introduction

Helicobacter species are spiral-shape or curved gram-negative bacteria inhabit the mucus, glands and parietal cells of the stomach. Since the initial isolation of *Helicobacter pylori* (*H. pylori*) from human gastric tissue in 1983 by Warren and Marshall, evidence implicating the bacterium as the causative agent of gastritis and duodenal ulcer has been established [16,34,53]. More recently, the bacterium also known to be a cofactor in the development of gastric adenocarcinoma and gastric lymphoma in humans [40,56].

Gastric spiral organisms in various animals, including pigs with gastric ulcers (*Helicobacter heimannii)*, cheetahs with severe gastritis (*Helicobacter acinonychis*), ferrets with gastritis and peptic ulcers (*Helicobacter mustelae*), monkeys (*Helicobacter nemestrinae*), rodents (*Helicobacter muridarum*) and dolphins have been also described [2,7,12,22,32,42]. In dogs and cats, several gastric spiral organisms also have been reported since the turn over the century, however, their presence has been largely ignored and even understanded as gastric commensals [13,54]. In now, these gastric organisms have received renewed attention because of the *H. pylori* has been proved as a strong gastric pathogen in humans.

Research works for gastric spiral organisms in domestic pets, especially dogs and cats was initially focused on the development of suitable animal model for studying *H. pylori* infection and extended to evaluate their clinical significance in these animals [32,43,44,46].

The main gastric spiral organisms described in dogs and cats are morphologically distinct from *H. pylori* with their more tightly coiled body shape and larger size [24,31]. Because these organisms cannot be distinguished when they are examined in gastric tissue by light microscopy, they are

*Corresponding author: Hong-Ryul Han, DVM, Ph.D.
Department of Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University. Republic of Korea
Tel: +82-2-880-8683, Fax: +82-2-875-5588
E-mail: hrhan@snu.ac.kr

collectively referred to as "gastiric *Helicobacter*-like organisms (GHLO)" [31]. To date, *Helicobacter felis* (*H. felis*), *Helicobacter heilmannii* (*H. heilmannii*), *Helicobacter pametensis*, *Helicobacter bizzozeronii*, *Helicobacter salomonis*, *Helicobacter bilis*, and *Flexispira rappini* have been identified on the basis of 16S ribosomal RNA sequencing, DNA hybridization, species specific polymerase chain reaction (PCR) analysis, electron microscopic appearance, and protein profiling analysis [5,9,20,27,28,31,36,37].

Infection of GHLO is highly prevalent in dogs and cats. The prevalence of GHLO in dogs has been reported to be between 61 to 100 %; it is seen in 61-82% of dogs with recurrent vomiting [15,25,57], 67-86% of clinically healthy pet dogs [8,57], and almost 100% of laboratory beagles and shelter dogs [6,8,24]. In cats, the prevalence of GHLO has been reported to be similar to that of the dogs between 41 to 100 % [15,18,21,25,30,36,38]. The observations that the high prevalence of GHLO in closely contacted cat groups like as research colonies and animal shelters have been identical with that of the dogs. *H. pylori* infection has been observed in group of laboratory cats and commercial vendor, but not in private owned pet cats [13,18,19].

In Korea, some studies for GHLO infection in dogs were also previously reported and showed that the infection rate was similar to that of foreign studies. These studies however, were only based on closely contacted healthy laboratory beagle colony and kennel dogs [1,35,39]. To date, the prevalence survey study for GHLO infection in private owned pet dogs and any group of cats in Korea has not been reported yet.

Despite the frequent occurrence of GHLO in dogs and cats, the relationships between these bacteria and clinical manifestation have not been clearly understood with gastritis accompanying infection in some but not all infected dogs and cats [8,21,25,57].

Several reports of GHLO infections in humans have lead to speculation that animals especially dogs and cats may serve as a source for human infection [6,23,50,51]. There is therefore a need to determine the prevalence of *Helicobacter* species in domestic pets in order to evaluate the possible risk to human health, and also to that of the host animals, in which gastritis and related complains can occur.

Therefore, this study was carried out to evaluate the prevalence and clinical characterizations of gastric *Helicobacter* spp. infection of dogs including private owned pet dogs and cats in Korea.

Materials and Methods

Experimental animals and gastric tissue sampling

Canine gastric tissues were collected at necropsy from 4 euthanized pet dogs having gastrointestinal signs (like intermittent vomiting with or without blood in vomitus and inappetence or anorexia which were not related with other non-gastric causes), 24 euthanized pet dogs had no gastrointestinal signs and 12 laboratory beagle dogs being used in another toxicological study. Feline gastric tissues were taken at necropsy from 24 cats being used in terminal procedure part of one research project. Up to 5 full-thickness gastric tissue samples were collected from the fudus, the cardia and the antrum of these animals with a sterile 6 mm skin biopsy punch. Collecting tissue samples for PCR analysis were frozen at -80℃ pending analysis and samples for histopathologic examinations were fixed in 10% buffered formalin. Samples from the fundus for cytokine analysis were collected, snap-frozen in liquid nitrogen and stored at −80℃ pending analysis. The remnants were used for urease mapping, direct smear examination and culture.

Endoscopic biopsies obtained from 4 clinically healthy pet dogs, 7 patient dogs with gastrointestinal signs and one clinically healthy pet cat using a pediatric endoscope and biopsy forceps. Endoscopic biopsies procured from the fundus (near cardia), the body (greater curvature), and the antrum (near pylorus). Two biopsies were taken from each site for urease testing, PCR analysis, direct smear examination and histopatologic examination. Additional fundus site biopsy for cytokine analysis was snap-frozen in liquid nitrogen and stored at −80℃ pending analysis.

Direct gastric tissue smear test

Gastric tissue samples were impressed on slide glass and were stained with Diff-Quick (International Reagent Co., Japan) and evaluated by light microscopy for the presence of *Helicobacter* spp.

Urease mapping

Urease mapping was performed to determine semi-quantitatively the density of colonization by *Helicobacter* spp. in different regions of the stomach. Gastric tissue samples were places in sterile tubes containing 200 μℓ of a solution composed of urea, sodium azide, phenol red, and phosphate-buffered saline (pH 6.5). Samples were incubated at 37℃ for 24 hours and observed at 1, 3, 12, and 24 hours for a change in the color of the indicator medium. A change from orange-red to bright pink was considered a positive results, and the time of color changes were recorded.

Culture of gastric tissue samples

Gastric fundic tissue samples were directly smeared on trypicase soy agar base (DIFCO, U.S.A.) supplemented with 5% bovine blood and containing trimethoprim, vancomycin, and polymyxin B. Culture plates incubated for 7 days at 37 ℃ in a moist microaerophilic atmosphere provided by anaerobic jar with a Campypak system (Campypak Plus; Beckton Dickinson Microbiology system, U.S.A.).

Bacterial strains

For evaluating the sensitivity of *Helicobacter*-genus and species-specific PCR assay, *H. felis* (ATCC 51211) purchased from the American Type Culture Collection (ATCC; U.S.A.)

and *H. pylori* (KCTC 2948) purchased from the Korean Collection for Type Cultures (KCTC; Korea) were used as standard *Helicobacter* strains.

PCR

DNAs from gastric tissue samples and standard bacteria were extracted with a Wizard® genomic DNA purification kit (Promega, U.S.A.) according to the manufacturer's instructions.

Helicobacter genus specific PCR assay was performed with C97 and C98 primers which amplify the 16S rRNA gene of *Helicobacter* species (Table 1.). DNA samples (5㎕) were added to a reaction mixture containing 400 μM dNTPs, 1X PCR buffer, 2.5 U of *Taq* DNA polymerase (ABgene, U.K.), 0.6 μM of each primer, and distilled water in a total volume of 50 ㎕. PCR samples were heated to 94℃ for 2.5 min once, followed by 40 cycles of denaturation at 94℃ for 1 min, primer annealing at 50℃ for 1 min, and extension at 72℃ for 1 min, with a final extension at 72℃ for 15 min in a Biometra (U.S.A.) personal thermocycler. PCR products were subjected to electrophoresis on a 2% agarose gel containing 0.5 ㎍ of ethidium bromide per ㎖ and visualized over UV light.

Helicobacter species-specific PCR assay was performed with fundic tissue samples as follows. *H. felis* and *H. pylori* specific PCR was performed with primers which amplify the urease B gene of *H. felis* and *H. pylori* (Table 1.). The PCR mixture and the cycle was the same as the *Helicobacter* genus specific PCR assay. *H. heilmannii* specific PCR assay was performed with primers which amplify the urease B gene of *H. heilmannii* (Table 1.). Two microliters of DNA was added to the PCR mixture described above in a total volume of 50 ㎕. The temperature and time schedule was as follows: 1 cycle of denaturation at 94 ℃ for 3 min, annealing at 52℃ for 2 min, and extension at 72℃ for 3 min followed by 30 cycles at 94℃ for 30 s, 52℃ for 30 s, and 72℃ for 1 min. Following completion of the 30 cycles, additional one cycle at 94℃ for 20 s, 52℃ for 20 s, and 72℃ 5 min was performed.

Cloning and nucleotide sequence analysis of PCR products

To confirm the identity of the *Helicobacter* species-specific PCR assay products with target genes, the PCR products were cloned with Topo TA Cloning® Kit (Invitrogen, U.S.A.) and the plasmids containing correct inserts were analysed.

Histopathologic examination

For evaluating the relation of *Helicobacter* spp. infection and clinical gastritis in dogs, formalin fixed gastric fundic tissue samples from 9 clinically abnormal infected dogs (Urease test positive, PCR assay positive and had clinically abnormal gastrointestinal signs), and 8 clinically healthy uninfected dogs (Urease test negative, PCR positive and had not clinically abnormal gastrointestinal signs) were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

For the histopathological assessment, the presence of lymphocyte aggregates, and the mean numbers of leukocytes at X400 fields were recorded under microscopy. Gastritis was grade as follows: "no gastritis", no lymphocyte aggregate or leukocytes; "mild gastritis", no lymphocyte aggregates and < 10 leukocytes per field; "moderate gastritis", lymphocyte aggreates present and/or 10 to 50 leukocytes per field; "severe gastritis". lymphocyte aggreates present and >50 leukocytes per field [37].

Analysis of cytokine gene expression in gastric fundic tissues

Gastric tissue samples from the fundus were collected from 9 clinically abnormal infected dogs and 8 uninfected healthy dogs described previously, snap-frozen in liquid nitrogen, and stored at -80℃ pending analysis. RNA was extracted from the tissues with an RNA extraction kit (NucleoSpin®RNA II; Macherey-Nagel, Germany) according to the manufacturer's instruction. mRNA expression for TNF-α, IL-1β, IL-8 was determined by reverse transcription (RT)-PCR. The RNA was reverse transcribed

Table 1. Primers used for PCR

Primer	Primer directiona and sequence	product size (bp)
C97	5′-GCTATGACGGGTATCC- 3′	**400**
C98	5′-GATTTTACCCCTACACCA- 3′	
H. felis	F, 5′-ATGAAACTAACGCCTAAAGAACTAG	**1,150**
	R, 5′-GGAGAGATAAAGTGAATATGCGT	
H. pylori	F, 5′-GGAATTCCAGATCTATGAAAAAGATTAGCAGAAAAG- 3′	**1,707**
	F, 5′-GGAATTCGTCGACCTAGAAAATGCTAAAGAGTTG- 3′	
H. heilmannii	F, 5′-GGGCGATAAAGTGCGCTTG- 3′	**580**
	R, 5′-CTGGTCAATGAGAGG- 3′	

a F, forward; R, reverse.

with cDNA synthesis kit (First Strand cDNA Synthesis Kit; MBI Fermentas, Lithuania) and resulting cDNA served as a template for PCR assay. PCR primers for canine TNF-α, IL-1β, and IL-8 were used to amplify their respective cDNAs (Table 2.). The PCR reaction was run for 94℃ for 5 min one time, followed by 35 cycles of 94℃ for 1 min, 45℃ for 1 min, and 72℃ for 1 min with a final extension at 72℃ for 10 min in a Biometra (U.S.A.) personal thermocycler. PCR products were subjected to electrophoresis on a 2% agarose gel containing 0.5 ㎍ of ethidium bromide per ㎖ and visualized over UV light. For the positive control, canine leukocyte-derived RNA from concanavalin A stimulated canine peripheral blood leukocytes was used. The RT-PCR results were judged as negative or positive only regardless of staining intensity.

Statistical analysis

Deferences in total positive rate of urease activity and total positive rate of *Helicobacter* genus-specific PCR assay of gastric region between clinically normal and abnormal dogs were evaluated by Bonferroni *t*-test. Deferences in these variables in cats and between gastric region in each dog groups were also evaluated by Bonferroni *t*-test. Deferences in total positive rate of urease activity between clinically normal and abnormal dogs and between gastric region in dogs were evaluated by logistic regression. All statistical analyses were performed with software package SAS (release 8.0, SAS Institute, Cary, NC, U.S.A.). A statistical significance level of 0.05 was used for analyses.

Results

Urease mapping

1) Dogs

Urease mapping results of clinically normal and abnormal dogs were summarized in Table 3. Most of positive results were detected within 12 hours incubation, but 6 of 11 fundic tissue samples from clinically abnormal dogs showed positive results within 1 hour. Forty of 51 tested dogs showed positive results at least one gastric region and the detection rate of *Helicobacter* spp. in dogs determined by urease test was 78.4%.

Total positive rate of tested tissues from clinically abnormal dogs was significantly higher than that from clinically normal dogs (p=0.0018; Odds ratio = 6.118; 95% Confidence Interval = 1.96~19.103). In comparison of gastric regions, total positive rate of the fundus was higher than that of other gastric regions and the deference between the antrum and the fundus was statistically significant (p=0.0013; Odds ratio = 4.4438; 95% Confidence Interval = 1.791~10.997). Positive rate of the fundus (100%) and the body (90.9%) of clinically abnormal dogs was significantly higher than that of same gastric regions from clinically normal dogs (72.5%, 57.5% respectively; $p<0.05$). In clinically normal dogs, positive rate of the fundus (72.5%) was significantly higher than that of the antrum (40%;

Table. 2. Primers used in the RT-PCR to detect mRNA of cytokines

Primer	Primer directiona and sequence	product size (bp)
IL-1β	F, 5′-GAGGTTCCAATGTGAAGTGC- 3′	291
	R, 5′-CCTGTAACTTGCAGTCCACC- 3′	
IL-8	F, 5′-ACTTCCAAGCTGGCTGTTGC- 3′	172
	R, 5′-GGCCACTGTCAATCACTCTC- 3′	
TNF-α	F, 5′-CCAAGTGACAAGCCAGTAGC- 3′	274
	R, 5′-TCTTGATGGCAGAGAGTAGG- 3′	

a F, forward; R, reverse.

Table 3. Urease mapping results of clinically normal and abnormal dogs

Group (No. of dogs)	Site	No. of urease activity (%)				
		< 1hr	1 - <3hr	3 -<12h	12-24h	Total positive
Clinically normal dogs (40)	**Fundus**	3 (7.5)	18 (45)	7 (17.5)	1 (2.5)	29 (72.5)
	Body	0 (0)	9 (22.5)	13 (32.5)	1 (2.5)	23 (57.5)
	Antrum	0 (0)	8 (20)	7 (17.5)	1 (2.5)	16 (40)
Clinically abnormal dogs (11)	**Fundus**	6 (54.5)	3 (27.3)	1 (9.1)	1 (9.1)	11 (100)
	Body	1 (9.1)	4 (36.4)	5 (45.5)	0 (0)	10 (90.9)
	Antrum	1 (9.1)	2 (18.2)	5 (45.5)	0 (0)	8 (72.7)

p<0.05) although there were no differences between gastric regions in clinically abnormal dogs.

2) Cats

Many samples showed positive results within 12 hours. Total positive rate of the fundus was 64% and this rate was higher than that of the body (32%) and the antrum (28%), but statistically significant difference (P<0.05) was only detected between the fundus and the antrum (Table 4).

Table 4. Urease mapping results of cats

Site (No. of samples)	No. of urease activity (%)				
	< 1hr	1 - <3hr	3 - <12h	12-24h	Total positive
Fundus (25)	2 (8)	3 (12)	8 (32)	3 (12)	16 (64)
Body (25)	1 (4)	2 (8)	3 (12)	2 (8)	8 (32)
Antrum (25)	1 (4)	4 (16)	2 (8)	0 (0)	7 (28)

PCR assay

1) *Helicobacter* genus-specific PCR assay in dogs

Helicobacter genus-specific PCR assay (Fig. 2) in dogs showed that positive rate of the fundus (100%) and the antrum (100%) of clinically abnormal dogs was significantly higher than that of same gastric region of clinically normal dogs (77.5% and 67.5%; P<0.05). Total positive rate of the fundus (82.3%) was highest but was not statistically significant compared with the other regions (P>0.05). There were also no significant deferences of positive rate between gastric region in each dog groups (P>0.05) (Table 5).

Table 5. Results of *Helicobacter* genus-specific PCR assay in dogs

Group (No. of dogs)	No. of Positive (%)		
	Fundus	Body	Antrum
Clinically normal dogs (40)	31 (77.5)	31 (77.5)	27 (67.5)
Clinically abnormal dogs (11)	11 (100)	10 (90.9)	11 (100)
Total (51)	42 (82.3)	41 (78.8)	38 (74.5)

2) *Helicobacter* genus-specific PCR assay in cats

Positive rate of the fundus, the body and the antrum of cat was 84%, 80% and 79% respectively, but these deferences were not statistically significant (p>0.05).

Table 6. Results of *Helicobacter* genus-specific PCR assay in cats

(No. of cats)	Site	No. of positive (%)
Cats (25)	Fundus	21 (84)
	Body	20 (80)
	Antrum	19 (79)

3) *Helicobacter* species-specific PCR assay in dogs

Each set of primers was shown to amplify the gene from which it was derived, without cross-hybridizing with the corresponding gene of the two other species (Fig. 3, Fig. 4). Thirty-two of 42 fundic tissue samples tested (76.2%) were positive for *H. heilmannii* and two samples (4.8%) from clinically normal dogs were positive for *H. felis* (Table 7). No amplification products corresponding to *H. pylori* were detected and 8 samples (19%) were negative for all species-specific PCR assay (Table 7). There were no significant differences of the results between clinically normal and abnormal dogs.

Table 7. Results of *Helicobacter* species-specific PCR assay in dogs

Group (No. of dogs)	No. of Species identified (%)			
	H. heilmannii	*H. felis*	*H. pylori*	non-identified
Clinically normal dogs (31)	23 (74.2)	2 (6,5)	0 (0)	6 (19.4)
Clinically abnormal dogs (11)	9 (81.8)	0 (0)	0 (0)	2 (18.1)
Total (42)	32 (76.2)	2 (4.8)	0 (0)	8 (19)

4) *Helicobacter* species-specific PCR assay results in cats

Eighteen of 21 fundic tissue samples (85.7%) tested were positive for *H. heilmannii* and 2 (9.5%) were positive for *H. felis*. *H. pylori* was not detected and 1 sample were not amplified by all species-specific PCR assay (Table 8, Fig. 3, Fig. 4).

Table 8. Results of *Helicobacter* species-specific PCR assay in cats

(No. of Cats)	No. of Species identified (%)			
	H. heilmannii	*H. felis*	*H. pylori*	*non-identified*
Cats (21)	18 (85.7)	2 (9.5)	0 (0)	1 (4.7)

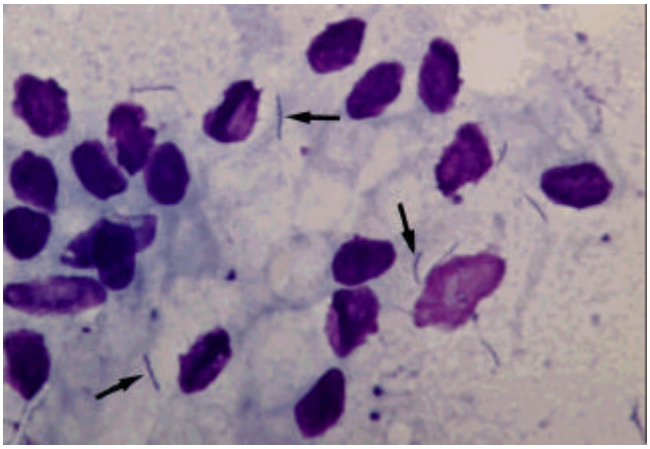

Fig. 1. Direct impression smear of gastric tissue from *Helicobacter* spp. infected dogs. Arrows indicate spiral shaped *Helicobacter* organisms.

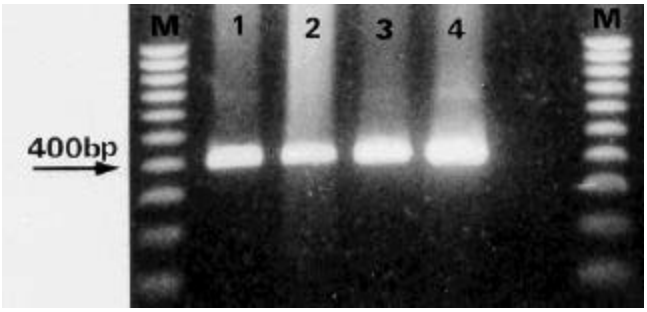

Fig. 2. Detection of *Helicobacter* spp. DNA (400bp) in gastric tissues by *Helicobacter* genus-specific PCR assay. Lanes: M, DNA ladder; 1, infected dogs; 2, infected cat; 3, DNA from *H. pylori* (KCTC 2948); 4, DNA from *H. felis* (ATCC 51211).

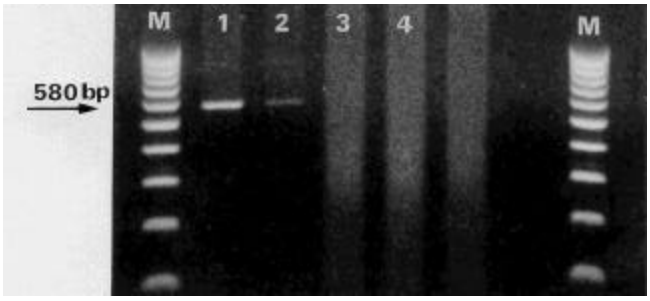

Fig. 3. Detection of *H. heilmannii* DNA (580bp) in gastric tissues by PCR assay. Lanes: M, DNA ladder; 1, *H. heilmannii* infected dog; 2, *H. heilmannii* infected cat; 3, DNA from *H. pylori* (KCTC 2948); 4, DNA from *H. felis* (ATCC 51211).

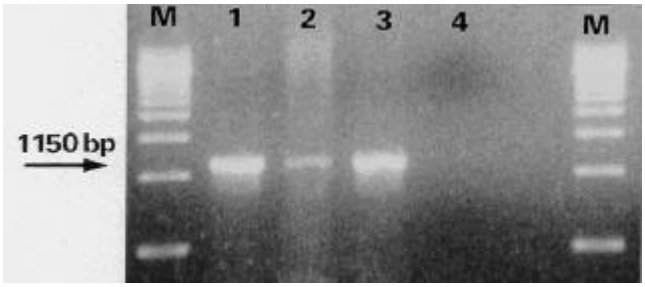

Fig. 4. Detection of *H. felis* DNA (580bp) in gastric tissues by PCR assay. Lanes: M, DNA ladder; 1, *H. felis* infected dog; 2, *H. felis* infected cat; 3, DNA from *H. felis* (ATCC 51211); 4, DNA from *H. pylori* (KCTC 2948).

5) Nucleotide homology of the Helicobacter species-specific PCR products

There was greater than 97% identity between the sequences of *H. heilmannii* specific PCR products and GeneBank sequences of the urease B gene of *H. heilmannii* (Accession No. L25079). In comparision of *H. felis* PCR products, greater than 98% identity was detected (Accession No. X69080).

Evaluation of *Helicobacter* spp. infection state by different detection methods

Thirty-nine of 51 (76.5%) dogs and 16 (64%) of 25 cats were positive for all test performed with gastric fundus tissues. Eight (15.7%) dogs and 4 (16%) cats showed negative in all tests. In direct tissue smear test, the results were concordant with the results of other tests in all dogs and cats but 4 dogs and 5 cats (2 dogs which were negative in direct tissue smear test were positive in other tests; 2 dogs and 5 cats which were positive in direct smear test were negative in urease test; Table 9).

Table 9. Evaluation of *Helicobacter* infection state in gastric fundic tissues by different detection methods

Smear[a]	Urease[b]	PCR[c]	No. of dogs with pattern	No. of cats with pattern
+	+	+	39	16
+	−	+	2	5
−	+	+	2	0
−	−	−	8	4

a: Direct tissue smear test : + = positive, − = negative.
b: Urease test; + = positive, − = negative.
c: PCR assay; + = positive, − = negative.

Culture result

Helicobacter organisms were not cultured from all gastric fundic tissue samples of dogs and cats.

Histopathologic findings

Most of clinically abnormal infected dogs had mild to moderate gastritis consisting of scattered leukocytes but similar degree of gastritis also was detected in clinically normal uninfected dogs. Whatever severe gastritis was only detected in one clinically abnormal infected dogs, there were no correlation between the presence of the bacteria with clinical signs and the gastritis score.

Table 10. Gastritis results for clinically abnormal *Helicobacter* spp. infected and noninfected normal dogs

Degree of gastritis	No. of dogs with result	
	Clinically abnormal infected (n = 8)	Clinically normal noninfected (n = 7)
None	1	2
Mild	2	1
Moderate	4	4
Severe	1	0

Analysis of cytokine gene expression in gastric fundic tissues

RT-PCR assay of gastric fundic tissues from dogs for detecting existence of cytokines did not show the evidence of upregulation of IL-1β, IL-8, or TNF-α mRNA in ether clinically abnormal infected or uninfected dogs. Appropriated reactions were only detected positive control samples (Fig. 5).

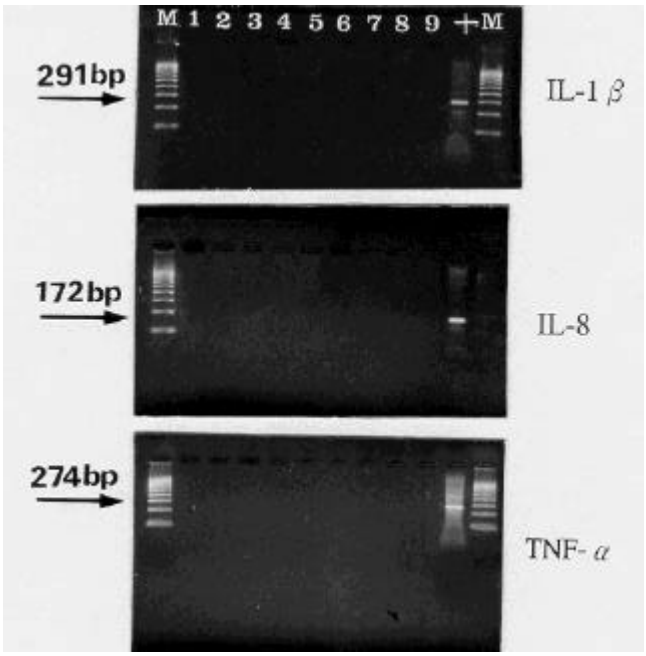

Fig. 5. Detection of mRNA for IL-1β, IL-8, and TNF-α in gastric tissues by RT-PCR. Agarose gel electrophoresis of DNA products. Lanes: M, DNA ladder; 1 to 7, Helicobacter spp. infected dogs; +, positive control (peripheral blood of dog).

Discussion

Since the discovery that *H. pylori* is a pathogen in humans, many studies have been evaluated the prevalence of *Helicobacter* infection and the relationship between infection and gastric pathology in other animals. This study was carried out with purpose of evaluating the prevalence and characterization of *Helicobacter* infection in domestic dogs and cats. In the present study, the prevalence of *Helicobacter* spp. in dogs and cats were evaluated with direct gastric tissue smear test, urease test which is commonly used in detecting *H. pylori* infection in humans and PCR assay. These test results in the present study showed that the prevalence of *Helicobacter* spp. in dogs (> 78.4%) and cats (> 64%) was as high as in previous reports [1,8,15,21,25,30,35,36,38,57].

Urease mapping based on urease test showed that total positive rate of tested tissues from clinically abnormal dogs was significantly higher than that from clinically normal dogs ($p=0.0018$; Odds ratio = 6.118; 95% Confidence Interval = 1.96~19.103). These findings were consistent with the results of *Helicobacter* genus specific PCR assay which showed that positive rate of the fundus (100%) and the antrum (90.9%) of clinically abnormal dogs was significantly higher than that of same gastric regions of clinically normal dogs (77.5 and 67.5% respectively). However, a previous report showed that there was no difference of the prevalence between clinically normal and abnormal dogs [57]. In spite of higher prevalence in clinically abnormal dogs, rate of showing positive urease activity within one hour in clinically abnormal dogs also higher than that in clinically normal dogs. It suggested that the density of *Helicobacter* colonization in clinically abnormal dogs was higher than that in clinically normal dogs based on the fact that urease activity is depended on the density of *Helicobacter* colonization.

The results of urease mapping in dogs also indicated that *Helicobacter* colonization in the fundus was more dense compared with the density in the antrum. These pattern of colonization was similar to that observed in previous reports conducted with naturally acquired helicobacteriasis and experimentally infected dogs and cats [5,21,38,46,57]. In comparison of gastric regions between clinically normal and abnormal dogs, positive rate of urease test for the fundus and body in clinically abnormal dogs was significantly higher than that in normal dogs ($p<0.05$). These results combined with the higher degree of colonization in clinically abnormal dogs may consider the possibility that high degree of *Helicobacter* spp colonization in the fundus and body can arise gastrointestinal signs in dogs.

To the best of our knowledge, this is the first report of evaluating the *Helicobacter* spp. infection of cats in Korea

although the number of cats evaluated was so small and limited to only one laboratory colony. The pattern of urease mapping results of cats was similar to that of dogs, which showed that colonization was less dense in the antrum of the stomach compared with the density in the fundus and body. Therefore, for reducing the possibility of false negative result in urease test, using biopsy tissues from the fundus and body rather than from the antrum is recommended. All of cats investigated in the present study had no gastrointestinal signs and the rate of showing positive urease activity within one hour was low. This result also supported the possibility as previously mentioned that high degree of *Helicobacter* spp. colonization might induce gastrointestinal signs.

In several recently developed PCR assays for detecting *Helicobacter* infection, two targets the urease and 16S rRNA genes, were appeared promising because partial or whole sequence information is available for both [3,4,14,17,26, 52,55]. In the present study, *Helicobacter*-specific primer pair C97 and C98 [14] which generate 16S rRNA amplicons of approximately 400 bases was used for *Helicobacter* genus-specific PCR assay. In *Helicobacter* genus-specific PCR assay in dogs and cats, *Helicobacter* spp. detecting rate (dog= 82.3%, cat = 84%) was slightly higher than that of urease test (dog = 78.4, cat = 64%). There were no significant deference of positive rate between gastric regions in dogs and cats. These results combined with the results of urease mapping indicated that *Helicobacter* spp. infection rate between gastric regions were not different but truly in colonization density.

For detecting *Helicobacter* spp. infection in gastric tissues, direct tissue smear test, urease test and PCR assay was used and each test results of fundic tissues were compared. Concordant results among the different diagnostic tests were reached for 92% of the dogs and 80 % of the cats evaluated. These results were similar to that of one previous report conducted in cats [36]. In direct tissue smear test, results were concordant with the results of other tests in all dogs and cats but 2 dogs which were negative although urease test and PCR asssy test showed positive. Two dogs and 5 cats were positive for direct tissue smear test and PCR assay but showed negative result in urease test. According to these results, direct tissue smear test and PCR assay appeared more sensitive than urease test. These observations concur with those in studies of experimentally *H. felis* infected cats and of dogs with naturally acquired helicobacteriasis [45,46]. The results of the present study also suggested that PCR assay is the most sensitive test for the detection of *Helicobacter* infection in dogs and cats. This observation is agreement with results obtained in studies of mice and dogs experimentally infected with *H. felis* and of humans and cats infected with *H. pylori*, which showed that PCR assay was more sensitive than histology, bacterial culture, and urease test [10,29,41,45].

PCR assay also has been known to be a specific methods to distinguish between *Helicobacter* species [36]. Primer pairs used for *Helicobacter* species-specific PCR assay in the present study were designed for amplifing urease B gene of *H. heilmannii*, *H. pylori* and *H. felis*. Each set of primers was shown to amplify the gene from which it was derived without cross-reaction with the corresponding gene of the two other species.

In *Helicobacter* species-specific PCR assay for dogs, 32 of 42 fundic tissues (76.2%) were positive for *H. heilmannii* and two (4.8%) were positive for *H. felis* (Table 7). In cats, 18 of 21 fundic tissues (85.7%) were positive for *H. heilmannii* and 2 (9.5%) were positive for *H. felis*. Observation that high prevalence of *H. heilmannii* in domestic dogs and cats is agreement with results obtained in previous foreign studies [36,37] and one study of domestic dogs [35]. *H. Pylori* infection in cats has been observed in group of laboratory cats and commercial vendor, but not in private owned pet cats [13,18,19]. In the present study, No amplification products corresponding to *H. pylori* were detected in both dogs and cats. This finding is important, because this may indicated that dogs and cats do not represent a source of *H. pylori* for the human population, at least in Korea. Eight fundic tissues (19%) from dogs and 1 tissue (4.7%) from cat were negative for all species-specific PCR assays although positive for genus-specific PCR assay. Possibly these negative results were due to yet another *Helicobacter* spp. with a urease that primer sets used in the present study were unable to amplify.

H. heimannii detected most frequently in this study is generally known to be unculturable by standard methods that have been successful with other *Helicobacter* species [47]. Similarly, all gastric fundic tissue samples from dogs and cats in this study were negative in culture although some *H. felis* which is usually culturable were only detected on *H. felis* specific PCR assay. The main problem was that contaminations with other bacteria were occurred frequently although some antibiotics were inserted in culture medium. These contaminations might prevent the growth of *Helicobacter* spp. or make the growing colonies of *Helicobacter* spp. to be undetectable by covering whole agar medium.

The relationship between *Helicobacter* spp. infection and clinical manifestation have not been identified in dogs and cats, because *Helicobacter* spp. infection have been found in clinically normal and abnormal dogs and cats [11,21,25,57]. This study and another previous studies of dogs and cats found no correlation between the severity of mucosal lesions of noninfected cases and that of infected cases [8,37,48]. Moreover, evidence of upregulation of IL-1β, IL-8, and TNF-α mRNA which is highly expressed in *H. pylori* infected human gastric tissues were not detected in gastric fundic tissues from clinically abnormal infected dogs even in one dogs showed severe gastritis.

This study suggested that *Helicobacter* spp. infection in domestic dogs including private owned pet dogs and cats is highly prevalent with no clinical sign but high density of

colonization can be related to gastrointestinal signs. Therefore, diagnostic tests for detecting *Helicobacter* spp. infection like PCR for gastric biopsies are highly recommended in dogs and cats having chronic gastritis signs (usually intermittent vomiting) and effective treatment for eradicating the organism should be applied if the animals were proved to be infected.

Acknowledgments

This study was supported by the 2000 SNU Research Fund.

References

1. **An, J. H., Nam, H. W., Han, J. H., and Kim, D**. The detection of *Helicobacter*-like organisms in dogs. Korean J. Vet. Clin. Med. 1999, **16**, 281-288.
2. **Bronsdon, M. A., Goodwin, C. S., Sly, L. I., Chilvers, T., and Schoenknecht, F. D**. *Helicobacter nemestrinae* sp. nov., a spiral bacterium found in the stomach of a pig tailed macaque (*Macaca nemestrina*). Int. J. Syst. Bacteriol. 1991, **41**, 148-153.
3. **Chuanfu, L. I., Tuanzuh, H. A., Donald, A., Ferguson, D. A., Chi, D. S., Zhao, R., Patel, N. R., Krishnaswamy, G., and Tomas, E.** A newly developed PCR assay of *H. pylori* in gastric biopsy, saliva, and feces; Evidence of high prevalence of *H. pylori* in saliva supports oral transmission. Dig. Dis. Sci. 1996, **41**, 2142-2149.
4. **Clayton, C. L., Kleanthous, H., Coates, P. J., Morgan, D. D., and Tabaqchali, S.** Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. J. Clin. Microbiol. 1992, **30**, 192-200.
5. **De Majo, M. M., Pennisis, M. G., Carbone, M., Fera, M. T., Masucci, M., Meil, F., and Cavallari, V.** Occurence of *Helicobacter* spp. in gastric biopsies of cats living in different kinds of colonies. Eur. J. Comp. Gastroenterol. 1998, **3**, 13-18.
6. **Eaton, K. A., Paola, J. P., and Johnson, S. E.** Gastritis associated with gastric bacteria in asymptomatic, random source dogs. Vet. Pathol. 1992, **29**, 454.
7. **Eaton, K. A., Dewhirst, F. E., Radin, M. J., Fox, J. G., Paster, B. J., Krakowka, S., and Morgan, D. R.** *Helicobacter acnonyx* sp. nov., isolated from cheetahs with gastritis. Int. J. Syst. Bacteriol. 1993, **43**, 99-106.
8. **Eaton, K. A., Dewhirst, F. E., Paster, B. J., Tzellas, N., Coleman, B. E., Paola, J., and Sherding, R.** Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: Animal and public health implications. J. Clin. Microbiol. 1996, **34**, 3165-3170.
9. **El-Zaatari, F. A. K., Woo, J. S., Badr, A., Osato, M. S., Serna, H., Lichtenberger, L. M., Genta, R. M., and Graham, D. Y.** Failure to isolate *Helicobacter pylori* from stray cats indicates that *H. pylori* in cats may be an anthroponosis-an animal infection with a human pathogen. J. Med. Microbiol. 1997, **46**, 372-376.
10. **Fabre, R., Sobhani, I., Laurent-Puig, P., Hedef, N., Yazigi, N., Vissuzaine, C., Rodde, I., Potter, F., Mignon, M., Etienne, J. P., and Braquet, M.** Polymerase chain reaction assay for the detection of *Helicobacter pylori* in gastric biopsy specimens: comparison with culture, rapid urease test, and histological tests. Gut 1994, **35**, 905-908.
11. **Feinstein, R. E., and Olsson, E.** Chronic gastro-enterocolitis in nine cats. J. Vet. Diagn. Invest. 1992, **4**, 293-298, 1992.
12. **Fox, J. G., Taylor, N. S., Edmonds, P., and Brenner, D. J.** *Campylobacter pylori* subsp. mustelae subsp. nov. isolated from the gastric mucosa of ferrets (*Mustela putorius furo*), and an emended description of *Camphylobacter pylori*. Int. J. Syst. Bacteriol. 1988, **38**, 367-370.
13. **Fox, J. G., Batcheder, M., Marini, R., Yan, L., Handt, L., Li, X., Shames, B., Hayward, A., Campbell, J., and Murphy, J. C.** *Helicobacter pylori*-induced gastritis in the domestic cat. Infect. Immun. 1995, **63**, 2674-2680.
14. **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., and Roa, I.** Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 1998, **114**, 755-763.
15. **Geyer, C., Colbatzky, F., Lechner, J., and Hermanns, W.** Occurrence of spiral-shaped bacteria in gastric biopsies of dogs and cats. Vet. Rec. 1993, **133**, 18-19.
16. **Graham, D.** *Campylobacter pylori* and peptic ulcer disease. Gastroenterology 1989, **96**, 615-625.
17. **Hammar, M., Tyszkiewicz, T., Wadstrom, T. and O'Toole, P. W.** Rapid detection of *Helicobacter pylori* in gastric biopsy material by polymerase chain reaction. J. Clin. Microbiol. 1992, **30**, 54-58.
18. **Handt, L. K., Fox, J. G., Dewhirst, F. E., Fraser, G. J., Paster, B. J., Yan, L. L., Rozmiarek, H., Rufo, R. and Stalis, I. H.** : *Helicobacter pylori* isolated from domestic cat: public health implications. Infect. Immun. 1994, **62**, 2367-2374.
19. **Handt, L. K., Fox, J. G. Stalis, I. H., Rosemarie, R., Lee, G., Lin, J., Li, X. and Kleanthous, H.** Characterization of feline *Helicobacter pylori* strains and associated gastritis in a colony of domestic cats. J. Clin. Microbiol. 1995, **33**, 2280-2289.
20. **Hänninen, M. L., Happonen, I., Saari, S. and Jalava, K.** Culture and characteristics of *Helicobacter bizzozeronii*, a new canine gastric *Helicobacter* sp. Int. J. Syst. Bacteriol. 1996, **46**, 160-166.
21. **Happonen, I., Saari, S., Castren, L., Tyni, O., Hanninen, M. L. and Westermarck, E.** Occurrence and topographical mapping of gastric *Helicobacter*-like

organisms and their association with histological changes in apparently healthy dogs and cats. J. Vet. Med. Asso. 1996, **43**, 305-315.

22. **Harper, C. M., Dangler, C. A., Xu, S., Feng, Y., Shen, Z., Sheppard, B., Stamper, A., Dewhirst, F. E., Paster, B. J. and Fox, J. G.** : Isolation and characterization of *Helicobacter* sp. from the gastric mucosa of dolphins, lagenorhynchus actus and delphinus delphis. Appl. Environ Microbiol. 2000, **66**, 4751-4757.
23. **Heilmann, K. L. and Borchard, F.** Gastritis due to spiral shaped bacteria other than *H. pylori*: clinical, hostological, and ultrastructural findings. Gut, 1991, **32**, 137-140.
24. **Henry, G. A., Long, P. H., Burns, J. L. and Charbonneau, D. L.** Gastric spirillosis in beagles. Am. J. Vet. Res. 1987, **48**, 831-836.
25. **Hermanns, W., Kregel, K., Breuer, W. and Lechner, J**. *Helicobacter*-like organisms: Histological examination of gastric biopsies from dogs and cats. J. Comp. Pathol. 1995, **112**, 307-318.
26. **Hoshina, S., Kahn, S. M., Jiang, W., Green, P. H. R., Neu, H. C., Chin, N., Morotami, M., LoGerfo, P. and Weinstein, I. B.** Direct detection and amplification of *Helicobacter pylori* ribosomal 16S gene segment from gastric endoscopic biopsies. Diagn. Microbiol. Infect. Dis. 1990, **13**, 473-479.
27. **Jalava, K., Kaartinen, M., Utriainen, M., Happonen, I. and Hänninen, M. L.** *Helicobacter salomonis* sp. nov., a canine gastric *Helicobacter* sp. related to *Helicobacter felis* and *Helicobacter bizzozeronii*. Int. J. Syst. Bacteriol. 1997, **47**, 975-982.
28. **Jalava, K., On, S. L. W., Vandamme, P. A. R., Happonen, I., SuKura, A. and Hänninen, M. L.** Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. Appl. Environ. Microbiol. 1998, **64**, 3998-4006.
29. **Kong, L., Smith, J. G., Bramhill, D., Abruzzo, G. K., Bonfiglo, C., Cioffe, C., Flattery, A. M., Gill, C. J., Lynch, L., Scott, P. M., Silver, L., Thompson, C., Kropp, H. and Bartizal, K.** A sensitive and specific PCR method to detect *Helicobacter felis* in a conventional mouse model. Clin. Diagn. Lab. Immunol. 1996, **3**, 73-78.
30. **Lavelle, J. P., Landas, S., Mitros, F. A., and Conklin, J. L.** Acute gastritis associated with spiral organisms from cats. Dig. Dis. Sci. 1994, **39**, 744-750.
31. **Lee, A., Hazell, S. L., O'Rourke, J. L. and Kouprach, S.** Isolation of spiral-shaped bacterium from the cat stomach. Infect. Immun. 1988, **56**, 2843-2850.
32. **Lee, A., Phillips, M. W., O'rourke, J. L., Paster, B., J., Dewhirst, F. E., Fraser, G. J., Fox, J. G., Sly, L. I., Romaniuk, P. J., Trust, T. J. and Kouprach, S.** *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. 1992, **42**, 27-36.
33. **Lee, A., Karakowka, S., Fox, J. G., Otto, G., Eaton, K. A. and Murphy, J. C.** Role of *Helicobacter felis* on chronic canine gastritis. Vet. Pathol. 1992, **29**, 487-494.
34. **Lee, A., Fox, J. G. and Hazell, S.** : Pathogenicity of *Helicobacter pylori* : a perspective. Infect. Immun. 1993, **61**, 1601-1610.
35. **Nam, H. W. and Kim, D.** Prevalence of *Helicobacter* species infection in dogs. Korea J. Vet. Res. 2000, **40**, 747-753.
36. **Neiger, R., Dieterich, C., Burnens, A., Waldvogel, A., Corthesy-Theulaz, I., Halter, F., Lauerburg, B. and Schmassmann, A.** Detection and prevalence of *Helicobacter* infection in pet cats. J. Clin. Microbiol. 1998, 36, 634-637.
37. **Norris, C. R., Marks, S. L., Eaton, K. A., Torabian, S. Z., Munn, R. J. and Solnick, J. V.** Healthy cats are commonly colonized with "*Helicobacter heilmannii*" that is associated with minimal gastritis. J. Clinic. Microbiol. 1999, **37**, 189-194.
38. **Otto, G., Hazell, S. H., Fox, J. G., Howlett, C. R., Murphy, J. C., O'Rourke, J. L. and Lee, A.** Animal and public health implications of gastric colonization of cats by *Helicobacter*-like organisms. J. Clin. Microbiol. 1994, **32**, 1043-1049.
39. **Park, J. H., Lee, B. J., Kim, C. K., Park, T. K., Park, J. H., Kim, C. H., Li, C. H. and Lee, Y. S.** Pathologic examination of stomach from beagle dogs spontaneously infected with Gastrospirillum sp. Korean. J. of the Lab. Anim. Sci. 1998, **14**, 121-126.
40. **Parsonnet, J., Friedman, G. D.,Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N. and Sibley, R. K.** *Helicobacter pylori* infection and the risk of gastric calcinoma. N. Eng. J. Med. 1991, **325**, 1127-1131.
41. **Perkins, S. E., Yan, L. L., Shen, Z., Hayward, A., Murphy, J. C. and Fox, J. G.** Use of PCR and culture to detect *Helicobacter pylori* in naturally infected cats following triple antimicrobial therapy. Antimicrob. Agents Chemother. 1996, **40**, 1486-1490.
42. **Queiroz, D. M. M, Rocha, G. A., Mendes, E. N., Moura, S. B., Oliveira, A. M. R. and Miranda, D.** Association between *Helicobacter* and gastric ulcers disease of pars esophagea in swine. Gastroenterology. 1996, **111**, 19-27.
43. **Radin, M. J., Eaton, K. A., Krakowka, S., Morgan, D. R., Lee, A., Otto, G. and Fox, J.** *Helicobacter pylori* gastric infection in gnotobiotic beagle dogs. Infect. Immun. 1990, **58**, 2606-2612.
44. **Rossi, G., Rossi, M., Vitali, C. G., Fortuna, D., Burroni, D., Pancotto, L., Capecchi, S., Sozzi, S., Rezoni, G., Ghiara, P. and Taccini, E.** A conventional beagle dog model for acute and chronic infection with *Helicobacter pylori*. Infect. Immun. 1999, **67**, 3112-3120.

45. **Simpson, K. W., McDonough, P. L., Strauss-Ayali, D., Chang, Y. F., Harpending, P. and Valentine, B. A.** *Helicobacter felis* infection in dogs: effect on gastric structure and function. Vet. Pathol. 1999, **36**, 237-248.
46. **Simpson, K. W., Strauss-Ayali, D., Scanziani, E., Straubinger, R. K., Mcdonough, P. L., Straubinger, A. F., Chang, Y., Domeneghini, C., Arebi, N. and Calam, J.** *Helicobacter felis* infection is associated with lymphoid follicular hyperplasia and mild gastritis but normal gastric secretory function in cats. Infect. Immun. 2000, **68**, 779-790.
47. **Solnick, J. V. and Tompkins. L. S. *Helicobacter pylori* and gastroduodenal disease: pathogenesis and host-parasite interaction.** Infect. Agents Dis. 1992, **1**, 294-309.
48. **Solnick, J. V., O'Rourke, J. L., Lee, A., Paster, B. J., Dewhirst, F. E. and Tompkins, L. S.** An uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. J. Infect. Dis. 1993, **168**, 379-385.
49. **Solnick, J. V., Canfield, D. R. and Parsonnet, J.** Seroprevalence of *Helicobacter pylori* infection in rhesus monkeys. Gastroenterology 1996, **110 (Suppl.)**, A261.
50. **Stolte, M., Wellens, E., Bethke, B., Ritter, M. and Eidt, H.** *Helicobacter heilmannii* (formerly *Gastrospirillum hominis*) gastritis: an infection transmitted by animals? Scand. J. Gastroenterol. 1994, 29, 1061-1064.
51. **Tomson, M. A., Storey, P., Greer, R. and Cleghorn, G. J.** Canine-human transmission of *Gastrospillium hominis*. Lancet 1994, **343**, 1605-1607.
52. **Van Zwet, A. A., Thijs, J. C., Kooistra-Smid, A. M. D., Schirm, J. and Snijider, J. A. M.** Senstivity and culture compared with that of polymerase chain reaction for detection of *Helicobacter pylori* from antral biopsy samples. J. Clin. Microbiol. 1993, **31**, 1918-1920.
53. **Warren, J. R. and Marshall, B. J.** Unidentified curved bacilli on gastric epithelium in active gastritis. Lancet 1983, **1**, 1273-1275.
54. **Weber, A. F., Hasa, O. and Sautter J. H.** Some observations concerning the presence of spirilla in the fundic glands of dogs and cats. Am. J. Vet. Res. 1958, **19**, 677-680.
55. **Weiss, J., Elvira, J. M., Silva, D. A. and Gassner, D.** Comparisom of PCR and other diagnostic techniques for detection of *Helicobacter pylori* infection in dyspeptic patients. J. Clin. Microbiol. 1994, 32, 1663-1668.
56. **Wotherspoon, A. C., Ortiz-Hidalgo, C., Falzon, M. R. and Isaacson, P. G.** *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991, **338**, 1175-1176.
57. **Yamasaki, K., Suematsu, H. and Takahashi, T.** Comparison of gastric lesions in dogs and cats with and without gastric spiral organisms. J. Am. Vet. Med. Assoc. 1998, **212,** 529-533.