Survey of Helicobacter infection in domestic and feral cats in Korea

Heh-Myung Ghil¹, Jong-Hyeon Yoo³, Woo-Sung Jung¹, Tae-Ho Chung¹, Hwa-Young Youn¹,², Cheol-Yong Hwang¹,²,*

¹Department of Veterinary Internal Medicine, and ²KRF Priority Zoonotic Disease Research Institute, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea
³BK21 Program of Integrative Network Systems for Veterinarians in Basic Science, Industrial Animals and Preventive Medicine, Konkuk University, Seoul 143-701, Korea

Discovery of Helicobacter (H.) pylori has led to a fundamental change in our understanding of gastric diseases in humans. Previous studies have found various Helicobacter spp. in dogs and cats, and pets have been questioned as a zoonotic carrier. The present study surveyed the Helicobacter infections and investigated the presence of H. felis and H. pylori infections in domestic and feral cats in Korea. Sixty-four domestic cats and 101 feral cats were selected from an animal shelter. Saliva and feces were evaluated by Helicobacter genus-specific polymerase chain reaction (PCR). Genus-specific PCR positive samples were further evaluated for H. felis and H. pylori using specific primer pairs. Thirty-six of 64 (56.3%) samples from domestic cats and 92 of 101 (91.1%) samples from feral cats were PCR positive; the positive rate of feces samples was higher than that of saliva samples in both groups. H. felis and H. pylori species-specific PCR was uniformly negative. The prevalence of Helicobacter spp. in feral cats was approximately two-fold higher than that of domestic cats. The fecal-oral route may be more a common transmission route not only between cats but also in humans.

Keywords: cat, Helicobacter, prevalence, zoonosis

Introduction

Since the discovery of Helicobacter (H.) pylori [28], gastritis has been studied from a whole new perspective. To date, spiral bacteria other than H. pylori found in stomach of humans, animals, dogs and cats have been considered a potential reservoir of zoonosis [7-9,13,17,19,20,31,32,36,41,44]. Two gastric Helicobacters, H. heilmannii and H. felis, are mostly associated with human gastric disease [14]. Nevertheless, eight other enterohepatic Helicobacters (H. canis, H. pullorum, H. cinaedi, H. fennelliae, H. canadensis, H. winghamensis, H. westmaedi, and H. rappini) have been isolated from humans [10].

In humans, Helicobacter spp. infections are associated with gastrointestinal diseases, cancers, and the immunocompromised. In dogs and cats, however, clinically healthy hosts are typically found. While H. felis is implicated as a potential pathogen in humans, many other species are still under research [14].

The route of transmission of Helicobacter spp. is uncertain, but is known to spread by direct contact. Oral-oral, gastro-oral, and fecal-oral routes are all possible [2]. Iatrogenic H. pylori infection transmitted by the endoscope or by contact with gastric fluid also has been reported [43]. H. pylori infection is predominant in the developing world, and low socio-economic status is associated with increased prevalence of the infection [27]. Fecal contamination of common sources including water [16] and soil [15] has been implicated in spread of the infection. This is supported by the findings that H. pylori infection rates are higher in developing countries, where untreated water and inadequately prepared vegetables contaminated with soil are common [4,15]. In animals, the DNA of Helicobacter spp. has been detected from sources other than gastric tissues, which include vomitus and saliva [37], dental plaques [37], and feces [18].

In Korea, Helicobacter spp. has been studied in many animals, for example, dogs [1,17,18,30,33,34], cats [17,22], pigs [35], mice [21], and Mongolian gerbils [24,25]. The DNA of several species of Helicobacter has been detected. ‘H. heilmannii’, formerly named Gastrospirillum hominis in humans [29], is the most predominant species known in cats [5,7,17,31,36,41]. H. felis [5,20,32,36,41] and H. canis [8,9] are also detected in cats. H. pylori was isolated from a group of cats from a commercial vendor of research animals [11,12], and the bacterial DNA has been detected in bile of cats [3]. In studies where specific pathogen free cats were experimentally inoculated with H. pylori or H. felis, the
bacteria induced mild gastritis associated with lymphoid follicles, with no gastric erosions or ulcers evident during upper gastrointestinal endoscopy or at necropsy [38,39]. The present study was surveyed the prevalence of Helicobacter infection and the specific presence of H. felis and H. pylori infection, as a means of clarifying the possible role of domestic and feral cats in Korea as a zoonotic source.

Materials and Methods

Animals and sampling
Saliva and feces samples of 165 domestic and feral cats were obtained. Cats were grouped by environmental criteria; domestic cats were those that were almost exclusively kept indoors and feral cats being those that had been captured roaming wild in suburban areas. In Korea, government policy dictates that overpopulating feral cats are euthanized to preserve the wild life in suburban forests. During weekly visits to an animal shelter operated by the Korea Animal Rescue and Management Association, saliva and feses feral cats (n = 101; 55 females, 46 males) were obtained. Ages and health status of the cats were not ascertained. Domestic cats (n = 64; 28 females, 36 males) were either admitted to the Veterinary Medical Teaching Hospital of Seoul National University (Korea) or were the pets of staff members. The cats had an average age of 3.1 years (range 3 months to 12 years). Twenty-three cats were healthy and 41 were clinically ill; of the latter, the clinical signs varied from simple anorexia to hepatic lipidosis, feline lower urinary tract disease, renal failure, diabetes mellitus, and lymphoma. Feces and saliva samples taken from each cat by swabbing with sterilized cotton swabs were merged in 500 μl of saline. DNA was extracted from 20 μl of each sample using DNeasy Tissue Kit (Qiagen, USA). The DNA samples eluted 100-200 μl were stored at -20°C until required.

Helicobacter genus-specific polymerase chain reaction (PCR)
Helicobacter 16S rRNA gene was amplified from each DNA sample using c97 and c98 primers (Table 1) [10]. The DNA sample using c97 and c98 primers (Table 1) [10]. The final reaction volume of 25 μl contained 2 μl of DNA sample, 12.5 pmole of each primer, ×1 PCR buffer (Takara Bio, Korea), 200 μM of deoxyribonucleoside triphosphates mixture (Takara Bio, Korea), and 0.75 U of recombinant Taq DNA polymerase (Takara Bio, Korea). The PCR cycle was 94°C for 2.5 min followed by 40 cycles of denaturation at 94°C, annealing at 50°C, extension at 72°C for 1 min each, and a final extension at 72°C for 15 min [17]. PCR was performed using a PC808 programmed temperature control system (Astec, Japan). PCR products were electrophoresed on ethidium bromide stained 1.5% w/v agarose gels in ×0.5 TBE buffer. The separated products were visualized on ultraviolet light illuminator. PCR sensitivity and specificity of fecal samples has been previously evaluated [18].

H. pylori and H. felis specific PCR
H. pylori and H. felis specific PCR was performed with primers (Table 1) that amplify the urease B gene of H. pylori and H. felis [31]. Two microliters of each DNA sample was added to a reaction mixture containing 12.5 pmole of each primer, ×1 PCR buffer (Takara Bio, Korea), 200 μM of deoxyribonucleoside triphosphates mixture (Takara Bio, Korea), and 0.75 U of recombinant Taq DNA polymerase (Takara Bio, Korea) to produce a total volume of 25 μl. For H. pylori specific PCR, samples were heated to 95°C for 5 min and 57°C for 5 min once, followed by 35 cycles of extension at 72°C for 1 min, denaturation at 94°C for 1 min, annealing at 72°C for 2 min, and a final extension at 72°C for 10 min [41]. The positive control (isolates purchased from the American Type Culture Collection; ATCC, USA) and the negative control (sterile distilled water) were carried out with every PCR. For H. felis specific PCR, samples were heated to 94°C for 2.5 min once, followed by 40 cycles of denaturation at 94°C, annealing at 47°C, extension at 72°C for 1 min each, with a final extension at 72°C for 15 min. The positive control (H. felis ATCC 49179) and the negative control (sterile distilled water) were carried out with every PCR. PCR products were electrophoresed on ethidium bromide stained 1.5% w/v agarose gels in ×0.5 TBE buffer. The separated PCR products were visualized using an ultraviolet light illuminator.

Table 1. Primer sequences for Helicobacter (H.) spp. polymerase chain reaction

| Target gene | Primer sequences | Product (bp) |
|-------------|-----------------|--------------|
| H. spp. 16S rRNA | F: 5'-GCT ATG ACG GGT ATC C-3'  
R: 5'-GAT TTT ACC CCT ACA CCA-3' | 400 |
| H. pylori urease B | F: 5'-GGA ATT CCA GAT CTA TGA AAA AGA TTA GCA GAA AAG -3'  
R: 5'-GGA ATT CGT CGA CCT AGA AAA TGC TAA AGA GTT G-3' | 1,707 |
| H. felis urease B | F: 5'-ATG AAA CTA ACG CCT AAA GAA CTA G-3'  
R: 5'-GGA GAG ATA AAG TGA ATA TGC GT-3' | 1,150 |

F: forward, R: reverse.
**Table 2.** PCR prevalence of *Helicobacter* spp. infection in domestic and feral cats

|                | Domestic cats (%) | Feral cats (%) |
|----------------|-------------------|----------------|
| Saliva         | 17 (26.6)         | 48 (46.5)      |
| Feces          | 29 (45.3)         | 85 (84.2)      |
| Saliva or Feces| 36 (56.3)         | 92 (91.1)      |
| Total          | 64 (100)          | 101 (100)      |

**Purifying and nucleotide sequence analysis**

A specific sized PCR product was extracted using a MEGAquick-spin gel extraction kit (Intron, Korea) to confirm the identity of the target gene PCR product. Purified PCR products were analyzed using an ABI 3100 automatic sequence analyzer (Applied Biosystems, USA).

**Results**

**Helicobacter genus-specific PCR**

On Helicobacter genus-specific PCR for 16s rRNA gene, 36 (56.3%) from 64 domestic cats were positive, and 92 (91.1%) from 101 feral cats were positive on either saliva or feces samples (Fig. 1). In domestic cats, 17 (26.6%) saliva samples and 29 (45.3%) feces samples were positive. Infection rates were higher in feral cats with 47 (46.5%) saliva samples and 85 (84.2%) feces samples being positive (Table 2). Among the 64 domestic cats for which the clinical status was known, 36 (56.3%) were positive for *Helicobacter* spp. infection. Clinically ill cats had a *Helicobacter* spp. infection rate of 63.4% (26/41), compared to 43.5% (10/23) of healthy cats, which was not statistically significant. Ill cats were not especially prone to gastrointestinal diseases, and their diagnoses mainly involved anorexia with or without hepatic lipidosis, feline urologic syndrome, diabetes mellitus, renal failure, feline infectious peritonitis, lymphoma, and otitis.

**H. pylori and *H. felis* specific PCR**

Species-specific PCR was performed on 17 saliva samples and 29 feces samples from the domestic cats and 47 saliva samples and 85 feces samples from the feral cats, which showed a positive result on genus-specific PCR. In *H. felis* specific PCR, which amplified a 1,200 bp fragment in the positive control (ATCC 49179), none of the samples were positive (Fig. 2). Also, no samples were positive on *H. pylori* specific PCR, which revealed a 1,700 bp fragment on the positive control (SS1 strain) (Fig. 3).
Heh-Myung Ghil

infection is typically acquired in early childhood in settings with overcrowding [2,23]. Moreover, since infection is predominant in developing countries and among intimate familial members [26], it seems likely that the cats in shelter environments are prone to *Helicobacter* spp. infection. Positive infection rates were higher on feces samples in both domestic and feral cats (46.5% and 84.2%, respectively) than on saliva samples (26.6% and 45.3%, respectively). This may suggest that under natural circumstances fecal-oral transmission is more likely than oral-oral transmission among cats. In a previous study, only fecal contact remained as a significant risk factor in an indirect study by questioning, and the seroprevalence for *H. pylori* increased significantly with age [6]. Nevertheless, healthy adult cats vomit naturally on occasion to spit out hairballs, which makes the gastro-oral route conceivable.

The incidence of positive outcome between saliva and feces samples was random, meaning positive feces samples did not always have positive saliva samples, nor did positive saliva samples not always have positive feces samples.

Clinical signs were unknown in feral cats, and domestic cats did not have apparent gastrointestinal signs. Forty-one domestic cats admitted to the hospital for sickness mostly had systemic diseases, and gastrointestinal signs were of simple anorexia coupled with stress or secondary mucosal bleeding due to azotemia. In clinically healthy domestic cats the rate of *Helicobacter* spp. infection was 43.5% (10/23) and in clinically ill domestic cats the rate was 63.4% (26/41). Correlation between the infection rate and the clinical illness including gastrointestinal signs was not confirmative in this study due to the small number of cats involved. Further study of a larger cat population would be needed.

In species-specific studies, neither *H. felis* nor *H. pylori* were found. This is consistent with the results of some previous studies. A Swiss study of 58 cats reported that no amplification of *H. felis* or *H. pylori* were detected in PCR [31]. However, other studies that utilized PCR for examination of gastric biopsy samples reported *H. felis* was in four of 17 [41], two of 21 [17], and one of 15 [5] cats, and one of 10 cheetahs [42]. Although the numbers of cats in the present study was higher than in previous studies, not a single *H. felis* positive sample was evident. Presently, direct sequencing of two 16S rRNA gene-specific PCR products was conducted from purified isolates of *genus-specific PCR*. One of the two products displayed 100% similarity to a *H. canis* 16S RNA sequence of 336 base pairs. The 16S rRNA sequences of *H. felis* and those of *H. bizzozeronii* and *H. salomonis* display 98.2-100% similarity [19], and *H. canis* differs by 8.1-10.1% from these species [40]. This implies that there is a significant genetic difference within the 16S rRNA gene of these Helicobacter species. *H. canis* also has been reported in...
cats in the United States [8,9].

In Korea, Helicobacter spp. studies in cats [17,22] have been fewer and not perceived as urgent a public health issue as similar studies conducted in dogs [1,17,30,33,34]. However, given the burgeoning population of domestic cats in Korea, and the likelihood that many of these cats are kept indoors in close contact with adults and children, careful study of the zoonotic potential of cats is warranted. While cats have not been regarded as a potential zoonotic threat for Helicobacter infections, the results of this study show prompt a re-examination of that view. It is suggested that care be taken especially when handling feces of domestic cats.

Acknowledgments
This work was supported by the Research Institute of Veterinary Science, College of Veterinary Medicine, Seoul National University and Korean Research Foundation Grant (KRF-005-E00078).

References

1. An JH, Nam HW, Han JH, Kim D. The detection of Helicobacter-like organisms in dogs. Korean J Vet Clin Med 1999, 16, 281-288.

2. Axon ATR. Is Helicobacter pylori transmitted by the gastro-oral route? Aliment Pharmacol Ther 1995, 9, 585-588.

3. Boomkens SY, Kusters JG, Hoffmann G, Pot RGJ, Spee B, Penning LC, Egberink HF, Van den Ingh TS, Ruthuizen J. Detection of Helicobacter pylori in bile of cats. FEMS Immunol Med Microbiol 2004, 42, 307-311.

4. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev 2000, 22, 283-297.

5. Chisholm SA, Owen RJ. Development and application of a novel screening PCR assay for direct detection of ‘Helicobacter heilmannii’-like organisms in human gastric biopsies in Southeast England. Diagn Microbiol Infect Dis 2003, 46, 1-7.

6. De Schryver A, Van Winckel M, Cornelis K, Moens G, Devlies G, De Backer G. Helicobacter pylori infection: further evidence for the role of feco-oral transmission. Helicobacter 2006, 11, 523-528.

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

8. Foley JE, Marks SL, Munson L, Melli A, Dewhirst FE, Yu S, Shen Z, Fox JG. Isolation of Helicobacter canis from a colony of Bengal cats with endemic diarrhea. J Clin Microbiol 1999, 37, 3271-3275.

9. Foley JE, Solnick JV, Lapointe JM, Jang S, Pedersen NC. Identification of a novel enteric Helicobacter species in a kitten with severe diarrhea. J Clin Microbiol 1998, 36, 908-912.

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

11. Handt LK, Fox JG, Dewhirst FE, Fraser GJ, Paster BJ, Van LI, Rozmiarek H, Rufo R, Stalis I. Helicobacter pylori isolated from the domestic cat: public health implications. Infect Immun 1994, 62, 2367-2374.

12. Handt LK, Fox JG, Stalis I, Rufo R, Lee G, Linn J, Li X, Kleanthes II. Characterization of feline Helicobacter pylori strains and associated gastritis in a colony of domestic cats. J Clin Microbiol 1995, 33, 2280-2289.

13. Hänninen ML, Happonen I, Saari S, Jalava K. Culture and characteristics of Helicobacter bizzozeronii, a new canine gastric Helicobacter sp. Int J Syst Bacteriol 1996, 46, 160-166.

14. Heilmann KL, Borchard F. Gastritis due to spiral shaped bacteria other than Helicobacter pylori: clinical, histological, and ultrastructural findings. Gut 1991, 32, 137-140.

15. Hopkins RJ, Vial PA, Ferreccio C, Ovalle J, Prado P, Sotomayor V, Russell RG, Wasserman SS, Morris GJ Jr. Seroprevalence of Helicobacter pylori in Chile: vegetables may serve as one route of transmission. J Infect Dis 1993, 168, 222-226.

16. Hulten K, Han SW, Enroth H, Klein PD, Opekun AR. Presence of multiple ‘H pylori’ strains and associated gastritis in a colony of domestic cats in Korea. J Vet Sci 2002, 7, 898-912.

17. Hwang CY, Han HR, Youn HY. Prevalence and clinical characterization of gastric Helicobacter species infection of dogs and cats in Korea. J Vet Sci 2002, 3, 125-133.

18. Hwang CY, Youn HY, Han HR. Development of non-invasive fecal PCR assay for detecting the Helicobacter species infection in dogs. J Vet Clin 2002, 19, 295-298.

19. Jalava K, Kaartinen M, Utriainen M, Happonen I, Hänninen ML. Helicobacter salomonis sp. nov., a canine gastric Helicobacter sp. related to Helicobacter felis and Helicobacter bizzozeronii. Int J Syst Bacteriol 1997, 47, 975-982.

20. Jalava K, ON SL, Vandamme PA, Happonen I, Sukura A, Hänninen ML. Isolation and identification of Helicobacter spp. from canine and feline gastric mucosa. Appl Environ Microbiol 1998, 64, 3998-4006.

21. Kim BH, Won YS, Lee CH, Hyun BH, Kim DY, Choi YK. Inflammatory large bowel disease due to Helicobacter hepaticus infection in BALB/c-A-Hfh11nu mice. Korean J Lab Anim Sci 1998, 18, 143-146.

22. Kim SK, Cho SJ, Kim O. Detection and identification of secreting Helicobacter species from cats. Lab Anim Res 2006, 22, 243-247.

23. Kivi M, Tindberg Y. Helicobacter pylori occurrence and transmission: a family affair? Scand J Infect Dis 2006, 38, 407-417.

24. Lee JU, Jung K, Kim O. Absence of vertical transmission of Helicobacter pylori in an experimental murine model. J Vet Sci 2006, 7, 225-226.

25. Lee JU, Kim O. Natural maternal transmission of H. pylori in Mongolian gerbils. World J Gastroenterol 2006, 12, 5663-5667.

26. Magalhães Queiroz DM, Luzzã F. Epidemiology of Helicobacter pylori infection. Helicobacter 2006, 11, 1-5.

27. Malaty HM, Kim JG, Kim SD, Graham DY. Prevalence of Helicobacter pylori infection in Korean children: inverse
relation to socioeconomic status despite a uniformly high prevalence in adults. Am J Epidemiol 1996, 143, 257-262.

28. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984, 1, 1311-1315.

29. McNulty CAM, Dent JC, Curry A, Uff JS, Ford GA, Gear MW, Wilkinson SP. New spiral bacterium in gastric mucosa. J Clin Pathol 1989, 42, 585-591.

30. Nam HW, Kim D. Prevalence of Helicobacter species infection in dogs. Korean J Vet Res 2000, 40, 747-753.

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

32. Norris CR, Marks SL, Eaton KA, Torabian SZ, Munn RJ, Solnick JV. Healthy cats are commonly colonized with "Helicobacter heilmannii" that is associated with minimal gastritis. J Clin Microbiol 1999, 37, 189-194.

33. Park JH, Lee BJ, Kim CK, Park TK, Park JH, Kim CH, Li GX, Lee YS. Pathological examination of stomachs from beagle dogs spontaneously infected with Gastrospirillum sp. Korean J Lab Anim Sci 1998, 14, 121-126.

34. Park JH, Park HM, Seok SH, Cho SA, Lee HY, Kim DJ, Park JH. Prevalence and pathological characteristics of Helicobacter spp. in gastric mucosa of domestic pet dogs. Korean J Lab Anim Sci 2002, 18, 120-124.

35. Park JH, Seok SH, Cho SA, Baek MW, Lee HY, Kim DJ, Park JH. The high prevalence of Helicobacter pylori in the gastric mucosa of beagle dogs. Vet Microbiol 2004, 104, 219-225.

36. Priestnall SL, Wuinberg B, Spohr A, Neuhaus B, Kuffer M, Wiedmann M, Simpson KW. Evaluation of "Helicobacter heilmannii" subtypes in the gastric mucosae of cats and dogs. J Clin Microbiol 2004, 42, 2144-2151.

37. Recordati C, Gualdi V, Tosi S, Facchini RV, Pengo G, Luini M, Simpson KW, Scanziani E. Detection of Helicobacter spp. DNA in the oral cavity of dogs. Vet Microbiol 2007, 119, 346-351.

38. Simpson KW, Strauss-Ayal D, Scanziani E, Straubinger RK, McDonough PL, Straubinger AF, Chang YF, Domenechnghi C, Arebi N, Calam J. Helicobacter felis infection is associated with lymphoid follicular hyperplasia and mild gastritis but normal gastric secretory function in cats. Infect Immum 2000, 68, 779-790.

39. Simpson KW, Strauss-Ayal D, Straubinger RK, Scanziani E, McDonough PL, Straubinger AF, Chang YF, Esteves MI, Fox JG, Domenechnghi C, Arebi N, Calam J. Helicobacter pylori infection in the cat: Evaluation of gastric colonization, inflammation and function. Helicobacter 2001, 6, 1-14.

40. Solnick JV, Schauer DB. Emergence of diverse Helicobacter species in the pathogenesis of gastric and enterohemorrhagic diseases. Clin Microbiol Rev 2001, 14, 59-97.

41. Strauss-Ayal D, Scanziani E, Deng D, Simpson KW. Helicobacter spp. infection in cats: evaluation of the humoral immune response and prevalence of gastric Helicobacter spp. Vet Microbiol 2001, 79, 253-265.

42. Terio KA, Munson L, Marker L, Aldridge BM, Solnick JV. Comparison of Helicobacter spp. in Cheetahs (Acinonyx jubatus) with and without gastritis. J Clin Microbiol 2005, 43, 229-234.

43. Tytgat GN. Endoscopic transmission of Helicobacter pylori. Aliment Pharmacol Ther 1995, 9 (Suppl 2), 105-110.

44. Wiinberg B, Spohr A, Dietz HI, Egelund T, Greiter-Wilke A, McDonough SP, Olsen J, Priestnall S, Chang YF, Simpson KW. Quantitative analysis of inflammatory and immune responses in dogs with gastritis and their relationship to Helicobacter spp. infection. J Vet Intern Med 2005, 19, 4-14.