J Vet Clin 19(3) : 295-298(2002)

Development of Non-Invasive Fecal PCR Assay for Detecting the *Helicobacter* Species Infection in Dogs

Cheol-Yong Hwang[1], Hwa-Young Youn and Hong-Ryul Han

College of Veterinary Medicine, Seoul National University

Abstract : This study was conducted to develope noninvasive fecal PCR assay for detecting the *Helicobacter* species in dogs. From the DNA isolated from fecal samples, and a region of the 16S rRNA gene conserved among *Helicobacter* spp. was amplified. In comparison with gastric biopsy test, the fecal PCR assay showed high specificity (100%) and sensitivity (96%). The prevalence of *Helicobacter* spp. infection in privately owned pet dogs in Korea determined by the fecal PCR assay was 72.1%. The fecal PCR assay determined in this study can be a new noninvasive test for detecting *Helicobacter* spp. infection in dogs.

Key words **:** *Helicobacter* species, Fecal PCR, Prevalence, Dog

Introduction

In dogs, several gastric *Helicobacter* species have been reported since the turn over the century, however their presence has been largely ignored and even understood as gastric commensals[4,15]. Recently, these gastric organisms have received renewed attention because not only the *Helicobacter pylori* (*H. pylori)* has been proved as a strong gastric pathogen in humans but also some clinical cases have been also reported in dogs and cats[6,9,16].

There are several methods being utilized for the diagnosis of *Helicobacter* spp. infection in human and animals. These tests can be categorized as invasive and noninvasive. With invasive tests, endoscopy must be done, which is then usually followed up by culture, histologic examination, and/or a rapid urease test of a gastric mucosal biopsy specimens. These tests have shown high sensitivity and specificity for diagnosis and considered gold standard for diagnosis. Noninvasive tests like urea breath test, which measures the amount of labeled CO_2 in breath produced by *Helicobacter* urease activity after oral administration of ^{14}C- or ^{13}C-labeled urea has been proved extremely useful diagnostic methods for *Helicobacter* spp. infection in humans. However, when urea breath test is conducted in animals, anesthesia or sedation is sometimes needed and exact collection of breath can be difficult[2,11].

Several reports of *Helicobacter* spp. infections in humans have lead to speculation that animals, especially dogs and cats may serve as a source for human infection[3,8,11]. Therefore there is a need to determine the prevalence of *Helicobacter* spp. 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.

[1]Corresponding author.
E-mail : doglover@chollian.net

Therefore, this study was conducted to develope noninvasive fecal PCR assay for detecting the gastric *Helicobacter* spp. in dogs and to evaluate of the prevalence of gastric *Helicobacter* spp. in privately owned pet dogs in Korea by using the fecal PCR assay.

Materials and Methods

Fecal sampling

Fecal samples were taken by swabbing of rectum with a sterile cotton swab and submerged in a 600 μl of Nuclei Lysis Solution of Wizard® genomic DNA purification kit (Promega) containing 120 μl of 0.5M EDTA.

Bacterial strains

For evaluating the sensitivity and specificity of the fecal PCR assay, *H. felis* (ATCC 51211) purchased from the American Type Culture Collection (ATCC) was used as standard *Helicobacter* strains. DNAs from *Escherichia coli* (*E. coli*; ATCC 25922) and *Camphylobacter jejuni* (*C. jejuni*; Culture Collection of Antibiotic Resistant Microbes, Seoul, Korea) were used for specificity evaluation.

DNA isolation

DNAs from fecal samples were isolated with Wizard® genomic DNA purification kit (Promega) according to it's protocol for mouse tail tissue. Bacterial DNAs were also isolated with the same kit as protocol for Gram negative bacteria.

PCR

Helicobacter genus-specific PCR assay was performed with C97 and C98 primers which amplify the 16S rRNA gene of *Helicobacter* species[5]. DNA samples (3 μl) were added to a reaction mixture containing 400 μM dNTPs, 1X PCR buffer, 2.5 U of *Taq* DNA polymerase (ABgene), 0.6 μM

of each primer, and distilled water in a total volume of 50 μl. PCR samples were heated to 94°C for 2.5 min once, followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 15 min in a Biometra personal thermocycler. PCR products were subjected to electrophoresis on a 2.0% agarose gel containing 0.5 μg of ethidium bromide per and visualized over UV light.

Specificity evaluation

To verify specificity of fecal PCR assay, fecal samples taken from 8 *Helicobacter* spp. uninfected live dogs or euthanized dogs were tested. These dogs were previously diagnosed as *Helicobacter* spp. unifected by endoscopic biopsy in live dogs or by necropsy with urease test, microscopy test with direct tissue smear and gastric tissue PCR assay. To evaluate primer specificity under condition of the fecal assay, DNAs from *E. coli*, and *C. jejuni* were added to separate PCRs containing pooled negative fecal DNA from *Helicobacter* spp. uninfected dogs.

Sensitivity evaluation

Fecal samples from 23 live dogs or euthanized dogs which were previously proved to be *Helicobacter* spp. infected by urease test, microscopy test with direct tissue smear and PCR assay of gastric tissue samples taken by endoscopic biopsy and necropsy were tested. The sensitivity of the fecal PCR assay for *H. felis* was determined by adding 10-fold serial dilutions of DNAs from cultured bacteria to seperate PCR mixtures in the presence of 3 μl of fecal DNAs from *Helicobacter*-free dog.

Evaluation of the prevalence of Helicobacter spp. in privately owned pet dogs in Korea by using the fecal PCR assay

For evaluating the infection state of *Helicobacter* spp. in privately owned pet dogs using the fecal PCR assay, fecal samples were taken from 61 patient dogs in Veterinary Medical Teaching Hospital of Seoul National University with owner's acceptance as previously described methods. Disease of the patients was not considered. Fecal DNA isolation and fecal PCR assay were performed as previously described methods.

Results

Specificity

Helicobacter genus-specific PCR assay of eight fecal samples from *Helicobacter* uninfected dogs produced no bands. These dogs were previously proved to be *Helicobacter* spp. uninfected by gastric tissue examination with a microscopic examination of direct tissue smear samples, urease test and PCR assay (gold standard of diagnosis). As a result, The specificity of fecal PCR assay for detecting *Helicobacter* spp. infection in dogs was 100% (Table 1). PCR assay with *E. coli* and *C. jejuni* DNA inserted fecal DNAs from uninfected dogs were negative (Fig 1).

Table 1. Comparison of results between gastric tissue examination and fecal PCR assay for detecting *Helicobacter* spp. infection of dogs.

Groups (No. of dogs)	No. of positive (%)	
	Gastric tissue exam.	Fecal PCR
Helicobacter spp. infected (25)	25 (100)	24 (96)
Helicobacter spp. uninfected (8)	8 (100)	8 (100)

Sensitivity

Of the 25 *Helicobacter* spp. infected dogs, 24 showed positive on *Helicobacter* genus specific fecal PCR assay and the sensitivity of this assay was 96% (Table 1). On PCR assay of 10-fold serial dilutions of *H. felis* DNA with fecal DNA from uninfected dog, the lower limit of detection of *H. felis* was 100 fg (Fig 2).

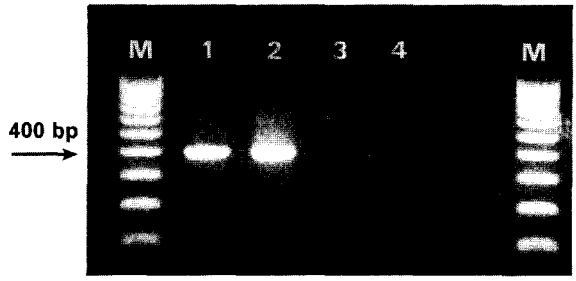

Fig 1. Detection of *Helicobacter* spp. DNA in feces by *Helicobacter* genus-specific PCR assay. Lanes: M, DNA ladder; 1, infected dog; 2, *H. felis* DNA inserted feces DNA from uninfected dog; 3, *E. coli* DNA inserted feces DNA from uninfected dog; 4, *C. jejuni* DNA inserted feces DNA from uninfected dog.

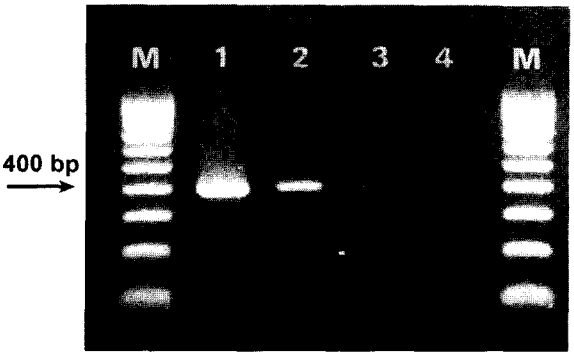

Fig 2. Fecal PCR amplification products of serial dilution of H. felis DNA in the presence of fecal DNA from Helicobacter spp. uninfected dog. Lanes: M, DNA ladder; 1, 10 pg of H. felis DNA; 2, 1 pg of H. felis DNA; 3 100 fg of H. felis DNA; 4, 10 fg of H. felis DNA.

Table 2. Prevalence of *Helicobacter* spp. of privately owned pet dogs in Korea evaluated by fecal PCR assay

Age range (No. of dogs)	No. of fecal PCR positive (%)
< 1 years (7)	5 (71.4)
1 - 3 years (22)	16 (72.7)
4 - 6 years (16)	12 (75)
7 - 9 years (10)	7 (70)
> 10 years (6)	4 (66.7)
Total (61)	44 (72.1)

Evaluation of the prevalence of *Helicobacter* spp. in privately owned pet dogs in Korea by using the fecal PCR assay

Helicobacter spp. were detected in 72.1% of tested privately owned pet dogs. Prevalence of *Helicobacter* spp. infection was very similar based on the age of the days (Table 2).

Discussion

The evaluation of the prevalence of *Helicobacter* spp. infection in privately owned pet dogs and cats is usually hampered by the lack of a noninvasive diagnostic tests, and reliance on endoscopic examination. Noninvasive test like urea breath test has been usually employed to diagnose *H. pylori* in humans and evaluated it's efficacy for detecting *Helicobacter* spp. infection in dogs and cats. However, when urea breath test conducted in animals, anesthesia or sedation is sometimes needed for collecting sufficient volume of breath if tested animals were not tolerant of the test procedures[2,11].

In the present study, efficacy of fecal PCR assay using primer pairs for generating 16S rRNA amplicons of *Helicobacter* species was evaluated with the purpose of developing new noninvasive diagnostic test for *Helicobacter* spp. infection in dogs. Fecal PCR assay described here showed high specificity (100%) and sensitivity (96%), which were concurred with those in previous study of laboratory rodents[1]. Fecal PCR assay tested with *E. coli* and *C. jejuni* DNA inserted fecal DNAs from uninfected dogs to verify primer specificity did not show positive results. These observations were important because these two organisms were believed to have the greatest potential to interfere with the assay; *E. coli* was included because it is commonly present in normal dog feces, and *C. jejuni* was included because members of the *Campylobacter* and *Helicobacter* genera have a high degree of homology in their 16S rRNA genes. The 100 fg sensitivity of fecal PCR assay in this study is comparable to that of previous study[1]and exceeds that of one study which showed sensitivity of 0.5 to 0.1 ng of *Helicobacter hepaticus* DNA[11]. Beckwith *et al*[1]considered that the lower sensitivity may be due to the method of DNA purification and developed fecal PCR assay with higher sensitivity using fecal dilution methods and commercial DNA purification kit. Using these two methods they overcame inhibition effects by inhibitor of Taq polymerase presented in feces. Fecal PCR assay described in the present study also used similar methods[1] for DNA purification and had same results. Based on these results, Fecal PCR assay developed in this study can be used as a noninvasive test for detecting *Helicobacter* spp. infection in dogs.

Evaluation the prevalence of *Helicobacter* spp. in privately owned pet dogs using fecal PCR assay showed that 72.1% of tested dogs were infected with *Helicobacter* spp. This observation would lead to consider the risk of transmission of *Helicobacter* spp. between house hold dogs and their owners by fecal-oral route. Although the risk of transmission of *Helicobacter* spp. between animals and humans in the developed countries has been known to be low, some researchers alarmed the significance of that mode of transmission because clinical *Helicobacter* spp. infection cases in human originated from animal sources have been reported[3,10].

Prevalence of *Helicobacter* spp. infection in dogs less than one year of age determined by fecal PCR assay was 71.4% and this rate was similar to the infection rate of older dogs. The tendency of increasing *Helicobacter* spp. infection with increasing age observed in *H. pylori* infection in humans was not detected (Table 2). One previous study reported that puppies may acquired gastric *Helicobacter* spp. infection from dams during the lactation period and puppies can infect each other during their early life[7]. This findings may explain the observation that high prevalence of *Helicobacter* spp. infection in dogs less than one year of age in the present study.

Conclusion

Fecal PCR assay developed in this study for detecting *Helicobacter* spp. in dogs showed high specificity (100%) and sensitivity (96%) and could be used as new noninvasive diagnostic test. Evaluation of the prevalence of *Helicobacter* spp. in privately owned pet dogs using fecal PCR assay showed that 72.1% of tested dogs were infected with *Helicobacter* spp. and the tendency of increasing *Helicobacter* spp. infection with increasing age was not observed.

References

1. Beckwith CS, Franklin CL, Hook RR, Besch-Williford CL, Riley LK. Fecal PCR assay for diagnosis of *Helicobacter* infection in laboratory rodents. J Clin Microbiol 1997; 35: 1620-1623.
2. Cornetta AM, Simpson KW, Strauss-Ayali D, McDonough PL, Gleed RD. Use of a [^{13}C]urea breath test for detection of gastric infection with *Helicobacter* spp. in dogs. Am J Vet Res 1998; 59: 1364-1369.

3. Dieterich C, Wiesel P, Neiger R, Blum A, Corthesy-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.
4. Fox JG, Batcheder M, Marini R, Yan L, Handt L, Li X, Shames B, Hayward A, Campbell J, Murphy JC. *Helicobacter pylori*-induced gastritis in the domestic cat. Infect Immun 1995; 63: 2674-2680.
5. Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, Paster BJ, Ericson RL, Lau CN, Correa P, Araya JC, Roa I. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 1998; 114: 755-763.
6. Geyer C, Colbatzky F, Lechner J, Hermanns W. Occurrence of spiral-shaped bacteria in gastric biopsies of dogs and cats. Vet Rec 1993; 133: 18-19.
7. H nninen ML, Happonen I, Jalava K. Transmission of canine gastric *Helicobacter salomonis* infection from dam to offspring and between puppies. Vet Microbiol 1998; 62: 47-58.
8. Heilmann KL, Borchard F. Gastritis due to spiral shaped bacteria other than *H. pylori*: clinical, hostological, and ultrastructural findings. Gut 1991; 32: 137-140.
9. Hermanns W, Kregel K, Breuer W, Lechner J. *Helicobacter*-like organisms: Histological examination of gastric biopsies from dogs and cats. J Comp Pathol 1995; 112: 307-318.
10. Lavelle JP, Landas S, Mitros FA, Conklin JL. Acute gastritis associated with spiral organisms from cats. Dig Dis Sci 1994; 39: 744-750.
11. 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.
12. Shames B, Krajden S, Fuksa M, Babida C, Penner JL. Evidence for the occurrence of the same strain of *Campylobacter pylori* in the stomach and dental plaque. J Clin Microbiol 1989; 27: 2849-2850.
13. Shames B, Fox JG, Dewhirst F, Yan L, Shen Z, Taylor NS. Identification of widespread *Helicobacter hepaticus* infection in feces in commercial mouse colonies by culture and PCR assay. J Clin Microbiol 1995; 33: 2968-2972.
14. Tomson MA, Storey P, Greer R, Cleghorn GJ. Canine-human transmission of *Gastrospillium hominis*. Lancet 1994; 343: 1605-1607.
15. Weber AF, Hasa O, Sautter JH. Some observations concerning the presence of spirilla in the fundic glands of dogs and cats. Am J Vet Res 1958; 19: 677-680.
16. Yamasaki K, Suematsu H, 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.

개의 *Helicobacter* 균속 감염 진단을 위한 비 침습적 분변 PCR 분석법

황철용[1] · 윤화영 · 한홍율

요 약 : 본 연구에서는 개의 *Helicobacter* 균속 감염을 비침습적 방법으로 진단하기 위해 분변을 시료로한 PCR 검사법을 확립하고 그 효능을 검정하고자 하였다. 분변 PCR 분석은 분변에서 채취된 DNA에서 *Helicobacter* 균속의 16S RNA의 특이적인 영역에 반응하는 primer를 이용해 수행하였다. 분변 PCR 분석법에 의한 결과와 위조직 검사법 결과를 비교해 본 결과 분변 PCR 분석법은 높은 특이도(100%)와 민감도(96%)를 나타내었다. 또한 확립된 분변 PCR 분석법을 이용해 국내 애완견들의 위내 *Helicobacter* 균속 감염율을 조사한 결과 감염율이 72.1%로 나타났다. 이상의 결과 본 연구에서 확립한 분변 PCR 분석법은 개의 *Helicobacter* 균 감염을 진단할 수 있는 새로운 비침습적 진단방법으로 이용될 수 있으리라 사료된다.

주요어 : 분변 PCR, 헬리코박터 균속, 개