

## Effect of Triple Therapy on Eradication of Gastric *Helicobacter* Species Infection in Dogs

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**Abstract:** For evaluating the effect of triple therapy on eradication of gastric *Helicobacter* species infection in dogs, 7 dogs that had naturally acquired *Helicobacter* spp. infections were administered amoxicillin, metronidazole and omeprazole orally for 14 days. Changes of infection state were determined by urease test for gastric biopsies and *Helicobacter* specific PCR analysis for gastric biopsies and fecal samples at 7, 14 days after triple therapy and 30 days after cessation of triple therapy. Although negative results for urease test were obtained 6 of 7 dogs at 14 days after starting triple therapy, PCR analysis for gastric biopsies and fecal samples showed negative results in 3 and 4 dogs respectively. At 30 days after cessation of triple therapy, all tests showed negative results in 3 dogs. Based on these results, diagnostic tests for detecting *Helicobacter* spp. infection are recommended in dogs having chronic gastritis sign (usually intermittent vomiting) and triple therapy described in this study can be applied for eradicating the organism if the animals were proved to be infected.

**Key words :** *Helicobacter* species, triple therapy, dog

### Introduction

Since the initial isolation of *Helicobacter pylori* (*H. pylori*) from human gastric tissues in 1983 by Warren and Marshall, evidence implicating the bacterium as the causative agent of gastritis and duodenal ulcer has been established<sup>1,3,22</sup>. The discovery of the association of *H. pylori* with gastritis and duodenal ulcers in humans has led to fundamental changes in the understanding of gastric disease. There has been a radical swing away from the use of antacids alone to the eradication of *Helicobacter* with antimicrobial agents<sup>1</sup>. More recently, the bacterium was also known to be a cofactor in the development of gastric adenocarcinoma and gastric lymphoma in humans<sup>16,22</sup>. Although infection of *Helicobacter* spp. is highly prevalent in dogs and cats ranged from 61 to 100%<sup>2,5-7,9,11,12,15,16,24</sup>, the role of *Helicobacter* spp. infection in gastrointestinal disease of small animals has not been clearly understood yet. Although evidence suggests that the usual consequence of *Helicobacter* spp. infection in dog and cat is a subclinical gastritis, *Helicobacter*-associated clinical signs reportedly include chronic vomiting and diarrhea, inappetence, pica, fever, and polyphagia<sup>24</sup>. Bloody diarrhea also has been described in a group of persian cats with chronic lymphocytic-plasmacytic gastroenterocolitis associated with a spiral shaped organisms<sup>3</sup>.

Various clinical trials using different antimicrobial treatments were initially conducted to assess their ability to eradicate *H. pylori* in humans. Combination therapies utilizing multiple drugs have been necessary in humans to eradicate *Helicobacter*<sup>21</sup>. Acid-inhibiting drugs (e.g., H<sub>2</sub> receptor blockers and proton-pump inhibitors) are usually used along

with antimicrobials like as amoxicillin, metronidazole and clarithromycin. In human medicine, the traditional bismuth-based triple therapy regimens consisting of bismuth, metronidazole, and either amoxicillin or tetracycline are associated with more than 90% cure rates in some studies<sup>20,21</sup>.

This study was conducted to evaluate effect of triple therapy (combination of amoxicillin, metronidazole, and omeprazole) which was based on a effective method in humans with *H. pylori* infection on eradicating of gastric *Helicobacter* spp. infection in dogs.

### Material and Methods

#### Animals

For this study, 7 dogs that had naturally acquired *Helicobacter* spp. infections were selected from blood donor dogs and teaching practice supporting dogs in veterinary medical teaching hospital of seoul national university. Four dogs were 2-6-year-old female and 3 were 2-7-year-old male. Their body weight was between 2-16 kg. One dog (Dog 2) had a history of vomiting sometimes early in the morning but the others didn't have a history of abnormal gastrointestinal signs. Dogs were housed in a separate aluminum cages fed a commercial diet twice a day and had a constant access to water throughout the study. The housing facility was cleaned daily and disinfected twice weekly with chlorhexidine during the treatment period.

#### Triple therapy

The effect of an antimicrobial and antisecretory regimen (AMO: amoxicillin 25 mg/kg PO q 12 h, metronidazole 30 mg/kg PO q 12 h, Omeprazole 1 mg/kg PO q 12 h for 14 days), which is efficacious in humans infected with *H. pylori*

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on eradicating *Helicobacter* spp. infection in dogs was evaluated.

### Gastric biopsy

Endoscopic biopsies of the stomach were obtained with a pediatric endoscope and biopsy forceps (2 mm) at 0, 7 and 14 days after starting AMO treatment and rebiopsied at 30 days after cessation of AMO treatment. Endoscopic biopsies were procured from the body (greater curvature) area of stomach. Two biopsies were taken for impression smear and urease test and one biopsy was taken for PCR assay. Endoscopic biopsy samples for PCR assay were frozen at  $-80^{\circ}\text{C}$  pending analysis. The endoscope was thoroughly cleaned and sterilized using an activated aldehyde solution. Biopsy forceps were sterilized in a similar fashion and the biopsy cups were immersed in chlorox (1:10 in water) for 10 minutes to destroy residual DNA.

### Urease test

Gastric mucosal biopsies were placed in sterile tubes containing 200  $\mu\text{l}$  of a solution composed of urea, sodium azide, phenol red, and phosphate-buffered saline (pH 6.5). Samples were incubated at  $37^{\circ}\text{C}$  for 24 hours. A change from orange-red to bright pink was considered a positive result after 24 hours incubation. Time of color changes were recorded and the results were additionally scored as follows: 0, negative at 24 hours; 4, positive at 1 hour; 3, positive at 3 hours; 2, positive at 12 hours; 1, positive at 24 hours.

### Fecal sampling

For evaluating the infection state using fecal PCR assay, fecal samples were taken at 0, 7 and 14 days after starting AMO treatment and 30 days after cessation of AMO treatment by swabbing of rectum with a sterile cotton swab and submerged in a 600  $\mu\text{l}$  of Nuclei Lysis Solution of Wizard<sup>®</sup> genomic DNA purification kit (Promega, U.S.A.) containing 120  $\mu\text{l}$  of 0.5 M EDTA.

### PCR

Gastric biopsies collected endoscopically and fecal samples from 7 dogs at 0, 7 and 14 days after starting AMO treatment and 30 days after cessation of AMO treatment were tested by *Helicobacter* genus-specific primers generating 16S rRNA amplicons<sup>4</sup>. DNA from gastric biopsy was extracted with a Wizard<sup>®</sup> genomic DNA purification kit (Promega) according to the manufacturer's instructions. DNAs from fecal samples were also isolated with a same kit according to its protocol for mouse tail tissue.

Biopsy tissue isolated DNA samples (100 ng) and fecal isolated DNA samples (100 ng) were added to a reaction mixture containing 400  $\mu\text{M}$  dNTPs, 1X PCR buffer, 2.5 U of *Taq* DNA polymerase (ABgene), 0.6  $\mu\text{M}$  of each primer, and distilled water in a total volume of 50  $\mu\text{l}$ . PCR samples were heated to  $94^{\circ}\text{C}$  for 2.5 min once, followed by 40 cycles of denaturation at  $94^{\circ}\text{C}$  for 1 min, primer annealing at  $50^{\circ}\text{C}$  for 1 min, and extension at  $72^{\circ}\text{C}$  for 1 min, with a final extension at  $72^{\circ}\text{C}$  for 15 min in a Biometra personal thermocycler. PCR products were subjected to electrophoresis on a 2.0% agarose gel containing 0.5  $\mu\text{g}$  of ethidium bromide per ml and visualized over UV light.

## Results

Negative results for urease test were obtained in 4 of 7 dogs at 7 days, 6 of 7 dogs at 14 days after starting AMO treatment. Thirty days after cessation of AMO treatment however, 4 of 7 dogs showed positive results again (Table 1).

PCR assays of gastric biopsies using *Helicobacter* genus-specific primers were positive in biopsies from 7 of 7 dogs at 7 days, 4 of 7 dogs at 14 days after starting AMO treatment (Fig. 1). There were no differences of test results between 14 days after starting AMO treatment and 30 days after cessation of AMO treatment.

PCR assays of fecal samples were positive in 7 of 7 dogs

**Table 1.** Evaluation of *Helicobacter* infection status in dogs before and after treatment with a combination of amoxicillin, metronidazole and omeprazole

Dog No.	Pretreatment			7 Days			14 Days			30 Days Posttreatment		
	GUA <sup>a</sup>	PCR(G) <sup>b</sup>	PCR(F) <sup>c</sup>	GUA	PCR(G)	PCR(F)	GUA	PCR(G)	PCR(F)	GUA	PCR(G)	PCR(F)
1	3	+	+	2	+	+	1	+	+	1	+	+
2	2	+	+	0	+	+	0	-	-	0	-	-
3	2	+	+	2	+	+	0	+	+	2	+	+
4	3	+	+	0	+	+	0	+	+	2	+	+
5	2	+	+	1	+	+	0	+	-	1	+	+
6	2	+	+	0	+	+	0	-	-	0	-	-
7	2	+	+	0	+	+	0	-	-	0	-	-

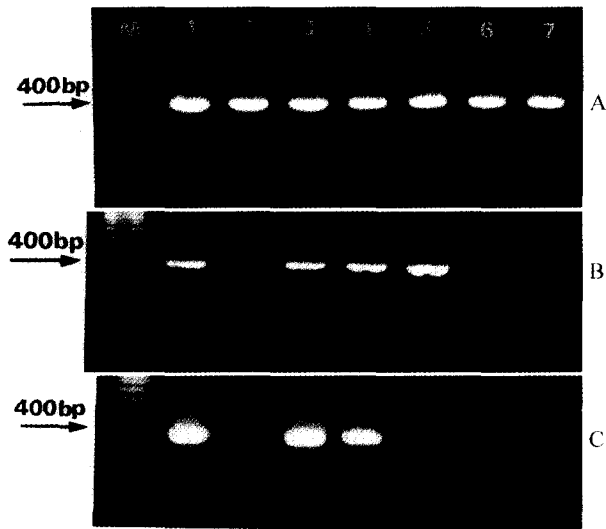
<sup>a</sup>Gastric urease activity: 0, negative at 24 hours; 4, positive at 1 hour; 3, positive at 3 hours; 2, positive at 12 hours; 1, positive at 24 hours.

<sup>b</sup>*Helicobacter* genus-specific PCR analysis of gastric tissue: +, positive; -, negative.

<sup>c</sup>*Helicobacter* genus-specific PCR analysis of fecal sample. +, positive; -, negative.

at 7 days, 3 of 7 dogs at 14 days after starting AMO treatment (Fig. 1). The results were consistent with the results of gastric biopsies except one dog show negative result at 14 days after starting AMO treatment although gastric biopsy test show positive. Thirty days after cessation of AMO treatment, 4 of 7 dogs show positive results again and the results were consistent with the results of gastric biopsies (Table 1).

In one dog (Dog No. 2) suffered from a history of chronic intermittent vomiting, *Helicobacter* spp. infection was suc-



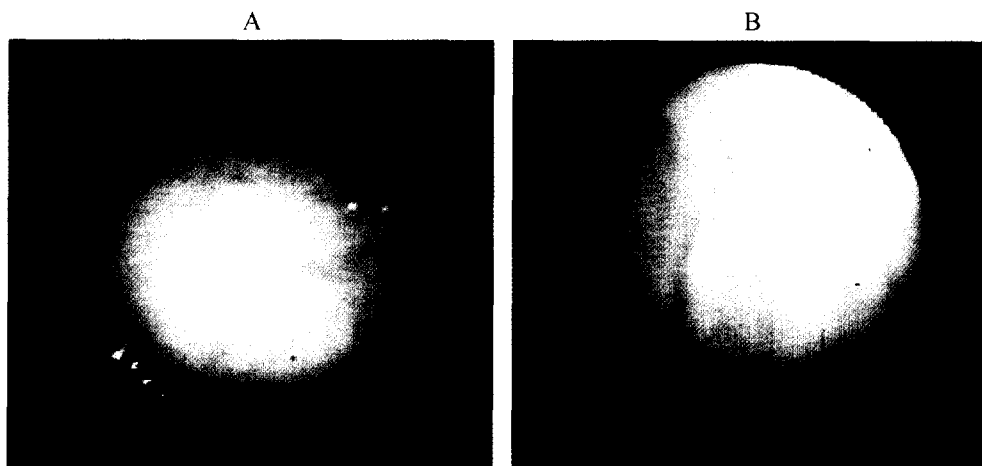
**Fig 1.** Detection of *Helicobacter* spp. 16s rRNA fragment DNA (400 bp) in gastric tissues and feces of dogs before AMO treatment and at 14 days after starting AMO treatment by *Helicobacter* genus-specific PCR assay. A, gastric tissues before AMO treatment; B, gastric tissues at 14 days after starting AMO treatment; C, feces at 14 days after starting AMO treatment. Lanes: M, DNA ladder (ABgene<sup>®</sup> 100bp ladder); 1-7 DNA from infected dogs.

cessfully eradicated concordant with recovering of the sign and disappearing of moderate gastritis lesions detected on endoscopy examination before AMO treatment (Fig. 2).

## Discussion

Although the traditional bithmuth-based triple therapy regimens for eradicating *H. pylori* infection in human medicine were consisted with bithmuth, metronidazole, and either amoxicillin<sup>20,21</sup>, acid-inhibiting drugs (e.g., H<sub>2</sub> receptor blockers and proton-pump inhibitors) used along with antimicrobials like as amoxicillin, metronidazole and clarithromycin were also effective for eradicating *H. pylori* infection<sup>10</sup>. In veterinary medicine, some studies for evaluating the efficacy of triple therapy regimens for *Helicobacter* spp. infection showed that a combination of ranitidine, bithmuth citrate and clarithromycin is effective in eliminating *H. mustelae* infection in ferrets<sup>14</sup>, a combination of amoxicillin, metronidazole and bithmuth subcitrate is effective in *Helicobacter* spp. infection in dogs<sup>8</sup>. The triple therapy regimen employed in this study in an attempt to eradicate gastric *Helicobacter* spp. in dogs was based on a method effective in humans with *H. pylori* infection<sup>10</sup>.

Results of the urease tests performed at 7 days and 14 days after starting AMO treatment showed 4 and 6 of 7 dogs were negative respectively. However, 4 of 7 dogs showed positive results again on 30 days after cessation of treatment. Moreover, results of PCR assays of gastric biopsies of treated dogs performed at 14 days after starting AMO treatment showed that 3 dogs were positive although results of urease test were negative. Among these 3 dogs, 2 dogs were also positive for fecal PCR assays performed at 14 days after starting AMO treatment and all 3 dogs showed positive results again for all three tests on 30 days after



**Fig 2.** Comparison of gastric endoscopic findings of one dog with chronic vomiting sign before starting and after finishing of AMO treatment. A, moderate gastritis was detected before starting AMO treatment; B, gastritis was disappeared after finishing of AMO treatment.

cessation of AMO treatment. The persistence of *Helicobacter* spp. after antibiotic therapy is similar to recent findings in dogs and cats with *Helicobacter* spp. infection<sup>18,19</sup>. Cats with *H. pylori* infection and dogs with unidentified *Helicobacter* spp. infection that were treated with similar antibiotic regimens as used in the present study showed initial clearance of the organisms, but reinfection or recrudescence was reported in the majority of animals 1 month after antibiotic treatment. The observation that positive PCR results remaining positive in the face of negative urease test results in the present study was also identical to that observed in these previous studies<sup>18,19</sup>. Those authors raised the possibility of the persistence of small numbers of bacteria during antimicrobial treatment, which were only detectable by use of PCR assay, and recrudescence of infection after cessation of treatment. In the present study, 3 treated dogs showed negative results for all tests at 30 days after cessation of treatment. This finding was differed to that of previous treatment studies<sup>18,19</sup> in dogs and cats and indicated that AMO treatment was sometimes effective for eradicating *Helicobacter* spp. infection in dogs. This finding may be due to higher antibiotic dosage than that of previous studies but this was not confirmed certainly. These variable efficacy of treatment in dogs with naturally acquired disease could possibly be related to infection with several species of *Helicobacter* organisms. For instance, it is possible that differences in antibiotic susceptibility of different *Helicobacter* spp. could have cause the variable response to treatment.

In one dog (Dog No. 2) suffered from a history of chronic intermittent vomiting, *Helicobacter* spp. infection was successfully eradicated concordant with recovering of the sign and disappearing of moderate gastritis lesions detected on endoscopy examination before AMO treatment (Fig. 2). This dog also had history of treatment with antacids and antiemetics before AMO treatment but the clinical sign was not resolved. This result suggested that *Helicobacter* spp. infection was a major cause of the chronic intermittent vomiting in this dog. Therefore, diagnostic tests for detecting *Helicobacter* spp. infection are recommended in dogs having chronic gastritis sign (usually intermittent vomiting) and effective treatment for eradicating the organism should be considered if the animals were proved to be infected.

### Conclusion

Triple therapy (combination of amoxicillin, metronidazole and omeprazole) caused suppression and sometimes eradication effect of gastric *Helicobacter* spp. in dogs. Therefore, diagnostic tests for detecting *Helicobacter* spp. infection are recommended in dogs having chronic gastritis sign (usually intermittent vomiting) and triple therapy described in this study can be applied for eradicating the organism of the animals were proved to be infected.

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## 개의 위내 *Helicobacter* 균속 감염에 대한 삼중요법의 효과

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**요 약 :** 위내 *Helicobacter* 균속에 자연 감염된 개에서 삼중요법의 효과를 알아보기 위해 *Helicobacter* 균속에 자연 감염된 것으로 판명된 7두의 개를 대상으로 amoxicillin, metronidazole과 omeprazole을 14일간 경구투여하였다. 삼중요법 실시에 따른 *Helicobacter* 균속 감염상태 변화를 평가하기 위해 투여 7일째, 14일째 그리고 투여 중지후 30일째의 위생검 조직에 대한 요소검사, *Helicobacter* 균속에 대한 중합효소 연쇄반응 검사와 함께 분변시료를 이용한 중합효소 연쇄반응 검사를 실시하였다. 삼중요법 14일째 위생검 조직의 요소검사에서는 7두중 6두가 음성이었으나 위생검조직과 분변시료의 중합효소 연쇄반응 검사에서는 각각 3두와 4두만이 음성이었다. 삼중요법 중지후 30일째 검사에서는 각 검사법이 모두 동일한 결과를 나타내었는데 7두중 3두에서 음성으로 나타났다. 이상의 결과를 바탕으로 위내 *Helicobacter* 균속에 자연 감염된 개에서 amoxicillin, metronidazole과 omeprazole을 이용한 삼중요법은 *Helicobacter* 균속 감염 정도를 낮추거나 때로는 완전 박멸할 수 있을 것으로 보여지기에 만성위염의 임상증상을 나타내는 개의 *Helicobacter* 균속 감염증에 적용할 수 있으리라 사료된다.