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Occurrence of suspected infection of *Campylobacter* spp and *Clostridium* spp in dogs with chronic diarrhea

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Abstract : Suspected infections of *Campylobacter* spp and *Clostridium* spp were observed in three dogs. The diagnosis was based on fecal cytology, Gram's stain, clinical signs and serum chemistry. The rectal swabs of diarrheic dogs were performed to confirm the enteropathogens. Suspected *Campylobacter* spp were a sea-gull shape and *Clostridium* spp had a large, clear endospore in rectal cytology. Treatment with appropriate antibiotics resulted in a complete resolution of all clinical abnormalities in three cases. The source of *Campylobacter* spp and *Clostridium* spp could not be found clearly in three cases, but gastrointestinal origin was most likely. When detecting the enteropathogens in feces, fecal smear with Wright's and Gram's stain should be made at first and also, if the patients have canine parvoviral enteritis, attention should be paid to confirm the *Campylobacter* spp and *Clostridium* spp. In addition, since *Campylobacter* spp and *Clostridium* spp as normal bacterial flora exists in canine intestines, it is thought that microbiological isolation should be performed to confirm the suspected *Campylobacter* spp and *Clostridium* spp as primary enteropathogens in subsequent study.

Key words : *Campylobacter* spp, *Clostridium* spp, diarrhea, dogs.

Introduction

The importance of enteric bacteria on the health of dogs was well documented[1]. These bacteria affect the function of the intestinal mucosa and play an important role in preventing colonization of pathogenic bacteria in the intestinal mucosa[1,2]. Therefore, it is clear that bacterial flora of in-

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testine is involved in diverse gastrointestinal diseases.

Especially, it was repoted that newborn puppies lack a normal bacterial flora in the intestines at birth[3]. The normal gastrointestinal microflora is usually established by three to four weeks of age and remains relatively stable throughout life[4]. Aerobic bacteria are together with anaerobic bacteria comprising the majority in the intestine. However, total numbers and distribution of bacteria vary considerably according to the regions of the gastrointestinal tract[5]. Bacterial numbers in the proximal small intestine are relatively low due to the influence of gastric acid and bile, and they gradually show increased trends than those of the distal large intestine. The duodenum and proximal jejunum have low bacterial numbers of Gram-positive bacteria, while anaerobic and Gram-negative bacteria exist mainly in the distal small intestine and colon. Anaerobic bacteria are consisting of the majority of colonic microflora.

Regulation in this normal microflora is required to prevent bacterial overgrowth by normal microflora and to prevent colonization by enteric pathogenic bacteria. The administration of antibiotics or steroids and the presence of gastrointestinal disease, such as canine parvovirus and coronavirus infection, may disrupt both the composition and distribution of this normal flora[6]. Intestinal bacteria such as *Campylobacter jejuni* and *Clostridium* spp may cause gastroenteritis by defeating host defense system. Bacterial diarrhea may be induced by direct mucosal injury or toxin production. Intestinal mucosa-invading bacteria could penetrate into epithelial cells and produce inflammation, mucosal necrosis, and hemorrhagic diarrhea. Enterotoxigenic bacteria remain on the epithelial surface and promote intestinal fluid fluxes without causing significant morphologic injury.

Recently, antibiotic treatment of campylobacter remains controversial and should be limited to patients with severe enterocolitis or evidence of septicemia or endotoxemia. Overuse of antibiotics enhances the susceptibility to infection, facilitates fecal shedding of pathogenic bacteria, and may prolong the carrier state[7]. *Campylobacter jejuni* and *Clostridium* spp, most commonly isolated from diarrheic feces, may cause mild, erosive enterocolitis. *Clostridium* spp are part of the normal anaerobic flora and occasionally may cause gasteroenteritis due to excess production of enterotoxin by overgrowth. It was reported that high numbers of *C perfringens* have been recovered from feces of dogs having parvoviral enteritis and hemorrhagic gastroenteritis[8-10].

This study reported the occurrence of suspected *Campylobacter* spp and *Clostridium* spp in dogs with chronic diarrhea.

Materials and Methods

History taking : Case no. 1 ; A 3-year-old, intact female Rottweiler was admitted for the Veterinary Medical Teaching Hospital of Seoul National University with history of vomiting and dirrhea during 15 days. There were no other recent medical problems observed by the owners. Vaccination and deworming were done. Case no. 2 ; A 1-year-old, intact, female, Jindo dog was evaluated for generalized lethargy, anorexia, severe vomiting and bloody diarrhea for 7 days. Vaccination was made three times. Case no. 3 ; A one and half year old, Pointer dog weighing 22.8kg was referred. Chief complaints were intermittent bloody diarrhea and coughing for 10 days. Deworming was not made. But vaccination was practived 4 times.

Physical examination : Case no. 1 ; Only mucoid diarrhea with an elevated rectal temperature was shown in physical examination. Case no. 2 ; Physical examination showed slightly depression, dehydrated, sunken eye and increased CRT (capillary refile time) and watery diarrhea. Rectal temperature was 38.9℃ and heart and respiratory rate per minute were 110 and 71, respectively. Case no. 3 ; This patient showed congested conjunctiva, harsh lung sound and increased abdomminal tension.

Hematological and serum chemistry : Case no. 1 ; Leukocytosis characterized by a left shift was observed. Lactate dehydrogenase was slighly increased and albumin was decreased. Other serum chemistry other than 2 parameters were normal. Case no. 2 ; Leukocytosis was observed. Especially monocytosis and lymphocytosis were also observed. Packed cell volume was increased and other test results were normal. Case no. 3 ; Complete blood counts showed leukocytosis and other serum chemistry profile appeared nor-

mal.

Rectal cytology : Case no. 1; Rectal swabs were taken from referred diarrhoeic dogs. Swabs were then smeared thin on the slide glass and stained by the method of Gram's and Wright's stain.

Detection of canine parvovirus infection : Canine parvovirus antigen detection kit (IDEXX) was used as the manufacture's instruction. Strong positive reaction of the test was observed only in case no. 1.

Treatment schedule : Case no. 1; Initial supportive care started with intravenous fluids and antibiotic therapy. Erythromycin (20mg/kg, q 8h) and metronidazole (20mg/kg, q 8h) were administered orally for 2 days. Case no 2; Antibiotic therapy was initiated as follows. Cephadroxil (20mg/kg, q 8h) and metronidazole (20mg/kg, q 8h) was administered orally for 3 days and also, enrofloxacin (5mg/kg, q 8h) was injected subcutaneously for 1 day. Case no 3; Treatment was initiated with antibiotics and antiparasitics. Metronidazole (20mg/kg, q 8h) and amoxicillin (20mg/kg, q 8h) was administered orally for 10 days.

Results

Fecal smear showed *Campylobacter* spp like a sea-gull shape and epithelial cells detached from the intestine. Case no. 2; Suspected *Clostridium* spp was observed in fecal cytology with Wright's and Gram's stain. *Ancylostoma* spp and *Trichuris vulpis* were detected on fecal flotation and direct examination. Case no 3; Fecal smear showed suspected *Clostridium* spp and other Gram positive and negative bacteria were shown in the same fecal sample. A tentative diagnosis of acute watery diarrhea was Clostridial enteritis based on the results of the initial diagnosis. Case no. 1; Because persistant diarrhea was observed, the same regime was continued for 14 days. After improvement of clinical signs, medication was stopped. The dog progressively improved and remained clinically asymptomatic at home. And then antibiotic therapy was discontinued after 14 days without further complication. Case no. 2; The dog was remained mildly febrile, anorectic, and depressed during initial supportive care. The rectal temperature returned to normal and no further vomiting episodes were observed after initial therapy. Appetite was gradually improved after 3 days. Case no. 3; After treatment, clinical signs were disappeared and feces was normal.

Table 1. Hematological profile in cases which were referred for diagnosis of vomitting and diarrhea

Test	Case No. 1	Case No. 2	Case No. 3
Hematocrit(%)	28.9	59.7	42
Red blood cells($\times 10^6$/uL)	ND	11	6.97
Hemoglobin(g/dl)	9.5	20.3	15.0
MCHC(g/dl)	32.9	34.3	ND
MCV(fL)	ND	61.3	51
MCH(pg)	ND	20.9	ND
White blood cells($\times 10^3$/uL)	24	19.54	18.9
Granulocytes($\times 10^9$/L)	21.7	ND	ND
Lymphocyte/monocyte($\times 10^9$/L)	2.3	ND	ND
Platelets($\times 10^9$/L)	252	220	ND

ND : not done MCV : mean corpuscular volume, MCHC : Mean corpuscular hemoglobin concentration, MCH : mean corpuscular hemoglobin.

Table 2. Serum chemistry profile in cases which were admitted for diagnosis of vomiting and diarrhea

Test	Case No. 1	Case No. 3
Albumin(g/dl)	1.8	2.9
Alkaline phosphatase(IU)	23	23
Alanine aminotransferase(IU)	12	34
Aspartate aminotransferase(IU)	28	43
Blood urea nitrogen(mg/dl)	11.2	24
Glucose(mg/dl)	100.3	107
Lactate dehydrogenase(IU)	391	ND
Triglyceride(mg/dl)	101	ND
Total bilirubin(mg/dl)	ND	0.3
Total protein(g/dl)	ND	8.5

ND : not done.

Discussion

Diarrhea is caused by overgrowth of enterotoxigenic *C perfringens* and it may be self-limiting but may require fluid, dietary, or antibiotic therapy, or a combination of the three[11]. Metronidazole administration appears useful in the treatment of debilitated dogs or those having chronic diarrhea. Similarly, metronidazole therapy resulted in prompt cessation of chronic diarrhea in three dog associated with *Clostridium* spp infection. However, two dogs required long-term therapy to prevent relapses. Addtional studies are required to define the roles of clostridial organisms as primary pathogens within the gastrointestinal tracts of dogs.

Canine parvovirus infections and parasitic infestations of the lower alimentary tract are also possible causes of diarrhea in dogs less than 1 year old. It is thought that common causes of chronic diarrhea include *Clostridium perfringen* enterotoxicosis. Once the diarrhea is localized to the large bowel an initial diagnostic plan includes the following; multiple fecal examinations for parasites, rectal cytology, highly digestible diet trial and therapeutic deworming for whipworms. If a diagnosis is not made and the diarrhea continues, analysis for *C perfringens* enterotoxin and a thorough endoscopic examination of the colon should be performed. A laboratory evaluation consisting of a complete blood count, biochemical profile, and urinalysis is indicated to rule out polysystemic disease. On occasion a fecal culture will help achieve a diagnosis.

One of the most common causes of chronic large bowel diarrhea seen in the author's experience was *C perfringens* enterotoxicosis. Diagnosis is suspected based on findings spores on a rectal cytology sample. Clinical signs include acute and chronic large bowel diarrhea, vomiting, and abdominal pain. The clinical signs can be self-limiting in some cases. Treatment with amoxicillin 22mg/kg q 12h~q 8h for 2 weeks was highly effective. Relapses can occur and will respond to a similar treatment.

Since *Campylobacter* spp require selective culture techniques for identification, this diagnostic method was not practical in local veterinary hospital. Therefore if bacterial enteritis or canine parvoviral enteritis was suspected, fecal cytology should be performed. Fecal cytology can be performed by staining a thin fecal smear with Wright's and Gram's stain. *Campylobacter* spp appeared sea gull-shaped. If animals shows signs of diarrhea, rectal cytology can be combined with this technique. A gloved finger or a cytology spatula can be inserted into the rectum and gently rubbed across the epithelial surface. This feces can be stained with Wright's stain. Normal fecal or rectal cytology should contain colonic epithelial cells and bacteria. Increased numbers of white blood cells or red blood cells may be indicative of inflammatory or hemorrhagic disorders. The presence of more than 3-5 spores of *Clostridium* spp suggested the possibility of enterocolitis. Spores appear as large rods with a clear center and dark staining ends.

Although these bacteria were not common in enteritis, these cases of the study demonstrated that *Campylobacter* spp and *Clostridium* spp enteritis should be considered as differential diagnosis in dogs showing clinical signs of acute canine parvoviral enteritis. Appropriate diagnosis, including fecal culture, should be made to identify whether bacterial infection is responsible for the clinical signs.

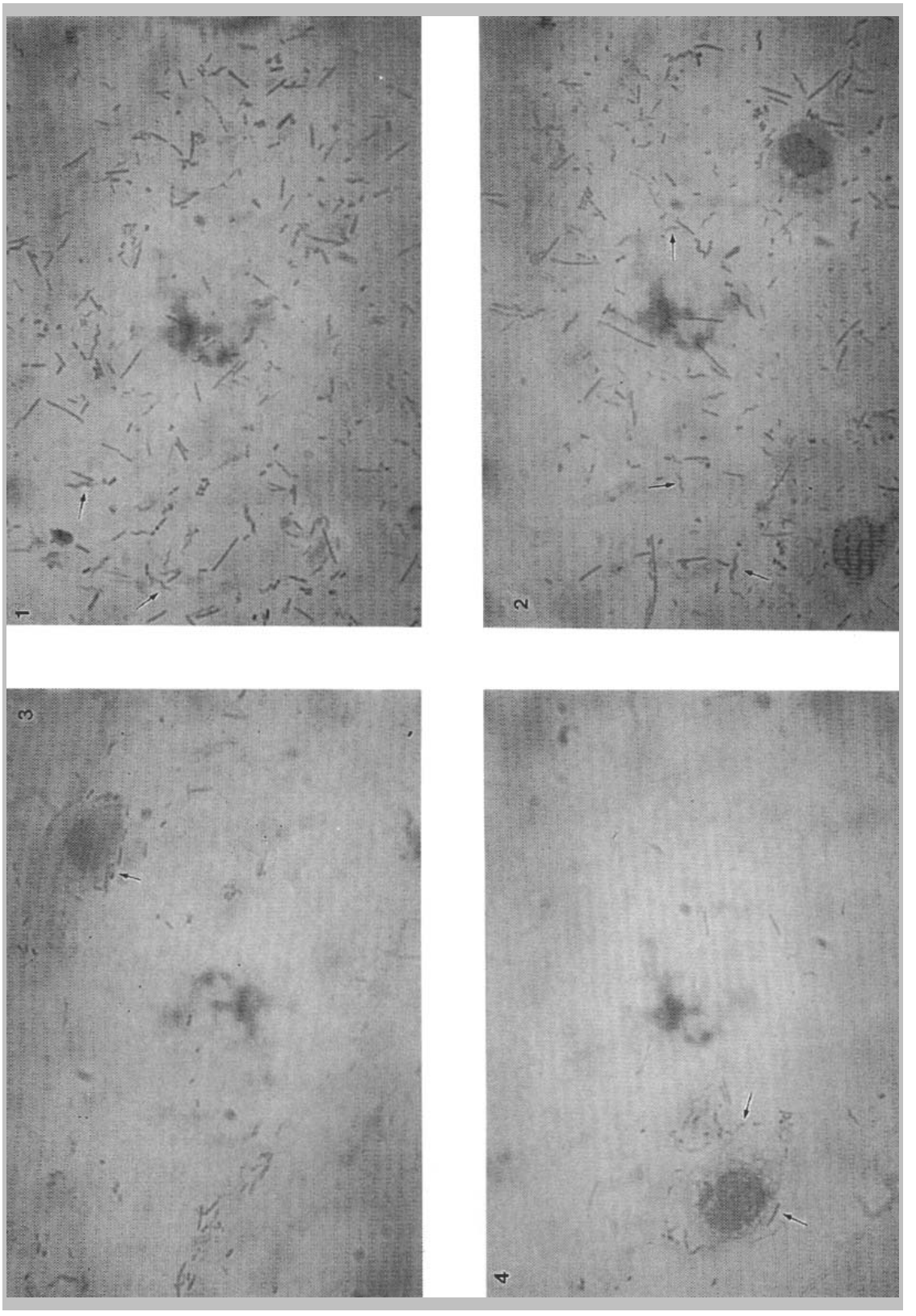
Legends for figures

Fig 1. Fecal smear showing increased fecal *Campylobacter* spp (arrows) in a 3-year-old dog with enteritis (Wright's stain ×1,000).

Fig 2. Fecal smear showing increased fecal *Campylobacter* spp (arrows) and epithelial cells in a 3-year-old dog with enteritis (Wright's stain ×1,000).

Fig 3. Fecal cytology from a 1-year-old dog with hematochezia and mucoid stools. Large endospore of *Clostridium* spp are present (Wright's stain ×1,000).

Fig 4. Fecal cytology showing the epithelial cell and adherent bacteria (arrows) (Wright's stain ×1,000).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References

1. Donaldson RM, Toskes PP. The relation of enteric bacterial populations to gastrointestinal function and disease. Sleisenger MH, Fordtran JS, eds. *Gastrointestinal disease pathophysiology diagnosis management*. Philadelphia: WB Saunders, 107-114, 1989.
2. Gorbach SL. Intestinal microflora. *Gastroenterology*, 60:1110-1129, 1971.
3. Smith WH, Crabb WE. The fecal bacterial flora of animals and man. its development in the young, *J Path Bact*, 82:53-66, 1961.
4. Simon GL. Gorbach SL. Intestinal microflora. *Med Clin North Am*, 66:557-574, 1982.
5. Strombeck DR. Microflora of the gastrointestinal tract and its symbiotic relatiohship with the host: Strombeck DR. ed. *Small animal gastroenterology*. Davis CA: Stonegate Publishing, 23-30, 1979
6. Isikawa H, Baba E, Matsumoto H. Studies on bacterial flora of the alimentary tract of dogs. Ⅲ. fecal flora in clinical and experimental cases of diarrhea. *Jpn J Vet Sci*, 44:343-347, 1982.
7. Grrene CE. Enteric bacterial infections. In: Greene CE. ed. *Clinical microbiol and infectious diseases of the dog and cat*. Philadelphia, WB Saunders, 617-632, 1984.
8. Prescott JF, Johnson JA, Patterson JM. Haemorrhagic gastroenteritis in the dog associated with Clostridium welchii. *Vet Rec*, 103:116-117, 1978.
9. Turk J, Fales W, Miller M. Enteric *Clostridium perfringens* infection associated with parvoviral enteritis in dogs: 74 cases (1987-1990). *J Am Vet Med Assoc*, 200-991-994, 1992.
10. Twedt DC, Jones RL, Collins JK. *Clostridium perfringens* infection associated with diarrhea in dogs. In proceedings: *7th Am Coll Vet Int Med Forum*, 1046, 1989.
11. Kruth SA, Prescott JF, Welch MK. Nosocomial diarrhea associated with enterotoxigenic *Clostridium perfringens* infection in dogs. *J Am Vet Med Assoc*, 195: 331-334, 1989.