Korean Journal
of Veterinary Service

Korean J Vet Serv (2006) 29(4):497-501

「CASE REPORT」

Clostridium perfringens type A associated enteritis in a Shitzu dog

Jae-Won Byun[1], Soon-Seek Yoon, Suk-Kyung Lim, O-Soo Lee

National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-824, Korea

(Received 25 October 2006, accepted in revised from 14 November 2006)

Abstract

Acute hemorrhagic enteritis was diagnosed in a seven-month-old male Shitzu dog dying of blood stained diarrhea and vomiting. Clinical findings were anorexia, dullness and sudden death after massive bloody diarrhea. At necropsy, main lesion was the hemorrhage in small intestine, mainly duodenum and jejunum. Microscopically, Gram positive long bacilli were massively detected on the mucose epithelial cells and necrotic debris of small intestine. Coagulative necrosis of epithelial cells and thrombosis of small intestine were also identified. However, there was no lesion of crypt epithelium. Mineral infiltration in both gastric mucosa and renal tubules was detected and proliferation of fibrous tissue was also shown in corticomedullary regions. In bacterial examination, *C perfringens* was isolated in anaerobic culture and it was confirmed to type A by multiplex PCR. Therefore, the dog was diagnosed as *C perfringens* type A associated enteritis with uremia.

Key words : *C perfringens* type A, Enteritis, Uremia, Dog.

[1]Corresponding author
Phone : +82-31-467-1751, Fax : +82-31-467-1800
E-mail : jwbyun@nvrqs.go.kr

Introduction

Clostridium perfringens is a Gram positive, spore forming bacterium, inhabiting the gastrointestinal tract of human and animals as well as terrestrial and marine environment[1-2]. It cause sudden death result from systemic effects of the

several toxins produced by *C perfringens* in the small and large intestine. *C perfringens* is divided into five biotypes from A to E, based on the possession of one or more of four major toxin gene: alpha(α), beta(β), epsilon(ε) and iota (ι). The enteric clostridial disease is developed in most of domestic animals such as cattle[3], sheep[4] and pig[5] but rarely in dog[6-7]. In dog, enterotoxigenic clostri dial infection is mainly associated with *C perfringens* type A and sometimes accompanied by parvovirus infection[1,8-9]. In Korea, Park et al.[10] reported the canine clostridial infection using spore detection method from feces of dogs showing chronic diarrhea. However, clostridial spore could be detected in normal feces because these bacteria are the one of normal inhabitants in intestine. As a result, C*lostridium* associated enteritis could be misdiagnosed with bacteriological results using the intestinal contents. Recently, it has been recommended that the several tools such as pathological findings, toxin detection and culture technique must be used to diagnose clostridial associated enteritis[1,6,11]. The aim of this study was to describe pathological and bacteriological findings of clostridial enteritis in a dog and confirm the molecular type of *C perfringens* enterotoxin.

Symptoms (Materials and Methods)

Historically, a seven-month-old male Shitzu dog was suffered from mild diarrhea and vomiting and died after massive bloody diarrhea. Clinical signs were emerged in two days after feed conversion. The necropsy was immediately carried out to diagnose the reasons for the death. Tissue samples collected from necropsy were fixed with 10% neutralized buffered formalin, processed routine procedure and embedded in paraffin wax. Three to four micrometer sections were stained with Hematoxylin and Eosin (H&E). For bacterial examination, duodenum and jejunal tissues were stained with Gram' s stain. Luminal contents of duodenum and jejunum were also smeared onto 5% defibrinated sheep blood agar (Komed Co., Ltd., Korea) and MacConkey agar, and incubated for 48 hours at 37℃ under aerobic and anaerobic conditions. The colony showing double hemolysis on the blood agar was shown and toxigenic types were determined by multiplex PCR as described Yoo et al[12]. Canine parvovirus was examined by indirect fluorescence antibody (IFA) technique using mouse monoclonal antibody (VMRD, Inc, USA) with frozen sections of jejunum. Virus particles were also determined by trans mission electronmicroscope (TEM, H-7100AF, Hitachi Co, Japan) using intestinal contents.

Grossly, the lesion was restricted in small intestine. Watery and bloody contents were filled in the intestinal lumen. Histopathologically, the main lesions of small intestine were coagulative necrosis and hemorrhage of mucosal layer (Fig 1). Thrombi were shown in intestinal blood vessels. The adherence of large Gram positive bacilli to the necrotic mucosal surface and intestinal debris were apparent (Fig 2). However, the change of crypt epithelium was not observed.

Canine parvovirus and enteric virus particles were not detected by IFA and TEM examination. Mineral infiltration was detected in midzone of gastric mucosa (Fig 3) and renal tubules in medullary regions of kidney. Fibrous tissues were also proliferated in corticomedullary regions. In anaerobic primary culture, numerous double hemolytic colonies were isolated and identified with *C perfringens*, indicating that *C perfringens* was the cause of the hemorrhagic enteritis. On PCR-based toxin typing, the isolates were identified as toxigenic type A of *C perfringens* harboring *cpe A* gene (Fig 4).

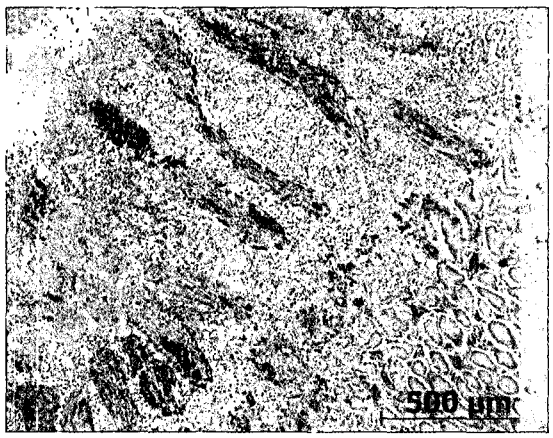

Fig 1. Jejunum. Coagulative necrosis and hemorrhage of intestinal mucosa (50×, H&E)

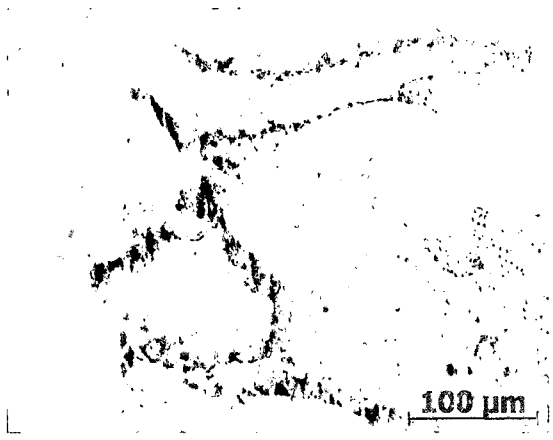

Fig 2. Jejunum. Numerous Gram positive bacilli adhered on the necrotic epithelial cells (200×, Gram's stain)

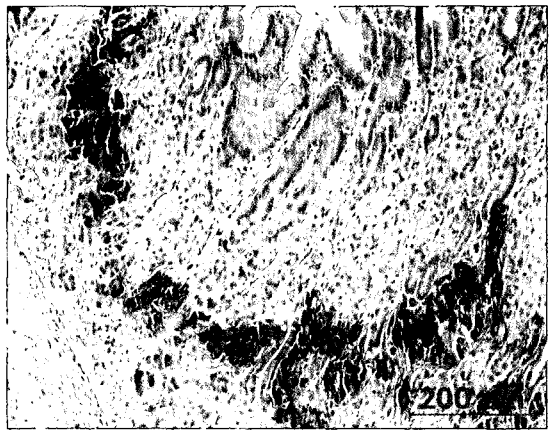

Fig 3. Stomach. Mineralization in middle part of gastric mucosa(100×, H&E)

Discussion

Hemorrhagic enteritis occurs rarely in dog compared with other domestic animals. *C perfringens* type A, usually causing peracute hemorrhagic enteritis have mainly been reported in dog, [1,6,11,13]. It was also reported that *C perfringens* frequently grows in the intestine of dog with canine parvovirus infection[8,9]. *C perfringens* is frequently isolated in normal intestine, so it is unfavorable to diagnose this disease only with bacterial detection. There is no gold standard to diagnose *C perfringens* associated enteritis However, the criteria such as clinical signs, pathologic findings, bacterial culture and toxin detection have widely

been recommended. It is also imperative that enterotoxin (ELISA) and toxin gene detection (PCR) are widely used as diagnostic tool for *C perfringens* in human and animals[1,11,14].

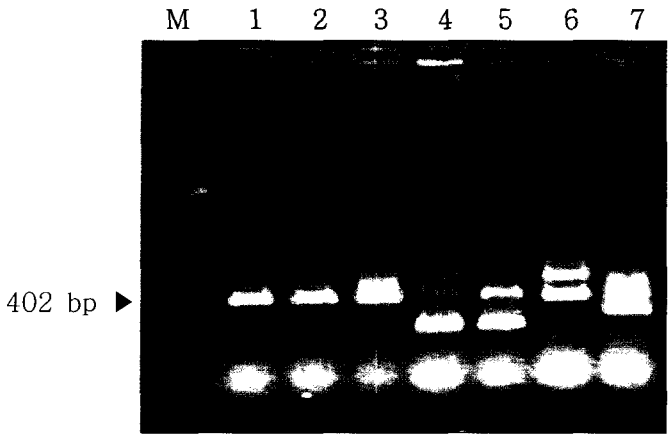

Fig 4. Electrophoretic analysis of *C perfringens* toxin genes amplified by a multiplex PCR

M: 100bp-ladder,
Lane 1: 2 isolates,
Lane 3: *C perfringens* type A,
Lane 4: *C perfringens* type B,
Lane 5: *C perfringens* type C,
Lane 6: *C perfringens* type D,
Lane 7: *C perfringens* type E

In this case, the characteristics of hemorrhagic enteritis with extensive adherence of Gram positive bacilli to the necrotic mucosa were similar to previous reports[6,9,13]. The bacteria isolated in this study were identified as *C perfringens* type A based on the results of multiplex PCR, indicating that *C perfringens* type A was the cause of the hemorrhagic enteritis. Alpha toxin produced by *C perfringens* type A is believed to be a major factor responsible for induction of the pathologic lesion. Main lesions caused by α-toxin are myonecrosis, hemolysis, an increase in vascular permeability, and platelet aggregation[7,12]. Predisposing factors such as stress, change of feed and deprivation of immune system may be responsible to induce bacterial over-growth in intestine and cause enteritis[1,13]. We supposed that feed conversion may be the predisposing factor for clostridium associated hemorrhagic enteri tis in this case. Mineralization was also detected in gastric mucosa and tubules of kidney. Mineralization in the dog is related with uremic symptoms and dietary imbalance of minerals[13,15]. These lesions are common in corticomedullary junction and gastric mucosa. Furthermore, fibrous tissue was proliferated in medullary regions of kidney. It indicated that the dog has been suffered from chronic renal disorder. It was supposed that the clostridium inducing enteritis could make worse the uremic condition. However, it was difficult that the uremic symptoms were considered as the main cause of the acute clinical signs. In conclusion, we diagnosed this case as hemorrhagic enteritis caused by *C perfringens* type A infection accompanied by uremia.

References

1. Marks SL, Kather EJ. 2005. *Infectious disease of the dog and cat,* 3th (Greene CE. eds.) WB Saunders

Press, St. Louis, Missouri : 363-365.
2. Quinn PJ, Carter ME, Markey BK, et al. 1994. *Clinical veterinary microbiology*. Mosby press, London : 191-208.
3. Dennison AC, Van Metre DC, Morley PS, et al. 2005. Comparison of the odds of isolation, genotypes, and *in vivo* production of major toxins by *Clostridium perfringens* obtained from the gastrointestinal tract of dairy cows with hemorrhagic bowel syndrome or left-displaced abomasum. *J Am Vet Med Assoc* 227: 132-138.
4. Uzal FA. 2004. Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *Anaerobe* 10 : 135-143.
5. Songer JG, Uzal FA. 2005. Clostridial enteric infections in pigs. *J Vet Diagn Invest* 17 : 528-536.
6. Sasaki J, Goryo M, Asahina M, et al. 1999. Hemorrhagic enteritis associated with *Clostridium perfringens* type A in a dog. *J Vet Med Sci* 61(2) : 175-177.
7. Songer JG. 1996. Clostridial enteric disease of domestic animals. *Clin Microbiol Rev* 9(2) : 216-234.
8. Tilton RC, Van Kruiningen HJ, Kwasnik I, et al. 1981. Toxigenic *Clostridium perfringens* from a parvovirus-infected dogs. *J Clin Microbiol* 14(6) : 697-698.
9. Turk J, Miller M, Pace L, et al. 1992. Enteric *Clostridium perfringens* infection associated with parvovirial enteritis in dogs: 74 case (1987-1990). *JAVMA* 200 : 991-994.
10. Park HM, Oh TH, Kim HU, et al. 1999. Occurrence of suspected infection of *Camphylobacter* spp and *Clostridium* spp in dogs with chronic diarrhea. *Korean J Vet Res* 39(4) : 819-824.
11. Tribe GW, Kerr MG. 2001. Toxicosis in dogs exposed to *Clostridium perfringens* type A. *Vet Rec* 149 : 310-311.
12. Yoo HS, Lee SU, Park KY, et al. 1997. Molecular typing and epidemiological survey of prevalence of *Clostridium perfringens* types by multiplex PCR. *J Clin Microbiol* 35 (1) : 228-232.
13. Barker IK, Van Dreumel AA, Palmer N. 1993. *Pathology of domestic animals.* 4 eds. (Jubb KVF, Kennedy PC, Palmer N eds.), Academic Press, San Diego : 237-247, 454-459.
14. Pritchet SJ. 1991. Enterotoxemia in dogs. *Vet Rec* 129 : 391.
15. Majeed SK. 1985. Mineralisation in kidney and stomach of Beagle dogs. *Vet Q* 79(2) : 162-164.