Hemorrhagic Enteritis Associated with *Clostridium perfringens* Type A in a Dog

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**ABSTRACT.** A female Shetland sheep dog died suddenly with hemorrhagic diarrhea and vomiting, and was examined pathologically and microbiologically. Gross pathological change was restricted to the intestinal tract. The intestine contained watery, blood-stained fluid. Histopathologically, the principal intestinal lesion was superficial mucosal hemorrhagic necrosis at the jejunoileum. Many Gram-positive bacilli were found adhering to the necrotic mucosal surface in parts of the intestinal tract. *Clostridium perfringens* in pure culture were isolated from jejunal contents by anaerobic culture. These results suggested that the typical lesion of this case coincided with canine hemorrhagic enteritis and enterotoxemia due to *C. perfringens* infection could be the cause of sudden death.—**KEY WORDS:** *Clostridium perfringens*, enterotoxemia, hemorrhagic enteritis.


Enterotoxemia attributed to *Clostridium perfringens* has been reported in the cattle, sheep, goat, pig and foal [1, 4, 6]. The disease causes sudden death as a result of systemic effects of several toxins, which are produced by *C. perfringens* in the small intestine of affected animals. Enterotoxemia in the dog is rare [5, 9], but canine hemorrhagic gastroenteritis caused by *C. perfringens* has been reported in the past [2, 3, 8]. The present report describes pathological and bacteriological findings of a case of peracute canine hemorrhagic enteritis due to *C. perfringens* infection.

A six-year-old, female Shetland sheep dog was referred to a local veterinary clinic after the sudden onset of hemorrhagic diarrhea and vomiting which continued from the previous night to early morning. She was lethargic and died during clinical examination. The dog was sent to our department for pathological diagnosis and autopsy was performed within one hour of death.

Tissue samples collected at necropsy were fixed in 10% formalin, processed routinely and embedded in paraffin wax. Sections were cut approximately 4 µm thick and stained with hematoxylin and eosin (HE). Selected sections were stained with Gram’s stain.

The etiological diagnosis was accomplished by indirect immunofluorescence staining technique. Paraffin-embedded sections of jejunal tissue were deparaffinized and rehydrated in xylene, graded ethanol, and distilled with phosphate buffered saline (PBS) pH 7.2. Specimens were incubated overnight at 4°C with a chicken polyclonal anti-*C. perfringens* type A sera prepared against *C. perfringens* [10]. A fluorescein isothiocyanate-conjugated anti-chicken IgG rabbit IgG (INC Pharmaceuticals, U.S.A.) was used as the secondary antibody and sections were incubated for 30 min at 37°C. Unbound conjugate was removed by rinsing with three changes of PBS. Finally, the sections were examined by UV microscopy.

Examination of jejunal content by anaerobic bacterial culture was performed with a gas pack system. The specimens incubated anaerobically for 24 hr at 37°C on Clostridium Welchii ager base-without kanamycin (CW ager, Nissui, Japan). In addition, biochemical tests were performed routinely for identification. Jejunal tissue was also examined virologically. Ten percent homogenized jejunal tissues were centrifuged, and the supernatant was filtered through a 450 nm membrane filter. The supernatant was inoculated on confluent monolayers of Crandell feline kidney (CRFK) cells and Madin-Darby canine kidney (MDCK) cells with coverslips. The cell cultures were incubated at 37°C and examined daily for cytopathic effects (CPE). The coverslips were removed at 7 days, fixed in Bouin’s fluid, stained with HE, and examined by light microscopy. This procedure, using the supernatant of culture was repeated twice at intervals of 7 days.

At necropsy, the dog was in good nutritional condition. The intestinal mucosa was dark and filled with watery, blood-stained fluid (Fig. 1). Hyperemia was apparent between posterior jejunum and ileum. The lymph nodes, spleen and Peyer’s pathes were atrophic. Both ventriculus were slightly dilated.

Histopathological changes were present in the intestinal

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**Fig. 1.** The mucosal surface showing dark-red color with watery, blood-stained fluid.
mucosa. The principal intestinal lesion was superficial mucosal hemorrhagic necrosis (Fig. 2). Although fibrin, debris and many bacteria adhered to the necrotic surface, few inflammatory cells were associated with these changes. A striking feature of the intestinal lesion was the adherence of large Gram-positive bacilli to the necrotic mucosal surfaces (Fig. 3). These bacilli were present only in the intraluminal necrotic debris. Necrosis of small intestinal crypt enterocytes and lymphoid tissues were not observed. No inclusion bodies were seen.

By an indirect immunofluorescence staining technique, many bacilli reacted positively with anti-*C. perfringens* type A sera in the necrotic area of jejunal villi (Fig. 4). Large numbers of *C. perfringens* in pure culture were isolated from the jejunal content. *C. perfringens* was identified by its characteristic stormy fermentation reaction in litomus milk and the production of acid from glucose, lactose and sucrose. Alpha toxin was produced, as was shown by lecithinase activity on CW agar. Using the *C. perfringens* differentiation strip (Nissui, Japan), inhibition of lecithinase activity was observed in circumference of strip. Neither CRFK nor MDCK inoculated with the inoculum developed any CPE during the observation period. No alterations were observed in any cells stained with HE.

Canine hemorrhagic enteritis has not been well documented, but the sudden onset of vomiting and passage of bloody diarrhea in previously healthy dogs has been described [3, 8]. The intestinal histopathology in the present case, characterized as hemorrhagic enteritis with extensive attachment of Gram-positive bacilli to the necrotic intestinal mucosa, is similar to previous reports [1, 4, 8]. The indirect immunofluorescent findings indicate that most of the positively staining bacilli were *C. perfringens*. The isolation
of *C. perfringens* from the intestine may also support the histological findings. However, the source of infection and predisposing factors could not be identified.

Enterotoxemia due to *C. perfringens* infection causes sudden death as a result of systemic effects of several toxins in many animals [1, 4, 6, 7]. In the present case, demonstration of *C. perfringens* toxins in the intestinal contents was not performed. Therefore, we couldn’t define the roles of the toxins in the present case.

*C. perfringens* isolated from the intestine of the present case showed inhibition of lecithinase activity with *C. perfringens* differentiation strip. *C. perfringens* type A is a Gram-positive spore forming anaerobic bacillus known to cause food poisoning in humans, necrotic enteritis in chickens and diarrhea in lambs, calves and piglets [1, 6]. However, *C. perfringens* usually forms a part of the normal intestinal flora and can be found in soil [6]. It was reported that *C. perfringens* frequently grows in the intestine with canine parvovirus infection [11]. Various physical stress, decrease of immune reaction and intestinal hypermotility reduces normal anaerobic bacterial flora, and may predispose to bacterial overgrowth of *C. perfringens* in the small intestine. Canine parvovirus infection causes hemorrhagic gastroenteritis similar to that caused by *C. perfringens* infection in clinical and gross pathological findings. There has been some confusion between *C. perfringens* infection and parvovirus infection in the dog, as there is much resemblance. However, there was no significant lesion in the intestinal crypt characteristic of canine parvovirus infection in this case. Therefore, pathological lesions of the intestine seemed to be attributable to the infection of *C. perfringens* type A.

REFERENCES