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Hemorrhagic Enteritis in Two One-month-old Dairy Calves Infected with Beta2-toxigenic *Clostridium perfringens* and Coccidium

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Abstract : Two one-month-old dairy calves which have *Eimeria* oocysts in their bloody diarrhea died acutely a few days after showing the first clinical signs. At necropsy, hemorrhagic and congestive gastrointestinal organs were observed in both calves, and abomasal ulcerations existed. As a prevalent agent in all of the collected intra-intestinal specimens, *Clostridium perfringens* was isolated and the strain was identified as type A possessing alpha and beta2-toxins. In these clinical cases, intercurrent infection by *C. perfringens* type A and *Eimeria* through contaminated environment may be responsible for acute hemorrhagic enteritis.

***Key words* :** Hemorrhagic enteritis, Calf, *Clostridium perfringens*, Coccidiosis, Intercurrent infection.

Introduction

Clostridium perfringens has been reported to be recovered from the intestinal organs in domestic animals and from the environmental areas (10), and the pathogen is responsible for hemorrhagic abomasitis in calves (11) and jejunal hemorrhagic syndrome in adult cattle (1). Based on the major toxins, five types of strains are suggested in the classification of *C. perfringens* (A to E type). *C. perfringens* type A is known to be associated with enterotoxemia in lamb (5) and poultry (13), and it is most commonly isolated from clostridial enteritis in calves (9). On the other hand, bovine coccidiosis is another cause of hemorrhagic diarrhea especially in young calves. In the most infections, the affected animals represent diarrhea with or without blood and recover normal condition within few days. However, if a calf intakes a large number of coccidium eggs, hemorrhagic diarrhea containing intestinal tissue may be continued more than one week which results in severe economic loss (2). In this case report, clinical and necropsy observations of two one-month-old calves affected by *C. perfringens* and bovine coccidium were described, and genotype of isolated *C. perfringens* was identified.

Case

Two one-month-old dairy calves which represented depression, anorexia, and bloody diarrhea were presented from one dairy farm in September and December 2016, respectively. The suckling calves which were separated from a dam at birth were raised individually in calf hutch units with sand and straw bedding.

The first calf showed clinical signs of intestinal hemorrhage with reduced concentrations of hemoglobin, sodium, and total protein in blood plasma analyzed by portable blood gas analyzer (epoc; Woodley, Lancashire, UK) and blood chemistry analyzer (BS-400; Mindray, Shenzhen, China). The 5 pathological antigens (*Escherichia coli* K99, Coronavirus, Rotavirus, Cryptosporidium, and Giardia) which frequently induce diarrhea in calves were ruled out by Ag test kit (Rapid BoviD-5 Ag Test Kit; Bionote, Hwaseong, Korea) using a rectal swab. Then, salt floatation method was utilized to identify the existence of coccidial oocysts in diarrhea sample. After detecting coccidial oocysts on the fecal smear under the microscope (Fig 1A), the coccidiostat toltrazuril (Baycox; Bayer, Leverkusen, Germany) was administered orally to the calf. Additional treatments for an iron supplementation, anti-inflammation, and vasoconstriction were injected repetitively, but the symptoms were not relieved, and the calf died after 10 days of the first treatment. At necropsy, all gastrointestinal organs were found to be filled with hemorrhagic contents, and ulceration was observed after removing the hair and bedding materials from abomasum (Fig 1B). Intra-jejunal samples were collected using sterilized cotton swab and cultured on blood agar in both anaerobic and aerobic condition. Colonies showing hemolysis were observed, and *C. perfringens* was isolated as a prevalent bacterium in anaerobic culture whereas *Escherichia coli* was isolated in aerobic condition (VITEK 2 Compact; bioMérieux, Marcy l'Etoile, France).

The second calf showing bloody diarrhea similar to the first one was requested two months after the first calf's death. The calf was housed in a hutch located in the same

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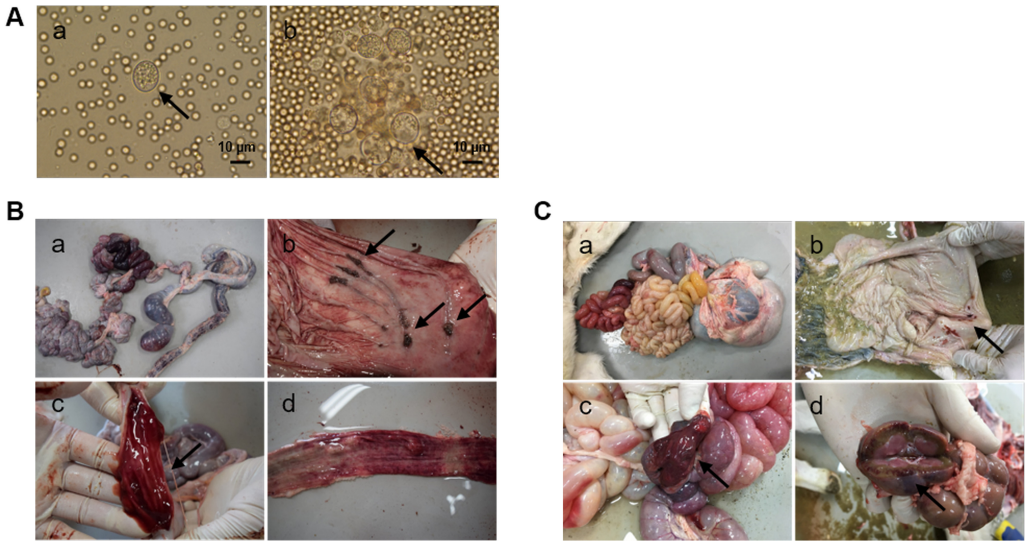

Fig 1. *Eimeria* oocysts observed on the fecal examination (A) and representative necropsy figures of two affected calves (B, C). The diarrhea samples from two affected calves were collected and examined under the microscope (scale bar represents 10 μm at magnification 1000×). *Eimeria* oocysts were detected either singly (A-a) or in combination (A-b). At necropsy, gastrointestinal hemorrhages were observed in both of the first (B) and the second (C) calf. The entire digestive organs were congested (B-a), and abomasal ulcers were distributed on the abomasal folds (B-b). The intra-jejunal specimen was collected for isolation of bacteria (B-c), and after elimination of bloody contents, hemorrhagic and congestive lesions were found on the intestinal wall (B-d). Hemorrhages through entire gastrointestinal organs were found also in the second calf (C-a), and small ulceration existed on the wall of the abomasum (C-b). Hemorrhagic intestinal contents from ileum, colon, and rectum were collected for isolation of causative agents (C-c). Petechial hemorrhages were observed on kidney (C-d).

area with the first calf, and the diarrhea symptoms were similar but watery. Mild decreases in sodium and hematocrit were observed by blood analysis, and *Eimeria* oocysts were also found on the fecal examination of direct smear (Fig 1A). Coccidiostats and probiotics were administered orally and injections of iron supplement, NSAIDs, vasoconstrictor, and antibiotics were repeated. Despite the clinical care, the calf was still lethargy accompanying rapid decreases in red blood cells, total proteins, and bicarbonate concentrations and an increase in lactate in the blood. Intravenous fluids were infused to adjust the blood pH to normal, but the calf was died 3 days after the onset of symptoms. Hemorrhagic ascites was found at subsequent necropsy, and abomasal ulceration, hemorrhagic enteritis in a range of an ileum to a colon, and petechial hemorrhages were also observed on the kidney (Fig 1C). Aseptic swabs from the ileum, colon, rectum, and kidney were smeared on blood agar plates, and by anaerobic culture, *C. perfringens* was isolated mainly from all of the culture plates.

The genotype of *C. perfringens* isolated from the two calves was analyzed by multiplex PCR according to the previous study (12). One single colony for a single organ was suspended in distilled water and used as a PCR template after lysis of bacteria cell wall at 95°C for 10 min. Primers for all of the toxin genes (alpha, beta, beta2, epsilon, iota, and enterotoxin) were used, and the target genes were amplified under the conditions of 95°C for 15 min, 40 cycles of 94°C for 30 sec/ 53°C for 90 sec/ 72°C for 90 sec, and finally 72°C

Table 1. Primer lists for *Clostridium perfringens* toxin typing

Toxin type	Sequence (5'-3')	Product size (bp)
α-toxin	GCTAATGTTACTGCCGTTGA CCTCTGATACATCGTGTAAG	324
β-toxin	GCGAATATGCTGAATCATCTA GCAGGAACATTAGTATATCTTC	195
β2-toxin	AAATATGATCCTAACCAAAAA CCAAATACTCTAATCGATGC	548
ε-toxin	TGGGAACTTCGATACAAGCA AACTGCACTATAATTTCCTTTTCC	376
ι- toxin	AATGGTCCTTTAAATAATCC TTAGCAAATGCACTCATATT	272
enterotoxin	TTCAGTTGGATTTACTTCTG TGTCCAGTAGCTGTAATTGT	485

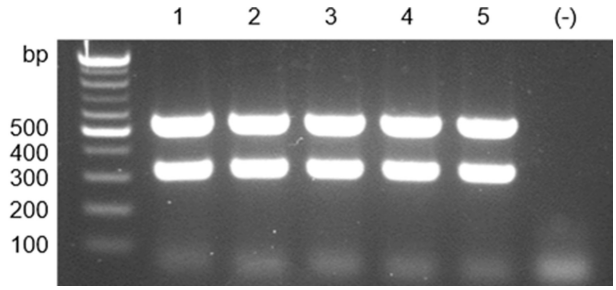

Fig 2. PCR result for *Clostridium perfringens* typing. *C. perfringens* genotyping by toxin detection was performed, and alpha and beta2 toxins were detected in all of the isolated colonies from dead calves' organs (1: jejunum of the first calf, 2: ileum of the second calf, 3: colon of the second calf, 4: rectum of the second calf, 5: kidney of the second calf).

for 10 min (Table 1). All of the *C. perfringens* colonies from the necropsy samples were genotyped as type A with alpha and beta2 toxins (Fig 2).

Discussion

The alpha toxin is the main cause of myonecrosis and hemolysis induced by *C. perfringens*, but the beta2 toxin is considered as more relevant for enterotoxemia in domestic animals (4,6,7). Though the role and the distribution of the *C. perfringens* strains which possess beta2 toxin is still controversial (3), the beta2-related enterotoxemia has been reported in both adult cattle and calves (1,9,11), and the strain was detected in all of the isolates consistently in this study.

In the early phase of diarrhea, oocysts of *Eimeria* spp. were detected in feces from both of two affected calves. Since the onset time of coccidiosis is known as 16 to 23 days after infections by *E. bovis* and *E. zuernii*, it is not considered as the main cause of neonatal diarrhea in calves. Generally, the coccidium-infected calves which develop species-specific immunity may show slight clinical signs, and metaphylactic treatment such as toltrazuril administration is proved to be effective (8). However, under the stressful conditions (e.g. weather, restriction, other infections, etc.), the infective form of coccidia can develop showing severe illness (e.g. bloody diarrhea, fever, dehydration, etc.) in naive calves, and the calves can die acutely with or without secondary complications (2). Thus, it seems that hemorrhagic enteritis in this report deteriorated due to toxins produced by *C. perfringens* in calves with coccidiosis. The route of infections was thought as an oral ingestion of oocysts and spores from the contaminated environment such as soil and bedding.

Conclusion

To our knowledge, hemorrhagic enteritis in calves induced by *C. perfringens* type A producing alpha and beta2 toxins and *Eimeria* spp. together has not been reported in Korea. Although the occurrence of hemorrhagic diarrhea is often requested, the rapid progress and lack of adequate treatment with antibiotics and anticoccidials may contribute to making the symptoms more severe. Early detection and subsequent cure of suffering animals are important, and above all, periodic disinfection of the housing environment, and adequate management such as a prophylactic administration of coccidiostat should be accompanied to control calf diarrhea.

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