Sudden Death Caused by *Clostridium perfringens* Type D Enterotoxemia in Feedlot Cattle


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Sudden deaths have occurred in feedlot cattle with marked necro-hemorrhagic enteritis of the jejunum, ileum and colon. Suckling beef calves are the most frequently affected. Over-consumption of large amounts of milk, inadequate colostrum intake, chilling and stress are conducive to the development of enterotoxemia. Enterotoxemia caused by *Clostridium perfringens* type D mostly occurs following a sudden change of diet, particularly to feeds made richer in order to grow the cattle to market weight in feedlots. During July 2006, sudden deaths of cattle occurred in the Youngcheon regional area of Gyeongbuk province. There were no significant clinical signs except anorexia, depression, intermittent diarrhea and mild respiratory failure. Histological findings revealed a prominent intranuclear inclusion as well as infiltration of the globular leukocytes in various organs including the heart, kidneys, liver, spleen and lymph nodes. Spleen and lymphatic tissues showed lymphatic necrosis and a starry sky appearance. In the submucosa of the small intestines, basophilic aggregation was detected with massive infiltration of the globular leukocytes and eosinophils. Gram staining for the tissue sections containing inclusions of the small intestines revealed a positive histochemical reaction. Taken together, we suggest that *Clostridium perfringens* type D-induced enterotoxemia is determined to be the cause of sudden death of feedlot cattle.

**Key words**: Feedlot cattle, *Clostridium perfringens*, enterotoxemia, intranuclear inclusion, sudden death

**Introduction**

Enterotoxemia caused by *Clostridium perfringens* in cattle, is sometimes referred to as ‘sudden death’ or ‘sudden death syndrome’. It implies that the animal was normal a short time before and then died in the space of an hour or two. The reason for these deaths is thought to be a toxemia produced by toxins of *Cl. perfringens*, a group of organisms multiplying in the gut under certain conditions [16]. Some easily identified causes of sudden deaths include atypical interstitial pneumonia, choking, acute tracheal edema, ruptured abscesses and systemic *clostridial* disease [4]. Pierson et al. examined animals that feedlot personnel had classified as sudden deaths and suggested that 47% of the sudden deaths were the result of respiratory disease, bloating and acidosis (20%), enteritis (9%), a riding injury (8%), pulmonary aneurysms (4%), and brisket disease (6%) [12]. Jensen and Mackey listed some specific diseases including enterotoxemia, acute indigestion or acidosis and bloating as causes of sudden death syndrome [6].

Bovine enterotoxemia is an acute to per-acute syndrome occurring mainly in calves and characterized by the sudden or very rapid death of the calf with colic convulsions and nervous disorders as clinical signs. *Cl. perfringens* strains are classified into five groups according to their specific toxins as follows; type A (α-toxin), type B (α-, β-and ε-toxin), type C (α- and β-toxin), type D (α- and ε-toxin), type E (α- and λ-toxin), type AE (α-and enterotoxin) [16]. Enterotoxin produced by *Cl. perfringens* type D causes enterotoxemia, a highly lethal disease in sheep, goat and other animal species [10]. Enterotoxemia caused by type D mostly occurs following a sudden change of diet, particularly to feeds rich in order to grow to market weight in feedlots [17,18]. Biological effects of these toxins include necrotizing action and marked increase in capillary permeability [19]. Laboratory diagnosis depends on the demonstration of various toxins in the intestinal contents and bloodstream of fresh specimens and overgrowth of *Cl. perfringens* organisms in the intestine [15]. The toxins of *Cl. perfringens* are detected and identified by serum-neutralization tests in mice and guinea pigs [5]. A polymerase chain reaction (PCR) has been

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applied for detection of low copy numbers of genes [19]. Immunohistochemistry represents a possible diagnostic alternative in those cases in which bacterial culture and PCR typing are not feasible. In this report, we demonstrated the identification of cattle enterotoxemia based on the histology and histochemical findings of _Clostridium perfringens_ type D that was a common cause of sudden death in feedlot cattle [4].

**Materials and Methods**

During July 2006, sudden deaths of cattle occurred in Youngcheon county, Gyeongbuk province. All the dead adult cattle (n=6; 3-6 yr.) and calves (n=3; 6 mo.-1 yr.) were being fed leftover food from local restaurants. The most common clinical signs were anorexia, depression, intermittent diarrhea and mild respiratory failure. During necropsy, severe hemorrhagic small intestines and colon were observed. The mesenteric lymph nodes and edematous lungs showed also severe hemorrhages. Formalin-fixed tissues including the kidneys, liver, lungs, spleen, heart, pancreas, parotid gland, supramammary lymph node, mesenteric lymph node, small intestine and large intestine, were submitted to the Laboratory of Pathology, College of Veterinary Medicine in Kyungpook National University for histopathological examination.

Histologic sections of formalin-fixed tissues, stained with hematoxylin and eosin (H&E), were examined microscopically for all 9 cases, respectively. Machiavello stain and Gram stain applied to the significant lesions detected tissues.

**Results**

Histological findings revealed pulmonary congestion and hemorrhage, necrotic intestinal mucosa accompanied with hemorrhages and congestion, and also increased globular leukocytes within the bronchial epithelium showing mild pneumonia. Intranuclear inclusions as well as infiltration of the globular leukocytes were prominent in various organs.

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**Fig. 1.** Histopathological findings. The glomeruli contained hyperchromatic cells with eosinophilic cytoplasm. Inset showed tubular degeneration and interstitial infiltrates of globular leukocytes. H&E, ×200, inset, ×100 (Fig. 1A). Multifocal inflammation was detected in the liver. Eosinophilic and granular cytoplasmic cells were infiltrated with other inflammatory cells. Machiavello stain, ×100 (Fig. 1B). The mucous of small intestines showed desquamation of epithelial cells in the lumen forming basophilic aggregations. Submucosa and lamina propria were also invaded by the globular leukocytes and eosinophils like the kidney. H&E, ×40, inset, ×100 (Fig. 1C). Globular leukocytes infiltrates were detected in the lymph node. The supramammary lymph node and parotid gland also revealed a depletion of lymphocytes and prominent starry sky appearance. H&E, ×100 (Fig. 1D).
including the heart, kidney, liver, spleen and lymph nodes. The spleen and lymphatic tissues showed lymphoid necrosis and a starry sky appearance. The kidney revealed tubular and glomeruli degeneration with interstitial infiltrates of globular leukocytes (Fig. 1A). Multi-focal inflammation with eosinophilic granular cytoplasmic cells with mononuclear inflammatory cells was detected in the liver (Fig. 1B). Mucus of the small intestines had basophilic aggregation with massive infiltration of the globular leukocytes and eosinophils in the submucosa layer. Lamina propria was also invaded by the globular leukocytes and eosinophils like the kidney. Gram staining for the tissue sections containing inclusions and the small intestines revealed highly positive reaction (Fig. 1C). Globular leukocytes infiltrates were detected in the most of lymph node; moreover the supramammary lymph node and parotid gland revealed depletion of lymphocytes and a prominent starry sky appearance (Fig. 1D). Machiavello staining for the supramammary gland and parotid gland sections showed various types of intracytoplasmic inclusions (Fig. 2A-a, 2A-b). Gram positive bacteria were diffusely infiltrated in the glomeruli and tubular epitheliums of kidney (Fig. 2B, white dotted circles), and intestinal mucosa (Fig. 2C, arrow).

Discussion

For many years, sudden deaths or the sudden death syndrome have been perceived as major concerns among cattle owners and veterinarians in Kyeongbuk province [7]. The local government set up a subcommittee to investigate the disease and take preventive measures. The major problems were that the cattle generally died of sudden recumbency with convulsions following anorexia, depression, ataxia, muscular tremor, tachycardia and dyspnea without any premonitory symptoms. Epidemiological surveys showed no causative agents such as related pesticide, fertilizer, or chemical drugs. Macroscopically, there were coagulation disorder in the abdominal cavity, and edematous hemorrhagic lung, and watery blood-tinged contents of the small intestine. All of the above gross lesions were compatible match with histopathologic findings. Microbiologically, *Cl. perfringens* were detected from the small intestinal contents, toxins were identified as *Cl. perfringens* type D toxin, and the cause of sudden deaths was proved to be enterotoxemia. The pathogenic tests

![Fig. 2. Histopathological findings II. The supramammary gland (Fig. 2A-a) and parotid gland (Fig. 2A-b) showed various types of intracytoplasmic inclusions (arrow). Machiavello, x100. Gram positive bacteria diffuse infiltrated in the glomeruli and tubular epithelium (white dotted circles). Gram staining, x100 (Fig. 2B). The intestinal mucosa also showed a gram positive reaction in the mucous (arrow). Gram staining, x100 (Fig. 2C).](image-url)
of mice and goats inoculated with *Clostridium perfringens* toxin have been demonstrated to be similar to the results of natural cases [3].

The useful method to prevent enterotoxemia of feedlot cattle was a dietary administration of antibiotics and probiotics [2,8]. Thereafter, it occurred sporadically and the incidence rate was reduced but the pathogenesis is still unknown. In this report, we tried to find out the histological changes of enterotoxemia based on the histology and histochemical findings of *Clostridium perfringens* type D. Histological findings revealed prominent intranuclear inclusions as well as infiltration of the globular leukocytes in various organs including the heart, kidney, liver, spleen and lymph nodes in our case. Gram staining for the tissue sections containing inclusions and the small intestines revealed positive histochemical reactions like previously reported [1]. The disease by *Clostridium perfringens* largely depends on the toxins having each pharmacological action cumulative in producing the final effect. There were differences in the changes caused by all three types of the *Clostridium perfringens* in cattle, which made it possible to distinguish them as causative agents. *Clostridium perfringens* type A induced slight icterus and a slightly manifested hemosiderosis of the liver, kidneys and spleen; *Clostridium perfringens* type D was responsible for severe injuries, hyaline dystrophy and a pulpy disease of the kidneys and CNS toxicity; *Clostridium perfringens* type C caused necrotic enteritis, pronounced hemorrhagic diathesis, degenerative changes in the ganglial cells, and demyelination of the brain [9,11,14]. The *Clostridium perfringens* type D epsilon-toxin causes a severe enterotoxemia characterized by cardiac, pulmonary, kidney and brain edema [14]. Experimentally, epsilon-toxin causes lethality in the mouse intravenous injection model [13].

Enterotoxemia development is pre-acute in most cases and treatment is possible only in very rare cases. The most practical way to handle *Clostridium perfringens* related illnesses is to prevent them. To combat the disease, however, accurate detection of *Clostridium perfringens* and diagnosis of the lesions of affected animals as well as anti-bacterial agent such as vaccines against type D toxin are needed. Our histological manifestation may help to identify the type D enterotoxemia and treat the disease thereafter.

In summary, histological findings showed prominent intranuclear inclusion bodies and infiltration of the globular leukocytes in various organs and lymphatic necrosis and a starry sky appearance in spleen and lymphatic tissues. Basophilic aggregation, coupled with massive infiltration of the globular leukocytes and eosinophils and positive responses for Gram staining in the tissue sections of small intestine were observed. On the bases of the results, *Clostridium perfringens* type D induced enterotoxemia may be a cause of sudden death of feedlot cattle in Youngcheon province.

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References


초록 : 비육 우의 Clostridium perfringens D 형 장독 혈증에 의한 폐사

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비육우의 공장, 회장 및 결장의 독성 적외출혈성 장염 증상은 폐사 증후군과 관련이 있는 것으로 인식되고 있다. 특히 끓어러 온이지들이 폐사 증후군에 가장 영향을 받는데, 포유식 동식물에게 파식을 시키곤, 비육생적 초예 무유, 체온 하강 및 스트레스는 장독혈증 발생의 원인들이다. 2006년 7월 중 경북 영천 지역에서 죽은 폐사가 발생하였다. 식육우전, 양기소해, 간업적인 설사 및 경미한 호흡곤란을 제외하고는 특별한 임상증상은 없었으나, 조직학적 소견에 의하면, 심장, 신장, 간, 비장 및 입피선을 포함한 각종 기관에서 globular leukocytes의 침윤을 나타내어 음성의 혈액생형체가 현저하게 나타났다. 비장과 간프 조직에서는 팽창 파괴와 팽창 부피로 인한 'starry sky appearance'를 나타내었다. 소장 장막 환자에서는 다양한 globular leukocytes와 호산구의 침윤과 함께 호염기성 세포 집단이 관찰되었다. 특히 폐쇄가 있는 소장 조직의 단면은 그램 염색에 대해 조직화학적 조직학적 분석 결과를 근거로 Clostridium perfringens D 형균에 의한 장독혈증으로 진단되었다.