Clostridium perfringens type A Myonecrosis in a Horse in Korea

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ABSTRACT. Acute hemorrhagic myonecrosis accompanied by severe inter- and intrafascicular edema and hemorrhage of the right gluteal area was diagnosed in a 13-year-old male thoroughbred horse. Once the muscular and fascicular changes were subsided, the horse then developed acute respiratory problem. Histologically, the lung had diffuse severe hemorrhage with mild neutrophilic infiltration. The cause of death was acute respiratory failure that is believed to occur secondary to toxæmic event. Alpha and β 2 toxin secreting *Clostridium perfringens* type A was isolated from the muscle and lung. The diagnosis was based on the light microscopic examination, bacterial toxinotyping and toxin genotyping from the muscular and pulmonary lesion. Also, susceptibility of the isolates to antimicrobial agents was determined.

KEY WORDS: *Clostridium perfringens*, equine, myonecrosis.

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Clostridium perfringens is a gram-positive anaerobic bacterium that is widespread in the environment and able to form spores [6]. *C. perfringens* is classified into five toxinotypes (A, B, C, D, and E) based on the production of four major toxins (α , β , ϵ , and ι). *C. perfringens* does not invade healthy cells but produces various toxins and enzymes that are capable of inducing associated symptoms and lesions.

Malignant edema, which is also known as clostridial myositis, occurs most frequently in cattle and sheep and *C. septicum*, *C. chauvoei*, *C. novyi*, and *C. perfringens* have been isolated most frequently [6]. Malignant edema caused by *C. chauvoei*, *C. septicum*, and *C. perfringens* type A has also been reported in horses [5, 12].

In this report, we describe clostridial myonecrosis in a horse that progressed to acute pulmonary hemorrhage, which is believed to be the cause of death.

A 13-year-old male thoroughbred horse suddenly developed lameness due to edematous swelling of the right hindleg. The horse was initially responsive to anti-inflammatory and anti-edema therapies, but then suddenly fell into acute respiratory distress, and died 3 days after the initiation of clinical signs. Postmortem examination was performed immediately after death to determine the cause of death.

At necropsy, there was a focally extensive dark reddish discoloration at the right gluteal area. Interfascicular and intrafascicular edema and hemorrhage were also noted in the same area. Inguinal lymph node was moderately enlarged and hemorrhagic on cross section. A moderate amount of blood-tinged fluid was present in the thoracic cavity. The trachea and bronchi were filled with blood-tinged frothy fluid. The lung was diffusely congested, edematous, and had numerous petechial hemorrhages. No

other significant gross abnormalities including both small and large intestines were noted during necropsy.

Tissue samples from the muscle, lung, lymph node, small and large intestines, heart, spleen, adrenal gland, liver, and kidneys were fixed in 10% phosphate-buffered formalin, routinely processed, and stained with hematoxylin and eosin for light microscopic examination. Tissue samples from the skeletal lesion and lung were collected aseptically and cultured on blood agar at 37°C under both aerobic and anaerobic conditions. Pure colonies grown under anaerobic condition were collected and basic biochemical tests such as Gram stain, hemolysis, and indole production were carried out. Final identification of the selected bacterium was performed with VITEK system (bioMérieux, MO, U.S.A.). Susceptibility to antimicrobial agents was determined by disc diffusion method based on the guidelines of National Committee for Clinical Laboratory Standard. Toxigenic type of the bacterium was determined by multiplex PCR with minor modifications as described previously [8, 15].

Microscopically, the muscle fibers in the right gluteal area were necrotic as characterized by homogenous eosinophilic sarcoplasm with loss of striation (Fig. 1). Mild neutrophilic exudation, hemorrhage, and edema were also noted (Fig. 1). Alveolar septa were thickened due to congestion and hemorrhage, and were occasionally necrotic. Alveoli were filled with proteinaceous fluid, red blood cells, a few neutrophils and macrophages. Subcapsular and medullary sinuses of the inguinal lymph node contained a large numbers of red blood cells and hemosiderin-laden macrophages. Hepatic sinuses were congested. Both small and large intestines were histologically unremarkable.

The bacteria were isolated as a pure culture from the skeletal muscle and lung, and were identified as *C. perfringens* based on the results of biochemical tests and VITEK system, indicating that *C. perfringens* was the cause of the myonecrosis and pulmonary hemorrhage in this case. Biochemical

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Fig. 1. Note a focal extensive area of myofibrillar necrosis and hemorrhage. H&E. $\times 150$.

Fig. 2. Electrophoretic analysis of *Cl. perfringens* toxin genes applied by multiplex PCR. M, 100 bp DNA marker; S, standard toxin (*etx*, 655 bp; *cpb2*, 567 bp; *iA*, 466 bp; *cpa*, 324 bp; *cpe*, 233 bp; *cpb*, 196 bp); Lane 1, toxin gene (*cpb2*, *cpa*) from muscle and lung; Lane 2, toxin gene (*cpa*) from only lung; Lane 3, negative control.

characteristics of the isolates from both skeletal muscle and lung were almost identical. The isolate was susceptible to ampicillin, bacitracin, chloramphenicol, carbenicillin, ceftiofur, enrofloxacin, nalidixic acid, norfloxacin, tylosin, and vancomycin; while they were resistant to amikacin, colistin, gentamicin, neomycin, streptomycin, and tetracycline.

On PCR-based toxinotyping, the isolate was identified as toxigenic type A of *C. perfringens* harboring *cpa* and *cpb2* genes (Fig. 2). Also, an additional strain harboring only *cpa* gene was isolated from lung. Recently, an unassigned type of *C. perfringens* that produces α -toxin and β 2-toxin was

reported [4].

Different *Clostridium* species are often isolated from horses with enteric typhlocolitis [1, 11, 14]. α -toxin is produced by all types of *C. perfringens* including nonpathogenic strains, and therefore is not considered to the primary cause of enteric disease [3]. A strain producing β 2-toxin has recently been isolated from a piglet that died of necrotic enteritis and the strain was originally classified as type C, but β 2-toxin is also produced by some type A strains, as identified by the mouse test [2, 10]. It was also found in horses with enterocolitis. Herholz *et al.* found a high incidence of β 2-toxigenic *C. perfringens* in samples of ingesta, biopsy specimens of the intestinal wall, and feces from horses suffering or dying from typhlocolitis compared to healthy horses that were all negative suggesting that β 2-toxigenic *C. perfringens* play an important role in the pathogenesis of typhlocolitis [4].

Recently, Peek *et al.* reported a retrospective study of 37 *Clostridial* myonecrosis in horses [9]. Of the 37 cases, 34 cases were associated with recent intramuscular injection and the remaining 3 cases were due to wound or laceration. Muscular injury secondary to intramuscular injection or blunt trauma can lead to necrotic and anaerobic conditions favoring spore germination, growth of vegetative organisms and elaboration of lethal toxins in clostridial infection. Furthermore, in humans, nontraumatic clostridial myonecrosis due to gastrointestinal malignancies, diabetes mellitus, and peripheral vascular disease have also been described. Immune-mediated hemolytic anemia suspected to have caused by *C. perfringens* septicemia and purulent pericarditis as a sequel to clostridial myositis were also reported in a horse [7, 13]. Even though, an additional strain harboring only *cpa* gene was isolated from the lung, since no definite evidence of *C. perfringens* septicemia was demonstrated in this case, acute pulmonary hemorrhage might have occurred secondary to toxæmic event.

In this case, no wounds were noted on the body during the necropsy and any of those primary causes mentioned above has found. No clinical signs related to gastrointestinal problem were noted and both small and large intestines were grossly and microscopically normal.

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