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SYSTEMIC CLOSTRIDIOSIS DUE TO *Clostridium perfringens* INFECTION IN *Equus asinus* – CASE REPORT

CLOSTRIDIOSE SISTÊMICA DEVIDO A INFECÇÃO DE Clostridium perfringens EM Equus asinus – RELATO DE CASO

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SUMMARY

Clostridial infections, particularly clostridial myonecrosis, can be fulminant and fatal; they often arise without an obvious history of trauma. The majority of equine clostridial myonecrosis cases are associated with intramuscular injection. This paper presents the first report of clostridial myositis by *C. perfringens* in a donkey after an injection of flunixin meglumine on the neck, describing the clinical, necroscopic, and histopathological events. Bacterial isolation and biochemical tests confirmed the definitive diagnosis of *C. perfringens*.

KEY-WORDS: *Clostridium perfringens*. *Equus asinus*. Myonecrosis. Systemic clostridiosis.

RESUMO

Clostridioses, particularmente a mionecrose clostridial, podem ser fulminantes e fatais; elas geralmente surgem sem um histórico óbvio de trauma. A maioria dos casos de mionecrose clostridial equina está associada à injeção intramuscular. Este artigo apresenta o primeiro relato de miosite clostridial por *C. perfringens* em jumento após injeção de flunixina meglumina no pescoço, descrevendo os achados clínicos, necroscópicos e histopatológicos. Isolamento bacteriano e testes bioquímicos confirmaram o diagnóstico definitivo de *C. perfringens.*

PALAVRAS-CHAVE: Clostridiose sistêmica. *Clostridium perfringens*. *Equus asinus*. Mionecrose.

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INTRODUCTION

Clostridial myositis, myonecrosis, cellulitis and malignant edema are all terms used by various authors to describe a syndrome of severe necrotizing soft tissue infection associated with *Clostridium* spp. (BREUHAUS et al., 1983; PEEK et al., 2003; SILVA et al., 2016). There are several species of clostridia associated with myonecrosis, *Clostridium perfringens* and *C. septicum* are the most common isolates in horses, followed by *C. chauvoei*, *C. novyi* and *C. fallax* (BREUHAUS et al., 1983; VALBERG & McKINNON, 1984; PEEK et al., 2003). *Clostridium perfringens* is a Gram-positive, spore-forming, non-motile, rod-shaped organism commonly found in soil and in the gut microbiome of humans and other animals (STEVENS, 2000). The major concern of *C. perfringens* are the toxins produced by this bacteria: alpha-toxin, which possesses hemolytic, dermonecrotic, and cytotoxic activities, and also suppresses myocardial contractility, and theta toxin that interfere in the response of tissue injury and induces cell lysis (AWAD et al., 1995; STEVENS, 2000; OCHI et al., 2003).

Most cases of equine myonecrosis occur due previous intramuscular injection as well as recent parturition, castration, or a puncture wound (BREUHAUS et al., 1983; VALBERG & McKINNON, 1984; PEEK et al., 2003; ANDERSON et al., 2013). It has also been shown that there are species of clostridia dormant in healthy horse muscle tissue that could be reactivated by trauma and begin to multiply (VENGUST et al., 2003). These animals usually develop rapid soft tissue swelling, subcutaneous and deeper soft tissue emphysema, and rapid toxemia that may progress to circulatory collapse and multiple organ failure over just a few hours (PEEK et al., 2003; STEWART, 2006).

Diagnosis of clostridial myonecrosis is based upon identification of characteristic symptoms, a detailed patient history, and a variety of specialized tests including a culture positive patient sample. Successful treatment depends on rapid diagnosis and an aggressive combination of antibiotics and surgical treatment (PEEK & SEMRAD, 2002).

CASE REPORT

A 3-year-old, 300kg donkey was treated with an unreported amount of flunixin-meglumine given intramuscular on the neck. Three days later the neck began to swell. Five days after the donkey was admitted in the Hospital Escola Veterinário do Centro Universitário Max Planck, Indaiatuba, SP, Brazil, presenting bilateral swelling of the neck. On admission the animal presented tachycardia (84 beats/minute), tachypnea (28 breaths/minute), mucous membranes were congested, capillary refill was 3 seconds, intestinal hypomotility and dysphagia. Hematocrit revealed dehydration (packed cell volume of 49%), and serum biochemistry evaluation revealed hypoproteinemia (5,2 g/dL). Presence of gas on the tissue was confirmed by ultrasound and needle punch. At physical examination, the area was firm on palpation, with subcutaneous crepitation and painful sensibility to touch.

Clostridial infection was suspected. A tracheotomy was performed to relieve the dyspnea. Lactated Ringer's solution was administered via a jugular catheter at a rate of 3 L/hr, and oxygen therapy of 7 L/min. An initial dose of metronizadol (IV, 15 mg/Kg) and ceftiofur (IM, 2,5 mg/Kg) was given. Even with all the efforts and the 3 hours of extensive care, the patient died.

RESULTS AND DISCUSSION

The definitive diagnosis was made by necropsy, histopathology and bacterial isolation. Necropsy examination was performed two hours after death. The animal had an extensive area of myonecrosis, and hemorrhage accompanied by severe emphysema in cervical musculature (Figure 1) that extended through the dorsal and bilateral scapular region. Macroscopic examination also showed multifocal hemorrhage in lungs, thoracic parietal pleura, renal cortex, liver and subendocardial muscle (Figure 2). Microscopically there was neutrophilic infiltration, diffuse congestion and areas of necrosis on the liver and lungs. The microscopic evaluation of the skeletal striated muscle fibers showed emphysema (Figures 3 and 4) with diffuse myofibrils fragmentation and necrosis accompanied by a severe neutrophilic inflammatory infiltrate with discrete macrophagic and lymphoplasmacytic infiltrates. The Gram staining of muscular tissue showed pleomorphic Gram positive bacilli, with some presenting central to subterminal spores adjacent to emphysema areas.

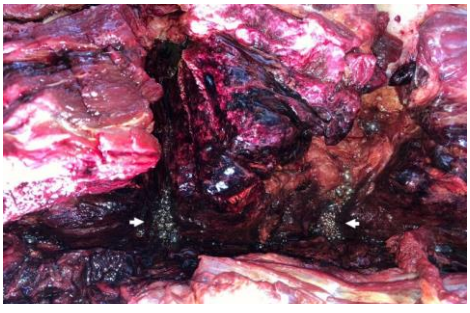

Figure 1 - Cervical musculature. Hemorrhagic myonecrosis due to *Clostridium perfringens*. Arrows show the presence of gas bubbles in musculature due to bacterial growth. *Equus asinus*.

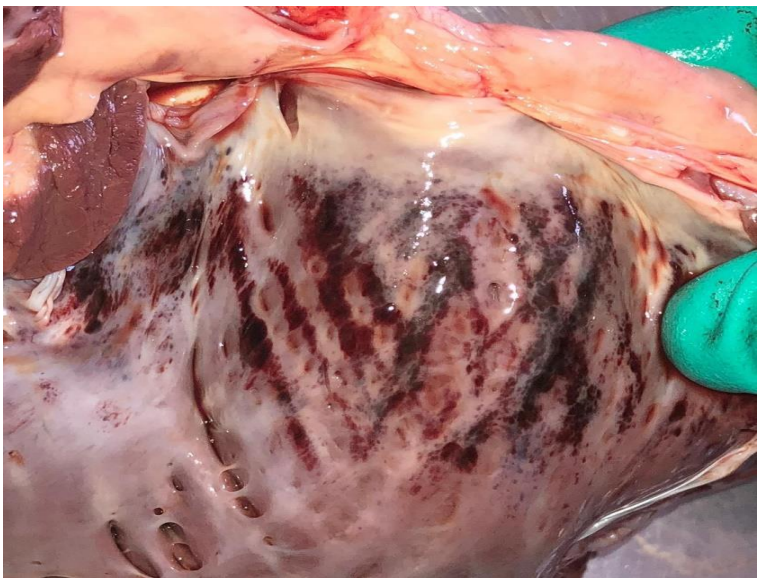

Figure 2 - Heart. Subendocardial left ventricular hemorrhage. *Equus asinus*.

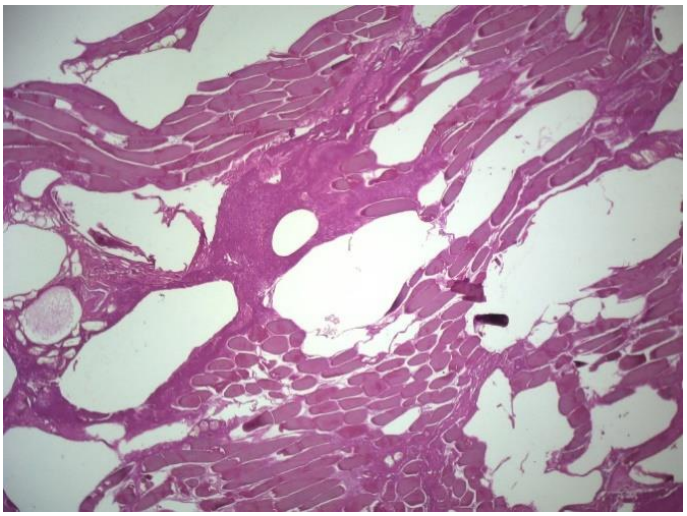

Figure 3 - Photomicrography of cervical musculature. Myonecrosis and emphysema evidenced by the formation of dilatations in the endomysial space. *Equus asinus*. HE. 40x magnification.

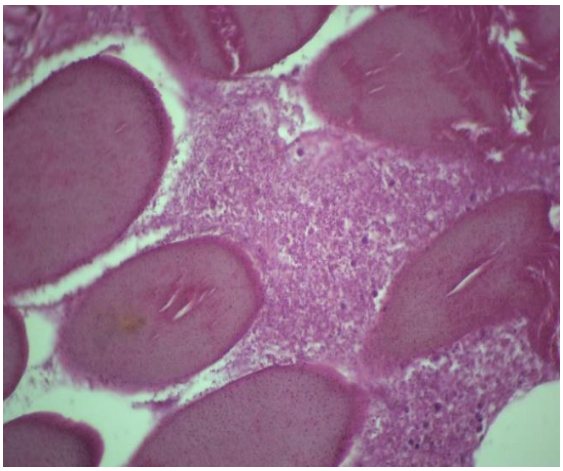

Figure 4 - Photomicrography of the cervical musculature. Myonecrosis with fibrinous exudate expanding the endomysium. *Equus asinus*. HE. 200x magnification.

Bacterial culture showed the presence of colonies with double halo in blood agar, sulfite-reducing anaerobic bacteria in agar TSC, bacterial growth on Shahidi-Ferguson perfringens agar and confirmation procedure with MUP-ONPG (BYRNE et al., 2008; HUSTÁ et al., 2020). Biochemical test fermentation was positive for lactose, maltose, sucrose (SCHRECKENBERGER & BLAZEVIC, 1976). All this data corroborated with the definite diagnosis of *C. perfringens* infection.

Clostridial myositis is a rapidly progressing disease that can lead to death of the animal, as in this case. The infection was due to the intramuscular injection of flunixin-meglumine. This has been the drug most associated with the occurrence of clostridial myositis in the cervical region (BREUHAUS et al., 1983; PEEK et al., 2003), because it is highly irritating, the injection site become anaerobic with focal necrosis, favoring the clostridial growth (PEEK et al., 2003). Bacterial growth is associated with gas production, necrotic and vasoactive toxins, with subsequent toxemia. Systemic infection has been proven at necropsy due to multiple organs hemorrhage and emphysema.

Due to the permanence of the bacteria in the sporulated form in the environment, the lack of hygiene during the application process favors the inoculation of the bacteria in the animal's musculature (CRUM-CIANFLONE, 2006). After the development of the vegetative form, the bacteria produces gas during the respiratory cycle and releases several toxins that have proteolytic activities, leading to hemolysis and myonecrosis (UZAL et al., 2014). As a result of tissue injury and bacterial growth, neutrophils are recruited to the site of infection (JUNIOR et al., 2020). The vascular alteration caused by toxigenic action and vascular flow changes related to the inflammatory process, favor bacteremia, leading to the development of bacterial colonization in different sites and diffusion of toxins inducing tissue damage in sites distant from the primary infection (UZAL et al., 2014).

When receiving a patient with suspected clostridiosis, emergency care is of fundamental importance for the stabilization of the patient and initiation of therapeutic treatment in order to control the constant production and dissemination of toxins and bacterial growth. In some conditions, it may be necessary to adjust fluid therapy consistent with shock cases, accompanied by fasciotomy to oxygenate the site of infection, improve local perfusion and treat compartmental syndrome. Chemotherapeutic management compatible with infection by an anaerobic organism and parenteral anti-inflammatory administration are essential for controlling the infection process and reducing the indirect damage resulting from the inflammatory process.

CONCLUSION

As far as we know from research in the reference literature, this is the first case report of systemic clostridiosis in a donkey. The present report identifies the occurrence of systemic clostridiosis with a clinical-pathological pattern similar to that in horses, raising awareness for careful management of these animals similar to that recommended for horses.

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