



## Myonecrosis caused by *Clostridium septicum* in a horse from Southern Brazil

Marlane Geribone Seeger<sup>1</sup>  Gabriele Biavaschi Silva<sup>2</sup>  Carolina Sleutjes Machado<sup>1</sup>   
Diego Rafael Palma da Silva<sup>2</sup>  Flávio Desessards De La Corte<sup>3</sup>   
Fernanda Silveira Flores Vogel<sup>4</sup>  Juliana Felipetto Cargnelutti<sup>4\*</sup> 

<sup>1</sup>Programa de Pós-graduação em Medicina Veterinária, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

<sup>2</sup>Clínica de Equinos Santa Maria, Santa Maria, RS, Brasil.

<sup>3</sup>Departamento de Clínica de Grandes Animais, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

<sup>4</sup>Departamento de Medicina Veterinária Preventiva (DMVP), Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: [jucargnelutti@gmail.com](mailto:jucargnelutti@gmail.com). \*Corresponding author.

**ABSTRACT:** *Clostridial myonecrosis is a highly fatal infectious disease of the muscle that is caused by pathogenic clostridia. When it occurs in equine muscle, the infection can cause a variety of clinical signs. The present report described a case of acute myonecrosis caused by Clostridium septicum in a horse, which had a favorable clinical evolution. A 6-year-old mare was admitted to a veterinary hospital due to increased volume and lameness in the left pelvic limb after an intramuscular injection of an anti-inflammatory drug. The diagnosis of myonecrosis caused by C. septicum was based on clinical and laboratory findings. The animal showed significant improvement after 5 months of treatment and was discharged from the hospital. There is very limited information regarding myonecrosis caused by C. septicum in horses in Brazil; our study showed that early diagnosis and prompt appropriate treatment can ensure significant improvement and recovery of affected animals.*

**Key words:** horse, myonecrosis, *Clostridium septicum*, infectious disease, intramuscular injection.

## Mionecrose causada por *Clostridium septicum* em um cavalo do sul do Brasil

**RESUMO:** *Mionecroses clostridiais são doenças altamente fatais, causadas por uma ou mais espécies de Clostridium sp. patogênicos. Entretanto, no músculo equino, a infecção pode produzir uma variedade de lesões e sinais clínicos. O presente relato descreve um caso de mionecrose aguda em um cavalo causada por Clostridium septicum, que apresentou uma evolução clínica favorável. Uma égua de seis anos foi internada em um hospital veterinário devido a um aumento de volume e claudicação no membro pélvico esquerdo, observado após injeção intramuscular de anti-inflamatório. O diagnóstico de mionecrose por C. septicum foi baseado nos achados clínicos e laboratoriais. Houve uma melhora significativa do animal após cinco meses de tratamento, permitindo que o mesmo fosse liberado do hospital veterinário. Considerando que as informações sobre C. septicum causadores de mionecroses em cavalos no Brasil são muito limitadas, este relato demonstra que o diagnóstico precoce e a implementação adequada do tratamento podem garantir uma melhora significativa e a recuperação dos animais afetados.*

**Palavras-chave:** cavalo, mionecrose, *Clostridium septicum*, doença infecciosa, injeções intramusculares.

Clostridial myonecrosis, or gas gangrene, is a necrotizing soft tissue infection caused by various pathogenic *Clostridium* species, including *Clostridium septicum*, *C. chauvoei*, *C. novyi* type A, *C. perfringens* type A, and *C. sordellii* (PEEK et al., 2003; SCOTT & MILLER, 2003). These species belong to a group of histotoxic clostridia, and the

infection may lead to toxemia with the worsening of the lesions (ASSIS et al, 2010).

Clostridial myonecrosis is most common in cattle, sheep, and goats, which are more susceptible than pigs and horses (RAYMUNDO et al., 2010). Few studies have reported cases of myonecrosis in horses in Brazil, but the animals died of the clostridial

infection in all of these cases (MACÊDO et al., 2007; RAYMUNDO et al., 2010; FARIAS et al., 2014; FERREIRA JÚNIOR et al., 2020).

*Clostridium* spp. are Gram-positive, anaerobic, spore-forming rods that exist as saprophytes in the environment. They produce a wide variety of toxins and cause numerous diseases (SCOOT & MILLER, 2003). *C. septicum*, *C. chauvoei*, and *C. perfringens* produce toxins that are important in the pathogenesis of myonecrosis, including  $\alpha$ -toxin, which is a lecithinase that is necrotizing, leukocidal, and hemolytic (QUINN et al., 2005; KENNEDY et al., 2009);  $\beta$ -toxin, which is a deoxyribonuclease that is leukotoxic; and  $\gamma$ -toxin, which is a hyaluronidase that promotes spread of the infection (QUINN et al., 2005).

Clostridial myonecrosis in horses has been reported to occur after infection of wounds in mucous membranes, orchietomy, and intramuscular injections (MACÊDO et al., 2007; ANDERSON et al., 2013; FARIAS et al., 2014). Most cases have been associated with recent intramuscular injection of commonly used therapeutic agents, and horses seem to be more vulnerable to clostridial myonecrosis (also known as gas gangrene) following this type of injection (PEEK & SEMRAD, 2002; PEEK et al., 2003).

Currently, there is limited information regarding myonecrosis-causing *C. septicum* in horses in Brazil. In addition, the reported survival rate of clostridial myositis is low. Thus, the objective of this study was to report a case of acute myonecrosis in a horse caused by *C. septicum* triggered by an intramuscular injection of flunixin meglumine in the left hindlimb, with a favorable clinical course. Our findings demonstrated the importance of early diagnosis and appropriate treatment to ensure the recovery of affected animals.

A 6-year-old mare was admitted to a veterinary hospital because of increased volume in the gluteal muscle region and lameness in the left hindlimb after receiving an intramuscular injection of flunixin meglumine 5 days earlier. The mare showed grade V lameness according to the AAEP scale I–V (AAEP, 1991). Clinical evaluation showed diffuse edema in the limb, with areas of crackling on palpation and percussion. A radiographic study of the hindlimb revealed the accumulation of a large amount of gas in the subcutaneous tissue (Figure 1A) within the patella region. In addition, ultrasonography showed an accumulation of gas in the regions around the main muscles of the pelvic limb.

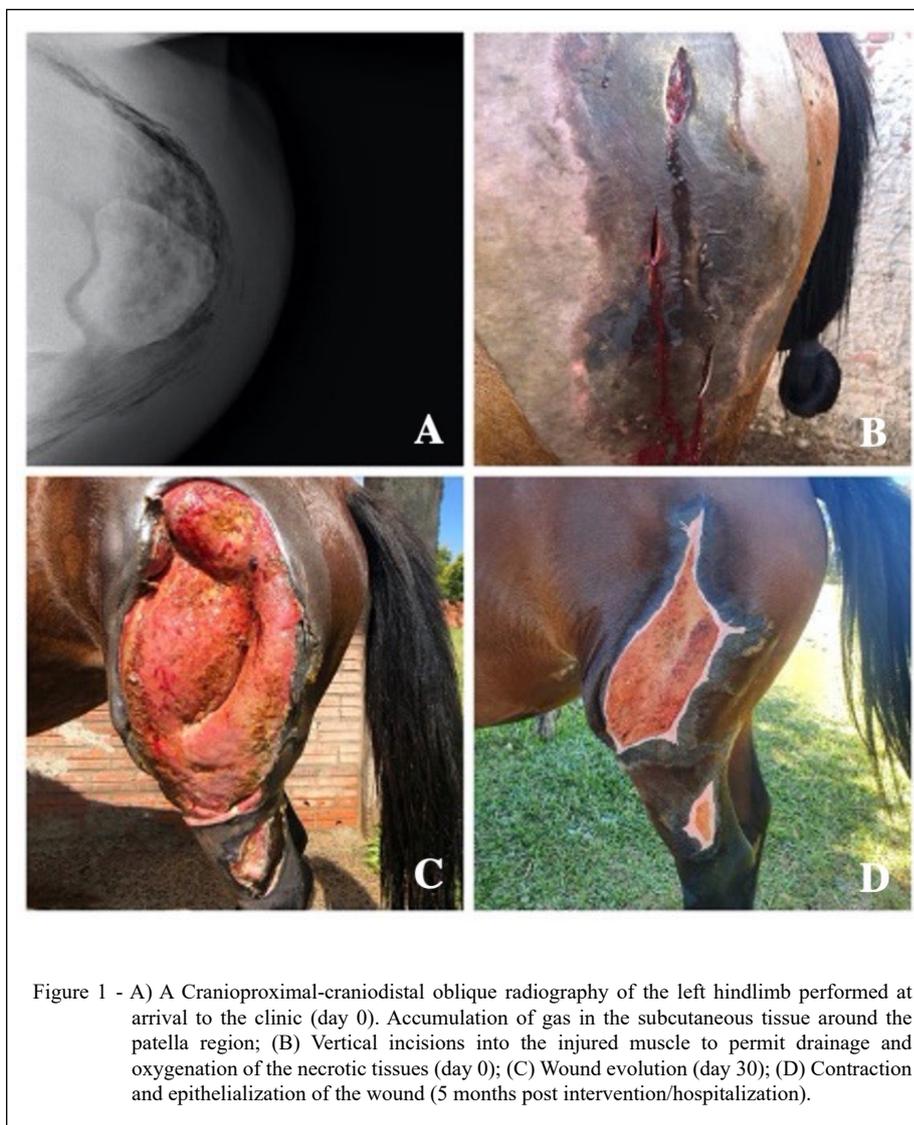
The hindlimb of the horse was subjected to trichotomy, and a large area of necrosis was observed. To promote aeration and facilitate wound cleaning, the treatment included vertical incisions into the injured

muscle to drain the foul exudate and gas (Figure 1B). Blood samples were collected for complete blood count (CBC) and biochemical analysis, and fluid and muscle biopsies were collected for bacteriological examinations (SB 82/20 protocol).

CBC performed on the first day of hospitalization showed only few changes, mainly related to fibrinogen (900 mg/dL), which was above the reference value (200–400 mg/dL). Systemic therapy was initiated on the first day at the clinic, which included intravenous potassium penicillin (40,000 UI/kg QID), phenylbutazone (4.4 mg/kg BID), intravenous fluid therapy, and oral omeprazole (1 mg/kg SID). Penicillin treatment was continued for 23 days. During the first days of hospitalization, wound cleaning and debridement were performed to remove necrotic tissue. Hydrogen peroxide was used to facilitate cleaning, and sugar was added to stimulate granulation tissue (Figure 1C). A full distal limb compressive bandage was placed to avoid distal migration of the edema. After completely removing the necrotic tissue, a 2% chlorhexidine degerming agent and silver sulfadiazine ointment were applied to the wound.

In the laboratory, fluid samples and muscle fragments were plated onto 5% sheep blood agar, MacConkey agar, and semisolid-reinforced clostridial medium (Thermo Scientific™ Oxoid™) and incubated anaerobically at 37 °C. After 48 h, small gray-white colonies were observed on blood agar, but no growth was observed on MacConkey agar. Microbiological growth was also verified in the semisolid-reinforced clostridial medium, which showed growth (turbidity) and gas formation. Gram staining of a smear from the culture on blood agar and reinforced medium showed pure large, Gram-positive rods. Antimicrobial testing by the disk diffusion method (CLSI, 2007) showed sensitivity of the bacteria to azithromycin, ceftiofur, enrofloxacin, penicillin, and trimethoprim-sulfamethoxazole and intermediate sensitivity to tetracycline.

The diagnosis was confirmed by polymerase chain reaction (PCR) and molecular analysis. DNA was extracted from the colonies (TAKEUCHI et al., 1997) and subjected to multiplex PCR for the identification of *Clostridium* species (SASAKI et al., 2002), based on the presence of the flagellin gene. A 294 bp amplicon was observed in isolate SB 82/20, corresponding to amplicon size of *C. septicum* flagellin gene. The amplicon was sequenced, and the consensus sequence obtained in the Staden package (STADEN, 1996) was compared with other *Clostridium* spp. sequences in GenBank using the Basic Local Alignment Search Tool. The



sequence was deposited in GenBank (SB 82/20, accession no. MZ508338). The nucleotide sequence of SB 82/20 showed high identity (94.9%–96.9%) with other *C. septicum* flagellin gene sequences in GenBank (GenBank accession numbers CP034358.1, AB058934.1, HQ650581.1, AB058935.1, CP023671.1, and AB058933.1) and less than 86% identity with those from other *Clostridium* spp.

In this report, the diagnosis of myonecrosis caused by *C. septicum* in a horse was based on clinical signs and biochemical and molecular findings. The applied treatment had significant effects, and the horse showed a considerable improvement in their clinical condition after 5 months of treatment (Figure 1D).

*C. septicum* is a highly virulent pathogen that is increasingly being recognized as a major contributor to serious clostridial infections (KENNEDY et al., 2005). The bacteria can infect the subcutaneous tissues, fascia, or muscle layers, and the infection is characterized by fever, depression, swelling, subcutaneous gas accumulation, rapid tissue necrosis, and extreme pain (KENNEDY et al., 2009).

There are several hypotheses about how *Clostridium* spores are introduced into the skeletal muscles of horses. According to BREUHAUS et al. (1983), spores can be introduced through medication or by a needle. In our case, the infection was probably

induced during an intramuscular injection of flunixin meglumine. Other drugs that have been associated with clostridial infection include ivermectin, antihistamines, phenylbutazone, dipyrone, vitamin B complex, aminopromazine, and synthetic prostaglandin (PEEK et al., 2003; ANDERSON et al., 2013). However, it has also been suggested that clostridia, can pass through lesions in the alimentary tract and colonize muscles, remaining inactive until stimulated by certain conditions. This corroborated the hypothesis that tissue damaged by injection of therapeutic agents can create a favorable environment for the activation of resident clostridial spores, resulting in the onset of myonecrosis (PEEK & SEMRAD, 2002; ANDERSON et al., 2013).

The horse was treated with antibiotics, cutaneous incisions, wound washing, and debridement. Earlier studies have suggested that bacteria associated with gas gangrene and myositis are typically susceptible to penicillin, provided adequate tissue levels are maintained (PERDRIZET et al., 1897). In the present case, penicillin therapy was initiated, and the animal showed significant improvement, emphasizing that the combination of antibiotic therapy with clinical management (skin incisions, fluid drainage, tissue debridement, and topical treatment) was essential to resolve the infection.

The prognosis of clostridial myonecrosis appears to be dependent on the *Clostridium* spp. involved (PEEK & SEMRAD, 2002). According to PEEK et al. (2003), clostridial myonecrosis due to *C. perfringens* has the most favorable prognosis, whereas there is a 50% survival rate following infection with *C. septicum* (PEEK et al., 2003). However, when dual infections occur, the fatality rate is 100% (VALBERG & MCKINNON, 1984). Thus, an accurate diagnosis and species identification are essential to improve the chances of recovery from the infection. Moreover, studies have shown that treatment is only effective when performed in the initial stages of the disease (PEEK et al., 2003). Our findings corroborated these results, as the mare received the treatment and showed significant improvement in its recovery from the infection.

In summary, the present study reported the isolation and molecular identification of *C. septicum* from a case of myonecrosis in a horse and discussed the clinical and therapeutic approaches that were essential for recovery. The trigger specific to the disease may have been an intramuscular injection. While there is a low incidence of *C. septicum* infection in horses, prophylaxis as well as preventive measures are important to avoid infection.

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## CONTRIBUTION'S AUTHORS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

## DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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