Case Report

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Gas Gangrene in a Horse in the Amazon Biome Caused by *Clostridium septicum*

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Simple Summary: Gas gangrene in horses, caused by the bacterium *Clostridium septicum*, is a serious and potentially life-threatening condition. This bacterium is commonly found in the environment and can infect wounds, particularly deep puncture wounds. Once the bacterium enters the body, it rapidly multiplies and produces toxins that destroy tissue and cause gas production within the affected area. This leads to swelling, severe pain, and a characteristic crepitus (crackling) sensation when the affected area is touched. Vaccination is the main form of prevention, when available for the animal category.

Abstract: Gas gangrene refers to severe necrotizing syndromes in soft tissues of animals caused by *Clostridium*. Five species of histotoxic clostridia are responsible for myonecroses. These agents require a port of entry; such as skin punctures with needles contaminated with bacterial spores. The present case report describes the clinicopathological findings of gas gangrene in a male Quarter horse living in the Brazilian Amazon biome. Clinical examination revealed clinical signs of toxemia and an enlarged left limb. The anamnesis found the administration of medication to this limb. The animal died five hours after its arrival at the veterinary hospital. At necropsy; histopathological lesions characterized by intense muscle necrosis; liquefaction; and gas formation were observed; suggestive of *Clostridium* lesions; which were confirmed by multiplex PCR. *C. septicum* was the etiological agent identified

Keywords: *Clostridium septicum*; alpha toxin; multiplex PCR; malignant edema; claudication

1. Introduction

Brazil has the third largest herd of horses in the world and the largest in Latin America, with approximately 5.8 million animals, moving about 16.5 billion reais and generating around 3.2 million direct and indirect jobs related to horse breeding [1]. Brazilian equine husbandry still faces many challenges, especially regarding poor sanitary management, which is associated with nutritional problems and favors the occurrence of many infectious diseases in a herd, resulting in damage to the equine production chain [2]. For example, clostridioses, though rarely reported in the literature in horses, have great economic importance because they cause pathologies that are difficult to diagnose and treat, leading to sudden death due to their acute clinical course [3,4].

Myopathies in horses are relatively common and have different etiologies for similar clinical manifestations. They can cause muscle necrosis in the worst cases, leading to an unfavorable prognosis [5]. Gas gangrene or malignant edema in horses, a clostridial myonecrosis, affects soft tissues and can be caused by one or more of the etiological agents *Clostridium chauvoei*, *C. septicum*, *C. novyi* type A, *C. perfringens* type A, and *C. sordellii* [6]. Penetrating wounds, open fractures, drug administration, and surgery without antisepsis are important entry ways for the development of gas gangrene, with mortality rates that can vary between 50% and 100% [7,8].

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Due to its resistance through endospores, clostridia remain viable in the environment for long times as a potential source of infection, and their eradication and control are difficult, mainly due to the lack of commercial vaccines specific for horses against clostridiosis, with the exception of tetanus therapy [2,4,6,8,9]. Clostridiosis in horses might be underreported since there are few publications on this disease in horses, but identifying and diagnosing these diseases early are important, considering the economic impact they cause [4,7]. This case report describes a case of gas gangrene in a horse in the Amazon biome.

2. Case Study

A five-year-old male Quarter horse weighing approximately 450 kg was treated at the Large Animals Sector of the Veterinary Hospital (HV) of the Federal University of Pará (UFPA), Castanhal, Pará, with a history of walking difficulties and an increase in the volume of the left pelvic limb for 4 days. The owner also reported that the animal was treated for *mal das cadeiras* (Trypanosomiasis) with diminazene, without informing us of the dose used, and that the day after giving the drug, the horse's leg increased in volume and sensitivity, with claudication. After 3 days, the animal was treated with 10 ml of 10 ml of flunixin meglumine, 10 ml of meloxicam, 50 ml of dimethyl sulfoxide in 500 ml of saline solution, 10 ml of phenylbutazone and dexamethasone, without informing us of the dosage), plus 20 ml of sorbitol diluted in 100 ml of saline solution and penicillin, without informing us of the dose used. The animal ate and drank normally, even in pain. Finally, the owner gave it antivenomous serum and furosemide, suspecting snakebite due to the constant presence of venomous snakes. He also told us that the animal was vaccinated against rabies, tetanus, herpesvirus, and influenza and was also up to date with deworming.

The clinical examination of the animal was performed as described by Feitosa (2008). In the general inspection, the animal was very agitated, was in a stationary position, and presented good overall body condition. In the inspection of the oculopalpebral and oral mucosa, these were yellowish, and in the presence of a toxemic halo, the capillary refill time and duration of skin turgor increased to more than 3 seconds. On palpation of the external lymph nodes, no changes were observed. On cardiac, respiratory and intestinal auscultations, a heart rate of 74 beats/minute, 32 respiratory movements/minute, and hypomotility were measured in the four quadrants of the abdominal auscultation. It was not possible to take the animal's temperature because it was very agitated and kicking, and we could not make it go to the containment trunk. In the specific physical examination of the locomotor system, left pelvic muscle edema was observed, with pain on palpation, associated with the presence of crackling in the muscles, which covered the entire limb (Figure 1A) and extended to the croup and lumbar region.

After the clinical examination of the animal and before the anamnesis, the main clinical suspicion was myonecrosis, possibly caused by bacteria of the genus *Clostridium*, with a poor prognosis defined due to the acute evolution of the condition. As the antibiotic had already been administered on the farm, we began a protocol of severe pain, with dipyrone (25 mg/kg intravenously, TID), tramadol (2 mg/kg intramuscularly, QID), and ketamine (0.2 mg/kg subcutaneously, QID) and intravenous fluids with lactated Ringer's, but the animal stayed very restless and reluctant. We removed the intravenous access, proceeded with surgery, and washed the area with oxygenated water. We decided to leave the animal to rest in the intensive care pen under continuous veterinary medical supervision. The animal was placed in lateral recumbency and died.

The animal was immediately taken to the Pathology Laboratory of IMV/UFPA for necroscopic examination. At necropsy, marked edema in the left hindlimb hip and thigh was observed, which extended to the pectoral and lumbar regions (Figure 2A and 2B), with large areas of intense muscle necrosis, liquefaction, and gas formation and separation of the muscle fibers (Figure 1B). To determine the definitive cause of death, fragments of intact muscle tissue with necrosis of the quadriceps were collected aseptically, frozen, and sent to the Anaerobic Laboratory of the Federal University of Minas Gerais (UFMG) for microbiological analysis via multiplex PCR as described by RIBEIRO et al. (2012)[10]. A portion of the collected tissue was placed in 10% buffered formalin, subjected to routine histopathological analysis, embedded in paraffin, cut, and stained by

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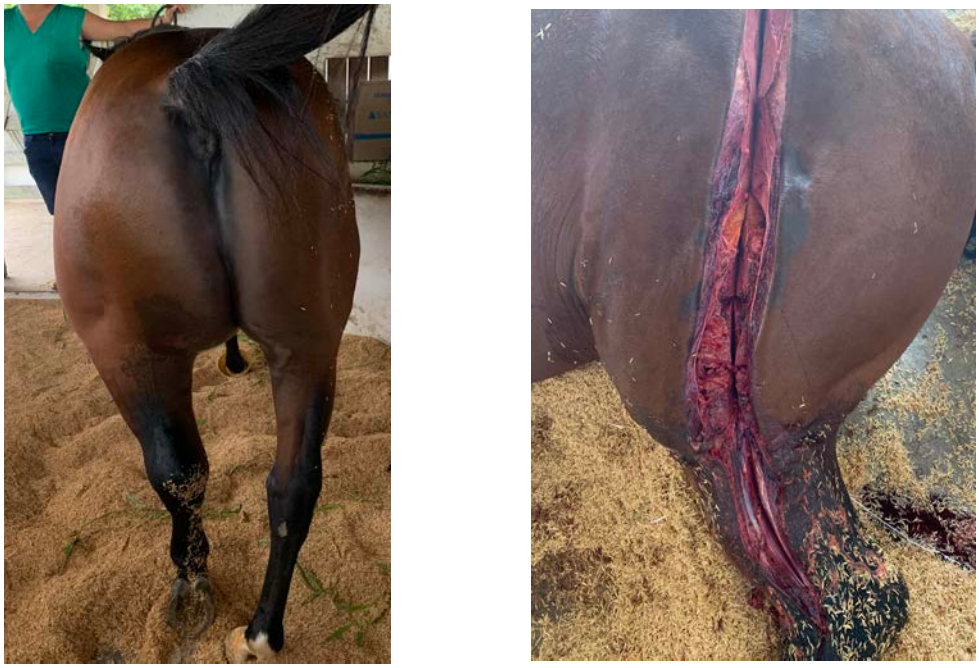
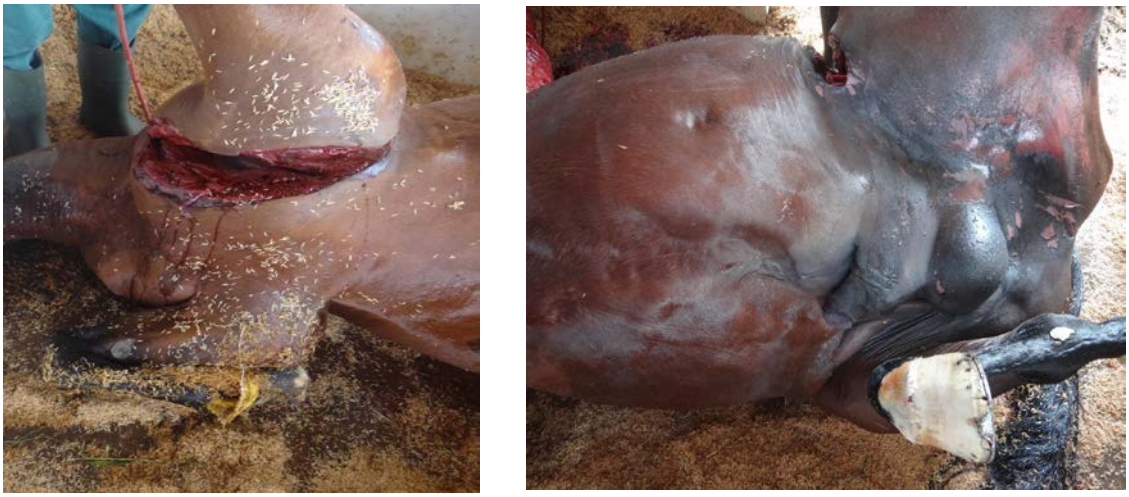
hematoxylin and eosin (H&E) [11]. Imaging was also performed on glass slides of healthy and necrotic muscles, which were fixed in heat and then stained with Gram's technique.



(**a**) (**b**)

Figure 1. Equine gas gangrene: (**a**) increased volume of the left hind limb; (**b**) hemorrhage and subcutaneous edema, with extravasation of serosanguineous fluid, liquefaction, and tissue necrosis (postmortem).



(**a**) (**b**)

Figure 2. Equine gas gangrene: (**a**) hemorrhage and subcutaneous edema, with extravasation of serosanguineous fluid, liquefaction, and tissue necrosis in the pectoral region; (**b**) ventral subcutaneous edema (postmortem).

Microscopically, there was diffuse floccular and hyaline muscle necrosis in the skeletal muscle, with many basophil bacilli and marked infiltration of neutrophils, vascular areas with thrombi, diffuse edema and hemorrhage (Figure 3A and 3B), and cytoplasmic vacuolization of hepatocytes in the central lobular region of the liver. Bacillary basophilic and Gram-positive bacterial aggregates were detected by Gram staining of muscle tissues. These lesions were compatible with clostridial

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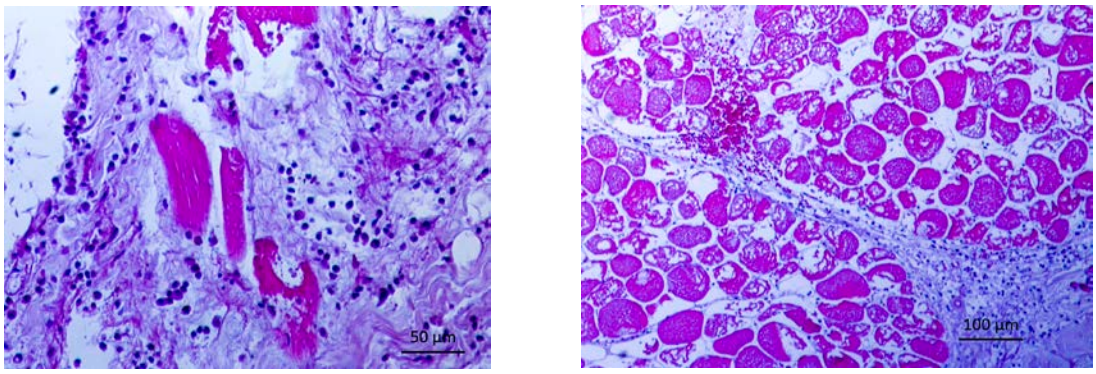
myonecrosis, and the microbiological laboratory diagnosis was confirmed by PCR as the etiological agent of the clinical picture of gas gangrene caused by *Clostridium septicum*.



(**a**) (**b**)

Figure 3. Equine gas gangrene: (**a**) Degeneration and hyaline necrosis of muscle fibers with intense inflammatory infiltrate [H&E obj. 10×]; (**b**) area of necrosis associated with edema with hemorrhage [H&E obj. 20×].

4. Discussion

In the present report, the diagnosis of gas gangrene or malignant edema caused by *C. septicum* infection in a horse was confirmed by clinicopathological examination, in addition to genotyping the muscle fragments of the skeletal muscle as described by RIBEIRO et al. (2012) [10] and FERREIRA JUNIOR et al. (2020) [12]. *C. sordellii* was diagnosed in all the cases reported by Sacco et al. (2020) [13], who used more than one technique, such as microscopy, immunohistochemistry, direct immunofluorescence, bacterial culture, and/or PCR. One of their cases was not detected by immunohistochemistry, which was supposedly due to the low bacterial load in the evaluated tissue, so the diagnosis relied on macroscopy and microscopy. The use of molecular biology is a popular technique because it detects a portion of the DNA of the bacterium existing in the tissue collected without external contamination and is analyzed, as in the case reported in question.

We diagnosed this horse with trypanosomiasis and treated it accordingly. Trypanosomiasis is a primary condition that may favor the systemic dissemination of enteric organisms to muscle tissue, as described in human medicine [14,15]. Earlier observations in animals with colic syndrome treated with subsequent administration of analgesics led to clostridial infection of the muscle, which, even with prolonged treatment and signs of improvement, died [16,17], as in this study.

The extent of tissue contamination, possibly due to the aseptic application of the drug against trypanosomiasis reported in the present case, is in agreement with that described in the literature [6,7]. Therefore, in a molecular-biological analysis of muscles of horses aseptically euthanized without infectious cause or trauma and to minimize contamination, skin swabs negative for clostridia were used. Several species of sporulated clostridia were detected in the muscle of clinically healthy animals [6,18]. *C. septicum* may also be aerotolerant, remaining dormant in a vegetative or spore form in muscle tissue [14,19,20] and, after muscle injury caused by blunt trauma or intramuscular injection leads to a pH and anaerobic environment that favor spore germination, leave the vegetative state and produce lethal myonecrotic toxins. This mechanism is well characterized in other species, but not in horses [17].

In turn, the clinical picture characterized by rapid development of muscle edema, claudication associated with pain, and the presence of crackles, as a function of gas production by the multiplication bacterial infection observed in the present case report, has also been described by other authors [11,21], and is the main clinical sign observed. Infection by *C. septicum* is described as having a much faster and more lethal course than infection by *C. perfringens*, with darkening of the tissues occurring much earlier and associated with interstitial hemorrhage, which does not occur in *C.*

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perfringens infection. This leads to ischemia due to destruction of the vasculature and faster deoxygenation of vital tissue, allowing *C. septicum* to establish infection in healthy tissue [22].

The death of the animal was probably caused by toxemic shock from a high concentration of *C. septicum* alpha toxin [23]. The clinical course is considered acute, ranging from 24 to 48 hours, with an unfavorable prognosis for the animal [7]. In a few cases, chronic myonecrosis caused by *C. perfringens* type A has been reported [24]. In a 15-year retrospective study, one author evaluated 37 patients and reported a survival rate of 73%. Aggressive treatment may be associated with survival rates of up to 81% for patients with *C. perfringens* alone, but survival rates for other clostridial species tend to be lower. The survival rate is also related to the survival rate of animals in which fasciotomy and myotomy were performed with surgical debridement; the percentage of survival may be related to the previous condition of the animals, considering that they worsen the general condition, since most of the deaths were caused by *C. septicum* and a history of colic in the animal (primary condition) [17]. In our case, trypanosomiasis was the primary condition, and the infection was caused by *C. septicum.*

The lesions and the autopsy report of the case corroborate those described in other reports associated with *C. septicum* [7,21], in which the alpha toxin, recognized as the main virulence of *C. septicum*, caused intravascular hemolysis, elucidating some lesions found in the histopathological analysis [6,23]. Molecular detection of alpha toxin was described in 2005, when strains lacking the coding fragment for alpha toxin protein were inoculated and were unable to cause myonecrosis in mice. Freitas et al. (2020) [4] and Freitas et al. (2021) [2] reported that the use of small amounts of this agent as a vaccine for the production of antitoxin alpha antibody significantly improved the response to subsequent infections [22].

The indirect immunofluorescence technique is the gold-standard method used worldwide for the diagnosis of clostridial myonecrosis, but it requires fresh and/or refrigerated material, which was unfeasible for us because HV-IMV/UFPA in Castanhal, Pará, was more than 2500 km away from the Anaerobic Laboratory of UFMG in Belo Horizonte, Minas Gerais, justifying the choice of frozen material for molecular-biological assays, which were recently developed and made feasible for the diagnosis of myonecrosis [12]. In addition, fragments of the agent within the material were better detected by PCR.

In view of the scarcity of diagnostic data on *Clostridium* spp. myonecrosis in horses, there is little evidence on the real economic impacts of this disease on Brazilian equine farms. Since the evaluation by Peek et al. (2003) [17], no studies have analyzed the real situation of cases of clostridial myonecrosis in horses. The large underreporting of cases thus hinders further analyses, with the exception of cases of *Clostridium tetani*, for which a characteristic clinical picture and diagnosis are available [6,25]. However, clostridiosis causes great losses in national agribusiness due to clinical conditions and mortality, especially in ruminants, swine and poultry [9,26–31]. And although *C. septicum* has been reported in cases of clostridial myositis in horses in other regions of Brazil [21,31], this is the first study to confirm it as a cause of gas gangrene in the Quarter horse in the Brazilian Amazon biome, in the northern region of the country.

There is a consensus that vaccination is the most efficient prophylactic method for the control and eradication of diseases caused by *Clostridium* spp. [7]. There are vaccines on the Brazilian market for commercial products against clostridioses that contain the toxoid alpha of *C. septicum*, but there are no vaccines specifically made for horses, with the exception of the tetanus monovaccine [23,25,31]. In the report by Morris et al. (2002) [33], in which only one animal among 12 that had the same management fell ill, the animals were vaccinated against *C. septicum.* The presence of this microorganism in the infection could have been related to a suboptimal vaccination procedure, to an individual lack of immune response, or to an unusually high dose challenge. In conclusion, vaccination reduces the mortality rate considerably when clostridia infection occurs. Freitas et al. (2020) [4] and Freitas et al. (2021) [2] evaluated the humoral immune response of horses immunized with recombinant toxoid alpha (CPA) and recombinant toxin beta (CPB) from *C. perfringens*. The efficacy of vaccine recombinants developed by these researchers for stimulating the production of anti-CPA and anti-CPB neutralizing antibodies, according to the requirements of international

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legislation, may be possible, and it may be warranted to develop new vaccines for horses that contain, for example, the alpha toxoid of *C. septicum*, which was diagnosed as the cause of the clinical picture of gas gangrene in the present report.

5. Conclusions

Myonecrosis caused by *C. septicum*, a producer of alpha toxin, can cause toxemic shock. This is the first case of myonecrosis caused by this agent reported in the Amazon biome. The low importance put on diagnosis and the use of a vaccine as the only method of prevention should be considered.

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Informed Consent Statement: They have obtained the informed consent from the participants.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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