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Article

Gas Gangrene in Horses: Retrospective Study of 20 Cases in the Amazon Biome

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Simple Summary: Gas gangrene in horses, caused by *Clostridium* species, is a severe and often fatal condition that presents significant challenges in the Amazon biome. 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. Therefore, is critical need for early recognition and swift, intensive treatment to manage gas gangrene in horses, especially in remote and resource-limited settings like the Amazon biome. Increased awareness among veterinarians and horse owners in such regions is essential to improve outcomes and reduce the mortality rate associated with this life-threatening condition. Remembering that vaccination is the main form of prevention, when available the animal category.

Abstract: This study describes, through a retrospective analysis, the epidemiological and clinicopathological findings of gas gangrene in horses in the Amazon biome. The study includes observations made in 20 animals from 2018 to 2023 by reviewing the clinical records of horses with muscle and mobility injuries treated at properties in the Amazon biome. The affected horses exhibited a sudden onset of severe lameness, swelling, and crepitus in the affected limb. The rapid progression of symptoms, including intense pain, fever, and toxemia, led to the clinical suspicion of gas gangrene. Immediate treatment was crucial, involving aggressive surgical debridement to remove necrotic tissue and high doses of broad-spectrum antibiotics to combat the infection. Despite these efforts, the condition of the horses deteriorated rapidly due to the aggressive nature of the disease and the challenging environment of the Amazon, which complicates timely veterinary intervention. The age range of the affected horses was from four months to 12 years, with a greater frequency in those over 12 months (80%, 16/20). Necropsy revealed histopathological lesions characterized by intense muscle necrosis, liquefaction, and gas formation, suggestive of *Clostridium* infection. The diagnosis was confirmed by multiplex PCR.

Keywords: malignant edema; *Clostridium septicum*; *Clostridium chauvoei*; *Clostridium novyi*; *Clostridium sordellii*; *Clostridium perfringens* type A; claudication; toxemia; multiplex PCR

1. Introduction

Brazil have to the third-largest horse population in the world and the largest in Latin America, with an estimated 5.8 million horses. This sector contributes significantly to the country's economy, generating approximately 16.5 billion reais and creating around 3.2 million direct and indirect jobs related to horse breeding [1]. However, the equine industry in Brazil faces numerous challenges, particularly regarding poor sanitary management practices. These challenges are often exacerbated

by nutritional deficiencies, which create favorable conditions for the occurrence of various infectious diseases within herds, thereby negatively impacting the equine production chain [2,3].

Among these infectious diseases, clostridiosis, although rarely reported in horses, no reports of their occurrence in the Amazon Biome were found in the literature, pose significant economic and health challenges due to their acute clinical course and the difficulties associated with their diagnosis and treatment [4]. Clostridial infections in horses can lead to severe conditions such as gas gangrene, also known as malignant edema. This condition, a type of clostridial myonecrosis, affects soft tissues and can be caused by several *Clostridium* species, including *Clostridium chauvoei*, *C. septicum*, *C. novyi* type A, *C. perfringens* type A, and *C. sordellii*. Typical entry points for these bacteria include penetrating wounds, open fractures, drug injections, and surgeries performed without proper antisepsis. The mortality rates for gas gangrene in horses are alarmingly high, ranging from 50% to 100% [2,3,5].

The persistence of *Clostridium* species in the environment through resilient endospores further complicates efforts to control and eradicate these infections. The lack of commercially available vaccines specific to horses against clostridial infections, with the exception of tetanus, underscores the need for improved preventive measures. Furthermore, the underreporting of clostridiosis in horses, due to the scarcity of detailed publications on the subject, emphasizes the importance of early identification and diagnosis to minimize economic losses and improve animal welfare [6,7].

The Amazon biome, with its unique environmental conditions, presents additional challenges for veterinary care. The region's vastness, coupled with its climatic and logistical difficulties, often hampers timely veterinary intervention, complicating the management of acute infections such as gas gangrene. This retrospective study aims to provide a comprehensive analysis of 20 clinical cases suspected of gas gangrene in horses treated at properties in the Amazon Biome of Pará between 2018 and 2024. By examining the epidemiological, clinical, and pathological findings of these cases, this study seeks to enhance the understanding of gas gangrene in horses within the Amazon biome, thereby contributing to better diagnostic and therapeutic strategies for this severe and often fatal condition.

2. Materials and Methods

2.1. Case Selection

The retrospective study was conducted focusing on the epidemiological data (age, race, sex, place of origin, clinical signs, necropsy findings) on cases of gas gangrene in 20 equines (identified numerically from 1 to 20) that presented between January 2018 and December 2023. The following procedures were employed for case selection and clinical evaluation: a). Case selection: a.1). Database Review: veterinary medical records from the Universidade Federal do Pará (UFPA) were reviewed to identify equine patients diagnosed with gas gangrene during the study period. Cases were included if they had a confirmed diagnosis of gas gangrene, based on clinical signs, microbiological testing, and histopathological findings; a.2). Inclusion criteria: the inclusion criteria for this study were equines that presented with clinical signs suggestive of gas gangrene, confirmed by the presence of *Clostridium* on microbiological culture and PCR, and histopathological evidence of myonecrosis and gas formation; a.3). Exclusion criteria: cases were excluded if the diagnosis was not confirmed by microbiological or histopathological analysis or if the medical records were incomplete or missing key diagnostic information.

2.2. Clinical Evaluation

The clinical examination of the animal was performed as described by Feitosa [8]. Initial Examination: upon presentation, each horse underwent a thorough clinical examination by a team of veterinarians. The examination focused on the identification of symptoms consistent with gas gangrene, such as sudden onset of severe lameness, swelling, crepitus in the affected area, and signs of systemic toxemia. Clinical signs documentation: clinical signs were meticulously documented, including the extent of swelling, presence of subcutaneous emphysema, pain on palpation, and the

general condition of the animal (e.g., fever, tachycardia, tachypnea). All available complementary tests of the animals included in the study were reviewed. Diagnostic imaging: in some cases, diagnostic imaging (radiography or ultrasound) was utilized to assess the extent of gas formation within the tissues. Laboratory testing: blood samples were collected for hematological and biochemical analysis, focusing on markers such as white blood cell count, creatine kinase levels, and lactate concentration, to assess the systemic impact of the disease. Microbiological sampling: tissue or fluid samples from the affected areas were collected aseptically for microbiological culture and sensitivity testing. Samples were processed under anaerobic conditions to isolate and identify *Clostridium*. Histopathological analysis: in cases where surgical intervention or necropsy was performed, tissue samples were collected for histopathological examination. These samples were fixed in formalin, processed, and stained with hematoxylin and eosin (H&E) to evaluate the extent of myonecrosis, inflammatory cell infiltration, and the presence of gas vacuoles and bacterial organisms [5].

2.3. Data Collection and Analysis

Data extraction: relevant clinical data, including patient history, clinical findings, laboratory results, and outcomes, were extracted from the medical records for analysis. Data analysis: descriptive statistics were used to summarize the demographic data, clinical signs, laboratory findings, and outcomes of the cases. The frequency of specific clinical signs and the correlation between clinical findings and diagnostic results were analyzed. This systematic approach ensured that only well-documented and confirmed cases of gas gangrene in equines were included in the study, providing a reliable basis for evaluating the clinical, microbiological, and histopathological characteristics of the disease in this population.

2.4. Complementary Tests: Hematological, Cerebrospinal Fluid Analysis, Histopathological Findings and PCR

To further characterize the clinical presentation and pathological impact of gas gangrene in equines, a series of complementary tests were conducted on affected horses. These tests included hematological analysis, cerebrospinal fluid (CSF) examination, histopathological evaluation and PCR each performed using the following methods: a). Hematological analysis [9]: a.1). Sample collection: blood samples were collected via jugular venipuncture into EDTA tubes for hematological analysis. A total of 5 mL of blood was drawn from each horse at the time of admission; a.2) Complete blood count (CBC): a CBC was performed using an automated hematology analyzer to assess key parameters, including total and differential white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, platelet count, and the presence of any morphological abnormalities in blood cells; a.3). Biochemical analysis: additional blood samples were collected in serum tubes for biochemical analysis, focusing on markers indicative of systemic involvement, such as liver enzymes (ALT, AST), creatine kinase (CK), lactate dehydrogenase (LDH), and electrolytes. These analyses were conducted using a standard clinical chemistry analyzer; a.4). Inflammatory markers: acute phase proteins, such as fibrinogen and serum amyloid A (SAA), were measured to evaluate the inflammatory response associated with gas gangrene. B). Cerebrospinal fluid (CSF) analysis [9]: b.1) Sample collection: CSF was obtained via atlanto-occipital puncture, performed under sterile conditions with the horse under general anesthesia. Approximately 5 mL of CSF was collected into sterile containers; b.2). Cytological examination: the CSF was immediately analyzed for cell count and differential, protein concentration, and glucose levels. A cytocentrifuge was used to prepare smears for microscopic examination, assessing for the presence of pleocytosis, increased protein, or other abnormal findings that might indicate systemic infection or neurological involvement; b.3). Bacteriological culture: CSF samples were cultured on both aerobic and anaerobic media to detect any bacterial pathogens, with a specific focus on isolating *Clostridium*. C). Histopathological examination [5]: c.1). Tissue sample collection: tissue samples for histopathological examination were obtained during necropsy or surgical debridement procedures. Samples were collected from the affected musculature, skin, and subcutaneous tissues, with special attention to areas showing gross

lesions, such as necrosis and gas formation; c.2). Fixation and processing: the tissue samples were immediately fixed in 10% neutral-buffered formalin for 24-48 hours, followed by routine processing, embedding in paraffin, sectioning at 4-5 microns, and staining with hematoxylin and eosin (H&E) for microscopic examination; c.3). Histopathological evaluation: the histopathological evaluation focused on identifying characteristic lesions of gas gangrene, such as extensive myonecrosis, the presence of gas vacuoles, infiltration by neutrophils and macrophages, hemorrhage, and the presence of large, rod-shaped, Gram-positive bacteria. Special stains, such as Gram stain and periodic acid-Schiff (PAS), were also utilized to enhance the identification of bacterial organisms and assess tissue damage. D). PCR [10]: 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) and Biological Institut of São Paulo (IB/SP) for microbiological analysis via multiplex PCR as described by Ribeiro et al. [10]. The multiplex-PCR reactions were performed with 5 ml of DNA added to a mixture containing 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, 200 μM of each dNTP, 3.5 U of Taq Polymerase and the 10 primers: *C. septicum* Forward AGAATAAACAGAGCTGGAGATG and Reverse CTTCACAAACACAATTCAATAAA; *C. chauvoei* Forward ATCGGAAACATGAGTGCTGC and Reverse AGTCTTTATGCTTCCGCTAG; *C. novyi* type A Forward AGAATAAACAGAGCTGGAGATG and Reverse CGCCTACTTGGAAAGTTACTC; *C. perfringens* type A Forward AATGTTACTGCCGTTGATAG and Reverse CAATCATCCCAACTATGACT; *C. sordellii* Forward GTAGTGCTTGGACACTCTGA and Reverse ATTGGATCTATTCCAGCTTC. E). Correlation with clinical findings: the histopathological findings were correlated with the clinical presentation, hematological results, and microbiological cultures to provide a comprehensive understanding of the pathogenesis and systemic effects of gas gangrene in these cases.

These complementary tests were essential in confirming the diagnosis of gas gangrene, characterizing the systemic effects of the disease, and providing insights into the pathophysiological processes at play in affected horses. The integration of hematological, CSF, and histopathological data contributed to a thorough understanding of the disease, aiding in the development of more targeted diagnostic and therapeutic strategies.

3. Results

During the study period of six years, 20 horses belonging to five different municipalities of the state of Pará (Castanhal, Novo Progresso, Santo Antônio do Tauá, São Félix do Xingú, and Soure). Data referring to the identification of the 20 animals, sex, age, such as municipality, PCR, treatment and outcome are detailed in Table 1. A higher number of cases was observed in males (80%, 16/20) than females (20%, 4/20). The age of the affected animals ranged from 10 months to 12 years, with a greater number of animals (90%, 18/20) in the age group of up to 12 months. Necropsies were performed on all 20 horses, always at postmortem. No animals were vaccinated against clostridiosis.

Table 1. Identification of animals, sex, age, city, PCR result, treatment and cases outcome of 20 horses suspected of gas gangrene in the state of Pará, Amazon Biome, Brazil.

Horses	Sex ¹	Age	City	PCR	Treatment ²	Outcome
1	F	2 years	Castanhal	<i>Clostridium septicum</i>	T2	Death
2	M	10 months	Castanhal	<i>Clostridium septicum</i>	T1	Death
3	M	7 years	Castanhal	<i>Clostridium septicum</i>	T1	Death
4	M	4 years	Castanhal	<i>Clostridium septicum</i>	T1	Death
5	M	3 years	Castanhal	<i>Clostridium septicum</i>	T2	Death
6	M	5 years	Novo Progresso	<i>Clostridium septicum</i>	T1	Death
7	M	6 years	Novo Progresso	<i>Clostridium septicum</i>	T1	Death
8	M	8 years	Novo Progresso	<i>Clostridium septicum</i>	T1	Death

9	F	2 years	Santo Antônio do Tauá	<i>Clostridium septicum</i>	T2	Death
10	M	9 years	Santo Antônio do Tauá	<i>Clostridium septicum</i>	T1	Death
11	F	9 years	Santo Antônio do Tauá	<i>Clostridium septicum</i>	T1	Death
12	M	9 months	Santo Antônio do Tauá	<i>Clostridium septicum</i>	T2	Death
13	M	3 years	Santo Antônio do Tauá	<i>Clostridium septicum</i>	T1	Death
14	F	11years	São Félix do Xingú	<i>Clostridium septicum</i>	T1	Death
15	M	10 years	São Félix do Xingú	<i>Clostridium septicum</i>	T1	Death
16	M	8 years	São Félix do Xingú	<i>Clostridium septicum</i>	T1	Death
17	M	9 years	São Félix do Xingú	<i>Clostridium septicum</i>	T2	Death
18	M	12 years	São Félix do Xingú	<i>Clostridium septicum</i>	T2	Death
19	M	2,5 years	Soure	<i>Clostridium septicum</i>	T2	Death
20	M	2 years	Soure	<i>Clostridium septicum</i>	T2	Death

¹ M = male; F = female; T1 = treatment 1 (Penicilin + Dexametasone); T2 = treatment 2 (Penicilin + Flunixin meglumine);

Gas gangrene, also known as clostridial myonecrosis, is a rapidly progressing and often fatal disease in horses, caused only *Clostridium septicum* and no detection other *Clostridium* species such as *C. perfringens*, *C. novyi*, *C. chauvoei*, and *C. sordellii*. The disease is most commonly associated with contaminated wounds, particularly deep puncture wounds, surgical sites, or areas of tissue necrosis. Gas gangrene in horses is reported worldwide but is particularly prevalent in regions where the bacterium's spores are common in the soil, such as tropical and subtropical areas. The Amazon Biome, with its warm and humid environment, provides ideal conditions for the proliferation of clostridial spores, making horses in this region particularly susceptible. The incidence of gas gangrene in horses tends to increase during wet seasons, when the soil is more likely to harbor clostridial spores, and horses are more prone to injuries from working in muddy or uneven terrain. Horses that suffer from penetrating injuries, particularly those involving muscle tissue, are at high risk for gas gangrene. Additionally, surgical procedures, injections with contaminated needles, or even parturition can create conditions favorable for infection. Poor hygiene, inadequate wound care, and improper management of surgical sites increase the likelihood of infection. While the exact incidence of gas gangrene in horses is difficult to determine due to underreporting, it is recognized as a significant cause of morbidity and mortality in equines, particularly in areas with endemic clostridial spores.

Gas gangrene in horses is characterized by a sudden onset of severe clinical signs (figures 1 and 2) due to the rapid multiplication of *Clostridium* bacteria and the production of toxins, which lead to extensive tissue necrosis, gas production, and systemic toxemia. The disease progresses swiftly, often within hours of infection. The affected area typically presents with severe pain, swelling, and edema. The swelling may rapidly increase in size and is often warm to the touch. A hallmark sign of gas gangrene is the presence of gas bubbles under the skin, which can be detected by palpation as a crackling sensation (crepitus). The skin over the affected area may change color, becoming pale, reddish, or darkened as the tissue becomes necrotic. As the infection progresses, horses may develop a high fever and an elevated heart rate. The horse may become increasingly lethargic, weak, and reluctant to move due to the severe pain and systemic effects of the toxins. If the wound is open, it may exude a foul-smelling, serosanguineous discharge, which may contain gas bubbles. Without prompt treatment, the horse may enter shock, characterized by cold extremities, rapid breathing, and pale mucous membranes. Systemic toxemia leads to multi-organ failure. The disease can progress rapidly to systemic toxemia, sepsis, and death within 24-48 hours if not treated aggressively. In many cases, the progression of gas gangrene is so swift that death occurs before treatment can be effectively administered.

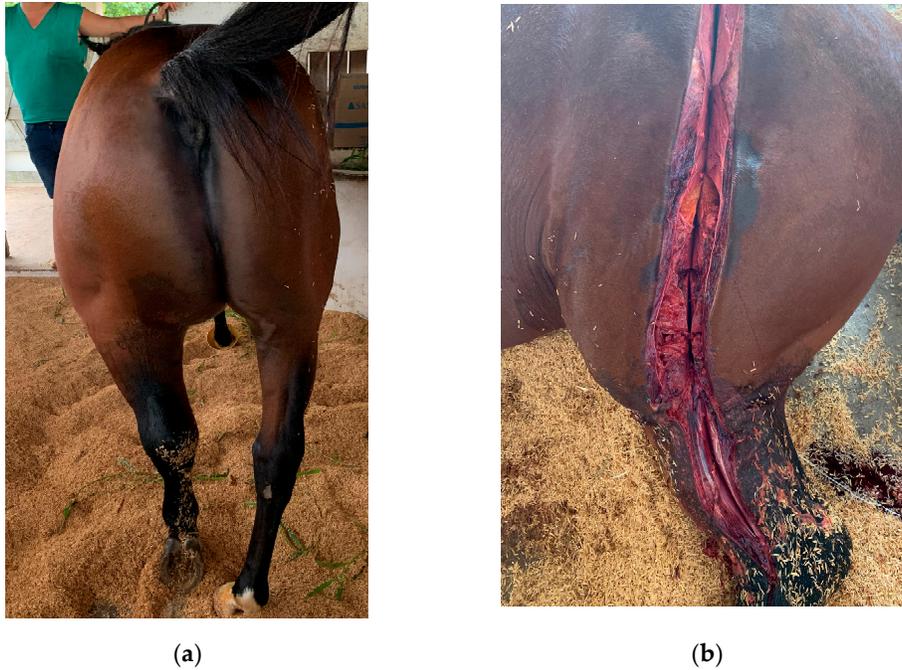


Figure 1. Equine gas gangrene: (a) increased volume of the left hind limb; (b) hemorrhage and subcutaneous edema, with extravasation of serosanguineous fluid, liquefaction, and tissue necrosis (postmortem).



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).

Gas gangrene in horses is a severe and often fatal condition that requires immediate veterinary intervention. The disease's rapid progression and the nonspecific nature of early signs make early diagnosis and treatment challenging, underscoring the importance of preventive measures, particularly in high-risk regions like the Amazon Biome. Understanding the epidemiological factors and clinical presentation of gas gangrene is crucial for effective management and improving the prognosis for affected horses.

In cases of gas gangrene, clinical pathology reveals profound systemic effects due to the rapid progression of infection and the widespread release of toxins by clostridial bacteria. Key clinical

pathology findings include: Horses with gas gangrene often present with a marked increase in white blood cell count (leukocytosis), accompanied by a left shift, indicating a response to severe infection. High levels of CK are common due to extensive muscle necrosis, as the enzyme is released into the bloodstream from damaged muscle tissue. Due to tissue hypoxia and necrosis, there is an accumulation of lactic acid, leading to elevated lactate levels in the blood. Loss of plasma proteins through damaged vessels and into necrotic tissues can result in decreased protein levels in the blood. The rapid tissue breakdown and systemic toxemia can lead to metabolic acidosis, characterized by decreased blood pH and bicarbonate levels. Hyperkalemia and hypocalcemia may occur due to muscle necrosis and cellular breakdown, contributing to the worsening of systemic clinical signs.

Gas gangrene in horses is most frequently associated with *Clostridium septicum*, although other *Clostridium* species can also be involved. These bacteria are anaerobic, spore-forming, and capable of producing potent exotoxins that cause tissue necrosis and gas production. Microbiological examination typically involves: Samples for microbiological analysis can be obtained from affected tissues, fluids from the site of infection, or aspirates of subcutaneous gas pockets. It's crucial to handle these samples anaerobically to prevent exposure to oxygen, which can inhibit the growth of *Clostridium* species. *Clostridium* species are cultured on specific anaerobic media. Colonies are typically large, with a characteristic foul odor. Identification is confirmed through biochemical tests, PCR, or MALDI-TOF mass spectrometry, which differentiate between the various *Clostridium* species. The identification of specific toxins, such as alpha, beta, and theta toxins produced by *C. perfringens* or the lethal toxins of *C. septicum*, may be performed to confirm the diagnosis and understand the pathogenesis.

Gas gangrene causes characteristic anatomical and histopathological changes, which can be observed during post-mortem examinations or from biopsy samples. The affected muscles are typically swollen, dark, and friable, with extensive necrosis. There may be gas bubbles within the muscle tissue, giving it a "bubble wrap" appearance on palpation. The necrotic tissue often exudes a foul-smelling, dark, serosanguineous fluid that may contain gas bubbles. The odor is typically pungent due to the anaerobic metabolism of *Clostridium* bacteria. Gas production by the bacteria leads to subcutaneous emphysema, with palpable crepitus in the overlying skin. This can extend over a significant area of the body, depending on the severity and extent of the infection. Extensive edema is often present, not only in the affected muscles but also in the surrounding tissues. This edema may contribute to circulatory impairment and further tissue damage.

Histopathology reveals severe myonecrosis, characterized by the loss of muscle fiber architecture, with extensive areas of coagulative necrosis. Muscle fibers appear fragmented, with loss of cross-striations, and may be replaced by eosinophilic, amorphous debris. Gas vacuoles may be observed within necrotic muscle fibers, a result of the gas-producing clostridial species. There is typically a mixed inflammatory response, including neutrophils and macrophages, although the infiltration may be limited due to the rapid progression of the disease and the extensive tissue destruction. Histological sections often show thrombosis and damage to blood vessels, contributing to ischemia and further exacerbating the necrosis. Gram-staining of histological sections can reveal the presence of large, gram-positive bacilli, consistent with *Clostridium* species, within the necrotic tissue. The bacteria are typically found in large clusters or chains. 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 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*.

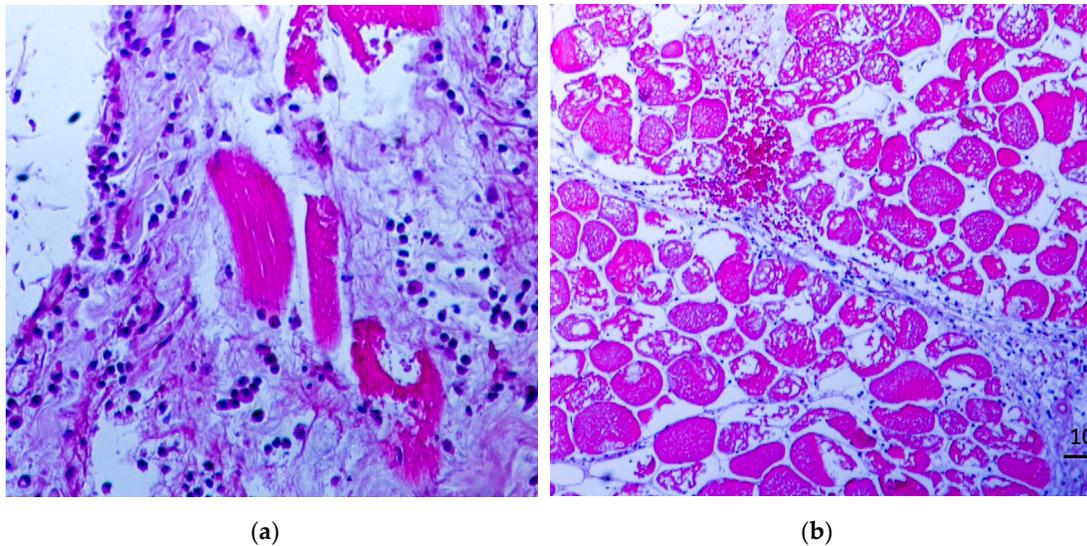


Figure 3. Equine gas gangrene: (a) Degeneration and hyaline necrosis of muscle fibers with intense inflammatory infiltrate [H&E obj. 10×]; (b) area of necrosis associated with edema with hemorrhage [H&E obj. 20×].

Gas gangrene in horses, caused primarily by *C. septicum*, presents with severe clinical pathology, microbiological, and histopathological changes. Understanding these findings is crucial for the accurate diagnosis, treatment, and management of this rapidly progressing and often fatal disease. The comprehensive examination of clinical samples, coupled with detailed anatomical and histopathological analysis, is essential for confirming the diagnosis and understanding the pathogenesis of gas gangrene in equines.

4. Discussion

The retrospective study conducted on equine gas gangrene in the Amazon Biome, which involved the analysis of 20 horses over a decade, offers critical insights into the prevalence and impact of *Clostridium septicum* as the causative agent. Although in horses the disease is most often caused by *Clostridium perfringens* type A [11], sporadic cases associated with other clostridial species have been described. All the horses studied succumbed to gas gangrene, underscoring the severe nature of the disease and the rapid progression that leads to high mortality rates. The exclusive identification of *C. septicum* [12] through PCR in these cases is particularly noteworthy, as it suggests a strong regional association of this pathogen with gas gangrene in horses. This finding raises important considerations for both diagnostic approaches and preventive strategies tailored to the Amazon Biome.

The consistent detection of *C. septicum* across all cases indicates that this pathogen may have a unique niche in the Amazon Biome, where environmental factors such as high humidity, warm temperatures, and organic-rich soils create ideal conditions for its survival and proliferation. Unlike other clostridial species, which are often more commonly associated with gas gangrene in temperate climates, *C. septicum* appears to dominate in this tropical region. This dominance could be due to the bacterium's ability to thrive in anaerobic conditions that are more prevalent in the warm, moist environment of the Amazon. Moreover, the rapid onset of gas gangrene symptoms following trauma or other predisposing factors in these horses highlights the need for immediate intervention upon the first signs of the disease [13–15].

The implications of this study are significant when compared to other Brazilian and global studies on clostridial infections [15]. While *C. perfringens* and *C. chauvoei* are often reported as primary agents of gas gangrene in livestock worldwide, the predominance of *C. septicum* in this study suggests a region-specific pathogen profile in the Amazon Biome. This contrasts with findings from other regions where different species of *Clostridium* are more prevalent. For example, in southern Brazil [12] and other parts of the world [16], *C. perfringens* is frequently implicated in clostridial myonecrosis

[11,17], particularly in cattle. However, the Amazon's unique ecological conditions appear to select for *C. septicum*, making it the leading cause of gas gangrene in horses in this area.

The high mortality rate observed in this study highlights the critical need for more effective prevention and control measures [18,19]. Current strategies, including vaccination, have not been fully developed or implemented for *C. septicum* in equines, particularly in the Amazon Biome. Given the rapid progression of gas gangrene and the high mortality associated with *C. septicum* infections, there is an urgent need for the development of equine-specific vaccines that target this pathogen [20]. Additionally, the study emphasizes the importance of early diagnosis and prompt treatment. The consistent use of PCR in this study underscores its utility as a diagnostic tool for clostridial infections. However, the reliance on advanced laboratory techniques also points to a gap in diagnostic capabilities in rural and remote areas, where access to such facilities may be limited [21].

Gas gangrene, also known as clostridial myonecrosis, is a highly lethal disease in equines, characterized by rapid onset and progression, leading to severe tissue necrosis, systemic toxemia, and often death. The importance of this disease for equine health cannot be overstated, particularly in regions like the Amazon Biome where environmental conditions favor the proliferation of clostridial bacteria, such as *C. septicum*. The study highlights the critical need for increased awareness, diagnostic capabilities, and preventive measures to mitigate the impact of this devastating disease on equine populations. In equines, the disease's rapid progression often leaves little time for effective intervention, underscoring the necessity for early detection and treatment, which are currently hampered by a lack of data and awareness among veterinarians and horse owners in the region [6,16,19].

In the context of Brazil, and especially within the Amazon Biome, the significance of gas gangrene in equines is magnified by several factors. The Amazon is a vast, remote, and ecologically unique region where veterinary services are often limited, and the infrastructure for disease surveillance and management is underdeveloped. This geographical and infrastructural challenge contributes to the lack of data on the occurrence of gas gangrene in equines, which in turn hinders efforts to understand the true prevalence and impact of the disease. The scarcity of epidemiological data on clostridial infections in the Amazon further complicates the development of targeted prevention and control strategies, leaving equine populations vulnerable to outbreaks with potentially devastating consequences. The study underscores the urgent need for comprehensive epidemiological surveys and the establishment of robust reporting systems to address this gap in knowledge [22-24].

Moreover, the lack of diagnosis and awareness among veterinarians and producers about gas gangrene poses a significant barrier to effective disease management in the Amazon Biome. Many veterinarians and equine breeders in the region may be unfamiliar with the clinical signs of gas gangrene, leading to misdiagnosis or delayed diagnosis, which reduces the chances of successful treatment. The disease's nonspecific early symptoms can be easily overlooked or mistaken for less severe conditions, further complicating timely intervention. This lack of knowledge not only affects animal health and welfare but also has significant economic implications [25-27]. The financial impact of gas gangrene on equine breeding in the Amazon is substantial, as the loss of valuable animals to the disease can result in direct financial losses for breeders, as well as broader economic effects on the equine industry in the region.

The findings of this retrospective study highlight the critical need for enhanced veterinary education and training focused on the recognition and management of gas gangrene in equines [28,29]. Additionally, there is an urgent need for the development and dissemination of practical diagnostic tools that can be used in the field by veterinarians and producers in remote areas of the Amazon. The study also calls for increased investment in research to develop effective vaccines [2,4,23,24] and treatment protocols tailored to the specific challenges of managing clostridial infections in equines within the Amazon Biome. By addressing these gaps in knowledge, diagnostics, and disease management, it is possible to reduce the incidence and impact of gas gangrene in equines, ultimately improving the health and productivity of equine populations in the Amazon and beyond.

Polymerase chain reaction (PCR) has emerged as the most widely used diagnostic tool for clostridial myonecrosis [10], primarily due to its high sensitivity, specificity, and rapid turnaround time. Unlike traditional culture methods, which can be time-consuming and often complicated by the fastidious nature of *Clostridium* species, PCR allows for the direct detection of clostridial DNA from clinical samples, leading to quicker diagnosis and treatment [6]. One of the main advantages of PCR is its ability to detect the presence of clostridial toxins or specific gene sequences that are indicative of pathogenic strains. This molecular approach bypasses the need for viable bacteria, which is particularly useful in cases where antibiotics may have already been administered, potentially killing the bacteria but leaving the DNA intact. The ability to identify specific *Clostridium* species, such as *C. perfringens*, *C. septicum*, and others, allows for more targeted treatment strategies, which can be critical in managing the rapid progression of gas gangrene. PCR's role in the diagnosis of clostridial myonecrosis is further solidified by its application in multiplex assays, which can simultaneously detect multiple pathogens or toxins from a single sample. This multiplexing capability is particularly beneficial in polymicrobial infections or when rapid differentiation between toxin-producing and non-toxin-producing strains is necessary [7,10]. Moreover, the adoption of PCR in veterinary medicine, particularly in regions like the Amazon Biome where clostridial diseases are prevalent, has enhanced the ability of veterinarians to manage outbreaks more effectively. PCR is not only faster but also more accurate than serological methods, reducing the likelihood of false positives or negatives. However, despite these advantages, the cost and requirement for specialized equipment and trained personnel limit PCR's accessibility in some field settings, particularly in rural or under-resourced areas. This has spurred ongoing research into the development of more portable and cost-effective molecular diagnostic tools that can be utilized in the field by farmers and veterinarians without compromising accuracy [6,16,19].

Gas gangrene, primarily caused by histotoxic *Clostridium* species such as *C. septicum*, is a severe and often fatal condition in equines [20,21,28]. Preventing gas gangrene is critical for maintaining the health and productivity of horses, especially in regions where these infections are prevalent. Various preventive measures can be employed: a). Prompt and thorough cleaning of wounds is essential to prevent the introduction and proliferation of *Clostridium* spores, which thrive in anaerobic conditions; b). Applying antiseptics to wounds and administering prophylactic antibiotics can help reduce the risk of infection, especially in deep or puncture wounds; c). Maintaining clean and dry stables reduces the risk of environmental contamination with *Clostridium* spores. Proper disposal of manure and organic waste is also crucial to minimize exposure; d). Ensuring that feed and bedding materials are free from contamination with *Clostridium* spores can prevent infections originating from the environment; e). Where available, regular vaccination schedules should be adhered to, especially in regions prone to clostridial infections; f). Regular veterinary examinations can help detect early signs of infection, allowing for prompt treatment before the disease progresses.

While vaccines for clostridial diseases are available for other livestock, such as cattle and sheep, there is a notable gap in the availability of equine-specific vaccines for gas gangrene [2,4,22–24]. The development of such vaccines is crucial for several reasons: 1). Species-specific immunogenicity: horses may respond differently to vaccines designed for other species, necessitating the development of vaccines tailored to their immune systems for optimal protection. 2). Disease prevalence: in regions where gas gangrene is common among equines, an equine-specific vaccine could significantly reduce the incidence and severity of the disease, thereby reducing mortality rates and improving animal welfare. 3). Economic impact: gas gangrene can cause significant economic losses in equine industries, particularly in areas where horses are used for labor, transportation, or competitive sports. Vaccines could mitigate these losses by preventing outbreaks and reducing the need for costly treatments.

The development of new vaccines and vaccine methodologies [2,4,22–24] is critical to advancing equine husbandry and preventing clostridial diseases like gas gangrene. Key areas of focus include: 1). Next-Generation Vaccines: a) Subunit vaccines: these vaccines use specific antigens, such as toxins or surface proteins, to elicit an immune response without using whole bacteria. They offer a safer alternative with fewer side effects; b). Toxoid vaccines: which use inactivated toxins, could be

particularly effective against gas gangrene, as the disease's pathology is largely driven by toxin production. 2). Adjuvants and delivery systems: a). Innovative adjuvants: the use of advanced adjuvants can enhance the immune response, allowing for lower doses of the antigen and potentially reducing the frequency of booster shots; b). Novel delivery systems: research into delivery systems such as nanoparticles or liposomes could improve the stability and efficacy of vaccines, making them more effective in challenging environmental conditions. 3). DNA and mRNA vaccines: these vaccines involve the introduction of plasmid DNA encoding the antigen into the host, prompting the production of the antigen and an immune response. DNA vaccines are stable, easy to produce, and offer the potential for rapid development. mRNA vaccines the success of mRNA vaccines in human medicine, particularly for COVID-19, suggests that similar technologies could be adapted for use in equines. These vaccines can be rapidly designed and produced, offering a flexible platform for responding to emerging clostridial strains. 4). Field-applicable vaccines thermostable formulations: developing vaccines that do not require refrigeration would be particularly beneficial in remote or resource-limited areas, where cold chain logistics are challenging. 5). Single-dose vaccines: single-dose formulations would simplify vaccination protocols, making them more practical for use by farmers and veterinarians in the field. Preventing gas gangrene in equines requires a multifaceted approach, combining proper wound care, environmental management, and vaccination. The development of equine-specific vaccines is a critical step in enhancing disease prevention strategies. Continued research and innovation in vaccine technologies are essential for creating more effective, accessible, and field-ready vaccines. These advancements will not only improve equine health and productivity but also contribute to the sustainability of equine industries world wide [24,22–24].

In conclusion, the findings from this study provide valuable epidemiological data on gas gangrene in horses in the Amazon Biome and highlight the need for region-specific interventions. The predominance of *C. septicum* in these cases suggests that this pathogen is a major threat to equine health in this region, necessitating targeted research, improved diagnostic tools, and the development of vaccines tailored to the unique challenges of the Amazon environment. The study also calls for increased awareness among veterinarians and horse owners about the risks of *C. septicum* and the importance of rapid response to prevent the high mortality associated with gas gangrene.

5. Conclusions

This is the first work in the world in which the consistent identification of *C. septicum* across all cases emphasizes the need for improved targeted preventive measures, including wound management and hygiene practices in equine care. Furthermore, this research calls for increased awareness among veterinarians and horse owners in the Amazon region regarding the risks of gas gangrene, particularly following traumatic injuries or surgical procedures. The study's conclusions contribute valuable knowledge to the understanding of gas gangrene in horses, advocating for enhanced prevention, rapid diagnosis, and effective treatment strategies to mitigate the devastating effects of this disease in the Amazon Biome.

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