Preprints.org

Review

Not peer-reviewed version

Clostridial Bovine Infections: A Review Focusing on Diseases in Brazil

Felipe Masiero Salvarani *

Posted Date: 21 May 2024

doi: 10.20944/preprints202405.1322.v1

Keywords: botulism; tetanus; blackleg; malignant edema; enterotoxemia.

Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

Clostridial Bovine Infections: A Review Focusing on Diseases in Brazil

Felipe Masiero Salvarani

Special Issue Editor of Diseases, Diagnosis and Treatment of Bovine Bacterial Infections. Instituto de Medicina Veterinária, Universidade Federal do Pará, Castanhal 68740970, PA, Brazil. felipems@ufpa.br

Simple Summary: Clostridial infections in cattle are a major veterinary concern in Brazil, significantly impacting the country's livestock industry. These infections are caused by various Clostridium species, which are anaerobic, spore-forming bacteria capable of producing powerful toxins. The main clostridial diseases affecting cattle in Brazil include botulism, tetanus, blackleg, malignant edema and enterotoxemia. Prevention and control measures of regular vaccination programs are crucial in preventing clostridial diseases. Ensuring proper hygiene, especially during surgical procedures, and minimizing injuries can help reduce the risk of infection. Surveillance and research are essential for understanding the epidemiology of these diseases and improving control strategies. Monitoring outbreaks and investigating new cases help in adapting vaccination protocols and management practices. The economic impact of clostridial infections cause significant eco-nomic losses in Brazil's cattle industry due to high mortality rates, decreased productivity, and the costs associated with vaccination and treatment. Effective prevention and control strategies are crucial to mitigate these losses and ensure the health and productivity of cattle herds.

Abstract: Clostridial infections in cattle are a significant concern for Brazilian livestock. These diseases are caused by various species of Clostridium, which are known for their ability to produce potent toxins. Botulism in cattle is a serious and often fatal condition caused by the ingestion of neurotoxins produced by *C. botulinum*. This bacterium thrives in decomposing organic matter, such as spoiled feed, carcasses, and contaminated water. Tetanus while less common, tetanus is a serious disease following contamination of wounds with *Clostridium tetani* spores. It results in muscle stiffness, spasms, and often death due to respiratory failure. Blackleg (*C. chauvoei*) is this disease primarily affects young cattle, leading to acute lameness, swelling, and high fever. Malignant edema (*C. septicum* and others) is characterized by rapid onset of swelling at wound sites, malignant edema can occur after injuries or surgical procedures. Enterotoxemia is triggered by the rapid growth of *C. perfringen*s in the gut following excessive carbohydrate intake. This leads to toxin production that causing sudden death. In conclusion, clostridial bovine infections remain a persistent challenge for Brazilian cattle farmers. With continued focus on vaccination, good management practices, and research, the impact of these diseases can be minimized, safeguarding the livestock industry's economic viability.

Keywords: botulism; tetanus; blackleg; malignant edema; enterotoxemia

1. Introduction

The genus Clostridium was first described by A. Prazmowski in 1880 and since then more than 225 species distributed in different geographic areas have been identified. Clostridium spp. They are Gram-positive rods, sporulating and strict anaerobes, the majority of which are part of the intestinal microbiota of animals and humans, but only a few species are capable of causing diseases in animals [1,2].

Many of the infectious processes and poisonings that affect domestic animals are caused by bacteria of the genus Clostridium. These diseases are called clostridioses, and have high mortality rates. Due to their high sporulation capacity, bacteria of this genus are capable of remaining potentially infectious in the soil for long periods, representing a significant risk to the animal and human population. Even though they are capable of producing disease in animals and humans, they are rarely considered zoonotic agents [3–6].

© 2024 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

The pathogenic bacteria that make up this genus cause diseases basically through two mechanisms: production of toxins and tissue invasion. Clostridia enter the body in sporulated form, through contaminated food, wounds or by inhalation. The toxins are produced in the animal's body or are ingested pre-formed. Among the toxins of clostridial origin, botulinum and tetanus neurotoxins and epsilon toxin produced by *C. perfringens* types B and D stand out as the most potent toxins of known microbial origin [7–9].

Clostridiosis is one of the main diseases that affect domestic animals in the country, with high morbidity and lethality rates, causing great economic losses to the production sector [10,11]. The main agents involved and the diseases caused by bacteria of the genus Clostridium in cattle are presented in table 1. This review covers the main clostridioses that affect cattle in Brazil and their impacts on one of the largest cattle producers and exporters in the world.

Table 1. Main clostridial diseases in cattle and their agents.

	Disease	Agent
Neurotropic Diseases	Tetanus	*Clostridium tetani*
	Botulismo	*Clostridium botulinum*
Enterotoxemias	Enterotoxemia of Cattle	*Clostridium perfringens* tipos B and D
Myonecrosis	Blackleg and Malignant edema	*Clostridium chauvoei*
		Clostridium septicum
		Clostridium sordellii
		Clostridium novyi tipo A
		Clostridium perfringens tipo A

2. Botulism

Botulism, derived from the Latin botulus, which can be translated as sausage, was first described in 1820, in Germany, after several cases of flaccid paralysis in humans associated with the ingestion of sausages and meat sauces. Initially considered to be a fungus, it was only between 1895 and 1897 that it was demonstrated that botulism was caused by the toxin of an anaerobic bacillus, known as Bacillus botulinus. During this same period, using a cell culture filtrate, free of bacilli and spores, signs of paralysis were reproduced in laboratory animals, confirming the existence of a toxin [12–14]

Currently called *Clostridium botulinum*, this anaerobic bacillus is classified into seven types (A, B, C, D, E, F and G), based on the antigenic specificity of the neurotoxin (BoNT) produced by each strain. Encoded by the BoNT gene, these toxins are produced as 150 kDa polypeptide chains, which are cleaved into two smaller chains, prior to release by the microorganism. Therefore, a heavy chain (HC), of 100 kDa, and a light chain (LC), of 50 kDa, are created, which remain linked through a disulfide bond. The origin of the BoNT gene varies between types of *C. botulinum*: types A, B, E and F, which cause human botulism, are of chromosomal origin; types C and D, responsible for the vast majority of cases of the disease in animals, originate from bacteriophages; For type G, the origin is plasmid. .Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code [1,5,8,12–14].

The mechanism of action of BoNTs is endowed with three steps: binding, translocation and enzymatic activity. In the first stage, HCs bind to the membranes of neurons, mainly cholinergic, through a double-receptor system, consisting of a ganglioside and a protein component. Then, BoNTs are translocated to the neuronal cytoplasm via endocytosis. It is believed that, during this step, HCs form pores in the membrane, through which LCs pass from the extracellular to the neuronal intracellular environment. Finally, LCs cleave one or more SNARE proteins, responsible for the docking and fusion of vesicles containing neurotransmitters at presynaptic terminals. As a result, there is a reduction in the release of acetylcholine at neuromuscular junctions, which leads to the inability to contract muscles or flaccid paralysis of skeletal muscles. Each serotype cleaves specific peptide bonds from one or more SNARE proteins [1,5,8,12–14].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Animal species have different susceptibilities to BoNTs and botulism. Horses appear to be sensitive to all serotypes. Cattle are susceptible to types C and D, rarely affected by types A and B, presenting acute or subacute conditions. The disease in sheep is commonly chronic. Dogs are less sensitive, with botulism being caused by serotypes A, B and C, but rarely. Birds are affected by types C and, more rarely, A and E, although they can eliminate all serotypes in their waste [1,5,8,12,13].

Botulism in cattle was first described in Brazil in 1970, in Piauí, and 13 years later in Rio Grande do Sul. From then on, botulism in domestic ruminants began to occur in an epizootic form. Animals with high nutritional requirements, such as pregnant or lactating females, raised in soils and pastures poor in minerals, especially phosphorus, without adequate mineral supplementation, developed the habit of osteophagy (eating bones) or sarcophagy (eating corpses), seeking to supply its mineral deficiencies. However, at the same time, they ingested pre-formed BoNTs during the decomposition of carcasses, which led to large epidemics of the disease with the death of thousands of animals [15–19].

Over the past two decades, ruminant botulism has occurred mainly in the form of sporadic outbreaks. In these outbreaks, BoNTs ingested by animals originate from food contaminated with decomposing organic matter, such as chicken litter, hay, grains, feed and silage of poor quality or poorly stored. In addition to these, water troughs with carcasses of small animals or other types of decomposing organic matter, as well as wells and ponds with stagnant water can serve as a source of BoNTs for animals [20,21].

The incubation period and severity of botulism will depend on the amount of toxin ingested and the susceptibility of the animal species. In ruminants, the course of the disease can last from hours to a few weeks and lethality is close to 100%. The initial clinical signs are difficulty in locomotion, incoordination of the hind limbs, with cranial progression of flaccid paralysis. The animal enters a pre-agonic state, and death, preceded by coma, occurs due to cardiorespiratory arrest. Throughout the symptomatology, the psyche of the animals remains unchanged. Injuries at necropsy are rare and limited to petechiae in the myocardium as a consequence of respiratory agony, which precedes death [12,13,16].

With high lethality and mortality rates, botulism is considered one of the most important diseases that affect wild birds and subsistence poultry farms. The following stand out as sources of BoNTs for birds: wells and lakes with stagnant water; larvae of flies and other invertebrates that develop in decomposing organic matter; food or water provided containing decomposing organic matter. Less commonly, endogenous botulism can occur, with the production of BoNTs in the intestine of birds or in wounds contaminated by spores. Clinically, botulism in birds is characterized by an ascending symmetrical paralysis, affecting legs, wings, neck and eyelids, concomitantly with loss of feathers and unchanged psyche. Death occurs due to cardiorespiratory arrest [1,5,8,12–14].

The diagnosis of botulism is based on epidemiological data, clinical signs of affected animals and the detection of BoNTs in clinical specimens and/or sources of poisoning. These may include rumen, gastric and intestinal contents, liver, serum, as well as samples of food and water that the animals may have ingested. The standard test for BoNTs research is the mouse bioassay. This test is based on the intraperitoneal injection of diluted samples into mice. If the toxin is present, the mice develop typical signs of botulism, such as raised hair, muscle weakness and dyspnea, which is manifested by a narrowing of the waist, called “wasp waist”. The type of BoNT is determined by neutralization of the toxin with its specific antitoxin. Although the mouse bioassay is highly specific and sensitive, with detection limits of up to 0.01 ng/ml of sample, other “in vitro” tests are available or under development. Highlights include enzyme-linked immunosorbent assays (ELISA), polymerase chain reaction (PCR), real-time PCR, chemiluminescence, electrochemiluminescence, radioimmunoassay, lateral flow immunoassay, endopeptidase assay and complement microfixation [1,5,15,19,21].

Vaccination with C. botulinum toxoids C and D is the main way to control botulism in domestic animals, with the only exception being birds. In association, other measures are extremely important, especially those that aim to prevent the ingestion of pre-formed BoNTs by animals. These measures include: adequate mineral supplementation of domestic ruminants; removal of mammal and bird carcasses from pastures and watercourse edges; adequate production, storage and supply of high-quality food; do not provide poultry litter to domestic ruminants under any circumstances; provide

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

good quality water, and prevent animals from accessing places with stagnant water or water of unknown quality [10,11,22,23].

3. Tetanus

Tetanus is a non-contagious infectious disease caused by the action of exotoxins produced by *C. tetani*, which cause functional changes in the central nervous system with increased excitability. *C. tetani* is cosmopolitan and is commonly found in soil in the form of spores and the intestinal tract of humans and domestic animals. Infection generally occurs through contamination by spores on the skin or mucosa with superficial or deep lesions of any nature. Improperly cutting and healing navels is a potential entry point for *C. tetani* spores in large animals. Factors such as the presence of devitalized tissues, foreign bodies, ischemia and infection contribute to the reduction of the redox potential in the lesion, which favors the germination of spores that multiply and produce the toxins tetanolysin and tetanospasmin, the latter being responsible for the clinical characteristics of tetanus [24,25].

Tetanospasmin is a potent neurotoxin encoded by the TeTx gene of non-conjugative plasmid origin. It is produced during the stationary phase of growth as a polypeptide with a molecular weight of 150KDa composed of a heavy chain (HC) and a light chain (LC) linked via a disulfide bond. HC has a binding domain in its C-terminal region capable of recognizing specific receptors in the presynaptic terminals of the central nervous system. The toxin is internalized and transported in endosomes to the cell body of the motor neuron, located in the spinal cord. Then, acidification of the endosome occurs, which results in a conformational change in the N-terminal domain of the HC, followed by its insertion into the endosome membrane and passage from the LC to the cell cytosol. Once in the cytosol, the LC is capable of cleaving SNARE proteins (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) responsible for exocytosis in neurons. As a result, there is a reduction in the release of inhibitory neurotransmitters such as gamma globulinic acid and glycine, resulting in spastic paralysis. Tetanolysin is a hemolysin capable of causing cell lysis through the formation of pores by hydrolysis of plasma membrane phospholipids. The clinical significance of this enzyme is unknown, however, it is inhibited by plasma oxygen and cholesterol [24,25].

Clinically, the disease manifests itself with low or absent fever and muscular hypertonia, causing jaw trismus, neck stiffness, protrusion of the third eyelid, dysphagia, hyperextension of limbs, opisthotonus and respiratory failure. Facial muscle spasticity (sardonic laughter) commonly observed in humans has already been described in dogs and cats. Initially, spasms and paroxysmal contractures are caused by tactile, sound, light stimuli or high ambient temperature and, as the disease progresses, they can be triggered spontaneously. In general, the animal remains conscious [26,27]

The diagnosis of tetanus is based on history, anamnesis and clinical signs, and does not depend on laboratory confirmation. At necropsy, no significant lesions are found and the presence of an entry point for the agent is not always observed, as in some reports of outbreaks of idiopathic tetanus in young cattle. Differential diagnosis for poisoning by metoclopramide and neuroleptics is important; strychnine poisoning with absence of trismus and generalized hypertonia during intervals of spasms and meningitis with presence of high fever from the beginning, absence of trismus and vomiting. In addition to these, rabies is included as a differential diagnosis, in which it is possible to observe the presence of convulsions, changes in behavior, absence of trismus in addition to a history of biting, scratching or licking by animals [24,25].

The basic principles of tetanus treatment are sedation, neutralization of circulating tetanus toxin by antitetanus serum, debridement of the infectious focus, eradication of the agent with administration of penicillin and general supportive measures. The animal must be kept in a dark and silent environment, with a stable and pleasant temperature in order to minimize the signs presented. Toxoid vaccination is the main form of tetanus prevention and the commercial product is available for cattle, sheep, goats, horses, pigs, dogs and cats. The administration of a booster vaccine prior to surgical procedures, as well as the administration of anti-tetanus serum at the time or after them, is essential for the prophylaxis of the disease [24–27]

4. Myonecrosis

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Blackleg is an acute disease of cattle and sheep caused exclusively by *C. chauvoei*. In cattle, the disease occurs endogenously and is frequently observed in young animals. The ingestion of spores present in the environment is the main route of transmission. Although most spores are excreted in feces, some may leave the intestine and be distributed to tissues where they remain dormant. The sequence of events that leads to the distribution of endospores in tissues is unclear, but it is believed that they are transported by macrophages. Gas gangrene is an exogenous and necrotizing infection of soft tissues that affects several species of domestic animals. This pathology is caused by one or the association of the following species of the genus Clostridium: *C. septicum, C. chauvoei, C. novyi* type A, *C. perfringens* type A and *C. sordellii*. The occurrence of this disease is related to strict contact between these agents and domestic animals, which favors the contamination of wounds resulting from surgical and/or sanitary practices carried out without aseptic care. Furthermore, tissue injury promotes a decrease in redox potential, alkalinization of pH and decomposition of protein products. These factors contribute to the penetration, germination and intense proliferation of clostridia, with consequent production of toxins responsible for the pathological condition of the disease [1,8,28–30].

Clostridium septicum was the first species of clostridium identified, being named Vibrion septicum by Pasteur and Jubert in 1877. The alpha toxin (Table 2), the main toxic factor of this agent, binds to receptors present in the cell membrane of the clostridia. hosts where they are activated by proteolytic cleavage. Activated monomers diffuse laterally across the membrane, forming an oligomer that undergoes conformational changes until an active pore spanning the cytoplasmic membrane is installed. The pore promotes changes in cell permeability, followed by water influx and consequent rupture, determining cell lysis and cytotoxicity [1,8,31].

Table 2. Histotoxic clostridia and characterization of the main virulence factors.

	Clostridium septicum	*Clostridium chauvoei*	*Clostridium novyi*	*Clostridium sordellii*		*Clostridium perfringens*
Toxin	Alpha	CctA	Alpha	Lethal	Hemorrhagic	Alpha
Origin	Plasmdial/ chromosomal	Chromosomal	Phage	Chromosomal	Chromosomal	Chromosomal
Molecular weight (Kda)	48	33	250	300	260	42
Action	Pore formation	Pore formation	Inactivation of GTPases	Inactivation of GTPases	Inactivation of GTPases	Phospholipase C

Although descriptions of gas gangrene and blackleg have been made since the mid-19th century, *C. chauvoei* was only described in 1880. Unlike other bacteria that cause myonecrosis, few studies have been carried out seeking to elucidate its toxic activity and mechanisms of action. of the toxins produced by *C. chauvoei*. The CctA toxin (Table 2) was recently identified as the main virulence factor of this microorganism, having the capacity to provide protection to animals vaccinated with its toxoid. However, it is considered necessary to carry out other studies to better characterize this and other proteins produced by *C. chauvoei* [1,8,28–30].

Clostridium novyi is classified into four types, from A to D, according to the production of toxins. Type A produces only alpha toxin and is the agent of gas gangrene in humans and domestic animals. Type B produces, in addition to the alpha toxin, the beta toxin. Type C does not produce toxins and type D, now called *C. haemolyticum*, causes bacillary hemoglobinuria in cattle. *C. novyi* alpha toxin (Table 2) is a glycosyltransferase that catalyzes the glycosylation of small GTPases, determining the inactivation of cytoskeletal proteins and disorganization of actin filaments, resulting in effects such as cell rounding, loss of intercellular junctions and increased of endothelial permeability, which is compatible with the edema observed in conditions caused by this bacterium [1,8,28–30].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Clostridium sordellii was first isolated in 1922 by Alfredo Sordelli, an Argentine microbiologist who named the bacteria *Bacillus oedematis sporogenes* and since 1928, the name C. sordellii has been adopted. Animals affected by C. sordellii tend to present less disseminated and painful lesions with vascular damage and reduced hemolysis. The virulence of this bacterium is attributed to numerous exotoxins, although only the lethal and hemorrhagic toxins are considered essential for the disease to occur (Table 2). Both catalyze the glycosylation of small GTPases, differing only in terms of target GTPases. These toxins have a glycosyltransferase action, modifying the GTPases that control the cell cycle, apoptosis, gene transcription and the structural functions of actin such as cell morphology, migration and polarity. The induced modifications cause actin condensation, culminating in cytoskeletal disorganization, cell rounding and eventual apoptosis [1,8,28–30].

The first description of *C. perfringens* was made from necropsy isolates from a cadaver with disseminated emphysema. This clostridium differs from other bacteria of the genus due to its relative aerotolerance, high and rapid growth rate and its genetic instability regarding the expression of toxin-coding genes. Alpha toxin (Table 2), the main toxic factor involved in cases of gas gangrene, interacts with membrane phospholipids where it has potent phospholipase C activity, hydrolyzing the membrane of eukaryotic cells. Furthermore, this toxin determines vascular collapse due to an exacerbation in the concentrations of arachidonic acid and its metabolites in the affected cells, which leads to a local inflammatory reaction and vasoconstriction. Alpha toxin activates protein kinase C, determining the production of platelet aggregation factor with consequent formation of intravascular thrombi, a fact that contributes to inflammation and local anoxia. Luminal obstruction of capillaries by neutrophils is pathognomonic of *C. perfringens* infection. The reasons for leukoestasis are unknown, but it is believed that it may be related to the necrosis of endothelial cells, which makes it impossible to rearrange the cytoskeleton that allows the transmigration of defense cells [1,8,28–30,32,33].

When gas gangrene or blackleg is not quickly controlled, the animal develops systemic toxemia and shock. Death occurs as a consequence of the effect of toxins on the hemodynamics of the vascular system. Clinically, the animal presents elevated or normal temperature, anorexia and depression. When a limb is hit, there is difficulty in locomotion and a variable increase in volume associated with subcutaneous crepitus, due to the large amount of gas produced by bacterial multiplication in the focus of infection, edema and hemorrhage. Sometimes, these changes are so intense that the skin appears tense, diffusely red or black, with bruises and suffusions. It is not uncommon to see a line delimiting the infected part from the healthy part. When cut, extravasation of liquid can be observed, with gas bubbles and subcutaneous hemorrhage. The fascia and muscle bundles can be separated when there is an intense amount of gas. The muscles appear intensely hemorrhagic, emphysematous and with gray areas, indicative of necrosis. Histologically, diffusely swollen, eosinophilic muscle fibers are observed, with loss of striations (hyaline degeneration) and/or diffusely fragmented (floccular necrosis), in the presence of bacilli. Inflammatory infiltration varies from absent to moderate, consisting mainly of neutrophils, with hemorrhage, edema and gas also observed in varying degrees in the tissue. Anthrax can also occur in its visceral and asymptomatic form, when internal organs, such as the tongue, diaphragm or heart, are affected. Consequently, the animal has no visible injuries, and cases of sudden death are frequent [34,35]

Clostridia are saprophytic microorganisms that promote the decomposition of the carcass of dead animals, which is why the material must be collected immediately after the death or euthanasia of the animal. The material can be shipped fresh, cooled, frozen or fixed in 10% neutral formalin. The laboratory diagnosis of myonecrosis is presented in table 3 [34,35].

Table 3. Methods for diagnosing clostridial myonecrosis.

Diagnostic method	Advantage	Disadvantage
Bacterial isolation	Relatively simple execution, with media and reagents common to anaerobic bacteriology laboratories.	Time-consuming (about 48h) and may result in inaccurate results due to the different growth ability and oxygen tolerance of histotoxic clostridia.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Direct immunofluorescence	Practical and quick protocol (about 4 h) that can be carried out directly from the imprint of the collected material.	Requires antibodies conjugated to fluorochromes and a special microscope for reading.
Immunohistochemistry	Shipping the material in formaldehyde prevents its autolysis, preventing saprophytic clostridia from multiplying. The execution is relatively simple, and the reading is carried out using light microscopy.	Relatively time-consuming, depending on histological processing prior to execution.
Multiplex PCR	Relatively simple and quick execution.	It requires the prior isolation step, adding to the disadvantages of the method and the costs of equipment and reagents

Affected animals must be quickly treated, due to the super-acute course of the disease, with intravenous administration of high doses of penicillin. The chances of recovery are greater for animals at the beginning of the infection or those whose muscle damage is not widespread, but, in general, treatments are not successful. The most important measure for controlling and preventing myonecrosis outbreaks is the systematic vaccination of the herd. The use of vaccines results in a marked reduction in the incidence of these diseases. However, it is essential that hygiene measures are adopted, such as disinfection of needles and surgical instruments, asepsis of vaccine application sites or surgical procedures, adequate handling of carcasses, among others [8,10,11,34–36].

5. Enterotoxemias

Conditions caused by *C. perfringens* occur under specific conditions and in the presence of certain predisposing factors. As a consequence, the agent multiplies in the gastrointestinal tract of animals and produces exotoxins, which are mainly responsible for the development of the nosological condition. *C. perfringens* is classified into five types (A-E) based on the production of four main toxins, alpha, beta, epsilon, and iota (Table 4) [5,37,38].

Table 4. Characterization of alpha, beta, epsilon and iota toxins from *C. perfringens.*

	Alpha toxin	Beta toxin	Epsilon toxin	Iota toxin
Codification	Gene *plc*	Gene *cpb*	Gene *etx*	Genes *iap* e *iab*
Origem	Chromosomal	Plasmidial	Plasmidial	Plasmidial
Molecular weight (kDa)	43	40	33,7	47,5 and 105
Main effect	Intravascular hemolysis, capillary damage and platelet aggregation.	Formation of pores and changes in vascular permeability.	Change in vascular permeability.	Change in the organization of the cellular cytoskeleton .

Clostridium perfringens type B is the causative agent of dysentery in newborn lambs, and can also affect goats, calves and foals. This condition affects lambs under two weeks of age and is mainly caused by the action of beta toxin, which is inactivated by proteolytic enzymes, such as trypsin. The fact that the disease occurs primarily in newborn animals comes from excess colostrum, as colostrum is a source of antitrypsinic factors, thus favoring the action of the toxin. The disease presents a morbidity rate of around 30%, with lethality reaching 100% of affected animals. The course is super acute, generally within a few hours the animals present severe abdominal pain, reduced suction of

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

colostrum and/or milk, semi-fluid and red-stained feces, in addition to developing enteritis, with extensive hemorrhage and ulceration of the small intestine [9,39].

Clostridium perfringens type D is the agent of enterotoxemia commonly called overfeeding disease or pulpous kidney disease. A disease with worldwide distribution, which primarily affects sheep of any age, with the exception of newborns, and, less frequently, goats and cattle. In Brazil there are reports of the disease in the three species mentioned. Changes in the rumen microbiota as a result of sudden changes in diet, provision of diets rich in carbohydrates and low in fiber, among other factors, lead to the multiplication of the agent in logarithmic proportions, producing large amounts of epsilon toxin, in the form of protoxin, being converted into a lethal protein by the action of digestive trypsin or by secondary toxins *from C. perfringens*. The activated toxin acts on the intestinal epithelium, causing increased vascular permeability, and, upon reaching the blood circulation, reaches organs such as the brain, kidneys, lungs, liver and heart, where it binds to specific receptors on endothelial cells, leading to degeneration. of these cells. With increased vascular permeability, extravasation of fluids and proteins occurs into the perivascular space, with consequent edema. When it occurs in brain tissue, it is called eosinophilic proteinaceous perivascular edema or microangiopathy [40–44].

Under natural conditions and in most cases, the death of animals occurs during the first six to 18 hours, but if they survive for more than 36 to 48 hours, necrosis of the brain tissue occurs, known as focal symmetric encephalomalacia. The most common clinical form is super-acute, with death within four to eight hours, and neurological changes such as opisthotonus and pedaling movements can be observed; and respiratory changes such as tachypnea and pulmonary edema. Goats can also present these three forms of the disease, but neurological lesions are uncommon, mainly enteric lesions, such as diarrhea and enterocolitis, with neurological lesions being restricted to histopathological findings of eosinophilic proteinaceous perivascular edema or microangiopathy. At necropsy, findings may not be very evident, but in some cases hydrothorax, hydropericardium, hydroperitoneum, pulmonary edema and cerebellar hernia are found [44].

The confirmatory diagnosis is directly dependent on the detection of the toxin(s) produced by the agents, directly in the intestinal contents. The conventional method for detecting these toxins is the serum neutralization test in mice. There are also different PCR techniques that, despite not directly detecting the toxin(s) produced by *C. perfringens* types A-E, but rather the genes that encode their production, allow the typification of the *C. perfringens* involved in a particular case or outbreak. Although the presence of toxins in intestinal contents is the most important indicator of enterotoxemia, it is necessary to combine this finding with others, especially the history of the animal and the property, clinical signs, necropsy findings and histopathology of the injured organs [40–43].

Control measures aim to ensure correct hygiene management, environmental disinfection and systematic vaccinations of the entire herd, as animals are in permanent contact with agents and factors that could trigger diseases. In diseases that affect young animals, vaccination of females, with the aim of transferring passive immunity to their progeny, is the main strategy. In animals over four months of age, primary vaccination is recommended, with a booster dose four to six weeks later and annual revaccination. However, the lack of specific vaccines on the Brazilian market, especially for poultry and pigs, requires the earlier use of antimicrobials [45–47].

6. Conclusions

Clostridiosis in cattle presents a significant challenge for the Brazilian livestock industry. These infections, caused by various species of the Clostridium genus, are notorious for their rapid onset and high mortality rates, leading to substantial economic losses. Key clostridial diseases affecting cattle in Brazil include botulism (*Clostridium botulinum),* tetanus (*Clostridium tetani*), blackleg (Clostridium chauvoei), malignant edema (*Clostridium septicum* and others) and enterotoxemia (*Clostridium perfringens*). The current challenges are high mortality and economic impact because Clostridial diseases often result in sudden death, causing immediate and significant losses to cattle herds. The high mortality rate directly translates to financial setbacks for farmers due to the loss of livestock and decreased productivity. The environmental persistence of Clostridium spores can survive in the environment for extended periods, making it difficult to predict and control outbreaks. This persistence in soil and organic matter means that even well-managed farms can experience sudden disease outbreaks. The diagnosis and treatment are hampered because the rapid progression of these

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

diseases often leaves little time for effective intervention. While antibiotics and antitoxins can be used in some cases, their efficacy is limited if not administered early in the disease course. Additionally, the cost and logistics of treatment can be prohibitive for many farmers. Therefore, prevention and control with regular use vaccination remains the cornerstone of clostridial disease prevention. Vaccination programs should be comprehensive, covering all major clostridial pathogens, and should be consistently administered to young and at-risk cattle. And also, the farm management practices that must focus on good hygiene and management practices are crucial. This includes proper disposal of carcasses, ensuring clean and uncontaminated feed and water sources, and minimizing injuries that could become infection sites. A continuous surveillance for clostridial disease outbreaks helps in early detection and response. This requires robust veterinary services and cooperation among farmers, veterinarians, and government agencies.

As future perspectives are expected, efforts should focus on developing more effective and long-lasting vaccines. Research into multivalent vaccines that protect against multiple clostridial species with a single shot could improve compliance and outcomes. Advances in diagnostic technologies, such as rapid on-farm tests, could enable earlier detection and treatment, improving survival rates. Development of portable, cost-effective diagnostic tools could greatly benefit small-scale and remote farmers. Increased education and training for farmers on best practices for disease prevention and management is essential. Extension services and veterinary outreach programs can play a critical role in disseminating this knowledge. Continued research into the epidemiology, pathogenesis, and control of clostridial diseases will provide new insights and solutions. Collaboration between research institutions, government agencies, and the private sector can drive innovation in disease management.

In conclusion, while clostridial diseases pose a formidable challenge to cattle farming in Brazil, a multifaceted approach involving vaccination, good management practices, surveillance, and research holds the key to mitigating their impact. By investing in future perspectives, the Brazilian livestock industry can enhance its resilience against clostridial infections, ensuring the health and productivity of cattle herds and the economic stability of the sector.

Author Contributions: Conceptualization, F.M.S.; methodology, F.M.S.; formal analysis, F.M.S; investigation, F.M.S.; data curation, F.M.S.; writing—original draft preparation, F.M.S.; writing—review and editing, F.M.S. The author has read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors are grateful to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPESPA (Fundação Amazônia de Amparo a Estudos e Pesquisas do Estado do Pará) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; finance code: 001.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Danilyuk, A.V.; Kapustin, A.V. The prevalence and species diversity of clostridia, the causative agents of anaerobic infections in cattle. *Trudi VIEV*, **2019,** 81, 19-26. http://doi.org/10.30917/ATT-PRINT-2019-10
2. Jerram, L. Clostridial disease in cattle. *Livestock,* **2019**, 24, 274–279. http://doi.org/10.12968/live.2019.24.6.274
3. Uzal, F.A.; Navarro, M.A.; Li, J.; Freedman, J.C.; Shrestha, A., McClane B.A. Comparative pathogenesis of enteric clostridial infections in humans and animals. *Anaerobe,* **2018,** 53, 11–20. http://doi.org/10.1016/j.anaerobe.2018. 06.002
4. Robi, D. T.; Mossie, T.; Temteme, S. A. Comprehensive Review of the Common Bacterial Infections in Dairy Calves and Advanced Strategies for Health Management. *Vet. Med.: Res. Repor.* **2024**, *15*, 1–14. https://doi.org/10.2147/VMRR.S452925
5. Popoff, M.R.; Bouvet, P. Clostridial toxins. *Future Microbiol.* **2009**, 4, 1021-1064. https://doi.org/10.2217/fmb.09.72
6. Santos, B.L.; Ladeira, S.R.L.; Riet-Correa, F.; Soares, M.P.; Marcolongo-Pereira, C.; Sallis, E.S.V.; Raffi, M.B.; Schild A.L. 2019. Clostridial diseases diagnosed in cattle from the South of Rio Grande do Sul, Brazil. A forty-year survey (1978-2018) and a brief review of the literature. *Pesq. Vet. Bras.* **2019,** 39, 435-446. https://doi.org/10.1590/1678-5150-PVB-6333
7. Rings, D.M. Clostridial disease associated with neurologic signs: tetanus, botulism, and enterotoxemia. *Vet. Cl.in North. Am. Food. Anim. Prac.* **2004**, 20, 379-91, vii-viii. http://doi.org/10.1016/j.cvfa.2004.02.006.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

8. Uzal, F.A.; Otter, A. Clostridial diseases in farm animals: 2. Histotoxic and neurotoxic diseases. *In Partice*, **2020**, 42, 279-288. https://doi.org/10.1136/inp.m1984
9. Uzal, F.A.; Otter, A. Clostridial diseases in farm animals: 1. Enterotoxaemias and other alimentary tract infections. *In Partice*, **2020**, 42, 219-232. https://doi.org/10.1136/inp.m1462
10. Zaragoza, N.E.; Orellana, C.A.; Moonen, G.A.; Moutafis, G.; Marcellin, E. Vaccine production to protect animals against pathogenic Clostridia. *Toxins*, **2019,** 11, 525. https://doi.org/10.3390/toxins11090525
11. Rodrigues, R.R.; Ferreira, M.R.A.; Kremer, F.S.; Donassolo, R.A.; Moreira Júnior, C.; Alves, M.L.F.; Conceição, F.R. Recombinant Vaccine Design Against Clostridium spp. Toxins Using Immunoinformatics Tools. *Methods Mol Biol*. **2022**, 2412, 457-470. http://doi.org/10.1007/978-1-0716-1892-9_25
12. Meurens, F.; Carlin, F.; Federighi, M; Filippitzi, M.E.; Fournier, M.; Fravalo, P.; Ganière, J.P.; Grisot, L.; Guillier, L.; Hilaire, D.; Kooh, P.; Le Bouquin-Leneveu, S.; Le Maréchal, C.; Mazuet. C.; Morvan, H.; Petit, K.; Vaillancourt, J.P.; Woudstra, C. *Clostridium botulinum* type C, D, C/D, and D/C: *Front. Microbiol.* **2023**, 5, 1099184. https://doi.org/10.3389/fmicb.2022.1099184
13. Silva ROS, Oliveira Junior CA, Gonçalves LA, Lobato FCF. Botulism in ruminants in Brazil. Cienc Rural . 2016Aug;46(8):1411–7. https://doi.org/10.1590/0103-8478cr20151486.
14. Păuna, A.M.; Crăciun, M.-D.; Sîrbu, A.; Popescu, R.; Enciu, B.G.; Chivu, C.-D.; Simoiu, M.; Piţigoi, D. Botulism Cases in Romania—An Overview of 14-Year National Surveillance Data. *Biomed.* **2024**, *12*, 1058. https://doi.org/10.3390/biomedicines12051058
15. Soares, M.C.; Gaspar, A.O.; Brumatti, R.C.; Gomes, D.C.; Neves, D.A.; Alcântara, L.O.B.; Leal, P.V.; Lemos, R.A.A. Economic impact of an outbreak of botulism in a cattle feedlot. *Pesq. Vet. Bras.* **2018,** 38, 1365-1370. https://doi.org/10.1590/1678-5150-PVB-5643
16. Helayel, M.A.; Lopes, S.P.; Ramos, A.T.; Cunha, I.M.; Ramos, L.F.C.S.; Dutra, I.S.; Carvalho, V.A.N.; Caldas, S.A. Epidemiological, clinicopathological, and economic aspects of an outbreak of botulism in beef cattle in the State of Tocantins, Brazil. *Braz. J. Vet. Med.* **2019,** *41*, e101919. https://doi.org/10.29374/2527-2179.bjvm101919.
17. Dutra, I.S.; Döbereiner, J.; Souza, A.M. Botulism in beef and dairy cattle fed with poultry litter. *Pesq. Vet. Bras.* **2005,** 25, 115-119. http://dx.doi.org/10.1590/S0100-736X2005000200009.
18. Dutra, I.S.; Döbereiner, J.; Rosa, I.V.; Souza, L.A.A.; Nonato, M. Botulism outbreaks in cattle in Brazil associated with contaminated water. *Pesq. Vet. Bras.* **2001,** 21, 43-48. http://dx.doi.org/10.1590/S0100-736X2001000200002.
19. Souza, A. M.; Marques, D. F., Döbereiner, J.; Dutra, I. S. Esporos e toxinas de *Clostridium botulinum* dos tipos C e D em cacimbas no Vale do Araguaia, Goiás. *Pesq. Vet. Bras.* **2006,** 26, 133-138. http://dx.doi.org/10.1590/S0100-736X2006000300001
20. Souillard, R.; Le Maréchal, C.; Ballan, V.; Mahé, F.; Chemaly, M.; Le Bouquin, S. A bovine botulism outbreak associated with a suspected cross-contamination from a poultry farm. *Vet Microbiol.* **2017**, 208, 212-216. https://doi.org/10.1016/j.vetmic.2017.07.022.
21. Le Maréchal, C.; Hulin, O.; Macé, S.; Chuzeville, C.; Rouxel, S.; Poëzevara, T.; Mazuet, C.; Pozet, F.; Sellal, E.; Martin, L. A Case Report of a Botulism Outbreak in Beef Cattle Due to the Contamination of Wheat by a Roaming Cat Carcass: From the Suspicion to the Management of the Outbreak. *Animals*, **2019**, *9*, 1025. https://doi.org/10.3390/ani9121025
22. Gil, L.A.; da Cunha, C.E.; Moreira, G.M.; Salvarani, F.M.; Assis, R.A.; Lobato, F.C.; Mendonça, M., Dellagostin, O.A.; Conceição, F.R. Production and evaluation of a recombinant chimeric vaccine against clostridium botulinum neurotoxin types C and D. *PLoS One*, **2013**, 31. e69692. https://doi.org/10.1371/journal.pone.0069692.
23. Moreira Júnior, C., Rodrigues, R.R.; da Cunha, C.E.P.; Donassolo, R.A.; Ferreira, M.R.A.; Finger, P.F.; Oliveira, H.G.S.; da Cruz, K.P., Moreira, Â.N.; Salvarani, F.M.; Conceição, F.R. Evaluation of long-term immune response in cattle to botulism using a recombinant *E. coli* bacterin formulated with Montanide™ ISA 50 and aluminum hydroxide adjuvants. *Microb. Pathog.* **2024**, 189, 106596. https://doi.org/10.1016/j.micpath.2024.106596
24. Popoff, M.R. Tetanus in animals. *J. Vet. Diag. Invest.* **2020**, 32, 184-191. https://doi.org/10.1177/1040638720906814
25. Malinovská, Z.; Čonková, E.; Váczi, P. "Tetanus in Animals — Summary of Knowledge". *Folia Vet.* **2020,** 64, 54-60. https://doi.org/10.2478/fv-2020-0027
26. Garber, J.R.O; Smith, B. I. (2011). Tetanus in cattle: Review and case description of clinical tetanus in a Holstein heifer. *The Bovine Pract.* **2011,** 45, 110–117. https://doi.org/10.21423/bovine-vol45no2p110-117
27. Quevedo, P.S.; Ladeira, S.R.L.; Soares, M.P.; Marcolongo-Pereira, C.; Sallis, E.S.V.; Grecco, F.B.; Estima-Silva, P.; Schild A.L. Tetanus in cattle in southern Brazil: study of 24 outbreaks. *Pesq. Vet. Bras.* **2011,** 31, 1066-1070. https://doi.org/10.1590/S0100-736X2011001200005
28. Sousa, A.I.d.J.; Galvão, C.C.; Pires, P.S.; Salvarani, F.M. Blackleg: A Review of the Agent and Management of the Disease in Brazil. *Animals* **2024**, *14*, 638. https://doi.org/10.3390/ani14040638

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

29. Oliveira Junior, C.A.; Silva, R.O.S.; Lobato, F.C.F.; Navarro, M.A.; Uzal, F.A. Gas gangrene in mammals: a review. *J. Vet. Diagn. Invest.* **2020**, 32, 175-183. https://doi.org/10.1177/1040638720905830.
30. Kapustin, A.V.; Laishevtcev, A.I.; Ivanov, E.V.; Danilyuk, A.V. Species diversity of Clostridia causing malignant edema in cattle. *IOP Conference Series: Earth Env. Sc.* **2020**, 548, 072041. http://doi.org/10.1088/1755-1315/548/7/072 041.
31. Jing, W.; Pilato, J.L.; Kay, C.; Feng, S.; Tuipulotu, D.E.; Mathur, A. *Clostridium septicum* α-toxin activates the NLRP3 inflammasome by engaging GPI-anchored proteins. *Sci Immunol.* **2022**, 7, eabm1803. https://doi.org/10.1126/sciimmunol.abm180
32. Monturiol-Gross, L.; Flores-Díaz, M.; Araya-Castillo, C.; Pineda-Padilla, M.J.; Clark, G.C.; Titball, R.W. Reactive oxygen species and the MEK/ERK pathway are involved in the toxicity of *Clostridium perfringens* α-toxin, a prototype bacterial phospholipase. *C. J. Infect. Dis.* **2012**, 206, 1218–26. https://doi.org/10.1093/infdis/jis496
33. Zhang, X.; Liu, Y.; Shen, Z. Osthole ameliorates myonecrosis caused by *Clostridium perfringens* type A infection in mice. *One Health Adv.* **2023,** 27. https://doi.org/10.1186/s44280-023-00028-6
34. Heckler, R.F.; Lemos, R.A.A.; Gomes, D.C.; Dutra, I.S.; Silva, R.O.S.; Lobato, F.C.F.; Ramos, C.A.N.; Brumatti, R.C. Blackleg in cattle in the state of Mato Grosso do Sul, Brazil: 59 cases. *Pesq. Vet. Bras.* **2018**, 38, 6-14. https://doi.org/10.1590/1678-5150-PVB-4964
35. Pires, P.S.; Ecco, R.; Silva, R.O.S.; Araújo, M.R.; Salvarani, F.M.; Heneine, L.G.D., Oliveira Júnior, C. A.; Lobato, F.C.F. A retrospective study on the diagnosis of clostridial myonecrosis in ruminants in Brazil. *Ciên. Rur.* **2017**,47, e20160492. https://doi.org/10.1590/0103-8478cr20160492
36. Alves, M.L.F.; Ferreira, M.R.A.; Donassolo, R.A.; Rodrigues, R,R,; Conceição, F.R. *Clostridium septicum*: A review in the light of alpha-toxin and development of vaccines. *Vaccine*, **2021**, 39, 4949-4956. https://doi.org/10.1016/j.vaccine.2021.07.019
37. Lobzin, Yu.V.; Kvetnaya, A.S.; Skripchenko, N.V.; Zhelezova, L.I. Current notions about etiopathogenic and genetics specific features of *Clostridium perfringens* toxins. *J. Microb. Epid. Immunob.* **2021,** 98, 91-103. http://doi.org/10.36233/0372-9311-37
38. Gohari, I.M.; Navarro, M.A.; Li, J.; Shrestha, A.; Uzal. F.; McClane. B.A. Pathogenicity and virulence of *Clostridium perfringens*. *Virulence*, **2021,** 12, 723–753. http://doi.org/10.1080/21505594.2021.1886777
39. Rood, J.I.; Adams, V.; Lacey, J.; Lyras, D.; McClane, B.A.; Melville, S.B.; Moore, R.J.; Popoff, M.R.; Sarker, M.R.; Songer, J.G.; Uzal, F.A.; Van Immerseel, F. Expansion of the *Clostridium perfringens* toxin-based typing scheme. *Anaerobe*, **2018, 5**3, 5–10. http://doi.org/10.1016/j.anaerobe.2018.04.011.
40. Geier, R.R.; Rehberger, T.G.; Smith, A.H. Comparative genomics of *Clostridium perfringens* reveals patterns of host-associated phylogenetic clades and virulence factors. *Front. Microbiol.* **2021,** 12, 649953. http://doi.org/10.3389/fmicb.2021.649953
41. Lu, W.; Sun, H.; Xu, Z.M.; Du, Z.; Si, L.; Yuan, S.; Jin, J.; Jin, C.H. Diagnostic and therapeutic strategy for *Clostridium perfringens* infection in postpartum dairy cows: a report of 14 cases. *J. App. An. Res.*, **2022,** 50, 350-354; https://doi.org/10.1080/09712119.2022.20783 29
42. Amini, M.; Kheirkhah, B.; Shamsaddini Bafti, M.; Rokhbakhsh Zamin, F. Molecular identification and sequence analysis of *Clostridium perfringens* virulence genes isolated from sheep and goats. *J Hellenic Vet. Med. Soc.*, **2023,** 74, 5345–5354; https://doi.org/10.12681/jhvms.29434
43. Nekrasov, R.V.; Lozovanu, M.I.; Laptev, G.Y.; Ilina, L.A.; Yildirim, E.A.; Tyurina, D.G.; Melikidi, V.C.; Gorfunkel, E.P.; Filippova, V.A.; Malahov, I.G.; et al. Bioactive Feed Additive for the Prevention of Clostridial Disease in High-Yielding Dairy Cattle. *Agriculture*, **2023**, *13*, 786. https://doi.org/10.3390/agriculture13040786
44. Filho, E.J.; Carvalho, A.U.; Assis, R.A.; Lobato, F.C.; Rachid, M.A.; Ferreira, P.M.; Nascimento, R.A.; Fernandes, A.A.; Vidal, J.E,; Uzal, F.A. Clinicopathologic features of experimental *Clostridium perfringens* type D enterotoxemia in cattle. *Vet Pathol.* **2009**, 46, 1213-20. https://doi.org/10.1354/vp.08-VP-0304-U-FL.
45. Moreira, G.M.; Salvarani, F.M.; da Cunha, C.E.; Mendonça, M.; Moreira, Â.N.; Gonçalves, L.A.; Pires, P.S.; Lobato, F.C.; Conceição, F.R. Immunogenicity of a Trivalent Recombinant Vaccine Against *Clostridium perfringens* Alpha, Beta, and Epsilon Toxins in Farm Ruminants. *Sci Rep.* **2016**,23,22816. https://doi.org/10.1038/srep22816.
46. Ferreira, M.R.A.; Moreira, G.M.S.G.; Cunha, C.E.P.d.; Mendonça, M.; Salvarani, F.M.; Moreira, Â.N.; Conceição, F.R. Recombinant Alpha, Beta, and Epsilon Toxins of *Clostridium perfringens*: Production Strategies and Applications as Veterinary Vaccines. *Toxins*, **2016**, *8*, 340. https://doi.org/10.3390/toxins8110340
47. Galvão, C.C.; Barbosa, J.D.; Oliveira, C.M.C.; Otaka, D.Y.; Silva, P.R.O.; Ferreira, M.R.A.; Moreira Júnior, C.; Conceição, F.R.; Salvarani, F.M. Measurement over 1 Year of Neutralizing Antibodies in Cattle Immunized with Trivalent Vaccines Recombinant Alpha, Beta and Epsilon of *Clostridium perfringens*. *Toxins*, **2021**, *13*, 594. https://doi.org/10.3390/toxins13090594

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 May 2024 doi:10.20944/preprints202405.1322.v1

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.