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CLOSTRIDIAL TOXINS - POTENT POISONS, POTENT MEDICINES

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ABSTRACT: *Clostridium* is an anaerobic bacterial genus. The clostridia produce more protein toxins than any other bacterial genus and are a rich reservoir of toxins for research and medicinal uses. Clostridia are widely spread in the environment: soil, dust and water, presenting more than 120 described species, although few can cause diseases. Diseases can grossly be divided into neurotropic disorders (nervous system is primarily affected), enterotoxemias (affecting intestinal tract and parenchymatous organs), and gas gangrene (myonecrosis with toxemia). Undoubtedly the most widely recognized infection due to anaerobes was clostridial myonecrosis, but recently interest has arisen for the role of clostridia in intestinal diseases. This report describes the most important species, the diseases caused by them, and their occurrence in Brazil, focusing on cattle raising.

KEY WORDS: Clostridiosis, *Clostridium* spp, toxins.

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INTRODUCTION

History

Lavoisier (1743-1794) discovered the respiration process and at that time just the oxygen-dependent creatures were known. Anaerobiosis was described in 1861, when Louis Pasteur proved that butyric fermentation occurred in the absence of oxygen, introducing the terms anaerobic and aerobic. Although Pasteur's idea of life without oxygen has faced much opposition, it was based on those studies that Anaerobic Microbiology was born (39).

The history of Clostridia begins in 1871 with Bottini's demonstration of the bacterial nature of Gas Gangrene (GG), although the microorganism was not isolated. Almost at the same time a similar type of infection, Black Leg (BL), was also recognized in cattle, and in 1879 it was shown to be due to an anaerobic bacillus named *Bacterium chauvoei* (today, *C. chauvoei)*, constituting thus the first naturally occurring anaerobic infection identified. After that, in 1891, Koch described an experimentally induced infection, the Malignant Edema (ME), very similar to BL, identifying the agent as *Oedembazillus,* the *Vibrion septique* described by Pasteur. Chauveau and Arloing (1884) proved that GG and ME were the same disease and *Oedembazillus* and *Vibrion septique* a unique bacterium. But it was only by 1919 that it became clear that *C. chauvoei* infections were exclusive of animals, and ME and GG were slightly different clinical manifestations that could be caused by many other *Clostridium* species (39).

Clostridium spp is one of the genera that comprise the group of anaerobic bacteria of considerable medical and economic importance. Clostridiosis is the general name given to a variety of diseases caused by the bacteria of this genus, which can occur in any part of the body that offers conditions for their development with consequent toxin production during multiplication.

Clostridia are widely spread in the environment: soil, dust and water, and are part of the flora. MacLennan (39) says that large number and wide distribution make clostridia one of the commonest types of bacteria found in wounds". More than 120 described species make up this genus, but few can produce diseases. Much of what is known today about clostridial toxins is due to GG cases during wartime, undoubtedly the most recognized infection due to anaerobes. More recently, interest has arisen for its role in intestinal diseases. The genus *Clostridium* consists of a diverse group of gram-positive bacteria which do not grow in the presence of oxygen,

being strict anaerobes, and having the ability to form heat-resistant endospores, an important factor in the epidemiology of the wide range of diseases they can cause (63).

Clostridia produce more protein toxins than any other bacterial genus, what makes them a source of knowledge for researchers in many areas, from pathology to molecular biology, allowing for the codification of gene proteins, and for medicinal uses (33). In the past, those toxins were designated by the first letters of the Greek alphabet in the order of their recognition; so for different species, despite the same letter, the toxins do not have the same biological property.

Virulence - the capacity to damage or kill the host - of clostridia is attributed to the properties of invasiveness and toxigenicity, expressed separately or in combination. Although not directly linked with virulence, spores play an important role in its expression, as they allow the bacteria to survive through adverse circumstances. Other pathogen structures are also of great value: adhesins (fimbriae or pili), permitting microorganisms adherence; and capsule, which confers resistance to phagocytosis by neutrophils. Invasiveness is another characteristic, although in general anaerobes are not invasive; in myonecrosis, when it is established, they can be fiercely aggressive. Exotoxins, though, are a relevant factor, responsible for the wide-ranging features of the diseases (16).

Based upon clinical aspects, this paper will consider clostridial species that can cause neurotropic disorders (*C. botulinum* and *C. tetani*), those that affect the intestinal tract and parenchymatous organs (*C. perfringens* and *C. difficile*), and finally those that determine myonecrosis (*C. chauvoei, C. septicum, C. haemolyticum, C. novyi*, *C. hystolyticum,* and *C. sordellii*). It also presents a review of the literature on the genus *Clostridium*, the pathogenic species, the toxins produced, their action, clinical aspects, and occurrence in Brazil livestock.

Clostridium botulinum

Clostridium botulinum spores commonly occur in soil, water, and marine sediments throughout the world and are normal inhabitants of the intestinal tract of animals (66). They produce one of the most highly toxic substances known, a neurotoxin with a high fatality rate: botulinum toxin type A is about 1,000 times more toxic than tetanus toxin at the myoneural junction. Seven toxinotypes (A, B, C, D, E, F, and G) are responsible for botulism symptoms. Herbivores are more susceptible to the toxin than

carnivores. The turkey vulture is known to be the only resistant species; it is able to resist 100,000 times as much type C toxin as it is required to kill a pigeon (35). Types A, B, E, and F are mainly involved in botulism in humans, while types C and D are mainly involved in animals (74). There has never been a confirmed outbreak of type G botulism. For Brazil, the Central Laboratory of Food Microbiology of Adolfo Lutz Institute has registered, from 1982 to 2001, 40 suspected cases/outbreaks. From those, 8 were confirmed to be botulism, and in 7 of them the toxin was identified as type A (21). Three categories of the disease are considered for human: foodborne, infant, and wound botulism. Human and animal botulism most often are foodborne, occurring after ingestion of toxin preformed in food (79). Human cases are associated with the ingestion of improperly preserved food, meat, fish, vegetables such as beans, peas or beets, and, more recently, of unusual food such as sautéed onions stored unrefrigerated (75), and bottled chopped garlic (76, 80). When botulinum toxin is ingested, part is destroyed by digestive processes and the part absorbed into the blood stream disseminates throughout the body and reaches peripheral nerve endings that have acetylcholine as a neurotransmitter. The central nervous system is protected by the blood-brain barrier. The flaccid paralysis results from the inhibition of the acetylcholine release at the neuromuscular junctions, and because of this no stimulus reaches the motor endplates, resulting in double vision and salivation as there is a difficulty in swallowing, leading to death by asphyxiation due to diaphragm paralysis. Although the toxin binds rapidly and irreversibly to tissue receptors, it does not immediately cause paralysis, a very important condition for the collection of samples for laboratory diagnosis (65).

The quantity of toxin ingested in foodborne botulism is not sufficient to trigger antibody formation, since recurrent cases in the same patient, with types B and C, have been reported in the literature (65).

Serological detection of the specific toxin remains the essential procedure for diagnosis in man and animals (73), and currently the most sensitive standard method of botulism toxin detection is the mouse bioassay (18). Botulinum toxin can also be produced by *C. butyricum*, *C. barati*, and *C. argentiniense*, causing infant botulism (27).

C. botulinum type C is responsible for botulism in waterfowl, poultry, mink, cattle and other animal species, although few human cases have been recorded (71).

Kalmbach (34) demonstrated experimentally that a variety of dead invertebrates and their debris can support the growth of *C. botulinum* type C and toxin production.

Bovine botulism, usually involving *C. botulinum* type D, is an intoxication which results from the eating (due to phosphorus deficiency) of carcasses left in the pasture or other decomposed animal matter, hay, or silage contaminated by decomposing carcasses of small animals or birds. It also results from the drinking of stagnant water containing decaying vegetation or carcasses in which a toxin has been elaborated (70). Less common sources are silage made of grains (brewer and corn) and grass, and also fowl litter (type C), used as a dietary supplement (37, 51).

Avian botulism causes huge losses in waterfowl throughout the world (37) and in fowl raising plants: layer (15, 20) and ornamental (58). The agent can colonize in the crop or fowl cecum, but fly larvae is the most important source of toxin (26, 32, 74).

All the serotypes of toxin can poison, but type A has caused the severest illness and many deaths. On the other hand, since the approval by the US Food and Drug Administration in 1989, the use of type A toxin for some disorders (strabismus, blepharospasm and hemifacial spasm) and the number of indications for treatment have increased (33), including cosmetic applications of which Brazil is considered to be the top user in volume injected according to Dr. Valcinir Bedin, President of the Brazilian Society of Medicine in 2005.

Clostridium tetani

C. tetani is a strict anaerobe found in soil samples of all parts of the world and in human and animal feces. It has terminal endospores with a characteristic drumstick form. *C. tetani* is not very invasive, but can establish in wounds and produce enough toxin to kill. Tetanus is an acute, often fatal, toxigenic disease that results from the production and absorption of tetanospasmin in the contaminated wound, as the participation of tetanolysin is questionable in the infection (45).

The tetanospasmin acts primarily in the central nervous system, blocks inhibitory synapses of the spinal cord, and causes hyperactivity of the motor system and spastic paralysis. The toxin spreads from the infected site by diffusing into the adjacent muscle tissue by transport via lymphatic system, or by passage through the nerves. The toxin enters the blood from the lymphatic system, attaches to a receptor on the nerve ending, and a fragment of the bound toxin is taken into the nerve cell and passes on to the central nervous system by retrograde movement through the

nerve axons (85). It is 2,000 times more toxic at central inhibitory nerves than at peripheral synapses (46).

In humans, symptoms begin with facial stiffness in the jaw and abdomen, progressing to the neck, trunk and extremities. The incidence of tetanus neonatorum is high in developing countries because mothers are not immunized and deliveries are at home. Recurrent sensitivity to tetanus toxin was observed in humans (65). Damage to the muscular junction appears to be permanent and recovery requires the formation of new synapses (46). In cattle, affected animals present muscular tremors, stiff gait and a slightly raised tail. The third eyelid becomes prominent; death comes in about a week with respiratory difficulty. Loud noises or touching elicit tetanic contractions (70,79).

Fowl are considered resistant, and among domestic mammals, equine are considered the most sensitive to tetanus (14), making their vaccination against tetanus a common practice. In dogs and cats, it is rare. Ruminants have a naturally acquired resistance but the disease can occur after delivery, castration or wounds by contamination with *C. tetani* spores (43, 61).

Clostridium perfringens

Among the species in this genus, *C. perfringens (Cp)* is the most widely distributed pathogen (62, 67), being recognized as the cause of many human and animal diseases (22, 55, 63). In humans, it causes GG, food poisoning, and several other diseases (5). In animals, various forms of acute enteritis, fatal enterotoxemias, hemorrhagic enteritis, and sudden death have been reported (7, 49, 57, 63, 68, 69). This species can produce up to 17 different antigens also named toxins, although only a selection of these enzymes is produced by a given strain of this agent (63). Four of these, alpha, beta, epsilon, and iota, are responsible for the tissue lesions and the hosts death (17, 63, 77) and are considered to be major toxins, being used to group the bacteria into their five toxigenic types, A, B, C, D, and E (Table 1) (77). All five types of *Cp* produce alpha toxin, but type A strains usually produce it in great quantity (55).

Table 1: Classification of *C. perfringens* into types based on the major toxins produced (40).

Type	Major Toxins
A	Alpha
B	Alpha, beta, epsilon
C	Alpha, beta
D	Alpha, epsilon
E	Alpha, iota

All the toxins are extracellular enzymes with the exception of the enterotoxin, which is a sporulation-related toxin. Many of these virulence factors are simple hydrolytic enzymes secreted by the organism as part of its saprophytic life in the soil, where they are probably involved in putrefaction processes. The genetic regulation of the expression of these virulence factors is important for the development of the infection (62, 67). Type A strains are the most commonly encountered under noninfectious conditions, and they are the only ones associated with the microflora of both soil and the intestinal tract. Types B, C, D, and E are invariably restricted to the intestinal tract, primarily of animals, and occasionally of man (55). In 1997, it was discovered another type, beta-2, associated with piglet gastrointestinal disease (22), and afterwards recognized as pathogenic for horses (30), dogs (81), African elephants (4), and bovine calves (42). Types A, C, and D are related to human diseases, and all types affect animals.

Cp type A is the causative agent of GG, food poisoning, and enterotoxemias in man. According to Waters *et al*. (87), beta-2 is type-A-toxin-dependent to produce damage. For animals, several types can cause enterotoxemias in neonates and adults. In neonates, the infection occurs when *Cp* colonizes in the intestine prior to the normal flora. On the other hand, in adults it happens when diet is changed abruptly, determining changes in the intestinal flora. Consumption of large quantities of carbohydrates elicits gas production that can lead to stasis, giving rise to *Cp* colonization and consequently toxin production. Enterotoxemia usually occurs in bovine populations when prophylactic measures are not observed.

The presence of *Cp* is also a public health problem for humans, who can be intoxicated by the ingestion of contaminated meat. It is most commonly associated with the consumption of cooked, uncured meat products that have been cooled

slowly or stored under inadequate refrigeration and then consumed without reheating. The prerequisite for diarrhea can be the consumption of large numbers of vegetative cells that survive the acidity in the stomach and reach the intestine, where they sporulate and thereby liberate the enterotoxin, which causes diarrhea. Necrotizing enteritis has also been observed with sudden dietary changes in Germany and Papua New Guinea due to type C *Cp* organisms consumed with the meat, or with the multiplication of part of the intestinal flora after the ingestion of meat (protein), producing beta-toxin. Because of dietary problems, low levels of proteases are not sufficient to destroy the toxin produced (86).

Clostridium difficile

C. difficile is considered commensal of 40-50% of neonate intestines and rarely of those of adults, where it is associated with antibiotic-induced enterocolitis, pseudomembranous colitis, and antibiotic-associated colitis (86). Two toxins are related to this organism, A and B, one is not produced without the other, and there is a correlation between the amounts of toxin produced (38). Both are lethal for mice, although type A potency is 50 to 400 times that of type B (36, 59, 64).

Toxin A has enterotoxic activity and is as cytotoxic as type B when tested in an appropriate cell line. Toxin B is potently cytotoxic, but active just in damaged intestine, and therefore not considered, by some, to be important in the disease. When the gut mucosa is compromised, the toxins can disseminate from the intestine to other target organs (38). The enterotoxic effect is seen when toxin A binds to its receptor. The toxin is internalized, causing tissue damage and influx of inflammatory cells. The inflammatory process leads to tissue damage and alteration of membrane permeability, resulting in the extravasation of serous fluid and hemorrhagic necrosis (38, 60, 82).

Other virulence factors, such as the motility-altering factor and other enterotoxins, besides type A toxin, are being studied to confirm their participation in the disease (38).

Clostridium chauvoei and *Clostridium septicum*

C. chauvoei and *C. septicum* are similar and considered, by some, to be members of the same species (39, 89).

C. chauvoei and *C. septicum* produce many toxic substances. The number and type of these vary with the strain, the culture medium, and the length of the incubation period (47).

C. chauvoei affects only animals, is presumably ingested, and seems to occur preferentially among the best calves in the herd. It produces four toxins: α-hemolisin, necrotoxin; β-deoxyribonuclease; γ-hyaluronidase; and δ-hemolysin.

C. septicum produces five toxins: α-lethal, necrotic, hemolytic; β-deoxyribonuclease; γ-hyaluronidase; δ-O_2-labile hemolysin (serologically related to that of *C. chauvoei* but not identical); and collagenase (86).

C. septicum was the first reported cause of GG due to war wounds (39). For a long time it was thought to be the most important agent in human pathology. Nowadays, it is associated with malignant disease, patient's intestinal tract probably being the source of infection (1, 53). It is also considered the agent of neutropenic enterocolitis (12).

Both bacteria are associated with cattle and sheep GG (29), the former being responsible for black leg, and the other causing ME (39). Black leg in cattle is a nontraumatic endogenous infection, and a considerable proportion of bovines may harbor *C. chauvoei* in their liver. Affected animals are anorectic, depressed, febrile, and lame (one side limb), presenting a hot, painful swelling that becomes cold, edematous with crepitation. Death is seen within 12 to 48 hours. This agent seems to have a preference for big muscles (thigh, diaphragm, and heart), which at necropsy are dark red, dry, and spongy (70). In sheep, it is associated with shearing, castration, and dehorning; the muscles affected are dark, the edema restricted, and death comes after 12 to 36 hours (29).

ME is characterized by fever and subcutaneous swelling; the infected muscles become deep red (dark) with bloody gelatinous fluid accumulation in the body cavities, not noted with *C. chauvoei* infection. In sheep, in which the disease is named Braxy, *C. septicum* invades the mucosa and submucosa of the abomasum, where it multiplies, producing toxin that leads to death (70, 79).

During the year of 1983, in the southern states of Brazil, there were many bovine deaths caused by *C. septicum*. This happened at the same moment as the Ministry of Agriculture has issued a law obliging the refrigeration of all products of animal origin. As the commercial establishments were committed to selling Foot and Mouth

Disease vaccines (economically more convenient), the clostridial vaccines were not available for use, thus the herds, not being protected, manifested the disease.

Clostridium novyi* and *Clostridium haemolyticum

C. novyi, formerly named *C. oedematiens,* is (together with *C. haemolyticum*) among the most O_2-sensitive bacteria and fastidious microorganisms for cultivation and isolation. This may be the reason why it is not so frequently recognized as an agent of histotoxic human cases (39). It is usually associated with *C. sporogenes* and considered by some to be the agent in one-third of the GG cases. It is responsible for infections in domestic animals, Bacillary Hemoglobinuria (BH) of cattle, and sheep necrotic hepatitis, liver parasitism being the infection origin (72).

C. haemolyticum is, by DNA studies, closely related to *C. novyi* type B (48). The diseases caused by both primarily involve β-toxin of *C. haemolyticum* and α-toxin of *C. novyi*. In human GG, *C. novyi* α-toxin produces capillary permeability that leads to massive edema, while β-toxin of *C. haemolyticum* in BH destroys circulating erythrocytes, leading to hemoglobin excretion in the urine and blood appearing in the intestinal lumen due to endothelium capillary destruction, being a highly fatal disease of cattle (72). These great differences in pathological aspects provide for the maintenance of the two species.

Nakamura *et al.* (48) showed a genetic relatedness among *C. novyi*, *C. haemolyticum* and *C. botulinum.*

Table 2 shows the toxins produced by these two species, used for their classification.

Table 2: *C. novyi* and *C. haemolyticum* toxins and their activities (28).

Toxin	Activity	*C. novyi*			*C. haemolyticum*
		A	B	C	(*C. novyi* D)
Alpha	Necrotizing, lethal	+	+	-	-
Beta	Lecithinase, necrotizing, lethal, hemolytic	-	+	-	+
Gamma	Lecithinase, necrotizing, hemolytic	+	-	-	-
Delta	O_2-labile hemolysin	+	-	-	-
Epsilon	Lipase	+	-	-	-
Zeta	Hemolysin	-	+	-	-
Eta	Tropomyosinase	-	+	-	-
Theta	Breaks egg yolk lecithin	-	Tr	-	+

C*lostridium sordellii* and *Clostridium bifermentans*

C. sordellii was first isolated from a human edematous wound, and from then on it was implicated in histotoxic diseases in cattle (25, 39). It can be a normal inhabitant, with other clostridia, in the liver and elsewhere without being the primary causative agent of the disease (70).

Recently, fatal human cases have been reported due to episiotomy, spontaneous endometritis with maternal death, and deep laceration of a thigh infection (13, 31, 44, 78).

C. bifermentans is related to *C. sordellii* but does not have the lethal toxin. *C. sordellii* produces four toxins, three in common with *C. bifermentans*: fibrinolysin, lecithinase, and O_2-labile hemolysin. Lethal toxin, responsible for the severe generalized gelatinous edema, shock, and sudden death, is also produced. *C. bifermentans* lecithinase has approximately 1/50 of the enzymatic and hemolytic activities and lethality of *C. perfringens* alpha-toxin (83).

Beta-toxin (*C. sordellii*) has two activities: dermonecrotic and hemorrhagic, when tested in the skin of laboratory animals. Dermonecrotic toxin causes massive edema, small areas of bright-red hemorrhage (subcutaneously or intramuscularly), and death in 24-36 hours, while the hemorrhagic toxin (intraperitoneally) produces little edema, but confluent areas of brownish hemorrhage in the skin that can propagate to other tissues. Lethality is not so common (3, 89).

Clostridium hystolyticum

C. hystolyticum was first described in 1916 in gangrenous and nongangrenous war wounds but rarely reported, maybe due to its low heat resistance and growth inhibition by the presence of sugar (50). In GG, its presence is easily identified clinically because it has seven collagenases, and as collagen is the most abundant protein in the animal body (constituting connective tissues) (88), there is a tissue digestion including the soft parts of bones (39, 72).

C. hystolyticum produces five toxins: alpha - (unstable), lethal and necrotizing; beta - a group of seven collagenases; epsilon - O_2-labile hemolysin; gamma - proteinase; delta - elastase (84).

The histopathologic aspect of lesions can suggest Clostridia species involvement in the infection (Table 3).

Table 3: Clostridia and pathology of lesions of cattle myonecrosis (79).

Clostridia	Lesion aspect
C. chauvoei and/or *C. septicum*	Blackened, dry, spongy, crepitant with pale yellow fluid, which later becomes bloodstained
C. novyi and/or *C. septicum*	Pronounced gelatinous edema subcutaneously and in connective tissues. Clear at first and then bloodstained
C. septicum	Dark-red muscle, bloodstained gelatinous edema with gas bubbles
C. sordellii	Edema similar to that of *C. novyi* but more bloodstained with foul odor
C. hystoliticum	Bloodstained with pronounced tissue digestion

Diagnosis and Treatment

Antibiotics have been used for clostridial infections treatment but the predictable patterns of clostridia susceptibility to antimicrobial drugs have been changed: resistance to antimicrobials is increasing (19). In addition, treatment for infections is useless when animals are sick and death is imminent. Botulism and tetanus are exceptions as these diseases last for some days, allowing the supportive treatment associated with the use of antitoxins when they are available. More important than the treatment are the prophylatic measures, vaccination being the relevant aspect. Vaccines confer a very high degree of immunity to clostridial diseases, and when the diagnosis is correct the disease can be easily controlled. For this, clinical samples must be properly collected and subjected to laboratory examination.

Good examples are the first diagnosis done by Biological Institute (Instituto Biológico). The study of anaerobes started in 1944 with a series of six papers written by Rodrigues & Guida (8), calling attention to the participation of anaerobes in animal diseases. After that, initially *C. chauvoei* was the only isolate (23, 24), from then on *C. chauvoei* and *C. septicum* occurred in higher number than in the previous year, with *C. chauvoei* occurrence almost three times that of *C. septicum* (8). Around 1985, botulism appeared as a big problem in cattle raising all over Brazil, although

laboratorial diagnosis showed that other clostridia could be involved with the high mortality, mainly *C. perfringens, C. novyi*, and *C. haemolyticum* (5, 9). From 1986 to 1989, in 517 suspected cases of botulism, toxin was detected in 37, leading to the supposition of participation of other diseases. During the period from 1986 to 1995 and thereafter, the isolation of *C. perfringens* surpassed the other two (10, 11, 52).

Laboratory diagnosis allow the isolation of new species or strains that can be studied and even added to the vaccines, which was achieved in Brazil in 1983, during the outbreak of GG due to *C. septicum*.

What must be considered is that clostridia will be present in almost every sample collected, mainly if it has suffered putrefaction. So the significance of the presence of any clostridia will depend on the time elapsed between the death and the sample collection. Once all diseases due to clostridia can be prevented by vaccination, if the problem continues despite vaccination, the diagnosis must be wrong. So samples must be collected as soon as possible after death or by sacrifice, selecting compromised tissues, which must be well-packed, in order to limit deterioration, and sent quickly to the laboratory.

Moreover, a case description is of fundamental importance to conduct laboratorial analysis. Diagnosis must be based on the history and clinical data, number of heads affected, type of feed, range of species involved, and age of diseased animals. Diagnosis is commonly made by serologic testing, the most commonly used screening. However, in Brazil, it is almost impossible to get antiserum for diagnosis or treatment due to importation problems, no availability, and price. Therefore, other forms of diagnosis, avoiding the use of both antitoxin and especially laboratory animals, have been tried with success, including esterase electrophoretic polymorphism for *C*. *perfringens* typing (6) and the widely used Polimerase Chain Reaction (PCR) test (54, 56)

CONCLUSION

The participation of clostridia in livestock diseases is well known, and vaccination against clostridial diseases must be a practice in animal husbandry (86).

Vaccination effectively prevents all clostridial diseases, so any problem that exists despite vaccination cannot be attributed to clostridia. Nevertheless, diseases caused by clostridia currently remain a great economical problem in Brazil, being responsible

for great losses for cattle producers, and decreases in meat exports and in protein availability to the population.

In veterinary practice, diagnosis of clostridiosis is based on history, clinical signs, and findings in the post-mortem examination. However, laboratory analyses are essential for confirmation of the presence of toxins, in some cases, toxigenic strains found in bacterial cultures of recently collected clinical samples are confirmatory proofs, since antitoxins are not available. In addition, PCR assay is being used to detect if isolated strains possess genes that codify toxin production.

Economic impact is not usually evaluated in medical studies, but Arnon (2), describing infant botulism, calls attention to the fact that the average hospital stay is usually one month, and as there is a need for intensive care, the costs exceed US$ 50,000 per case. He also mentions a complicated case of 10 months hospitalization, which costs US$ 635,000. Beyond medical assistance, legal expenses must be considered as these can reach 84.4% of the total expenses, what was well documented by Man *et al*. (41) on a foodborne outbreak of botulism.

Molecular biology studies are so progressive that, certainly, more factors of pathogenicity will be discovered as well as other uses for them. One example is botulinal toxin type A, which, despite being the cause of a wide range of neurologic disorders, is being used for treatment and relief of several human dystonias; and as Schantz & Johnson (65) pointed out, in the future, a combination of botulinal toxin and tetanospasmin will also be used to control neurologic disorders.

Researchers should take in mind Claude Bernard phrase written in 1875 (65): “Poisons can be employed as a means for the destruction of life or as agents for the treatment of the sick”, let’s not follow those sick minds that have used them as biological weapons!

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