

Botulism in Cattle Associated with Osteophagy in the State of Acre, Brazil

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ABSTRACT

Background: Botulism is a non-febrile intoxication resulting from the ingestion of *Clostridium botulinum* neurotoxins manifested by partial or complete flaccid paralysis of the musculature of locomotion, swallowing and respiration. The objective of this study was to report the first case of botulinum intoxication associated with osteopathy in the state of Acre, as well as to alert breeders and veterinarians to the incidence of this disease in cattle farming.

Case: The present report is an outbreak of botulism in the municipality of Acrelândia, in the state of Acre, which resulted in the death of 16 Nelore beef cattle in approximately 30 days. The affected animals were females in reproductive phase maintained under extensive breeding system. The main clinical signs presented were weakness in the pelvic limbs, prostration, recumbency and death in less than 48 h. Only one animal, with similar symptomatology, was found alive and submitted to emergency therapeutic measures, but without success. During the necropsy of this bovine, no significant changes were found, only related to the decubitus and agony time, except for fragments of long bones visualized in the reticulum. Samples of bone particles, ruminal contents, reticulum, rumen and intestine fragments were collected for the detection of botulinum toxins by the mouse bioassay method, as well as brain and brain stem for differential diagnosis of rabies and bovine spongiform encephalopathy by direct immunofluorescence and immunohistochemistry, respectively. The samples were sent to the Laboratory of General Bacteriology of the Biological Institute, São Paulo, Brazil, and all the analyzes presented negative results.

Discussion: One of the main risk factors for the occurrence of botulinum toxin is the presence of carcasses in the pastures, added to the deficiency of phosphorus and other minerals. The cattle develop the habit of gnawing bones and tissues from dead animals in the field. With this exercise, animals can ingest pre-formed toxins in corpses, as decomposing remains offer an anaerobic environment conducive to spore development and toxin production. Bovine females, in breeding, present greater nutritional need than adult males and younger animals. This is because the reproductive phase increases the demands of minerals, vitamins and organic compounds that participate in the female's reproductive physiological process. In this case, the inadequate supply of minerals may stimulate the osteopagic practice of cadaveric remains in the pastures, making the bovids susceptible to botulism. Diagnosis is established by means of clinical signs, epidemiological characteristics and absence of specific anatomopathological findings. Confirmation is given by the isolation of the toxin in the body of the diseased animal. However, the lack of detection of this does not rule out the possibility of occurrence of the disease, in view of the rapid passage of the neurotoxin through the hematogenous route and through the tissues before reaching the neuromuscular junctions. In suspect cases, it is important to perform a differential diagnosis for other diseases that demonstrate clinical symptoms of neurological or acute neuromuscular character. The description of this outbreak, besides exposing the first notification of botulism in the state of Acre, shows the epidemiological relationship between the main risk factors and the occurrence of the disease in the Brazilian herds.

Keywords: *Clostridium botulinum*, mineral deficiency, neurotoxins, Amazon.

INTRODUCTION

Botulism is a non-febrile intoxication that results from the ingestion of *Clostridium botulinum* neurotoxins. It manifests through a partial or complete flaccid paralysis of the locomotion, swallowing, and breathing muscles. In cattle, this disease is caused by type C or D toxins, which are ingested by the animal through food, water or even by osteophagy [1,8].

Botulinum intoxication is one of the leading causes of bovine mortality, causing losses in the productive chain and damaging the sanitary indexes of the livestock activity [14]. The neuronal lesions are virtually irreversible, and the therapeutic protocols available through antitoxins are a distant option in developing countries, and their efficacy depends on the course of the disease [11].

Botulism episodes in cattle are described worldwide, especially in extensive rearing systems. Brazil has edaphoclimatic conditions favorable to the occurrence of this disease, but there are still few reports on its incidence in the federative units of the country. The lack of diagnosis and absence of description of outbreaks hinders the real perception of the occurrence of this disease in the herds [9]. In the state of Acre, Brazil, the situation is similar, with no reports on the intoxication in cattle.

The objective of this study was to describe the clinical course and diagnosis of a botulism outbreak in cattle, associated with osteopathy, in a rural property in the municipality of Acrelândia, Acre, Brazil.

CASE

The outbreak occurred in a rural property in the municipality of Acrelândia (latitude 10°04'23"S - longitude 67°03'14"W), located in the Vale do Acre mesoregion, Acre, in July 2017. The disease afflicted 16 female, Nelore beef cattle, in the reproductive phase. The municipality is bordered to the north by Amazonas and Rondônia, to the south and southwest by the municipality of Plácido de Castro, to the east by Bolivia, and to the west by the municipality of Senador Guiomard.

The request for veterinary support occurred after the death of 15 animals in approximately 30 days. The producer narrated a scenario of weakness in the pelvic limbs, prostration, decubitus, and death within a few days. During the anamnesis, we evaluated several sanitary parameters such as rearing

characteristics, herd immunization, hygienic conditions of the troughs, pasture quality, storage and type of salt used in the mineral supplementation, and water supplied to animals.

The property consisted of 60 beef cattle, between males and females, of different ages, submitted to the extensive rearing system. The animals were periodically wormed and vaccinated against rabies, symptomatic carbuncle, foot-and-mouth disease, and brucellosis. We performed no quarantine for newly acquired animals, isolation of diseased animals, disinfection of facilities or adequate carcass disposal.

The troughs showed poor hygiene, presenting moisture and small animal feces, as well as rodents. The water from three dams, used for the watering of the herd, was of poor quality and difficult access to the animals. The pasture consisted of the *Brachiaria brizantha* forage under poor management conditions. The forage cutting height and rest period were not respected after grazing (Figure 1).

Throughout the property, we verified cadaverous remains from the animals affected by the disease and by other causes before the outbreak, at various stages of decomposition. These carcasses were not subjected to any elimination process and remained for months or even years in the pastures until their complete decomposition (Figure 2).

The type of mineral salt (Supremax 65®)¹ supplied to the animals was not recommended for adult and reproductive females but young, growing animals. Thus, the affected cows were not supplemented with iron and received lower doses of phosphorus, calcium, methionine, manganese, selenium, and fluoride.

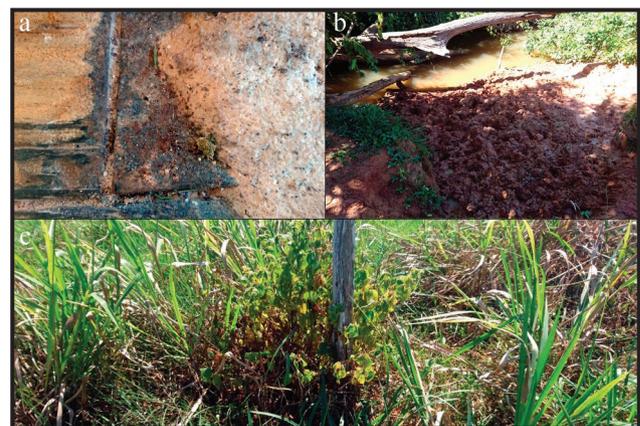


Figure 1. Sanitary and nutritional characteristics of the property. a- Feces in the troughs with the mineral salt; b- Water source used by the herd for drinking; c- *Brachiaria brizantha* pasture in bad management conditions.



Figure 2. Bovine carcass decomposing in the pasture. a- Animal in decomposition; b- Cadaver remained for months or even years in the pastures until their complete decomposition.



Figure 3. Bovine in lateral decubitus and respiratory distress.

After the anamnesis and evaluating the facilities, we examined the last affected bovine, which presented clinical signs similar to those of the others, approximately 24 h earlier. This animal was found in lateral decubitus in a forest area, away from the herd (Figure 3).

The physical examination revealed significant changes in vital parameters, such as bradycardia (40 bpm), bradypnea (16 mpm), abdominal breathing, 8 to 10% dehydration (capillary filling time > 2 s/skin turgor > 5 s), ruminal hypotonia (0 movement/5 min), and pale mucous membranes. The animal also presented decreased tongue tonus and skeletal muscle paralysis but with a normal state of consciousness. There was no hyperthermia (37.2°C), and the superficial lymph nodes were morphologically normal.

The animal died the next day, 36 h after the beginning of the clinical signs. We found no specific anatomopathological changes during the necropsy, only related to the time of decubitus and agony, apart from the presence of long bones fragments found in the reticulum (Figure 4).



Figure 4. Bone fragments found in the reticulum with clinical signs of botulinum intoxication.



Figure 5. Carcasses clustered to the wood for incineration.

We sent the bones found in the reticulum, as well as ruminal contents, reticulum, rumen, and intestine fragments, under refrigeration to the Laboratory of General Bacteriology of the Biological Institute, São Paulo, SP, to detect the botulinum toxins by the mouse bioassay method. Furthermore, we collected the encephalon and brainstem, which was maintained under refrigeration and formaldehyde, to perform the differential diagnosis of rabies and bovine spongiform encephalopathy through direct immunofluorescence and immunohistochemistry, respectively. All analyses presented negative results.

However, based on epidemiological considerations, we indicated emergency measures such as the removal of animals from the pasture; vaccination against botulism; adequate mineral supplementation; cleaning and disinfection of the troughs with sodium hypochlorite (10%); incineration of the carcasses from the pasture (Figure 5), and disinfection of the incineration site with virgin lime in the proportion of 5 kg per m². After conducting these procedures, there were no more deaths on the property.

DISCUSSION

The first descriptions of botulism in Brazilian cattle occurred in the 1970s and 1980s, in the state of Piauí and Rio Grande do Sul, respectively [5]. Since then, the occurrence of this disease in the Brazilian herd has presented an epizootic character. The episodes of this disease are reported in ruminants for over approximately 50 years in some regions of the country [15]. However, there is no record of botulism in the animals in Acre. Therefore, this work is the first description of the disease in cattle for this state.

The morbidity rate of this disease can range from 0.1 to 100%, and the number of animals affected may depend on the vaccination program performed by the producer [10]. However, the lethality rate is always very high, close to 100% [9,15]. In this report, the morbidity of intoxication and lethality were of approximately 26.66% and 100%, respectively.

One of the main risk factors for the occurrence of botulinum intoxication is the presence of carcasses in the pasture, added to the deficiency of phosphorus and other minerals such as calcium, sodium, zinc, magnesium, selenium, and iron. The cattle develop the habit of gnawing on bones and tissues from the dead animals in the field, therefore ingesting toxins pre-formed in the cadavers since decomposing remains provide an anaerobic medium favorable to spore development and toxin production [1].

Bovine females in breeding present greater nutritional need than adult males and younger animals given that the reproductive phase increases the demands of minerals, vitamins, and organic compounds that participate in the female's reproductive physiological process. In this case, the inadequate supply of minerals can stimulate the osteophagy of the cadaveric remains in the pastures, making the bovines susceptible to botulism, as described above [11].

Animals with botulism can show locomotor difficulty, excitation, tongue protrusion, respiratory distress, permanent decubitus, and death. Such signs occur due to the pathogenic mechanism of the botulinum toxins, which inhibit the acetylcholine exocytosis in the nerve endings of the peripheral nervous system (PNS), decreasing muscle contraction [8,13].

The evolution of the disease can be hyperacute, with the death of the animal in less than 24 h; acute, with death occurring in one to three days; subacute, with death in three to seven days; or chronic, with a

clinical course occurring over more than one week, evolving to death or a cure. The flow of the disease is related to the amount of toxin ingested and the animal organism [12], justifying the deaths that occurred over several days in this report.

Botulinum intoxications do not, generally, produce considerable pathological (macroscopic and histological) changes, which is characteristic of the disease. The findings of the necropsy were due to the decubitus period and respiratory agony before the death of the animal. It is known that the more acute the intoxication, the fewer post-mortem findings will be identified [2,8].

However, the presence of long bones in the reticulum is exceptionally relevant to the association with botulism, but only indicates the osteophagy habit of the herd. The animals can also consume toxins pre-formed in cadaverous remains and contaminated water, food, and salt. Therefore, during an outbreak, not all bovines may have become intoxicated with the same source of contamination [3].

The diagnosis is established through clinical signs, epidemiological characteristics, and the absence of specific anatomopathological findings. The confirmation is given by isolating the toxin in the body of the diseased animal. However, not detecting this disease does not rule out the possibility of its occurrence, given the quick passage of the neurotoxin through the hematogenous pathway and tissues before reaching the neuromuscular junctions [7].

Thus, in suspected cases, it is necessary to perform a differential diagnosis for other diseases that demonstrate clinical symptoms of acute neurological or neuromuscular character, such as forage intoxications, encephalopathies, hypocalcemia, listeriosis, polioencephalomalacia and rabies [4,6,9].

The emergency measures performed during this outbreak, such as vaccination against botulism, the correct disposal of decomposed organic matter in pastures, adequate mineral supplementation, and provision of good quality water and food were decisive to control the disease at the farm [10]. Vaccination is still considered the most effective prophylactic measure, especially when added to other preventive actions [5,8].

The purpose of this case report is to alert producers and veterinarians to botulinum toxicity in cattle farming in Acre, which is essential to strengthen

animal health programs since the absence of reports of a disease does not mean that there is none and may cause the underestimation of the incidence of the disease in the country.

The description of the botulism outbreak in Acre exposes the first notice of this intoxication in the state and indicates the epidemiological relationship between the main risk factors and the occurrence of the disease in Brazilian herds. The presence of decaying carcasses in the pasture, the reproductive phase of the females, the lack of adequate mineral supplementation,

and the practice of osteophagy may have favored the ingestion of pre-formed botulinum toxins and led to the clinical manifestation of the disease. Botulism should always be included in diagnostic protocols to investigate sudden deaths of unapparent causes in herds, given the severity of an outbreak.

MANUFACTURER

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- 1 Camara A.C.L., Oliveira C.M.M., Vale A.M., Batista J.S. & Soto-Blanco B. 2014. Epidemiologia, sinais clínicos, achados laboratoriais e patológicos em oito surtos de botulismo em bovinos no Rio Grande do Norte. *Acta Scientiae Veterinariae*. 42: 1200.
- 2 Cereser N.D., Costa F.M.R., Rossi Júnior O.D., Silva D.A.R. & Sperotto V.R. 2018. Botulismo de origem alimentar. *Ciência Rural*. 38(1): 280-287.
- 3 Galiza G.J.N., Silva M.L.C.R., Dantas A.F.M., Simões S.V.D. & Riet-Correa F. 2010. Doenças do sistema nervoso de bovinos no semiárido nordestino. *Pesquisa Veterinária Brasileira*. 30(3): 267-276.
- 4 Guizelini C.C., Lemos R.A.A., de Paula J.L.P., Pupin R.C., Gomes C.D., Barros C.S.L., Neves D.A., Alcântara L.O.B., Silva R.O.S., Lobato F.C.F. & Martins T.B. 2019. Type C botulism outbreak in feedlot cattle fed contaminated corn silage. *Anaerobe*. 55: 103-106.
- 5 Lobato F.C.F., Salvarani F.M., Gonçalves L.A., Pires P.S., Silva R.O.S., Alves G.G., Neves M., Oliveira Júnior C.A. & Pereira P.L.L. 2013. Clostridioses dos animais de produção. *Veterinária e Zootecnia*. 20: 29-48.
- 6 Lucena R.B., Pierezan F., Kommers G.D., Irigoyen L.F., Figuera R.A. & Barros C.S.L. 2010. Doenças de bovinos no Sul do Brasil: 6.706 casos. *Pesquisa Veterinária Brasileira*. 30(5): 428-434.
- 7 Maboni F., Monego F., Dutra I., Costa M.M. & Vargas A.C. 2010. Ocorrência de botulismo em bovinos confinados no Rio Grande do Sul. *Ciência Animal Brasileira*. 11(4): 962-965.
- 8 Maréchal C., Woudstra C. & Fach P. 2016. Botulism. In: Uzal F., Prescott J., Songer G. & Popoff M. (Eds). *Clostridial Diseases of Animals*. Ames: John Wiley & Sons, pp.303-330.
- 9 Mariano V., Nardi A., Gradassi S., De Santis P., Anniballi E., Bilei S., Scholl F., Auricchio B., Bielli C., Culicchi M. & Rosa G.L.C. 2019. A severe outbreak of botulism in cattle in Central Italy. *Veterinaria Italiana*. 55(1): 57-62.
- 10 Moreira C.J., Ferreira M.R.A., Cunha C.E.P., Donassolo R.A., Dedo P.F., Moreira G.M.S.G., Otaka D.Y., Sousa L.A., Barbosa J.D., Moreira Â.N., Salvarani F.M. & Conceição F.R. 2018. Immunogenicity of a bivalent non-purified recombinant vaccine against botulism in cattle. *Toxins*. 10(10): DOI 10.3390/toxins10100381.
- 11 Pandian S.J.G., Subramanian M., Vijayakumar G., Balasubramaniam G.A. & Sukumar K. 2015. Therapeutic management of botulismo in dairy cattle. *Veterinary World*. 8(11): 1305-1309.
- 12 Quevedo P.S. 2015. Clostridioses em ruminantes - Revisão. *Revista Científica de Medicina Veterinária*. 25: 1-16.
- 13 Rossetto O., Megighian A., Scorzeto M. & Montecucco C. 2013. Botulinum neurotoxins. *Toxicon*. 67: 31-36.
- 14 Rulff R., Schrödl W., Basiouni S., Neuhaus J. & Krüger M. 2015. Is Downer cow syndrome related to chronic botulism? *Polish Journal of Veterinary Sciences*. 18(4): 759-765.
- 15 Silva R.O.S., Oliveira Junior C.A., Gonçalves L.A. & Lobato F.C.F. 2016. Botulism in ruminants in Brazil. *Ciência Rural*. 46(8): 1411-1417.