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PATHOLOGY

Botulism outbreak in cattle due to ingestion of accidental deteriorated feed

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ABSTRACT: Botulism is a condition caused by the ingestion of pre-formed toxins produced by the bacterium *Clostridium botulinum*. This study described an outbreak of type D botulism with a high mortality rate (67%) in a cattle herd, caused by the consumption of fermented silage. The affected cattle presented with a hyperacute to acute clinical course with progressive flaccid paralysis. Five animals underwent necropsy with no significant macroscopic and microscopic alterations. A most probable source of contamination was deemed to be an inadequately stored silage; although, the toxin was not detected in the feed. The diagnosis of botulism was based on both clinical and epidemiological findings and the detection of botulinum toxin in the intestinal contents of two of the affected cattle. The present study emphasized the high number of fatally affected cattle, an unusual source of botulinum toxin and the importance of proper management of animal feed supply.
Key words: bovine, botulinum neurotoxins, *Clostridium botulinum*, mouse bioassay, neurotoxin type D.

Surto de botulismo em bovinos devido à ingestão de silagem acidentalmente deteriorada

RESUMO: Botulismo é causado pela ingestão de toxinas pré-fromadas produzidas por *Clostridium botulinum.* Este estudo descrevre um surto de botulismo tipo D em um rebanho bovino, com alta taxa de mortalidade (67%), devido ao consumo de silagem mal acondicionada. Os bovinos afetados apresentaram curso clínico hiperagudo e agudo de paralisia flácida progressiva. Cinco animais foram necropsiados e não foram visualizadas alterações macroscópicas e microscópicas. Embora a toxina não tenha sido detectada no alimento, a fonte mais provável de contaminação foi considerada a silagem armazenada inadequadamente. O diagnóstico de botulismo foi baseado nos achados clínicos, epidemiológicos e pela detecção da toxina botulínica no conteúdo intestinal de dois bovinos. O presente estudo enfatiza a morte de muitos bovinos por uma fonte incomum de toxina botulínica e a importância do manejo adequado no fornecimento de ração aos animais.
Palavras-chave: bovino, neurotoxina botulínica, *Clostridium botulinum*, bioensaio em camundongo, neurotoxina tipo D.

Botulism is a neuroparalytic infectious disease caused by the ingestion of botulinum neurotoxins (BoNTs) produced by *Clostridium botulinum* in decomposed organic plants or animal substances that may affect domestic animals, wildlife, and humans (LE MARÉCHAL et al., 2019). *Clostridium botulinum* is a gram-positive, anaerobic, spore-forming rod with worldwide distribution (LE MARÉCHAL et al., 2019). Eight antigenically distinct BoNTs with the same neurotoxic effect have been identified: A, B, C, D, E, F, G, and H (BARASH & ARNON, 2014), of which there are over 40 subtypes (BARASH & ARNON, 2014). In cattle, only types A, B, C and D have been shown to cause disease (FRYE et al., 2020; KELCH et al., 2000; MYLLYKOSKI et al., 2008).

In cattle, the disease predominantly occurs by the ingestion of pre-formed botulinum toxins in carcass contamitaded feed (UZAL et al., 2016). The clinical signs of progressive flaccid paralysis is related to the amount of toxin ingested and generally appear 3 to 17 days after ingestion (QUINN et al., 2011). Necropsy and histopathological examinations do not show morphological changes, and diagnosis should be confirmed by the detection of botulinum toxin in tissues as well as potential contaminants (UZAL et al., 2016).

Botulism is an endemic disease in Brazil, representing a significant cause of death in cattle (DUTRA et al., 2001; DUTRA et al., 2005; GUZELINNI et al., 2019). This disease can lead to

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significant economic losses, (SOARES et al., 2018) and botulism is an important differential diagnosis for neurological diseases and associated with high mortality rates that does not present with significant anatomopathological lesions (DUTRA et al., 2001; DUTRA et al., 2005; GUZELINNI et al., 2019). We now describe an outbreak of type D botulism in a cattle herd. The present study is relevant because of the high number of fatally affected cattle and poor reports of botulism outbreak owing this source of contamination.

The epidemiological and clinicopathological data were obtained during a visit to the farm by the referring veterinarian. A botulism outbreak was confirmed in the municipality of Viamão (30° 04' 51" S 51° 01' 22" W), Rio Grande do Sul, Brazil, in November 2021. Out of a batch of 475 beef cattle, 320 animals became ill (67.4% morbidity), all of whom succumbed to infection (100% lethality). The animals had not been vaccinated against botulism or any other clostridial diseases. All the cattle were castrated males of undefined breed of approximately 1.5 years old. The animals were raised semi-extensively, and the mortality outbreak began after the supply of a feed mixture comprising corn silage, sorghum, moist barley, wheat flour, feed, and minerals, which were stored at room temperature in at mixer wagon for five days due to mechanical problems. The affected cattle showed hyperacute to acute clinical course, ranging from a few hours to 36 hours following ingestion. The animals primarily exhibited excessive salivation, difficulty swallowing, respiratory distress, paresis, and muscular paralysis of the forelimbs (Figure 1A), which rapidly progressed to sternal and lateral recumbency and loss of muscle tone in the tongue (Figure 1B) and death. During the technical visit, the mixture was inspected, revealing a dark color, significant moisture content, and an unpleasant odor (Figure 2). No animal carcasses were observed for any of mixtures. An independent water dispenser was used at each picket.

Of the 320 affected cattle, five that died spontaneously were submitted to necropsy. During necropsy, fragments from different organs (brain, spinal cord, skeletal muscle, bone marrow, liver, kidney, adrenal gland, heart, lung, esophagus, pancreas, small and large intestines, and spleen) were collected, fixed in 10% formalin, and routinely processed for histology, and stained using the hematoxylin and eosin (HE) histochemical technique. Additionally, fresh samples of liver, intestinal and ruminal contents, and serum of the five necropsied cattle, and mixture from the feed mixer were submitted for detection of BoNTs by standard mouse bioassay and seroneutralization (SMITH, 1977) at the Universidade Federal de Goiás (UFG). Biological samples (liver, intestinal, and ruminal contents) were processed and inoculated (0.5mL) intraperitoneally into mice with body weights ranging from 20 to 25 g, which remained under observation for seven days, in accordance with the procedures described by SMITH (1977). Subsequently, typing of the positive samples in the mouse bioassay was carried out through seroneutralization with botulinum antitoxin types C and D, standardized and provided by the Laboratório Federal de Defesa Agropecuária (LFDA/MG), which was inoculated as previously described. Additionally, feed samples were seeded in Wright's medium and incubated at 37 ºC for 5 days for indirect detection of *Clostridium botulinum* spores. Subsequently, the mice were inoculated with the supernatant (0.5, 0.4, and 0.3mL) and monitored for seven days (SMITH, 1997).

Figure 1 - Botulism outbreak in cattle. The affected cattle showing clinical signals. A. Muscular paralysis of the forelimbs. B. Decreased tongue tone, apathy, and sternal recumbency.

Figure 2 - Deteriorated feed offered to the cattle.

On necropsy, macroscopic examination revealed that all cattle had markedly distended urinary bladders. No significant macroscopic alterations were observed in any other organs. Botulinum toxin was detected in the intestinal contents of two of the five necropsied animals. The diagnosis of botulism in cattle was based on epidemiological and clinical findings, including the absence of significant macroscopic and microscopic lesions, and the detection of botulinum toxin in the intestinal contents of the two affected cattle. Botulinum toxin was not detected in any of the feed samples. Most botulism outbreaks in cattle are caused by the consumption of feed contaminated with decaying carcasses (DUTRA et al., 2005; GALEY et al., 2000; LOBATO et al., 2008; MYLLYKOSKI et al., 2008). In our study, no animal carcasses were found in the pasture where the animals lived or in the silage. We therefore hypothesized that the outbreak was triggered by the consumption of inadequately stored feed in the feeding wagon, comprising a mix of insufficiently fermented grains, and stored at the ideal temperature for the production and maintenance of botulinum toxin, in anaerobiosis. We believe that the time of the year when the outbreak occurred (late spring) may have also favored food deterioration in the wagon, due to the rising temperatures. Corn was one of the primary components of the mix provided to animals and is an excellent substrate for bacterial growth (LEMOS & RIET-CORREA, 2023). In such cases, silage is usually harvested from fields with high of *C. botulinum* (KELCH et al., 2000). Acidification of silage to a pH of 3.8 to 4.0 inhibits the growth of *C. botulinum*, even under anaerobic conditions (DRIEHIUS et al., 2018); however, in the present study, we did not measure the pH. In outbreaks associated with osteophagia, morbidity was lower (9.19%) than that in outbreaks associated with contaminated food (30%), with a lethality rate above 85% (DUTRA et al., 2001). The mortality rate in the outbreak was 67%, with a lethality rate of 100%. Our morbidity rate of 71% is similar to results found in other reports (64,7%) associated with the consumption of inadequately stored silage (GUZELLINI et al., 2019). Outbreaks associated with the consumption of contaminated food can lead to the death of many animals within a short period of five days, thus reinforcing the possible source of the toxin (LEMOS & RIET-CORREA, 2023). However,

the morbidity and lethality coefficients of botulism outbreaks are variable, because the impact caused by this disease depends on the amount, concentration, and period of ingestion of the neurotoxin consumed by the animals (COLBACHINI et al., 1999).

The clinical signs observed in the outbreak animals were similar to those described in previous studies and were predominantly characterized by a loss of hindlimb movements, decreased tongue tone, and recumbency (GUZELLINI et al., 2019). Signs of flaccid paralysis are associated with inhibition of presynaptic acetylcholine release at the neuromuscular junction (BURGEN, 1949). As such, signs of autonomic dysfunction can coexist with skeletal neuromuscular dysfunction (BURGEN, 1949), suggesting mechanism of urinary bladder distension (WENHAM & COHEN, 2008). The absence of macroscopic and histopathological changes in the animals in the present case is also commonly reported in cases of botulism in domestic animals, as botulinum toxin causes only functional changes without tissue lesions.

The diagnosis of botulism can be confirmed through laboratory tests involving the detection of the botulinum toxin in the serum and tissues, predominantly the liver and ruminal and intestinal contents of affected animals, in water, and in suspected food sources (QUINN, 2011). Mouse bioassay is the gold standard for confirming botulism (UZAL et al., 2016). In the bioassay, mice inoculated with the suspicious material will develop paralysis and dyspnea ("wasp waist"), subsequently progressing to death (MOELLER et al., 2009). Despite being considered the gold standard test, a low epidemiological sensitivity of the mouse bioassay has been identified for botulism diagnosis because some animal species, such as cattle, are more sensitive to botulinum toxin than mouse, such as cattle, or because there is no more circulating toxin at the time of collection (ALLISON et al., 1976; UZAL et al., 2016). Thus, the detection of botulinum toxin in only two of the five animals was sufficient to confirm the diagnosis of botulism. The fact that the toxin was not detected in the other three necropsied animals does not rule out the diagnosis of the disease because, as mentioned above, it is not always possible to detect the toxin in the sample analyzed.

The non-detection of botulinum toxin in food does not exclude silage as a source of infection. As the detection of botulinum toxin has been shown to be related to the place and time at which the material is harvested, the dilution of the toxin in sources such as water and the low sensitivity of the mouse bioassay (SEIFERT & BÖHNEL, 1994). As such, the negative results for the detection of botulinum neurotoxin in the feed could have been expected because most of the contaminated food stored in the mixer wagon had already been consumed. Since other animals consumed the same water provided to the cattle and did not show clinical signs, water was ruled out as a source of the toxin. Thus, a possible source of the toxin was feed that was stored incorrectly in the mixer wagon and offered to the animals.

The primary differential diagnoses for botulism in cattle, include diseases that present with neurological alterations, including rabies, infection by bovine herpesviruses (BoHV-1 and BoHV-5), listeriosis, pesticide intoxication (mainly organophosphates), urea poisoning, polioencephalomalacia, cerebral babesiosis, and mycotoxin poisoning by the fungus *Aspergillus clavatus* (CANTILE & YOUSSEF, 2016; BRUST et al., 2015; MOELLER & PUSCHNER, 2007). Toxic diseases that induce necrotic myopathy, such as poisoning by *Senna* spp. and ionophore antibiotic poisoning, should also be considered (HORN et al., 2011; MOELLER & PUSCHNER, 2007). However, these differential diagnoses were ruled out in the present case based on epidemiological, clinical, and pathological findings.

The detection of botulinum toxin type D in the tissues of animals that died of clinical signs confirmed the clinical, pathological, and epidemiological evidence of botulism recorded during the outbreak. We suspect that, in this outbreak, the toxin was probably introduce by improperly stored and supplied food to the cattle, highlighting the importance of proper management of the feed provided to the animals and the importance of vaccinating the herd against clostridial diseases. In the present outbreak, 73.6% of the total herd was lost due the consumption of feed contaminated with botulinum toxins. Overall, the outbreak resulted in the loss of most of the herd, leading to significant economic losses, emphasizing the negative economic impact of the disease.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the

collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled "Botulism outbreak in cattle associated with ingestion of accidental deteriorated feed" declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation by the Ethics Committee of the Universidade Federal do Rio Grande do Sul. However, we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assumed full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

REFERENCES

ALLISON, M. J. et al. Inactivation of *Clostridium botulinum* Toxin by Ruminal Microbes from Cattle and Sheep. **Applied and Environmental Microbiology**, v.32, p.685-688, 1976. Available from: <https://pubmed.ncbi.nlm.nih.gov/984838/>. Accessed: Aug. 10, 2023. doi: 10.1128/aem.32.5.685-688.1976.

BARASH, J. R.; ARNON, S. S. A novel strain of *Clostridium botulinum* that produces type B and type H botulinum toxins. **Journal Infection Diseases**, v.209, n.2, p.183-191, 2014. Available from: <https://pubmed.ncbi.nlm.nih.gov/24106296/>. Accessed: Aug. 03, 2023. doi: 10.1093/infdis/jit449.

BRUST, L. A. C. et al. Cattle diseases associated with consumption of beer residues. **Pesquisa Veterinária Brasileira**, v.35, n.12, p.956-964, 2015. Available from: <https://www.scielo.br/ /j/pvb/a/hZ6fCHSdrXNMx8WLFPmmfhS/>. Accessed: Aug. 10, 2023. doi: 10.1590/S0100-736X2015001200004.

BURGEN, A. S. V.; ZATMAN D. L. J. The action of botulism toxin on the neuro-muscular junction. **The Journal of Physiology**, v.109, p.10-24, 1949. Available from: <https://physoc.onlinelibrary.wiley.com/doi/10.1113/jphysiol.1949.sp004364>. Accessed: Sept. 13, 2024. doi: 10.1113/jphysiol.1949.sp004364.

CANTILE, C.; YOUSSEF, S. Nervous System. In: Maxie M.G. (Ed). **Jubb, Kennedy, and Palmer's Pathology of Domestic Animals**. 6.ed. St. Louis: Elsevier, 2016. p.250-406.

COLBACHINI, L. et al. Bovine experimental intoxication with botulinic toxin type D. **Arquivo Brasileiro de Medicina Veterinária e Zootecnica**, v.51, n.3, p.229-233, 1999. Available from: <https://www.scielo.br/j/abmvz/a/Hxj6C4tfXQf8YktHZcRBxBS/>. Accessed: Aug. 12, 2023. doi: 10.1590/S0102-09351999000300005.

DRIEHIUS, F. et al. Silage review: Animal and human helath risks from silage. **Journal Dairy Science**, v.101, n.5, p.4093-4110, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S002203021830328X#>. Accessed: Aug. 18, 2023. doi: 10.3168/jds.2017-13836.

DUTRA, I. S. et al. Botulism outbreaks in cattle in Brazil associated with contaminated water. **Pesquisa Veterinária Brasileira**, v.21, n.2, p.43-48, 2001. Available from: <https://www.scielo.br/j/pvb/a/gv7GBP99gtQFbzjnR55krhG/>. Accessed: Aug. 07, 2023. doi: 10.1590/S0100-736X2001000200002.

DUTRA, I. S. et al. Botulism in beef and dairy cattle fed with poultry litter. **Pesquisa Veterinária Brasileira**, v.25, n.2, p.115-119, 2005. Available from: <https://www.scielo.br/j/pvb/a/L6nVzZsTy4gbwHVgNhXrBdz/?lang=pt>. Accessed: Aug. 07, 2023. doi: 10.1590/S0100-736X2005000200009.

FRYE, E. A. et al. Outbreak of botulism type A in dairy cows detected by MALDI-TOF mass spectrometry. **Journal Veterinary Diagnostic Investigation**, v.32, n.5, p.722-726, 2020. Available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7488966/>. Accessed: Aug. 04, 2023. doi: 10.1177/1040638720943127.

GALEY, F. D. et al. Type C botulismo in Dairy cattle from feed contaminated with dead cat. **Journal Veterinary Diagnostic Investigation**, v.12, n.3, p.204-209, 2000. Available from: <https://journals.sagepub.com/doi/10.1177/10406387000120030>. Accessed: Aug. 10, 2023. doi: 10.1177/104063870001200302.

GUZELLINI, C. C. et al. Type C botulismo outbreak in feedlote cattle fed contaminated corn silage. **Anaerobe**, v.55, p.103-106, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/30408576/>. Accessed: Aug. 06, 2023. doi: 10.1016/j.anaerobe.2018.11.003.

HORN, J. F. et al. Outbreak of Intoxication by Senna occidentalis in Cattle. **Acta Scientiae Veterinariae**, v.39, n.1, p.954, 2011. Avaliable from: <https://seer.ufrgs.br/index.php/ActaScientiaeVeterinariae/article/view/133706>. Accessed: Aug. 18, 2023. doi: 10.22456/1679-9216.133706.

KELCH, W. J et al. Fatal Clostridium botulinum toxicosis in eleven Holstein cattle fed round bale barley haylage. **Journal of Veterinary Diagnostic Investigation**, v.12, p.453-455, 2000. Available from: <https://journals.sagepub.com/doi/10.1177/104063870001200511>. Accessed: Aug. 18, 2023. doi: 10.1177/104063870001200511.

LE MARÉCHAL, C. et al. 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**, v.9, p.1025, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/31775272/>. Accessed: Aug. 03, 2023. doi: 10.3390/ani9121025.

LEMOS, R. A. A.; RIET-CORREA, F. Botulismo. In: RIET-CORREA, F. et al. **Doença de Ruminantes e Equinos** (4ed). São Paulo, Editora Medvet LTDA, 2023. p.200-211.

LOBATO, F. C. F. et al. Botulism in ruminants being fed with poultry litter. **Ciência Rural**, v.38, n.4, p.1176-1178, 2008. Available from: <https:// www.scielo.br/j/cr/a/nGX3wSGHdbDn9zsLmgqw7YK/?lang=pt#>. Accessed: Aug. 10, 2023. doi: 10.1590/S0103-84782008000400046>.

MOELLER, R. B.; PUSCHNER, B. Botulism in Cattle – A Review. **The Bovine Practitioner**, v.41, n.1, p.54-58, 2007. Available

from: <https://bovine-ojs-tamu.tdl.org/bovine/article/view/2876>. Accessed: Aug. 18, 2023. doi: 10.21423/bovine-vol41no1p54-58.

MOELLER, R. B. et al. Short communication: Attempts to identify Clostridium botulinum toxin in milk from three experimentally intoxicated Holstein cows. **Journal of Dairy Science**, v.95, n.6, p.2529-2533. Available from: <https://www.sciencedirect.com/science article/pii/S0022030209705673. Accessed: Sept. 13, 2024. doi: 10.3168/jds.2008-1919.

MYLLYKOSKI, J. et al. Type C bovine botulism outbreak due to carcass contaminated non -acidified silage. **Epidemiology and Infection**, v.137, n.2, p.284-293, 2009. Available from: <https://pubmed.ncbi.nlm.nih.gov/18606025/>. Accessed: Aug. 03, 2023. doi: 10.1017/S0950268808000939.

SEIFERT, H.; BÖHNEL, H. Clostridioses. In: BLOBEL, H.; SCHLIESSER, T. **Handbuch der bakteriellen Infektionen bei Tieren.** Gustav Fischer Verlag, Stuttgart, 1994. p.89-153.

SOARES, M. C. et al. Economic impact of an outbreak of botulismo in a cattle feedlot. **Pesquisa Veterinária Brasileira**, v.38, n.7, p.1365-1370, 2018. Available from: <https://www.scielo.br/j/pvb/a/zgYvN3LqmqB3xBFQzcy67hD/>. Accessed: Aug. 06, 2023. doi: 10.1590/1678-5150-PVB-5643.

SMITH, L. D. S. **Botulism**: the organism, its toxins, the disease. Thomas Publisher, Illinois. 1977.

UZAL, F. A. et al. In: **Clostridial diseases of animals**. Wiley-Blackwell, 2016. p.336.

QUINN, B. K. et al. Clostidium species. In: **Veterinary Microbiology and Microbial Disease**, 2th ed. Iowa: Wiley-Blackwell, 2011. p.618-663.

WENHAM, T.; COHEN, A. Botulism. **Continuing Education in Anaesthesia Critical Care & Pain**, v.8, n.1, p.21-25, 2008. Available from: <https://academic.oup.com/bjaed/article/8/1/21/277773>. Accessed: May, 10, 2024. doi: 10.1093/bjaceaccp/mkm051.