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# Botulism in Cattle – A Review

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## Abstract

Botulinum toxins are considered the most lethal toxic substances known to man. Eight botulinum toxins are produced by different strains of *Clostridium botulinum*, *C. baratii* and *C. butyricum*. Cattle are primarily affected by botulinum toxins B, C and D. Although these botulinum toxins can be found worldwide, certain botulinum toxins have predominance in certain geographical regions. Poisoned cattle often show muscle weakness, constipation, colic, ataxia, muscle fasciculation, decreased tongue tone and dirty noses. Currently no treatments other than supportive care are available for affected cattle.

**Keywords:** cattle, botulinum toxin, botulism, review

## Résumé

Les toxines botuliniques sont parmi les substances toxiques les plus létales connues à ce jour. Huit toxines botuliniques sont produites par différentes souches de *Clostridium botulinum*, *C. baratii* et *C. butyricum*. Les bovins sont principalement affectés par les toxines botuliniques B, C et D. Même si ces toxines botuliniques se retrouvent partout dans le monde, certaines toxines botuliniques sont plus prévalentes dans certaines régions géographiques. Les bovins empoisonnés montrent souvent les signes cliniques suivants: faiblesse musculaire, constipation, colique, ataxie, fasciculation musculaire, tonicité réduite de la langue et naseau souillé. Le traitement de soutien est la seule alternative disponible présentement pour les bovins affectés.

## Introduction

Botulinum toxins, considered the most lethal toxic substances known to man, are produced under anaerobic conditions by certain strains of *Clostridium botulinum*, *C. baratii* and *C. butyricum*. These clostridial organisms have the ability to produce eight antigenically distinct botulinum toxins: A, B, $C_1$, $C_2$, D, E, F and G.[4,9,12] All are paralytic neurotoxins except for $C_2$, which is cytotoxic and interferes with adenosine diphosphate, resulting in changes in membrane permeability.[9,12,19] Each of the seven botulinum neurotoxins has the potential to affect many different species of animals, including man. The bacterial strains that produce the different botulinum toxins are found in a variety of environments. Botulinum intoxication in man is usually caused by subtypes A, B, E and F, while cattle intoxication is more common with subtypes B, C and D. Animals poisoned with *C. botulinum* toxin type B are commonly found in the eastern United States and Europe,[9,12,20] while type C is commonly seen in the western United States[9,12,20] and type D is most common in South Africa, South America and Australia.[9,12]

## Pathogenesis

Typically, botulism results from ingestion of preformed toxin-contaminated feed sources. Bacterial spores of the organism are commonly found in all soils and have the potential to contaminate most plant material. In order for the bacteria to multiply and produce toxin, an anaerobic environment must be maintained. Ingestion of spoiled hay or silage (particularly oat, rye and barley silage) is a common source for botulinum toxin type B.[6,13,19,20,21,22] It is believed that wet, cool spring weather prevents rapid fermentation of silage, resulting in elevated pH. This can result in vegetation of *C. botulinum* spores and production of the toxin.[6,19] Ingestion of dead animals inadvertently picked up in hay and silage, or ingestion of poultry litter containing dead bird parts, are common sources of botulinum toxin type C intoxication.[1,8,10,11,16,18] Botulinum toxin type D intoxication is common in phosphorus deficient areas where toxin contaminated bones are ingested. There is evidence that the toxin can be found for years in bone marrow.[7,12,15] Ingestion of contaminated water (usually type C and D botulinum toxins) from shallow, warm nutrient-rich ponds has also been a source of intoxication. Wound infections can also lead to botulinum intoxication, but are rare in ruminants and more commonly reported in horses.[12]

In cattle, botulism occurs after consumption of the preformed toxin that is absorbed by the intestinal tract and distributed to the nerves via the blood stream. The toxin enters primarily through cholinergic neurons, and exerts its effect at the neuromuscular junction of skeletal muscle by preventing the release of the neurotransmitter acetylcholine. Failure to release acetylcholine results in weakness and ultimately flaccid paralysis of the animal. Death is usually due to paralysis of the muscles of the diaphragm, leading to respiratory arrest.[4,5,9,12,14]

Botulinum toxins are composed of a 150 kDa dichain molecule consisting of a 100 kDa heavy chain (responsible for membrane targeting and attachment to the target cells) linked by a disulfide bond to a 50 kDa light chain (responsible for the toxic damage in the nerve). The botulinum toxin molecule attaches to the neuron via receptors, and enters the cell by endocytosis. The endocytotic vesicle is acidified, which causes the 50 kDa light chain to detach from the 100 kDa heavy chain and escape the endosome by pH-dependent translocation and transport into the cytoplasm of the neuron. Once in the cytoplasm of the neuron, the 50 kDa light chain acts as zinc-dependent metalloproteases that freely cleave and inactivate vital intracellular docking proteins important in the release of acetylcholine at the neuromuscular junction. These critical docking proteins are located on the synaptic vesicle, which contains the neurotransmitter acetylcholine, or the cell membrane at the neuromuscular junction. These docking proteins are synaptobrevin-2, located on the synaptic vesicle, and syntaxin-1A and SNAP (synaptosomal-associated protein)-25, located on the neuromembrane at the neuromuscular junction. Botulinum toxins B, D, G and F cleave certain sites on synaptobrevin-2. SNAP-25 is cleaved by botulinum toxins A, $C_1$, and E. Syntaxin 1A is cleaved by botulinum toxin $C_1$.[5,14]

Most cases of botulism in cattle involve adult or young growing animals, while calves are rarely involved. Cattle have been determined to be 12.88 times more sensitive to botulinum toxin C (on a kilogram basis) than the mouse, with a median toxic dose in lactating dairy cows of 0.38 ng/kg body weight.[17]

### Clinical Signs and Diagnosis

Diagnosis of botulism in cattle is very difficult due to the non-specific clinical signs. In most cases, a diagnosis of botulinum toxin intoxication is made after other causes of muscle weakness are ruled out. The rapidity of clinical signs depends on the amount of toxin ingested by the affected animal. Clinical signs often develop between 48 and 96 hours after ingestion of the toxin, but may appear as early as 24 hours or as late as 10 to 18 days after exposure.[1,15] The typical clinical presentation is the development of progressive muscle weakness in a large number of animals followed by recumbency over a period of several days. Cattle often appear to be constipated, and raise their tail while straining to defecate (Figure 1). Cattle also appear uneasy on their feet, with their feet placed closer together than normal; they often have a "hunched up" appearance suggesting colic or abdominal pain (Figure 2). Over the next few hours or days, affected cattle become weaker with some ataxia. When standing for an extended period of time, animals may develop muscle fasciculations of large muscle groups. Affected cattle may lean against walls of buildings or stay close to fencing for support (Figure 3). When lying down, they may remain alert to their surroundings while remaining in sternal recumbency. Slight ear drooping may be noted (Figure 4). As animals become weaker, they frequently have difficulty holding their heads upright, and lay with their head stretched out. They eventually weaken to the point of prostration.[1,2,6,8,11,13,15,16,17,18,21]

For a thorough and accurate diagnostic workup, it is important to examine multiple animals and compare them to clinically normal ones. A classic feature of botulism is loss of tongue tone. Extraction of the tongue from the mouth of the affected cow is fairly easy–often a difficult task in normal animals–and in advanced cases

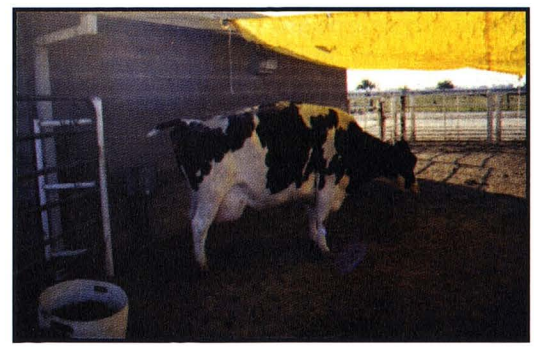

**Figure 1.** Cow with tail raised and straining to defecate.

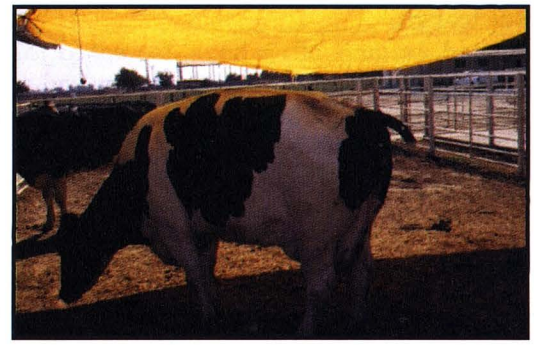

**Figure 2.** Cow with feet close together and "hunched back" as if suffering from back or abdominal pain.

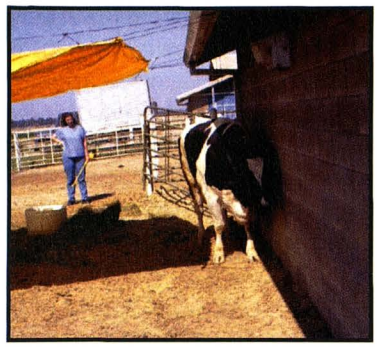

**Figure 3.** Cow supporting itself by leaning against the wall.

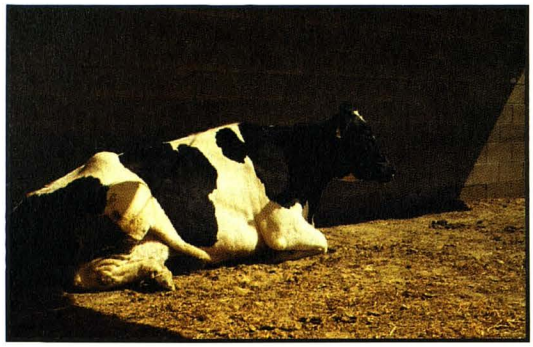

**Figure 4.** Cow in sternal recumbency and alert. Note slight ear drooping.

the tongue may hang from the mouth for several seconds before it is retracted (Figure 5). In early cases or in cattle less severely affected, loss of tongue tone may be subtle and not noticed. As a result, direct comparison of tongue tone in affected and non-affected cattle is critical. With poor tongue control, cattle have difficulty cleaning their nose, resulting in a dirty, dry appearance (Figure 6). Additionally, loss of tongue control and laxity in jaw muscles can result in dropping of food and difficult mastication. Difficulty in swallowing and excessive drooling may also be noted. When attempting to drink water, cattle may submerge their nose deep into the water trough.

Affected animals often have decreased tail tone, upper eyelids tend to droop and the palpebral reflex is decreased. Pupils are commonly dilated and respond poorly to light stimuli, however this can be difficult to determine. Ruminal motility may either be normal or decreased, depending on the stage of intoxication. Labored breathing is more frequently seen in later stages of the disease. Once the animal becomes prostrate, death ensues due to ruminal expansion and respiratory paralysis.[1,2,6,8,11,13,15,16,17,18,21] On necropsy, no gross lesions are noted, however some animals may have excessively dry feces and a dilated rectum (Figure 7).

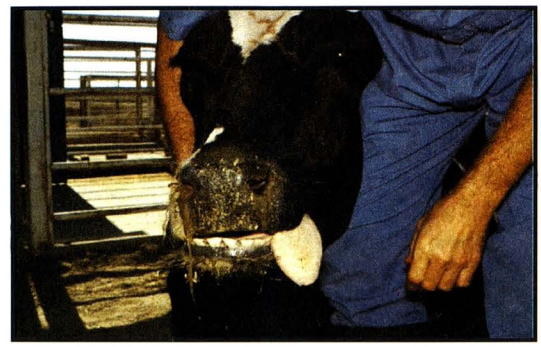

**Figure 5.** Cow with marked laxity in the tongue with failure to retract the tongue.

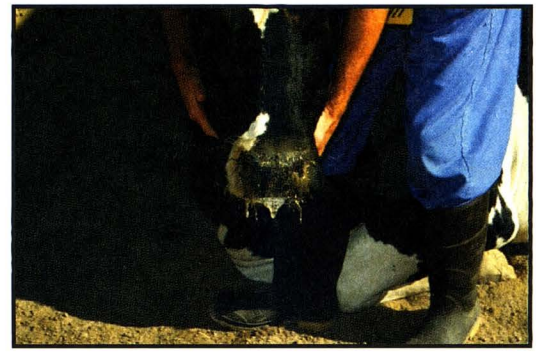

**Figure 6.** Cow with dirty nose due to inability to control the tongue for nose cleaning.

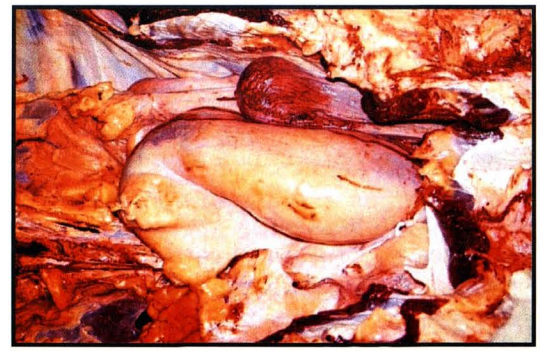

**Figure 7.** Dilated rectum from cow that died of botulinum toxin intoxication.

Biochemical and hematological findings are usually normal in cattle poisoned by botulinum toxin.[2] If the animal has been down for an extended time, muscle enzymes, such as aspartate aminotransferase, may be elevated. If dehydrated, hematocrit and total protein may be elevated; serum electrolytes are usually normal.

No commercially available routine diagnostic tests for detection of botulinum toxin in animals exist today. During a suspected botulism outbreak, blood samples

(both serum and whole blood) should be collected and serum separated for storage. Postmortem liver, rumen material and cecal content should be collected for toxicological evaluation. Anaerobic bacterial cultures from feces of a freshly dead animal may also aid in identifying the organism. Large numbers of *C. botulinum* organisms in feces or rumen content is suggestive of botulism. Using current standard testing procedures (mouse bioassay), serum and blood samples often fail to identify the presence of botulinum toxin. This is likely due to: 1) cattle are more sensitive to botulinum toxins than the mouse, which makes the mouse bioassay less sensitive in cattle, and 2) because the toxin can be cumulative, long-acting and intracellular, minute amounts of toxin present in the circulatory system may not be detected using standard testing procedures.

## Differential Diagnosis

Botulinum toxin is difficult to identify in intoxicated animals, therefore ruling out other causes of weakness and prostration in cattle is essential. Other possible causes of muscle weakness and prostration include: 1) hypocalcemia, hypokalemia or hypomagnesemia, 2) ionophore toxicity, 3) gossypol, 4) organophosphorus and carbamate insecticides, and 5) heavy metals.

Usually, multiple animals are affected in a botulism outbreak, which decreases the likelihood of calcium, magnesium or potassium deficiency as the cause of severe muscle weakness and collapse in the animals. Testing for deficient levels of calcium, magnesium and potassium in serum can quickly rule these out as potential sources of muscle weakness and collapse.

Ionophore and gossypol poisoning are usually associated with weakness due to cardiac insufficiency. Affected animals often develop myocardial lesions noted on histopathology. Analysis of feed for toxic ionophore or gossypol levels should be performed immediately. Organophosphorus and carbamate poisoning lead to muscular weakness, but other clinical signs of cholinergic stimulation may aid in differentiating these from botulism. Determination of blood cholinesterase activity is a useful diagnostic tool in suspected organophosphate and carbamate poisoning cases. Heavy metal toxicity (lead) can also cause clinical signs similar to botulism. Blood, urine and liver samples can be analyzed for lead levels. It is possible for individual animals to ingest botulinum toxin, although it is uncommon.

## Treatment

Treatment of poisoned cattle is often difficult and unsuccessful. The toxin is long-acting, and affected cows require hospitalization for weeks to months. Intense supportive measures should include oral and IV fluids, hand feeding and maintaining the animal in sternal recumbency. In a study of 30 cattle poisoned with botulinum toxin (type C and D), 13 of 30 animals (43%) treated with intravenous fluids (glucose/saline–10 to 20 liters per day) and daily administration of rumen fluid survived. However, these animals were less severely affected and remained ambulatory. Animals that became recumbent (down cows) and showing severe clinical signs of botulinum intoxication did not respond to this treatment, and died or were euthanized.[2] It is critical that animals be moved every few hours to prevent muscle and nerve damage. Movement and handling of affected animals may cause excitement and death of the animal due to respiratory failure.

Administration of pentavalent antitoxin to cattle has not proven beneficial when given to animals clinically affected with botulism. Antibiotic treatments are of little use. In some countries where botulism is prevalent, vaccination of cattle with a toxoid against botulinum toxin types B, C and D is common. There are currently no approved toxoids for immunization of cattle in the United States. However, there are botulinum toxin type B and C toxoids available for equine and mink species. Immunization of horses with botulinum toxin type C toxoid has been effective in stopping outbreaks caused by type C botulinum toxin, and may reduce the number of deaths in cattle if given soon after a diagnosis (R.B. Moeller Jr., personal experience). However, efficacy and safety of using the equine product in cattle has not been established. Use of equine type B botulinum toxoid would be experimental if given to cattle affected with type B botulinum toxin.

## Carcass Disposal and Public Health

When a diagnosis of botulism has been established, proper disposal of dead animals becomes a major concern and must be discussed with regulatory agencies. There is little risk to humans when working with or handling intoxicated animals; likewise, carcasses of dead animals pose little threat to individuals handling them. Also, sick cattle cannot directly pass the toxin to normal herd mates. The most common method of carcass disposal is rendering, which poses no threat to workers at the rendering plant; the rendering process (high heat) inactivates any toxin present in tissues.

One paper reported finding botulinum toxin in milk (type B toxin) from a cow with both mastitis and clinical signs of botulinum toxin intoxication;[3] it was suspected that this cow had *C. botulinum* bacteria in the infected mammary gland, resulting in secretion of the toxin into the milk. To date, the toxin has not been identified in milk from healthy cows (R.B. Moeller Jr., personal experience). Affected animals should not be slaughtered for human consumption.

## Conclusions

Diagnosis of botulism in cattle can be difficult to confirm in sick animals. With thorough examination of the animal, proper sample collection, and rule-out of other possible causes, botulism can be confirmed or at least suspected in affected animals. Unfortunately, there are no definitive treatments for the disease, therefore, animals affected with botulism often have a poor prognosis for recovery.

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# Abstract

## Lying Behavior and Activity of Early Lactation Holstein Dairy Cattle Measured Using an Activity Monitor

Blackie N., Scaife J.R., Bleach E.C.L.

*Cattle Practice* (2006) 14(2):139-142

In this study we compared the lying behaviour and activity of 25 early lactation Holstein dairy cows over the first 12 weeks of lactation. The lying behaviour of the dairy cows was recorded over 4 days using IceTag™ activity monitors during weeks 1, 6 and 12 of lactation. The activity monitors measure the proportion of time dedicated to lying down, standing up and activity. Cows in week 6 spent significantly time less ($P<0.05$) lying and more time ($P<0.05$) standing up than cows in week 12. This may be attributed to more time spent feeding than cows in week 1 and 12 of lactation. The lying behaviour of cows was not different between weeks 1 and 6 or weeks 1 and 12. Cows in week 1 were significantly more active ($P<0.05$) than cows in week 12. Activity of cows in week 6 was not different to weeks 1 and 12 of lactation. The increased activity of cows in week 1 could be attributed to stress and the cows adapting to their new environment following calving.

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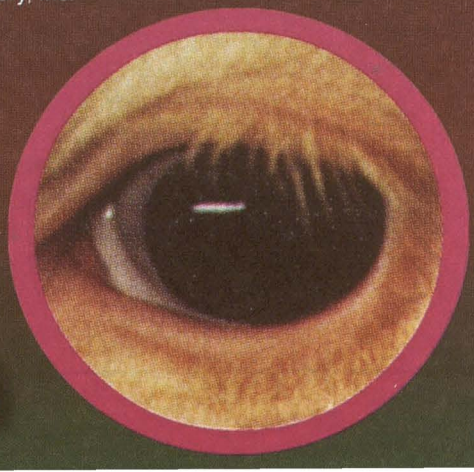

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