Presumptive diagnosis of *Clostridium botulinum* type D intoxication in a herd of feedlot cattle

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Abstract — Fifty-two feedlot cattle exhibited clinical signs suggestive of botulism. *Clostridium botulinum* type D organisms were recovered from ruminal fluid of 4 of the 5 affected animals tested and were isolated from bakery waste fed to the cattle. *Clostridium botulinum* type D has not been reported previously in Canadian cattle.

Résumé — Diagnostic présumé d'intoxication à *Clostridium botulinum* de type D dans un troupeau de bovins en parc d'engraissement. Cinquante-deux bovins en parc d'engraissement présentaient des signes cliniques évocateurs du botulisme. Du *Clostridium botulinum* de type D a été retrouvé dans le liquide ruminal de 4 animaux atteints sur les 5 qui ont été testés en plus d'être isolé dans les sous-produits de boulangerie servis aux bovins. Le *Clostridium botulinum* de type D n'a pas été rapporté auparavant chez les bovins canadiens.

(Traduit par Docteur André Blouin)

Can Vet J 2001;42:210–212

A herd of feedlot cattle suffered a disease outbreak characterized by clinical signs of progressive weakness, ataxia, recumbency, and death. Fifty-two out of 795 animals, ranging in age from 8 to 18 mo, were in sternal or lateral recumbency but were alert and aware of their surroundings. Before progressing to recumbency, the feedlot operator and referring veterinarian noted that many of the affected animals walked slowly and that their hind limb gait was stiff and ataxic. According to the feedlot operator, the feed intake in the affected pens was decreased by 50% and the manure of affected cattle was malodorous. No significant gross lesions were found on postmortem examination of 5 dead steers performed in the field by the referring veterinarian. Tissues from animals necropsied in the field were sent to the New Brunswick Provincial Veterinary Laboratories; no significant histopathological lesions were found. The referring veterinarian treated some of the animals empirically with calcium and magnesium solutions, IV, as well as with thiamine, without clinical improvement.

Two feedlot steers, approximately 9 to 12 mo of age, 1 live and 1 dead, were presented to the Atlantic Veterinary College Teaching Hospital on the 3rd day of the outbreak. A postmortem examination on the dead steer revealed no significant gross or histologic lesions. Liver and kidney tissues were evaluated for lead concentrations and ruminal fluid was tested for the presence of organophosphates or other pesticides, but all results were negative. The 310-kg, Limousin crossbred steer that was alive presented in lateral recumbency but was assisted into a sternal recumbency position and maintained in that position by using a bale of hay for support. The steer was hypothermic (36.2°C), tachycardic (100 beats/min), and had a respiratory rate of 20 breaths/min. Muscle fasciculations were noted over the cervical area. The steer had decreased tongue tone and ate slowly; grain fell from its mouth while chewing. All 4 limbs were hyporeflexive, tone in the tail and the anus was decreased, and pupillary light responses were poor. However, peripheral pain sensation was normal. Prior to our receiving the laboratory results, the steer was treated with 500 mL of 20% calcium borogluconate, IV, but no clinical response was noted. Serum biochemistry results indicated a mild hypokalemia (3.8 mmol/L; reference range, 3.9 to 5.9 mmol/L), mild hypomagnesemia (0.69 mmol/L; reference range, 0.80 to 1.32 mmol/L), hyperglycemia (8.2 mmol/L; reference range, 1.8 to 3.8 mmol/L), and an increased creatine phosphokinase (CPK) concentration (3881 U/L; reference range, < 350 U/L). Results of a complete blood cell (CBC) count performed the next day were within normal limits. The steer was moved to a heavily bedded stall and administered magnesium sulphate (60 g) and potassium chloride (34 g) in 25 L of water, PO. The steer did not respond to therapy and was euthanized 2 d later. Postmortem examination revealed a bronchopneumonia localized to the right middle lung lobe, suggestive of aspiration pneumonia. No other significant findings were found on postmortem. Lead concentrations in the liver and kidney were within normal limits.

An investigative team from the Atlantic Veterinary College visited the feedlot on the 5th day of the herd outbreak and examined 8 recumbent steers. All of the steers exhibited clinical signs similar to those of the steer admitted to the teaching hospital. Complete blood cell counts and serum biochemical profiles were performed on 5 recumbent cattle. One animal had a neutrophilic leukocytosis with a left shift, but results from the other animals were within normal limits. Serum biochemical profiles of 4 animals demonstrated mild to moderate elevations in CPK concentrations (591 to 9460 U/L) and 2 animals demonstrated mild increases in aspartate aminotransferase (AST) concentrations (127.5 to 340 U/L; reference range, 46 to 118 U/L). Two of the recumbent cattle had mild hypomagnesemia (0.51 to 0.69 mmol/L). Serum calcium concentrations were normal in all of the animals tested.

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Table 1. Summary of suspect cases by diet and pen location

Pen	No. recumbent[a]	No. dead[b]	Total[c]
Low concentration diet			
1	0	0	85
2	0	0	25
4	0	0	45
9	0	0	60
10	2	0	80
11	0	0	80
Total	2	0	375
Prevalence	0.5%		
High concentration diet			
3	6	2	80
5	10	4	30
6	10	6	80
7	16	2	80
8	2	0	150
Total	50	14	420
Prevalence	12%		

[a]Number of cattle in lateral or sternal recumbency
[b]Number of recumbent cattle that died at the feedlot
[c]Total number of cattle in the pen

Most diseases involving the central nervous system (CNS) in cattle are associated with changes in mentation or blindness, neither of which was present in any of the affected animals. Furthermore, postmortem examination of the CNS tissue was unremarkable in necropsied animals. These findings suggested that the cause of recumbency was muscular or neuromuscular in origin. Exclusions for peripheral neuromuscular weakness include nutritional myopathy, organophosphate toxicity, tick paralysis, *Clostridium botulinum* intoxication, and metabolic disturbances, such as hypocalcemia, hypomagnesemia, hypokalemia, and hypophosphatemia. Although mild hypokalemia and hypomagnesemia were noted in 3 of 6 animals tested, these mild deficiencies were unlikely the cause of the profound muscle weakness. Also, several animals failed to respond to calcium, magnesium, phosphorus, and potassium therapy. There was no evidence of organophosphate exposure and no ticks were found on any of the affected animals. Nutritional myopathy was unlikely to cause a large outbreak of recumbency and death in feedlot-aged cattle, and it was ruled out on the postmortem examinations. Therefore, *Clostridium botulinum* intoxication was the most likely explanation for the acute outbreak of weakness, recumbency, and death in the feedlot.

There were 11 pens on the feedlot, but affected animals were confined to 6 pens. All pens with affected cattle, except for one, were fed the finishing diet. The one pen not on a finishing diet had only 2 of 80 steers affected and was fed a diet that was a transition from the low concentrate diet to the finishing ration (Table 1). Overall, the prevalence of the disease among cattle fed a finishing diet was 12% compared with a prevalence of 0.5% among cattle being fed a lower concentrate diet. The finishing total mixed ration (TMR), fed ad libitum, consisted of 14% silage, 35% potato waste, 45% oats, and 6% bakery waste. Free choice hay was also offered. The low concentrate TMR consisted of 72% silage and only 19% potato waste, 7% oats, and 2% bakery waste. This indicated that one of the carbohydrate sources, namely oats, potato waste, or bakery waste, was the likely source of the toxin.

Oats were stored in a grain bin and had been fed to the cattle for months prior to the outbreak. A new shipment of bakery waste, containing mainly sliced bread and other unrecognizable bakery products, had been received 3 d prior to the outbreak. A new shipment of potato waste had been received 2 d prior to the outbreak and comprised a different type of potato waste then had previously been fed to the cattle. Both the bakery waste and the potato waste were stored on the floor of an uncovered bunker silo and were saturated by rain. On the 2nd day of the outbreak, on recommendations from the referring veterinarian, the feedlot operator discontinued feeding the potato waste and added more hay to the diet, but new cases continued to develop for the next 3 d. Based on this information, the bakery waste was considered the likely source of the toxin. The bakery waste was discontinued 5 d after the onset of the outbreak; 2 d later there were no new reported cases.

Liver and ruminal fluid samples from 5 affected cattle were sent to a Health Canada diagnostic laboratory. Neurotoxin was not identified by using the mouse inoculation test, but *Clostridium botulinum* was isolated from the ruminal fluid of 4 affected cattle. Typing of the isolates via mouse inoculation tests showed that all 4 isolates were *C. botulinum* type D. Bakery waste, potato waste, and the finishing TMR diet were submitted for neurotoxin assay and anaerobic culture. *Clostridium botulinum* type D was isolated from both the bakery waste and the TMR diet; however, neurotoxin assays were negative. A presumptive diagnosis of botulism was made based on the isolation of *C. botulinum* type D organisms in 4 of 5 ruminal samples and the 2 feed sources. The contaminated bakery waste was considered the likely source of the toxin.

Clostridium botulinum is a gram-positive, spore-forming, rod-shaped, ubiquitous organism. It is an obligate anaerobe and is saprophytic on animal and plant material. It elicits a potent neurotoxin, which irreversibly binds at the presynaptic site of the neuromuscular junction (1,2). This binding inhibits the release of acetylcholine, resulting in flaccid paralysis. Death is often caused by respiratory paralysis (2). The clinical signs seen in this case included hyporeflexia, decreased tongue tone, decreased pupillary light response, ataxia, recumbency, and death. Other signs include decreased salivation, constipation, and incontinence. Affected animals are unable to reach feed or water and have difficulty swallowing, resulting in dysphagia and adipsia. The steer that was presented to the Veterinary Teaching Hospital consumed the feed and water placed in front of it, but the steer had difficulty with mastication and swallowing. Also, the feedlot operator reported a 50% decrease in feed intake during the outbreak. Feed consumption returned to normal levels approximately 1 wk after the bakery waste was removed from the diet. While most intoxicated animals die of respiratory paralysis, some may recover weeks later, after the neurotoxin has been degraded. In this outbreak, 2 of 4 recumbent animals kept on the feedlot recovered completely, following 2 wk of supportive care.

Cattle with botulism exhibit no consistent hematologic abnormalities (3,4). As reported in this outbreak, elevated serum CPK and AST concentrations are common and are attributed to muscle damage from prolonged recumbency. The mild hypokalemia and mild hypomagnesemia were likely a consequence of decreased feed consumption and were not severe enough to cause recumbency. Consistent with botulism, postmortem gross and histological evaluations of tissues were unremarkable.

There are 8 types of *C. botulinum.* Types A, B, C, and D have been reported in cattle (1,2). Type A was associated with spoiled silage in an outbreak in Idaho (2). Type C is typically associated with carrion-contaminated silage. Type B is the most common type found in the eastern United States (2). Types C and D have been implicated in outbreaks where poultry litter was fed to cattle or in cases of carrion ingestion (1,5). Most cases of type C and D botulism have been reported in the southern hemisphere and in Europe (2,6,7), although type C has been reported in Canada (1). In this case, type D botulism was isolated from a previously unreported source, bakery waste. To the authors' knowledge, only 3 confirmed or suspected cases of type D botulism have been reported in North America, all within the US (2,4). This is the first time an outbreak of type D botulism has been reported in Canadian cattle.

To definitively diagnose a suspected outbreak of *C. botulinum* intoxication, the neurotoxin must be detected in serum, ruminal fluid, or tissue of affected animals. The gold standard is the mouse inoculation test. This test has limited sensitivity because it only detects active, nonbound toxin. Furthermore, a negative mouse bioassay does not eliminate botulism from the diagnosis, because the neurotoxin concentration in the serum of clinically affected cattle may be below the threshold of detection of the bioassay (2,8). Isolating neurotoxin from ruminal fluid is further hampered due to low neurotoxin concentrations in the rumen and neurotoxin biodegradation by ruminal microbes (2). These limitations of the mouse inoculation test can explain the negative results on ruminal fluid and feedstuffs in this herd outbreak. Enzyme-linked immunoassays (ELISA) are available for identifying neurotoxins but are currently less sensitive than the mouse inoculation test (2,9,10).

Presumptive diagnosis of botulism can be made by isolation of *C. botulinum* from the ruminal fluid of animals exhibiting appropriate clinical signs and from the feed (2,8). Although *C. botulinum* can be a normal inhabitant of the gastrointestinal tract, it is rarely isolated from ruminal contents of normal cattle (2). In this outbreak, *C. botulinum* type D was isolated from ruminal fluid of 4 of 5 affected cattle, the bakery waste, and TMR. The positive culture from the TMR sample probably resulted because the TMR contained bakery waste. The recent shipment of bakery waste was the most likely cause of botulism in this outbreak. How the bakery waste became contaminated was not pursued, but contamination with botulism spores by carrion or bird feces is often incriminated in cases of *C. botulinum* type D (1,5). The bakery waste was likely contaminated prior to delivery to the feedlot, because a 2nd feedlot, feeding the same source of bakery waste, reported 2 steers dying with clinical signs consistent with botulism. The heavy rains that saturated the bakery waste may have provided an adequate anaerobic environment for the botulinum spores to proliferate.

During this outbreak, the feedlot operator sent 93 animals to slaughter. Thirty-eight of those animals were recumbent on arrival at the slaughter plant. Although the carcasses passed inspection, they were quarantined initially, based on the suspicion of botulinum intoxication. After a diagnosis of botulism was supported by laboratory tests, all detained carcasses were condemned and disposed of appropriately. Various papers have stated that botulinum-intoxicated animals are not fit for human consumption, but the true risk to public health is unknown (1,2). Botulism due to types A, B, and E is most common in humans (2), but there is potential risk of human intoxication with all types of *C. botulinum,* including type D.

In this feedlot outbreak, a strong presumptive diagnosis of botulism was made based on the clinical signs of neuromuscular weakness and the isolation of *C. botulinum* type D from the bakery waste and 4 ruminal fluid samples. *Clostridium botulinum* should be considered as a rule-out for outbreaks of weak and recumbent cattle. To our knowledge, this case is unique because bakery waste appears to be the cause of the intoxication and it is the first case of *C. botulinum* type D reported in Canadian cattle. CVJ

References

1. Jean D, Fecteau G, Scott D, Higgins R, Quessy S. *Clostridium botulinum* type C intoxication in feedlot steers being fed ensiled poultry litter. Can Vet J 1995;36:626–628.
2. Whitlock R. Botulism toxicosis of cattle. Proc Annu Conf Am Assoc Bovine Pract, 1999;32:45–53.
3. Rings DM. Bacterial menigitis and diseases caused by bacterial toxins. Vet Clin North Am Food Animal Pract 1987;3(1):93–94.
4. Abbitt B, Murphy MJ, Ray AC, et al. Castastrophic death losses in a dairy herd attributed to type D botulism. J Am Vet Med Assoc 1984;185:798–801.
5. Williams J, Whitlock R. Botulism in cattle: A review of 31 outbreaks, 1984–1997. Proc Annu Conf Am Assoc Bovine Pract 1998;31:218–219.
6. Popoff MR, Lecoanet J. Botulinum type D toxin and *Clostridium perfringens* enterotoxin in a bull calf. Vet Rec 1987;121:591–592.
7. Brizuela CM. Bovine botulism in Paraguay. Trop Anim Health Prod 1996;28:221–222.
8. Trueman KF, Bock RE, Thomas RJ, et al. Suspected botulism in three intensively managed Australian cattle herds. Vet Rec 1992;130:398–400.
9. Gregory AR, Ellis TM, Jubb TF, Nickels RJ, Cousins DV. Use of enzyme-linked immunoassays for antibody to types C and D botulinum toxins for investigations of botulism in cattle. Aust Vet J 1996;73:55–61.
10. Main DC, Gregory AR. Serological diagnosis of botulism in dairy cattle. Aust Vet J 1996;73:77–78.