Clostridium botulinum type D intoxication in a dairy herd in Ontario

Sarah Martin

Abstract — Thirty-four Holstein cows died after exposure to *Clostridium botulinum* type D toxin, presumably from contaminated haylage. The presence of type D toxin in ruminal contents was confirmed by mouse inoculation. This is the first confirmation by direct toxin isolation of *C. botulinum* type D toxin in cattle in North America.

Résumé — Intoxication par *Clostridium botilinum* type D dans un troupeau laitier de l'Ontario. Trente-quatre vaches Holstein sont mortes après avoir été exposées à la toxine *Clostridium botilinum* type D, présumément à partir d'ensilage de foin. La présence de toxine du type D dans le contenu du rumen à été confirmée par l'inoculation de souris. Il s'agit de la première confirmation par isolement direct de la toxine *C. botilinum* type D chez des bovins en Amérique du Nord.

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A well-managed dairy herd in southern Ontario experienced a severe outbreak of botulism, with 38 cows affected. New clinical cases developed over 17 d and deaths continued until day 23. Thirty-four of the affected cows died or were euthanized for humane reasons, and the remaining 4 made a prolonged but full recovery.

The herd consisted of 85 Holstein cows and heifers. The affected group included milking cows and dry cows that were nearing their calving dates. The unaffected group included dry cows at earlier stages of gestation and heifers. The 2 groups were housed in separate areas of the barn and fed different rations.

On day 1, veterinary care was sought for a downer cow, #180, a high producer that had calved 6 mo earlier and was near estrus. Physical examination revealed no cause for recumbency, and the cow was treated empirically for hypocalcemia and mild dehydration. She remained recumbent after treatment but continued to eat and drink. The same day, cow #115 died with no apparent clinical signs, after being moved to a different stall earlier that day.

On day 2, cow #180 died. She had remained recumbent, tended to push forward into the manger, and had an "s" bend in her neck. She had maintained her appetite and had good tongue tone, but her respiration was rapid and shallow. When 4 other cows at various stages of lactation became recumbent on day 2, botulism was suspected on the basis of clinical signs. Rations for the affected and unaffected groups differed in that haylage (from a concrete tower silo), bicarbonate, and mineral were fed to the lactating cows. Haylage was removed from the ration in the late morning of day 2. The bicarbonate and mineral were removed on day 3. The herd owners recalled removing a small portion of an animal's hide from the first-cut haylage months earlier. Abnormalities on a complete blood cell (CBC) count (VetAutoread; Idexx Laboratories, Westbrook, Maine, USA) and serum biochemical profile (VetTest; Idexx Laboratories) from cow #180 before treatment on day 1 included leukopenia (0.6×10^9 cells/L; reference range, 2.0 to 6.0×10^9/L), hyperglycemia (7.42 mmol/L; reference range, 3.11 to 4.89 mmol/L), mild hypokalemia (3.23 mmol/L; reference range, 3.90 to 6.40 mmol/L), and hypophosphatemia (0.87 mmol/L; reference range, 1.29 to 2.77 mmol/L). These biochemical abnormalities were not helpful in identifying a specific reason for acute illness and death.

Cows #115 and #180 were submitted to the Animal Health Laboratory (University of Guelph, Guelph, Ontario) for complete postmortem examination. Differential diagnoses included botulism, myopathy (ionophore toxicity), hypokalemia, and, possibly, a peripheral nervous system toxin (1). Necropsy findings included pulmonary congestion, edema, and emphysema (cows #115 and #180), and acute subepicardial hemorrhage and acute hemorrhagic enteritis (cow #180). Ruminal contents were saved and frozen for assay of botulinum toxin.

By day 7, a total of 20 cows had died. Serum samples from 2 other cows showing clinical signs and 2 clinically normal cows were submitted to the Animal Health Laboratory. One of 2 affected cows was hyperglycemic (serum glucose 4.5 mmoI/L; reference range, 2.1 to 3.8 mmol/L). Creatine kinase was increased in both

Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Dr. Martin's current address is Paris Veterinary Clinic, 321 Grand River Street North, P.O. Box 21120, Paris, Ontario N3L 4A5.

Address all correspondence and reprint requests to Dr. Martin.

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cows (2251 and 3976 U/L; reference range, 65 to 234 U/L). No specific biochemical abnormalities were found in the samples from the 2 cows that appeared normal. By day 9, an additional 9 cows had died (total 29). Five more cows died by day 23, bringing the total deaths to 34. Herd mortality was 40% and mortality in the group at risk was 77%.

Clinically affected cows exhibited generalized muscle weakness, evidenced by recumbency, but remained bright and eating. Clinical signs progressed rapidly, with the average duration of illness 4 d (range, 3 h to 14 d). Tongue tone, assessed by grasping mid-tongue, remained normal. Abdominal breathing, decreased ruminal sounds, and decreased tail tone were noted. Some affected cows appeared to be staring, but a palpebral reflex could be elicited. As clinical signs became severe, neck chains were released to prevent strangulation. Four cows recovered after prolonged recumbency (up to 7 d) and weakness (up to 1 mo). One cow aborted twins at approximately 6 mo gestation, and the others maintained their pregnancies.

Clostridium botulinum type D toxin was identified by mouse inoculation bioassay (Animal Health Laboratory) by using the filtered ruminal content from cows #115 and #180. Botulism was confirmed when mice injected with the filtered ruminal contents developed the 'wasp-waist' sign and died. Further typing of the toxin was performed by combining filtered ruminal contents with specific antitoxins A through E. Only type D antitoxin afforded protection, evidenced by survival of the mice. The haylage tested negative for botulinum toxin by mouse inoculation. Culture of *C. botulinum* was not attempted.

Demonstration of the preformed toxin in serum, feed materials, or ruminal contents by mouse inoculation remains the gold standard for the diagnosis of botulism (1). A negative mouse inoculation test does not eliminate the possibility of botulism, because the toxin may be present at a level below the threshold of detection. In addition, botulinum neurotoxin is rapidly biodegraded by microbes in the rumen (1,2). An enzyme-linked immunosorbent assay (ELISA) has been developed for types C and D toxin (3) and for antibodies to types C and D toxin (4). Both ELISAs have lower sensitivity than the mouse inoculation test and are considered supplemental diagnostic tests (3).

Clostridial vaccination was initiated on day 6, using a bivalent C and D botulinum toxoid (CSL Limited, Victoria, Australia), available in Canada by emergency drug release. Heifers, dry cows, and all milking cows still standing were vaccinated and then revaccinated on day 20. All herd introductions were vaccinated with a primary series on arrival. Two cull dairy goats were brought in as sentinel animals, and 1 received the toxoid. Neither goat developed clinical signs, presumably because contaminated haylage had already been removed from the silo. A localized high concentration of botulinum toxin in the haylage is considered the most likely explanation as the point source for the following reasons: haylage samples collected on day 2 were negative for botulinum toxin; an unvaccinated goat did not die from botulism after being fed the haylage; and the only difference between the affected and the unaffected cows was the haylage, bicarbonate, and mineral in the diet of the affected group. The vaccinated goat received the bicarbonate and mineral in her water prior to her booster dose and did not develop botulism. After the outbreak, the silo was completely emptied and the haylage was spread on the fields with manure.

Botulism is caused by a neurotoxin produced by *Clostridium botulinum,* a gram-positive, spore-forming anaerobe. Botulinum toxin is an exotoxin produced during the growth and autolysis phase of the organism under anaerobic conditions. It is the most potent toxin known to man. One gram of pure toxin, adequately distributed in feed, could kill 400 000 adult cows (5). Botulinum toxin is absorbed from the intestinal tract, or from a wound, and is carried in the blood to neuromuscular endings, where the toxin binds to receptors on nerve endings, is internalized, and interferes with acetylcholine release at the neuromuscular function (1,6). Flaccid paralysis occurs, and death results from respiratory failure.

Treatment of affected cattle consists primarily of nursing care and restriction of muscular activity. Recovery requires regeneration of new nerve endings rather than metabolism or elimination of the toxin (7). Neither polyvalent nor type-specific antitoxin is efficacious once the toxin has translocated into the nerve ending (1,7). However, antitoxin will stabilize the animal by neutralizing toxin still in circulation. Antitoxin was not available during this outbreak.

Eight known botulinum toxins, A, B, Ca, Cb, D, E, F, and G, have been identified (1). Disease in farm animals is produced primarily by types B, C, and D (6). Type B spores are common in the soil in the mid-Atlantic states and are usually responsible for botulism in cattle reported in North America (1,6). Type E botulinum is a fish-associated strain and has been responsible for outbreaks in waterfowl on the Great Lakes.

Botulism occurs in several forms. Forage and carrion-associated botulism result from ingestion of the preformed toxin. Wound and toxicoinfectious botulism result from proliferation of *C. botulinum* in situ. Forage botulism occurs when the pH (> 4.5), moisture, and anaerobic conditions within the forage allows proliferation of *C. botulinum.* Carrion-associated botulism is commonly associated with type C and D strains, occurring when the carcass of a dead animal is invaded by *C. botulinum* and toxin is produced, contaminating feedstuff. Type C strains are usually associated with a decomposing carcass, for example, cats (5), or waterfowl (8), or with poultry litter (7). Type D botulism occurs more commonly in South Africa and South America when phosphorous-deficient range cattle chew on bones of decaying carcasses (6). Botulinum toxin can persist in carrion for at least 1 y (6).

Two published cases of botulism type D in North America have been confirmed by isolating *C. botulinum* spores, culturing and proliferating the organism, and typing the toxin produced (1,2). The most recent of these 2 cases occurred in New Brunswick and affected 52 of 795 feedlot cattle. *Clostridium botulinum* spores were isolated in the bakery waste fed to these animals (2). Toxicoinfectious botulism, which develops after spores are ingested, and toxin is produced and absorbed from the

gastrointestinal tract (6), causes the 'shaker foal syndrome' and human infant botulism associated with ingestion of unpasteurized honey. The safety of milk and meat from cattle affected with botulism has not been fully evaluated. In the New Brunswick outbreak of type D botulism, carcasses from affected cattle passed inspection but were held back pending laboratory confirmation of botulism. Once botulism was confirmed, all carcasses were condemned (2). In a case of type C botulism in California, in which 427 of 441 Holstein cattle died, results of ELISAs for the toxin in milk were negative (5). It is possible for the toxin to be present in meat or milk at less than currently detectable limits. Most recommendations state that the toxin is denatured by 10 min of boiling, but spores require more than 30 min for denaturation (9). The passage of botulinum toxin by milk in germ-free rats to 2- to 3-day-old rat pups has been demonstrated (10).

Clinical signs of botulism intoxication in cattle include loss of tongue tone, decreased upper eyelid and tail tone, dilated pupils, dysphagia and subsequent salivation, decreased ruminal motility, and, occasionally, bradycardia and hyperglycemia (1). Although loss of tongue tone was not demonstrated in this outbreak, it is considered to be the most specific and sensitive clinical sign for botulism in cattle (1). Tongue tone often characterizes an outbreak, all of the cases either having or not having tongue paralysis (6).

This is the first case known to the author in which the diagnosis of type D botulism was confirmed by the accepted gold standard test; namely, by identifying and typing the toxin directly from affected animals.

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