Research Summaries

Don Schlafer, *Presiding*

Botulism in Cattle: Clinical and Diagnostic Approaches

R. H. Whitlock*, J. Messick*, T. Divers*, J. Ott, A. Oliver***, and R. Bartholomew***

Botulism in cattle is nearly always associated with ingested carrion contaminated *Clostridium botulinum* toxin (Blood and Henderson). Botulism in cattle in Europe, Australia and South Africa was reported due to the ingestion of carrion or forages contaminated with dead animals where *Clostridium botulinum* Type C or D was responsible for toxin production (Enfors et al 1975, E Telvedt and Hanssen 1974, Theiler et al 1927). Suspected Type D botulism was recently reported from a herd of cattle in Texas (Abbitt et al 1984).

Non-carrion associated botulism (forage poisoning) in cattle due to forages contaminated with *C. botulinum* Type B toxin has been reported in Europe (Haagsman and Terlacck 1978, Breukink et al 1978). A similar episode was reported in the United States associated with ingestion of contaminated silage but dead animals were not found, nor was the toxin type identified (Gray and Bulgin 1982). Because of the difficulty identifying the toxin, a clinical diagnosis could not be confirmed in other reports of botulism in cattle presumably due to the ingestion of contaminated silage (deLahunta 1977).

This report describes the clinical signs and diagnostic approaches in three outbreaks of botulism in cattle where *Clostridium botulinum* Type B organisms were identified in each herd and specific toxin detected in the forage of one herd.

Case History for Herd I:

During the summer of 1984 oatlage was ensiled on top of alfalfa haylage in a concrete silo. The added amount was a 4-5 ft layer in a 20′ x 60′ silo. During the period of ensiling the weather was poor, thus requiring several days for the harvest. During August 1985 haylage was fed to a herd of 65 milking cows with no untoward effects. A fetid odor was noted as the oatlage was fed in a bunk. The oatlage was first fed on a Sunday evening feeding. The following morning three cows of the 65 adult cows fed the oatlage were recumbent. One cow had calved 6 weeks earlier, one was 6 months pregnant and the other was 70 days pregnant. Physical examinations were nonremarkable except for poor to absent rumen motility, muscular weakness and obvious recumbency. Atypical milk fever was suspected, however, the serum calcium values were only mildly abnormal: 13.1; 9.5 and 6.5 mg/dl. Each cow received intravenous calcium (8 gms) but only the cow with a serum calcium of 9.5 mg/dl responded; however, she was again recumbent one hour later. Subsequently each cow received 5 gallons of oral electrolyte solution and laxatives. Later in the day the owner reported the cows were not eating the oatlage and 3 more cows were weak and wobbly while the original three animals had not improved. Late in the day the oatlage was removed from the bunk and the cows were fed hay as the only roughage.

Tuesday morning two of the original 3 recumbent cows were found dead and the other three weak ataxic cows were recumbent and unable to rise. The milk production of all 65 cows was severely reduced. Further examination of the herd found several other weak and ataxic cattle that were completely anorexic and barely able to rise. The rumen motility was weak or absent on each affected cow and minimal feces were passed. Later Tuesday evening a rumenotomy was performed on the two recently recumbent cows because of a concern for "toxic indigestion" and to remove the possibly offending material in the rumen. Each rumen had firm ingesta dorsally and adequate rumen liquour ventrally. These two cows received fluids and antibiotics. The other original recumbent cow died and a field necropsy was nonremarkable.

On Wednesday morning (Day 3) both cows with the rumenotomy and another cow had died. Other cows seemed to be eating better and milk production was increasing.

**Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348.*

***Franklin Veterinary Associates, 2380 Buchanan Trail West, Greencastle, PA 17225.*

****Greencastle Veterinary Hospital, 9587 Brownsmill Road, Greencastle, PA 17225.*

However one other cow became recumbent and 4 others were obviously weak and ataxic which were sent to slaughter. On Friday morning the 7th cow had died. On the following Monday (Day 8) the remaining cows were back on full feed and nearly normal milk production. However, 7 cows had died and 4 others were sent for salvage out of a total of 65 affected. The attack rate (morbidity) was 100% and the fatality rate 7/65 or 18%. Some of the weak, ataxic, but not recumbent cows did survive. None of the routine treatments appeared to alter the course of the illness.

Samples of oatlage, haylage, and rumen contents from one cow at necropsy and feces from two downer cows and one weak cow were submitted to our laboratory for culture of *Clostridium botulinum* and toxin testing. Each sample was *positive* for *Clostridium botulinum* Type B organisms but extracts of each sample were negative for toxin.

Case II:

During July 1985 the concentrate ration was changed from 16% to 13% protein. Rye silage was free choice which has been stored as high moisture round bales. Each cow received an average 6 lbs alfalfa hay. The herd of 50 Jersey and 100 Holstein milking cattle were housed as two separate herds on the same farm and only share the same milking facilities. Cattle from both groups became ill as the last round bales of rye haylage stored in plastic bags were fed.

Six cows were reported ill on July 17, 1985, three Jerseys were down and the others were weak and ataxic. Two days later three others had developed the same signs and all were afebrile with normal respiratory rates. One cow died while receiving intravenous calcium. The rye forage was withdrawn and replaced with alfalfa haylage. The next day, July 20, 1985, two others were weak and "off feed" which eventually recovered.

The clinical signs of affected animals included anorexia; profound decrease in rumen motility; no diarrhea, bloat or fever; progressive weakness; and ataxia leading to recumbency. The heart rate was either slow (45-55/minute) or normal. Despite symptomatic therapy with parenteral antihistamines and calcium gluconate, activated charcoal and mineral oil orally and other supportive therapy, the more severly affected cows progressively became weaker, recumbent and died over a period of 4-5 days. Less severely affected cows, i.e., those that were able to remain standing, gradually improved over a period of 3-5 days and recovered completely.

The hematologic and biochemical determinations did not reveal any obvious organ dysfunction. The electrolytes were essentially normal with a tendency toward alkalosis which is typically associated with acute gastrointestinal stasis.

Over the 12-day period affected animals had evidence of generalized muscular weakness, 13 of 150 cows had clinical signs, one of which died. Thus the attack rate was 9% and the mortality was 47%, or 7 deaths of the 15 obviously affected.

Rumen contents and fecal samples from 4 affected cows along with two samples of high moisture silage were submitted to our laboratory for toxin testing and culture for *Clostridium botulinum.* Extracts of all the samples were negative for any *Clostridium botulinum* toxin, however, each sample was *positive* for *Clostridium botulinum* Type B organisms. Thus, the finding of botulinum spores in the feces and rumen contents of affected cows helps confirm the diagnosis of botulism. The presence of spores in the high moisture alfalfa haylage would suggest this was the source of the disease. The fact the silage was excessively dry at the time of ensiling may have prevented adequate fermentation, thus the silage pH did not become sufficiently acid (<5.0) to inhibit sporulation and toxin production by *Clostridium botulinum.*

Case III:

This outbreak occurred on a 30-cow dairy farm in Lancaster County, Pennsylvania. The farm also had 4 heifers, 1 bull, 78 pigs and 6 mules. The owner began feeding rye silage from the top of an upright silo on May 28 which had been ensiled 3 weeks earlier. On May 30 one cow was anorectic and weak. The following day 3 more cows were anorectic and appeared weak. On June 1, 2 more cows were similarly affected and most of the cows in the herd had decreased feed intake and milk production. Two cows dribbled urine when they walked. By June 4 the first 4 clinically affected cattle had died. They became progressively weaker after the onset of clinical signs and became recumbent and had refused to eat two days before death.

Five of the six mules entered the feeding area on May 31; that evening one mule was found to be weak, staggering and died 2 hours later. By the evening of June 1, all 5 mules that had eaten the silage had died. One mule that could not be made to enter the silage feeding area survived. On June 4 two additional cows were recumbent and could not rise. Both cows hads tachycardia and no detectable rumen contractions. The tongue tone was assessed as weak in both cows in that the tongue could easily be pulled from the mouth. Ten other cows were weak and wobbly but could stand. Each of these cows had decreased rumen contractions and had passed no feces in the previous 4 hours they had been in the barn. During rectal examination two of the most severely affected cows had distended urinary bladders. Most of the cows had persistent chewing movements and during oral examination each cow was found to have poorly chewed hay in its mouth. Despite the fact all ten could drink water, each cow had a small pool of saliva in front of the cow. All ten had a normal heart rate except one which was 52 beats/minute. Both recumbent cows died the next day. An autopsy performed on one cow found no gross lesions.

Fecal samples from 4 cows with clinical signs compatible with botulism, samples of cecum and colon contents from one cow at necropsy and 3 silage samples were collected for laboratory analysis. Additionally, 6 soil samples from the

field where the silage was harvested was submitted. One silage sample contained "preformed" *Clostridium botulinum* Type B toxin (30 mouse LD_{50}/gm). *Clostridium botulinum* producing Type B toxin was identified in 2 of the 3 silage specimens. The 4 fecal specimens, 6 soil samples, and 2 postmortem specimens were negative for preformed toxin but were all positive for *Clostridium botulinum* Type B in enrichment cultures. Subsequent testing of silage samples obtained 3 weeks after the episode of botulism continued to demonstrate both preformed toxin and *Clostridium botulinum* Type B in enrichment cultures. However, one week later no preformed toxin was detected but botulinum organisms were present in the silage.

Materials and Methods

The fecal, haylage and oatlage specimens were extracted by mixing with an equal amount (weight to volume) of sterile gelatin phosphate diluent in a sterile motor (CDC Handbook 1978). The toxin was allowed to elute from the solid phase for 4 hours at 4°C. A portion of the tissue suspension was centrifuged at 12,000g for 20 minutes to clarify the suspension and the supernatant was removed for botulism toxin testing.

Mouse toxicity and neutralization testing was performed on the supernatant as described by Hatheway for the testing of serum (Hatheway 1979). All the mice injected intraperitoneally with botulism toxin containing supernatant died within the 96 hour test period. Those mice receiving a mixture of the trypsinized tissue extract containing the monovalent antitoxin corresponding to the type of toxin present in the tissue survived the 96 hour test period.

Culturing of the tissue specimens for detection, isolation and identification was performed using procedures outlined by the CDC (CDC Handbook 1978, Dowell and Hawkins 1977). Fecal, haylage and rumen contents were cultured in chopped meat-glucose starch medium (CMGS) and the supernatant of these enrichment cultures was tested for botulism toxin activity following 4 days of anaerobic incubation at 35°C. Further, the CMGS enrichment cultures were streaked onto *Clostridium botulinum* isolation agar (CBI) for isolation of the organisms (Dezfulian et al 1981).

Discussion

Botulism is caused by a neurotoxin produced by *C. botulinum* that can affect all mammals.[14] The organism is a strict anaerobe spore forming, gram-positive rod that elaborates a potent exotoxin during growth and autolysis. There are 8 known types of the toxin; types A, B, C_a, C_b, D, E, F, and G.[15] Each type is unique in its geographic distribution and species susceptibility.[15 16]

Infection occurs by three routes: a) Ingestion of the preformed toxin, b) wound botulism, and c) toxicoinfectious botulism.[17] The ingestion of preformed toxin in decaying foods (animal or plant) is the most common form of exposure in man, and probably in most adult animals. Confirmed wound botulism is rarely reported, but may develop when the organism infects a wound, sporulates, and releases its toxin under anaerobic conditions. Toxicoinfectious botulims develops after spores are ingested and the toxin produced in the gastrointestinal tract is absorbed. Toxicoinfectious botulism occurs in children less than 6 months of age as one cause of sudden infant death syndrome and in foals up to 8 months of age. The reason for this unusual intestinal colonization and toxin release is unknown, but is thought to be related to high exposure to the organism and a change in intestinal flora.[18]

Type B organisms are common in the soil in the mid-Atlantic states (Smith 1979). Two forms of Type B toxin occur, a proteolytic toxin which has maximal toxicity and a nonproteolytic toxin which must be activated by trypsin to be fully activated. The proteolytic Type B toxin occurs most commonly in the soil of the mid-Atlantic states and Kentucky (Smith 1979). Type B toxicoinfectious botulism in foals is almost completely restricted to those regions (Klyza 1983).

The definitive diagnosis is very difficult as this requires the demonstration of toxin in the patient's serum. Occasionally this is possible in man, which as a species is more resistant to the toxin than cattle or horses which are exquisitely sensitive to the toxin. The suspect serum sample must be toxic to mice then be neutralized by the specific antitoxin. Circumstantial or a tentative diagnosis can be made by finding the organism and toxin in feedstuffs which have been recently consumed and the animals have compatible clinical signs.

Botulism has been reported in dogs, cattle and horses in the United States (Abbitt 1984; Gray 1982; Barsanti 1978; MacKay 1982 and Swerczek 1980). Although toxicoinfectious botulism has been experimentally reproduced in foals, there has not been any natural cases where toxin has been recovered from blood or serum (Swerczek 1980). Circumstantial evidence suggests that the "Shaker foal disease" in the mid-Atlantic states and Kentucky is botulism (Swerczek 1980). Horses may be so incredibly sensitive to botulinum toxin, the serum concentration is below the detection limit by the time clinical signs develop. Botulism associated with the consumption of feed containing a carcass is most often Type C or D (Enfors 1975, Etelvedt 1974) while forage poisoning is typically Type B (Haagsman 1978 and Breukink 1978). There is no evidence that toxicoinfectious botulism occurs in cattle. Both reported outbreaks from the Netherlands were due to silage made from brewers grain and grass silage containing the Type B toxin (Haagsman 1978), Breukink 1978).

The initial therapeutic objective should be the neutralization of circulating toxin with specific or multivalent antiserum. The antitoxin has no effect on the toxin after it has been translocated in the cells (Simpson 1979). The patient should have muscular activities restricted

and be observed closely for signs of respiratory failure. Antimicrobics may be given for specific secondary complications (i.e., aspiration pneumonia). Antimicrobics have been ineffective in eradicating the organism from the intestinal flora (Beaty 1983). If antimicrobics are used, those that may potentiate neuromuscular weakness should be avoided, i.e., aminoglycosides, tetracyclines, and procaine penicillin. After the specific antitoxin is administered, continued absorption from the intestine should have minimal or no additional effect on the clinical condition. Additionally, some form of cathartic usually is recommended because of the ileus. Magnesium products should be avoided, as these may potentiate the neuromuscular weakness. Mineral oil or sodium sulfate may be used as cathartics. Parasympathomimetics, although they may provide temporary improvement in clinical signs, have been shown to increase mortality. Adherence to general principles of good nursing care and nutrition are of utmost importance because of the prolonged time required for recovery. Recovered patients are thought to regain normal nervous and muscular function.

In each of these outbreaks Type B organisms were isolated from the silage which may have been improperly fermented. The key factor may be the lack of adequate fermentation to reach a low pH, thus inhibiting sporulation and toxin production by *Clostridium botulinum*. Botulinum organisms were also isolated from the rumen contents and feces of affected cattle which help confirm the diagnosis because normal cattle do not have botulinum organisms in the gastrointestinal contents. The exception to this is the animal at risk, i.e., exposed to a herd outbreak but not showing clinical signs. Botulism does occur sporadically and the practitioner needs to be kept aware of that possibility.

References

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Engineering Tomorrow's Cow: Embryo Sexing, Splitting and Gene Insertion

R. H. Foote, *Ph.D.*
Department of Animal Science
Cornell University
Ithaca, New York

Introduction

We live in a world of technology, including biotechnology such as genetic engineering. While several "buzz" words have become popular recently, we have lived in a world of biotechnology for at least a generation.

Artificial insemination (A.I.) is an example of the greatest single biotechnology applied to livestock, particularly dairy cattle (1). This opened the field of sperm physiology and the study of fertilization and embryo mortality, as well as the control of certain infectious diseases. Artificial insemination and dairy record keeping provided information for the geneticists to develop effective dairy sire selection programs. Computers were used extensively, starting in the 1950's.

The development of frozen semen, with glycerol as a cryoprotectant, heralded the beginning of a major emphasis on cryobiology—freezing blood, tissues and organs followed. Today, this has developed so that frozen embryos