

Type C botulism in dairy cattle from feed contaminated with a dead cat

F. D. Galey, R. Terra, R. Walker, J. Adaska, M. A. Etchebarne, B. Puschner, E. Fisher, R. H. Whitlock, T. Rocke, D. Willoughby, E. Tor

Abstract. Four hundred twenty-seven of 441 adult Holstein dairy cattle from a 1,200-cow dairy died over a 1-week period during early spring 1998. Affected animals were from 4 late lactation pens, one of which included the bull string. Signs included weakness, recumbency, watery diarrhea, and death. Eighty animals from the 4 pens were dead approximately 8 hours after the first ill cows were noted. Affected cows would collapse on stimulation and extend all 4 limbs with moderate rigidity. Several lacked lingual tonus and had abdominal breathing patterns. The animals had been fed a load of total mixed ration that included a rotten bale of oat hay containing a dead cat. No common toxicants were identified, and pathologic examination revealed no consistent lesions. Testing of tissue from the cat carcass found in the feed sample using mouse protection bioassay identified the presence of type C botulinum toxin. Samples of feed, tissue from affected animals, cat tissue from feed, milk, and serum were also tested using an enzyme-linked immunosorbent assay (ELISA) specific for type C botulinum. Two samples of rumen contents were tested and found to be positive for botulism by ELISA, and 1 of 3 liver samples had a weak positive finding. No botulinum toxin was found in milk or sera using the ELISA.

Botulism is a disease that is characterized by progressive, flaccid paralysis in all species of animals.¹⁴ The paralysis is caused by toxins produced by *Clostridium botulinum*. *Clostridium botulinum* is an obligately anaerobic, spore-forming, gram-positive rod.¹⁴ The bacterium is ubiquitous and is found in soils and organic matter worldwide. Eight different botulinum toxins are produced and are designated as types A, B, C₁, C₂, D, E, F, and G.^{13,14}

With the exception of type C₂, which causes changes in membrane permeability, the botulinum toxins are paralytic neurotoxins that act by preventing release of acetylcholine by the presynaptic, cholinergic neuron. Paralysis occurs at the neuromuscular junction, parasympathetic end plates, and cholinergic ganglia of the sympathetic nervous system and within the adrenal glands.¹⁴

A clinical syndrome of progressive paralysis frequently leading to death is characteristic of botulism in animals. Definitive diagnosis of botulism is elusive

because circulating toxin levels are often low, source material may be absent, and current analytic methods (such as bioassay) are of insufficient sensitivity relative to livestock to screen for clinically relevant levels of the toxin. A definitive diagnosis is difficult primarily because of the high toxicity of botulinum toxin.^{14,16}

Exposure to botulism toxin generally occurs in 1 of 3 scenarios. Ingestion of preformed toxin associated with carcasses or decayed organic matter (e.g., poorly ensiled small grain haylages) and coprophagy (in the case of poultry) are the most common methods of exposure. Toxicoinfectious botulism is another type in which the organism grows in the gut, leading to toxin production. This form of botulism occurs in infants¹⁸ and possibly in poultry. The third form of botulism is wound botulism,¹⁹ which results from infection of an anaerobic wound, leading to toxin production. For example, the “shaker foal syndrome” results from botulism in ulcers or other necrotic lesions in 2–4-week-old foals.¹⁹

Preformed toxin is the most common cause of botulism in cattle; the toxin is usually type B, C, or D botulinum.¹³ Type B botulism in cattle is usually associated with feeding of poorly ensiled small grain haylages. Type C botulism results from ingestion of feeds containing carcasses or poultry litter contaminated with the bacterium and toxin. Type D botulism may result from ingestion of bones from dead animals by animals with pica.¹⁴ Mortality in cattle may range from 8% to 64%; 30–45% mortality is most common.^{1,21}

From the Wyoming State Veterinary Laboratory, 1174 Snowy Range Road, Laramie, WY 82070 (Galey); the California Veterinary Diagnostic Laboratory System, Davis, CA 95616 (Walker, Puschner, Tor); Lander Veterinary Clinic, Turlock, CA 95381 (Terra, Fisher); California Veterinary Diagnostic Laboratory System, Tulare, CA 93274 (Adaska); Modesto, CA 95352 (Etchebarne); Department of Clinical Studies, University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348-1692 (Whitlock); the National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711 (Rocke); and the California Department of Food and Agriculture, Animal Health Branch, Modesto, CA 95351 (Willoughby).

Received for publication June 17, 1999.

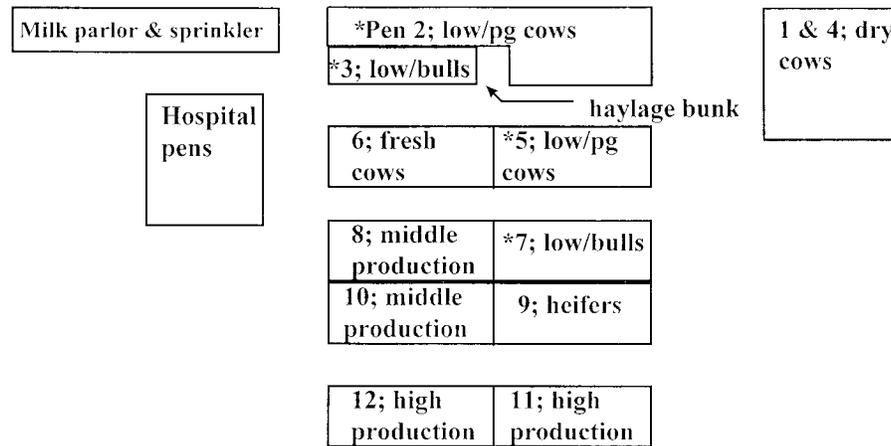


Figure 1. Layout of a dairy that experienced large death losses in adult Holstein cows due to type C botulism. * = affected pens. One load of contaminated feed was known to have been fed only in those pens the evening before death losses were first noticed.

Here, we describe the catastrophic loss of cattle in a dairy herd due to botulism. The intensive diagnostic challenge of such a case and the high mortality rate in this herd are discussed.

Materials and methods

Case history. A Holstein dairy herd in the Central Valley of California containing approximately 1,200 head of cattle lost 80 of 441 cows on 1 morning in mid-April 1998. Cows died after having developed signs of weakness, recumbency, and watery diarrhea. Affected animals were from 4 late lactation pens, one of which housed the bull string (Fig. 1).

Pathology. Five live cows and tissues from 5 others were submitted to the California Veterinary Diagnostic Laboratory System (CVDLS) for examination the day after the onset of signs. Four of the 5 live cows were recumbent. When encouraged to stand, cows went into lateral recumbency and suffered brief spastic rigidity followed by abdominal breathing and development of a cyanotic appearance. Tongues of animals were moderately flaccid. Tissues from 2 other animals that died on day 1 along with environmental samples were also submitted for testing. Samples of major tissues were fixed in 10% neutral buffered formalin for histologic examination. Fresh samples of brain, liver, kidney, fat, rumen contents, and cecal contents were obtained for microbiologic and toxicologic assays. Two samples of rumen content were also evaluated for cat hair at the Texas Veterinary Medical Diagnostic Laboratory by microscopy.

Mouse bioassay. Testing for botulism was done in 2 laboratories (CVDLS and University of Pennsylvania) using modifications of the traditional mouse protection bioassay.⁶ Either 0.5 ml serum or gelatin buffer^a extract from 10–25 g of liver or gut content (1:1 w:v refrigerated overnight at 4–8 C) was given via intraperitoneal injection to each of 2 Swiss-Webster mice.^b Samples of ingesta were also tested with and without pretreatment with trypsin and were tested for heat inactivation after finding a positive result. The mice were observed for 72–96 hr for signs of botulism (waspwaist syndrome, death). If signs were noted, another group of 2 mice were tested using 1 ml of sera (or extract) that was

mixed with 0.25 ml of polyvalent antitoxin.^c If those mice survived, then 2 mice each were tested using serum (or extract) aliquots that had been mixed with no antitoxins or with monovalent types A, B, C, D, or E.^c The surviving group of 2 mice indicated the type of toxin that was present. For this case, samples of cat tissue found in feed, rumen content ($n = 8$), total mixed ration (TMR) (pooled, plus 2 pens), serum ($n = 5$), liver ($n = 7$), premixed grain ration ($n = 1$), and milk from 1 affected cow were tested in the CVDLS laboratory. The cat tissue, TMR, rumen content, and serum samples were also tested at the University of Pennsylvania.

Enzyme-linked immunosorbent assay for type C botulinum. Samples of cat tissue, pooled TMR, rumen content ($n = 2$), serum ($n = 3$), liver ($n = 3$), and milk ($n = 2$) were also examined using an enzyme-linked immunosorbent assay (ELISA) developed to test samples for type C botulinum.¹⁵ Negative control samples of rumen content, serum, liver, and milk from unrelated cases submitted to the CVDLS were also tested using ELISA. This assay utilizes a simple antigen-capture system that employs polystyrene immunosticks as a backing, chicken antitoxin (IgY) as the coating antibody, rabbit antitoxin as the primary antibody, and peroxidase-labeled goat anti-rabbit as the secondary antibody.¹⁵ Sample (fluid or filtered homogenate of tissue in buffer; 3 ml) is pipetted into 3.8 ml polypropylene vials.^d The immunosticks impregnated with the chicken antitoxin are inserted into the vials. The vials are then refrigerated overnight at 4 C using a rotator to mix. Sticks are removed, washed, and transferred to clean tubes containing rabbit antitoxin and incubated at 37 C, for 1.5 hr while shaking gently. After incubation, sticks are removed, washed, and incubated in the peroxidase-labeled goat anti-rabbit antibody again for 1.5 hr at 37 C. The washed paddles are then transferred to new tubes and allowed to react with TMB peroxidase substrate^e for 20 min. Positive results are indicated by a purple color on the bottom half of the immunostick.¹⁵

Other testing. Samples of liver and kidney were tested for heavy metals and selenium by ICP analysis. Brain was tested for sodium using ICP and for organochlorine and pyrethrin insecticides using gas chromatography with mass spectrom-

Table 1. Progression of death losses from botulism in Holstein cows from a large dairy in central California.

Day of outbreak	No. cows affected	
	Dead	Recumbent
1, PM (24 hr after feeding contaminated feed)	150	...
2, PM	300	...
3, AM	305	...
6	390	25
7	416*	...
8	418	9
13	420	...
18	427/431	...

* Two of the additional dead animals were from previously unaffected pens. Affected individuals both were known by the dairy owners to reach into one of the affected pens next door.

etry (GC/MS) as a detection method.^g Blood and brain samples were examined for cholinesterase activity using a modification of the Ellman method.²⁰ Blood samples were also assayed for lead using atomic absorption spectroscopy.^h Samples of rumen contents were assayed for common plant alkaloids, oleander, ammonia, carbamate and organophosphorus insecticides, common drugs, avitrol, ionophore antibiotics, metaldehyde, strychnine, and zinc phosphide. A sample of rumen contents was also extracted using ethanol in ethyl acetate (5%) after basic extraction and screened for peaks using the GC/MS. Rumen contents were examined for plant parts and other foreign bodies. Feeds, including TMR, and environmental samples were assayed for ionophore antibiotics, gossypol, heavy metals, selenium, salts, nitrate/nitrite, and mycotoxins (including trichothecenes, aflatoxins, ochratoxin, and zearalenone). Heavy metal levels were assessed in water samples using the ICP.

Treatments. Selected animals were injected subcutaneously using polyvalent antiserum for botulinum toxin (made available through R. H. Whitlock).

Results

Clinical course. Three days after the initial outbreak of diarrhea and weakness, over 300 animals died. After the first day, gastrointestinal signs became less significant, and affected cows tended collapse on stimulation. Several lacked lingual and palpebral tonus. Severely affected animals developed abdominal breathing patterns before death. Ultimately, 14 days after onset of the initial clinical signs, 427 died (Table 1). At that point, 14 animals from the affected group remained alive, 2 of which were recumbent and 12 were clinically normal.

The affected pens were fed a TMR that consisted of approximately 25% grain premix, silage, haylage, alfalfa hay, oat hay, mineral mix, and whey. Historic review suggested that a load of TMR, fed at noon the day before the first cases appeared (18 hours previously), may have included a rotten bale of oat hay that contained a dead cat. That feeding had been complete-

ly consumed. The subsequent evening feeding was not consumed. Feed obtained from a pile that was generated when the bunks were cleaned immediately after initial signs were observed contained a scapula and pieces of spine with tissue (Fig. 2). Additionally, all samples of feed had evidence of the discolored oat hay. The same feed ingredients, minus the carcass and rotten bale, made up the TMR for the unaffected high-producing strings.

As the case progressed, milk from affected pens was discarded. Carcasses were rendered at temperatures of >120 C (250 F) for more than 30 minutes. Affected feed was incinerated. Care was taken not to dispose of the manure from affected pens in fields where crops were going to be harvested.

Pathology and toxicology testing. Gross examination of tissues from the 5 animals that were submitted live revealed no significant gross findings other than mild multifocal pyloric ulcerations in 2 animals. One animal had moderate mucosal thickening in the terminal jejunum and ileum. All animals were in excellent nutritional condition. Histologic examination of selected tissues revealed mild hepatic lipidosis in 2 of 5 sets of tissues that were submitted and a minimal suppurative rumenitis in another animal. Among the animals that were submitted live, 3 animals had no significant histopathologic changes. One had a granulomatous enteritis and lymphangitis with intracellular acid-fast organisms, suggesting that animal had been infected with *Mycobacterium paratuberculosis* (Johne's disease). A second animal had a marked, acute, locally extensive suppurative bronchopneumonia. Testing of selected tissues and ingesta for infectious diseases revealed no significant pathogens, although *Salmonella* serotype *montevideo* was cultured from the colon contents of one of the animals submitted live.

Extensive toxicology testing revealed no significant toxicants. The rumen content from 1 animal contained hair that matched a known cat hair when viewed under a microscope.

Mouse bioassay. Tissue from the cat that was found in the feed caused signs of a "wasp waist" abdominal breathing pattern and death in mice within 24 hours after injection of buffer extract in 2 laboratories (CVDLS and University of Pennsylvania). Injected mice were protected from signs with antitoxin specific for type C botulinum. Samples of rumen contents from 8 cattle, TMR (3 samples), serum from 5 cattle, and milk from 2 affected cows caused no signs of botulism in mice in both laboratories. The CVDLS laboratory also tested 7 liver samples and 1 sample of premix, with negative results.

ELISA. Once the type C botulinum was identified, immunostick ELISA paddles specific for type C botulinum were used for further testing. Strongly positive



Figure 2. Remains of a cat carcass found in feed that was removed from the bunks of 4 pens of adult Holstein dairy cattle as soon as death losses began to occur.

(purple coloration of the distal end of the stick) results were obtained for the cat tissue and a known high standard culture solution containing type C botulinum (Fig. 3). Positive results were also found for a diluted standard, the pooled TMR, 2 samples of rumen contents from cows that died on day 2 of the outbreak, and 1/3 samples of liver from 3 affected cows. The immunoassay paddles indicated a negative response for 3 serum samples and milk samples from 2 cows. Samples of rumen contents, liver, serum, and milk from normal cows also produced no reaction (no color change) in the paddles.

Treatment. Two affected cows were treated with equine-origin polyvalent antitoxin serum via subcutaneous injection. Later, several more cattle were similarly treated. No clinical improvement was noted in any of the animals, and all of those treated animals ultimately died.

Discussion

The history of carcass contamination in the feed, clinical signs, lack of consistent and specific lesions, negative testing for infectious and toxic agents, and identification of botulinum toxin in the carcass, feed, and rumen contents all support a diagnosis of type C botulism. Signs of ataxia, weakness, and paralysis, all of which were aggravated by stimulation, are typical

of poisoning due to exogenous botulinum poisoning.¹³ Whether ingestion of spores with subsequent gastrointestinal production of toxin occurred to contribute to the duration and severity of the outbreak was not studied. The gastrointestinal signs with diarrhea that were observed initially in the outbreak may have been related to a type C₂ toxin. Type C₂ botulinum has ADP ribosyl transferase activity and clinically can cause increased fluid movement across membranes and subsequent colic and diarrhea.¹⁴

Findings of type C botulinum in the cat and feeds using bioassay and ELISA support contaminated oat hay as the likely source of exposure. Cat carcasses have been reported as a source of type C botulism in livestock.¹⁸ Type C botulism has also caused large numbers of deaths in cattle in association with feeding of poultry litter^{7,9,12} and has caused die-offs of waterfowl.²²

This case resulted in catastrophic death losses (99% mortality in exposed pens). Deaths of increasingly large numbers of livestock appear to be occurring due to this syndrome. Cases of type C and D botulism from feeding of poultry litter resulted in deaths of 42/67 cattle in 1 case¹ and 68/150 exposed cattle in another.¹² The reason for these high death losses is not known. These losses may be a result of a combination of factors that include feeding of larger herds in a drylot

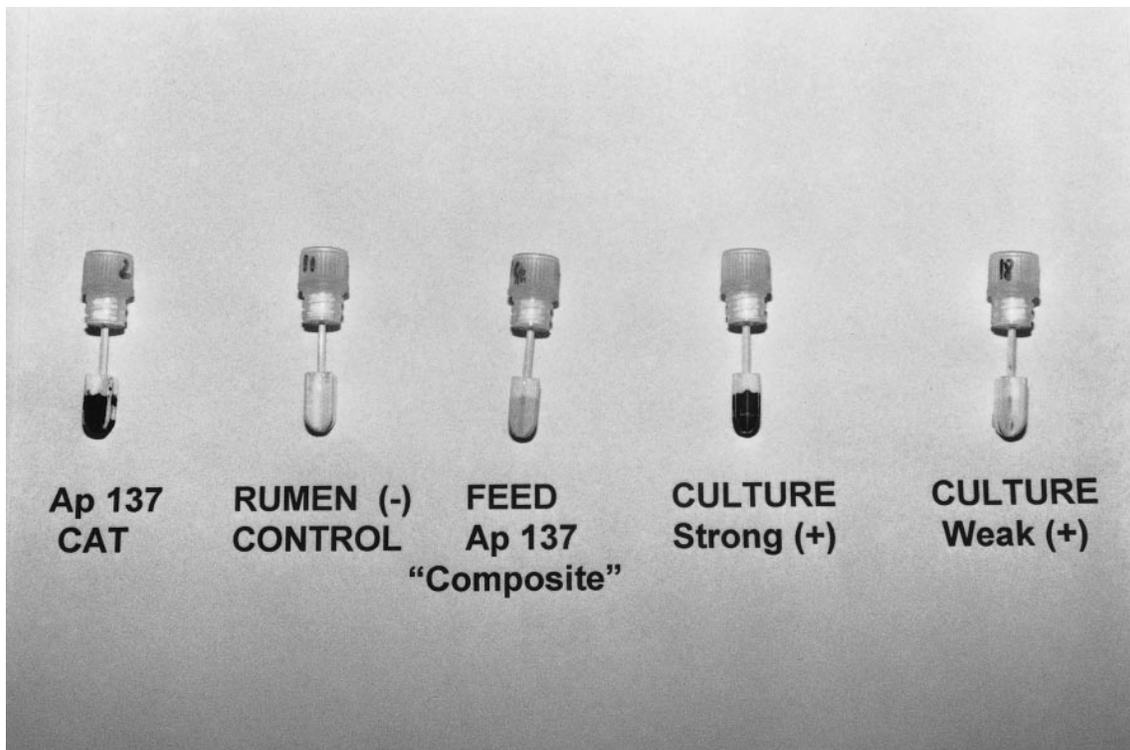


Figure 3. Experimental ELISA specific for type C botulinum applied to buffer extracts of the cat carcass and a random sample of the feed removed from bunks in affected pens. The distal portion of the paddle turns purple if positive. The top portion of the paddle would only change color for nonspecific reactions. Left to right: cat (strongly positive), rumen control (negative, no nonspecific reactions), feed composite (weak positive), and culture materials with known type C botulinum (concentrated strong positive and diluted weak positive reactions).

situation, improved mixing of feeds, the known extreme toxicity of the botulinum itself, and the lack of a rapid, easy assay for the toxin that is of sufficient sensitivity. Additionally, the potential impact of feeding of various feedstuffs and additives such as cotton (gossypol) and monensin (an antibiotic), both of which may cause muscle damage with a degree of weakness if present in excess, is not known.

The numbers of cattle in dairy herds in California have continued to grow to the point that milking herds of >1,000 cows are not unusual. This industrial type of production means that more cows are being fed from single mixings of feed and/or from similar feed constituents. Thus, the impact of a mixing error or contaminant in feed can be greatly magnified.

In this case, the presence of oat hay in every sample of the feed and the apparent uniform mixing of monensin in every sample of feed suggest that the TMR was very thoroughly mixed before feeding. Botulinum is one of the most toxic compounds known. An approximate median lethal dose for botulinum in mice may be as low as 0.005 $\mu\text{g}/\text{kg}$ of body weight.⁵ For comparison purposes, the median lethal dose for VX (a very toxic nerve gas) in the mouse is 22 $\mu\text{g}/\text{kg}$ by parenteral injection.¹¹ Putting that information into hy-

pothetical perspective, 1 g of pure toxin, if properly mixed, could kill 400,000 adult cows, assuming the toxicity was similar to mice on a microgram per kilogram body weight basis.

Although botulism is a common food safety topic, the disposition of botulinum in tissues and products from affected food animals has not been well characterized. Milk taken from affected cattle in this study contained no botulinum toxin when tested using ELISA and mouse protection bioassay. A person exposed to type A botulism had no toxin in her milk, and her 8-month-old breast-fed baby had no evidence of botulism.² Axenic rats experimentally dosed with *C. botulinum* spores developed severe botulism 4 days after dosing and then nursed unrelated 2–3-day-old rat pups for 3 hours. The rat pups were killed, and the milk was removed from the gastrointestinal tracts. That milk had very low but detectable levels of botulinum (type not specified).¹⁰ Although the potential for milk contamination from affected cows is apparently low, this potential remains to be studied. The botulinum toxin is sensitive to heat, and toxin levels have been decreased by up to 5 orders of magnitude in salmon paste by subjecting the material to temperatures >80 C for 1–5 minutes.¹⁷ At this affected dairy,

contaminated carcasses were rendered at a heat sufficient to destroy both the toxin and the spores. In general, the spores are destroyed at temperatures of >120 C for 5 minutes under moist conditions.³

Diagnosis of botulism remains a difficult task. Unfortunately, the classical mouse protection bioassay continues to be the most useful screen for diagnosis.¹⁴ However, this method requires the inoculation of live animals, and it is not particularly sensitive. One species of livestock (horses) may be anywhere between 1 and 10,000 times more sensitive to botulinum than is the bioassay mouse.⁸ When toxin is taken up and bound it is much less likely to be present in the circulation in appreciable amounts. Thus, diagnosis of botulism using this assay in livestock cases is often limited by the ability of the investigator to find sources of botulinum in the feed or environment. For that reason, many cases, including some that involve large numbers of animals,²¹ may go undiagnosed other than ruling out other causes and noting the presence of appropriate clinical signs.

ELISA testing has promise as a useful diagnostic tool. Although apparently still not of optimal sensitivity, the assay applied in this case was able to detect botulinum in a liver sample, the mixed feed, and the cat carcass. This method is highly specific for only 1 type of toxin (C₁)¹⁵ and therefore is of little use as a general screen, when other types of toxin could also be present.

Botulism can cause deaths in a large number of cattle, as illustrated by this case. Modern feed mixing capabilities and the feeding of larger herds may result in catastrophic losses due to the extremely high toxicity of botulinum toxin. Diagnosis of botulism still requires thorough investigation to rule out many possible causes of weakness and death in livestock. The mouse protection bioassay is useful to the diagnostician primarily in cases where a possible source is identified and present. New, more specific ELISAs are helpful once the type of botulinum is known, but at this time, more sensitive and specific diagnostic methods are needed.

Acknowledgements

We thank Dr. John Reagor, Texas Veterinary Medical Diagnostic Laboratory, College Station, for microscopy of the rumen contents, C. Hult, CVDLS, Davis, for technical assistance in carrying out mouse bioassays, and Dr. Dirk Holstege for coordinating analytic chemistry test methods.

Sources and manufacturers

- a. Sigma Chemical Co., St. Louis, MO.
- b. Simonsen, Gilroy, CA.
- c. CDC Biological Products, Atlanta, GA.
- d. Nunc, Naperville, IL.
- e. Kirkegaard & Perry, Gaithersburg, MD.

- f. Inductively coupled plasma emission spectrometer, Fisons, Deerborne, MI.
- g. Model 5890 and model 5980 MSD, Hewlett Packard, Palo Alto, CA.
- h. Z 5100 Perkin-Elmer, Norwalk, CT.

References

1. Abbitt B, Murphy MJ, Ray AC, et al.: 1984, Catastrophic death losses in a dairy herd attributed to type D botulism. *J Am Vet Med Assoc* 185:798–801.
2. Anonymous: 1976, Morbid Mortal Weekly Rep 25:399–400.
3. Biberstein EL, Hirsh DC: 1999, The clostridia. *In: Veterinary microbiology*, ed. Hirsh DC, Zee YC, pp. 233–245, Blackwell Science, Malden, MA.
4. Bonventre PF: 1979, Absorption of botulinum toxin from the gastrointestinal tract. *Rev Infect Dis* 1:663–667.
5. Chao LP: 1986, The mechanism of botulism. *Med Hypotheses* 19:83–87.
6. Hathaway CL: 1979, Laboratory procedures for cases of suspected infant botulism. *Rev Infect Dis* 1:647–651.
7. Jean D, Fecteau G, Scott D, et al.: 1995, Clostridium botulinum type C intoxication in feedlot steers being fed ensiled poultry litter. *Can Vet J* 36:626–628.
8. Kinde H, Betsey RL, Ardans A, et al.: 1991, Clostridium botulinum type-C intoxication associated with consumption of processed alfalfa hay cubes in horses. *J Am Vet Med Assoc* 199:742–746.
9. McLoughlin MF, McIlroy SG, Neill SD: 1988, A major outbreak of botulism in cattle being fed ensiled poultry litter. *Vet Rec* 122:579–581.
10. Moberg LJ, Sugiyama H: 1980, The rat as an animal model for infant botulism. *Infect Immun* 29:819–821.
11. National Institute of Occupational Safety and Health: 1999; Phosphonothioic acid, methyl-, S-(2-(diisopropylamino) ethyl) O-ethyl ester. *In: Registry of toxic effects of chemical substances*, RTECS TB1090000. National Institute of Occupational Safety and Health, Cincinnati, OH.
12. Neill SD, McLoughlin MF, McIlroy SG: 1989, Type C botulism in cattle being fed ensiled poultry litter. *Vet Rec* 124:558–560.
13. Rings DM: 1987, Bacterial meningitis and diseases caused by bacterial toxins. *Vet Clin North Am* 3:85–97.
14. Rocke TE: 1993, Clostridium botulinum. *In: Pathogenesis of bacterial infections*, ed. Gyles CL, Thoen CO, 2nd ed., pp. 86–96. Iowa State University Press, Ames, IA.
15. Rocke TE, Smith SR, Nashold SW: 1998, Preliminary evaluation of a simple in vitro test for the diagnosis of type C botulism in wild birds. *J Wildl Dis* 34:744–751.
16. Saguchi G: 1983, Clostridium botulinum toxins. *Pharmacol Ther* 19:165–194.
17. Siegel LS: 1993, Destruction of botulinum toxins in food and water. *In: Clostridium botulinum, ecology and control in foods*, ed. Hauschild AHW, Dodds KL, pp. 323–341. Marcel Dekker, New York, NY.
18. Smith L, Holdeman L: 1968, Pathogenic anaerobic bacteria. Charles C Thomas, Springfield, IL.
19. Swerzek TW: 1980, Toxicoinfectious botulism in foals and adult horses. *J Am Vet Med Assoc* 176:217–220.
20. Tor ER, Holstege DM, Galey FD: 1994, Determination of cholinesterase activity in brain and blood samples using a plate reader. *J Assoc Off Anal Chem* 77:1308–1313.
21. Truman KF, Bock RE, Thomas RJ, Taylor JD: 1992, Suspected botulism in three intensively managed Australian cattle herds. *Vet Rec* 130:398–400.
22. Wobeser G, Baptiste K, Clark EG, Deyo AW: 1997, Type C botulism in association with a botulism die-off in waterfowl in Saskatchewan. *Can Vet J* 38:782.