Review of Equine Botulism

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This review of botulism includes a brief history of the disease. The discussion includes the different types of neurotoxin produced by Clostridium botulinum, the clinical manifestation of the disease, and current information regarding the toxin's mechanism of action at the molecular level. Authors' address: Dept. of Medicine and Epidemiology, Veterinary Medical Teaching Hospital, University of California at Davis, Davis, CA 95616. © 1997 AAEP.

1. Introduction
Botulism causes neuromuscular blockade and flaccid paralysis in all warm-blooded animals. Though a diagnosis must frequently be made based only on clinical signs,\(^1\) treatment with specific antiserum must be instituted early in the course of the disease to produce a positive outcome. The toxin enzymatically disrupts neuroexocytosis at the presynaptic membrane. The purpose of the review is to discuss elements of diagnosis and treatment that can be influenced by the practitioner. New information identifying the neurotoxins of Clostridium botulinum as zinc-dependent endopeptidases is presented.

2. Discussion
Botulism is the clinical manifestation of intoxication with any of seven neurotoxins elaborated by Clostridium botulinum. Cranial nerve paresis and generalized weakness, progressing to flaccid paralysis, are hallmarks of the disease. Eight antigenically distinct toxins (A,B,C\(_1\),C\(_2\),D,E,F,G) have been identified and are used to classify the bacteria. C\(_2\) does not cause the classic neuroparalytic signs and is not considered a neurotoxin.\(^2\) Two other clostridial species, C. barati and C. butyricum, also produce botulinum neurotoxin.

Clinical descriptions of botulism from Greek and Roman authors indicate that the disease has been known since antiquity. Only in the last century, however, has the bacterial origin of the disease been established. The term botulism is derived from its association with the consumption of sausage in 19th century Europe (botulus, Latin for sausage). Like other clostridial organisms, C. botulinum is a strictly anaerobic, gram positive, spore-forming rod. The bacterium's ability to exist in both active and spore forms enables it to survive for prolonged periods of time in the environment, a selective advantage for an organism whose disease manifestation is almost invariably fatal to the host. Like C. tetani, C. botulinum attacks and disrupts neuromuscular function. Whereas C. tetani produces a single toxin responsible for clinical tetanus, C. botulinum produces seven immunologically distinct neurotoxins. Because animals in the wild rarely survive botulism and thus do not develop resistance, the selective pressures leading the organism to produce multiple types of toxin remain speculative. The toxins play no role in the physiology or growth of the bacterium.\(^2\)

Dysphagia, muscle trembling, and recumbency are frequent signs that prompt owners to seek veterinary intervention. These signs arise as a
result of the interruption of nerve transmission across cholinergic synapses. Nerves conducting high efferent traffic will be most affected by the toxin. Therefore, cranial nerves and nerves supplying anti-gravity muscles are often the earliest affected. Decreases in palpebral, anal, tail, jaw, and hypoglossal tone, as well as neck weakness, are other frequent signs. Intoxication of autonomic neurons may result in impaction colic secondary to ileus, and pupillary mydriasis. Death from unmitigated botulism results from failure of the diaphragmatic and intercostal musculature. Sensory functions remain unaffected.

Ingestion of preformed toxin (forage poisoning) in contaminated feed is the most common route of intoxication in horses. Elaboration of toxin by organisms colonizing the intestinal tract (toxicoinfections botulism) or growing in an infected wound are less common. Shaker foal syndrome is a well-described manifestation of toxicoinfectious botulism caused by type B organisms in young foals. C. botulinum has a worldwide, but asymmetric, geographic distribution. In general, types A, B, E, F and G principally inhabit soil and seawater or freshwater sediments, whereas types C and D seem to be obligate parasites in animals and birds. Type B organisms are endemic in soils of the mid-Atlantic states and Kentucky. Type C botulism has been identified in California, Canada, Florida, and the New England states. Type D spores have a worldwide distribution in alkaline soils, and they cause disease principally in cattle that are driven to chew on carcasses by trace mineral deficiency or starvation. Types B, C, and D are responsible for nearly all cases of animal botulism. The type of botulism involved in an outbreak or sporadic case reflects the geographic prevalence of the toxin type in that particular area, or in the region from which the contaminated feed originated. In a 1989 type C outbreak in southern California, contaminated hay cubes had been produced and processed in Utah prior to being distributed to horse owners in the Los Angeles area. Differences have been described between the clinical disease entities caused by types B and C.

The primary therapeutic objective is to neutralize toxin prior to neuronal binding and internalization. Expedient administration of antiserum containing antitoxin specific to the type of toxin present may be lifesaving if given before the horse is in a state of obligate recumbency. Treatment with antiserum will not reverse clinical signs, and progression of signs following treatment may occur. Inactivation of intoxicated neurons is permanent, and the gradual course of convalescence is dictated by the host's synthesis of new synapses. Recovery in surviving animals appears to be complete. Additional therapeutic modalities include gastrointestinal catharsis to remove additional toxin and prevent impaction, antimicrobial therapy targeted at secondary complications such as aspiration pneumonia, and enteral feeding via nasogastric tube. Aminoglycosides, tetracycline, and procaine may exacerbate neuromuscular blockade and should be avoided if possible. The administration of metronidazole can predispose hosts to dosorial overgrowth in the intestine, and its use should likewise be avoided.

Recently it has been demonstrated that C. botulinum neurotoxins are zinc-containing endopeptidases having a molecular mass of approximately 150 kDa. After binding with high affinity to specific receptors on the presynaptic nerve membrane, the toxin enters the neuron by receptor-mediated endocytosis, translocates across the endosome membrane into the cytosol, and enzymatically cleaves polypeptides essential for exocytosis of neurotransmitter vesicles. Tetanus toxin and botulinum neurotoxin types B, D, F, and G are zinc endopeptidases specific for vAMP/synaptobrevin, one component of the multiprotein complex involved in vesicle docking and fusion. Types A and E toxin cleave SNAP-25, and type C acts on syntaxin. The retroaxonal transport of tetanus toxin from an infected wound to inhibitory neurons in the spinal cord elicits spastic paralysis, a clinical picture markedly different from the flaccidity demonstrated by botulism patients, even though its intraneuronal mechanism of action is virtually identical.

Attempts to diagnose botulism are frequently unrewarding. A definitive diagnosis requires demonstration of toxin in plasma, the liver, or gastrointestinal contents. The mouse bioassay is the most sensitive analytic test available, but the amount of toxin present in the serum of even a severely affected horse may still be insufficient to cause mortality in mice. By the time clinical signs have become apparent, much of the absorbed dose of toxin has bound to neurons and may not be circulating in the plasma. A tentative diagnosis can be rendered if C. botulinum spores can be demonstrated in gastrointestinal contents or in feed consumed by the horse. Lastly, some animals that survive the disease will have a measurable serum antibody titer to C. botulinum. All analytical tests are more likely to yield positive results when samples are obtained early in the disease course.

3. Conclusions
Despite the difficulties inherent in the positive identification of botulism, a rapid presumptive diagnosis is the most important determinant of case outcome. Although botulism is not an uncommon disease in North America, in many cases it is not considered in the differential diagnosis of generalized weakness or recumbency. An awareness of the disease's presenting signs is essential to the equine veterinarian for early recognition and intervention. Until an approved vaccine for type C botulism is available for horses and the disease can be prevented, emphasis must continue to be placed on expedient treatment with specific antiserum. Hyperimmune plasma can be obtained from several sources. The ability to
offer this treatment to equine patients depends upon the product being affordably priced and delivering protective levels of antitoxin. At the University of California at Davis, an inventory is maintained of polyvalent plasma\textsuperscript{a} and a commercially available bivalent product\textsuperscript{b} containing antibodies against types B and C toxin.

The vaccination of broodmares with inactivated toxin has proven to be effective at preventing shaker foal syndrome in Kentucky.\textsuperscript{8} The development of a type C toxoid is needed for use in Florida, California, and other states where type C botulism occurs. No cross-protection exists between types B and C, although type C\textsubscript{1} antitoxin does confer protection against type D toxin.\textsuperscript{11} A type C vaccine is available for use in commercial mink operations, but no product is currently labeled for use in horses.

Directions for treatment of botulism in the future include the possible development of zinc endopeptidase inhibitors. One such agent is captopril: a widely used antihypertensive drug, it inhibits angiotensin-converting enzyme, a zinc endopeptidase located in pulmonary endothelial cells. Though captopril has been shown to be too weakly active against botulism neurotoxin to provide clinical efficacy,\textsuperscript{9} it may pave the way for the development of pharmaceutical agents that will prove useful in the future. Botulism toxin is the most potent biotoxin known; it is speculated that a single molecule of toxin can cleave every molecule of substrate in an intoxicated neuron.\textsuperscript{10} Realization of the toxin's endopeptidase structure and function has important clinical implications, because agents that may interrupt the enzymatic activity of the toxin can be investigated and developed. A drug that antagonized the action of botulism toxin intracellularly would lengthen the window of time available for effective therapeutic intervention and could significantly impact our ability to positively influence the outcome for horses with this disease.

References

\textsuperscript{a}Pentavalent hyperimmune plasma (A, B, C, D, E), R. Whitlock, 382 West St. Rd., New Bolton Center, Kennett Square, PA 19348.
\textsuperscript{b}Clostridium botulinum antitoxin of equine origin: types B and C, Veterinary Dynamics, Inc., 1535 Templeton Rd., Templeton, CA 93465.