Botulism is a severe neuroparalytic disease that affects humans, all warm-blooded animals, and some fishes. The disease is caused by exposure to toxins produced by Clostridium botulinum and other botulinum toxin–producing clostridia. Botulism in animals represents a severe environmental and economic concern because of its high mortality rate. Moreover, meat or other products from affected animals entering the food chain may result in a public health problem. To this end, early diagnosis is crucial to define and apply appropriate veterinary public health measures. Clinical diagnosis is based on clinical findings eliminating other causes of neuromuscular disorders and on the absence of internal lesions observed during postmortem examination. Since clinical signs alone are often insufficient to make a definitive diagnosis, laboratory confirmation is required. Botulinum antitoxin administration and supportive therapies are used to treat sick animals. Once the diagnosis has been made, euthanasia is frequently advisable. Vaccine administration is subject to health authorities’ permission, and it is restricted to a small number of animal species. Several measures can be adopted to prevent or minimize outbreaks. In this article we outline all phases of management of animal botulism outbreaks occurring in wet wild birds, poultry, cattle, horses, and fur farm animals.

Botulism is a severe neuroparalytic disease that affects humans, all warm-blooded animals, and some fishes. The illness is caused by exposure to botulinum neurotoxins (BoNTs), which are produced by anaerobic, spore-forming, ubiquitous microorganisms belonging to the genus Clostridium, referred to as BoNT-producing clostridia. BoNTs act on nerve endings blocking acetylcholine release. Their potency depends on 2 factors: their enzymatic activity and their selective affinity for binding neurons.1,2 Human disease does not differ fundamentally
in clinical features, diagnosis, supportive laboratory testing, management, or therapeutic measures from that seen in veterinary practice, but the rarity of human botulism contrasts with the frequent occurrence of animal botulism.\textsuperscript{3,4} On a worldwide basis, avian botulism probably represents the most important disease of migratory birds. More than a million deaths have been reported in relatively localized outbreaks in a single year, and outbreaks with losses of 50,000 birds or more are relatively common.\textsuperscript{5}

Botulism in animals represents a serious environmental and economic concern because of the high mortality observed during the outbreaks. BoNTs and BoNT-producing clostridia represent an additional concern because of their potential use as biological weapons.\textsuperscript{6,7} A bioterrorist attack directed at a livestock population by means of viruses, microorganisms, or toxins could instill fear among the population and cause major economic consequences; thus, livestock agroterrorism has caught the attention of government policymakers in most countries.\textsuperscript{8} It may be difficult to attribute an animal disease outbreak to a biological attack. In fact, unlike bioterrorism attacks directed toward humans, bioterrorism attacks on livestock or poultry would have fewer indications that could be used to distinguish them from natural outbreaks.\textsuperscript{8} The countermeasures to prevent a possible agroterrorism attack include sharing information among government agencies, improving veterinary and public health awareness, and supporting research to identify new, more efficient and rapid methods to combat these threats.\textsuperscript{8}

This article outlines phases of management of animal botulism outbreaks from clinical suspicion to practical countermeasures to prevent or minimize outbreaks occurring in waterfowl, poultry, cattle, horses, and fur farm animals, in the framework of the activities promoted by the European research project AniBioThreat (www.anibiothreat.com).

**BoNT-Producing Clostridia and Their Toxins**

The species *Clostridium botulinum* and some strains of *Clostridium baratti* and *Clostridium butyricum* are currently classified as BoNT-producing clostridia. On the basis of their genotypic, phenotypic, and biochemical characteristics, these strains are divided into 6 groups: *C. botulinum* (groups I–IV), *C. butyricum*, and *C. baratti*. Groups I and II, *C. butyricum*, and *C. baratti* are mainly associated with human botulism, whereas group III organisms are mainly responsible for animal botulism.\textsuperscript{1,2} Group IV organisms, also known as *Clostridium argentinense*, are associated with wound botulism.\textsuperscript{9} On the basis of the BoNTs produced, these strains can additionally be classified in 7 groups, from A to G.\textsuperscript{1,2} Group I and II organisms are capable of producing type A, B, E, and F toxins; group III organisms can produce type C, D, and their mosaic C/D and D/C toxins.\textsuperscript{10–12} Group IV, *C. butyricum*, and *C. baratti* produce type G, E, and F toxins, respectively. Some strains are also capable of producing 2 toxins simultaneously or may carry a second silent nonexpressed gene.\textsuperscript{13,14}

*C. botulinum* strains play a vital role in the natural carbon recycling process, growing in decaying organic matter (eg, animal carcasses) and producing high levels of BoNTs. High protein substrates are essential because *C. botulinum* lacks the ability to synthesize certain essential amino acids. Also, pH and salinity in the sediments together with temperature play a crucial role for growth and toxin production, with an optimum growth temperature between 25°C and 42°C.\textsuperscript{4,5,15} Type D strains can produce toxin in carrion at a temperature as low as 9°C, whereas a type C strain failed almost completely to do it at 16°C.\textsuperscript{16}

BoNT-producing clostridia secrete the toxins during their vegetative growth as inactive single-chain polypeptides that subsequently are activated by bacterial or tissue protease. In nature BoNTs are found as progenitor toxins containing the neurotoxin and nontoxic associated proteins, probably responsible for protecting the neurotoxin from environmental factors.\textsuperscript{17} Genes encoding BoNTs can be located on the chromosome or on extrachromosomal elements, such as plasmids or bacteriophages.\textsuperscript{18–20} Specifically, toxin genes for group III organisms are carried by bacteriophages that exert an unstable lysogenic cycle. Molecular and genomic analysis of the bacteriophage genome revealed that this phage exists as a circular plasmid prophage in the lysogenic state, and it does not integrate into the host chromosome.\textsuperscript{21}

The sensitivity to BoNTs seems to be widely variable among the different animal species. Cattle botulism is most frequently caused by type D or D/C toxin, followed by type C toxin, although type A, B, and C/D toxin types have also been reported. In wild birds, the most common toxin isolated is type C and for fish-eating waterfowl type E. In contrast, in poultry the most common toxins are type C and C/D, although type A has been reported. The mosaic toxin type C/D seems to be more lethal to chickens than type C toxin.\textsuperscript{10} In addition, birds may harbor type D *C. botulinum* strains in their gut without showing symptoms.\textsuperscript{22} Equine botulism is caused by type B, C, and A toxins.\textsuperscript{22–25} Fur farm animals (eg, fox, ferret, mink) seem to be susceptible to type C and type C/D toxins, but rare outbreaks due to type A and E toxins have also been reported.\textsuperscript{22,26,27}

**Pathogenesis and Pathophysiology**

BoNT is the etiological agent of human and animal botulism. As extensively reported elsewhere, the BoNT mechanism of action consists of the following steps: The active toxin is absorbed in the small intestine by binding to the receptors on the apical surface of gut epithelial cells. It is then released into the general circulation, reaching all peripheral cholinergic nerve endings. In these sites, the toxin
hypothesized to occur when C. botulinum is variable, resulting in peracute, acute, and chronic forms of cumbency and often results in death. The speed of progression quarters with weakness, muscle tremors, stumbling, and re-

Clinical Manifestations

In birds, the flaccid paralysis progresses cranially from the legs, to include wings, neck, and eyelids. Initially, affected birds are unable to sustain flight or show uncoordinated flight and may be found sitting and reluctant to move. They seem lame, and the wings may droop when paralyzed. Paralysis of the inner eyelid or nictitating membrane and neck muscles follows, resulting in the inability to hold the head erect. Birds may also appear lateral or ventral recumbent and comatose or dead.34,41 Recumbent birds intermittently stand and walk a few steps. Often they cannot stand up completely and even walk on their tarsometatarsi. More severely affected water birds are unable to walk and drag themselves forward using their wings and beak.5,40,41 Broilers generally appear ruffled and often show defecated areas on the back. Affected chickens may have soiled vents caused by diarrhea.40

Clinical Manifestations in Cattle

Cattle in the early stage of the disease appear listless, reluctant to move, and stiff. Constipation and signs of colic may be present at this stage. Weakness in the hind limbs results in difficulty rising, and affected cattle therefore often appear recumbent. Dysphagia is present in most, but not all, cases of botulism in cattle. Decreased masseter tone, decreased tongue strength, and pharyngeal paralytic disorders may contribute to dysphagia, each in variable degree. At this stage the animals remain bright and alert and usually are able to eat the grass around them and drink water from a bucket. As the paralysis progresses, they may become unable to eat and drink, they may be laterally recumbent with terminal abdominal breathing, and the tongue may lose reaction strength and become completely paralyzed. Other clinical signs include photophobia, sluggish rumen movements, reduced anal tone, and tail paralysis.34 Atypical clinical presentation can appear in cattle because of the ingestion of BoNT/B, including diarrhea, profuse salivation, regurgitation, and vomiting.40

Volume 11, Supplement 1, 2013 S193

ANNIBALI ET AL.
Clinical Manifestations in Horses

The clinical signs in horses are similar to those in cattle. The main signs in adult horses are generalized muscle weakness and dysphagia; decreased tail, eyelid, and tongue tone; mydriasis; and prolonged pupillary light reflex, frequently followed by recumbency, respiratory failure, and death. Further signs such as anorexia, weight loss, colic, hyper-salivation, and tachycardia are common.22–25 Horses with type C botulism may have more prominent mydriasis, more labored breathing, or less dysphagia than horses with type A or B botulism.23

In foals, the “shaker foal syndrome” appears to be similar to infant botulism. The initial presenting sign is often the onset of muscle tremors leading to recumbency. Dysphagia; constipation; reduced eyelid, tongue, and tail tone; mydriasis; sluggish pupillary light reflexes; and frequent urination may also be seen.25

Clinical Manifestations in Fur Farm Animals

In fur farm animals, the most typical clinical picture includes the same clinical signs as those in large domestic animals. Paralysis of the hind legs, total paralysis, and recumbency are common. Foxes with milder symptoms often take a sitting position, dragging the rear part of their bodies.26 In ferrets, clinical signs include weakness, ataxia, ascending paralysis, blepharospasm, photophobia, and urinary incontinence, with death resulting from respiratory failure. The progression of botulism can be very fast in fur animals. In some cases, animals that have appeared well have died only 2 or 3 hours later.44

Diagnosis

Clinical Signs

Clinical signs of animal botulism are often strongly indicative but not specific. A presumptive diagnosis is based on a combination of signs observed in sick animals, the duration of the outbreak, and the postmortem findings and by ruling out other differential diagnoses.

Avian botulism most often afflicts waterfowl in the season when they are flightless because of wing molt. It is therefore essential to distinguish between birds in molt and birds with early stages of botulism, since the behavior of these birds may be similar. Molting birds are very difficult to catch, whereas birds that are suffering from botulism can easily be captured when they lose the ability to dive to escape pursuit. Birds at this level of intoxication still have a high probability of surviving if they are properly treated. During outbreaks it is common to find healthy, sick, and dead animals in the same area.5

In cattle, clinical signs (especially flaccid paralysis), the epidemiology of the outbreak, the clinical chemistry (especially hyperglycemia, although this can occur in any cow with a severe life-threatening disease), and neutrophilia support the diagnosis.28,30 Differential diagnosis includes hypocalcemia, hypomagnesemia, carbohydrate overload, and toxicosis, including from mycotoxin, lead, nitrate, organophosphate, atropine or atropine-like alkaloid, tick paralysis, and paralytic rhabdomyolysis.3,34,45

In horses, weakness, decreased eyelid tone, decreased or absent tongue tone, decreased tail and anal tone, sluggish pupillary light reflex, and occasional episodes of slow and uncoordinated padding support the diagnosis. Differential diagnosis includes toxic plant poisoning, organophosphate intoxication, equine viral encephalitis, central nervous system trauma, equine protozoal myeloencephalitis, aberrant larval migration, and hyperammonemia.35 In horses that feed in a group, the dominant horse eats first and is therefore usually the first one to develop clinical signs and shows most rapid progression of the neurotoxic syndrome.35

Postmortem Examinations

Postmortem examination is usually unremarkable, although in more protracted cases, secondary pathologies may be present. Negative postmortem results can be used as a diagnostic tool per exclusionem.22 Diffuse intestinal hemorrhage may be observed on postmortem examination, as some strains of *C. botulinum* type C and D also produce an enterotoxin, but these changes are not consistent or sufficiently specific to confirm a diagnosis. Nevertheless, a full necropsy should be carried out on as many cases as possible to help rule out other conditions. In particular, stomach contents should be examined for evidence of suspicious material (eg, decayed carcass material, bones, maggots).34,41

Avian botulism should be suspected when maggots are found as part of the ingesta of gizzard contents of dead birds; however, such findings are rare.5 A presumptive diagnosis is often based on a combination of signs observed in sick animals.

Specific neurophysiological studies such as repetitive nerve stimulations have been particularly useful as a diagnostic aid for neuromuscular disease. In large animal neurology, the use of electrodiagnostics has been limited compared with other species. Aleman and colleagues summarized neurophysiological studies conducted on foals.46

In horses, edema of the head and neck may be a prominent, although inconsistently observed, necropsy finding associated with botulism. In some cases, edema might be attributable to simple hydrostatic mechanisms, secondary to the unnaturally prolonged static posture.35

Laboratory Confirmation

Laboratory confirmation can be done by demonstrating: (1) BoNTs in serum, gastrointestinal content, liver, and wound; (2) BoNT-producing clostridia in gastrointestinal

Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science
content, liver, and wound; (3) BoNTs or BoNT-producing clostridia in feed or the close environment of the sick animal; or (4) an antibody response in an animal with symptoms of botulism. Demonstration of BoNTs in serum, feed material, or intestinal content by the mouse bioassay remains the gold standard for laboratory confirmation of botulism. However, a negative mouse bioassay does not exclude the botulism diagnosis, because the toxin may be present at a level below the limit of detection or may have been biodegraded by microbes in the intestinal tract of the animals. Since BoNT-producing clostridia are not a normal inhabitant of the gut, but are present in the alimentary tract of animals that have recently ingested contaminated material, demonstration of spores in the gastrointestinal contents or tissue of animals with symptoms is indicative of botulism. This is supported by the clinical diagnosis, as well as demonstration of neutralizing antibodies, and may allow a retrospective diagnosis in recovered animals. Demonstration of BoNT-producing clostridia or BoNTs in feed given to animals that show symptoms of botulism may also help to identify the source of contamination in order to prevent future cases.

Because the use of in vivo assays is discouraged for ethical reasons, a number of alternative methods have been developed, and some of them have been validated. Serum, ruminal or gastrointestinal content, fecal material, tissue samples, and feed for laboratory investigation should be collected as soon as possible after the onset of the illness, or after death, to enable efficient detection of C. botulinum and/or its toxins.

Treatment

When botulism is suspected, the first critical therapeutic step is to give polyvalent antitoxin to affected animals. Antitoxin treatment should be initiated as soon as possible, because it is effective only against circulating toxin and not when toxin is fixed at the neuromuscular junction. Antibiotic administration is indicated only when there is a suspicion of inhalation pneumonia or wound infection. Clostridiocidal drugs may lyse vegetative cells of BoNT-producing clostridia, thereby increasing the amount of free toxin in the intestinal tract. Aminoglycosides may potentiate neuromuscular weakness and a nondepolarizing type of neuromuscular block. Although antibiotic treatment is discouraged, beta-lactams have been successfully used to treat poultry affected by the toxicoinfection form of botulism. Other therapies include supportive care (oral water and electrolytes) and reduced physical activity. For cattle, vaccination can be considered to be effective as a therapeutic treatment in an outbreak situation.

Waterfowl can recover from botulism by being administered antitoxins or being provided fresh water and shade. Treated birds should be maintained in pens that provide free access to fresh water, shade, the opportunity for recovered birds to fly out, and minimal disturbance. Once the diagnosis has been made, euthanasia is frequently advised to avoid problems in maintaining the animals’ welfare.

Prevention

Countermeasures to prevent or minimize feedborne botulism are based on: (1) providing safe and high-quality feed to farm animals; (2) properly storing animal feed; (3) inspecting water sources for dying or dead small animals and birds; (4) avoiding spreading poultry litter that contains birds or dead animals on pastures; (5) avoiding using poultry litter as bedding material; and (6) vaccinating animals. These measures are described in more detail below.

Ensuring High-Quality Feed for Farm Animals

Feed producers and suppliers should apply good manufacturing practices and good agricultural practices to ensure that fields are not spread with poultry litter containing carcasses or others materials that may support the growth of C. botulinum, as requested by EU legislation. Farmers should check the quality of the raw materials used for silage and eliminate those presenting traces of contamination with molds or that appear rotten or spoiled. The current methods of mixing and distributing feeds, especially in cattle farms, mean that a small quantity of a contaminant can easily be distributed. Farmers must take precautions to avoid contamination of feed ingredients.

Animal feed prepared without acidification is particularly liable to growth and toxin production of BoNT-producing clostridia. However, acidification does not necessarily eliminate the risk of toxin formation in a carcass, unless it is chopped into small pieces and the cells or spores present in the carcass are exposed to the acid. Moreover, decomposing carcasses may contain large amounts of preformed toxin that may be further spread to the feed on processing.

Mitigating Environmental Conditions

Preventing avian botulism outbreaks in waterbirds depends on a thorough understanding of the interactions between the agent, the host, and the environment. Because C. botulinum spores are ubiquitous in wetlands, attempts to reduce or eliminate the agent are not currently feasible. However, some actions can be carried out to mitigate environmental conditions that increase the likelihood of outbreaks. Wetland flooding and draining, pesticides, and other agricultural pollutants, as well as anoxia of the wetland bottom, may kill or change aquatic life and provide more substrates for the growth and toxin production of BoNT-producing clostridia. Reduction of organic inputs into wetlands and elimination of factors that introduce
large amounts of decaying matter, as well as a prompt re-
moval and proper disposal of vertebrate carcasses (appli-
cable also in farms), are efficient measures to remove
substrate, thus reducing growth and toxin production of
BoNT-producing clostridia.5,43,57,58

**Vaccination**

Preventing botulism can be efficiently achieved by vacci-
nation, which generates neutralizing antibodies against
BoNTs. Vaccine administration is subject to health au-
thorities’ permission and in the EU to Directive 2001/82/
EC of the European Parliament and of the Council.59 A list
of available vaccines is reported in Table 1.

In some countries in which botulism is endemic, cattle
have been routinely vaccinated against type C and type D
toxins. For example, in Israel the vaccination program for
cattle starts when they are 2 months old and consists of 2
doses 4 weeks apart followed by an annual booster. Vac-
cination of cattle with bivalent toxoids conferred consid-
erable immunity following exposure to BoNTs for
extended time periods.60

Immunization has been successfully adopted for broilers
grown on farms with recurrent cases of the disease and for
pheasants and ducks. In waterfowl, immunization might be
used to reduce the risk of reintoxication. Usually, 2 doses of
vaccine administered about 14 days apart are used.61 The
degree of protection afforded by toxoid vaccination is

### Table 1. List of Botulism Vaccines

<table>
<thead>
<tr>
<th>Product Name</th>
<th>BoNT Type</th>
<th>Animal Species</th>
<th>Dosage</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultravac botulinum</td>
<td>C, D</td>
<td>Cattle, Sheep</td>
<td>2.5 ml cattle: 1.0 ml sheep</td>
<td>Pfizer Animal Health</td>
</tr>
<tr>
<td>Longrange</td>
<td>C, D</td>
<td>Cattle</td>
<td>2.5 ml</td>
<td>Pfizer Animal Health</td>
</tr>
<tr>
<td>Singvac 1-year botulinum</td>
<td>C, D</td>
<td>Cattle</td>
<td>2.0 ml</td>
<td>Virbac Animal Health</td>
</tr>
<tr>
<td>Singvac 3-year botulinum</td>
<td>C, D</td>
<td>Cattle</td>
<td>2.0 ml</td>
<td>Virbac Animal Health</td>
</tr>
<tr>
<td>Websters LV Bivalent botulinum</td>
<td>C, D</td>
<td>Cattle, Sheep, Mules, Sheep, Goats</td>
<td>2.0 ml cattle: 1.0 ml sheep</td>
<td>Virbac Animal Health</td>
</tr>
<tr>
<td>Botulism Vaccine</td>
<td>C, D</td>
<td>Cattle, Horses, Mules, Sheep, Goats</td>
<td>1.0 ml sheep and goats: 2.0 ml cattle, horses, and mules</td>
<td>Onderstepoort Biological Products</td>
</tr>
<tr>
<td>Botvax B</td>
<td>B</td>
<td>Horses</td>
<td>2.0 ml</td>
<td>Neogene Corporation</td>
</tr>
<tr>
<td>Febrivac 3 plus</td>
<td>C</td>
<td>Mink</td>
<td>1.0 ml</td>
<td>Impfstoffwerk-Dessau-Tornau Gmbh</td>
</tr>
<tr>
<td>Biocom- D</td>
<td>C</td>
<td>Mink</td>
<td>1.0 ml</td>
<td>United Vaccines, Inc.</td>
</tr>
<tr>
<td>Biocom –P</td>
<td>C</td>
<td>Mink</td>
<td>1.0 ml</td>
<td>United Vaccines, Inc.</td>
</tr>
<tr>
<td>Biocom</td>
<td>C</td>
<td>Mink</td>
<td>1.0 ml</td>
<td>United Vaccines, Inc.</td>
</tr>
<tr>
<td>Botumink</td>
<td>C</td>
<td>Mink</td>
<td>1.0 ml</td>
<td>United Vaccines, Inc.</td>
</tr>
</tbody>
</table>
influenced more by the time and number of inoculations than by the amount of toxoid injected.\textsuperscript{62}

Foals can be vaccinated as early as 2 weeks of age. Their immunization can be achieved by vaccinating pregnant mares, considering the high titer of antitoxin antibodies found in the colostrum. Vaccinated foals or adult horses have to receive an annual booster.\textsuperscript{63}

In the US, minks have been vaccinated against botulism since the 1950s.\textsuperscript{64} In Europe, mink are the only animal species routinely treated, although cattle, horses, and pheasants are sometimes vaccinated in outbreak situations.\textsuperscript{65}

**Conclusion**

According to EU legislation, botulism is a communicable disease to be covered by the European epidemiologic surveillance network, as well as a zoonosis to be monitored according to the epidemiologic situation.\textsuperscript{62} Member states annually report the number of human cases that have occurred in their countries to the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Agency (EFSA), but no notification procedures are foreseen for animal disease. The lack of compulsory notification of the disease in animals hampers an accurate knowledge of European cases and outbreaks and results in an underestimation of the incidence. Although botulism in animals is not reported, the illness can be classified in Europe as an emerging disease.\textsuperscript{60} The increase in the number of cases of animal botulism during the past decade is probably due to the lack of use of antimicrobial drugs in feed and the publication of the European Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents.\textsuperscript{66} As a consequence of the increased awareness by competent authorities, animal botulism has been included in the differential diagnosis in cases of recovery of sick or dead birds. Further efforts are needed to reduce the underreporting of outbreaks and improve awareness among veterinarians and other competent authorities. Finally, the availability of antidotes and vaccines to treat sick animals is essential to prevent additional cases.

**Acknowledgments**

This research was supported by the framework of the EU project AniBioThreat (Grant Agreement: Home/2009/ISEC/AG/191) with financial support from the Prevention of and Fight against Crime Programme of the European Union, European Commission—Directorate General Home Affairs. This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use that may be made of the information contained therein.

**References**


MANAGEMENT OF ANIMAL BOTULISM OUTBREAKS


63. Sprayberry KA. Botulism: a perfect killer. *Horse* 2007;article 20150.

Manuscript received December 27, 2012; accepted for publication April 23, 2013.

Address correspondence to:
Dario De Medici, PhD
Senior Researcher
Istituto Superiore di Sanita (ISS)
Department of Veterinary Public Health and Food Safety
Viale Regina Elena 299
Rome 00161 Italy
E-mail: dario.demedici@iss.it