Botulism

Shaker Foal Syndrome,
Limberneck,
Western Duck Sickness,
Bulbar Paralysis,
Loin Disease,
Lamziekte

Last Updated: June 2010

the Center for
Food Security
&Public Health

IOWA STATE UNIVERSITY®

College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011
Phone: 515.294.7189
Fax: 515.294.8259
cfsph@iastate.edu
www.cfsph.iastate.edu

INSTITUTE FOR
INTERNATIONAL
COOPERATION IN
ANIMAL BIOLOGICS

Iowa State University
College of Veterinary Medicine
www.cfsph.iastate.edu/IICAB/

Importance

Botulism is caused by botulinum toxins, neurotoxins produced by *Clostridium botulinum* and a few other species of *Clostridium*. By binding to nerve endings, these toxins cause progressive flaccid paralysis in humans and animals. Many untreated cases end in death from paralysis of the respiratory muscles. *C. botulinum* spores are common in the environment, but they can germinate and grow only in anaerobic environments under specific conditions. Foodborne botulism results from the ingestion of the preformed toxin after the organism has grown in food. Botulinum-producing organisms may also grow in the immature gastrointestinal tracts of human infants and foals, in human gastrointestinal tracts with certain abnormalities, and in anaerobic wounds. In addition, these toxins are a concern in bioterrorism.

Sporadic cases and outbreaks of botulism occur in both humans and animals. This disease is an important cause of death in unvaccinated ranched mink, and it can cause large outbreaks among wild birds such as waterfowl and gulls. Livestock may be accidentally fed the toxin in contaminated feed. Botulism seems to be increasing in cattle, possibly due to the increased use of plastic-packaged grass silage, and these outbreaks can cause significant economic losses. Cattle in areas with phosphorus-deficient soils may also chew on toxin-contaminated bones and scraps of flesh in the environment to satisfy the deficiency. In Senegal, which has such soils, botulism is thought to cause more deaths than any other cattle disease. In addition, botulinum has been reported in a variety of other species including poultry, dogs, cats, foxes, captive lions and sea lions, turtles, farmed fish and wild bighorn sheep. Botulism can be treated successfully, but patients may require weeks or months of intensive care, sometimes including mechanical ventilation, while the nerve endings regenerate. Treatment may be impractical in adult livestock unless the case is mild.

Etiology

Botulism is caused by botulinum toxin, a potent neurotoxin produced by *Clostridium botulinum*, a few strains of *C. baratii* and *C. butyricum*, and the recently reclassified species *C. argentinense* (formerly known as the type G toxin producing strains of *C. botulinum*). All of these organisms are anaerobic, Gram-positive, spore-forming rods.

The organisms that produce botulinum toxin are diverse, and can produce seven types of toxins (A through G). Researchers have also described a mosaic C/D toxin from outbreaks in birds. Most clostridial strains only produce one toxin type. All of the botulinum toxins cause the same clinical signs, although there may be some differences in the severity of the disease. However, knowing the toxin type is important in selecting an antiserum for treatment; antiserum produced against one type is not protective against others. Different toxin types also tend to cause botulism in different species. In people, botulism is usually caused by types A, B and E, although rare cases or outbreaks caused by types C, D, F and G have been described. Types C and D are the most common causes of disease in other mammals and birds, but types A, B and E can also be involved. Type C is especially common in birds, mink, horses in most parts of the world, cattle that have been fed poultry litter, and dogs that have eaten contaminated bird carcasses. Type C or type D may be found in livestock that have eaten feed contaminated with the carcasses of small animals. Types B and A are reported regularly from horses in the U.S., and can also affect other species. Type E toxin is often associated with aquatic environments, and can cause botulism in farmed rainbow trout (*Oncorhynchus mykiss*) and other fish, as well as in fish-eating birds. In addition to botulinum toxin, *C. botulinum* type C can produce a C2 toxin, which is an enterotoxin and causes gastrointestinal signs.

The species *C. botulinum* is very diverse, and has been divided into four genotypically and phenotypically distinct groups. In humans, botulism is usually caused by group I or group II organisms. Group I contains proteolytic strains that produce toxins A, B or F, while group II consists of nonproteolytic strains that make B, E or F toxins. Group I and II *C. botulinum* strains differ in heat resistance (spores from group I organisms are more heat resistant), growth temperatures, and other characteristics, which can influence the types of foods where they tend to grow.

Group III *C. botulinum* strains, which produce toxins C or D, are usually associated with botulism in animals. Group IV *C. botulinum* produces the type G toxin and has been reclassified as the new species *Clostridium argentinense*. This organism has caused a single outbreak among humans in Switzerland. *C. butyricum* produces type E toxin, and *C. baratii* produces type F.

Equine grass sickness

Botulinum toxin has also been implicated in equine grass sickness, a neurodegenerative disease that occurs among grazing equids. This disease, which is often fatal, is seen most often in the United Kingdom, but it has also been reported from other locations. A very similar disease occurs in South America, where it is called mal seco. Equine grass sickness affects the gastrointestinal tract and some other organs, can be acute or chronic, and tends to be seen in young horses on pastures in the spring. Its cause is still uncertain, but *C. botulinum* toxins have been implicated.

Geographic Distribution

C. botulinum is found worldwide. Although botulism can occur anywhere, the distribution of the organism is not homogeneous, and cases tend to be more common in certain geographic areas. Environmental factors can also influence where botulism is seen. For example, this disease tends to common in cattle from areas with phosphorus-poor soils, such as those in southern Africa.

The distribution of strains can also vary with the geographic area. In one study from the U.S., strains that produce type A toxin were detected mainly in neutral or alkaline soils in the western states, while type D-producing strains were found in some alkaline soils in these states. The distribution of type B strains was more uniform, but these organisms were especially common in the eastern states. Type C strains were detected in acidic soils in the Gulf Coast states. In North America, type E strains are most common along the shores of the Great Lakes and in the Pacific Northwest. In one study from the former U.S.S.R., strains that produced type E toxin accounted for 61% of the isolations. Knowing the toxin types that are prevalent in an area may be helpful in selecting an antitoxin, as this must often be done before the laboratory results are complete.

Transmission

All species of *Clostridium* can produce spores, dormant forms of the organism that are highly resistant to disinfectants, heat and environmental conditions that kill vegetative cells. These spores can survive for many years until favorable conditions allow them to germinate and grow. *C. botulinum* spores are common in the environment. In soil studies, this organism was detected in approximately 18-23% of the samples in the U.S. and 10% of the samples in the former U.S.S.R. In addition to soils, *C. botulinum* can be found in sediments in lakes, streams and coastal waters. This organism has been reported from the intestinal tracts of some healthy fish, birds and mammals, and the gills and viscera of shellfish such as crabs. Botulinum toxin has been detected in snails, earthworms, maggots feeding on contaminated carcasses, and nematodes. Because invertebrates are not affected by the toxin, they can be important in transmitting it to species such as birds.

The vegetative (active) form of *C. botulinum* can only grow and produce toxins under anaerobic conditions. A wide variety of plant and animal material can support its growth, but the conditions it needs are strict. In addition to the food or tissue being anaerobic, it must have a relatively high water content, the pH must be greater than 4.6, and ingredients such as salt or preservatives must not be present at high enough levels to inhibit germination and/or growth. Different strains or groups of *C. botulinum* may have somewhat different requirements. For instance, the acidity necessary to inhibit *C. botulinum* type C strains is reported to be pH 5.1 to 5.6, but other organisms can survive a lower pH. Each group also has an optimal, minimal and maximal temperature for growth. For example, some group II organisms may be able to grow in foods at refrigeration temperatures (3-4°C/ 37-39°F), but group I organisms and type C toxin-producing strains are inhibited. Because *C. botulinum* does not compete well with other microorganisms, growth is more likely to occur if other bacteria and molds have been killed or inhibited. *C. botulinum* spores can survive cooking and some food-processing conditions that kill vegetative cells, then germinate and grow in the cooked food.

Botulism usually occurs when people or animals ingest the toxins in food or water, or when the spores germinate in anaerobic tissues and produce toxins as they grow. Botulinum toxin does not pass through intact skin, but it can cross mucous membranes and broken skin. Laboratory accidents can cause botulism by inhalation or other means, and bioterrorism is a possibility.

Botulism in humans

In humans, the three major forms of disease are foodborne, wound, and infant or intestinal botulism. Foodborne botulism occurs when humans ingest preformed toxins in various foods. Modern industrial canning techniques were designed to kill *C. botulinum* spores, and most cases are caused by home-canned, low acid foods (pH > 4.6), as well as meat products such as sausages and ham, and fermented fish, seal and whale meat. However, many different foods can be involved if the conditions are favorable; botulism has been caused by products as diverse as yogurt, garlic oil and foil-wrapped baked potatoes. Baked foods that have been left at room temperature or in a warm oven overnight can cause this disease if the baking kills competing microorganisms, and anaerobic conditions occur in the interior of the food. Commercial foods are occasionally involved.

Wound botulism occurs when an anaerobic wound is contaminated with *C. botulinum*, and the organism is able

to grow and produce toxin. Wound botulism is rare except in injecting drug abusers, where it is caused by contaminated needles or drugs. It is especially common among people who inject "black tar heroin" directly into the subcutaneous tissues.

Infant botulism is seen in children less than a year of age. In this form, *C. botulinum* spores germinate in the intestinal tract and produce toxin. Infants are thought to be predisposed because their intestinal flora is immature and because they produce reduced amounts of bile acids, which inhibit clostridial organisms. Honey has been associated with some cases of infant botulism, but spores can also be found in many other sources including dust. Botulinum spores from the environment can be ingested by most older children and adults without harm; these spores simply pass through the intestines without germinating. However, there are rare cases of intestinal colonization botulism in people who have altered intestinal conditions from gastrointestinal surgery, intensive antibiotic therapy or abnormalities such as achlorhydria.

Botulism can also occur from laboratory accidents (e.g., by inhalation or accidental injection of the toxin). The toxin is used therapeutically to treat some muscle movement disorders and other conditions, and a few cases of iatrogenic botulism have been reported.

Botulism in animals

Preformed toxins in a variety of sources including decaying vegetable matter (e.g., grass, hay, haylage, grain, spoiled silage), meat and fish, carcasses, invertebrates and contaminated water can cause botulism in animals. Carnivores are usually fed the toxins in contaminated meat or fish, or ingest the toxins in carcasses (possibly including iguanas) or decaying, high protein garbage. Cattle in phosphorus-deficient areas may develop pica and chew bones and scraps of attached meat; a gram of dried flesh may have enough botulinum toxin to kill a cow. Similar cases occur in Australia, where protein-deficient sheep sometimes eat the carcasses of rabbits and other small animals. Herbivores also become ill when they are fed the toxin in forage such as hay or insufficiently acidified silage. These feeds may be contaminated by the toxin-containing carcasses of birds and mammals, or from other sources of *C. botulinum*. The feeding of poultry litter containing type C spores has been linked to some outbreaks in ruminants. Birds can ingest botulinum toxins in maggots that have fed on contaminated carcasses or invertebrates from water with decaying vegetation. Fish have been suggested as the source of the toxin in some outbreaks among birds. Contaminated feed can also result in cases in poultry. In addition, botulism has been described in animals that drank water contaminated by a carcass or other source of toxin.

The toxicoinfectious form of botulism in animals corresponds to the wound and intestinal forms in humans.

Similarly to human infants, botulism in foals (the shaker foal syndrome) seems to be caused by the growth of *C. botulinum* in the gastrointestinal tract. Rare cases of wound botulism have been seen in some species such as horses. Toxicoinfectious botulism is also seen in chickens, when broilers are intensively reared on litter; the cause of this phenomenon is unknown.

Botulism is not contagious by casual contact, but it can be transmitted between animals by predation or cannibalism. Contaminated foods usually contain spores as well as the toxin. Spores that are passing through the gastrointestinal tract may germinate and grow if the animal dies. This can perpetuate the cycle, and may result in large outbreaks in birds or other species. Outbreaks of botulism can also contaminate the environment with spores, making future outbreaks more likely.

Botulinum and Bioterrorism

In a bioterrorist attack, botulinum toxin could be delivered by aerosols, as well as in food or water. Aerosolization is the most likely form. After aerosol transmission, the clinical disease is expected to be similar to foodborne botulism.

Disinfection/Inactivation

Botulinum toxins are large, easily denatured proteins. Toxins exposed to sunlight are inactivated within 1 to 3 hours. They can also be inactivated by treating with 0.1% sodium hypochlorite or 0.1 N NaOH, as well as by heating to 80°C (176°F) for 20 minutes or to greater than 85°C (185°F) for at least 5 minutes. The toxin's heat resistance varies with the medium, its pH and the concentration of the toxin. In beef broth, type E toxin was reported to be inactivated within 1 to 9 minutes at 80°C (176°F), with the longest survival time at pH 5.0 and the shortest survival time at the pH extremes (pH 3.5 or 6.8). The World Health Organization (WHO) recommends boiling food for a few minutes to inactivate botulinum toxins. Chlorine and other disinfectants can destroy the toxins in water.

The vegetative cells of *Clostridium botulinum* are susceptible to many disinfectants, including 1% sodium hypochlorite and 70% ethanol. In contrast, spores are very resistant to environmental conditions. The spores of group I *C. botulinum* strains are highly heat resistant; 121°C (250°F) for 3 min is used to destroy them during commercial canning. Spores of group II strains are often damaged by heating to 90°C (194°F) for 10 min, 85°C for 52 min, or 80°C for 270 min; however, these treatments may not be sufficient in some foods. For example, lysozyme or other lytic enzymes present in the food may help damaged spores germinate. *C. botulinum* spores can be destroyed by autoclaving with moist heat (120°C/ 250°F for at least 15 minutes).

Botulism in Humans

Incubation Period

The incubation period for foodborne botulism can be a few hours to 8 days; most cases become symptomatic in 12 to 72 hours. Wound infections may become evident within a few days to two weeks, with an average incubation period of 10 days. The incubation period for adult intestinal colonization or infant botulism is unknown; some cases in adults have occurred up to 47 days after ingesting food that contained the organism. Botulism acquired by inhalation usually develops 12 to 36 hours after exposure, but in some cases the incubation period may be as long as several days.

Clinical Signs

The neurological signs caused by botulinum toxin are similar in all forms of the disease. Additional symptoms (e.g., gastrointestinal signs in foodborne cases) may also be seen in some forms.

Foodborne botulism

In foodborne cases, gastrointestinal disturbances such as nausea, vomiting and abdominal pain are often the first signs. The neurotoxin causes constipation; however, diarrhea may also occur with contaminated food. As the disease progresses, a symmetric, descending flaccid paralysis develops in the motor and autonomic nerves. The clinical signs may include blurred or double vision, photophobia, drooping eyelids, an expressionless face, slurred speech, dysphagia, urine retention, a dry mouth, somnolence and muscle weakness. Untreated cases may progress to descending paralysis of the respiratory muscles, arms and legs. Fatal respiratory paralysis can occur within 24 hours in severe cases. The pharynx may also collapse from cranial nerve paralysis, resulting in respiratory dysfunction even if the respiratory muscles are not affected. Fever is not usually seen, and cognitive function and the senses are almost always unaffected. Death is usually the result of respiratory compromise. Recovery can take weeks or months. In some cases, survivors report fatigue and shortness of breath for years.

Wound botulism

Wound botulism is very similar to the foodborne form; however, gastrointestinal signs are not usually present and patients may have a wound exudate or develop a fever. The abscess can also be minor (e.g., a small furuncle or mild cellulitis).

Infant botulism

Most cases of infant botulism occur in 2-week to 6-month-old babies, but infants up to a year of age can be affected. The first symptom is usually constipation, which can persist for several days before flaccid paralysis develops. Lethargy, weakness, excessively long sleep periods, difficulty in suckling and swallowing, diminished gag reflexes, dysphagia with drooling, drooping eyelids and poor pupillary light reflexes may also be seen. Some babies develop a weak or altered cry. In progressive cases, the infant may develop flaccid paralysis; a "floppy head" is typical. In severe cases, there may be respiratory dysfunction or arrest. Botulism might also be responsible for some cases of sudden death in infants. The symptoms and severity of this disease vary considerably in different babies. In infants that must be hospitalized, supportive care is usually required for several weeks; however, some mildly affected babies may recover quickly. Relapses are seen occasionally after the clinical signs have resolved.

Intestinal colonization botulism in adults

The initial symptoms of intestinal colonization botulism in adults may include lassitude, weakness and vertigo. As the disease progresses, patients may experience blurred or double vision, progressive difficulty speaking and swallowing, descending flaccid paralysis, and other symptoms characteristic of botulism. Abdominal distention and constipation may also be seen. Although this form of botulism resembles foodborne botulism, the symptoms may be prolonged, and relapses may be seen.

Inhalational botulism

Inhalational botulism was reported in laboratory workers in 1962. It resembled foodborne botulism.

Communicability

Person–to–person transmission has not been reported. Nevertheless, care should be taken when handling clinical samples that may contain botulinum toxin, such as feces, gastric contents or body fluids.

Diagnostic Tests

Botulism can be tentatively diagnosed by the clinical signs and the exclusion of other neurologic diseases. The definitive diagnosis relies on identifying the toxin and/ or bacterium in feces, blood/ serum, vomitus, gastric aspirates, wounds or food samples.

Botulinum toxin is usually identified with a mouse bioassay (the mouse neutralization test), but enzyme-linked immunosorbent assays (ELISAs) can also be used. Because ELISAs detect both active and inactivated (e.g., heat treated) toxins, false positives are possible with this test. Only active toxins are detected in mice. Polymerase chain reaction (PCR) assays that detect the neurotoxin genes can be helpful in identifying *C. botulinum*; however, some genes (silent toxin genes) do not produce active toxin, and the results from this assay are confirmed using the mouse bioassay. Botulinum toxins can be typed with neutralization tests in mice. Serology is not used in diagnosis, as small amounts of toxin are involved and survivors rarely develop antibodies.

C. botulinum can be isolated from food or clinical samples in anaerobic culture. Heat or ethanol treatment can aid recovery in highly contaminated samples such as food or feces. These treatments destroy competing

microorganisms while allowing clostridial spores to survive. The temperature used varies with the group; 80°C (176°F) can be used for 10 minutes with group I spores, but 60°C (140°F) for 10 to 20 minutes is less likely to injure group II spores. Some solid media that may be used are blood or egg yolk agar, *Brucella* agar with 5% sheep blood, and phenyl ethyl alcohol blood agar. Suitable liquid media include chopped-meat-glucose-starch medium, cooked-meat medium, reinforced clostridial medium, anaerobe broth and others. On solid media, *C. botulinum* colonies are usually grayish-white with an irregular edge. The colonies are generally beta-hemolytic on blood agar, while on egg yolk medium, they usually display surface iridescence that extends beyond the colony (lipase positive), and are variable for lecithinase activity.. The iridescent zone around the colony tends to be larger for C, D and E toxins. (Lipase is not specific for *C. botulinum*; many other *Clostridium* species and other bacteria also produce this enzyme.) The stained organism is a Gram positive rod that develops oval subterminal spores, especially on media such as chopped meat medium incubated for 5 to 7 days at 30°C (86°F). Biochemical tests and the detection of volatile metabolic products, using gas-liquid chromatography, are helpful in identification. The metabolic patterns and other characteristics vary with the strain/ group. Definitive identification is by demonstration of the toxin. Molecular genetic techniques are helpful in tracing the source of an outbreak.

Other diagnostic or clinical tests may be helpful in excluding other causes of flaccid paralysis, or supporting the diagnosis. Electromyography suggests neuromuscular junction blockage, normal axonal conduction, and potentiation with rapid repetitive stimulation in the affected muscles.

Treatment

The binding of botulinum toxins to the presynaptic endplates of neurons cannot be reversed; however, axons can produce new endplates if the patient can be kept alive while they regenerate. Supportive treatment is the cornerstone of treatment. Depending on the severity of the illness, the respiratory system may need to be sustained with oxygen treatment, intubation to keep the airway open and/or mechanical ventilation. Supportive care may be necessary for up to several weeks or months.

Botulinum antitoxin, given while the toxin is still circulating in the blood, may prevent the disease from progressing and decrease the duration of the illness. Once the toxin has bound to the nerve endings, antitoxin cannot reverse the binding. For this reason, it should be given as soon as possible, preferably within the first 24 hours. Recent studies and cases suggest that botulinum toxin may be found in the circulation for up to 12 days in some patients with the foodborne form. How late the administration of antitoxin should be considered is uncertain. Botulinum antitoxin is usually produced in equines, and it can produce adverse effects such as serum sickness and sensitization to equine proteins, with the possibility of anaphylaxis. Equine source antitoxin is not used in infants for this reason; instead, infant botulism can be treated with human-derived antitoxin (BIG-IV/ Baby-BIG). This antitoxin is produced in human donors immunized with pentavalent botulinum toxoid (A to E). BIG-IV/ Baby-BIG can decrease the hospitalization time significantly in infants. It may also be used for older patients who cannot tolerate equine serum. In the U.S., equine botulinum antitoxin is available from the Centers for Disease Control and Prevention (CDC) through state health departments, and Big-IV/Baby-BIG is available from the California Department of Health Services' Infant Botulism Treatment and Prevention Program.

Additional treatments depend on the form of the disease. In foodborne illness, the amount of toxin in the gastrointestinal tract may be reduced with stomach lavage, emetics, enemas and/or cathartics. Treatment for wound botulism includes surgical debridement of the wound and antibiotics. Aerobic conditions may be induced in the wound by the use of hydrogen peroxide or hyperbaric oxygen therapy. Antibiotics are not recommended in infant botulism because the death of the microorganisms might release additional toxins from lysed cells. If antibiotics are used to treat botulism, drugs that have neuromuscular blocking properties, such as aminoglycosides, should be avoided.

Prevention

Procedures used to heat treat foods in commercial canning can destroy *C. botulinum* spores. The risk of botulism can also be reduced by acidification, reductions in the amount of moisture in the product, and treatment with salt or other compounds known to inhibit the organism's germination and/or growth. Refrigeration can prevent the growth of group I strains, but some nonproteolytic group II strains may grow at 3-4°C (37-39°F). Foods with "off" odors or flavors should not be eaten, but *C. botulinum* may grow without changing the food's flavor, odor or appearance. Preformed toxins in foods can be destroyed by boiling the food before serving.

Because some batches of honey may contain *C. botulinum* spores, this food should not be fed to children less than a year of age. To prevent toxins in sick animals from affecting people, meat from affected animals is not used for food. Whether the toxin can be transported from the bloodstream to the milk in ruminants is uncertain, but milk from these animals is not allowed to enter the human food chain.

In laboratories, *C. botulinum* must be handled under BSL-2 conditions at a minimum, with BSL-3 precautions used for some procedures. Investigational vaccines may be available for people who have a high risk of exposure (e.g., laboratory workers), and improved vaccines are in development. Person-to-person transmission of botulinum

has never been described, but precautions should be taken to avoid exposure to toxins in body fluids and feces, and anyone who has been exposed should remain alert for the onset of symptoms.

Morbidity and Mortality

Botulism tends to occur as sporadic cases or small outbreaks that affect a few people, but large outbreaks can also be seen, especially when commercially prepared foods are involved. Since 1980, infant botulism has been more common than foodborne botulism in the U.S. In 2006, 107 cases of infant botulism, 19 cases of foodborne botulism and 45 cases of wound botulism were reported to the CDC. Wound botulism, which was once very rare, has been increasing with certain types of drug abuse.

Untreated cases are often fatal, but supportive care has a high success rate when the disease is diagnosed in time. Before 1950, the case fatality rate for foodborne botulism was 60-70%; currently, it is approximately 5-10% in developed countries. Patients in some risk groups, such as those older than 60 years of age, have a higher case fatality rate. The severity of the illness and time before recovery may also be influenced by the dose of the toxin and the toxin type. Cases caused by type A toxin tend to be more severe than those caused by types B or E. People may also have differing sensitivity to the toxin. In one case, a person had detectable toxin in the circulation but was asymptomatic.

The case fatality rate for infant botulism is 2%. In infants that must be hospitalized, recovery usually requires several weeks in the hospital. Antitoxin (Baby-BIG/ BIG-IV) can significantly decrease this time. Estimates of the case-fatality rate for wound botulism vary widely from 1% to 15%.

People who survive botulism do not become immune to the disease. Even in the most severe cases, the amount of toxin in the body is usually too low to stimulate antibody production.

Botulism in Animals

Species Affected

Botulism has been reported in a variety of vertebrates including mammals, birds, reptiles and fish. This disease occurs in horses, cattle and sheep, as well as in ranched mink and foxes. It has also been documented in ferrets, laboratory rodents, nonhuman primates, captive mammals including lions and sea lions, and wild species such as bighorn sheep. Dogs, cats and pigs are relatively resistant to the ingestion of this toxin. Nevertheless, there are occasional reports of botulism in dogs and pigs, and an outbreak was reported in cats that had eaten highly contaminated tissues from a pelican. Botulism has also been seen in more than a hundred species of birds in 22 families, including chickens, pheasants, turkeys, ducks, geese, gulls, loons, mergansers, herons, horned grebes and cormorants.

Outbreaks have been reported in farmed rainbow trout, and other fish are susceptible in experimental studies. Botulism has also been documented in turtles.

Incubation Period

The incubation period can be 2 hours to 2 weeks; in most cases, the clinical signs appear in 12 to 48 hours. Mink are often found dead within 24 hours of ingesting the toxin, and in an outbreak among foxes, the incubation time was 8 to 96 hours. In dogs, it is reported to be 24-48 hours, but in one experiment, dogs fed the toxin became ill in 2-4 days.

Clinical Signs

Botulism is characterized by progressive motor paralysis. In animals, botulism usually affects the hind legs first and ascends. In addition to muscle paralysis, animals may have difficulty chewing and swallowing, experience visual disturbances, and develop generalized weakness and incoordination. Autonomic dysfunction may also be seen. Death usually results from paralysis of the respiratory muscles. Mildly affected animals may recover with minimal treatment.

Ruminants

Muscle weakness and incoordination, progressing to paralysis, is the most apparent sign in ruminants. Weakness is seen in the hind legs first. The animal may also have difficulty chewing and swallowing food, the tongue may protrude, drooling may be seen, and the head may be held abnormally low. Restlessness and urine retention can also occur. In cattle that become recumbent, the head is often turned toward the flank, similarly to a cow with hypocalcemia. Laterally recumbent animals are usually very close to death. Some sheep and goats have been found dead.

In Germany, a cattle disease characterized by lethargy, constipation alternating with diarrhea, edema, decreased milk yield, non-infectious chronic laminitis, engorged veins, a retracted abdomen and emaciation has been linked to the presence of botulinum toxin in the colon and cecum. Affected cattle may die unexpectedly. Most cases have been seen during the peripartum period, but slow growth and wasting was reported in heifers. This disease has been tentatively been named "visceral botulism."

Horses

The clinical signs in horses are similar to cattle. They may include restlessness, knuckling, incoordination, dysphagia, paralysis of the tongue, drooling, decreased muscle tone in the tail and/or tongue, and recumbency. The muscle paralysis is progressive; it usually begins at the hindquarters and gradually moves to the front limbs, head and neck. As in other species, paralysis of the respiratory muscles can result in death.

The "shaker foal syndrome" appears to be similar to botulism in human infants. The most characteristic signs are

a stilted gait, muscle tremors and the inability to stand for more than a few minutes. Dysphagia, constipation, reduced eyelid, tongue and tail tone, mydriasis, sluggish pupillary light reflexes and frequent urination may also be seen. In the later stages, foals usually develop tachycardia and dyspnea. Without treatment, death from respiratory paralysis generally occurs 24 to 72 hours after the initial signs. Some foals may be found dead.

Pigs

Pigs are relatively resistant to botulism. Reported clinical signs include anorexia, refusal to drink, vomiting, pupillary dilation and muscle paralysis.

Foxes and Mink

During outbreaks of botulism in mink, many animals may be found dead, while others have various degrees of flaccid paralysis and dyspnea. The clinical picture is similar in commercially raised foxes. In some mildly affected foxes, only the hind legs are paralyzed. These animals may sit and drag the hind part of their bodies.

Dogs

Limited studies in dogs suggest that this species is relatively insensitive to the ingestion of toxin. Clinical signs reported in dogs include vomiting and anterior abdominal pain, as well as signs related to the effects of the toxin on nerves, such as salivation, incoordination, weakness of the hind legs, flaccid paralysis, a depressed gag reflex, and a diminished withdrawal reflex and/or pupillary reflexes. Congestion of the mucous membranes of the mouth, brownish fetid saliva, cheilitis and an unusual, hoarse, suppressed bark or whine were reported in some experimentally exposed dogs. Some dogs recover, but others have died of respiratory failure.

Cats

In the single outbreak described in cats, anorexia and mild depression were the first signs, followed by flaccid paralysis, and in some cases, dyspnea. Similarly to other animals, the paralysis was evident first in the hindlegs, followed by the forelegs. Some severely affected cats died, others recovered spontaneously and rapidly.

Ferrets

Experimentally exposed ferrets developed botulism signs including weakness, ataxia, ascending paralysis, blepharospasm, photophobia and urinary incontinence, with death resulting from respiratory failure.

Sea lions

Inactivity and dysphagia, followed in some cases by unexpected deaths, were reported in sea lions. Although some animals appeared to be hungry, chewing fish and attempting to swallow, they eventually released the partially chewed fish from their mouths.

Birds

In poultry and waterfowl, botulism is an ascending flaccid paralysis and affects the legs first, followed by the wings and neck. Mild cases may have only paresis or leg paralysis. In gulls, the wing muscles seem to be affected before the legs, and delayed or uncoordinated flight may be an early sign. Mildly affected gulls are able to stand and run, but not fly. Diarrhea with excess urates and paralysis of the nictitating membrane have also been reported in some species. The feathers of chickens may be ruffled, and they may be shed easily when the birds are handled. Birds may die from respiratory dysfunction, and waterfowl with paralyzed necks may drown.

Reptiles

Loss of equilibrium and flaccid paralysis of the legs, followed by drowning, have been reported in green sea turtles (*Chelonia mydas*).

Fish

Loss of equilibrium and erratic swimming have been seen in fish. Increased swimming bursts were the first sign of botulism in experimentally exposed rainbow trout (*Oncorhynchus mykiss*). Some fish including rainbow trout, walleye (*Stizostedion vitreum*) yellow perch (*Perca flavescens*), tilapia and coho salmon (*Oncorhynchus kisutch*) may attempt to swim in a head up/ tail down orientation, with breaching of the water surface. Hyperpigmentation, which can be dramatic, occurs in some species. In round goby (*Neogobius melanostomas*), the first sign of botulism was a faint, black band behind the pectoral fins, which darkened and spread toward the tail until the entire posterior of the fish was darkened. This was followed by darkening of the anterior body, until the entire fish was almost black. Goby did not develop abnormal swimming behavior until the late stages of hyperpigmentation. Hyperpigmentation was also an early sign in yellow perch, and it has been reported in carp (family Cyprinidae). In contrast, decreased color intensity was seen in tilapia and rainbow trout in one experiment, while rainbow trout did not have obvious changes in pigmentation in another study. Similarly to other vertebrates, death occurs in the late stages from respiratory compromise. Fish are usually immobile at this stage. A few fish with mild clinical signs such as slight loss of equilibrium and increased swimming bursts may recover completely.

Communicability

Botulism is not communicable by casual contact, but tissues from dead animals can be toxic if ingested by other animals.

Diagnostic Tests

Botulism can be difficult to diagnose, as the toxin is not always found in clinical samples or the feed. Diagnosis is often a matter of excluding other diseases. A definitive diagnosis can be made if botulinum toxin is identified in the

feed, serum/ blood, stomach, crop or intestinal contents, vomitus, feces or tissues. The toxin can usually be found in the blood or serum only in the early stages of the disease. Botulinum toxin is typically detected with a mouse bioassay. ELISAs may also be used, but they detect both active and inactivated (e.g., heat treated) toxins, and false positives are possible with this test. Botulinum toxins can be typed using neutralization tests in mice.

C. botulinum organisms may also be isolated from the feed. In toxicoinfectious botulism, the organism may be cultured from the gastrointestinal contents, feces, wounds or other tissues. Because healthy animals can have *C. botulinum* spores in the gastrointestinal tract, finding them in this location should be interpreted with caution.

C. botulinum must be isolated in anaerobic culture. Heat or ethanol treatment can aid recovery in highly contaminated samples such as food or feces. These treatments destroy competing microorganisms while allowing clostridial spores to survive. Some solid media that may be used are blood or egg yolk agar, *Brucella* agar with 5% sheep blood, and phenyl ethyl alcohol blood agar. Suitable liquid media include chopped-meat-glucose-starch medium, cooked-meat medium, reinforced clostridial medium, anaerobe broth and others. On solid media, *C. botulinum* colonies are usually grayish-white with an irregular edge. The colonies are generally beta-hemolytic on blood agar, while on egg yolk medium, they usually display surface iridescence that extends beyond the colony (lipase positive), and are variable for lecithinase activity.. The iridescent zone around the colony tends to be larger for C, D and E toxins. (Lipase is not specific for *C. botulinum*; many other *Clostridium* species and other bacteria also produce this enzyme.) The stained organism is a Gram positive rod that develops oval subterminal spores, especially on media such as chopped meat medium incubated for 5 to 7 days at 30°C (86°F). Biochemical tests and the detection of volatile metabolic products, using gas-liquid chromatography, are helpful in identification. The metabolic patterns and other characteristics vary with the strain/ group. Definitive identification is by demonstration of the toxin. Molecular techniques can be been used for the genetic characterization of *C. botulinum*, and may be helpful in determining the source of an outbreak.

Serology is not used routinely in diagnosis, but antibodies to botulinum toxin have been reported in some animals that recovered, including horses, cattle and a dog. In the dog, paired serum samples revealed a fourfold increase in titer. Antibodies to type C and/or D botulinum toxin have also been reported in a few golden jackals (*Canis aureus syriacus*) in Israel.

Treatment

Treatment is supportive, and may include nursing, nutritional support, oxygen and the use of mechanical ventilation until the nerve endings regenerate. Mechanical ventilation has significantly reduced the death rate in foals,

but it is impractical and/or unavailable for some animals such as adult livestock. In one study, foals that required hospitalization could be discharged in approximately 2 weeks, although they were not fully recovered and stall rest or confinement in a small paddock was recommended for an additional period. Feed that might be contaminated should be removed. Gastric lavage, emetics, cathartics and/or enemas may be used to eliminate some of the toxin from the gastrointestinal tract, and. activated charcoal or other substances may help prevent absorption. Where the water supply has high salinity, giving fresh water to prairie wildfowl can improve their condition. The supraorbital gland in these birds, which functions in osmoregulation, is innervated by nerves affected by the toxin.

Monovalent or polyvalent botulinum antitoxin is sometimes used in animals, but it can be expensive, especially in adult livestock. Antitoxin against one toxin type does not provide any significant cross-protection to other types. Decisions on antitoxin treatment must often be made before the results from typing are available. The use of guanidine hydrochloride, which might help the neuromuscular blockade, could also be considered.

Various treatments including antibiotics and citric acid (which chelates the iron needed by *C. botulinum* to grow) have been used in the toxicoinfectious form in birds, with varying success. In foals, antibiotics may be given to prevent complications such as aspiration pneumonia. If antibiotics are used, drugs that have neuromuscular blocking properties, such as aminoglycosides, should be avoided.

Some animals with mild disease can survive with minimal treatment, or recover on their own.

Prevention

In areas where botulism is relatively common, vaccines may be used in animals including horses, cattle, sheep, goats, mink and birds. Vaccine availability varies with the country. In the U.S., commercial vaccines are licensed for horses and mink. There is no cross-protection between toxin types. Surviving a case of botulism does not protect an animal from later exposure to this toxin or eliminate the need for vaccination. Foals born to vaccinated dams have occasionally developed the shaker foal syndrome.

During an outbreak, carcasses should be collected to prevent animals from eating contaminated tissues or the invertebrates that feed on them. Flies should be controlled to prevent the occurrence of "toxic" maggots (maggots that have ingested botulinum toxin), which may be eaten by birds. If possible, litter should also be removed from poultry houses in an outbreak. If this cannot be done, commercial acid disinfectant or granular sodium bisulfate treatment may help suppress the growth of the organism. During outbreaks in chickens, it may also be helpful to clean and disinfect the environment with products effective against spore-forming bacteria. Waterfowl should be chased away from contaminated areas when botulism occurs in

wild birds. It may also be helpful to stabilize water levels (fluctuations have been linked to the proliferation of *C. botulinum*) and eliminate large shallow areas where vegetation decays.

Feed for mink and other ranched animals may be heat processed and/or acidified to reduce the risk of botulism. Care should be taken in the preparation of feed for herbivores. Carcasses should not be allowed to contaminate the feed, and silage should be monitored for proper acidification. Ruminants should be given feed supplements to reduce the incidence of pica when dietary deficiencies exist.

Morbidity and Mortality

Outbreaks of botulism occur regularly in wild waterfowl and shorebirds. They can be preceded by fluctuations in the temperature and/or level of the water, which may increase the proliferation of *C. botulinum*. An estimated 10 to 50 thousand wild birds , especially ducks, are killed annually. In some large outbreaks of type C botulism in western North America, a million or more birds may die. Large numbers of gulls and other birds have been affected in some other areas, including coastlines in Europe. Between 2000 and 2004, more than 10,000 seabirds, mainly herring gulls (*Larus argentatus*) died from type C outbreaks in Sweden. Since 1999, type E botulism affecting large numbers of fish-eating birds, such as gulls and loons, has been reported regularly in the Great Lakes of North America.

The incidence of botulism in domesticated animals is not known with certainty. Carnivores are said to be relatively resistant to this disease; however, contaminated feed can cause outbreaks affecting hundreds or thousands of mink or ferrets. Mink are often vaccinated. Botulism seems to be uncommon in ranched foxes, but one outbreak resulted in the death of more than 44,000 animals. The mortality rate in this outbreak was 22%, and some lots of contaminated feed caused the death of more than 40% of the foxes. The majority of the animals affected were blue foxes (*Alopex lagopus*) and shadow foxes (a color variant of this species), while silver foxes and blue silver foxes, which are color variants of *Vulpes vulpes,* had mortality rates of less than 4%. Cats were relatively resistant to the ingestion of botulinum toxin in limited experimental studies. In the only outbreak reported in this species, the dose of toxin appeared to very high. Four of eight cats fed the contaminated meat died, but the surviving cats recovered quickly. Limited studies in dogs also suggest that this species is relatively insensitive to toxin ingestion. In one experiment, dogs did not become ill after feeding botulinum toxin unless food was withheld first for 48 hours.

In most parts of the world, botulism seems to be relatively uncommon in herbivores; however, it can occur more frequently where conditions such as phosphorus-deficient soils are conducive to this disease. Large numbers of animals may be affected in some outbreaks. The prognosis is poor in recumbent adult livestock. In cattle, death generally

occurs within 6 to 72 hours of sternal recumbency. Mortality rates of up to 90% have been described in adult horses. Toxicoinfectious botulism in foals also had a case fatality rate of 90% at one time; however, the use of intensive care, mechanical ventilation and antitoxin has significantly improved survival. In two recent series, 87.5% of the mechanically ventilated foals in one study and 96% of the treated foals in another study (some of which did not require respiratory support) recovered.

Outbreaks of botulism in broiler chickens are uncommon. These birds become less susceptible to botulism with age, and most cases occur in intensively reared flocks between the ages of 2 and 8 weeks. The mortality rate varies from a few birds to 40% of the flock.

Among fish, susceptibility seems to vary with the species. In one experiment, the mortality rate at various oral doses was 92-100% in round goby, 83-92% in walleye, 42-92% in rainbow trout, and 25%-67% in yellow perch. Yellow perch also survived significantly longer than the other three species.

Post Mortem Lesions   ⬛ **Click to view images**

There are no pathognomonic lesions; any lesions are usually the result of general muscle paralysis, debilitation, the inability to eat and drink, or other secondary effects, and may include signs such as congestion in a variety of tissues. Respiratory paralysis may cause nonspecific signs in the lungs. In shaker foal syndrome, the most consistent lesions are excess pericardial fluid with strands of fibrin, pulmonary edema and congestion.

Internet Resources

Botulism Toolkit
http://botulismtoolkit.com/

California Department of Health Services' Infant Botulism Treatment and Prevention Program
http://www.cdph.ca.gov/programs/ibtpp/Pages/default.aspx

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/nczved/divisions/dfbmd/diseases/botulism/

eMedicine.com. Botulism
http://emedicine.medscape.com/article/829125-overview

Food and Drug Administration (FDA). Bacteriological Analytical Manual Online
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

FDA. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (Bad Bug Book)
http://www.fda.gov/Food/FoodSafety/FoodborneIllness

/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm

Manual on Meat Inspection for Developing Countries
http://www.fao.org/docrep/003/t0756e/t0756e00.htm

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627/

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

References

Abe Y, Negasawa T, Monma C, Oka A. Infantile botulism caused by *Clostridium butyricum* type E toxin. Pediatr Neurol. 2008;38(1):55-7.

Acha PN, Szyfres B [Pan American Health Organization (PAHO)]. Zoonoses and communicable diseases common to man and animals. Volume 1. Bacterioses and mycoses. 3rd ed. Washington DC: PAHO; 2003. Scientific and Technical Publication No. 580. Botulism; p. 28-40.

Böhnel H, Schwagerick B, Gessler F. Visceral botulism--a new form of bovine *Clostridium botulinum* toxication. J Vet Med A Physiol Pathol Clin Med. 2001;48(6):373-83.

Bruchim Y, Steinman A, Markovitz M, Baneth G, Elad D, Shpigel NY. Toxicological, bacteriological and serological diagnosis of botulism in a dog. Vet Rec. 2006;158(22):768-9.

Caya JG, Agni R, Miller JE. *Clostridium botulinum* and the clinical laboratorian: a detailed review of botulism, including biological warfare ramifications of botulinum toxin. Arch Pathol Lab Med. 2004;128(6):653-62.

Centers for Disease Control and Prevention (CDC). Botulism [online]. CDC; 2009 Jul. Available at: http://www.cdc.gov/nczved/divisions/dfbmd/diseases/botulism/technical.html. Accessed 24 May 2010.

Center for Food Safety and Applied Nutrition (CFSAN). Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. U.S. Food & Drug Administration, CFSAN; 2009 Sept.. *Clostridium botulinum*. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070879. Accessed 24 May 2010.

Chin J, editor. Control of communicable diseases. Washington, D.C.: American Public Health Association; 2000. Botulism; p. 70-75.

Coppock RW, Mostrom MS. Intoxication due to contaminated garbage, food and water. In: Kirk RW, editor. Current veterinary therapy IX. Small animal practice. Philadelphia, PA: WB Saunders Co; 1986. p. 221-225

Elad D, Yas-Natan E, Aroch I, Shamir MH, Kleinbart S, Hadash D, Chaffer M, Greenberg K, Shlosberg A. Natural *Clostridium botulinum* type C toxicosis in a group of cats. J Clin Microbiol. 2004;42(11):5406-8.

Fagan RP, McLaughlin JB, Middaugh JP. Persistence of botulinum toxin in patients' serum: Alaska, 1959-2007. J Infect Dis. 2009;199(7):1029-31.

Graham R, Eriksen S. Experimental botulism in dogs. J Infect Dis. 1922;31(5):402-406.

Herenda D, Chambers PG, Ettriqui A, Seneviratna P, da Silva TJP. Manual on meat inspection for developing countries [online]. FAO animal production and health paper 119. Publishing and Multimedia Service, Information Division, FAO; 1994 (reprinted 2000). Botulism. Available at: http://www.fao.org/docrep/003/t0756e/T0756E03.htm#ch3.3.2.* Accessed 12 Dec 2002.

Johnson AL, McAdams SC, Whitlock RH.Type A botulism in horses in the United States: a review of the past ten years (1998-2008). J Vet Diagn Invest. 2010;22(2):165-73.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2006. Clostridial diseases. Botulism. Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/50707.htm. Accessed 26 May 2010.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2006. Clostridial diseases. Foxes: diseases. Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/170502.htm. Accessed 26 May 2010.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2006. Mink: bacterial diseases. Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/170902.htm. Accessed 26 May 2010.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2006. Poultry: botulism.Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/205400.htm. Accessed 26 May 2010.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2006. Toxic disorders. Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/100608.htm. Accessed 26 May 2010.

Kortepeter M, Christopher G, Cieslak T, Culpepper R, Darling R, Pavlin J, Rowe J, McKee K, Eitzen E, editors. Medical management of biological casualties handbook [online]. 4th ed. United States Department of Defense; 2001. Botulinum. Available at: http://www.vnh.org/BIOCASU/17.html*. Accessed 10 Dec 2002.

Licciardello JJ, Ribich CA, Nickerson JT, Goldblith SA. Kinetics of the thermal inactivation of type E *Clostridium botulinum* toxin. Appl Microbiol. 1967;15(2):344-9.

Lindström M, Korkeala H. Laboratory diagnostics of botulism. Clin Microbiol Rev. 2006;19(2):298-314.

Lindström M, Myllykoski J, Sivelä S, Korkeala H. *Clostridium botulinum* in cattle and dairy products. Crit Rev Food Sci Nutr. 2010;50(4):281-304.

Lindström M, Nevas M, Kurki J, Sauna-aho R, Latvala-Kiesilä A, Pölönen I, Korkeala H.Type C botulism due to toxic feed affecting 52,000 farmed foxes and minks in Finland. J Clin Microbiol. 2004;42(10):4718-25.

Lúquez C, Dykes JK, Yu PA, Raphael BH, Maslanka SE. First report worldwide of an infant botulism case due to *Clostridium botulinum* type E. J Clin Microbiol. 2010;48(1):326-8.

Moeller RB Jr, Puschner B, Walker RL, Rocke TE, Smith SR, Cullor JS, Ardans AA. Short communication: Attempts to identify *Clostridium botulinum* toxin in milk from three experimentally intoxicated Holstein cows. J Dairy Sci. 2009;92(6):2529-33.

Morishita TY. Common diseases in backyard ducks and geese. Semin Avian Exot Pet Med. 2004;13(4):191-196.

Neimanis A, Gavier-Widén D, Leighton F, Bollinger T, Rocke T, Mörner T. An outbreak of type C botulism in herring gulls (*Larus argentatus*) in southeastern Sweden. J Wildl Dis. 2007;43(3):327-36.

Powere LV. Bacterial and parasitic diseases of ferrets. Vet Clin Exot Anim. 2009;12:531–561.

Promed Mail. Botulism, canine – USA (Florida) suspected. Feb 8, 2010. Archive Number 20100208.0430. Available at http://www.promedmail.org. Accessed 7 Jun 2010.

Public Health Agency of Canada. Material Safety Data Sheet – *Clostridium botulinum*. Office of Laboratory Security; 2001 Jan. Available at: http://www.phac-aspc.gc.ca/msds-ftss/msds35e-eng.php. Accessed 24 May 2010.

Rings DM. Clostridial disease associated with neurologic signs: tetanus, botulism, and enterotoxemia. Vet Clin North Am Food Anim Pract. 2004;20(2):379-91.

Roberts TA, Collings DF. An outbreak of type-C botulism in broiler chickens. Avian Dis. 1973;17(3):650-8.

Sobel J. Botulism. Clin Infect Dis. 2005;41(8):1167-73.

Solomon HM, Lilly T. Bacteriological analytical manual [online] 8th ed. U.S. Food and Drug Administration (FDA);2001 Jan. *Clostridium botulinum*. Available at: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070879. Accessed 24 May 2010.

Smith LA. Botulism and vaccines for its prevention. Vaccine. 2009;27 Suppl 4:D33-9.

Steinman A, Millet N, Frenkel C, King R, Shpigel NY. Presence of antibotulinum neurotoxin antibodies in selected wild canids in Israel. J Wildl Dis. 2007;43(3):548-50.

Smith LDS. *Clostridium botulinum*: Characteristics and occurrence. Rev Infect Dis 1979;1(4):637-41.

Stewart JS. Anaerobic bacterial infections in reptiles. J Zoo Wildl Med. 1990;21(2):180-4.

Taillac PP, Kim J. Botulism. eMedicine; 2010 Mar: Available at: http://emedicine.medscape.com/article/829125-overview. Accessed 7 Jun 2010/

Takeda M, Tsukamoto K, Kohda T, Matsui M, Mukamoto M, Kozaki S. Characterization of the neurotoxin produced by isolates associated with avian botulism. Avian Dis. 2005;49(3):376-81.

Trampel DW, Smith SR, Rocke TE. Toxicoinfectious botulism in commercial caponized chickens.Avian Dis. 2005;49(2):301-3.

van der Burgt GM, Mitchell ES, Otter A, Whitaker KA, Hogg R. Seven outbreaks of suspected botulism in sheep in the UK. Vet Rec. 2007;161(1):28-30.

Wagner JE, Mann PC. Botulism in California sea lions (*Zalophus californianus*): A case report. J Zoo Anim Med. 1978;9(4):142-6.

Weber JT, Hatheway CL, St. Louis ME. Botulism. In: Hoeprich PD, Jordan MC, Ronald AR. Infectious diseases, 5th ed.. Philadelphia: JB Lippincott Company; 1994. p. 1185–1194.

Wells CL, Wilkins TD. *Clostridia*: sporeforming anaerobic bacilli. In: Baron S., editor. Medical microbiology. 4th ed. New York: Churchill Livingstone; 1996. Available at: http://www.gsbs.utmb.edu/microbook/ch018.htm.** http://www.ncbi.nlm.nih.gov/books/NBK7627/. Accessed 10 Dec 2002.

Wilkins PA, Palmer JE. Botulism in foals less than 6 months of age: 30 cases (1989-2002). J Vet Intern Med. 2003;17(5):702-7.

Wilkins PA, Palmer JE. Mechanical ventilation in foals with botulism: 9 cases (1989-2002). J Vet Intern Med. 2003;17(5):708-12.

World Health Organization (WHO). Botulism [online]. WHO; 2002. Available at: http://www.who.int/mediacentre/factsheets/fs270/en/. Accessed 7 Jun 2010.

Wylie CE, Proudman CJ. Equine grass sickness: epidemiology, diagnosis, and global distribution.Vet Clin North Am Equine Pract. 2009;25(2):381-99.

Yule AM, Barker IK, Austin JW, Moccia RD. Toxicity of *Clostridium botulinum* type E neurotoxin to Great Lakes fish: implications for avian botulism. J Wildl Dis. 2006;42(3):479-93.

* Link defunct as of 2010

** Link defunct as of 2012