BOTULISM

Aetiology Epidemiology Diagnosis Prevention and Control
Potential Impacts of Disease Agent Beyond Clinical Illness References

AETIOLOGY

Classification of the causative agent

Botulism is the most common name given to the clinical presentation that arises in animals exposed to *Clostridium botulinum* neurotoxins. Other names include “limberneck”, “Western duck sickness”, “duck disease”, and “alkali poisoning”. *C. botulinum* is a large, Gram-positive anaerobic rod that is found in the environment most commonly as a spore. The species is organised into four groups based on toxin type and proteolytic ability.

The genes that encode for botulinum toxins are chromosomal, located on plasmids, or obtained from bacteriophages. There are seven distinct toxins, A-G, and bacterial toxinotypes are named according to the toxin they produce (e.g., *C. botulinum* toxinotype A produces botulinum toxin A) after germinating. That is, the bacterial spores do not actively produce toxin.

Botulinum neurotoxins are exceedingly potent and induce flaccid paralysis by inhibiting acetylcholine release at the neuromuscular junction. Mammalian and avian species alike are susceptible to toxicity, which can occur via toxicoinfection (infection with or ingestion of toxin-producing bacteria) or intoxication (ingestion of the toxin itself).

Resistance to physical and chemical action

Temperature: Spores are very heat-resistant; germinated bacteria grow optimally between 25°-40°C; non-proteolytic toxinotypes can replicate and produce toxins at temperatures as low as 5°C; toxins are inactivated at 80°C for >10 minutes

pH: Growth of *C. botulinum* is inhibited at pH <4.5; toxin production is optimized at pH 5.7-6.2

Chemicals/Disinfectants: Chlorine dioxide and mixed oxidants are effective at inactivating spores; chlorine is not a reliable spore inactivator but is capable of inactivating toxin; ozone is effective but requires impractical pH and concentration parameters for common use.

Survival: Spores are very stable in the environment and can remain viable for years to decades

EPIDEMIOLOGY

Affected species

Many avian and mammalian species can be affected by botulism; this list is non-exhaustive:

- Over 100 species of wild birds have been documented with botulism worldwide - toxins C, E
    - Waterfowl, shorebirds, and migratory birds are particularly at risk
    - Vultures (families *Cathartidae*, *Accipitridae*) are resistant to type C
    - Toxin type E more commonly affects piscivorous birds
    - Outbreaks in raptors are associated with improper poultry carcass disposal
- Cattle (*Bos taurus*) - toxins C, D
    - Type C is more common in North America, whereas D is more common in South America and South Africa

- California sea lions (*Zalophus californianus*)
    - Outbreaks in captive animals associated with endemic waterfowl outbreaks
- Humans (*Homo sapiens*) - toxins A, B, E, and F
- Horses (*Equus ferus caballus*) - toxins A, B, C, D
- Mink (*Neovison* and *Mustela* spp.) - toxins A, C, D, E
- Poultry, namely chickens (*Gallus gallus domesticus*) and pheasants (*Phasianus colchicus*) - toxins A, C
- Sheep (*Aries ovis*) - toxins C, D

Routes of exposure

- Ingestion of decaying tissue containing toxin
- Ingestion of toxin-accumulating invertebrates, especially maggots, associated with decaying organic matter such as carcasses
- Proliferation of germinating *C. botulinum* in:
    - the intestinal tract, which permits subsequent absorption of neurotoxin (toxicoinfection, also called “gut toxigenesis”)
    - wounds (including gastrointestinal ulcers), where neurotoxin is deposited directly into the bloodstream (toxicoinfection)

Sources

- Germinated/vegetative (i.e., non-sporulated) *Clostridium botulinum*
    - Spores may be found within the tissues of wetland invertebrates and birds and, when ingested, can germinate. Spores may also be shed from the gastrointestinal tract in faeces.

Occurrence

C. botulinum toxinotypes A and B are most commonly found in the soil, whereas toxinotypes C, D, E, F, and G are more common in environments with high moisture, such as wetlands. Toxinotype G has only been identified in soil samples in Argentina; it has not been associated with outbreaks. Geographic “hotspots” of botulism are not uncommon and may persist for a number of years. These toxins have some degree of species associations due to different target site specificity on synaptic vesicle membrane proteins.

Environmental factors that encourage the development of waterfowl outbreak events are not entirely characterised due to their complex, multifactorial relationships, but some abiotic factors are believed to be of particular significance. Mortality events are more common in the summer when ambient temperatures are high and water temperatures are >20°C. A salinity of >2 parts per million is associated with increased risk of outbreak occurrence, as is a water pH of approximately 7.5-9.0. A negative redox potential in the water and decreased oxygen availability are also favorable conditions. Biotic factors such as the substrate on which the bacteria persist are also significant; *C. botulinum* is incapable of synthesizing all essential amino acids and therefore requires a high protein substrate to persist in a vegetative form. This is likely the reason for the association between botulism outbreaks and the presence of large volumes of decaying organic matter. *C. botulinum* is also associated with filamentous algae and aquatic plants in some ecosystems.

Anthropogenic factors likely impact the amount and quality of substrate for *C. botulinum*, and therefore production of toxin in the environment. For example, wetland flooding/draining, pesticide use, and other pollutants such as agricultural runoff can kill aquatic organisms and therefore provide additional appropriate substrate for bacterial growth. Additionally, deposition of raw sewage, decomposing vegetation, et cetera, into the environment provides further substrate and encourages oxygen depletion secondary to the effects of nutrient enhancement. At domestic and captive facilities, silage and haylage fermentation failure may result in the proliferation of *C. botulinum.*

Wild migratory birds are among the most notable species affected by large-scale botulism-induced mortality events. Waterfowl and shorebird outbreaks are believed to be caused by ingestion of small aquatic invertebrates and maggots that contain toxins C and/or E. Because invertebrates are not affected by botulinum toxin, they serve as toxin accumulators and contribute to what is called the “carcass-maggot” cycle of avian

botulism. In short, this cycle is characterised by the following: 1) toxin production in decaying animal carcasses which are fed upon by maggots, 2) maggots concentrating toxin, 3) waterfowl ingesting maggots, and 4) death of the animal, carcass accumulation, and further toxin production. Once this cycle begins, focal die-offs can rapidly amplify into massive, large-scale mortality events that may involve tens of thousands to millions of birds. This is because one carcass can support a large number of maggots, but ingestion of only a few maggots can cause death.

For more recent, detailed information on the occurrence of this disease worldwide, see the OIE World Animal Health Information System - Wild (WAHIS-Wild) Interface [http://www.oie.int/wahis_2/public/wahidwild.php/Index].

DIAGNOSIS

Clinical signs in susceptible species typically occur within hours to days. Time to onset and progression to death is believed to be dose-dependent, but can vary by species. Some animals may recover, but they are not protected against future toxin exposures.

Clinical diagnosis

Field indications of an avian botulism die-off typically include clusters of sick and/or dead birds at the water's edge, usually near vegetation. In areas where water levels are stable (e.g., impoundments, lakes, large rivers), congregations are seen near flooded vegetation and vegetated peninsulas/islands. Birds of various stages of illness are often found together, as are carcasses at various stages of decay and decomposition. Multiple species may be affected simultaneously. If botulism is suspected, crop and gizzard contents of dead birds should be inspected for the presence of maggots. The presence of maggots in the gastrointestinal tract may increase suspicion of botulism, but their absence should not decrease it; culpable ingesta may have been eliminated by the time clinical signs developed.

Intoxication in waterfowl causes an inability to sustain flight due to weakness in the early stages. Birds develop flaccid paralysis in their legs and will propel themselves over land or through water with their wings. The third eyelid or nictitating membrane may protrude due to paralysis. Eventually, flaccid paralysis of the neck develops and the head cannot be held above water; at this stage, birds typically drown before toxin-induced respiratory failure occurs.

In mammalian species, clinical signs of botulism are also characterised by progressive paresis but may also include dysphasia, drooling, dysuria, respiratory distress, and ultimately respiratory or cardiac paralysis. Generally, both avian and mammalian species are found in sternal recumbency with low head carriage or neck outstretched.

Lesions

- There are commonly no lesions appreciable in cases of botulism
- Waterfowl and shorebirds may have evidence of drowning (fluid in airways, etc.)

Differential diagnoses

- Algal bloom toxicity
- Hypokalaemia
- Central nervous system trauma or infection
- Mammals
    - Anthrax
    - Rabies
    - Idiopathic polyradiculoneuritis
    - Tick paralysis
    - Parturient paresis ("milk fever")
- Birds

    - Seasonal wing molt is a common cause of flightlessness and should be considered during the summer; attempted capture of the animal often successfully elucidates its general health status and fitness
    - Lead poisoning
    - Castor bean poisoning
    - Marek's disease
    - Ionophore toxicity
    - Pasteurellosis ("fowl cholera")
    - Duck viral enteritis ("duck plague")
    - High pathogenicity avian influenza (HPAI)

Laboratory diagnosis

Samples

For identification of toxin

- Whole blood or serum
- Gastrointestinal contents
- Only samples obtained from moribund or freshly dead animals should be utilised for testing; *C. botulinum* can proliferate rapidly on decaying tissue and produce toxin that was not present ante-mortem.
    - Samples should be refrigerated or frozen quickly after collection and shipped to diagnostic facilities on ice for the same reason

Serological tests

- Serology is not used to diagnose botulism.

Procedures

Identification of toxin

- Inoculation of serum from suspected animals into mice
    - Two groups of mice are used, one of which is treated with type-specific antitoxin. If the serum contains botulinum toxin, the non-treated mice alone will develop clinical signs attributable to botulinum toxicity and die.
- Antigen-capture enzyme-linked immunosorbent assay (ELISA)
    - Detects both active (proteolytic) and inactive (non-proteolytic) toxins
- Polymerase chain reaction (PCR) assays are available for *C. botulinum* toxinotype differentiation, but they cannot detect the presence of botulinum toxins in blood or gastrointestinal content.

Serological tests

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PREVENTION AND CONTROL

Sanitary prophylaxis

- *C. botulinum* spores are both hardy and common in the wetland environment, and the conditions that encourage germination into the vegetative state are complex and multivariate. Therefore, control efforts should be focused towards risk reduction and removal of contributing factors that encourage toxin production.

    - Prompt collection and disposal of carcasses (incineration, burial) is strongly recommended to break the carcass-maggot cycle of transmission, especially where waterfowl and shorebirds are common.
    - Reduce the amount of organic input into wetlands and other managed bodies of water. This includes reflooding or rapidly draining bodies of water, especially in the summer.
    - Management of water pH, salinity, and oxygenation may be necessary in at-risk areas.
    - Routing wastewater effluent into wetlands is discouraged.
- Fly control near captive facilities may decrease the risk of toxic maggot deposition.
- Captive facilities should disinfect the premises with agents known to be effective against spore-forming bacteria, especially if the premises have a history of botulism outbreaks.

Medical prophylaxis

- There are toxin-specific vaccines utilised for domestic horses, farmed mink, and commercial pheasant flocks, but their use is not cost-effective for wild ducks or commercial chicken facilities.
- Antitoxins are available and effective for most species.
    - Exceptions include coots, shorebirds, gulls, and grebes, which are likely to succumb to botulism despite antitoxin treatment and supportive care.
- In captive facilities, any nutritional deficiencies should be addressed as rapidly as possible to prevent animals from compulsory consumption of decaying materials in the environment.
    - Phosphorus-deficient cattle and sheep tend to chew on bones, which increases their risk of ingesting spores.

POTENTIAL IMPACTS OF DISEASE AGENT BEYOND CLINICAL ILLNESS

Risks to public health

- Humans are susceptible to botulinum toxins but they do not commonly ingest them from wildlife or environmental sources. There have been cases where type E toxin ingestion was linked to improperly prepared fish. The toxins are generally inactivated by properly cooking food.
- Humans are primarily affected by types A, B, and E; the effects of type C toxin are not well described in humans but are appreciated in multiple non-human primate species.

Risks to agriculture

- Poultry and livestock species, including farmed mink, are susceptible to botulinum neurotoxins. The source is not always clear or discernable, and if a large proportion of the herd or flock is affected, farmers can face significant animal and financial losses.

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Science Department (scientific.dept@oie.int). Last updated 2020. Written by Samantha Gieger and Erin Furmaga with assistance from the USGS National Wildlife Health Center.