Outbreak of Botulism (Clostridium botulinum type C) in Wild Waterfowl: Seoul, Korea

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ABSTRACT: Over a 6-day period beginning on October 15, 2008, 93 dead or sick wild waterfowl, including Mallards, Spotbills, and teal species, were found along the shore of a branch stream of the Hangang River, which flows through Seoul, Korea, and were submitted to the National Veterinary Research and Quarantine Service (NVRQS) for diagnosis. Clinically, the affected birds showed flaccid paralysis of the legs and wings and paralysis of the neck. Grossly, no bird showed any lesions, but all had almost empty stomachs. Histopathologic findings included mild lymphocytic hepatitis and mild lymphocytic interstitial nephritis. Clostridium botulinum type C toxin was identified in sera collected from the birds using a mouse bioassay for botulinum toxins; however, no bacteria were isolated from any of the affected birds. In addition, a low-pathogenic avian influenza virus was isolated from two Spotbills, and pesticides such as diazinon and phorate, were detected in seven Mallards. The cause of this outbreak is not clear, but an increase in organic materials from sewage due to drought, increased temperatures, and an increased number of aquatic carcasses resulting from pesticide contamination may have increased the replication of C. botulinum, contributing to the release of botulinum toxins into the waterfowl food chain.

Key words: Botulism, flaccid paralysis, Mallard, pesticide, Spotbill, Teal.

Botulism is a fatal paralytic disease of birds and some mammals caused by the ingestion of toxins produced by Clostridium botulinum. Although seven types of toxin have been demonstrated, type C botulism is the one that typically causes disease and mortality in wild birds (Forrester et al., 1980; Wobeser et al., 1983; Shayegani et al., 2007), shorebirds (Charadriiformes; Forrester et al., 1980), gulls (Larus argentatus; Neimanis et al., 2007), chickens (Gallus gallus; Dohms et al., 1982), turkeys (Meleagris gallopavo; Smart et al., 1983), cattle (Bos primigenius; Wobeser et al., 1997), and horses (Equus ferus; Bernard et al., 1987). In this study, we describe botulism in wild ducks caused by C. botulinum toxin type C near Seoul, Korea.

On October 15, 2008, three dead Spotbills (Anas poecilorhyncha) were found in the Anyangcheon (37°33′13″N, 126°52′40″E), a small branch stream of the Hangang River (37°33′13″N, 126°52′40″E), which flows through Seoul, Korea. The Anyangcheon rises from Uiwang (37°20′23.84″N, 126°59′16.67″E) south of Seoul and then flows through Anyang (37°23′21.98″N, 126°56′29.21″E) into the Hangang (Fig. 1). The stream is used as a stopover by migratory birds, but no previous mass deaths were reported prior to this outbreak. Over the 6 days after the discovery of dead birds, 93 migratory wild birds were found paralyzed (Spotbills, n = 2) or dead (n = 91) on the shore or in the water of the same stream. Approximately 51% of the affected waterfowl were Mallards (Anas platyrhynchos, n = 48). Other affected species included Spotbills (n = 21), Green-winged Teals (Anas crecca, n = 18), Baikal Teals (Anas formosa, n = 4), Shovelers (Anas clypeata, n = 4), and Pintails (Anas acuta acuta, n = 1). All wild ducks were...
The two Spotbills affected showed depression, flaccid paralysis of the legs and wings, and paralysis of the neck. Maggots were found rarely in some carcasses. Upon necropsy, blood or blood clots, intestinal contents, and maggots were collected for use in a mouse bioassay for botulinum toxins; in addition, portions of the gastrointestinal contents were analyzed for pesticides by gas chromatography (HP6890N, GC/NPD, FPD; Hewlett Packard, Palo Alto, California, USA) and gas chromatography/mass spectrometry (HP6890N and HP5973N; Hewlett Packard). The limit of detection was about 0.2 ppm, although it differed among the pesticides. Various organs and tissues (e.g., brain, lungs, heart, liver, spleen, kidneys, trachea, proventriculus, gizzard, and intestine from birds or brain, spinal cord, lungs, heart, liver, spleen, kidneys, and intestine from mice) were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for histopathology. Intestine and liver samples were cultured on blood agar and MacConkey agar for bacterial isolation. For viral examination, samples of trachea, kidney, and cecal tonsil were inoculated in chicken embryos. Grossly, stomachs were typically almost empty and contained only a small amount of sand and a few seeds. No gross lesions were seen in any of the birds examined.

For the mouse inoculations, pooled sera and filtered supernatants of intestinal contents and maggots were prepared by submitted date and species. For each assay, two mice were injected intraperitoneally with 0.2 ml of the sample (sera and filtered supernatants of the intestinal contents from sick animals, and sera and filtered supernatants of the intestinal contents and/or maggots from dead animals) and observed for up to 4 days for characteristic signs of botulism, including a “wasp-waist” appearance. Then, the botulism type was confirmed by mouse neutralization tests using antitoxin types A, B, C, and E (National Institute for Biological Standards and Control, UK). Type C botulinum toxin was identified in serum samples collected from Spotbills (four of 21), Mallards (18 of 48), and Baikal Teals (four of four). However, no botulinum toxin was detected in intestinal contents or maggot samples. In addition, no histopathologic lesions were observed in any of the mice showing characteristic signs of botulism or the mice injected with toxin-neutralized serum.

No bacteria were cultured from the intestinal contents or livers. A low pathogenic avian influenza virus (H3) was isolated from two Spotbills. Histopathologically, a few lymphocytes and macrophages had infiltrated the livers and kidneys in most birds. Although parasites were not classified, they were observed in blood vessels and the bile duct of the liver in one Spotbill and one Mallard. In organophosphate residue tests of gastrointestinal contents, diazinon and phorate were detected in seven Mallards with type C botulinum toxin. The concentration of diazinon in the birds ranged from 0.34 to 0.57 mg/kg, while that of phorate ranged from 0.51 to 0.79 mg/kg.
During the outbreak, the weather in October was warmer than normal (mean temperature for this time of year for the past 36 years); the monthly average maximum temperature was 22.4 °C, 1.7 °C higher than normal, and the minimum temperature was 10.7 °C, 1.9 °C higher than normal. The highest daily temperature in October was 28.3 °C in Seoul (37°34′18″N, 126°57′59″E; Fig. 2). Further, the monthly precipitation amounted to 32.7 mm in Korea, only 57% of that recorded in previous years (Table 1). In particular, there was no rainfall in the beginning or middle of October, suggesting that the proportion of sediments may have increased due to an influx of sewage and decreased precipitation. The optimal temperature for the multiplication of *C. botulinum* is between 25 and 40 °C (Rocke and Bollinger, 2007). However, Botulinum type C toxin is produced at temperatures as low as 15.6 and 12.8 °C (Segner, 1971). Botulism occurs frequently in the summer and fall because ambient conditions are near the optimal temperature for bacterial growth (Dohms et al., 1982; Wobeser et al., 1983; Rocke and Bollinger, 2007).

In Korea, three outbreaks of botulism in wild waterfowl, including the present outbreak, have been reported: the first botulism event occurred in October of 2007 in the Tancheon (37°33′12.17″N, 126°52′40.69″E; Fig. 1), a small branch stream of the Hangang. Species affected by the Tancheon stream event were similar to those affected by the Anyangcheon stream event and included 20 species of migratory and resident wild birds, such as Spotbills, Teal species, Mallards, Pintails, and Mergansers (Kang et al., 2008). The second botulism event occurred in the Namdonggongdan reservoir (37°23′28.40″N, 126°40′35.76″E) in October and November of 2008. About 1,200 wild birds were sickened and died, including waterfowl and shorebirds (Inchon Metropolitan Health and Environmental Research Institute, unpubl. data). The causes of the botulism outbreak in the Namdonggongdan reservoir may have been similar to those in wetlands (i.e., shallow water, a decrease in the amount of dissolved oxygen, an increase in the sediment temperature, and decaying organic matter; Rocke and Bollinger, 2007).

*Clostridium botulinum* is an anaerobic organism found in soils, the intestines of animals, and aquatic environments throughout the world, and it multiplies in decaying organic materials, where toxins are released. Invertebrates play an important role in mediating the transfer of toxins from organic materials to wild ducks (Rocke and Bollinger, 2007). The carcass-and-maggot cycle is a well-known scenario through which botulism outbreaks occur in waterfowl (Wobeser, 1997; Rocke and Bollinger, 2007). Botulinum toxin has also been identified in extracts of maggots collected from carcasses that died of botulism (Shayegani et al., 1984). However, in this outbreak, no botulinum toxin was detected in maggots.

Although the rate of toxin detection was low in this outbreak, type C botulinum toxin was confirmed by mouse inoculation from 26 affected birds. Some studies
reported that the toxin was not detected via the mouse assay in some birds affected by botulism (Thomas, 1991; Neimanis et al., 2007). However, a mouse assay was used for diagnosis because of the irreversible reaction of botulinum toxin at neuromuscular junctions (Swerczek, 1980; Rossetto and Montecucco, 2003).

In this study, low-pathogenic avian influenza viruses and organophosphates such as diazinon and phorate were detected in two Spotbills and seven Mallards, respectively. Although avian influenza was isolated from two birds, lack of lesions and the isolated nature of this incident do not implicate this virus in this epidemic.

The acute oral LD50 of diazinon is 2.7 mg/kg in Mallard ducklings, while that of phorate is 0.62 mg/kg in Mallards (Tomlin, 2006). Diazinon was present at a lower concentration than would cause acute toxicity, whereas the detected concentration of phorate was above the threshold for acute oral toxicity in Mallard ducks, suggesting that the death of Mallards was caused by phorate. Although not all of poisoned birds show clinical signs and lesions associated with organophosphate toxicity, tracheobronchial secretion, lung edema, and cyanosis are observed in affected animals (Nettles, 1976), and their crops and gizzards are filled with feed (Quick, 1982; Reece and Handson, 1982). However, there were no gross and histopathologic findings associated with organophosphate toxicity in this study, suggesting that botulinum toxin may have been the main cause of death.

We identified botulism based on the characteristic clinical signs of flaccid paralysis and the detection of *C. botulinum* toxin in sera from wild waterfowl. Although the source of the toxin was not determined, an influx of pesticides may kill aquatic animals including vertebrate and invertebrate, and the decaying carcasses would provide increased substrate for bacterial growth, multiplication, and toxin production. Furthermore, the concentration of raw sewage may have been increased due to drought. As a result, the water became shallow, the current was slow, and sediment deposition was increased, thereby decreasing dissolved oxygen in the water. The botulinum toxin thus produced would then have been released into the food chain, resulting in an outbreak of botulism.

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**LITERATURE CITED**


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### Table 1. Precipitation, maximum (Max.) and minimum (Min.) temperatures for Seoul, Korea for the fall of 2007 and 2008.

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