An Outbreak of Type C Botulism in Waterbirds: Incheon, Korea

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ABSTRACT: Five outbreaks of botulism in waterbirds were encountered over a 5-yr period from 2004 to 2008 in Korea. In October 2008, an outbreak of avian type C botulism affected approximately 2,000 wild waterbirds in the Namdong flood control basin, Incheon, South Korea. Ecologic conditions, clinical signs exhibited by moribund birds, and lack of gross pathology and microbial evidence of infectious disease, suggested botulinum intoxication. Type C botulinum toxin was demonstrated in duck sera, liquid culture of intestinal tissue, and an extract of maggots taken from the carcasses. Additionally, 34 of 40 (85.0% ) sediment samples from the same area were positive for botulinum toxin by mouse bioassay using multivalent (types A–F) antiserum, indicating that toxigenic Clostridium botulinum was present in the environment. This is the most severe case of avian botulism documented in Korea.

Key words: Korea, outbreak, type C botulism, waterbirds.

Avian botulism is the most common cause of death for waterbirds worldwide. The disease is most frequently caused by the type C toxin, one of seven antigenically different neurotoxins (types A–G) produced by Clostridium botulinum (Mitchell and Rosendal, 1987). Since large outbreaks of type C botulism were first documented in the western USA and Canada in the early 1900s (Hobmaier, 1932), type C botulism has been diagnosed in wild waterbirds in at least 28 countries on every continent except Antarctica (Pullar, 1934; Jensen and Price, 1957; Rocke, 2006). In Asia, botulism outbreaks in wild birds rarely have been reported except in a few countries including Japan (Ono et al., 1982; Rocke, 2006). We report an outbreak of botulism involving approximately 2,000 wild birds at the Namdong basin and Weoam sewage treatment waterway in Incheon, South Korea, in October 2008. We also present diagnostic findings and a summary of previous outbreaks in Korea.

The Namdong flood control basin is a 613,800-m² artificial impoundment adjacent to the Yellow Sea in Incheon City (37°29′N, 126°38′E; Fig. 1). A stream called Seunggi-cheon flows from the north into this basin. A sewage disposal plant is located in the proximity of the Namdong basin and treated water is drained to the Yellow Sea through the muddy Weoam drainage area (Fig. 1). Some of the untreated sewage discharges directly into Seunggi-cheon. Despite the presence of sewage, mud flats near the Namdong basin are used by over 100 species of birds. The average temperatures in Incheon City were 21.9 C and 16.6 C in September and October 2008, respectively (http://www.kma.go.kr/sfc/sfc_03_02.jsp). The average annual precipitation (1995–2005) in Incheon City was 1,271 mm (Table 1; Ham and Kim, 2007).

In the Weoam waterway, a few dead birds were observed by birdwatchers on 28 September 2008. Mortalities increased on 18 October around the Namdong basin, and approximately 2,000 birds were dead by the end of the year. We collected 230 dead and 11 live, sick birds (10 ducks and one shorebird) from Namdong basin during 18–19 October. Of 241 individuals, 96.3% were filter-feeding and dabbling.
waterfowl such as the Shoveler (*Anas clypeata*, 44.8%), Green-winged Teal (*Anas crecca*, 32.4%), Spot-billed Duck (*Anas poecilorhyncha*, 11.6%), Pintail (*Anas acuta*, 2.9%), Mallard (*Anas platyrhynchos*, 2.5%), and Eurasian Wigeon (*Anas penelope*, 2.1%). The remainder (3.7%) were diving ducks, shorebirds, and gulls, including the Tufted Duck (*Aythya fuligula*, 2.5%), Pochard (*Aythya ferina*, 0.4%), Dunlin (*Calidris alpina*, 0.4%) and Black-tailed Gull (*Larus crassirostris*, 0.4%). Most affected birds were found at the water’s edge. Live birds showed flaccid neck paralysis and paralysis of the inner eyelid and some showed inability to sustain flight. Various degrees of maggot infestation were observed in the dead birds. Blood from three moribund birds was taken from the heart or the wing vein and maggots from three carcasses were collected and pooled into a sterile 50-ml conical tube. Additionally, 40 sediment samples (100 g each) were taken from the soil at a depth of 10–30 cm and a spacing of ~10 m around the Namdong basin, stored under anaerobic conditions at room temperature, and rapidly sent to National Institute of Health, Seoul, Korea.

Eleven live birds and 44 carcasses of various wild birds were transported to the Laboratory of Avian Disease, College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea, for laboratory investigation. At necropsy, bodies of the affected birds were somewhat dehydrated; there were no specific gross lesions and most birds had empty alimen-
tary tracts. Microscopic observation revealed no specific lesions indicative of cause of death in any of the tissues examined, including brain, liver, heart, kidney, spleen, gizzard, pancreas, and duodenum. We saw no evidence of virus or bacterial infection such as avian influenza or Pasteurella multocida from trachea, cecum, tonsil, or liver samples in any bird (Munch et al., 2001).

Serum, gizzard, and small intestine contents from three ducks, maggots from the carcasses, and sediment samples were pretreated for detection of botulinum toxins at the National Institute of Health, Seoul, Korea, as described by Austin and Blanchfield (1997) and the presence of botulinum toxins was determined using the mouse bioassay (Hatheway, 1988). We detected type C neurotoxin in sera of the three live birds, maggots from the bird carcasses, and an intestinal culture supernatant (Table 2). The contents from three gizzard samples and three intestinal extracts from one duck were negative. Thirty-four of 40 sediment samples (85.0%) were positive for botulinum neurotoxins A–F.

Although botulism is the most significant disease of waterbirds on a worldwide basis, avian botulism was not reported in Korea until recently (Table 3). In October 1999, the death of dozens of herons on Geoje Island was suspected but not confirmed to be due to botulism. More recently, five outbreaks of botulism in waterbirds were diagnosed over a 5-yr period from 2004 to 2008 in Korea. The first documented outbreak of avian botulism occurred in September 2004 around Yeong-am Lake in Haenam-gun, southwestern Korea (Fig. 1A; Shin et al., 2007). Yeong-am Lake is an artificial lake formed by the construction of a seawall for land reclamation and is one of the major habitats for migratory birds in Korea. During the outbreak, over 1,000 wild birds were affected and diagnosed with avian botulism. Most affected birds were herons (86.0%) including the Great Egret.
(Egretta alba, 54.4%), Gray Heron (Ardea cinerea, 21.2%), and Intermediate Egret (Egretta intermedia, 5.2%). Others (≈14%) belonged to dabbling ducks (12.9%) and shorebirds (1.1%). Clostridium botulinum toxin was detected in sera from herons but was not typed. Type C botulinum toxin was first demonstrated in October 2007 during a mortality event of ducks near Tan-cheon in Seongnam City (Fig. 1A). Type C toxins were detected from sera of the Common Teal and Spot-billed Duck (Kang et al., unpubl.).

In this study, we described the most severe case of avian botulism in Korea to date. Most of the dead birds were filter-feeding and dabbling waterfowl, suggesting they were among the species at greatest risk. Die-offs caused by type C botulism in these species occur almost annually throughout the world (Rocke and Friend, 1999). The neuroparalytic clinical signs, lack of gross or microscopic pathology indicating cause of death, and laboratory findings were consistent with botulism type C as a cause of this mortality event (Galvin et al., 1985; Neimanis et al., 2007). Presence of toxigenic C. botulinum in the environment was also documented in 85.0% of sediment samples, confirming environmental conditions conducive to botulinum toxin production in this wet-

### Table 2. Summary of samples used in mouse bioassay for detection of botulinum toxins.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antitoxin type used</th>
<th>No. positive samples/No. samples examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sera</td>
<td>C</td>
<td>3/3</td>
</tr>
<tr>
<td>Gizzard contents</td>
<td>C</td>
<td>0/3</td>
</tr>
<tr>
<td>Extract of maggots</td>
<td>C</td>
<td>1/1</td>
</tr>
<tr>
<td>Maggot culture supernatants&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C</td>
<td>5/5</td>
</tr>
<tr>
<td>Intestinal extracts&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C</td>
<td>0/3</td>
</tr>
<tr>
<td>Intestinal tissue culture supernatants&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C</td>
<td>1/3</td>
</tr>
<tr>
<td>Sediments&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ABCDEF</td>
<td>34/40</td>
</tr>
</tbody>
</table>

<sup>a</sup> A pellet of homogenized maggots was divided into five tubes; each was cultured in deaerated cooked meat medium (CMM) at 30°C for 3 days under anaerobic conditions. Each supernatant was applied to the mouse bioassay for detection of botulinum toxin.

<sup>b</sup> Supernatants of homogenized small intestines were applied to mouse bioassay after sterilization using 0.2-μm filters. Pellets were cultured in deaerated CMM at 30°C for 3 days under anaerobic conditions and supernatants were prepared by centrifugation followed by filtration.

<sup>c</sup> Sediment suspended in distilled water was mixed 1:1 (v:v) with twofold concentrated CMM (25 g/100 ml) after heat treatment at 80°C for 20 min and incubated in an anaerobic chamber (Sheldon Manufacturing, Cornelius, Oregon, USA) at 30°C for 5 days. Culture supernatant was centrifuged at 14,000 × g for 20 min at 4°C and passed through a 0.2-μm filter.

### Table 3. Outbreaks of avian botulism in wild birds in Korea.

<table>
<thead>
<tr>
<th>Month and year</th>
<th>Location</th>
<th>No. bird deaths</th>
<th>Species affected</th>
<th>Pathogen identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1999</td>
<td>Geoje Island</td>
<td>Dozens</td>
<td>Herons</td>
<td>Botulism suspected&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>September 2004</td>
<td>Yeong-am Lake, Haenam-gun City</td>
<td>~1,000</td>
<td>Herons, dabbling ducks</td>
<td>Botulism&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>August 2006</td>
<td>North Han River, Chuncheon City</td>
<td>Dozens</td>
<td>Ducks</td>
<td>Botulism&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>October 2007</td>
<td>Tan-cheon, Seongnam City</td>
<td>Dozens</td>
<td>Ducks</td>
<td>Botulism type C</td>
</tr>
<tr>
<td>October 2008</td>
<td>Namdong retarding basin, Incheon City</td>
<td>Thousands</td>
<td>Ducks, gulls, shorebirds</td>
<td>Botulism type C</td>
</tr>
<tr>
<td>October 2008</td>
<td>Anyang-cheon, Seoul City</td>
<td>~100</td>
<td>Ducks</td>
<td>Botulism type C</td>
</tr>
</tbody>
</table>

<sup>a</sup> Type not confirmed.
land. Zechmeister et al. (2005) demonstrated a correlation between the prevalence of botulinum neurotoxin C1 and its corresponding gene in environmental samples and the frequency or likelihood of botulinum epizootics in the environment. Spores of *C. botulinum* in sediment are frequently ingested by birds and remain latent in their intestines or livers (Reed and Rocke, 1992). When these birds die, the carcasses provide a good environment for growth and *C. botulinum* toxin production. In fact, we identified the existence of *C. botulinum* type C in the culture supernatant of an intestinal tissue. Taken together, these findings suggest that this outbreak corresponds to the epizootiology of the well known carcass-maggot cycle of avian botulism. The high prevalence of botulinum spores and maggots as a means of toxin transfer to birds is expected to be a critical factor precipitating the outbreak (Duncan and Jensen, 1976; Reed and Rocke, 1992; Wobeser, 1997).

Epidemiologically, the area of the Namdong outbreak exhibits environmental conditions suitable for growth of *C. botulinum*. Because it lacks the ability to synthesize certain essential amino acids, *C. botulinum* requires a high concentration of protein substrate as an energy source for growth and multiplication (Rocke and Friend, 1999). Sewage from Seunggi-cheon has flowed in this area for several years (Fig. 1B) and resulted in the destruction of aquatic life and provision of decaying organic matter for toxin production (Rocke and Friend, 1999). The quality of the aquatic environments in this region appears to be related to the amount of rainfall (Table 1). Rainfall decreases after a maximum in August; biochemical oxygen demand and chemical oxygen demand, indicators of water pollution level, sharply increase through September and October (Ham and Kim, 2007). The sudden deterioration of aquatic conditions might have enhanced spore germination, bacterial growth, and toxin production by *C. botulinum*. These data explain why the outbreak occurred in autumn. Air temperature in this period also seems to play a critical role in *C. botulinum* multiplication and toxin production. In Incheon City, the high temperature (ranging from 17.8 to 30.1 C) was maintained until 24 September, satisfying the temperature requirement of 20–23 C required for botulinum toxigenesis (Segner et al., 1971).

Outbreaks of type C botulism in waterfowl are a natural and classical phenomenon in ecosystems worldwide. However, we hypothesize that the recent frequent occurrence of avian outbreaks in Korea is associated with ecosystem stress caused by sudden changes in environmental conditions, including climate fluctuations, changes in the soil microbial composition, changes in bird population densities, and environmental pollution following human development. Investigation into these causes could contribute to decreasing the frequency of botulism outbreaks in wild birds in the future.

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**LITERATURE CITED**


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