Journal of
ZOO and WILDLIFE
MEDICINE

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Journal of Zoo and Wildlife Medicine 43(2): 388–390, 2012

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Abstract: In late summer 2010, an outbreak of type C botulism affected the birds kept in a dam at a southern Brazilian zoo. A total of 14 (10 black-necked swans, *Cygnus melancoryphus*; 3 Muscovy ducks, *Cairina moschata*; and 1 fulvous whistling-duck, *Dendrocygna bicolor*) out of 100 birds died after showing flaccid paralysis of the skeletal muscles characterized by general locomotion deficit, flight and swimming disorders, dropped neck, and severe dyspnea. Carcasses of dead birds (some infested by larvae of sarcophagus fly) scattered in the bird enclosure, and oxygen-free, organically rich mud and/or shallow standing waters present at the edges of the weir were identified as possible toxin sources. Postmortem examinations revealed no significant pathological changes. Epidemiologic and clinical findings indicated the diagnosis of type C botulism toxin, which was confirmed by mouse bioassay and seroneutralization.

Key words: Avian botulism, *Clostridium botulinum*, type C botulism, black-necked swan, *Cygnus melancoryphus*, captive wild birds.

BRIEF COMMUNICATION

Botulism is caused by the ingestion of toxins produced by *Clostridium botulinum*.[9,11] In an anaerobic medium, the spores of *C. botulinum* proliferate and produce the neurotoxins that are classified as eight different serotypes (A, B, C1, C2, D, E, F, and G).[9,11] Type C toxin is associated with most outbreaks in birds.[3,5,9,12] Oxygen-free and organically rich conditions in shallow standing water during hot summer months,[1,7,9] and fly larvae or other invertebrates feeding on avian carcasses, are among the major sources of toxin associated with the outbreaks of botulism in wild birds.[2,3,4,9] Affected birds develop signs of progressive weakness, paresis, and flaccid paralysis of skeletal muscles characterized by general locomotion deficit, flight and swimming difficulties, dropped neck, and protrusion of the nictitating membranes.[2,6] Diagnosis of the disease in birds is based on history, clinical signs, and absence of significant changes at necropsy[9] and is confirmed by the mouse bioassay with serum neutralization[8] or the enzyme-linked immunosorbent assay.[10] This communication describes an outbreak of type C botulism in captive wild birds kept at a southern Brazilian zoo.

Four black-necked swans (*Cygnus melancoryphus*) and one Muscovy duck (*Cairina moschata*) were found moribund and were euthanized and necropsied after unsuccessful treatment at the Sapucaia do Sul Zoological Park in Rio Grande do Sul, Brazil. Clinical information was retrieved from the veterinarians in charge. At necropsy, tissue samples were fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut in 5-μm sections, and stained with hematoxylin and eosin (H&E). Samples from intestinal contents, liver, and serum were collected, stored under refrigeration, and sent to the Laboratório Nacional Agropecuário (LANAGRO-MG), where mice were inoculated intraperitoneally with liver extract and serum from necropsied birds, seroneutralization was subsequently performed with antitoxins C and D.

In late summer 2010, a total of 14 out of approximately 100 birds, kept in the same dam (Fig. 1), died after showing comparable clinical signs that included flaccid paralysis of the skeletal muscles characterized by general locomotion deficit, flight and swimming difficulties, dropped neck, and severe dyspnea. Some birds showed deep depression, but when stimulated to move, presented the aforementioned signs, sometimes culminating in death. Three days before the die-off began, the birds were not fed their normal commercial diets. Avian carcasses, some infested by fly larvae (maggots), were found in the enclosure of the birds. At the edges of the dam were mud and shallow standing waters (Fig. 2),

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Figure 1. Enclosure in which birds were affected by type C botulism.

associated with persistent hot summer. At necropsy, based on muscle development and fat stores, the birds were in a good body condition; the livers were diffusely yellow. There were petechial hemorrhages in the epicardium, and a large collection of fibrin on the small intestinal mucosae. In one swan, hemorrhagic content was observed in the initial portion of the duodenum. All dead birds had dark red lungs with sanguinous fluid at cut surfaces. Microscopic findings included moderate fatty degeneration of the liver and marked pulmonary congestion. Mouse intraperitoneal inoculation of liver extract and serum from dead birds produced clinical disease indistinguishable from botulism. Antitoxin seroneutralization identified type C botulism toxin.

Presumptive diagnosis was established by the association of major epidemiological factors in outbreaks of botulism in wild birds[3,9] with the absence of significant pathological changes. It was confirmed through the detection of type C botulinum toxin after intraperitoneal inoculation of liver extract and serum from dead birds in mouse. The 3 days of nonfeeding may have induced an additional risk factor, because the birds were forced to search for food in contaminated water. There is a long list of avian species that have been affected by type C botulism;[9] however, no report of the disease in black-necked swan (*Cygnus melancoryphus*) could be found. Especially in a zoo, but also in other scenarios, daily inspection of the environment and removal of carcasses from the enclosure plays a fundamental role in the prevention of the disease.[4] The actual cause behind the higher number of cases in *C. melancoryphus* is unknown; however, this high prevalence could be associated with a higher species' susceptibility or some risk factor linked to the species' feeding behavior.

Acknowledgments: This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. The authors are grateful to Professor Cláudio Estêvão Farias Cruz for reviewing this manuscript.

Figure 2. Shallow standing waters in the edges of the weir were associated with persistent high summer temperatures and created additional risk factor for botulism in wild birds.

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Received for publication 14 May 2010