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Botulism type C outbreak in free-ranging wild birds in a public urban park in Ribeirão Preto, São Paulo state, Brazil

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ABSTRACT

Background: Botulism is a neurological disease caused by the ingestion of the toxin produced by *Clostridium botulinum*. This bacterium is globally distributed, infects mainly mammals and birds, and is present in both the intestinal microbiota and the environment. The most frequently observed clinical manifestation is flaccid paralysis of the skeletal muscles, which can rapidly progress to death due to cardiorespiratory failure. Diagnosis is performed through clinical, environmental, and laboratory findings, mainly by mouse bioassay seroneutralization, which serves as the gold standard.

Case Description: During the seasonal period, approximately 30–60 dead or sick animals were found in an urban park in Ribeirão Preto, São Paulo, Brazil, between late 2020 and 2021. However, only four wild ducks (*Cairina moschata*) and one great egret (*Ardea alba*) were evaluated. All five animals presented with neurological signs of limb paralysis, dyspnea, and neck flaccidity and were unable to fly. After clinical evaluation, the animals were euthanized, and necropsies were performed. Although we observed discrete hemorrhagic enteritis in the three evaluated ducks, no other relevant lesions were found. Samples were sent to the laboratory, where type C *botulinum* toxin was detected.

Conclusion: The presence of botulism in urban areas, especially in leisure areas, raises public health concerns. To the best of our knowledge, this is the first report of type C botulism in a public urban park in São Paulo, Brazil. Although this toxin does not pose a direct risk to human health, it is a strong indicator of environmental health, affecting wild free-ranging birds.

Keywords: Environmental health indicator, Flaccid paralysis, Type C toxin, Urban area, Water birds.

Introduction

Botulism is a neurological disease that causes paralysis and is frequently fatal in animals, especially in birds (Rocke and Friend, 1999), including wild aquatic birds (Crisley *et al.*, 1968). It occurs through the ingestion of the neurotoxin produced by the bacterium *Clostridium botulinum*, a motile, gram-positive, obligate anaerobic rod widely recognized for causing mortality, particularly in wild aquatic birds (Crisley *et al.*, 1968). Botulinum toxins are classified into nine distinct serotypes: A, B, C (1 and 2), D, E, F, G, and H (Barash and Arnon, 2014). Although birds can excrete all types

of toxins in their feces, serotypes C and D are the most commonly found in avian outbreaks, but types E and A have also been associated with disease in these animals (Linares *et al.*, 1994; Neimanis *et al.*, 2007; Chipault *et al.*, 2015; Badagliacca *et al.*, 2018; Lima *et al.*, 2020; Rogers *et al.*, 2021; Rosciano *et al.*, 2021; Quevedo *et al.*, 2022). In humans, serotypes A, B, E, and F are pathogenic (BRASIL, 2024). *Clostridium botulinum* is commonly found in the intestinal microbiota of animals and can contaminate soil, water, and food. In favorable conditions for development (anaerobiosis in decomposing organic matter of animal or plant, proper temperature, and humidity), the spore shifts to

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Fig. 1. Urban park in Ribeirão Preto, São Paulo State, Brazil. A) On the upper right corner is a small white square showing (in red) the location of São Paulo state, and in the São Paulo state map, the municipality of Ribeirão Preto highlighted in red; B) Perimeter of the municipality of Ribeirão Preto outlined in white. The white arrow indicates the urban park's location (21°12'54.14"S; 47°49'6.06"W). C) Aerial view of the public urban park, showing all three retention ponds (P1, P2, and P3). Source: A) Raphael Lorenzeto de Abreu, 2006. (https://commons.wikimedia.org/wiki/File:SaoPaulo_Municip_RibeiraoPreto.svg); B and C) Google Earth, 2025.

its germinative form, initiating the production of the botulinum toxin (Rocke and Friend, 1999).

The clinical signs are characterized by flaccid paralysis of the skeletal muscles. Affected birds show ascending symmetrical paralysis, affecting the legs, wings, neck, and eyelids. Additionally, these birds lose the ability to sustain flight at the beginning of intoxication, and since ducks with botulism cannot fly and their legs are paralyzed, they often propel themselves through the water using their wings (Rocke and Friend, 1999; Olinda *et al.*, 2015). However, no behavioral changes are observed, and death occurs due to cardiorespiratory arrest. The beginning of the clinical signs and their severity depend on the amount of toxins ingested and the susceptibility of the affected species (Olinda *et al.*, 2015).

A presumptive diagnosis of botulism is based on the clinical history with characteristic clinical signs and the absence of macroscopic *postmortem* lesions. Laboratory confirmation can be performed by detecting and serotyping botulinum toxin using the mouse bioassay, which is considered the gold standard test (Grenda *et al.*, 2014).

In Brazil, botulism was first reported in domestic chickens and ducks in 1971 (Brada *et al.*, 1971). Since

then, outbreaks have been recorded throughout the country, including in the states of Goiás (Martins *et al.*, 2022), Espírito Santo (Quevedo *et al.*, 2022), and Rio Grande do Sul (Raymundo *et al.*, 2012). However, botulism cases have not been reported in the state of São Paulo. Therefore, this study reports the first known outbreak of type C botulinum intoxication in four wild ducks (*Cairina moschata*) and a great egret (*Ardea alba*) in an urban park in the city of Ribeirão Preto, São Paulo, Brazil.

Case Description

On January 26, 2021, two wild ducks (*C. moschata*) were admitted to “Dr. Fábio de Sá Barreto” Municipal Zoo (BZM-RP) for veterinary care. The animal rescue occurred in an urban park located in the southern region of Ribeirão Preto, São Paulo (Fig. 1). The birds showed severe neurological signs, including loss of postural maintenance, sternal recumbency, dyspnea, apathy, flaccid neck, and complete absence of limb movement. Due to the severity of their condition, both animals were euthanized, and necropsy was performed. The only lesion observed was hemorrhagic enteritis with a moderate number of petechiae distributed throughout the intestine. Brain, stomach, intestinal,



Fig. 2. Photographs taken from an urban park after sick ducks were admitted to the zoo. A and B) Retention pond 1. A) unmaintained vegetation with buildings in the background; B) water without movement, with dirt on the surface and organic matter. C and D) Retention pond two. C) Decomposed ducks on the shore of pond two; D) Drain of the first lagoon with large amounts of organic matter and garbage on the surface. The presence of a floating plastic container and unmaintained vegetation is also noted. E and F) Third and final retention ponds. E) Presence of neotropic cormorants (*Nannopterum brasilianus*) on piles of stones in the lake. Four drainage pipes of the dam can be seen in the background; F) A peninsula with a large bamboo grove. There are rubbish and plastic bags scattered around the area.

and proventriculus contents samples were collected for further diagnostic investigation. When the birds were admitted to the BZM-RP, it was reported that in the past 2 months, approximately 30–60 birds had died in that exact location, most of them domestic or wild ducks. Due to the high mortality rate within a short period, the Official Veterinary Service (OVS) was notified.

On February 5, 2021, an inspection was performed at the site to evaluate the local fauna and environmental conditions. The area consists of three large retention ponds arranged in a cascading system that are interconnected and receive rainwater from surrounding neighborhoods (Fig. 2). Several animal species were observed, including domestic ducks (*Anas* sp.),



Fig. 3. A) Terrestrial and aquatic birds exhibiting normal behavior and occupying different areas of the lakes. A) roosters (*Gallus gallus domesticus*). B) Four black swans (*Cygnus atratus*) and one white duck (*Cairina moschata*). C. and D. Ducks (*Cairina moschata*).

roosters (*Gallus gallus domesticus*), black swans (*Cygnus atratus*), neotropic cormorants (*Nannopterum brasiliense*), wild ducks (*C. moschata*), black-bellied whistling ducks (*Dendrocygna autumnalis*), native turtles, and several fish species (Fig. 3).

During the inspection, two decomposed carcasses of wild ducks that had been deceased for at least 48 hours, with the presence of larvae (Fig. 4A and B), were found, as well as the decomposed carcass of an armored catfish (Loricariidae family). Two other birds were observed presenting neurological signs, including a duck (Fig. 4C) with pelvic limb paralysis, sternal recumbency, and flaccid necks, showing no escape response to the human approach. A great egret (*Ardea alba*) was also found in the water, with open wings and no pelvic limb movement (Fig. 4D).

The park's vegetation was poorly maintained, with a significant amount of waste, including plastic bags and cans, and decomposing organic matter throughout the area. The first pond also had an oil layer on its surface,

most likely of synthetic origin. The OVS collected samples to investigate neurological syndromes relevant to public health and commercial poultry farming, such as Avian Influenza (AI) and Newcastle Disease (ND). Additionally, samples were taken for differential diagnosis of West Nile virus (WNV) and Saint Louis encephalitis virus (SLEV) at the Molecular Virology Laboratory of the Ribeirão Preto Medical School, University of São Paulo (FMRP/USP). Although only the birds collected by the OVS were tested for AI and ND, all four bird samples were tested for WNV and SLEV. Only two duck samples (stomach, intestinal, and proventriculus contents) were sent for botulism diagnosis (mouse bioassay and seroneutralization in mice) at the Biological Institute of São Paulo (LBG/IB-SP). All tests were negative, and it was not possible to conclude the outcome.

On December 13, 2021, another wild duck from the same area was brought to BZM-RP. This animal had clinical signs similar to those observed in the cases



Fig. 4. Dead and sick birds found during the sanitary inspection. A) dead wild duck in the park's walking area, with many flies. B) Another dead wild duck was found in the drain area from the first retention pond, with soil and organic matter accumulated, as well as a large amount of garbage (plastic bottles and bags). C) Sick duck presenting flaccid paralysis in legs and wings, and difficulty supporting the neck. Nearby, you can see the carcass of a catfish. D) Great egret floating in the water, showing open wings and paralyzed legs.

from January and February 2021, including severe dyspnea, flaccid neck, uncoordinated wing movements, and an inability to maintain postural balance. The bird was euthanized on December 14, 2021, and during necropsy, gastrointestinal alterations similar to those seen in previous ducks, with additional hemorrhaging in the dorsal lung region, were observed.

In this case, in addition to brain and gastrointestinal samples collected during necropsy, blood serum and plasma (EDTA/heparin) were collected before euthanasia. The samples were stored at -20°C and sent to the LBG/IB-SP for botulism testing via mouse bioassay. Investigations for WNV and SLEV diseases were also performed and returned negative. The botulism test result, however, was positive in blood serum, indicating botulinum toxin type C. Although

only one animal had laboratory confirmation and was aware of the low analytical sensitivity of the bioassay, the other animals were also considered positive for botulism by epidemiological criteria.

Discussion

Ribeirão Preto is a regional hub in different segments, playing a significant role in various sectors, particularly agriculture, healthcare, and services. Given this, urban expansion is a constant reality, and areas that were previously natural and had water springs are increasingly beginning to receive large housing projects in their surroundings. An example is the park studied in this case report, which did not exist before the 2000s, and until then, its water sources were unprotected. Because of real estate pressure in the surrounding

area, an intervention was necessary, and with it, the implementation of the park and the creation of wet retention ponds to collect rainwater from neighboring areas.

The increasing global temperature and the persistence of these high temperatures throughout the year without evident seasonal variations have contributed to more considerable occurrences of disease outbreaks, many of which are significant to human and animal health. Regardless of dry periods, the park maintains a humid basin with saturated soil and water emergence zones, creating stagnant water areas. Due to the low renewal rate of water, organic residues, waste, and animal excrement accumulate, contaminating the environment and cultivating high microbial loads that favor the proliferation and maintenance of pathogens like *C. botulinum*.

Anaerobic conditions must be present for *C. botulinum* to thrive, allowing spore germination and neurotoxin release into the environment. Multiple substrates can support botulinum toxin production, but outbreaks require birds to have an opportunity to ingest the toxin. In some cases, birds can directly consume decomposing organic matter. In other cases, an intermediary mechanism may facilitate toxin transmission from the substrate to the susceptible animal, presumably through zooplankton or invertebrates (i.e., larvae) that consumed botulinum toxin (Rocke and Friend, 1999; Espelund and Klaveness, 2014).

As observed in this report, the environment where the animals were found met all the conditions for the complete germination of *C. botulinum* and the production of neurotoxins, given the presence of debris, mud, abundant organic matter, and dead animals. Thus, the environment plays a crucial role in the development of an outbreak and can, therefore, be used as an environmental health indicator, considering the specific conditions required for its occurrence (i.e., decomposition of plants, algae, and animals). These environmental characteristics create an anaerobic environment where *C. botulinum* may grow and enter food webs, leading to animal intoxication (Espelund and Klaveness, 2014).

We observed discrete hemorrhagic enteritis in some carcasses, probably due to concurrent involvement of other conditions of infectious, parasitic, or environmental origin. The period of non-feeding may also have induced an additional risk factor because the birds were forced to search for food in contaminated environments (Raymundo *et al.*, 2012). Although histopathological analysis could not be performed, the differential diagnosis of neurological disorders excluded other infectious causes.

The primary source of the botulinum toxin was not identified. However, it has been reported in wetlands that some *C. botulinum* strains can be associated with toxin-unaffected organisms (including algae, plants, and invertebrates) in which the bacteria appear to

germinate and stay in the vegetative form for longer periods (Hubalék and Halouzka, 1991; Espelund and Klaveness, 2014). Another critical source of botulinum toxin is water collections, such as lakes, ponds, and dams, where high levels of toxin can be found, especially when the water level begins to decrease, concentrating all types of waste and organic matter, which is an ideal substrate for *C. botulinum* (Rocke and Friend, 1999). Despite this, other reports have also detected the toxins in areas where water collections remain stable, whether in dry or rainy periods (Raymundo *et al.*, 2012; Włodarczyk *et al.*, 2014; Martins *et al.*, 2022). For this reason, proper cleaning, appropriate disposal of excess organic material and, most importantly, removal of animal carcasses from these areas are essential to prevent outbreaks like this from emerging and, therefore, to preserve both environmental health and local wildlife.

Botulism is a clear example of how the environment's health can significantly affect individuals' health. In other words, an unhealthy environment can lead to illness in all living beings. Therefore, preserving the environment means taking care of everyone. This is supported by the One Health concept.

Conclusion

To the best of our knowledge, this is the first outbreak report of type C botulism in a public urban park in the State of São Paulo affecting at least 60 birds, including wild ducks (*C. moschata*) and the great egret (*A. alba*). This study highlights the importance of environmental management in preventing botulism and raises public health awareness. Proper management of aquatic habitats, rapid removal of organic matter sources (mainly decomposition plants, algae, and animal carcasses), and implementation of appropriate hygiene practices are essential measures for reducing the risk of botulism intoxication. Furthermore, education and awareness among those involved in bird breeding and maintenance are crucial for maintaining healthy environments and preventing botulism outbreaks.

An outbreak of botulism in birds represents not only an animal health problem, but also a public health concern, especially in urban natural recreational areas, highlighting the need to maintain a clean and healthy environment to preserve the well-being of all living organisms.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Author contributions

MJLS: clinical care, field investigation, necropsy and sample collection, conceptualization, drafting, and review of the manuscript's final version; JCAD: assistance in necropsy, sample collection, and manuscript drafting; SML: manuscript drafting; BALF and KW: conceptualization and review of the manuscript's final version; SM: laboratory diagnosis, conceptualization, drafting, and review of the manuscript's final version.

Data availability

The data and supporting findings of this report are available in the manuscript.

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