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# First record of mass wild waterfowl mortality due to *Clostridium botulinum* in Brazilian semiarid

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**Abstract:** In 2008, 270 wild birds from aquatic environments were found dead or debilitated on the banks of smaller lakes that had been formed due to the decrease in the level of the holding lake of the Sobradinho Dam located on the São Francisco River in the Caatinga of Bahia, Brazil. The outbreak occurred months after the dam's partial drainage, with the formation of puddles that accumulated decomposing organic material. Amongst the 270 individuals examined and/or found dead, the majority (50%) of the birds found belonged to the Anatidae family. The debilitated birds presented neurological clinical signs including lack of motor coordination, weakness, grave flaccid paralysis in the legs, wings, neck and eyelids, diarrhea, and dyspnea. Tissue samples of the birds were collected, as were water samples and samples of the substrate of the lakes. Zoonotic arboviroses or heavy metals were not detected. Analyses of liver and digestive tract content samples through bioassay and serum neutralization in mice revealed the presence of type C botulinic toxin in the viscerae samples, and type D in sediment samples. According to our knowledge, this is the first record of an outbreak of botulism in wild birds in natural conditions in Brazil.

**Key words:** botulinum toxins, *Clostridium botulinum*, wild birds, Caatinga, aquatic environment.

## INTRODUCTION

Large scale mortality events in wild birds can be attributed to different causes, such as viral (Deliberto et al. 2009), bacterial infection (Wobeser 1997), as well as intoxication from heavy metals (Jones et al. 1983). Die-off events of considerable scale (i.e. when a large part or all of a local population dies) demand timely assessment and study due to the possibility of epizootic outbreak (Morner et al. 2002), which would pose a threat to human health due to the risk of spillover events (Zeppelini et al. 2016).

Botulism is a disease caused in animals and humans by ingestion of with one of the most potent neurotoxins found in nature, botulinum toxins, produced by the Gram-positive, anaerobic bacteria *Clostridium botulinum* (Austin 2001). On a world-wide basis, avian botulism is the most significant disease of waterbirds and has potential to cause significant population declines in some species (Rocke 2006). Eight types of botulinic toxins, currently classified from A to H, with each bacterial strain producing one or two toxins in different levels (Barash & Arnon 2014): the types A, B, E and F are associated with human cases, while C, D, E and both C/D and D/C neurotoxin mosaics are found in animal botulism (Nakamura et al. 2010, Chipault et al. 2015). The spores of *C. botulinum* can be found in terrestrial or aquatic (fresh and salt water) environments throughout the world (Austin 2001). In aquatic environments, there are several reports of outbreaks of botulism in fish farms (Cann & Taylor 1982, Huss & Eskilden 1974), with the largest outbreaks occurring during the summer, due to high temperatures (Yule et al. 2006). High temperature of water during avian botulism outbreaks in Spain (Vidal et al. 2013) and South Korea (Son et al. 2018) have also been reported. Type C botulism is considered the main cause of botulism in birds (Wobeser 1997), has been diagnosed in wild waterbirds in at least 28 countries and on every continente except in Antarctica (Rocke 2006) and mortality can occur in sporadic outbreaks in birds that feed in water environments, parting from the mobilization of pathogenic spores incubated in the sediments (Espelund & Klaveness 2014). The collection of deceased animals can reduce the risk of further mortality and contamination (Evelsizer et al. 2010). Type E is more geographically restricted compared to type C, causing high mortality of waterbirds in the Great Lakes region of the USA (Chipault et al. 2015).

The lack of specific legislation about the appropriate final destination of cadavers in animal husbandry in Brazil is considered one of the main causes of environmental contamination by *C. botulinum* (Dutra et al. 2005, Dutra & Döbereiner 1995), and additionally possibly the source of wildlife infection in mass mortality events. The absence of information about proper sanitation procedures from the official government and little knowledge on the part of producers about the sanitary and economic consequences of leaving decomposing cadavers in the field favor environmental contamination and intoxication events (Curci et al. 2007). It is important to stress, however, that natural aquatic environments often harbor the ideal conditions for outbreaks (Espelund & Klaveness 2014), as well as areas of extensive cattle herding that cross migratory bird routes.

In Brazil few studies reported outbreaks of botulism in wild birds and when happened, there described in the South of the country in zoo conditions (Schonhofen & Ferreira 1981, Cubas 1996, Raymundo et al. 2012) or in establishment with domestic or wild captivity birds (Oliveira Junior et al. 2016). However, in wild birds under natural conditions, to our knowledge, there is no report in Brazil. Botulism as a differential diagnosis to arboviruses is a relevant condition for sanitary surveillance, which may have wild birds as reservoirs. The current study investigates an event of mass mortality and morbidity of wild birds on an aquatic environment registered in the state of Bahia, Brazil, which has been attributed to a Botulism outbreak.

## MATERIALS AND METHODS

The study was performed in the area surrounding the holding lake of the Sobradinho Dam, located on the São Francisco River in the municipality of Remanso, Bahia, Brazil. The municipality of Remanso has an area of 4,694 km², and a population of 38,957 inhabitants (IBGE 2016). The region is part of the Caatinga biome, and its economy is based on commerce and farming (cutivation and livestock).

Throughout 2008, the lake retained only 19.0% of its holding capacity due to an extended drought. This led to the formation of small, shallow and isolated lakes, with a large amount of decomposing organic material and low free oxygen content, where the dead and debilitated birds were found. Five of these small lakes (EPI 1 to 5) were included in the study (Figure 1). The inquest about the mortality and morbidity of the birds was performed by the Ministry of Health, as part of the actions of the federal program on epidemiological vigilance, with the participation of specialized technicians, and had a primary goal of investigating possible risk for human populations, other wildlife, and the environment, as well as the possible involvement of emerging viruses such as Avian Influenza and West Nile Fever having infected the birds.

The general survey for the ave-fauna in the study area, including those found dead or debilitated, was performed during January and February of 2008, through observation with the naked eye or using *Swarovski* 8 X 30 binoculars. Morphological, behavioral and vocalization characteristics were considered for the identification of species. The birdsongs were recorded with a Sennheiser ME-66 directional microphone and a digital recorder MP3 Edirol. The technique of audio recognition was used, reproducing a recording of the audio of the birdsong or the calling of a particular species to stimulate the approaching of the respective bird or a vocal response (Motta-Junior et al. 2010). The taxonomy and nomenclature of the registered species followed the norms of the Brazilian Committee for Ornithological Registers (Comitê Brasileiro de Registros Ornitológicos/ CBRO 2014), and for the subspecies level, the taxonomy proposed in the "Guia Completo para Identificação das Aves do Brasil" by Granstau (Grantsau 2010) was used. Characteristics such as endemism and feeding habits followed the classifications presented by Sick (Sick 1997).

A total of 270 birds were recorded, comprising both the dead (N=225) and debilitated (N=45). Biological samples of 45 debilitated birds were collected: blood (N=40), cloacae swabs (N=27), and viscera (brain, lung, liver and kidneys) (N=43). The previous samples were stored in liquid nitrogen. Aliquots of the

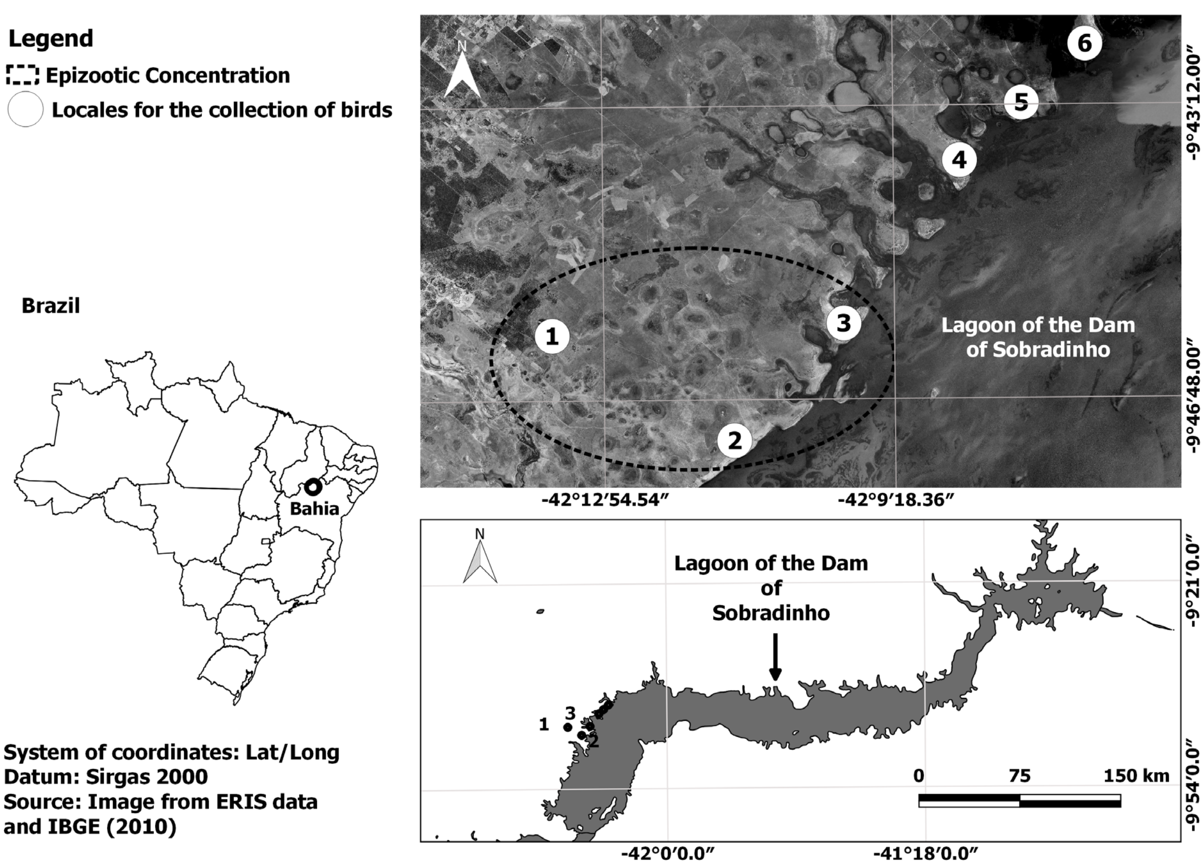


**Figure 1. Map of the study area. The black polygon marks the area of concentration of the epizootic episode. In the left, the position of the area within the country and the Caatinga Biome; in the right, a detailed view of the sampling area.**

biological samples were sent to the Evandro Chagas Institute, for isolation research of the 19 different arboviruses and/or their serology. The presence of avian influenza was also investigated by LANAGRO (National Agricultural Laboratory/Ministry of Agriculture) given the high number of birds which were found dead or debilitated belonging to the Anseriformes order (ducks, geese, swans), which are sensitive to the influenza virus (Deliberto et al. 2009). Cell cultures were used for viral isolation (cells C6/36-clone *Aedes albopictus* and VERO – kidney of a green monkey), in which the samples collected from the birds were inoculated, and examined daily to visualize the cytopathogenic effect. The presence of viral infection was confirmed by indirect antibody fluorescent test (IFI) (Gubler et al. 1984), using polyclonal antibodies from the main antigenic groups of the parvoviruses that occurs in Brazil, including representatives from the five principal families (and principal genera) of the arboviruses: (*Togaviridae*, *Alphavirus*, Group A); (*Flaviviridae*, *Flavivirus* group B); (*Bunyaviridae*, *Orthobunyavirus*; group C), (*Bunyaviridae*, *Phlebovirus*; Phlebotomus); (*Reoviridae*, *Orbivirus*; Changuinola); (*Rhabdoviridae*, *Vesiculovirus*; VSV).

In order to evaluate the possible involvement of water contamination in this bird mortality event from mining, agricultural or urban sewage contamination in the areas surrounding the lake, water samples, substrates, algae and snails were collected and sent for bacteriological and physicochemical analysis at CETREL Laboratory (Environmental Protection Company for the Petrochemical Pole in Camaçari, Bahia). To test for heavy metals, 4.5 liters of water from each of the studied lakes were collected (EPI 1 -5). The water samples were stored in 1.5 L amber flasks, identified, and refrigerated. The samples were analyzed in an atomic absorption spectrometer using the SMEWW 3120 B method (for boron, barium, beryllium, calcium, cadmium, cobalt, chrome, copper, iron, potassium, lithium, magnesium, molybdenum, manganese, sodium, nickel, lead, silicon, tin, strontium, thallium, vanadium, zinc, arsenic, mercury, antimony and selenium).

Sediment (silt), algae, and snail samples were collected from the five lakes and stored in plastic bags, identified, and conserved in ice. The material was sent to the CETREL Laboratory within 24 hours. For microbiological and physicochemical exams, 500 milliliters of water from three of the five sampled lakes were selected at random. The levels and measurements of the following were analyzed: free cyanide, conductivity, DQO-RF, the phenols index, pH, surfactants, CR. IONIC, chloride, fluoride, N-nitrate, N-nitrite, and sulfate.

Given the clinical symptoms observed in the debilitated birds suggested botulism, samples from different duck species (of the family Anatidae), in which mortality was more expressed, as well as samples from sediment (silt), algae, and snails, were collected from the five lakes, conserved at -20 °C, and analyzed at the Laboratory of Infectious Diseases of Animals, at the College of Veterinary Medicine and the State University of São Paulo (UNESP). These samples (liver, gizzard and hindgut contents) were processed and analyzed for botulinum toxin through the standard bioassay technique in mice as recommended by the *Centers for Disease Control and Prevention* – CDC/USA and described by Dutra (2001), using monovalent antitoxins (types C and D, provided by the Ministry of Agriculture, Livestock and Supply). The presence of *C. botulinum* in sediment, algae and snail samples from the puddles were evaluated using the filtered portion of the supernatant of the anaerobic bacterial culture in Wright's medium inoculated with 1g of each material; following five days of incubation at 37 °C (Souza et al. 2006). The presence of the toxin in the supernatant followed the technique described for the avian samples.

## RESULTS

The general ave-fauna registered at the study area comprised of 64 species from 24 families, of which 17 species (26.6%) are considered migratory, 43 (67.2%) residents, and 4 (6.3%) with resident and/or migratory characteristics depending on changes in the humid environment (Table I). Two hundred and seventy wild birds, either debilitated (N = 225) or dead (N = 45), were found belonging to 16 species, of which 68.8% (11/16) were migratory, 3.1% (2/16) were resident and two species, *Aramus guarauna* and *Podiymbus podiceps* (3.1% 2/16) which could be resident or migratory depending on the environmental conditions.

Regarding feeding guild division, species that feed on small invertebrates and filter-feeders such as Anatidae, Charadriidae and Recurvirostridae were the most affected, followed by vertebrate predators (e.g. Ardeidae: 10.3%, 28/270). Those of the family Anatidae were the most affected with debilitated or dead individuals registered of all the eight species identified in the ave-fauna survey in the area studied (Table I). Of the nine species of Ardeidae identified, the snowy egret (*Egretta thula*) represented almost all dead or debilitated individuals (N = 27, 10%). Of the two species of Charadriidae identified, only the southern

**Table I. Families and species of birds identified in the study area, discriminated by number of individuals debilitated and dead, feeding habits and foraging locale in relation to the water depth of the small lakes near to the Holding Lake of the Sobradinho Dam; in the municipality of Remanso, Bahia in 2008.**

| Family/Species | Debilitaded and dead | Diet | Foraging locale |
|---|---|---|---|
| **Podicipedidae** | | | |
| *Podilymbus podiceps* | 1 | Fish and insects | L R* |
| **Phalacrocoracidae** | | | |
| *Phalacrocorax brasilianus* | 2 | Fish | “ |
| **Ardeidae** | | | |
| *Egretta thula* | 27 | Fish and amphibians | “ |
| *Bubulcus ibis* | 0 | “ | “ |
| *Nycticorax nycticorax* | 1 | “ | “ |
| *Butoridis striatus* | 0 | “ | “ |
| *Ardea cocoi* | 0 | “ | L F** |
| *Ardea alba* | 0 | “ | “ |
| *Tigrisoma lineatum* | 0 | “ | “ |
| *Mycteria americana* | 0 | “ | “ |
| *Jabiru mycteria* | 0 | “ | “ |
| **Anatidae** | | | |
| *Dendrosygna autumnalis* | 96 | Filter feeders | L R |
| *Dendrosygna viduata* | 68 | “ | “ |
| *Amazonetta brasiliensis* | 35 | “ | “ |
| *Dendrosygna bicolor* | 4 | “ | “ |
| *Cairina moschata* | 3 | “ | “ |

**Table I (continuation)**

| Family/Species | Debilitaded and dead | Diet | Foraging locale |
|---|---|---|---|
| *Netta erythrophthalma* | 3 | " | " |
| *Anas bahamensis* | 2 | " | " |
| *Sarkidiornis melanotos* | 2 | " | " |
| **Aramidae** | | | |
| *Aramus guarauna* | 2 | Aquatic mollusc | L R |
| **Rallidae** | | | |
| *Gallinula chloropus* | 0 | Omniverous | S*** |
| *Porphyrio flavirostris* | 0 | " | " |
| **Jacanidae** | | " | |
| *Jacana jacana* | 2 | Insects and amphibians | L R |
| **Recurvirostridae** | | | |
| *Himantopus mexicanus* | 6 | Insects/larvae | " |
| **Charadriidae** | | | " |
| *Vanellus chilensis* | 16 | " | " |
| *Charadrius collaris* | 0 | " | " |
| **Sternidae** | | | |
| *Phaetusa simplex* | 0 | Fish | S |
| **Cathartidae** | | | |
| *Coragyps atratus* | 0 | Scavenger | M**** |
| *Cathartes aura* | 0 | " | " |
| *Cathartes burrovianus* | 0 | " | " |
| **Accipitridae** | | | |
| *Rupornis magnirostris* | 0 | Predacious | M |
| *Rostrhamus sociabilis* | 0 | Aquatic mollusc | S |
| **Falconidae** | | | |
| *Caracara plancus* | 0 | Predacious | M |
| *Mivalgo chimachima* | 0 | " | " |
| *Falco femoralis* | 0 | " | " |
| *Falco sparverius* | 0 | " | " |
| *Falco peregrinus* | 0 | " | " |
| **Columbidae** | 0 | Seeds | |
| *Columbina minuta* | 0 | " | M |
| *Columbina picui* | 0 | " | " |
| *Columbina squammata* | 0 | " | " |
| *Columbina talpacoti* | 0 | " | " |
| *Leptotila verreauxi* | 0 | " | " |

**Table I (continuation)**

| Family/Species | Debilitaded and dead | Diet | Foraging locale |
|---|---|---|---|
| *Patagioenas picazuro* | 0 | " | " |
| *Zenaida auriculata* | 0 | " | " |
| **Cuculidae** | | | " |
| *Crotophaga ani* | 0 | Omniverous | " |
| *Guira guira* | 0 | " | " |
| **Nyctibiida** | | | " |
| *Chordeiles pusilus* | 0 | Insects | " |
| **Picidae** | | | |
| *Ceryle torquatus* | 0 | Fish | S |
| **Furnariidae** | | | |
| *Certhiaxys cinnamomeus* | 0 | Insects and larvae | S |
| *Furnarius rufus* | 0 | " | S/ M |
| *Furnarius figulus* | 0 | " | S |
| **Tyrannidae** | | Insects | " |
| *Fluvicola albiventer* | 0 | " | " |
| *Fluvicola nengeta* | 0 | " | " |
| *Pitangus sulphuratus* | 0 | Omniverous | " |
| *Tyrannus melancholicus* | 0 | " | " |
| *Tyrannus savanna* | 0 | " | M |
| **Hirundinidae** | | | |
| *Progne tapera* | 0 | " | S/ M |
| *Progne subis* | 0 | " | " |
| *Hirundo rustica* | 0 | " | " |
| *Tachycineta albiventer* | 0 | " | " |
| **Mimidae** | | | |
| *Mimus saturninus* | 0 | Omniverous | M |
| **Cardinalidae** | | | |
| *Paroaria domicana* | 0 | Seeds | " |
| **Passeridae** | | | |
| *Passer domesticus* | 0 | Omniverous | " |
| **Icteridae** | | | |
| *Sturnella superciliaris* | 0 | Omniverous | S/ M |
| *Families =24. Species = 64* | N = 270 of 16 species | | |

**Key: * LR = the depth of shallow water, ** LF = depth of deep water, *** S = on the water surface and ****M = on the banks.**

**Table II.** **Result of direct research of botulinum toxin through the bioassay test, following the Centers for Disease Control and Prevention, USA, assay, and neutralization in mice of the biological material from the different species of ducks found dead.**

| Species of Ducks | Toxin | Gizzard | Intestinal content | Liver |
|---|---|---|---|---|
| *Amazoneta brasiliensis* | Type C | - | + | + |
| *Amazoneta brasiliensis* | Type C | + | + | + |
| *Dendrocygna autumnalis* | Type C | - | + | + |
| *Denseicygna autumnalis* | | - | - | -* |
| *Dendrocygna autumnalis* | | - | - | - |
| *Dendrocygna viduata* | | - | - | - |
| *Dendrocygna viduata* | | - | - | - |
| *Dendrocygna viduata* | | - | - | - |

***The samples marked in red contained botulinum toxin to provoke symptoms in mice but without causing death. By the technique, they are considered negative types even having been neutralized by the anti-toxin C.**

lapwing (*Vanellus chilensis*) appeared among the debilitated or dead individuals.

The 45 birds of the 11 species found debilitated presented good nutritional states; however, there were signs of apathy, inability to move or fly, dry ocular conjunctivitis, paralysis of the nictate membrane and ocular swelling, open wings, fallen necks, and died within a short period of time. The biological samples tested by the Evandro Chagas Institute, were negative to the presence of the West Nile or other arboviruses, as well as for H5N1, and any other virulent strains of the avian influenza.

The heavy metal analysis did not detect any parameters above the limits recommended by CONAM (National Environment Council – Ministry of the Environment). These results, despite referring to the admissible toxicity levels for humans, indicate no causal role in the death of the birds. Moreover, aside from the birds, no other animal species associated with the small lakes studied were found dead. The microbiological and physicochemical exams showed that the lakes were eutrophicated.

The C type botulinum toxin was identified in 3 of the 8 biological samples from the different duck species observed dead, which is normally involved in botulism in birds (Table II). In the 3 sediment samples from the lakes, presence of *C. botulinum* were detected, as well as the C (1) and D (1) toxins, the latter normally involved in cattle outbreaks (Woudstra et al. 2012). Furthermore, one sample was not neutralized by type C or D monovalent antitoxins, remaining unclassified. The samples of molluscs and algae collected in the study area were negative for the presence of both the bacteria and botulinum toxins.

## DISCUSSION

The results indicate a local *C. botulinum* outbreak causing mass mortality and morbidity amongst the wild bird fauna. The decomposition of organic matter in natural or artificial aquatic environments (such as the case in the Sobradinho Dam, which was drained and formed small puddles) can create favorable conditions for the reproduction and proliferation of *C. botulinum*. In fact, the detection of the type C botulinic toxin in the bioassays prove the cause of the mortality event as observed and delimitated by clinical and epidemiological observations. The presence of type D toxin *C. botulinum* spores in the sediment does not affect the diagnosis, as the co-detection is common in natural environments (Souza et al. 2006). The analysis discards the possibility of a mass death event due to heavy metal poisoning, the neurological manifestations and symptoms of which could be mistakenly attributed to botulism (e.g. weakness, walking in circles, rhythmic balancing of the head, convulsions, ataxia, and paralysis) (Jones et al. 1983). Despite the presence of mining companies in the region of the São Francisco River, the levels of heavy metals found in the samples were within admissible parameters. However, it is not possible to completely discard or associate the possibility of interactions between these substances and other causal agents in the increase of the susceptibility and weakening of the animals. It is also important to stress that tests were performed only for type C and D toxins, meaning that the negative results of the algae and mollusk samples could be due to the specificity of the monovalent antitoxins.

The mice bioassays detecting the type C toxin in the birds' viscerae gives solid proof for the suggested diagnosis of the outbreak. Together with the clinical observations and epidemiologic data, they support the diagnosis of mass mortality by botulism in this report. Although type D *C. botulinum* spores in the sediment samples were found, it does not conflict with the diagnosis of a type C outbreak, as it is a plausible finding in natural/seminatural environments and also shared by several animal species (Souza et al. 2006). In the present study only the techniques for detection of toxins in the viscerae, stomach and intestinal content of the birds, sediment cultivation, and bioassays for the C and D toxins, making it impossible to evaluate the presence of C, D,D/C mosaic toxins by PCR as performed by Nakamura et al. (2010), or to other types of *C. botulinum*. In this context, we cannot exclude the possibility of existence of environmental contamination by other strains; as well as the non-detection of *C. botulinum* in the algae and mollusk samples is plausible, as few samples were used for this assay.

The analyses performed at the Evandro Chagas Institute did not identify the presence of antibodies or arboviruses, and nor did the LANAGRO detect the presence of pathogen strains of the avian influenza virus. The occurrence of mortality in birds is always a warning of potential risks to human health and for the poultry production of a region, and the corresponding possible involvement of emerging viruses is a priority in investigations, since birds are the third most frequent source of parvovirus isolation, following humans and rodents (Araujo et al. 2012).

A differential diagnostic is important where outbreaks occur in bird populations accompanied by high mortality, considering that this can be related to several causes including West Nile Virus and other arboviruses (which can infect birds, horses and humans), avian influenza, avian botulism (Manarolla et al. 2009, Spackman et al. 2002, Włodarczyk et al. 2014), as well as the presence of more species from the genus *Flavivirus* (Scaramozzino et al. 2001) which also causes phenomena of mortality. Despite the greatest mortality of birds having occurred during the period of the current study in 2008, other episodes of bird death have occurred in the region in 2012, 2013 and 2015. In Brazil, there are no programs focused on environmental sanitation such as carcass collection in mass mortality events such as the one investigated here. According to Evelsizer et al. (2010), such measures are important to mitigate the risk of new outbreaks, mass mortality and environmental contamination.

In the study area, the presence of large flocks of vultures (family Cathartidae) was observed, from the following species: black vulture (*Coragyps atratus*), turkey vulture (*Cathartes aura*) and the lesser yellow-headed vulture (*Cathartes burrovianus*) which feed on dead bird carcasses. The members of this family are scavenger and, resistant to botulism, cholera and anthrax. Possible explanations for the resistance of vultures to pathogens were previously described (Carvalho et al. 2003). In the current study, no members of this avian family were found debilitated or dead, which corroborates the observations in literature (Cann & Taylor 1982, Hauschild & Dodds 1993, Huss & Eskilden 1974). Two species of raptors were observed, the yellow-headed caracara (*Milvalgo chimachima)* and the southern caracara (*Caracara plancus*), which feed on fresh bird carcasses. Despite this, none of these species were found dead or debilitated.

There were no reports on deaths of other animals in the region, even though production animals (horses, cattle and sheep) regularly consume water from the lakes where there were debilitated and dead birds; this is possibly due to the standardized practice of vaccinating domestic animals against botulism in the region, due to its endemicity. The ingestion of water contaminated with botulinum toxin could cause water-borne botulism in production animals (Dutra et al. 2001, Souza et al. 2006). The link between contaminated water ingestion and botulism outbreaks has been observed in prior literature (Dutra et al. 1990). Water-borne botulinum intoxication have affected cattle in Senegal and South Africa (Doutre 1969, Kriek & Odendaal 2004, Thiongane et al. 1984), and buffalo in Brazil (Langenegger & Döbereiner 1988).

Although *C. botulinum* is naturally found in soil and sediment, which create the baseline possibility of outbreaks, it is important to take in consideration human uses in the area such as livestock rearing, that can contribute to the environmental contamination. Independent of vaccination against type C and D toxoids, bovines are known for carrying *C. botulinum* spores and shedding them with their feces, with the risk of contaminating water reservoirs (Souza et al. 2006). Under such circumstances, considering that there is no policy on removing bovine carcasses (which represent a source of contamination) from grasing sites, and the management of the water reservoirs such as the Sobradinho Dam, an environmental and sanitary surveillance practice makes itself fundamental to prevent and/or mitigate such events (Evelsizer et al. 2010, Espelund & Klaveness 2014).

The samples of molluscs and algae collected in the study area were negative for spores and botulinum toxin. The sediment samples from the small lakes revealed the presence of spores of *C. botulinum,* and types C and D of botulinum toxin, as well as an unidentified botulinum toxin. Tests on the samples taken from the three species of ducks revealed the presence of type C botulinum toxin in gizzard samples, stomach content, and the liver (Table II); this toxin is generally associated to avian botulism (Yule et al. 2006), while type D are associated with the contamination and mortality of cattle.

The shallowness of the bodies of water, consequent decrease in dissolved oxygen, increase in temperature, and presence of decomposing sediments are favorable for the growth of *C. botulinum*, which benefits from these conditions in its multiplication and liberation of its toxins (Espelund & Klaveness 2014). Invertebrate fauna seems to play an important role in the availability of the toxin for wild ducks, forming a bioaccumulation chain following a environment, invertebrate fauna and duck predator route (Rocke & Bollinger 2007). This could serve as a potential explanation as to why birds of the Anatidae family were most affected in the current outbreak.

The results presented in this study reveal the first case of avian botulism in wild birds in an aquatic environment in Brazil in natural conditions. Despite the fact that this disease is well studied in commercial animals, its impact on wild animals is not yet known in the country and should be researched, both for the conservation of wild animals, as well as for the update of the sanitary guidelines governing the discard of carcasses by the agrobusiness, which would contribute to the reduction of environmental contamination of *Clostridium botulinum*.

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PCL, RL, ISD were responsible for conducting the fieldwork and taxonomical identifications, CRF, CGZ and FAAA performed the lab assays. All authors contributed equally for the writing of the original manuscript in portuguese. CGZ was responsible for the article translation and editorial submission.