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Botulism in wild birds and its association with water quality at the Pelican Harbor Seabird Station, Miami, Florida

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Abstract: Botulism, caused by a toxin produced by Clostridium botulinum, is a disease of great importance in public and animal health, being the most common cause of death in wild birds worldwide, mainly affecting aquatic and shore birds. In October 2022, a significant increase in clinical cases of avian botulism was identified at the Pelican Harbor Seabird Station, in Miami, Florida, the United States of America. The objective of the present study was to analyze report cases of avian botulism and its association with water quality from Biscayne Bay of Miami, Florida. Between June and October 2022, 130 animals with a presumptive clinical diagnosis of botulism were received at the Wildlife Center of the PHSS. The clinical histories were obtained, which were systematized in a database to perform descriptive analyses and estimate prevalence, lethality, and mortality indicators. The association of the presence of botulism and water quality was carried out using Spearman´s correlation coefficient for non-parametric data and risk analysis with the calculation of chi-squared test (X^2) and odds ratio (OR). The results showed that the prevalence of avian botulism was 20.7% in the period, with the mortality varying between 9% and 19% and lethality between 54% to 90%. The most affected species were Eudocimus albus (American white ibis) and *Leucophaeus atricilla* (laughing gull). The main clinical signs observed were limb paralysis (92%). An association was found between the disease and combined water quality, water clarity, and nitrogen. The risk analysis was statistically significant for combined water quality (OR=1.96) and nitrogen (OR=0.21). A possible association between the disease and water was identified, however, more studies are needed to determine the influence of different water quality parameters and the bird botulism.
Keywords: Avian botulism, Clostridium Botulinum, Birds, Water quality.

1. Introduction

Botulism is a disease of great importance for public and animal health and is the most common cause of death in wild birds worldwide, mainly affecting aquatic and shore birds, with high mortality rates (Rasetti-Escargueil et al., 2018, Hatheway, 1995). The wide range of bird species affected, and the annual frequency of this disease, have an impact on the populations and their reproductive success (Rocke, 2006, Degernes, 2008). Botulism in birds is caused by a toxin produced by *Clostridium botulinum,* specifically by a botulinum neurotoxin (BoNT) type C and E. Clostridium *botulinum* is an anaerobic bacterium, transmitted mainly by the larva-corpse cycle through the ingestion of contaminated food (Skarin et al., 2010, Coffield et al., 2007). Once the birds ingest the toxin, it travels through the lymphatic or bloodstream to the peripheral nervous system, preventing the neuromuscular synapse, and generating flaccid paralysis in the voluntary muscles (Heredia, 2018). Paralysis begins in the hind limbs, followed by paralysis of the nictitating membrane and neck muscles. The disease presents signs such as the inability to lift the neck and head, the lack of blinking, and the inability to fly or walk. In the most advanced cases of the disease, it can lead to death due to drowning, respiratory failure, and dehydration (Rocke, 2006).

The first reports of avian botulism date back to the beginning of the 20th century, in the United States of America and Canada (Rocke and Bollinger, 2007). The disease is widely spread and has been reported in more than 28 countries, however, there are still no measures to prevent the occurrence of botulism in wild birds or sufficient information for adequate management of the fauna (Rocke, 2006). Avian botulism is related to environmental conditions, the bacteria are found mainly in soil and water that has favorable anaerobic conditions. Various studies have addressed the relationship of water in the occurrence of avian botulism outbreaks, showing the influence of water contamination on the disease. However, more specific studies are still required considering the other water contaminants (Vidal et al., 2013). In South Korea, outbreaks of botulism have been associated with high water temperatures, increased biomass, presence of dead invertebrates, alkaline pH, low redox potential, decreased turbidity, and low salinity (Son, 2018). In the Macha Humeda Biosphere Reserve wetland located in south central Spain, the botulism outbreak was associated with high water temperatures, sediment 5-day biochemical oxygen demand (BOD5), water redox potential, chlorophyll a level, sulfate levels, and water inorganic carbon levels (Anza et al., 2014).

The National Wildlife Health Center of the United States analyzes waterfowl mortality, reporting that the main cause of bird mortality is botulinum neurotoxin types C and E (Newman et al. 2007). The first outbreaks of avian botulism in Florida were reported in the 1980s; the outbreak was caused by type C botulism in a settling pond at a phosphate mine in northern Florida (Forrester et al., 1980). The Southern coast of Florida has experienced the impact of anthropogenic interventions since the beginning of the 20th century, potentially affecting the condition of the fauna, flora, and environment (Briseño, 2011). At the Pelican Harbor Seabird Station Wildlife Rehabilitation Center, an increase in cases with a positive clinical diagnosis of avian botulism was observed. The objective of this study was to characterize the clinical cases of botulism reported at Pelican Harbor Seabird Station and explore its association with water quality.

2. Materials e Methods

An epidemiological study was carried out at the Pelican Harbor Seabird Station located in the city of Miami. It is a tropical coastal region located at an average altitude of 2 meters above sea level with a temperature between 23 °C and 17 °C and precipitation between 1227 mm and 1621 mm (National Weather Service, 2022) (Table 1).

Month	Temperature	Dew point	Precipitation	Wind (mph)	Sea level	Humidity (%)
June	29.88	26.11	0.51	6.39	29.97	78
July	28.07	23.52	0.16	9.37	30.06	72
August	28.07	23.52	0.16	9.37	30.06	72
September	28.59	23.54	0.43	6.38	29.91	76
October	26.49	20.72	0.16	6.39	29.77	72

Table 1 – Environmental conditions in area Miami between June to October 2022.

The wild birds (n = 627) that entered the consultation between June and October 2022 were analyzed. The database was systematized and stored in data banks. Subsequently, descriptive analysis was carried out using statistics and the construction of epidemiological indicators. To explore the association between botulism and water quality was carried a risk analysis study type of case control. The cases were birds that presented presumptive clinical diagnoses, with signs such as difficulty blinking, absence, or presence in cloaca tone, who could or could not hold their head, paralysis in the hind limbs, dehydration, moderate or severe, with moist or sticky/dry mucous membranes, regardless of age and species. The control population was considered birds with a negative clinical diagnosis of avian botulism, which presented for consultation for other reasons, including different species and ages.
Information on water quality was obtained, considering the location where the wild birds were collected; state water quality databases were searched (Figure 1). Considering the 2022 Biscayne Bay report produced by Miami-Dade County, which qualifies the water quality as poor, fair, or good, based on annual evaluation of physical, chemical, and microbiological parameters such as nutrients, water clarity, bacteria, aquatic vegetation, chlorophyll, combined quality, and frequency of sponges (Table 2). Each parameter was classified into 5 levels; levels 4 and 5 were considered high-risk concentrations.

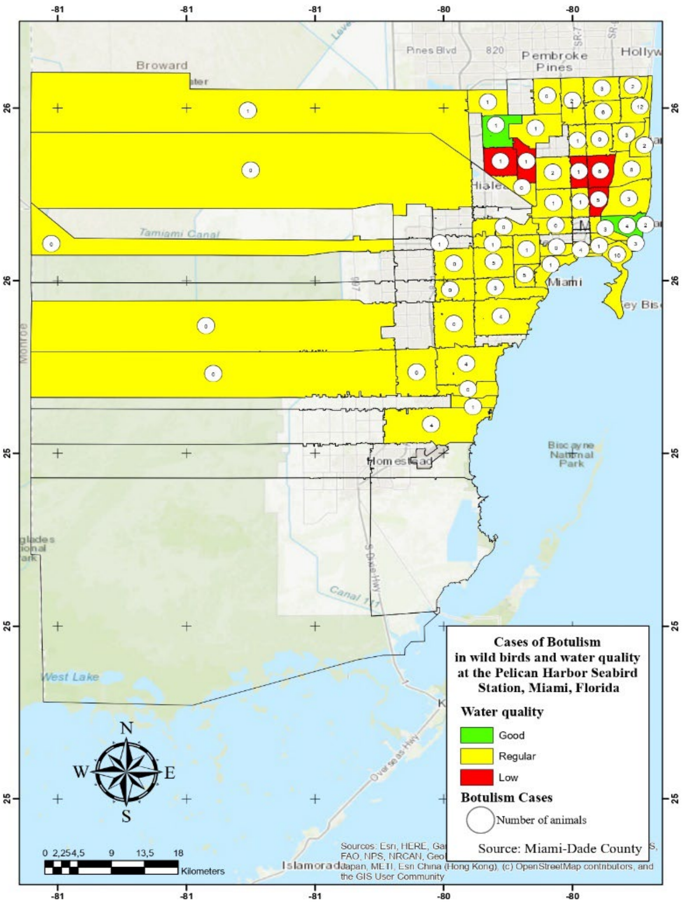

Figure 1 – Cases of botulism in wild birds and water quality at the Pelican Hearbor Seabird Station. Location of origin botulism in wild birds' cases and number birds by area code is presented, as well as the water quality reported for each area form 2022 Biscayne Bay report produced by Miami-Dade County.

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The risk analysis was carried out by constructing 2×2 tables, calculating Chi-square (χ^2) and Odds Ratio. Likewise, water quality variables were associated with the presence of the disease using Spearman's correlation coefficient for non-parametric data. Analysis was performed using SPSS software version 20 (SPSS Inc., Chicago, USA).

Component	Qualification				
	1	2	3	4	5
Nutrients nitrogen	<25	25th percentile - long-term	Long term limit- (0.8)	(0.8) *upper limit to upper	upper limit
Water clarity	< Base	base- 10% above base	10% - 20% above base	20%-30% above base	>30% above base
Escherichia coli (126 MPN/100 ml)	>252	126-252	100.8- 125	75.6-100.8	75.6
Enterococci (35 MPN/100 ml)	>70	35-70	28-35	21-28	<21
Combined water quality (Phosphorus, nitrogen, chlorophyll, water clarity, bacteria)	>4.5	3.51-4.5	2.51-3.5	1.51-2.5	<1.5
Submerged aquatic vegetation	0- 1.50	1.51-2.50	2.51-3.50	3.51-4.50	4.51-5
Sponge frequency	0%-20	20.1%-40	40.1%-60	60.1%-80	80.1%-100

Table 1 – Classification of the physical, chemical and microbiological parameters of the water from the basins of Miami, Florida

3. Results

A presumptive clinical diagnosis for botulism was observed in 130 (20.7%) birds, evidencing the high presence of the disease. A progressive increase in cases of avian botulism was observed between June with 15% positivity (19/123) and October with 25% (41/167). Mortality in the period was 20.7%, showing variations over time, being lower in June at 9% and higher in September and October at 19%, the lethality was 66.9%, with higher lethality in September and August at 85% and 90% respectively. 53% of the birds were categorized as adult birds and 47% as juvenile. The species reported with avian botulism were *Anhinga anhinga* (2), *Dendrocygnid autumnalis* (1), *Nycticorax nycticorax* (4), *Sula leucogaster* (1), *Pelecanus occidentalis* (10), *Bubulcus ibis* (2), *Calonectris borealis* (1), *Phalacrocorax auratus* (7), *Ardea Herodias* (2), *Ardea alba* (6), *Butorides virescens* (11), *Larus argentatus* (3), *Leucophaeus atricilla* (38), *Calidris minutilla* (2), *Sternula antillarum* (1), *Larus fuscus* (4), *Thalasseus maximus* (1), *Egretta tricolor* (3), *Rallus limicola* (1), *Eudocimus albus* (25), *Nyctanassa violacea* (5) (Figure 1).

The clinical signs observed in wild birds were paralysis of limbs in 92% of cases, absence or partial presentation of blinking in 68%, not holding the head in 58%, dry or semi-dry membranes in 57%, absence of cloaca tone in 55%, thin condition in 48%, mild dehydration in 45%, moderate dehydration in 37%, alert attitude in 37%, calm attitude in 31%, depressed 31%, severe dehydration in 18%, stupors 7%. Paralysis of the limbs and the absence of blinking were the most frequent symptoms of bird mortality. Birds diagnosed with avian botulism were treated with subcutaneous fluid therapy with B complex, activated carbon, ocular lubricants, and hydrotherapy for paralyzed birds. Their recovery was difficult, as was their reintegration into the ecosystem.

The cases came from areas of Miami Bay categorized by zip code, the areas with the most cases were Sunny Isles (12), Key Biscayne (10), Miami Beach (9), Coral Way (9), and North Bay Village (8). The quality of the water in the different areas showed that 79% had regular water quality, 6% good and 15% showed a lack of water quality. Considering the disaggregated physical, chemical and microbiological parameters, the percentage of areas with high ranges (category 4 and 5) was calculated: 15.6% presented high concentrations of phosphorus, 8.33% nitrogen, 41.6% water clarity, 15.5% *E. coli* (126 MPN/100 ml), 47.9% *Enterococci* (35 MPN/100 ml), 58.3% combined water quality, 8.33% aquatic vegetation and 8.33% sponge frequency. Different water quality variables were studied, and an association was found between the combined water quality with an OR of 1.96 ($P \leq 0.049$) and nitrogen with an OR of 0.218 ($P \leq 0.033$). The correlation analysis found a low relationship with combined water quality (r: 0.146), water clarity (r: 0.18), and nitrogen (r: -0.145).

4. Discussion

At the Pelican Harbor Seabird Station Wildlife Rehabilitation Center a high presence of cases of avian botulism is observed with a prevalence of 20.7%, the results are consistent with what was reported by Anza et al., (2016), where a prevalence of up to 23% was found during the outbreak period. An avian botulism mortality rate of 13% was observed, studies have reported mortality rates between 7 to 17%, as well as survival rates varying between 75% and 90%, data consistent with our results (Hidalgo et al., 2008). The data show variations in the occurrence of cases, mortality, and lethality in temporal terms, however, the study of climatic conditions was analyzed for a short time to identify a trend. The bird families predominantly affected during the study were *Leucophaeus atricilla* (38), *Butorides virescens* (11), *Eudocimus albus* (25), *Elecanus occidentalis* (10), in Chile the species of the family Laridae and Anatidae were also reported to be affected by avian botulism (Hidalgo et al., 2008). On the other hand, in an outbreak in Korea, the bird species mainly affected were *Anas poecilorhyncha* and *Ardea cinerea* (Son, 2018). In Poland, the species reported in an outbreak were *Anas platyrhynchos* and *Fulica atra* (Wlodarczyk et al., 2014). Vulnerability of the species may be related to different factors, probably related to feeding habits, season of arrival, rotation, and, possibly, phylogenetic resilience (Aza et al., 2016). Studies in Michigan mainly reproduce the effects of botulism on migratory birds, presenting multiple routes of exposure of birds with botulism in Lake Michigan (Chipault et al., 2015). It is important to know the most vulnerable species to prioritize fauna management.

Studies have reported that the main symptom of botulism is flaccid paralysis, which means that one cannot stand, or maintain a sternal position, as well as the head falling to one side, also called "flexibility", being the classic symptoms to identify the disease in animals. The birds can subsequently survive for three days, before recovery or death (Duff, 2017). The signs predominated were neurological, where the most common were the absence of blinking, and paralysis of the lower limbs, signs that occurred in 100% of the mortality cases. In an outbreak of avian botulism in Korea, symptoms such as paralysis of the flaccid neck and paralysis of the inner eyelid were also reported, and some showed an inability to maintain flight (Son, 2018). Likewise, dead birds with worm infestation are reported (Shin et al., 2010). Likewise, Sonne et al., (2011), based on a study on paralysis in wild birds, state that there are several negative effects at the population level, and multifactorial. Knowing the clinical presentation of the disease is essential, considering that the diagnosis of botulism in birds is not a routine test, due to the number of birds received and the difficulty of identifying the toxin.

Several studies report cases and outbreaks of avian botulism, however, few have explained the relationship of the disease with water quality making it essential to conduct studies that address the interdependent relationship of fauna with ecosystems to promote effective animal and environmental health. This study explores on a small scale and through data level the relationship between water quality and botulism, finding associated water parameters such as combined water quality, water clarity, and nitrogen. The increase in the presence of nutrients in the water, such as nitrogen, can lead to the loss of marine vegetation and generate anaerobic environments that favor the presence of *C. botulinum* (Brand, 2001). Nitrogen data is also included in the calculation of combined water quality, which could influence the association of this indicator with cases of Avian botulism. However, it is necessary to do the analyses for each parameter independently. Water clarity is also associated with an overabundance of nutrients that leads to eutrophication, producing turbidity in the water. The lack of clarity of the water can result in shading over seagrass habitat, affecting the aquatic vegetation (Briceño et al., 2011).

Multiple studies have investigated the correlation between the presence of various nutrients and physical-chemical characteristics in water and avian botulism. In Korea, outbreaks were linked to water temperature and nutrient loads, particularly nitrogen and phosphorus; the opening of dams helped reduce these factors and minimize botulism cases (Son, 2018). A retrospective study in the La Macha wetland in Spain identified a potential association between avian botulism and increased levels of inorganic carbon, decreased redox potential, chlorophyll and sulfate levels, as well as water temperature and biochemical oxygen demand (BOD5) (Aza et al., 2014). In a Hungarian wetland habitat, a suggested relationship was found between water quality parameters such as temperature, pH, Water-Soluble Oxygen (WSO), and Chemical Oxygen Demand (COD) with avian botulism outbreaks (Babinszky et al., 2008). In Spain, an outbreak in the wetlands of La Mancha was associated, with the low concentrations of chloride ions and high organic matter content, with the presence of *C. botulinum* identified in sediments (Vidal et al., 2013). Modeling studies in the USA indicated that pH, redox potential, temperature, and salinity measured just above the sediment-water interface were associated with the risk of botulism outbreaks in wetlands (Rocke and Samuel, 1999).

In Miami Bay, problems with water turbidity, nutrient loading and enrichment, bacterial enrichment, and other variables such as the presence of chemical compounds derived from runoff, pesticides, and industrial and stormwater pollutants have been reported (Brand, 2001, Briceño et al., 2011). Urban discharges can increase the organic load of the water, providing nitrogen and phosphorus, which favors the growth of phytoplankton, which initially favors aquatic birds, but which contributes to pollution and produces eutrophication (Perez et al., 2001). Polluted runoff and groundwater can be sources of contaminants (pathogens and toxins) in the Miami-Dade County canal system, which ultimately reach the Bay (Long et al., 2005). More studies are required on the causes of paralysis and mortality of birds in Florida, as well as considering interventions in wildlife habitats. Any proposed intervention must evaluate the possible impacts and quickly identify the possible risks of changes in wildlife. (Circella et al., 2019). Researchers propose that environmental water management strategies that aim to maintain water quality by providing flow to waterfowl colonies could mitigate the risk of botulism outbreaks (Brandis et al., 2019). Prediction studies considering water quality could generate valuable information for intervention (Rocke and Samuel, 1999).

5. Conclusion

A high number of cases of avian botulism was identified at the Pelican Harbor Seabird Station, Miami, Florida with a prevalence of 20.7% between June and October 2022, with a mortality rate of 9 and 19% and lethality between 54 and 90%. The most affected species were *Eulogiums albus* and *Leucophaeus atricilla*. The main clinical sign observed was limb paralysis (92%). An association of botulism cases with the reported water quality data was made, finding an association between the disease and the combined quality of the water, water clarity, and nitrogen. More studies are recommended on the relationship between water quality and avian botulism, as well as measures to preserve fauna and the environment.

Figure 2 – Species reported with avian botulism at the Pelican Harbor Seabird Station. A y B*) Pelecanus occidentalis*, C*) Eudocimus albus*, *D) Leucophaeus atricilla.*

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