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Simultaneous occurrence of type C botulism in poultry and dogs[1]

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ABSTRACT.- Martins A.S., Alves L.R., Silva T.A., Santos R.M., Almeida A.M.S., Saraiva J.R., Dutra I.S. & Borsanelli A.C. 2025. **Simultaneous occurrence of type C botulism in poultry and dogs.** *Pesquisa Veterinária Brasileira 45:e07615, 2025.* Setor de Medicina Veterinária Preventiva, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Rodovia Goiânia-Nova Veneza Km 8, Goiânia, GO 74690-900, Brazil. E-mail: anaborsanelli@ufg.br

Botulism is a serious disease caused by neurotoxins produced by *Clostridium botulinum*, an anaerobic spore-forming bacterium commonly found in the environment and the intestinal tract of animals. The disease occurs through the ingestion of food or water contaminated with preformed toxins, leading to progressive flaccid paralysis across various susceptible species. This study reports the clinical, epidemiological, and laboratory aspects of the simultaneous occurrence of type C botulism in domestic birds (chickens and ducks) and dogs on a farm in Rio Pomba, Minas Gerais, Brazil. The farm housed 47 chickens, two ducks, and seven dogs. Over approximately 15 days, 40 chickens (85.1%) and one duck (50%) fell ill and died. Among the seven dogs, five (71.4%) exhibited clinical signs, and two (28.6%) ultimately died. Diagnosis of botulism was confirmed through bioassay technique and neutralization using homologous antitoxin in mice, which identified botulinum toxin type C in serum samples from two chickens with clinical signs, in samples from two necropsied chickens and in one water sample. The most probable transmission route was likely the remains of a decomposing bovine carcass, and the water that had accumulated around it, to which the chickens, ducks and dogs had access, highlighting the importance of proper carcass disposal to prevent disease outbreaks.

INDEX TERMS: Botulinum neurotoxins, chickens, *Clostridium botulinum*, mouse bioassay, dogs.

RESUMO.- [**Ocorrência simultânea de botulismo tipo C em aves e cães.**] Botulismo é uma doença grave causada por neurotoxinas produzidas por *Clostridium botulinum*, uma bactéria anaeróbica formadora de esporos comumente encontrada no ambiente e trato intestinal de animais. A doença ocorre pela ingestão de alimentos ou água contaminados com toxinas pré-formadas, levando à paralisia flácida progressiva em várias espécies suscetíveis. Este estudo relata os aspectos clínicos, epidemiológicos e laboratoriais de um surto de botulismo tipo C que afetou aves domésticas (galinhas e patos) e cães em uma fazenda no município de Rio Pomba, Minas Gerais, Brasil. A fazenda abrigava 47 galinhas, dois patos e sete cães. Ao longo de aproximadamente 15 dias, 40 galinhas (85,1%) e um pato (50%) adoeceram e morreram. Entre os sete cães, cinco (71,4%) apresentaram sinais clínicos e dois (28,6%) acabaram morrendo. O diagnóstico de botulismo foi confirmado por meio da técnica de bioensaio e neutralização com antitoxina homóloga em camundongos, que identificou toxina botulínica tipo C em amostras de soro de duas galinhas com sinais clínicos, em amostras de duas galinhas necropsiadas e em uma amostra de água. A rota de transmissão mais provável foi provavelmente os restos de uma carcaça bovina em decomposição e a água que havia se acumulado ao redor dela, à qual as galinhas, patos e cães tiveram acesso, o que destaca a importância do descarte adequado de carcaças para prevenir surtos da doença.

TERMOS DE INDEXAÇÃO: Neurotoxinas botulínicas, galinhas, *Clostridium botulinum,* bioensaio em camundongos, botulismo tipo C, cães, aves.

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INTRODUCTION

Botulism is a disease that affects humans, birds, and mammals, caused by neurotoxins produced by *Clostridium botulinum*, a Gram-positive, ubiquitous, spore-forming bacillus (Takeda et al. 2005). This microorganism can produce eight distinct toxin types (A, B, C, D, E, F, G, and H), which are classified into four groups (I, II, III, and IV) based on their cellular biochemical activity (Harris 2016). In animals, the primary neurotoxins associated with botulism are types C and D, along with the mosaic forms C/D and D/C. Susceptibility to these toxins varies significantly across species (Le Maréchal et al. 2017).

In all species, botulism progresses to a state of flaccid paralysis, which can lead to respiratory and cardiac failure. The disease develops due to the ingestion of botulinum toxin, which was previously formed in decomposed plant or animal matter (Barros et al. 2006). These neurotoxins inhibit the release of acetylcholine at neuromuscular junctions and are resistant to intestinal activity, being absorbed through the mucosa (Barros et al. 2006).

Botulism is reported on all continents, with distribution varying based on environmental factors, climatic events, neurotoxins presence, and the species involved (Rocke & Bollinger 2007, Le Maréchal et al. 2017). In Brazil, botulism in cattle has significant economic and sanitary importance, with numerous outbreaks documented across extensive regions. Decomposing cattle carcasses in pastures is a key source of environmental contamination and transmission, as animals ingest preformed toxins from these sources. Thus, the presence of carcasses, along with other epidemiological factors, creates conditions for the occurrence of outbreaks among cattle (Dutra et al. 2001, Souza et al. 2006).

In addition to cattle, botulism is a significant cause of mortality in waterfowl and shorebirds (Anza et al. 2016). Recently, an outbreak of botulism in southern lapwings (*Vanellus chilensis*) in southern Brazil resulted in the death of 130 adult birds, likely due to the ingestion of larvae present in fresh chicken litter (Santos et al. 2024). Waterborne botulism outbreaks have also been documented in free-ranging waterfowl in the Central-West region (Martins et al. 2022) and on a large scale in the country's semi-arid region (Lima et al. 2020). Clinical signs, such as flaccid paralysis of the neck, wings, and eyelids, are commonly observed in both wild and domestic birds affected by the disease (Circella et al. 2019, Rasetti-Escargueil et al. 2019).

Although carnivores are generally less susceptible to botulinum toxin, cases of botulism in dogs are not uncommon. Typical clinical signs in affected dogs include reduced superficial and deep pain reflexes, ascending flaccid paralysis, absence of eyelid reflexes, and decreased muscle tone in the cervical region (Rosa et al. 2022). The amount of toxin ingested usually influences the incubation period and clinical progression in dogs (Barros et al. 2006). In Brazil, an outbreak of type C botulism simultaneously affected dogs, domestic chickens, and a nonhuman primate; while the source of this outbreak was not definitively identified, contaminated carcass ingestion was suspected (Silva et al. 2018).

Although botulism is relatively common in birds and dogs, simultaneous outbreaks involving both species are rare. This study describes an occurrence of type C botulism on a farm in Minas Gerais, Brazil, which affected dogs and domestic birds, including chickens (*Gallus gallus domesticus*) and Muscovy ducks (*Cairina moschata*). The occurrence was associated with the presence of a decomposing carcass at the edge of a pond, which served as a water source for the animals.

MATERIALS AND METHODS

Ethical approval. The use of the bioassay in mice was approved by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal de Goiás" (UFG), Protocol No. 024/20.

Outbreak presentation. A botulism outbreak affecting dogs and poultry occurred on a farm in Rio Pomba, Minas Gerais, Brazil. Clinical and epidemiological data were collected by the referring veterinarian. At the time, the farm housed 47 chickens, two ducks, and seven dogs. Approximately 20 days before the outbreak, a young bovine was found dead in a pond near the animal enclosure. The cause of death was unknown, and the carcass was removed from the pond and left on the bank, where it began to decompose.

Several birds started showing clinical signs of weakness a few days later, including flaccid paralysis of the legs, wings, neck, and eyelids, which progressed to death (Fig.1). The birds roamed freely on the farm with unrestricted access to the pond. They were fed a mixture of sorghum, corn, and bran directly on the ground, with the pond as one of their water sources. Five of the farm's dogs also began displaying signs of flaccid paralysis and respiratory difficulty. The owner reported that these dogs had access to the decomposing carcass on the pond's bank and were also observed eating the remains of chickens that had died with similar clinical signs.

During the 15-day outbreak, 40 chickens (85.1%) and one duck (50%) became ill and died. Among the seven dogs, five (71.4%) showed clinical signs; of these, two (28.6%) died. After the bovine carcass was removed from the pond's edge and buried in a remote location, animal mortality on the farm ceased.

Fig.1. Frontal view of one chicken with flaccid paralysis of the legs, wings and neck.

Laboratory diagnosis. Given the clinical signs observed in the dogs and domestic birds, along with the history of a decomposing bovine carcass near the pond where the animals drank, botulism was suspected. Serum samples were collected from two dogs and two chickens showing clinical signs to investigate the presence of botulinum toxin. Additionally, two adult chickens that exhibited clinical signs and later died were necropsied, with samples of liver, stomach contents, and intestinal contents collected. Water samples from around the bovine carcass and from the birds' drinking water were also collected.

All samples (liver, stomach contents, intestinal contents, serum, and water) were processed and analyzed for the presence of botulinum toxin using the standard mouse bioassay technique at the "Laboratório de Bacteriologia" (Bacteriology Laboratory) of the "Escola de Veterinária e Zootecnia" (School of Veterinary Medicine and Animal Science – EVZ) of the UFG. Suspected samples were injected intraperitoneally into mice (0.5 mL) weighing 20 to 25 grams. Inactivated samples (heated at 85 °C for 20 min) were inoculated into additional mice as described by Smith (1977). Mice were observed for seven days. Positive samples were then typed using seroneutralization with botulinum antitoxin types C and D, standardized and provided by the "Laboratório Federal de Defesa Agropecuária" (LFDA/MG).

RESULTS

The necropsied chickens were in good nutritional condition, and no macroscopic lesions were observed. Botulism was confirmed by the detection of botulinum toxin in serum samples from two chickens (*Gallus domesticus*) displaying clinical signs and in samples from the two necropsied chickens (Table 1). Botulinum toxin was also identified in a water sample collected from the area surrounding the decomposing carcass near the pond (Table 1).

In mice inoculated with these samples, signs of paralysis and dyspnea (characterized by a "wasp waist" appearance) were observed, with death occurring on average 12 to 24 hours post-inoculation. None of the mice inoculated with the heat-treated samples exhibited clinical signs. It was not possible to detect botulinum toxin in the serum sample from the symptomatic dogs. Botulinum toxin type C was confirmed in both the water and bird samples (Table 1).

DISCUSSION

In this study, botulinum toxin type C was detected in a water sample collected around a decomposing bovine carcass accessible to various animals on the farm. Botulism cases in birds have been documented in approximately 264 species across 39 families (Soos & Wobeser 2006, Le Maréchal et al. 2016), with waterborne outbreaks reported in species such as chickens (*Gallus domesticus*), mallards (*Anas platyrhynchos*), guineafowl (*Numida meleagris*), garganeys (*Anas querquedula*), and turkeys (*Meleagris* sp.), often linked to contaminated water sources (Olinda et al. 2015, Quevedo et al. 2022). In the present outbreak, it is suspected that animals became sick by both consuming remnants of the decomposing carcass and drinking contaminated water from the area around it.

Botulinum toxin types C, D, C/D and D/C are frequently associated with environments rich in organic matter. Ingestion of contaminated food and water can easily trigger botulism outbreaks in poultry (Meurens et al. 2023). Decomposing carcasses provide an ideal substrate for *Clostridium botulinum* multiplication and toxin production, with fly larvae potentially increasing the risk of botulism when ingested by poultry (Le Maréchal et al. 2016). This was evident in the present outbreak, where the presence of a bovine carcass significantly heightened the animals' vulnerability to botulism.

The diagnosis of botulism in cattle is highly challenging due to the absence of characteristic macro and microscopic lesions, which makes laboratory confirmation difficult and often leads to inconsistencies depending on the diagnostic methodologies applied (Döbereiner & Dutra 2004, Lima et al. 2024). Botulism diagnosis relies on a combination of clinical signs, epidemiological history, and necropsy findings, often with minimal species-specific variations. The mouse bioassay is considered the gold standard for botulinum toxin detection, providing high accuracy in identifying toxins (Hedeland et al. 2011, Mazuet et al. 2017, Meurens et al. 2023). However, this technique has several limitations, including long result times, ethical concerns due to high animal usage (Hedeland et al. 2011), limited epidemiological sensitivity, and the potential absence of detectable toxins at the time of sampling (Allison et al. 1976). Nevertheless, it is still the only laboratory test accessible in Brazil to confirm the diagnosis of botulism. It has the advantage of detecting the activity of the toxin, not just its presence (Lima et al. 2024).

These diagnostic challenges are not limited to cattle but also affect other species, as observed in this study, where botulinum toxin was detected in birds and water samples but not in dogs' serum. The absence of detectable toxin should not rule out a clinical diagnosis, as false negative results may occur due to low sensitivity of mice to botulinum toxin, toxin degradation by autolysis, or its rapid binding to myoneural junctions, which hinders detection (Döbereiner & Dutra

Table 1. Results of the direct search for botulism toxin in biological sample of domestic chickens, dogs and water by mouse bioassay and serum neutralization in mice

Tested sample (N*)	Mouse bioassay (N**)	Seroneutralization in mice
Intestinal content *Gallus gallus domesticus* (2)	Positive (1)	Botulinum toxin type C
Stomach contents *G. gallus domesticus* (2)	Positive (1)	Botulinum toxin type C
Liver tissue *G. gallus domesticus* (2)	Positive (1)	Botulinum toxin type C
Serum *G. gallus domesticus* (2)	Positive (2)	Botulinum toxin type C
Canine serum (2)	Negative	-
Water around carcass (1)	Positive (1)	Botulinum toxin type C
Birds' drinking water (1)	Negative	

N* = number of samples tested, N** = number of positive samples for the detection of botulinum toxin.

2004, Barros et al. 2006, Le Maréchal et al. 2016, Lima et al. 2024). The lack of standardization in diagnostic approaches across different regions also contributes to discrepancies in epidemiological data and disease prevalence estimates. Recent studies have proposed a diagnostic protocol tailored to Brazilian conditions to address these challenges, aiming to establish standardized criteria and enhance diagnostic accuracy (Lima et al. 2024).

Reports of botulism among wild birds in Brazil highlight the disease's complex epidemiology. Documented outbreaks have been linked to environmental factors, such as the partial drainage of water bodies (Lima et al. 2020). This study's detection of type C botulinum toxin in domestic birds aligns with previous Brazilian reports, which have noted sporadic botulism outbreaks in domestic birds since 1971 (Brada et al. 1971, Silva et al. 2018).

Generally, high levels of circulating toxin are observed in the blood of birds after poisoning, therefore making serum the primary sample for toxin identification via mouse bioassay (Lobato et al. 2008). Domestic birds appear more sensitive to type C toxin than to type D, consistent with this and previous studies (Brada et al. 1971, Meurens et al. 2023). In wild birds, toxin C has also been described in botulism outbreaks in Brazil and was detected in species like the Southern lapwing (*Vanellus chilensis*), *Amazonetta brasiliensis*, and *Dendrocygna autumnalis* (Lima et al. 2020, Santos et al. 2024).

In dogs, the incubation period can vary up to 48 hours, and clinical progression depends on the ingested toxin amount. Botulism cases in dogs have been reported in several countries, often linked to ingestion of contaminated carcasses (Farrow et al. 1983, Wallace & McDowell 1986, Bruchim et al. 2006, Uriarte et al. 2010, Silva et al. 2018, Rosa et al. 2022). In this study, access to a decomposing bovine carcass in a pond was a key factor in the occurrence of botulism. Additionally, botulinum toxin was detected in the water sample collected around the carcass. Although *C. botulinum* is ubiquitous in the environment, decomposing carcasses exacerbate environmental contamination by dispersing spores (Souza & Langenegger 1987, Souza et al. 2006). These spores are highly resistant to environmental conditions and can remain viable in fluid media for up to 30 years (Smith & Sugiyama 1988).

In Brazil, the lack of regulations for carcass disposal on farms, combined with limited awareness of the sanitary risks posed by botulism, significantly increases the likelihood of outbreaks in domestic and wild animals. This case highlights the critical role of proper carcass management in outbreak prevention, as animal mortality ceased promptly after the carcass was removed and buried. Effective disposal of carcasses not only prevents environmental contamination but also minimizes the risk of toxin exposure to other animals. Although the cause of death of the bovine remains unknown, it is well established that carcasses of animals that died from botulism are particularly hazardous, as they are potentially more toxic than those that died from other causes (Oliveira Júnior et al. 2016).

Botulism in cattle is characterized by variable morbidity and lethality, depending on the amount of toxin ingested and other epidemiological factors (Barros et al. 2006). In this study, mortality rates were 85.1% in chickens and 28.6% in dogs, highlighting the greater susceptibility of birds to botulism compared to carnivores; despite the high morbidity observed, with 71.4% of the dogs on the farm exhibiting clinical signs, most survived, underscoring the importance of early intervention. While vaccination remains a key preventive measure in cattle health programs, no vaccines are currently available for dogs or poultry. This limitation highlights the critical need for effective carcass management and awareness of the disease's risks. Implementing routine preventive strategies, such as proper carcass disposal and environmental monitoring, is essential to reduce the occurrence of botulism outbreaks in both domestic and wild animals.

CONCLUSION

The epidemiological challenges posed by botulism in dogs and birds, coupled with the associated risks in diverse production systems and natural environments, underscore the need for effective preventive measures. Proper carcass disposal, ensuring safe access to food and water, and efforts to reduce mortality on farms are critical components of botulism control. *Clostridium botulinum* is a ubiquitous microorganism, and while its eradication is impossible, the implementation of sound sanitary practices can significantly reduce the risk of outbreaks across various animal species. This emphasizes the urgent need for regulations governing proper carcass disposal and the importance of raising awareness to prevent future outbreaks, thus safeguarding animal health.

Conflict of interest statement. - The authors declare that there is no conflict of interest.

Credit author statement.- All authors contributed to the conception and design of the study. Ronald M. Santos was responsible for sample collection and clinical assessments. Material preparation, data collection, and analysis were performed by Andressa S. Martins, Tamires A. Silva, Ana Maria S. Almeida, and Ana Carolina Borsanelli. Andressa S. Martins, Lisandra R. Alves and Ana Carolina Borsanelli organized the data. Ana Carolina Borsanelli, Júlia R. Saraiva, and Iveraldo S. Dutra interpreted the data. Ana Carolina Borsanelli, Andressa S. Martins, Lisandra R. Alves, and Tamires A. Silva prepared the final manuscript. All authors read and approved the final manuscript.

Data availability statement.- The data are available from the corresponding author upon reasonable request.

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