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Type C botulism in dogs from rural properties located in Goiânia, Brazil

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ABSTRACT

Botulism is a disease usually fatal, caused by the ingestion of neurotoxins produced by *Clostridium botulinum*. In dogs, intoxication is caused by the ingestion of botulinum toxin type C, and animals often recover spontaneously. The present study describes the occurrence of type C botulism in two dogs domiciled on neighboring rural properties in the municipality of Goiânia, state of Goiás, Brazil, probably associated with ingestion of decomposing bovine carcass. Upon clinical evaluation, the dogs were alert in the lateral decubitus position with ascending flaccid paralysis, absence of eyelid reflexes, and reduced muscle tone. Due to their worsening clinical symptoms, the animals died within 12 h and 3 days after supportive treatment. Botulinum toxin type C was identified, in the serum and feces of both dogs, by seroneutralization in mice with homologous monovalent antitoxin. The results of the high-throughput gene sequencing showed that the abundance of *C. botulinum* in the fecal microbiota of one of the affected dogs was low (0.53%). In this way, the present study highlights the need of sanitary practices related to the appropriate collection and disposal of bovine carcasses in rural areas since they represent a risk factor for the occurrence of botulism in dogs domiciled on rural properties.

Keywords: *Clostridium botulinum* type C; botulinic toxin; flaccid paralysis; mice bioassay; 16S rRNA gene sequencing.

Botulism is nonfebrile intoxication, usually fatal, caused by the ingestion of *Clostridium botulinum* neurotoxins, previously formed in decomposed animal or plant organic matter (DUTRA et al., 2001; SOUZA et al., 2006). Botulinum toxins are considered the most potent and feared microbial toxins in nature due to their biological effects and lethality in both humans and animals (KRIEK; ODENDAAL, 2004).

Currently, eight immunologically distinct types of botulinum toxins are recognized (A, B, C, D, E, F, G, and H), indicated by letters of the alphabet and in chronological order of their classification (KRIEK; ODENDAAL, 2004; BARASH; ARON, 2014). In animals, toxin types C and D are the primary causative agents of botulism (BARSANTI et al., 1978; DUTRA et al., 2001, 2005; MONEGO et al., 2006; URIARTE et al., 2010).

After ingestion, botulinum toxin is absorbed in the digestive tract, distributed throughout the body, and enacts pharmacological action on peripheral nerve endings for which the neurotransmitter is acetylcholine (SUGIYAMA, 1980). Although patients may experience flaccid paralysis, resulting from the inhibition of the release of acetylcholine in the neuromuscular junctions, the central nervous system is not involved (SMITH; SUGIYAMA, 1988). Despite

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having almost the same pharmacological action, the various types of botulinum toxin are antigenically different and can be neutralized by homologous sera.

Carnivores are considered more resistant to botulinum toxins, although several studies have reported the occurrence of botulism in dogs in various countries (BARSANTI et al., 1978; BRUCHIM et al., 2006; URIARTE et al., 2010). The incubation period and evolution of the disease are inversely proportional to the amount of toxin ingested, that is, the more botulinum toxin ingested, the shorter the incubation period and the faster the clinical course (KRIEK; ODENDAAL, 2004; BARROS et al., 2006).

In Brazil, scientific reports on the occurrence of botulism in dogs and the epidemiology of these cases are uncommon. Therefore, the present study aimed to describe two cases of botulism in dogs from the municipality of Goiânia, state of Goiás, Brazil. Two dogs from neighboring rural properties, with free access to the pastures of nearby properties, were treated at a veterinary clinic in Goiânia. The first dog (dog 1), a 2-year-old mixed breed male weighing 38 kg, presented to the clinic with a lack of movement in the pelvic and thoracic limbs. The second dog (dog 2), a 4-year-old mixed breed female weighing 25 kg, presented to the clinic 9 days after the former, with similar clinical symptoms. Dog 1 had a known history of ingesting part of a bovine carcass in the pasture of the rural property; however, dog 2 was not seen ingesting any of the carcass. Both dogs presented with flaccid paralysis of the thoracic and pelvic limbs.

On physical examination, they were normohydrated, with normal colored mucosa, tachypnea, and tachycardia. For the neurological evaluation, the dogs were alert, in lateral decubitus, and were noted to have reduced superficial and deep pain reflexes, ascending flaccid paralysis, an absence of eyelid reflexes, and reduced muscle tone in the cervical region. Only dog 1 had a swallowing reflex. The hematological examination of dog 1 revealed a slightly lower-than-normal mean corpuscular hemoglobin concentration, thrombocytopenia, and mild hyperproteinemia. A SNAP 4Dx Plus test (IDEXX Laboratories, Westbrook, USA) was also performed on dog 1, and the results were positive for *Ehrlichia* spp. and *Anaplasma* spp. Dog 2 had normal hematological results.

Both animals received the same supportive treatment with maintenance fluid therapy using simple Ringer's solution in conjunction with glucose, ondansetron, tramadol, and dipyrone. Dog 1 also received oxytetracycline and imidocarb dipropionate due *Anaplasma* spp. and *Ehrlichia* spp. infections. Nutritional support was provided in the form of hypercaloric solution administered through a nasogastric tube, and the urinary bladder was emptied with the aid of a urethral catheter every 2 h. An enema of activated charcoal diluted in saline solution was performed on dog 1, and numerous bone fragments were found in the fecal matter. A simple gastric lavage was performed on dog 2, with activated charcoal diluted in saline solution. Dog 1 remained hospitalized for 3 days, but died from increasing cardiorespiratory and neurological symptoms. Due to financial restrictions, dog 2 was hospitalized for only 12 h, and died 2 h after leaving the hospital.

To detect botulinum toxin, serum and feces samples collected from both dogs on their first day of care were processed and inoculated (0.5 mL) intraperitoneally into mice with body weights ranging from 20 to 25 g, according to procedures described by SMITH; SUGIYAMA (1988). At the same time, specimens from the same samples were heated to 85 °C for 20 min, and were inoculated into additional mice. The mice inoculated with the untreated serum and feces samples developed paralysis and dyspnea (*wasp waist*), and subsequently died between 12 and 24 h after inoculation. None of the animals inoculated with the heat-treated samples showed any clinical symptoms.

The typing of positive samples in the mouse bioassay was performed through serum neutralization (SMITH; SUGIYAMA, 1988) with botulinum antitoxin types C and D, which were inoculated as previously described. Through the seroneutralization technique in mice, the toxin was identified as type C, and was qualitatively detected in the serum and feces samples from both dogs. Additionally, high-throughput bacterial 16S rRNA gene sequencing was used to determine the microbial profile in the remaining stool sample from dog 1. Preparation of the libraries followed a proprietary protocol (Neoprospecta Microbiome Technologies, Brazil). The bioinformatics pipeline used for sequence analysis was Neotools and a proprietary database (NeoRef16S v.1.0) composed of complete gene sequences (mostly), which contained sequences recovered from genomes, unambiguous and filtered for chimera sequences.

The clinical symptoms observed and the histories of the dogs reinforced the initial suspicion of botulism, especially due to the report that dog 1 was seen ingesting the remains of a bovine carcass. Although there was no evidence that dog 2 ingested the same remains, the two animals shared the same environment, as the two rural properties were approximately 200 m apart. Therefore, due to the proximity of the two properties and the short interval between the two cases, it was suspected that the same bovine carcass was the means of transmission of the botulinum toxin to the two dogs. Animal carcasses are reported to be one of the primary means of transmission of botulinum toxins in cases of botulism in dogs in Brazil (MONEGO et al., 2006; SILVA et al., 2008). However, a definitive diagnosis of botulism must be based on the detection and classification of botulinum toxin(s) in biological samples. The mouse bioassay is still considered the gold standard for the detection and identification of botulinum toxins, and is the method used by 80% of European laboratories

(UZAL et al., 2016). Seroneutralization in mice identified botulinum toxin type C in the serum and fecal samples of the two dogs discussed in the present study. This, together with the clinical symptoms and the epidemiological characteristics of the two dogs, confirmed the diagnosis of botulism.

Although canids are considered relatively resistant to botulinum toxin (BARSANTI et al., 1978), cases of botulism in dogs and other canids have been reported in several countries. LINDSTRÖM et al. (2004) described the largest outbreak of botulism in fur-producing animals, resulting in the deaths of 52,000 animals on 83 farms. At that time, the animals were fed bird remains, in which botulinum toxin type C was subsequently identified. In dogs, botulism is usually due to botulinum toxin type C, and cases of botulism associated with this toxin have previously been described in dogs in New Zealand, Canada, Israel, the United States, and Brazil (BARSANTI et al., 1978; WALLACE; MCDOWELL, 1986; BRUCHIM et al., 2006; MONEGO et al., 2006; SILVA et al., 2008; URIARTE et al., 2010).

In a study carried out on rural properties in the state of Goiás, SOUZA et al. (2006) identified spores of *C. botulinum* in 31% of the samples of bovine feces collected. The elimination of spores by feces contributes to an increase in environmental contamination, generating a situation of potential risk. In the present study, the results of the high-throughput gene sequencing showed that the abundance of *C. botulinum* in the fecal microbiome of one of the affected dogs was 0.53% (Fig. 1), thus suggesting that the presence, in quantitative terms, of *C. botulinum* in the feces of dogs with botulism is low.

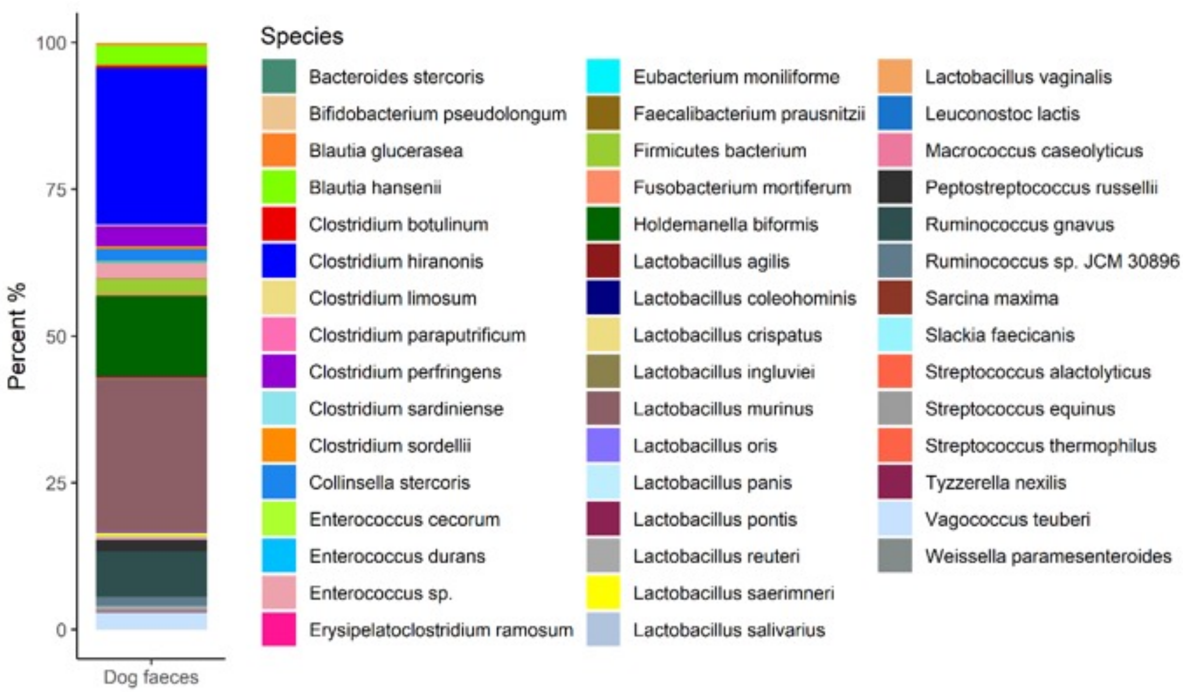

Figure 1. The relative abundance of the bacterial communities at the species level, identified in the fecal microbiome of dog 1, by high-throughput bacterial 16S rRNA gene sequencing.

As in other species, the evolution and prognosis of botulism in dogs are associated with the amount of toxin ingested by the animal (BARROS et al., 2006). The average incubation period varies anywhere from 12 to 24 h, although it can be as long as 5 days after the ingestion of the toxin. The earlier the clinical symptoms appear, the greater the severity of the disease (BARSANTI, 2012). In the most severe cases, the prognosis is reserved and death occurs due to respiratory failure, as was observed in the two animals in the present study.

There is no specific treatment protocol for cases of canine botulism, and supportive treatment is essential to maintain the animal's basic needs while preventing possible secondary infections. Commercially available antitoxins are difficult to acquire and act only on circulating toxins. In cases of suspicion of the recent ingestion of suspicious food, gastric lavage and enemas are indicated (BARSANTI, 2012), as was performed on the dogs in the present study. Antibiotic therapy is recommended to avoid secondary infections, as in the case of dog 1, in which drugs were chosen in an effort to fight infections from *Anaplasma* spp. and *Ehrlichia* spp. In cases of severe intoxication, supportive treatment is not very effective because its objective is to control clinical symptoms and to prevent secondary infections (BARSANTI, 2012).

Even though the two dogs described in the present study died, the prognosis of botulism in dogs is usually favorable in mild and moderate cases, and recovery occurs in around two or three weeks (BARSANTI, 2012). Based on the severity of the clinical outlook of the two dogs described here, it can be inferred that the animals ingested a large amount of botulinum toxin. Additionally, the coinfection by *Anaplasma* spp. and *Ehrlichia* spp. in dog 1 likely contributed to the worsening of respiratory condition due to the destruction of erythrocytes.

Due to the lack of mandatory reporting in most countries, and the subsequent lack of accurate information on outbreaks of botulism in animals, incidence of this disease is likely underestimated in several species, including dogs (UZAL et al., 2016). Difficulties in confirming cases of botulism are due to the lack of laboratories carrying out diagnostic analyses along with the limitations of the gold standard technique.

In Brazil, the fact that botulism represents the main cause of bovine mortality in several regions was due to several epidemiological components present in livestock. One such component is the intensification of environmental contamination by *C. botulinum* spores due to the ongoing presence of decaying carcasses in pastures (DUTRA et al., 2001, 2005; BARROS et al., 2006). In a study carried out on rural properties in southern Goiás, high concentrations of *C. botulinum* type C and D spores were identified within a radius of up to 30 m from decomposing bovine carcasses (SOUZA; LANGENEGGER, 1987). Furthermore, the presence of these carcasses in pastures also represents a risk factor for the occurrence of botulism in dogs domiciled on these rural properties, as reported in the present study. Therefore, this study highlights the need for sanitary practices related to the appropriate collection and disposal of bovine carcasses in rural areas to prevent canine botulism.

AUTHORS' CONTRIBUTIONS

Conceptualization: Borsanelli, A.C.; Santos e Silva, D.; Dutra, I.S. **Data curation:** Athayde, F.R.F. **Formal analysis:** Athayde, F.R.F. **Investigation:** Rosa, I.C.C.R.; Santos e Silva, D.; Leopoldino, A.G.R.; Borsanelli, A.C. **Methodology:** Rosa, I.C.C.R.; Santos e Silva, D.; Leopoldino, A.G.R.; Borsanelli, A.C.; Dutra, I.S. **Project administration:** Borsanelli, A.C. **Supervision:** Borsanelli, A.C. **Writing – original draft:** Rosa, I.C.C.R.; Santos e Silva, D.; Leopoldino, A.G.R.; Athayde, F.R.F.; Botelho, A.F.M.; Dutra, I.S. **Writing – review & editing:** Rosa, I.C.C.R.; Santos e Silva, D.; Leopoldino, A.G.R.; Athayde, F.R.F.; Botelho, A.F.M.; Dutra, I.S.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL APPROVAL

This study was approved by the Ethics Committee on Animal Use of Universidade Federal de Goiás (Protocol UFG No. 025/20).

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