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Outbreak of botulism in horses associated with toxin A and D in São Paulo state, Brazil[1]

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ABSTRACT.- Miyashiro S, Valim AC, Roncati NN, Paretsis NF, Nassar AC, Cerri FM, Andrade DGA, Oliveira-Filho JP, Amorim RM, Borges AS. **Outbreak of botulism in horses associated with toxins A and D in São Paulo state, Brazil.** *Pesquisa Veterinária Brasileira 45:e07686, 2025*. Departamento de Clínica Veterinária, Faculdade de Medicina e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Prof. Doutor Walter Mauricio Corrêa s/n, Cx. Postal 560, Botucatu, SP 18618-681, Brazil E-mail: alexandre.s.borges@unesp.br

Botulism is a neuromuscular disorder caused by neurotoxins produced by *Clostridium botulinum*. Equine botulism has been frequently reported worldwide; however, descriptions of cases in South America remain uncommon. This report describes a botulism outbreak in horses due to the ingestion of big bale silage at a stud farm in Brazil. Eight horses consuming silage hay provided in large round bales in the pasture presented progressive muscular weakness, decreased tongue and tail tone, dysphagia, fasciculations, and difficulty raising their head. Five horses died on the farm, and three were referred to a veterinary hospital. Two of the hospitalized horses were discharged, while the third died, and *post mortem* samples were collected for the detection of *C. botulinum* toxins. Analysis of a cecum liquid sample confirmed the presence of both botulinum toxins, BoNT/A and BoNT/D, via the bioassay method. Given the nonspecific nature of initial clinical signs, laboratory confirmation becomes imperative, facilitating the implementation of preventive measures. Notably, confirmed diagnoses of botulism are relatively uncommon in horses in Brazil.

INDEX TERMS: Recumbency, *Clostridium botulinum*, neurotoxins, bale silage.

RESUMO.- [Surto de botulismo em equinos associado com toxina tipo A e D no estado de São Paulo, Brasil.] O botulismo é um distúrbio neuromuscular causado por neurotoxinas produzidas pelo *Clostridium botulinum*. O botulismo em equinos tem sido frequentemente relatado em todo o mundo, no entanto descrições de casos na América do Sul permanecem escassas. Este relato descreve um surto de botulismo em cavalos devido à ingestão de silagem em fardos grandes em um haras no Brasil. Oito cavalos, após consumirem feno ensilado fornecido em fardos redondos grandes, apresentaram fraqueza muscular progressiva, diminuição do tônus da língua e da cauda, disfagia, fasciculações e dificuldade para manter o pescoço levantado. Cinco cavalos morreram na propriedade, e três foram encaminhados a um hospital veterinário. Dois dos cavalos hospitalizados receberam alta, enquanto o terceiro morreu, sendo coletadas amostras *post mortem* para a detecção de toxinas de *C. botulinum*. A análise de uma amostra de líquido do ceco confirmou a presença das toxinas botulínicas BoNT/A e BoNT/D utilizando o método de bioensaio. Dada a natureza inespecífica dos sinais clínicos iniciais, a confirmação laboratorial torna-se imprescindível, facilitando a implementação de medidas preventivas. Vale destacar que diagnósticos confirmados de botulismo são relativamente incomuns em equinos no Brasil.

TERMOS DE INDEXAÇÃO: Decúbito, *Clostridium botulinum*, neurotoxinas, silagem.

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INTRODUCTION

Botulism is a disease associated with *Clostridium botulinum* and its botulinum neurotoxins (BoNTs). *C. botulinum* is classified into eight types (A to G) based on BoNT production (Swink & Gilsenan 2022, Uzal et al. 2022). This disease affects humans (Lonati et al. 2020), cattle (Lima et al. 2024), birds, fish, dogs, and cats (Oliveira-Júnior et al. 2016, Meurens et al. 2023, Uzal et al. 2024, Paz et al. 2025) and horses (Sprayberry & Carlson 1997, Whitlock & Buckley 1997, Ostrowski et al. 2012), as well as other domestic and wild animal species that may also be affected (Silva et al. 2016, Circella et al. 2019, Lima et al. 2024). Botulism has been extensively described in other species in Brazil, particularly cattle (Silva et al. 2016, Lima et al. 2024), while only two outbreaks have been reported in horses in Brazil (Coelho et al. 2000, Argenta et al. 2017).

In horses, the primary route of infection is through the ingestion of toxins via contaminated feed, such as silage or hay, or water (foodborne botulism) (Ricketts et al. 1984, Divers et al. 1986, Wichtel & Whitlock 1991, Lindtröm et al. 2001; Böhnel et al. 2003, Coelho et al. 2000, Whitlock & McAdams 2006, Ostrowski et al. 2012). Other modes of disease acquisition have also been described: ingestion of *C. botulinum* spores, which subsequently germinate in the gastrointestinal tract of horses and produce toxins (toxicoinfectious botulism) (Wilkins & Palmer 2003a, 2003b), and, less frequently, the contamination of wounds with *C. botulinum*, which leads to bacterial proliferation and toxin production (wound botulism) (Mitten et al. 1994, Liguori et al. 2008).

At the molecular level, BoNTs act at the neuromuscular junction by inhibiting acetylcholine release (Rings 2004, Gerber et al. 2006, Meurens et al. 2023). The mechanism of action involves the cleavage of SNARE complex proteins, which are essential for neurotransmitter exocytosis, resulting in its inhibition (Rings 2004, Gerber et al. 2006). The different *C. botulinum* serotypes are classified based on their target proteins: BoNT/A and E cleave SNAP-25; BoNT/B, D, F, and G act on VAMP; and BoNT/C cleaves both SNAP-25 and syntaxin (Meurens et al. 2023).

Clinically, affected horses may develop progressive, symmetrical flaccid paralysis, fasciculations, decreased tongue and anal sphincter tone, dysphagia, recumbency, and death (Kelly et al. 1984, Ricketts et al. 1984, Haagsma et al. 1990, Johnson et al. 2010, 2015).

This study aimed to describe the epidemiological, clinical, and laboratory aspects of a type A and D botulism outbreak in horses in Brazil.

MATERIALS AND METHODS

Ethical approval. All procedures on the farm and during sample collection were performed by a licensed veterinarian who adhered to animal welfare principles, and, with the owner's consent. Similarly, for animals admitted to the veterinary teaching hospital, all procedures were authorized by the owner. The study was approved by the Animal Experimentation Ethics Committee (Protocol 000.282/2024) of the "Faculdade de Medicina Veterinária e Zootecnia" at "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (FMVZ-Unesp).

Data collection. Eight horses raised in an extensive farming system on a farm in the Itu (23° 15′ 51″ S, 47° 17′ 57″ W), São Paulo state, Brazil, presented clinical signs compatible with botulism. Three patients were referred to the Veterinary Teaching Hospital, FMVZ-Unesp, Botucatu (22° 53′ 09″ S, 48° 26′ 42″ W), Brazil. The medical history and epidemiological data of the animals were collected, and clinical examinations, necropsy, and sample submissions for complementary testing were performed.

Laboratory tests. A complete blood count, blood biochemistry, blood gas and cerebrospinal fluid (CSF) analysis were performed in the clinical pathology laboratory of the veterinary hospital. During necropsy, the cecal and intestinal contents were sampled for enteropathogen (*Salmonella* spp., *Clostridioides difficile, Clostridium perfringens*, *Cryptosporidium* spp., *Giardia* spp. and rotavirus) detection via quantitative polymerase chain reaction (qPCR) in a commercial Veterinary Molecular Diagnostics Laboratory, and samples of intestinal segments (duodenum, jejunum, cecum, and colon) and central nervous system (telencephalon, diencephalon, cerebellum, brainstem, and spinal cord) were collected for histopathological analysis. Three fecal samples and serum (Horses 4, 7, and 8) and cecal contents (*post mortem* Horse 4) were collected for the mice inoculation bioassay (performed at the Animal Bacteriology Department of Instituto Biológico/SP, following Centers for Disease Control guidelines – CDC 1998). Briefly, 25 g of the suspected sample was diluted 1:2 (w/v) with sterile gelatin phosphate buffer, homogenized and then refrigerated at 4 °C for 12 h to allow toxin elution from solid material. The supernatant fluid was subsequently centrifuged at 12,000 × g for 20 min, and a 1 mL aliquot was treated with trypsin for 30-60 min before the intraperitoneal injection of 0.5 mL into Swiss Webster mice, which weighed 18-25 g. The *Clostridium botulinum* serotype was determined by adding 0.25 mL of serotype-specific monovalent antitoxins (A, B, C and D). Inoculated mice were periodically observed for signs of botulism, including ruffling of the fur, labored but not rapid breathing, weakness of the limbs and gasping for breath.

RESULTS

Setting and feeding management

There were 89 horses on the property, 72 of which grazed on tifton pasture (*Cynodon dactylon*), and the others (n = 17) were housed in stables; both groups received the same dry tifton hay. In addition, all horses were supplemented with commercial feed specifically formulated for horses and mineral salt. The horses grazing on tifton pasture (*C. dactylon*) were supplemented with ensiled big bales (*C. dactylon*). Only horses in the group supplemented with ensilage big bales presented clinical abnormalities.

Outbreak description and clinical findings

Eight horses supplemented with ensiled big bales (*C. dactylon*) exhibited weakness, dysphagia, tremors, and spontaneous episodes of nasogastric reflux. No brain abnormalities, including changes in the level of consciousness, head position, or behavior, were observed in these horses. There were no signs of ataxia or muscle atrophy. Five horses became recumbent within 2-5 days and were euthanized at the farm. Necropsy was performed on two of them, and no significant macroscopic or histological changes were observed in the central nervous system (CNS) or muscles. Rabies was ruled out through direct immunofluorescence and mouse inoculation using encephalic and spinal cord samples from these horses.

Clinical management

The three hospitalized Horses (4, 7, and 8) presented locomotion difficulties due to weakness (flaccid paresis),

muscle tremors, difficulty standing unaided, reduced tongue and tail tone, dysphagia, nasal discharge of saliva, and difficulty in raising their heads. The clinical signs observed in all eight horses are detailed in Figure 1-4 and Table 1. Blood samples were collected for a complete blood count, serum biochemistry, upper airway endoscopy (Horses 7 and 8), blood gas analysis and CSF analysis (Horse 4).

Based on epidemiological data and clinical signs, botulism was suspected. Supportive treatment was initiated, including frequent assisted standing using a sling, fluid therapy with Ringer's lactate for fluid replacement and maintenance, electrolyte correction, analgesia (dipyrone 25 mg/kg IV SID – Lemma-Injex Biologic, Vespasiano, Minas Gerais, Brazil), anti-inflammatory therapy (flunixin meglumine 1.1 mg/kg IV SID – JA Saúde Animal, Patrocínio Paulista, São Paulo, Brazil – or meloxicam 0.6 mg/kg IV SID – Vetnil, Louveira, São Paulo, Brazil), recumbency management with wound cleaning and frequent repositioning, as well as physiotherapy and rehabilitation. Owing to the presence of food content in both nostrils resulting from dysphagia, antibiotic therapy (ceftiofur 5 mg/kg IV SID – Vetanco, Vinhedo, São Paulo, Brazil – and metronidazole 20 mg/kg IV BID – JP Farma, Ribeirão Preto, São Paulo, Brazil) was administered to prevent pneumonia (Horse 4).

One of the referred horses (Horse 4) died after six days of hospitalization and presented severe colitis (greenish, liquid feces), persistent lateral recumbency, cyanotic mucous membranes, and fever (39.3 °C). Immediately after death, CSF was collected.

Laboratory results and pathological findings

The complete blood count revealed leukocytosis in Horse 4 and neutrophilia in all horses. Serum biochemistry analysis revealed elevated AST activity in Horses 4 and 7 and increased total bilirubin in all horses. For Horse 4, CSF analysis revealed a normal pattern: protein levels of 22 mg/dL, red blood cells (2 cells/μL), and two nucleated cells/μL. Blood gas analysis of Horse 8 revealed hypochloremia (98 mmol/L).

On endoscopy, grade three lymphoid hyperplasia was observed, along with focal laryngitis (above the opening of the guttural pouch on the left side), the presence of water and feed on the floor of the larynx (below the epiglottis),

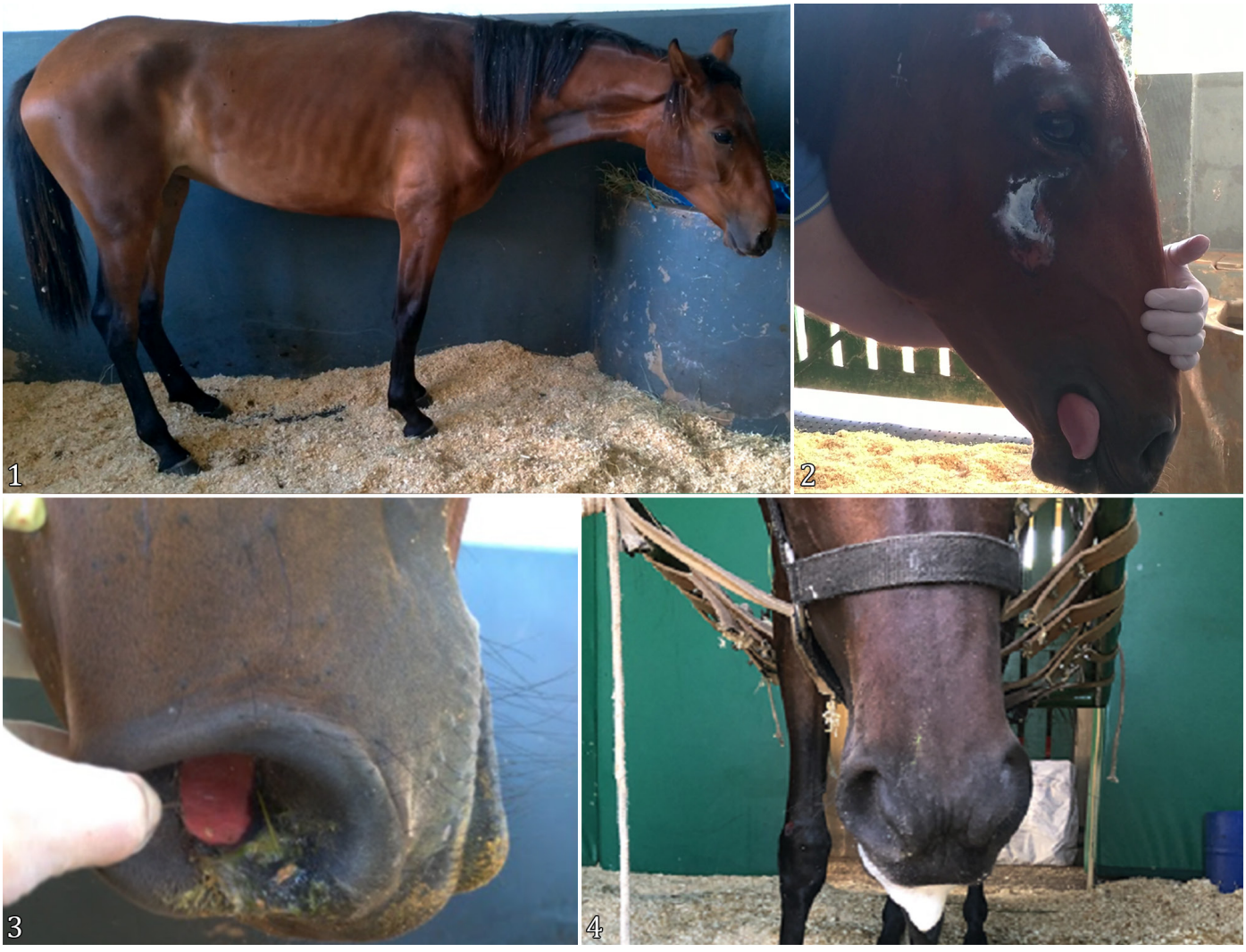

Fig. 1-4. Clinical signs observed in horses with botulism (types A and D). (**1**) Horse 8 extended neck (inability to raise the head). (**2**) Dysphagia (inability to protrude the tongue). (**3**) Presence of food content in the nostrils. (**4**) Sialorrhea.

mild chondritis of the corniculate processes of the arytenoid cartilages, mild chondritis of the epiglottis, and the presence of feed in the esophagus. In Horse 7, intermittent dorsal displacement of the soft palate was observed.

During the necropsy of Horse 4, gastric mucosa showed moderate multifocal hemorrhage in the small intestine and cecum. Histology revealed a moderate diffuse mononuclear inflammatory infiltrate in the mucosa and a mild infiltrate in the submucosa. qPCR analysis detected the presence of *Salmonella* spp. and non-toxigenic *Clostridioides difficile* DNA in fecal samples from this horse. No degenerative, vascular or inflammatory abnormalities were found in the central nervous system.

Unprotected mice exposed to the trypsinized toxin-containing extract died within 24 h after inoculation with classic signs of respiratory tract musculature failure (respiratory muscle paralysis syndrome). Botulinum toxin could be detected in cecum content samples from Horse 4, and seroneutralization was positive for BoNT/A and BoNT/D.

DISCUSSION

Botulism outbreaks in ruminants are highly relevant in Brazil (Lima et al. 2024). Equine botulism has been widely reported in both the United States and Europe (Table 2), and this outbreak represents the third documented case of botulism in horses in Brazil. Previously, two outbreaks involving the identification of BoNT/C in the states of Goiás (Coelho et al. 2000) and Rio Grande do Sul (Argenta et al. 2017) had been reported. These Brazilian outbreaks were likely caused by contamination from hay-containing carcasses (Coelho et al. 2000) or the presence of stagnant water with a large accumulation of organic matter (Argenta et al. 2017).

Among the possible differential diagnoses initially considered for the cases were botulism, hyperkalemic periodic paralysis, equine motor neuron disease (EMND), equine herpes virus-1 (EVH-1), West Nile virus, rabies, atypical myopathy and ionophore intoxication (Stratford et al. 2014, Johnson 2020). Although individual cases may present with similar signs, the collective assessment of the cases in this report, along with the observed findings, allows for the exclusion of these differential diagnoses. Briefly, hiperkalcemic periodic paralysis (HYPP) typically occurs in isolated cases, and as an inherited genetic disease is associated with Quarter Horses, and manifests as transient episodes of weakness; EMND has a chronic course characterized by muscle atrophy and specific histopathological changes in spinal cord motor neurons; EHV-1 usually presents as an outbreak, often preceded by respiratory abnormalities, and is associated with ataxia and, in some cases, tail and bladder paralysis, as well as distinct lesions in the CNS; West Nile virus infection leads to encephalic neurological signs and ataxia, with suggestive changes in both histopathology and CSF analysis. Atypical myopathy and ionophore intoxication both involve muscle lesions, elevated muscle enzymes, and characteristic histological findings. Ionophore intoxication, in particular, is linked to muscle damage and prior exposure to the toxic agent (Stratford et al. 2014, Johnson 2020). Rabies was ruled out in the necropsied animals. Considering the breed, epidemiological conditions, clinical history, number of animals affected, disease progression, laboratory results, and *post mortem* findings, these diseases were ruled out, and botulism was considered the primary differential diagnosis. Due to the strong epidemiological indicators and the presence of *Clostridium botulinum* toxins, the definitive diagnosis was confirmed.

The observation of botulism cases is correlated with the microbiological characteristics of the agent, as the bacillus is strictly anaerobic and spore-forming, allowing it to survive for long periods in the environment (Huss 1980, Espelund & Klaveness 2014). The formation of spores can lead to the contamination of forage crops intended for animal feed (Graham & Brueckner 1919, Divers et al. 1986, Whitlock & Buckley 1997). Considering that the big bale silage was the only feed offered exclusively to the animals that showed clinical signs, it is highly possible that the source of the outbreak was the contamination of this feed. Environmental food contamination of feed supplied to horses has been reported in other cases. It is associated with silage (Haagsma et al. 1990, Franzen et al. 1992, Hutchins 1994, Dietz 1998), hay (Switzer et al. 1984, Wichtel & Whitlock 1991, Szabo et al. 1994, Galey 2001, Johnson et al. 2010) and big bale silage (Ricketts et al. 1984, Divers et al. 1986, Franzen et al. 1992, Goehring & Sloet van Oldruitenborgh-Oosterbaan 2002, Goehring et al. 2005).

Frequent clinical signs in horses include dysphagia, muscle tremors, decreased tail tone, and recumbency, resulting from

Table 1. Clinical signs of horses with botulism (types A and D) in Brazil

Clinical signs	Horse							
	1[a]	2[a]	3[a]	4[a,b]	5[a]	6[a]	7[b]	8[b]
Apathy	X			X	X			
Flaccid paralysis				X	X	X	X	X
Extended neck (inability to raise the head)	X	X	X	X	X	X	X	X
Recumbency		X	X	X	X	X	X	X
Dysphagia	X			X	X	X	X	X
Sialorrhea	X			X	X	X		
Saliva secretion through the nostrils				X			X	
Muscle tremors				X				
Decubitus bedsores				X			X	X
Decreased tongue tone				X			X	X
Decreased tail tone				X			X	X
Anal sphincter and flaccidity					X			

[a] Dead horses, [b] hospitalized horses.

Table 2. Information on published outbreaks of botulism in horses

Author	BoNT	Affected/ dead horses	Country	Probable source of intoxication	Sampling	Method
Krumholz (1962)	B	12/ND	Israel	Contaminated food	Supplied vegetables	Isolation and mouse bioassay
Muller (1962)	C	ND/ND	Denmark	Contaminated food by carcass	Liver	Isolation, microscopy and mouse bioassay
Doutre & Chambron (1966, Doutre 1969)	D	ND/ND	Senegal	Water contaminated by carcass	Water	Isolation and mouse bioassay
Chambron et al. (1971)	D	3/3	Senegal	ND	Liver	Isolation and soroneutralization
Doutre & Toure (1978)	D	7/7	Senegal	Water	Liver	Isolation and soroneutralization
Swerczek (1980)	B	8/8	USA	ND	Gastric ulcers and abscess in the lung	Isolation and mouse bioassay
Kelly et al. (1984)	C and D	11/8	Australia	Hay straw	Hay	Isolation and mouse bioassay
Thiongane et al. (1984)	D	5/5	Senegal	Water	Liver	Soroneutralization
Ricketts et al. (1984)	B	13/9	England	Big bale silage	Serum and feces	Mouse bioassay
Switzer et al. (1984)	D	ND/ND	USA	Hay		Mouse bioassay
Divers et al. (1986)	B	6/6	USA	Silage	NP	Mouse bioassay
Bernard et al. (1987)	B	1/0	USA	ND	Necrotic wound	Mouse bioassay
Heath et al. (1988)	C	1/1	Canada	Silage	Gastrointestinal content	Mouse bioassay
Heath et al. (1990)	C	4/3	Canada	Contaminated food	Intestinal content	Mouse bioassay
Vaala (1991)	ND	53/ND	USA	NF	ND	
Franzen et al. (1992)	B	7/6	Sweden	Big bale silage	ND	Mousse bioassay
Dietz (1998)	C and D	1/1	Germany	Silage	Silage	Mouse bioassay
Haagsma et al. (1990)	B	12/4	Belgium	Silage	Serum and feces	Mouse bioassay
Mayhew & Ricketts (1991)	B	2/0	UK	ND	Feces	Isolation
Kinde et al. (1991)	C	38/31	USA	Alfalfa cubes	Liver, serum and intestinal contents	Mouse bioassay
Crane (1991)	B	2/0	USA	ND	Feces	Mouse bioassay
Wichtel & Whitlock (1991)	B	8/7	USA	Alfalfa hay	Intestinal content	Mousse bioassay
Mitten et al. (1994)	B	1/1	USA	Intramuscular injection	Necrotic wound	Mousse bioassay
Hutchins (1994)	C	42/31	Australia	Silage and hay	Serum, feed, intestinal and liver	Mouse bioassay
Szabo et al. (1993)	A and E	1/1	Australia	Hay	Feces, liver, and spleen	PCR and ELISA
Gudmundsson (1997)	B	13/8	Iceland	Carcass	ND	Mouse bioassay
Hunter et al. (1999)	C	13/13	Scotland	Grass	Feces and intestinal content	ELISA
Coelho et al. (2000)	C	7/7	Brazil		ND	Mouse bioassay
Böhnel et al. (2003)	C and D	2/2	Germany	Green grass blades	Spleen and intestinal content	Mouse bioassay and ELISA
Schoenbaum et al. (2000)	C	13/13	USA	Contamination with feces and carcass	Feces and intestinal content	Mouse bioassay
Galey (2001)	A	15/14	USA	Alfalfa hay	Hay	Mouse bioassay
Goehring & Sloet van Oldruitenborgh-Oosterbaan (2002)	B	59/59	Netherlands	Big bale silage	Silage	Mousse bioassay
Wilkins & Palmer (2003a, 2003b)	B	30/27	USA	ND	ND	Mouse bioassay
Goehring et al. (2005)	B	4/3*	Netherlands	Big bale silage	Serum	Elisa
Jahn et al. (2008)	B	2/1	Czech Republic	ND	Serum and gastrointestinal content	Mouse bioassay
Liguori et al. (2008)	B	1/1	Italy	N/A	Gastric ulcer	Mouse bioassay
Roest et al. (2009)	B	2/2	Netherlands	ND	Gastrointestinal content	Mouse bioassay
McCann (2010)	Suspect	1/1	UK	ND	ND	ND
Johnson et al. (2010)	A	ND	USA	Wet hay	Feces, gastrointestinal content, and umbilical cord	Mouse bioassay
Ostrowski et al. (2012)	A	54/49	USA	Grass clippings	Grass clippings	Mouse bioassay
Johnson et al. (2015)	B	92/44	USA	ND	Feces, gastrointestinal content, serum and wound	Mouse bioassay

Author	BoNT	Affected/ dead horses	Country	Probable source of intoxication	Sampling	Method
Prutton et al. (2016)	A	1/0	USA	ND	Feces	PCR
Kasap et al. (2016)	A	1/1	Turkey		Serum	ELISA
Argenta et al. (2017)	C	7/7	Brazil	Water	ND	Mouse bioassay
Shnaiderman-Torban et al. (2018)	D	12/10	Israel	Contamination by carcass	Gastrointestinal content	Mouse bioassay
Lanci et al. (2019)	B	1/1	Italy	ND	ND	Mouse bioassay and PCR

* Four different outbreaks; BoNT = botulinum neurotoxins, ND = not determined, N/A = not available, PCR = polymerase chain reaction, ELISA = enzyme-linked immunosorbent assay.

the interruption of nerve transmission at cholinergic synapses (Rings 2004). Dysphagia, a clinical sign usually observed in botulism cases, was present in these horses and confirmed by the presence of food material in the nostrils and endoscopy (Schoenbaum et al. 2000). Reduced palpebral, anal, tail, jaw, and hypoglossal tone, as well as neck weakness, are other common signs (Johnson et al. 2010, 2015, Argenta et al. 2017, Shnaiderman-Torban et al. 2018). The clinical signs observed were similar to those described in cases reported in Brazil (Coelho et al. 2000, Argenta et al. 2017) and other countries (Kelly et al. 1984, Ricketts et al. 1984, Divers et al. 1986, Kinde et al. 1991 Schoenbaum et al. 2000, Galey 2001, Ostrowski et al. 2012). Death occurs due to failure of the diaphragmatic and intercostal musculature, resulting in respiratory paralysis (Whitlock & Buckley 1997, Galey, 2001, Whitlock & McAdams 2006, Johnson et al. 2010, Stratford et al. 2014). The complementary exams excluded other neuromuscular or primary neurologic diseases.

A definitive diagnosis requires demonstration of toxins in the plasma, liver, or gastrointestinal contents. In this study, it was possible to identify BoNTs in the cecal contents, confirming the diagnosis. In equids, cases were associated with BoNT/A-E, with descriptions of BoNT/A (Johnson et al. 2010, Ostrowski et al. 2012, Kasap et al. 2016, Prutton et al. 2016) and BoNT/D (Chambron et al. 1971, Doutre & Toure 1978, Switzer et al. 1984, Shnaiderman-Torban et al. 2018), and BoNT/E (Kelly et al. 1984, Szabo et al. 1994). This outbreak represents, to our knowledge, the first reported co-occurrence of BoNT/A and D in horses.

Therapeutic management should be guided by physical examination findings and clinical complications, such as dehydration, which necessitates correction through the administration of balanced polyionic solutions. The presence of dysphagia is a predisposing factor for the development of pneumonia in horses with botulism (Wilkins & Palmer 2003a, 2003b, Johnson et al. 2010). Flaccid paralysis and prolonged lateral recumbency were observed in most animals studied. Under these conditions, the use of a lifting system to assist in placing horses into a quadrupedal stance is essential (Ishihara et al. 2006, Schatzmann 1998). Another therapeutic option to be considered in these cases is mechanical ventilation, which has demonstrated efficacy in foals (Wilkins & Palmer 2003a, 2003b). However, its application in adult horses is limited by technical constraints.

Horses with clinical signs of botulism should immediately be confined to a stall, and physical activity should be minimal. Muscular activity rapidly depletes acetylcholine stores, thus worsening progressive weakness. A mild laxative is indicated to promote colonic evacuation and to prevent the impact of colic. Further treatment is directed at the maintenance of hydration by oral alimentation. Therapeutic modalities include gastrointestinal catharsis to remove residual toxins and prevent impaction, antimicrobial therapy targeting secondary complications such as aspiration pneumonia, and enteral feeding via a nasogastric tube (Whitlock & Buckley 1997). If antimicrobials are used, aminoglycosides should be avoided, as they may potentiate neuromuscular weakness. Horse 4 also presented with colitis, which was probably a result of antimicrobial treatment resulting in dysbiosis.

Recovery from botulism is dependent on the toxin dose and the resulting severity of the clinical disease. Dysphagic horses that can stand will gradually regain the ability to swallow. Most adult horses can eat hay and swallow grain and water for 7-10 days following treatment with antitoxin. The use of antitoxins is a challenge in cases of botulism in horses, as in Brazil, only commercial options against BoNT/A and BoNT/B are available. In North America, both polyvalent and BoNT/B-specific botulinum antitoxins are available (Sprayberry & Carlson 1997, Whitlock & McAdams 2006, Johnson et al. 2010, 2015).

In the present study, the observed recovery rate (25%, 2/8) was higher than that reported in outbreaks where animals did not receive treatment at specialized veterinary medical centers (Swerczek 1980, Divers et al. 1986, Kinde et al. 1991, Coelho et al. 2000, Argenta et al. 2017). Compared with cases admitted to veterinary teaching hospitals, the recovery rate can range from 10% to 53%, depending on the therapeutic approach and the amount of toxin ingested (Whitlock & Buckley 1997, Wilkins & Palmer 2003a, 2003b, Johnson et al. 2015).

Prevention of botulism can be based on vaccination with *C. botulinum* toxoids, with horses in endemic regions requiring annual boosters to maintain immunity (Wichtel & Whitlock 1991). However, the absence of commercially available botulism vaccines for horses in Brazil represents a significant gap in disease prevention strategies, and the development of polyvalent vaccines would be necessary in order to target the different toxins involved. This limitation highlights the need for rigorous quality control measures in equine nutrition, particularly concerning feed storage and contamination risk mitigation, to reduce the incidence of toxicosis. Contamination by *C. botulinum* should be considered within the production chain of ensiled bales, especially when it is intended for horses (Maslanka et al. 2013). Its wide environmental distribution is a determining factor for the contamination of these foods. Measures such as acidification, reduction in water activity, high concentrations of chlorides, and the presence of sodium nitrite should be implemented to prevent botulism (Bischoff &

Moiseff 2018). Proper feed preparation for horses is essential, especially when fermentation processes and the creation of an anaerobic environment are involved.

CONCLUSION

The horses in this study presented with flaccid paralysis, recumbency, and dysphagia as the main clinical signs. The identification of two types of botulinum neurotoxins (BoNT/A and D) in horse biological material confirmed the diagnosis of botulism. Educating horse owners and producers on appropriate silage handling practices is a key strategy to reduce the risk of feedborne intoxications and ensure animal health.

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