ACTA VET. BRNO 2016, 85: 071–076; doi:10.2754/avb201685010071

Botulism (type A) in a horse - case report

Sevim Kasap[1], Hasan Batmaz[1], Meric Kocaturk[1], Frank Gessler[2], Serkan Catık[1], Onur Topal[1]

[1]Uludag University, Faculty of Veterinary Medicine, Department of Internal Medicine, Bursa, Turkey
[2]Miprolab GmbH, Göttingen, Germany

Received August 3, 2015
Accepted February 10, 2016

Abstract

This paper presents the case of a six year-old, male, thoroughbred horse with clinical signs of inappetence, weakness, and incoordination when walking. Clinical examination showed that the horse staggered and leaned to the left side. Feedstuff was present inside and around its mouth. Salivation was increased and there was no reflex at the palpebrae and tongue. The horse had difficulty swallowing and the tone of its tail was reduced. Botulism was diagnosed based on the clinical signs. Antibiotic (ceftiofur) and fluid-electrolyte treatment was commenced. Next day, neostigmin was added to the horse's treatment, and it became recumbent. The horse's palpebral, tongue and tail reflexes returned partially after neostigmine methylsulphate treatment on the same day and it stood up on day four. However, it could not swallow anything during the whole week, so after getting permission from the owner, the horse was euthanized on day 10. Samples of the colonic content and blood serum were sent by courier to the laboratory for toxin neutralization, however, botulinum neurotoxins could not be detected. After that, serum samples from days 6 and 10 were sent to another laboratory for testing for botulinum neurotoxin antibodies by ELISA. Specific antibodies against botulinum neurotoxin type A were measured, indicating a previous, immuno-relevant contact with the toxin. This seroconversion for type A supports the clinical botulism diagnosis. Type A botulism is rarely seen in Europe and has been detected in a horse in Turkey for the first time.

Equine, Clostridium botulinum, botulinum neurotoxin, Turkey

Botulism is a fatal, progressive flaccid paralysis caused by the toxins of *Clostridium botulinum* (Sakaguchi 1983; Whitlock 1997). *Clostridium botulinum* is a gram-positive, obligate anaerobic spore-former (Sakaguchi 1983; Mitten et. al. 1994; Whitlock 1997; Bartlett 2000). Botulism can occur in all species of mammals and birds. The horse is extremely sensitive to botulinum neurotoxins (BoNT) (Whitlock 1997; Galey 2001). There are eight distinct BoNTs designated as types A, B, C1, D, E, F, G and recently H (Coffield and Whelchel 2007). *Clostridium botulinum* can be found in the environment, especially soil and in the gut content of animals (Sakaguchi 1983; Whitlock 1997; Galey 2001; Coffield and Whelchel 2007). It is considered to be ubiquitous and occurs worldwide. Types A and B botulisms are associated with forage or hay. Most reported cases of botulism in horses result from types B, C, and D (Adam-Castrillo et al. 2004; Whitlock and McAdams 2006). Three types of botulinum intoxication have been described in horses (Whitlock 1997; Coffield and Whelchel 2007; Wilkins 2007): 1) Forage poisoning (ingestion of the preformed toxin present in feedstuffs); 2) Wound botulism (sporulation of *C. botulinum* in wounds followed by production and systemic absorption of toxin); 3) Toxoinfectious botulism (ingestion of spores with subsequent production and absorption of toxin from the gastrointestinal tract of foals). One case of iatrogenic botulism during an experimental trial using botulinum toxin type B in horses has been reported, but this is not a common means of infection (Adam-Castrillo et al. 2004). Types A and B botulisms usually result from direct proliferation of *C. botulinum*

Address for correspondence:
Dr. Sevim Kasap
Department of Internal Medicine
Faculty of Veterinary Medicine
Uludag University, 16190 Bursa –Turkey

Phone: +90 224 294 08 14
E-mail: cetins@uludag.edu.tr
http://actavet.vfu.cz/

and subsequent exotoxin production in decaying vegetable matter. The most common types of this infection are toxicoinfectious (in foals) and food-borne (in adults) botulisms (Whitlock and McAdams 2006).

In the US many studies were conducted on botulism and the relevant toxin types. Most cases of equine botulism result from serotypes B, although type A cases have also been reported. There is significant geographical variation in serotype prevalence and associated disease. Type B predominates in the mid-Atlantic USA, whereas type A is more common in the western USA and its growth, sporulation and toxin release occur most readily in an alkaline environment (Whitlock and McAdams 2006; Whitlock 2009; Johnson et al. 2010). Outbreaks in Australia have been associated with serotype C and serotype D (Kelly et al. 1984; Hutchins 1994). In Europe, studies have shown that most cases of equine botulism result from serotypes B, C, and D (Gerber et al. 2006). In Israel type C antibodies were also reported (Steinman et al. 2007).

First clinical signs may be observed from 12 h to 10 days following toxin ingestion, with the onset and magnitude of the signs being toxin dose-dependent (Stratford et al. 2014). In peracute cases death may occur quickly within hours. Regardless of the toxin type and source of contamination, the clinical signs are the same: progressive flaccid paralysis, cranial nerve deficits such as dysphagia, poor tongue and eyelid tone, and slow pupillary light reflexes (Wilkins 2007). However, minor differences related to the toxin type may be observed (Vaala 1991; Whitlock 1997; Jahn et al. 2008).

A presumptive diagnosis of botulism can be made based on a characteristic history, clinical signs of dysphagia, reduced tongue tone and generalised myasthenia progressing to recumbency, with appropriate consideration and exclusion of common differential diagnoses (Whitlock 1997).

Case description

A six-year-old, male thoroughbred horse was referred to the Equine Clinic, Faculty of Veterinary Medicine in Bursa on June 9, 2014. The reason for admission to the clinic was inappetence, weakness, staggering gait, increased salivation and lethargy. The owner reported that the horse could not keep its neck straight, reclined toward the left side, could get the feed but could not swallow all of it. The first signs were observed five days before. The horse was fed oats, barley, corn and haylage kept in storage. The owner reported a musty smell of the barley.

Depression, staggering, leaning constantly to the left side (Plate VII, Fig. 1), and moving with a shuffling gait were the first clinical signs observed after admission. Feedstuff was present inside and around its mouth and saliva drooled. It dragged its hooves along the ground and stood with the head and neck hanging below the horizontal position. Decreased pupillary reflex was noted to light source. Decreased tongue, swallowing and tail reflexes were noted but the anal tone was normal. The body temperature was 38.6 °C, the heart rate 60/min, and the respiratory rate 24/min. Mucous membranes were hyperaemic, capillary filling time (CFT) was 3 s.

On the first day endoscopy revealed the presence of saliva and feedstuff in the nasopharynx. Hyperaemic areas and oedema were seen in the epiglottis mucosa. Haematology and biochemical indicators except mild leukocytosis (white blood cell count (WBC): 14,800/µl) and neutrophilia (neutrophil (NEU): 85.1%) were within the expected physiological ranges.

Botulism was diagnosed based on the clinical findings. Antimicrobial therapy was administered as ceftiofur 2 mg/kg intramuscularly once daily (Seftivet®, Vetaş, Istanbul). In addition, the clinical signs and haematocrit value (packed cell volume (PCV): 44%) about the hydration status were within normal ranges so intravenous (IV) hydration was

started using fluid therapy with lactated Ringer's solution with 5% dextrose (minimum 5 l. for a day). The horse became recumbent and pupillary, palpebral reflexes, and tongue (Plate VII, Fig. 2) and tail tones ceased the following day. So, neostigmine methylsulphate (Peristor®, Provet, Istanbul) was given subcutaneously at 0.025 mg/kg × 3 a day for two days. Some of the symptoms began to change on the same day including the palpebreal reflex, tail and tongue tones. The horse tried to stand up and began to drink some water but it could not masticate the feed. On the third day, defecation was observed, but aspiration pneumonia had developed. The total leukocyte count was increased (WBC: 24,500/µl). Therefore, metronidazole (Polifleks®, Polifarma) was added to the antibiotic treatment. It was given at 10 mg/kg, IV, twice a day for four days. On the fourth day, the patient stood up (Plate VIII, Fig. 3), and continued to drink water. However, the swallowing reflex did not recover. Nasogastric tube could not be swallowed; we administered intravenous fluids.

In the following days, the ability to swallow feed and water was not restored and decubital lesions occurred after lying for prolonged time due to weakness. The patient was euthanized on day 10 with the permission of the owner. On the same day, necropsy was made; no specific results were found.

Samples of the colonic content and blood serum were sent by courier to the Pendik Veterinary Control and Research Institute for toxin neutralization but BoNT could not be detected. Then the serum samples from days 6 and 10 were sent to Miprolab, Germany for the detection of specific BoNT antibodies by ELISA. In the samples antibodies against BoNT/A were detected indicating a previous, immuno-relevant contact with the antigen.

Discussion

Botulism is a disease characterised by generalized muscle weakness, dysphagia, decreased tail, eyelid and tongue tone, prolonged pupillary light reflexes (Sakaguchi 1983; Whitlock 1997). Normal laboratory rates in light of neurological deficits support the diagnosis of botulism (Whitlock 1997; Galey 2001; Coffield and Whelchel 2007; Wilkins 2007). The clinical symptoms for type A are generalized muscle weakness, dysphagia, decreased tail, eyelid and tongue tone, prolonged pupillary light reflexes, midriasis, recumbency, respiratory failure, and eventually, death (Johnson et al. 2010). Similar symptoms were observed in this case. Another report described that type A toxins resulted in cervical muscle weakness in four horses (Ostrowski et al. 2012) and also for this case the same symptom was observed. At the same time, secondary respiratory clinical signs resulting from neurological malfunction in botulism such as aspiration pneumonia should be closely investigated (Stratford et al. 2014). Johnson et al. 2015 described that pneumonia, the second-most frequent complication (24% of cases), can be caused by aspiration secondary to dysphagia and potentially worsened by compression atelectasis secondary to recumbency. In this case, aspiration pneumonia resulted from dysphagia and lack of the pharyngeal reflex. The horse stood up on day 4, however, it was euthanized on day 10 because pharyngeal reflexes did not recover and the horse became recumbent.

Diagnosis of botulism is primarily made on the basis of history and clinical signs after the exclusion of other diagnostic possibilities (Whitlock 1997; Galey 2001; Stratford et al. 2014). Differential diagnoses include but are not limited to severe electrolyte imbalance (hyponatraemia), tick paralysis, and postanaesthetic myasthenic syndrome. Definitive diagnosis is usually based on detection of the toxin in serum, gastrointestinal contents or feed (Wilkins 2007; Whitlock 2009; Stratford et al. 2014) by enyzme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), passive haemagglutination, and polymerase chain reaction (PCR). All these tests have been described for identification of the botulinum toxin (Wilkins 2007). However, due to the high affinity of the toxin to the cholinergic synapses, the BoNT concentration in serum is often below the limit of detection

of the assays even in clinically evident cases. In this case the serum and gastrointestinal contents were shipped to the Pendik Veterinary Control and Research Institute for toxin neutralization. However, BoNT could not be detected in these sample matrices. The use of ELISA for detection of antibody in the serum has been helpful to detect type C and D toxins in horses and cattle (Whitlock 1997). A number of studies about ELISA are available. In the study of Krüger et al. (2014) BoNT/C and BoNT/D were detected using ELISA in 53% and 18%, respectively, of the cows that exhibited clinical symptoms of chronic botulism. On the other hand, in the study of Steinman et al. (2007), 31% of 198 healthy horses in Israel tested positive by ELISA for BoNT type C IgG antibodies. In unvaccinated horses, testing of specific antibodies against BoNT may be used as a surrogate to indicate a previous contact with the antigen confirming the clinical diagnosis (Mayhinney et al. 2012). Therefore, ELISA is a useful method to detect botulism for confirmation of the clinical suspicion. In this case antibodies against type A were detected.

We applied symptomatic therapy (Whitlock and McAdams 2006; Wilkins 2007; Reed 2010) and added neostigmine methylsulphate. Because neostigmine methylsulphate is an acetylcholinesterase inhibitor, it is thought to restore motility of the large colon in cases of large colon impaction in the horse and to restore gastrointestinal motility and its effect on selected hypodynamic gastrointestinal disorders in horses and cattle (Steiner and Roussel 1995; Nieto et al 2013). Another clinical sign is generalised myasthenia progressing to recumbency (Whitlock 1997; Whitlock and McAdams 2006). The aim of using neostigmine methylsulphate was to decrease these signs. Additionally, a low dose of neostigmine methylsulphate was administered for another effect, as it increases saliva secretion and the patient did not have the swallowing reflex.

Several reports of equine botulism have been published in many parts of the world over the last decade (Hunter et al. 2002; Goehring et al. 2005; Gerber et al. 2006; Steinman et al. 2007; Jahn et al. 2008; Whitlock 2009; Johnson et al 2010; Ostrowski et al. 2012). In the United States, nine reports describing naturally occurring cases of type B or C botulism were identified. Three reports described individual cases of toxicoinfectious or wound botulism (Mitten et al. 1994; Mackay and Berkhoff 1982; Swerczek 1980). Two reports described case series of type B toxicoinfectious botulism in foals from Kentucky and the mid-Atlantic region (Hunter et al. 2002; Wilkins and Palmer 2003), and two described outbreaks of type B food-borne botulism in groups of horses in states east of the Mississippi River (North Carolina and Tennessee) (Wichtell and Whitlock 1991; Kinde et al. 1991). Finally, two reports described outbreaks of type C botulism in groups of horses in western states (California and Arizona) linked to carrion contamination of feed or feeding areas (Gudmundsson 1997; Schoenbaum et al. 2000). Several outbreaks of botulism have been reported in Europe. Gudmundsson (1997) described two suspected outbreaks of BoNT/B intoxication in Iceland. Bakos et al. (2000) reported a case in Hungary; McCann (2000) reported a suspected case in England. Wollanke (2004) described one botulism outbreak in Germany. Goehring et al. (2005) described suspected cases of botulism between 2000 and 2004 in the Netherlands. Jahn et al. (2008) reported two cases of botulism in the Czech Republic. Gerber et al. (2006) conclude that the incidence of equine botulism in Europe is increasing because no cases of botulism in Switzerland had been reported in the literature in the previous century but 38 horses were admitted to the Equine Clinic of the Veterinary Faculty in Bern since 2001. And also, Steinman et al. (2007) detected type C antibody in serum samples in Israel. Johnson et al. (2010) reported the first outbreaks of type A botulism in horses in the United States. Although we lack the demonstration of BoNT/A in the clinical samples, the clinical signs and the positive BoNT/A antibody titre strongly support the assumption of a BoNT/A intoxication in this case, which would be the first BoNT/A case observed in horses in Turkey.

References

Adam-Castrillo D, White NA, Donaldson LL, Furr MO 2004: Effects of injection of botulinum toxin type B into the external and sphincter on anal pressure of horses. Am J Vet Res **65**: 26-30

Bakos Z, Varos K, Bodo G, Biksi I, Sztojkov V 2000: Clinical diagnosis of botulism in a horse. Case report (in Hungarian). Magy Allatorvosok **122**: 79-83

Bartlett JG 2000: Botulism. In: Goldman L, Bennet JC (Eds): Cecil Textbook of Medicine. WB Saunders, London.

Coffield JA, Whelchel DD 2007: Botulinum neurotoxin. In: Gupta RC (Ed.): Veterinary Toxicology. Elsevier Saunders, London, pp. 755-770

Galey FD 2001: Botulism in the horse. Vet Clin North Am Equine Pract **17**: 579-588

Gerber V, Straub R, Frey J 2006: Equine botulism and acute pasture myodystrophy: New soil-borne emerging disease in Switzerland? Schweiz Arch Tierheilk **148**: 553-559

Goehring LS, van Maanon C, van Oldruitenborghoosterbaan MMS 2005: Neurological syndromes among horses in the Netherlands. A 5 year retrospective survey (1999-2004). Vet Quart **27**: 11-20

Gudmundsson SH 1997: Type B botulinum intoxication in horses: case report and litareture review. Equine Vet Educ **9**: 156-159

Hunter JM, Rohrbach BW, Andrews FM, Whitlock RH 2002: Round bale grass hay: a risk factor for botulism in horses. Compend Contin Educ Pract Vet **24**: 166-169

Hutchins R 1994: Preliminary report on an outbreak of botulism. Aust Equine Vet **12**: 54-55

Jahn P, Ludvikova E, Chmelar D, Kalova L 2008: Botulism in horses: a case report. Vet Med-Czech **53**: 680-684

Johnson AL, McAdams SC, Whitlock RH 2010: Type A botulism in horses in the United States: a review of the past ten years (1998-2008). J Vet Diag Invest **22**: 165-173

Johnson AL, McAdams-Gallagher SC, Aceto H 2015: Outcome of adult horses with botulism treated at a veterinary hospital: 92 cases (1989-2013). J Vet Intern Med **29**: 311-319

Kelly AR, Jones RJ, Gillick JC. Outbreak of botulism in horses 1984: Equine Vet J **16**: 519-521

Kinde H, Bettey RL, Ardans A, Galey FD, Daft BM, Walker RL, Eklund MW, Byrd JW 1991: *Clostridium botulinum* type-C intoxication associated with consumption of processed alfalfa hay cubes in horses. J Am Vet Med Assoc **199**: 742-746

Krüger M, Neuhaus J, Herrenthey AG, Gokce MM, Schrödl W, Shehata AA 2014: Chronic botulism in a Saxony dairy farm: sources, predisposing factors, development of the disease and treatment possibilities. Anaerobe **28**: 220-225

MacKay RJ, Berkhoff GA 1982: Type C toxicoinfectious botulism in a foal. J Am Vet Med Assoc **180**: 163-164

Mayhinney I, Palmer D, Gessler F, Cranwell M, Foyle L, Otter A, Payne J, Stugnell B 2012: Investigation of serology for diagnosis of outbreaks of Botulism in cattle. Vet J **192**: 382-384

McCann JL 2000: A suspected case of botulism in a horse. Equine Vet Educ **12**: 114-119

Mitten LA, Hinchcliff KW, Holcombe SJ, Reed SM 1994: Mechanical ventilation and management of botulism secondary to an injection abscess in an adult horse. Equine Vet J **26**: 420-423

Nieto JE, Morales B, Yamout SZ, Stanley SD, Harmon FA, Synder JR 2013: In vivo and in vitro effects of neostigmine on gastrointestinal tract motility of horses. Am J Vet Res **74**: 579-588

Ostrowski SR, Kubiski SV, Palmero J, Reilly CM, Higgins JK, Cook-Cronin S, Tawde SN, Crossley BM, Yant P, Cazarez R, Uzal FA 2012: An outbreak of equine botulism type A associated with feeding grass clippings. J Vet Diagn Invest **24**: 601-603

Reed SM 2010: Botulism. In: Reed SM, Bayly WM, Sellon DC (Eds): Equine Internal Medicine. Saunders, St. Louis, pp. 641-642

Sakaguchi G 1983: *Clostridium botulinum* toxins. Pharmacol Ther **9**: 164-194

Schoenbaum MA, Hall SM, Glock RD, Grant K, Jenny AL, Schiefer TJ, Sciglibaglio P, Whitlock RH 2000: An outbreak of type C botulism in 12 horses and a mule. J Am Vet Med Assoc **217**: 365-368

Steinman A, Kachtan I, Levi O, Shpigel NY 2007: Seroprevalence of antibotulinum neurotoxin type C antibodies in horses in Israel. Equine Vet J **39**: 232-235

Steiner A, Roussel AJ 1995: Drugs and restoring gastrointestinal motility and their effect on selected hypodynamic gastrointestinal disorders in horses and cattle. Zentralbl Veterinarmed A **42**: 613-631

Stratford CH, Mayhew IG, Hudson NPH 2014: Equine botulism: A clinical approach to diagnosis and management. Equine Vet Educ **26**: 441-448

Swerczek TW 1980: Toxicoinfectious botulism in foals and adult horses. J Am Vet Med Assoc **176**: 217-220

Vaala WE 1991: Diagnosis and treatment of *Clostridium botulinum* infection in foals: a review of 53 cases. Proc Am Vet Intern Med **9**: 379-381

Wichtell JJ, Whitlock RH 1991: Botulism associated with feeding alfalfa hay to horses. J Am Vet Med Assoc **199**: 471-472

Whitlock RH 1997: Botulism. Vet Clin North Am Equine Pract **13**: 107-128

Whitlock RH, McAdams S 2006: Equine Botulism. Clin Tech Equine Pract **5**: 37-42

Whitlock RH 2009: Botulism (Shaker Foals, Forage Poisoning). In: Smith BP (Ed.): Large Animal Internal Medicine. Mosby Elsevier, St. Louis, pp. 1096-1101

Wilkins PA, Palmer JE 2003: Botulism in foals less than 6 months of age: 30 cases (1989-2002). J Vet Intern Med **17**: 702-707
Wilkins PA 2007: Botulism. In: Sellon DC, Long MT (Eds): Equine Infection Disease. WB Saunders, London, pp. 372-376
Wollanke B 2004: Botulism in einem Bestand mit 16 Preferden und Ponys. Praktischer Tierartz **85**: 252-261

Fig. 1. The horse was leaning its head to the left side on the first day at the clinic.

Fig. 2. Paralysis was seen in its tongue on the second day.

Fig. 3. After treatment with neostigmine, the horse stood up on day 4 of hospitalization. Salivation continued to increase, because swallowing was not restored.