Botulism in horses: a case report

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ABSTRACT: Two cases of botulism in horses are described in the article. In the first case two horses died, one survived and recovered after four weeks. Botulotoxin type B was detected using a mouse bioassay in the gastrointestinal content of both dead horses; *Clostridium botulinum* bacteria were cultivated from one of them. In the second case two horses were affected. One of them was euthanized because of persistent recumbency, the second one recovered after six weeks. Detection of botulotoxin in the serum of the dead horse using the mouse bioassay was not successful.

Keywords: intoxication; *Clostridium botulinum*; botulotoxin

Botulism is a disease characterised by progressive flaccid paralysis. It can occur in all species of mammals and birds. The horse as a species is extremely sensitive to botulotoxin (Whitlock and Buckley, 1997; Galey, 2001). The disease is caused by exotoxins produced by *Clostridium botulinum*, an obligate anaerobic, spore-forming, gram-positive rod. The bacterium is ubiquitous and is found in soils and organic matter worldwide. Eight different botulinum toxins are produced and are designated as types A, B, C_1, C_2, D, E, F and G (Coffield and Whelchel, 2007). Three types of botulinum intoxication have been described in horses (Whitlock and Buckley, 1997; Coffield and Whelchel, 2007; Wilkins, 2007):

(1) Forage poisoning (ingestion of the preformed toxin present in feedstuffs).

(2) Wound botulism (sporulation of *Cl. 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).

Several reports of equine botulism in Europe have been published during the last decade. Gudmundsson (1997) reported two suspected outbreaks of botulotoxin type B intoxication in Iceland. In the first of them four horses died and one was euthanized, in the other 14 horses displayed clinical signs of botulism and three of them were euthanized. Bakos et al. (2000) described a case of equine botulism in Hungary; McCann (2000) described one suspected case in England. Wollanke (2004) reported on a botulism outbreak in a group of 16 horses and ponies in Germany of which nine died or had to be euthanized. Goehring et al. (2005) reported suspected cases of equine botulism in at least four premises in The Netherlands during a five year period (2000 to 2004). Gerber et al. (2006) argue that the incidence of equine botulism in Europe is increasing because 38 horses with symptoms highly suggestive of botulism had been referred to the equine clinic of the veterinary faculty in Bern since November 2001 whereas no cases of botulism in Switzerland were described in the literature of the last century.

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Botulism in horses has never occurred in the Czech Republic to the best of our knowledge. Two cases of this disease are described in the present article.

CASE 1

History

A two-year-old thoroughbred colt was referred to the Equine clinic, University of Veterinary and Pharmaceutical Sciences in Brno on 5th February 2002. The horse originated from a racing stable, where he was stabled with another 20 horses. The reason for admission to the clinic was lethargy, inapetence, weakness and shivering of pectoral muscles and front limbs which had lasted three days.

Similar clinical signs had been shown by one filly and one colt from the same stable two days before the onset of signs in this patient. Progress of the disease was very rapid in these two horses. Both of them became recumbent and unable to stand during the following 24 hours, therefore both were euthanized. Non specific results were found during necropsy. Because of the suspicion of intoxication, samples of the stomach and colonic content were taken from both horses and frozen. All horses were fed on oats, barley, corn and haylage stored in plastic bales.

Clinical course and outcome

Depression, a wide based stance and shivering of front limbs were the first clinical signs observed in our patient after admission. Weakness with a shortened stride, especially in the front limbs, was observed during movement. Feedstuff was present in both nostrils. The body temperature was 38.8°C, heart rate 60, respiratory rate 28. Mucous membranes were injected, CRT 3 s, gut sounds bilaterally decreased. Symmetrical mydriasis and decreased pupillary light response were noted.

Endoscopy revealed the presence of feedstuff in both nasal passages and nasopharynx. There were no pathological changes in the trachea and guttural pouches. Gastroscopy revealed that the content of the stomach was dehydrated. Visible portions of the gastric as well as oesophageal mucosa were normal. Rectal examination revealed only dehydrated content in the small colon and rectum. Haematological and biochemical tests were within the reference ranges. On the basis of history and clinical signs botulism was suspected. Twenty litres of normal saline were administered intravenously to the patient. General status of the horse improved and gut sounds increased after this treatment. The horse was able to chew hay and tried to swallow it but most of the feed was expectorated. Also any attempt to drink was followed by the water being discharged from the nostrils. Therefore an indwelling oesophageal tube was inserted and the horse was hydrated by this tube during the consecutive three days.

The ability to swallow feed and water returned on the third day of hospitalization and improved progressively. The horse was discharged from the hospital on the tenth day. According to information from the trainer the horse recovered fully after four weeks in the home stable and returned to race training.

Laboratory confirmation of botulism

The trainer of the horse was informed about the suspected diagnosis of botulism immediately after the suspicion was pronounced on the basis of clinical examination. The frozen samples of stomach and colonic content of both euthanized horses from the same racing stable were sent by messenger to the Czech National Reference Laboratory for Anaerobic Bacteria in Ostrava.

The samples of the stomach and colonic content of the euthanized filly and colonic content of the colt were cultivated using Wilkins-Chalgren Agar in the MACS 1000 box for anaerobic cultivation (Don Whitley Scientific Ltd.); cultivation time was 48 hours. *Cl. perfringens* and *Cl. bifermentas* were found in the colonic content and *Cl. perfringens* in the stomach content of the euthanized filly, *Cl. botulinum* and *Cl. perfringens* in the colonic content of the colt. Using the identification kit ANAEROtest 24 (Pliva-Lachema, Brno, Czech Republic), production of botulotoxin type B (BoNT type B) was confirmed in the *Cl. botulinum* strain. BoNT type B production was evidenced by mouse inoculation using monovalent antisera (Imuna Sarisske Michalany, Slovakia).

The presence of botulotoxin in prepared stomach and colonic content of the euthanized filly and colonic content of the colt was identified by a mouse bioassay. Botulotoxin was further specified as type B (BoNT type B) using monovalent antisera (Imuna Sarisske Michalany, Slovakia) in all of the three samples.

CASE 2

History

A six-year-old akhal-teke stallion was referred to the Equine Clinic University of Veterinary and Pharmaceutical Sciences in Brno on 27th June 2007 after twelve days of eating difficulties (slow chewing and swallowing) and weight loss. On the day of admission muscle trembling was observed by the owner.

The horse shared a stable with six other horses. Slow chewing and swallowing had been observed in one of them (an eleven-year-old akhal-teke stallion) nine days before. Muscle trembling and weakness appeared four days later in this horse. The horse became recumbent after two days but was still alert and with a good appetite. Heart rate was elevated, body temperature was normal. The horse was euthanized on the seventh day because of persisting recumbency.

Both horses were imported in November 2006 from Russia and stabled in the same facility but were not in close contact. The horses were in separate paddocks but two weeks previously they had shared the same paddock. Five other horses in the stable were without clinical signs of disease. All horses were fed on the same hay, grain and commercial vitamin supplement. Neither silage nor haylage were fed in this stable.

Clinical course and outcome

The horse was alert, with a good appetite. Body condition worsened with a resulting wasp-tail shaped abdomen. The body temperature was 38.2°C, heart rate 40, respiratory rate 16. Muscle trembling of the hind legs was observed at the time of admission but it disappeared after a short rest. Clinical examination did not reveal any pathologic changes. Pupils were of normal size and responsive to light. Tail tonus was normal and the eyelids were symmetric without ptosis. Also, tonus of the tongue was normal and the horse was able to retract it into the oral cavity. However, chewing was very slow and with an extended neck. Therefore the standard Whitlock and Buckley (1997) test for the amount of grain consumed was performed. The time needed for uptake of 250 ml of oats was four minutes. Five control horses that are kept at the clinic for teaching purposes were able to eat the same amount in less then two minutes.

Endoscopic examination did not reveal any pathology within the respiratory tract and oesophagus (including guttural pouches). During endoscopy muscle tremor was observed. Routine haematological and biochemical tests did not reveal any pathologic changes.

During the next six days no changes in the clinical status were observed. Slow chewing of oats and hay persisted but the horse did not lose weight. The horse was discharged from the clinic on the eighth day. A private practitioner examined the horse including its haematology and biochemistry after one month. The clinical status of the horse was without improvement, haematological and biochemical tests did not reveal any pathologic findings. Improvement in the chewing ability and body condition was reported two weeks later. One year after discharge from the clinic the horse is used successfully for endurance riding.

Laboratory confirmation of botulism

A suspicion of botulism was pronounced on the basis of history and clinical signs observed in the horse after admission to the clinic. A sample of the serum of the euthanized horse from the same stable was sent to the laboratory of the State Veterinary Institute in Prague and examined by a mouse bioassay but the presence of botulotoxin was not confirmed.

DISCUSSION

Clinical signs of botulism include dysphagia, flaccid paralysis, diminished pupillary reaction, decreased eyelid, tongue and tail tone and progressive flacid tetraparesis and tetraplegia progressing to recumbency. The onset of clinical signs begins a few hours or days after botulotoxin ingestion. Normal laboratory values in light of neurological deficits support the diagnosis of botulism (Whitlock and Buckley, 1997; Galey, 2001; Coffield and Whelchel, 2007; Wilkins, 2007).

Most of the above clinical signs were present in Case 1. Clinical signs in Case 2 were milder and included weakness, slow intake of hay and grain despite a good appetite and muscle trembling. The pupil size, tongue and tail tone were normal. All euthanized horses suffered pronounced weakness and muscle trembling leading to recumbency.

The onset and severity of clinical signs are toxin-dose dependent. A small amount of botulotoxin (10^3 IU) causes the onset of clinical signs within three to seven days. Affected horses can show only mild dysphagia and recovery time is about one week without therapy. The development of clinical signs is very rapid in cases of a large amount of botulotoxin (10^8 IU) intake. Affected horses may be recumbent within 8–12 hours of toxin intake. Rapid development of clinical signs has a poor prognosis (Whitlock and Buckley, 1997). Because of the different clinical course of botulism in horses from Case 1 we can suppose an unequal distribution of *Cl. botulinum* toxin in the haylage. The largest amount of toxin was probably ingested by the two euthanized horses. The horse from Case 1, which was admitted to the clinic probably ingested a smaller amount of toxin and recovered with only supportive therapy. Other horses in the stud farm were affected very slightly or not at all because no clinical signs were noticed by the trainer. They could have been limited to very mild dysphagia or slow intake of feedstuff.

A tentative diagnosis of botulism in a horse can be made on the basis of history and comprehensive neurological assessment after exclusion of other diagnostic possibilities (Coffield and Whelchel, 2007; Whitlock and Buckley, 1997). It is possible to support the clinical diagnosis of botulism by needle electromyography (Wilkins, 2007) but this method was not available in our clinic at the time of hospitalization of both of these cases. The differential diagnoses of botulism includes various encephalitides, EHV 1 infection, exposure to a number of toxicants like heavy metals, insecticides, cholinesterase inhibitors, ionophore antibiotics, mold toxins, pharmaceutic agents (Galey, 2001), severe electrolyte imbalances (hyponatremia), tick paralysis, postanethetic myasthenic syndrome (Wilkins, 2007), guttural pouch mycosis, listeriosis, equine motor neuron disease, hyperkalemic periodic paralysis, white muscle disease and pharyngeal ulceration (Coffield and Whelchel, 2007).

Botulotoxin intoxication was suspected on the basis of history, clinical signs and course of the disease in both hospitalized horses. All the clinical signs that were observed in the case 1 horse are typical for botulism. Also, the information about the feeding of haylage in the history is important as this is a potential source of the botulotoxin. If the grass that is used for the haylage production is cut shorter than 10 cm from the ground, there is a higher risk of contamination of the foodstuff by the soil containing spores of *Cl. botulinum* (Wollanke, 2004; Gerber et al., 2006). If the ensiling process in the plastic bag is incomplete or circumvented, the pH level does not fall to below 6 and the environment becomes suitable for anaerobic growth of *Clostridium* sp. (Galey, 2001). It was not possible to identify the source of botulotoxin in our case because of the prompt burning of suspected feedstuff by the trainer.

In Case 2 the clinical signs were not expressed as intensively as in Case 1. Diagnosis was supported by the test for consumption of a standard amount (250 ml) of oats. Four minutes were necessary for our patient despite the brightness and good appetite of the horse, whereas five control horses needed less than two minutes which is the time required for a healthy horse according to Whitlock and Buckley (1997).

The probable source of toxin in Case 2 was not found. The surviving horse and the dead horse shared the same paddock two weeks before the onset of clinical signs in the surviving horse. *Cl. botulinum* spores could be present in the soil of the paddock. However, toxoinfectious botulism in which the microorganism grows in the gut leading to toxin production occurs in foals (Galey, 2001) and is not typical for adult horses. The source of the botulotoxin was most probably hay contaminated with spores.

Definitive diagnosis of botulism is very often difficult to establish because of the absence of gross pathognomic and histopathologic lesions. The gold laboratory standard for botulism diagnosis is mouse bioassay. It is usually based on the detection of toxin in serum, faeces, gastrointestinal contents or feed. The test is highly specific but the sensitivity may be limited due to higher sensitivity of horses to botulotoxin in comparison with the mouse (Galley, 2001; Coffield and Whelchel, 2007). The diagnosis was confirmed by mouse bioassay in two euthanized horses from Case 1 by identification of the toxin in their stomach and colonic contents but not in the serum of the euthanized horse from Case 2. The plasma/serum concentration of circulating toxin is usually very low in the horse. Because the horse is more sensitive to botulotoxin than the mouse, a mouse bioassay using serum is most valuable in early, peracute equine botulism when a higher concentration of toxin may be present in the bloodstream. Therefore false negative results must be taken into account when using this method (Whitlock and Buckley, 1997; Galey, 2001).

From the cases mentioned above, only Bakos et al. (2000) were successful in confirming botulism in the plasma of horses with clinical signs of botulism. None of the cases diagnosed clinically in Switzerland could be confirmed by serum testing (Gerber et al., 2006).

Wollanke (2004) also did not confirm botulotoxin in the blood or serum of four horses with botulism.

A greater diagnostic success may be achieved through the detection of botulotoxin and/or spores of *Cl. botulinum* in foodstuff in association with clinical signs (Galley, 2001). Gudmundsson (1997) supported the diagnosis of botulism by botulotoxin detection in a bird carcass that was found at the feeding place of horses with clinical signs. Other possibilities of laboratory diagnostics include ELISA detection of serum antitoxin antibodies in unvaccinated horses with clinical signs (Whitlock and Buckley, 1997).

The anaerobic cultivation of the gastrointestinal tract content for the detection of spores is not considered to be a reliable method for the confirmation of botulism in the horse since spores may be present in the faeces of healthy horses (Galey, 2001; Coffield and Whelchel, 2007). On the other hand the percentage of spore shedders was higher in the group of horses with clinical signs of botulism than in the control group in one study (Whitlock and Buckley, 1997). Because of the higher sensitivity of the horse to botulotoxin in comparison to mouse and low sensitivity of current laboratory methods for botulotoxin, the diagnosis of botulism is only clinical in many cases (Galey, 2001).

Samples that cannot be shipped to the laboratory for several days can be frozen. Freezing may compromise the detection of *Cl. botulinum* bacteria but will not affect toxin detection (Coffield and Whelchel, 2007). This way of sample storage was also used in our Case 1.

Toxin types A, B, C and D are found in clinical cases of botulism in horses (Wilkins, 2007). The botulotoxin found in the gastrointestinal content of two horses in Case 1 was identified as type B. This type of botulotoxin was determined as a cause of botulism in Europe in Iceland (Gudmundsson, 1997). Bakos et al. (2000), McCann (2000), Wollanke (2004) and Gerber et al. (2006) did not identify the type of toxin in the cases of equine botulism that they described. Goehring et al. (2005) found antibodies against type A, B and D in horses from premises suspected of having cases of equine botulism. Botulotoxin type B is also the most commonly identified type of botulotoxin in horses in the USA (Coffield and Whelchel, 2007).

Coffield and Whelchel (2007) stated that adult horses that ingest low doses of toxin may show only mild dysphagia and recover with minimal treatment. Some muscle wasting may take months to resolve (Galey, 2001). However, surviving horses in our cases recovered fully but it took at least four weeks in Case 1 and seven weeks in Case 2.

The two cases of botulism in horses described here highlight the increasing incidence of this disease in Europe.

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