Outbreak of botulism in a dairy herd in Turkey

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In this study, the clinical findings and results of haematological and biochemical analyses of 26 cattle with botulism were evaluated. The most important clinical signs in the affected cattle included: decreased appetite, ataxia, difficulty to rise, loss of tongue tone, salivation and bradycardia. A definitive diagnosis of botulism was based on demonstration of the preformed toxin in ruminal and intestinal contents and feed materials including poultry litter, by mouse inoculation test. This study is the first confirmation, by direct toxin isolation, of Clostridium botulinum type C and Clostridium botulinum type D in cattle, in Turkey.

Key words: Cattle, botulism, toxin, type C, type D

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Introduction

Botulism is caused by a neurotoxin produced by *Clostridium botulinum*, a gram-positive, spore-forming anaerobe. Botulinum toxin is an exotoxin produced during the growth and autolysis phase of the organism under anaerobic conditions (Radostits *et al.,* 1989; Smith, 1990). Eight known botulinum toxins, A, B, Ca, Cb, D, E, F and G, have been identified. Disease in cattle is produced primarily by types C and D. *Clostridium botulinum* types C and D produce potent toxins in carrion, feed contaminated with dead rodents, birds or reptiles, or any rotting material (Radostits *et al.,* 1989; Smith, 1990; Böhnel, 1999). This study is the first confirmation, by direct toxin isolation, of *Clostridium botulinum* type C and *Clostridium botulinum* type D in cattle, in Turkey.

Materials and methods

The study was conducted on a Holstein Friesian breeding farm near the town of Bandirma in Balikesir. The herd consisted of 105 cattle. Clinically, 26 cattle including milking cows were found to be suffering from different degrees of suspected botulism. They ranged in age from four to eight years and had been ill for between two and eight days. A routine clinical examination of the animals, including body temperature, pulse, respiratory rates and ruminal movements, was performed. The neurological examination included an assessment of each affected animal's mental status, gait, pupillary light reflexes, anal reflexes, tongue reflexes, swallowing reflexes, tail tone and sensitivity to pricking with a needle. In the detailed history, the owner reported that the milking cows' feed, in addition to grain, haylage and silage, included ensiled poultry litter. It was reported that dry cows, heifers and calves were unaffected. These animals were fed different rations, without poultry litter. Routine haematological values, including haematocrit, haemoglobin, erythrocyte, total white cell and platelet counts, were determined by a haemocell counter (Cell Dyn 3500; Abbott Inc., USA). The concentrations of serum urea, creatinine, aspartate aminotransferase (AST), creatinine kinase (CK) and, potassium (K) were assessed (Reflotron; Boehringer&Monnheim Inc., Germany). The levels of total protein, calcium (Ca) and phosphorus (P) were determined in serum by spectrophotometer (Labospec; Germany).

As a first step in the definitive diagnosis of botulism, ruminal contents and serum from all affected animals and their feed materials were collected, immediately cooled and frozen as soon as possible. In addition, the contents of the small and large intestines were sampled from five dead animals. Samples were sent to the Veterinary Control and Research Institute (Pendik, Istanbul). A definitive diagnosis was performed by mouse inoculation test, as described by Gessler *et al.* (2005), on samples of feed material including grain, haylage, silage, ensiled poultry litter, serum, ruminal and intestinal contents from affected animals. For toxin typing, a neutralisation test was performed by demonstrating mouse lethality on intraperitoneal injection of the specimen extracts with specific botulinum antitoxins A through E. The mice were observed for clinical signs of botulism including respiratory distress, progressive paralysis or death over a period of four days (Böhnel *et al.,* 2001; Gessler *et al.,* 2005). The neutralisation was considered successful if the animals with the homologous antiserum survived and if those with the heterologous antiserum died.

Initially, feed contaminated with poultry litter was removed from the meal. The cattle were treated with the intravenous administration of 10 to 15 litres of lactated Ringers solution and 5% dextrose per day (Eczacibasi, Istanbul,

Figure 1: Recumbent cattle adopted a posture where the head was turned back against the flank.

Turkey), and oral administration of activated charcoal (Eucarbon; Santa Farma Inc., Istanbul, Turkey) at a rate of 1g/kg PO. After about 30 minutes, sodium sulphate (1g/kg PO) was administered. In addition, 0.005 mg/kg neostigmine (Neostigmin; Adeka Inc., Istanbul, Turkey)was administered subcutaneously. In view of the risk of aspiration pneumonia, cefquinome (Cobactan, Intervet Inc., Istanbul, Turkey) was administered intramuscularly to each animal at a dosage of 1 mg/kg body weight, each day for five days.

Results

In all the affected cattle, the appetite, ataxia, inability to rise, loss of tongue tone, decreased upper eyelid and tail tone, salivation, decreased ruminal movements were observed to varying degrees (**Table 1**).

Eleven of the cattle, based on auscultation of the lungs. In addition, cattle that were recumbent adopted a posture wherein the head was turned back against the flank, similar to that seen with hypocalcaemia (**Figure 1**). In the terminal stages of the disease, all the cattle were recumbent, had hypothermia and their respiration was laboured. Haematological results were within normal limits, except for haematocrit, total white cell count and differentials in some animals. Twelve of the cattle had leucocytosis (mean: 15.2 ± 0.9 x 10^9 cells/L, normal reference range: 4-12 x 10^9 cells/L) along with neutrophilia (mean: 13.1 ± 0.9 x 10^9 cells/L , normal reference range 0.6-4 x 10^9 cells/L). In addition, 15 of the cattle had a high hematocrit (mean: 47.5 ± 2.1 %, normal reference range: 24-46%). There was a slight increase in the activities of AST (272.5 ± 21.3 U/L, normal reference range: 60-150 U/L) and CK (mean 315.9 ± 25.8 U/L, normal reference range: 46-169 U/L) in 14 of the recumbent animals. In addition, the concentrations of serum urea (mean: 68.2 ± 6.9 mg/dL, normal reference range: 6-27 mg/dL), creatinine (mean: 3.4 ± 0.3 mg/dl, normal reference range: 1-2.7 mg/dL), total protein (mean: 8.1 ± 0.4 g/dL, normal reference range: 6.6-7.8 g/dL) and potassium (mean: 6.4 ± 0.4 mEq/L, normal reference range: 3.9-5.8 mEq/L) were high in recumbent cows. Other biochemical parameters were normal.

Serum samples, and feed materials such as grain, haylage and silage, were negative for botulinum toxin by mouse inoculation bioassay. However, botulism was confirmed when mice were injected with specimen extracts including the ruminal, intestinal contents, and the ensiled poultry litter. These mice developed the 'wasp-waist' sign, respiratory distress, progressive paralysis and death within 24 hours. Only type C and type D antitoxins afforded protection, evidenced by survival of the mice. Consequently, *C. botulinum* type C and D toxins were identified in all affected animals by mouse inoculation bioassay (Veterinary Control and Research Institute, Pendik, Istanbul) by using the filtered ruminal, intestinal contents and the feed material containing ensiled poultry litter.

In spite of therapy, there was no clinical improvement in the animals. Progressive paralysis, with difficulty swallowing, occurred. By day eight, a total of 26 cows had died. No pathognomic changes were observed at necropsy, except for aspiration pneumonia and pulmonary oedema.

Discussion

Botulism is a potentially lethal paralytic disease caused by *Clostridium botulinum*. This bacterium grows best in neutral or alkaline conditions and produces toxins in anaerobic environments such as decaying vegetable matter and animal carcasses. Where cattle subsist on a phosphorus-deficient diet, exhibit osteophagia and ingest carrion, the disease is likely to occur in outbreak form. Types C and D are particularly well associated with carrion feed contaminated with dead rodents, cat, birds, or reptile, or any rotting material, and types C and D cases occur as outbreaks in cattle fed poultry litter (Smart *et al.,* 1987; Neill *et al.,* 1989; Radostits *et al.,* 1989, Smith, 1990; Jean *et al.,* 1995, Galey *et al.,* 2000). In this study, *C botulium* type C and D toxins were found in the ruminal, intestinal contents, serum and the ensiled poultry litter. Ensiled poultry litter was considered as the likely source of the toxins.

The most distinctive symptoms of botulism type C or D intoxication are ataxia, progressive paralysis, dysphagia, salivation, bradycardia, loss of tongue tone, decrease in the muscle tone of the tail and rumen, dilated pupils presence of dry and hard faeces in the rectum and a characteristic recumbency (Haagsma and Ter Laak, 1979 ; Radostits *et al.,* 1989; Smith, 1990 ; Van Der Lught *et al.,* 1995; Braun *et al*, 2005). Loss of tongue tone is considered to be the most specific and sensitive clinical sign for botulism in cattle.

These signs are associated with the irreversible binding of botulism neurotoxins at the presynaptic site of the neuromuscular junction. This binding inhibits the release of acetylcholine, resulting in flaccid paralysis. Cattle affected from botulism in this report had similar clinical signs including hyporeflexia, decreased tongue tone, decreased pupillary and anal reflexes, ataxia, recumbency, salivation, constipation and difficulty swallowing (**Table 1**).

In this clinical study, tentative diagnosis of botulism was based on the animals' history, and clinical findings. Other diseases that have similar clinical signs, such as listeriosis, paralytic rabies, tick paralysis, hypocalcaemia, poisoning by organophosphates or carbamates, were considered in the differential diagnosis (Radostits *et al.,* 1989; Smith, 1990). To definitively diagnose a suspected outbreak of *C. botulinum* intoxication, however, the neurotoxin must be detected in serum, ruminal fluid, or the tissue of affected animals. The mouse inoculation test is still the most reliable method for diagnosing botulism (Böhnel *et al.,* 2001). Therefore, a definitive diagnosis was confirmed via the mouse toxicity test, by the identification of type C and type D toxins from ensiled poultry litter and ruminal and intestinal contents of affected cattle. Enzyme-linked immunoassays (ELISA) are also available for identifying neurotoxins but are currently less sensitive than the mouse inoculation test. An enzyme-linked immunosorbent assay (ELISA) has been used for types C and D toxin (Thomas, 1991) and for antibodies to types C and D toxins (Gregory *et al*, 1996).

None of haematology results were indicative of botulism (Radostits *et al.,* 1989; Smith, 1990, Van Der Lugt *et al.,* 1995; Braun *et al.,* 2005). Serum urea and creatinine concentrations, indicators of glomerular filtration, were moderately high in cattle with botulism in this case, probably due to loss of normal ability to urinate, atonic bladders (Smith, 1990) and dehydration as a result of reduction in water intake (Braun *et al.*, 2005). Similarly, a high haemotocrit and total protein levels may have resulted from dehydration. Slight increases in the activities of AST and CK, which source from muscle, may be associated with recumbency. Twelve of the cattle in this study had leucocytosis along with neutrophilia. These changes probably resulted from complications such as aspiration pneumonia associated with respiratory paralysis and stress.

Recovery from botulism types C and D is extremely rare. Therapy is mainly palliative and should include fluid and nutritional support, along with general nursing care. Antibiotics should not be routinely administered in animals with botulism unless a secondary infection such as aspiration pneumonia is suspected. Antibiotics that have been associated with neuromuscular weakness, including aminoglycosides, tetracyclines and penicillin, should be avoided (Smith, 1990). In addition to supportive care, the cattle in this study received activated charcoal, sodium sulphate and neostigmine. By day eight of treatment, a total of 26 cows had died. Death usually occurs between one and four days after the onset of clinical symptoms, but can take up to 14 days. The prognosis in cattle with botulism is poor (Radostits *et al.,* 1989). Although polyvalent antitoxin is useful in the early stages of botulism (Jean *et al.,* 1995; Braun *et al*, 2005), it was not administered to the affected cattle in this study, because *Clostridium botulinum* type C and D antitoxins are not produced in Turkey and were unavailable.

Clinical reports describing outbreaks of type C and type D botulism in cattle, sheep and goats have come from many countries: the Netherlands (Haagsma and Ter Laak, 1979), South Africa (Van der Lugt *et al.,* 1995), Canada (Jean *et al.,* 1995; Heider *et al.*, 2001), Germany (Böhnel *et al.*, 2001), Ireland (Neill *et al.*, 1989), Brazil (Ortolani *et al.*, 1997), the US (Galey *et al.*, 2000) and Switzerland (Braun *et al.*, 2005). *C. botulinum* type C and type D, however, has not been reported previously in cattle in Turkey. Therefore, this study, which represents the first confirmation by direct detection of *C. botulinum* type C and type D toxins in cattle in Turkey, is important.

Table 1: Clinical and neurological findings in 26 cattle with botulism

Variable	Finding	Number of cattle
Appetite	Decreased	9
	Absent	17
Ruminal motility	Reduced	9
	Absent	17
Salivation	Mild	12
	Marked	14
Tongue strength	Moderately reduced	7
	Severely reduced	19
Swallowing	Difficult	8
	Unable to swallow	18
Pupillary light reflexes	Decreased	22
	Absent	4
Anal reflexes	Reduced	26
Jaw and tail tone	Low	6
	Absent	20
Sensitivity to pricking with a needle	No reaction	26
Body temperature[a] (reference range: 37.8 – 39.2 °C)	Normal	15
	High	11
	Low	26
Heart Rate[b] (reference range: 60 – 80 / beats/minute)	Normal	4
	Bradycardia	18
	Tachycardia	4
Respiratory Rate[c] (reference range: 10 – 30 breaths / minute)	High	26

a Body temperature: normal (mean: 38.5± 0.4 °C); high (mean: 39.6 ± 0.2°C); low (mean: 35.6 ± 0.3° C)

b Heart rate: normal (mean: 72±3 beats/minute); bradycardia (mean: 48± 4 beats/minute); tachycardia (mean: 96±6 beats/minute)

c Respiratory rate: high (mean 42 ± 4 breaths/minute)

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