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TYPE-C BOTULISM IN A CALVING DAIRY HERD IN NORTH WEST-SPAIN

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ABSTRACT: Botulinum intoxication from B and C types have been reported in cattle as a result of feed intake contaminated with animal putrefied carcasses or poultry litter, but plastic-packaged grass silage may also be a source of botulinum intoxication. In the present work, conducted at a calving dairy herd from North-West Spain, the veterinary care was sought because 21 heifers showed a sternal recumbency with their heads in lateral flexion, listless, reluctant to move and stiff limbs. In a few animals could be observed excessive salivation and difficulty in retracting the tongue. Physical examination revealed no cause for recumbency and heifers were treated symptomatically. A week after the outbreak eight heifers died, five animals were euthanized for human reasons, and only eight heifers survived the outbreak. Heifers fed a mixture of *ad libitum* ration constituted for a new grass silage, straw and a commercially concentrate. It could verify that the plastic covering the grass silage was damaged on the sides, permitting mould and spoilage. In posterior analysis *Clostridium botulism* type C toxins was detected from mouldy silage. These results together with the history and clinical signs suggest type C botulism.
Key Words: *Clostridium botulinum*; grass silage

BOTULISMO TIPO C EM UM REBANHO DE NOVILHAS LEITEIRAS NO NOROESTE DA ESPANHA

RESUMO: Intoxicações por toxina botulínica dos tipos B e C têm sido relatadas em bovinos como consequência do consumo de alimento contaminado com carcaças putrefatas de animais ou cama de frango, mas silagem de pasto embalada em fardos com cobertura plástica também pode ser fonte de intoxicações botulínicas. No presente trabalho, realizado em um rebanho de novilhas leiteiras no Noroeste da Espanha, a assistência veterinária foi procurada devido a que 21 novilhas apresentavam decúbito com suas cabeças flexionadas lateralmente, letárgicas, relutantes a movimentar-se e com os membros contraídos. Em poucos animais foi possível observar salivação excessiva e dificuldade para retrair a língua. No exame físico não se encontrou motivo para o decúbito e as novilhas foram tratadas para aliviar os sinais clínicos. Uma semana depois do surto, 8 novilhas morreram e 5 novilhas foram eutanasiadas por motivos éticos, tendo sobrevivido apenas 8 novilhas. As novilhas haviam sido alimentadas com ração à vontade constituída por silagem fresca, feno e ração comercial. Foi verificado que a cobertura plástica da silagem estava danificada nas laterais, deteriorada e com mofo. Em análise posterior foi detectada toxina tipo C de *Clostridium botulinum* a partir de amostras de silagem mofada. Os resultados junto com a história clínica e os sinais clínicos levaram ao diagnóstico de botulismo tipo C.
Palavras-chave: *Clostridium botulinum*; silagem de pasto

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INTRODUCTION

Clostridium botulinum is an anaerobic Gram-positive microorganism that produces a neurotoxin (exotoxin) associated with clinical signs of botulism in all mammals (Smith, 1979). Eight toxin types have been identified: A, B, C_1, C_2, D, E, F, and G. Only Types B, C and D were generally associated with cattle botulism, and most outbreaks described were of C and D types (Divers *et al.*, 1986; Neill *et al.*, 1989). When cattle ingest the toxin, it causes a potentially lethal neuroparalytic disease, due to interference with the release of acetylcholine at the neuromuscular junction, resulting in progressive flaccid paralysis of striated muscle (Wilson *et al.*, 1995).

Traditionally, type C and D toxins were associated with putrefied carcases of birds, cats and poultry litter that have contaminated feed and water (Lindström *et al.*, 2010). In Brazil, Döbereiner *et al.* (1992) described botulism in cattle with phosphorus deficiency that had eaten bones of dead animals contaminated with botulinum toxin, and Ortolani *et al.* (1997) mentioned an outbreak of botulism in three cattle herds associated with the ingestion of poultry litter. However, other reports of botulism in cattle have shown that contaminated forages can also serve as a source for botulinum toxin (Wilson *et al.*, 1995; Heider *et al.*, 2001). Currently, most farms use plastic-packaged grass silage, and the plastic covering may be damaged, permitting mould and spoilage, which may lead to anaerobic conditions that allow botulism spores to produce toxins (Notermans *et al.*, 1981).

The prevalence of *C. botulinum* spores type C is still unknown in Spain, in spite of suspected outbreaks of botulism, and the toxin type has not been determined. However, type B botulinum intoxication associated with the consumption of a dairy liquid by-product was described in a group of Holstein calves from North East-Spain (Partida *et al.*, 2010). The present work describes a type-C botulism occurred in a calving dairy farm in North West-Spain associated with the ingestion of plastic-packaged silage.

MATERIAL AND METHODS

The present work was done on a field-case basis at a calving dairy herd of 62 Holstein-Friesian cows and 21 heifers located in Galicia (North West-Spain).

The veterinary care was initially sought for a downer heifer. The heifers in the herd were separated in two batches and they ranged in age from 11 to 24 months. Batch one consisted of 9 animals between 11 and 14 month-old and batch two of 12 animals between 15 and 24 month-old. Both batches received a mixed *ad libitum* ration (grass silage, straw and commercial feed) according to their requirements. Five days before the veterinary search, heifers were fed with new grass silage. It was verified that the plastic covering the grass silage was damaged on the sides, permitting mould and spoilage.

The affected heifer was found in sternal recumbency with its head in lateral flexion, and others two heifers were listless, reluctant to move and with stiff limbs. Physical examination revealed no cause for recumbency and heifers were treated supportively and symptomatically (fluids, nutritional supplementation and good nursing care).

The following day, three more heifers were found in sternal recumbency and two animals were found dead, despite being treated empirically. Over the day, other animals showed progressive muscular weakness and most developed paraparesis, which leads to recumbency. A few animals showed excessive salivation and difficulty of tongue retraction, although

no animals were seen with complete tongue paralysis. By day seven, 8 heifers had died and 5 animals were euthanized for humane reasons (T-61 Euthanasia Solution, Intervet). The course of the disease lasted 15 days, with the loss of 13 animals (62% of total heifers). Only 8 animals survived the outbreak.

Whole blood and serum were collected before euthanizing three affected heifers for haematological and biochemical analyses. Haematocrit, total leucocytes count, and concentrations of total protein and fibrinogen were determined in EDTA blood. A biochemical profile (glucose, calcium, phosphorus, aspartate transaminase and creatine phosphokinase), was performed in the serum samples (Vet-Test, Idexx Laboratories).

Samples from liver, kidney and rumen content were taken for further microbiological cultivation and isolation. Liver and kidney tissues were evaluated by spectrometry of atomic emission inductively coupled plasma (ICP-AES) for evaluating arsenic, lead and copper concentrations. Liver and ruminal fluid were tested by gas chromatography to determine the presence of organophosphates or other pesticides. Necropsy was done on five affected animals that were euthanized.

Samples of mouldy silage were obtained and sent to a specialized laboratory for anaerobic culture of *Clostridium botulinum* and for detection of neurotoxin type through the Mouse Neutralization Test.

RESULTS

The results of the haematological and biochemical analyses in blood collected before euthanasia from the three affected heifers are showed in Table 1. A moderate leukocytosis and increase in fibrinogen were observed in all three animals. Serum biochemical profile revealed hyperglycemia and hypophosphatemia and moderate elevations in AST and CPK activities.

Any alteration was observed in the serum concentrations of calcium and total proteins.

Table1- Haematological and biochemical analyses of affected heifers.

Parameter	Heifer-1	Heifer-2	Heifer-3	Reference range[1]
Haematocrit (%)	42	41	39	24-46
WBC[2] (cells/µL)	18000	20000	25000	4000-12000
Fibrinogen (g/L)	7.25	8.15	9.45	1.00-6.00
Glucose (mmol/L)	7.5	7.06	6.39	2.5-4.17
Phosphorus (mmol/L)	0.87	1.05	1.19	1.29-2.77
AST[3] (U/L)	140	129	136	43-127
CPK[4] (U/L)	735	970	645	105-409
Calcium (mmol/L)	2.4	2.45	2.47	2.42-3.1
Total proteins (g/L)	76	74	72	67-75

[1]Smith (2002); [2]WBC: white blood cells; [3]AST: aspartate transaminase; [4]CPK: creatine phosphokinase.

In the animals that were examined post-mortem any macroscopic or histological lesion were founded. Only non-specific lesions were observed, like dehydration, hyperaemia and congestion of gastrointestinal tract without gas, subepicardial haemorrhages and congestive lungs and kidneys.

Toxins of *Clostridium botulinum* were not detected in the examined samples from liver, kidney and rumen content. However, anaerobic culture of mouldy silage yielded growth of *Clostridium botulinum.* Mouse neutralization test identified it as *C. botulinum* type C. Botulinum toxins were not detected in water or commercial feed.

Normal limits of arsenic, lead and copper concentrations were found in the samples from liver and kidney tissues. Liver and ruminal fluid showed negative results for the presence of organophosphates or other pesticides.

DISCUSSION

In the present case, a diagnosis of botulism type C was done based on the history, clinical signs and identification of *C. botulinum* type C toxin in the spoiled mouldy silage. The clinical signs were highly consistent with botulism, dismissing other causes like nutritional myopathy (the appearance of clinical signs in a large number of animals and normal calcium levels in heifers), organophosphate toxicity (the results of the ruminal fluid test were negative), and tick paralysis (no evidence of ticks was found in the post-mortem examination). Plants known to induce a myopathy in cattle were not found in the grass silage, and there was no history of ionophores having feed.

Despite not finding any *Clostridium botulinum* toxins in samples from liver, kidney and rumen content, *C. botulinum* type C toxins were found from mouldy silage. These results together with the history and clinical signs lead to a type C botulism diagnosis. Demonstration of the toxin in the serum and internal organs is often difficult because the interval between the ingestion of the toxin and the analysis and also because the high susceptibility of cattle to small amounts of the toxin (Wilson *et al.*, 1995).

Current methods of harvesting and storing grass and silage using heavy machinery that contaminates grass with soil in addition to humid plastic wrapping create the conditions for growing of *Clostridium botulinum*.

The rate of progression of clinical signs in botulism depends on the dose of toxin ingested (Whitlock, 2002). Massive concentrations of toxin may lead to clinical signs within 12 to 24 hours after ingestion (event uncommon) and low toxin concentrations may not cause any clinical signs for seven to ten days. In the present case, it is probable that animals that absorbed moderate toxin concentrations were those founded with clinical signs of listless, reluctant to move and stiff before recumbent. But within 24 hours, the clinical signs became severe, including depression, dehydration and progressive paralysis.

Abdominal breathing, decreased ruminal sounds, and decreased tail tone were also noted. At this stage, they were unable to eat and drink, and became laterally recumbent. Most recumbent heifers died due to respiratory failure, dehydration or complications of recumbency.

Increases in AST and CPK suggest muscle damage due to trauma and/or recumbency. The slight increase in muscle enzymes in this work, combined with the absence of muscle lesions on histological examination ruled out the nutritional myodegeneration initially suspected. Hyperglycemia and hypophosphatemia (by phosphorus consumption in the cells) may be indicating profound stress by pain and discomfort situations. Also, a moderate inflammatory condition was revealed by leukocytosis and high fibrinogen concentrations. However, those haematological and biochemical alterations were not helpful in identifying a specific reason for acute illness and death.

Treatment of botulism consists of supportive care leading to the relief of the symptoms, basically maintaining hydration and adequate nutrition. The neutralization of the toxin is possible with polyvalent antitoxin but the cost makes its use limited only to very valuable animals (Wilson *et al.*, 1995).

Also, antitoxin often may be not effective due to the fact that it only stops the progression of the clinical disease. Antibiotics must not be used because the disease is caused by the preformed toxin and their use may potentiate neuromuscular weakness (Whitlock, 2002).

CONCLUSION

The present case is the first type C botulism due to contaminated silage intake reported in dairy heifers in Spain. The botulinum intoxications, mainly of B and C types, are being more often recognized in other countries that use mechanical harvesting and storing of grass and silage with plastic-packaged bales. The most affected farms that applied such technology are dairy herds, which until a few years ago did not show botulinum intoxication from grass or silage round balers and that must be alerted to avoid a potential outbreak.

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