

Signs of Neurotoxicity in a Belgian Blue Herd After Ingestion of Moulded Silage

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ABSTRACT

After ingestion of moulded beet pulp silage, cases of cerebro-cortical necrosis (CCN) and mortalities were observed in a dual purpose Belgian Blue (BB) herd. Contamination with *Paecilomyces* spp., a mould that produces byssochlamic acid, malformins and patulin, was proven.

Twenty-five days after progressive introduction of beet pulp silage into the ration, most of the animals showed diminished appetite, excessive salivation and decreased milk production. Some of them showed anorexia, head pressing and blindness while 4 animals died within 1 week after onset of neurological symptoms. The survivors had been treated successfully with thiamine and recovered completely within five days. Once the beet pulp silage had been identified as causative agent, it was removed from the animals' ration and no more clinical cases were observed.

Silage was obviously moulded and analysis revealed the presence of 1.6 million CFU *Paecilomyces* spp./g of silage. Although no further investigation was undertaken to identify the mycotoxins, intoxication with patulin was suspected, since other mycotoxins produced by these species are less toxic. Although it has not been described that CCN can be induced by ingestion of *Paecilomyces* spp., it seems that there is a close relation between ingestion of *Paecilomyces*-contaminated silage and clinical signs observed in this herd.

INTRODUCTION

Silages made from a variety of forage crops constitute important components of ruminant diets in many areas of the world. In order to ensure good animal health and performance, it is essential to produce silages with high feeding value and good hygienic quality. Apart from contamination of silages with undesired or even pathogenic microorganisms, e.g. *Clostridium botulinum* or *Listeria monocytogenes*, the occurrence of filamentous fungi (moulds) and their secondary toxic metabolites (mycotoxins) have attracted considerable attention as potential causes for poor performance and health disorders in domestic livestock. Although it is well recognised that a significant proportion of cereal crops worldwide is affected by mycotoxins annually and results in economic losses (Moss 1998), the contamination of forage crops and silages with toxic fungal metabolites is not well documented.

The term "mould" refers to a diverse group of microorganisms that are ubiquitous in nature and exist as saprophytes or plant pathogens. Mycotoxins are fungal metabolites, representing a great variety of chemical families. Many mycotoxins found in silage are produced in the field prior to harvest and ensiling. Fungal infestation and mycotoxin formation in crops before harvest is a likely explanation for the contamination of silages with field-derived toxins because, under appropriate ensiling conditions, the toxin-producing species are normally replaced by the characteristic silage mycoflora during the early stages of fermentation.

Cases of acute toxic syndromes and even fatal poisoning of unknown etiology in beef, dairy cattle and sheep after consuming moulded silage are described in the literature (Müller & Amend 1997). Mycotoxicosis in ruminants is often associated with neurotoxic symptoms (Cheeke 1994). This paper reports neurological clinical signs following ingestion of moulded beet pulp silage in a cattle herd.

CASE REPORT

This case report describes the repeated appearance of fatal neurotoxicity symptoms after the ingestion of moulded silage in a dual purpose Belgian Blue herd in Belgium.

The herd consists of 100 cows of the dual purpose Belgian Blue breed, of which 35 were lactating (milk yield of about 5,000 litres/cow). In July, cows were at grass and received maize silage as a nutritional supplement. The maize silo contained, in the background, a proportion of beet pulp silage. In August, the beet pulp silage appeared in the supplement fed at pasture. Over time, the proportion of maize silage decreased and the proportion of beet pulp silage increased. In the middle of August, the first clinical cases appeared.

Firstly, two cows died at pasture. One day later, a cow showed anorexia and weakness and died one week later. Necropsy revealed hardware disease, peritonitis and fatty liver syndrome. The day after, 4 cows were diagnosed with anorexia, hyper-salivation, blindness, ataxia of the hind limbs, decreased milk production and general weakness.

The following day, 2 new cases with similar clinical signs occurred. These 2 cases were brought to the University of Liège for a complete examination.

Both cows showed similar clinical signs of teeth grinding, circling, star-gazing and head pressing (Figure 1). Both had a decreased visual acuity, absence of the blink reflex and reduced pupillary reflex (direct and indirect) bilaterally. Mydriasis and dorso-medial strabismus were observed. Atony of the rumen was present in the 2 cows. On the basis of these clinical findings, cerebro-cortical necrosis (CCN or polioencephalomalacia) was suspected. Cows were treated, intravenously, with 10 mg/kg body weight of thiamine (vitamin B1), three times a day (TID). One week after the beginning of the treatment, the animals had completely recovered.

Figure 1.



Four days after the beginning of the treatment of these 2 cows, a new one presented the same clinical pattern in the farm. This cow was treated on the field with high dosages of Vitamin B complexes and slowly recovered.

Three weeks after the beginning of the onset of CCN, animals were kept in the barn and did not have access to the pasture. They received grass silage, beet pulp silage and minerals. The farmer observed that cows were reluctant to consume the beet pulp silage but continued to eat grass silage voluntarily. The farmer decided to remove the beet pulp silage from the ration. The cows returned in pasture, with addition of protein concentrates as nutritional supplement. No new case was observed from this moment.

ANCILLARY EXAMS

Inspection of the feed revealed that the beet pulp silage was moulded while grass silage presented no abnormal macroscopically aspect (Figures 2 and 3). The beet pulp silo was a walled-concrete-surface silo with an airtight cover. Beet pulps were brown-grey and presented numerous areas of mould. Some were of blue-brown colour, and others were of white-yellow colour. Some parts of the beet pulps had a grey-paste aspect. The beet pulps were warm ($> 40^{\circ}\text{C}$) in numerous localisations at the surface and in

Figure 2.

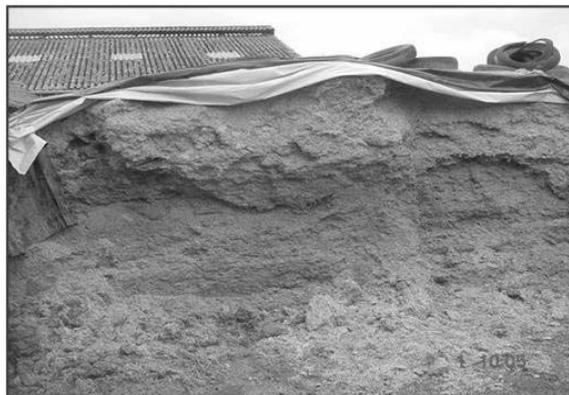
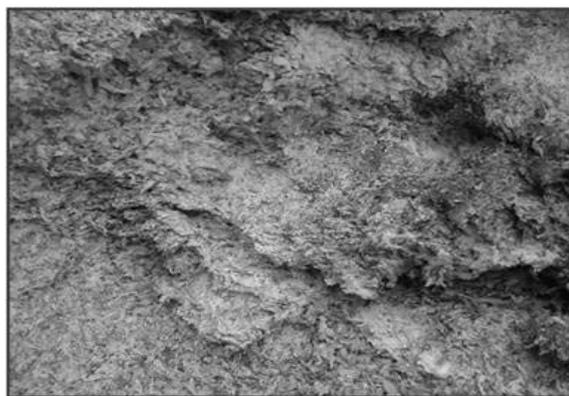


Figure 3.



depth. Samples (surface and depth, areas of mould) of beet pulps were analysed. The pH ranged between 3.98 and 4.15. Cultures showed the presence of mould and yeast in all samples (ranging between $1.6 \cdot 10^6$ cfu/g and $3.0 \cdot 10^6$ cfu/g). *Paecilomyces* spp was identified in the beet pulp samples (Figure 4). Detection of antimicrobial substances in the beet pulp revealed negative result.

Figure 4.



Beet pulp silage had been removed from the animals' ration and no more clinical cases have been observed. Four weeks later, the same beet pulp silage has been reintroduced into the animals' ration and provoked again diminished appetite, salivation and a decrease in milk production in most of the animals. The farmer distributed the beet pulp silage to calves

and observed the day after a decreased appetite and salivation in 80% of the calves. He immediately stopped the distribution of the beet pulps to all animals. There is a close relation between ingestion of *Paecilomyces*-contaminated silo and the observed clinical signs in this herd.

Lead poisoning, inducing similar symptoms, was excluded based on negative blood lead assays. Furthermore, there was no evidence of lead contamination in the environment of the animals.

DISCUSSION AND CONCLUSIONS

After ingestion of moulded beet pulp silage, cases of neurotoxicity and mortalities have been observed in a Belgian Blue herd. Contamination with *Paecilomyces* spp., a mould that potentially produces byssochlamic acid, malformins and patulin, has been proven. Among these toxins, patulin is known to have cancerogenic, immunosuppressive and tremorgenic effects, but also acts on the respiratory and digestive systems. Although no further investigation has been made to identify the mycotoxins, intoxication with patulin has been suspected, since the other mycotoxins produced by these species are less toxic.

Patulin is one of the smallest of the group of toxic metabolites known as polyketides. It may be produced by a number of species of *Aspergillus*, *Penicillium* and *Paecilomyces*. Patulin was first isolated as an antimicrobial active principle during the 1940s from *Penicillium patulum* (now called *Penicillium griseofulvum*) but then turned out to be toxic to plants and animals which precluded its use as antimicrobial. The same metabolite was also isolated from other species and given the names clavacin, claviformin, expansin, mycoin c, and penicidin (Ciegler *et al.* 1971).

The oral LD₅₀ of patulin in the rat is reported to be 32.5mg/kg body weight and a 2-year study on Wistar rats gave no observed effect at the level of 43µg/kg body weight. It is very rapidly excreted and a single dose is excreted in the faeces (49%), urine (36%) and expired air as carbon dioxide (1-2%). It has also been shown to have some immunosuppressive activity (Sharma 1993) and this, combined with its antibiotic activity, could be of concern in some clinical situations. Even though reported for centuries, there is no evidence of carcinogenic activity in man when consumed orally (Ciegler *et al.* 1971).

Neurological symptoms similar to those observed in this herd have already been described in cattle after ingestion of patulin (Sabater-Villar *et al.* 2004). In these animals, histopathological examination of the CNS revealed clear multifocal neuronal degeneration. Neuronal degeneration in the grey matter of the spinal cord appeared in all its length with the exception of the cervical portion. Furthermore, nerve fibre degeneration in the white matter was observed in the thoracical, lumbal and

sacral spinal cord. These lesions are similar to those observed in animals succumbed due to *Aspergillus clavulatus* toxicosis. *Aspergillus clavulatus* is capable of producing a variety of neurotoxins, including patulin. However in these reports the toxin was not identified (Gilmour *et al.* 1989, Van der Lugt *et al.* 1994).

With respect to clinical signs of patulin intoxication, Capitaine and Balouet (1974) described incoordination, paralysis and neuronal degeneration in the cerebral cortex when the toxin was injected into mice. Kellerman *et al.* (1984) cited experimental evidence that patulin poisoning in mice gave similar clinical signs as the bovine *Aspergillus clavatus* intoxication, although no toxin was identified. Rats receiving patulin intra-peritoneally showed convulsions, tremors, impaired locomotion, and stiffness of the hindlimbs and wagging of the head (Devaraj *et al.* 1982). Yamamoto (1954) related the development of nervous signs, cerebro-cortical haemorrhage and death in mice and mature bulls to patulin exposure. Based on the analysis of the reported data, patulin was suspected to be one of the main toxins involved in the presented case, even though this could not have been proven specifically.

Nevertheless, other hypothesis can also explain, to some extent, the clinical signs. A rapid alimentary transition between maize silage (that contains starch) and beet pulp silage (that contains pectin and soluble sugars) can induce a flora disturbance. The presence of antimicrobial substance such as patulin in the feed plays a similar role for the rumen flora. Both can decrease the production of thiamine in the rumen, or increase the consumption of thiamine by several bacteria in the rumen fluid, and lead to polioencephalomalacia by vitamin B1 deficiency. In this case report, the transition between maize and beet pulp was very progressive and the rumen had enough time to adapt. Further, no antimicrobial substance was detected in the beet pulp silage. However, it is not an evidence for the absence of patulin in the beet pulp silage. Beet pulp is known to contain sometimes large amounts of Sulphur (S). An excess of S in the ration (S or SO₄) can decrease the utilisation of vitamin B1 by cells and generate polioencephalomalacia as well. Unfortunately, S was not measured in the ration. Finally, thiaminase could exist as a product of moulds but it has never been described until now.

Moulds and mycotoxins are common contaminants of forage crops and silages in many areas of the world. They pose a potential health hazard to domestic livestock. If silage mycotoxins are suspected causes for health disorders and poor performance in cattle, the use of adsorbents may be an attractive and very practical option to alleviate their effects. Nevertheless, the prevention may be more effective than the treatment. Finally, in some

heavily infested silage, the only solution is unfortunately to discard the silage.

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