Case Report

Using roquefortine C as a biomarker for penitrem A intoxication in a beef herd

F Bonelli[1,2], V Meucci[1], L Turini[1,2*], M Sgorbini[1,2]

[1]Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy.

[2]Centro di Ricerche Agro-Ambientali "E. Avanzi", University of Pisa, via Vecchia di Marina 6, 56122, San Piero a Grado (PI), Italy

***Corresponding Author**

Dott. Luca Turini

Department of Veterinary Sciences

University of Pisa

Viale delle piagge 2, 56124, Pisa, Italy

Tel. +390502210115

Email: luca.turini@phd.unipi.it

State of novelty

- Incidence of Roquefortine C in livestock' food is high reported, especially in visibly molded areas.
- Penitrem A is a well-recognized neurotoxic mycotoxin produced by *Penicillium* spp. which could induce ataxia, tachypnea, and sustained tremors in dogs, cattle, sheep, rabbits, poultry and rodents. Penitrem A is difficult to isolate in laboratory conditions.
- Roquefortine C has been associated with Penitrem A in tremorgenic toxicosis in dogs and it might be considered a valuable diagnostic marker for Penitrem A intoxication.
- Roquefortine C has been found in forage of cattle affected by tremorgenic syndrome.

Abstract

Fifteen grazing beef cattle and calves presented a history of neurological signs like ataxia, intentional head tremors, muscle twitching. Animals were fed with the same alimentary ration. Nervous ketosis, nervous BVD, BHV-1,5, tremorgenic intoxication from hay, and Listeriosis were considered as differential diagnosis.

Blood samples were collected for CBC count, biochemistry panel, infectious diseases tests. Inspection of hay bales showed large white dusty and moldy areas. Samples were taken and analyzed. Altered hay was immediately removed in all animals' stock.

No alterations were found in blood tests. Food analysis showed high concentrations of Roquefortine C (RC) (345 μg/kg DM). Tremorgenic syndrome has been reported in Penitrem A (PA) intoxication, but PA is difficult to isolate in laboratory conditions. Both RC and PA are produced by *Penicillum* spp. RC has been associated with PA in tremorgenic toxicosis in dogs and it might be considered a valuable diagnostic marker for PA intoxication.

Key words: bovine, mycotoxin, Penitrem A, Roquefortina C.

Introduction

Mycotoxins are low molecular weight compounds, metabolic products of the fungi growing on plants in the field, or on forages during storage periods (Driehuis et al., 2008). Animal feed are naturally in contact with yeasts and filamentous fungi present in the field, but contamination can also occur during harvesting, transport and storage. The most common genera that are implied in ruminant mycotoxicosis are *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* (Keller et al., 2013). Exposure to mycotoxins through contaminated feed is one of the major risks affecting ruminant health and toxic syndromes are different depending on the toxin involved (Keller et al., 2013). Roquefortine C (RC) has been primarily associated with *Penicillium roqueforti* and *Penicillium crustosum*. Presence of RC in livestock' feed is highly reported, especially in silage because is both acid tolerant and able to grow at low oxygen levels (Driehuis et al., 2008). RC intoxication causes anorexia, paralysis and ketosis in cattle. High concentrations of RC seem also able to induce a shift in the rumen microflora composition (Driehuis et al., 2008). Despite has been showed that neurotoxic properties to RC (Shell, 2000), literature did not demonstrate that RC could cause a tremorgenic syndrome. Penitrem A (PA) is a well-recognized neurotoxic mycotoxin produced by *Penicillium* spp. which could induce ataxia, tachypnea, and sustained tremors in dogs, cattle, sheep, rabbits, poultry and rodents (Shell, 2000; Tiwary et al., 2009). In mice, experimental intoxications with PA caused seizures by increasing the release of glutamate, GABA and aspartate at cerebrocortical synapses and biochemical reversible lesions (Barker et al., 2013). Diagnosis is based on clinical signs, demonstration of the mycotoxins (RC and PA) in the feed, gastric fluid, urine, blood or feces (Barker et al., 2013).

History

The owner of an organic beef farm reported that several calves have been showed head tremors and stiffness gait in the last two days (March 2016). Affected calves belonged to different "grazing groups" but were all kept in neighbouring area. The owner did not report changes in the animals' diet.

The herd was composed by a total 98 cows and their calves, kept in three different herds allocated by breed: Limousine, Mucco Pisano and Chianina. A bull of each breed was also present, for a total of three bulls. The animals were grazed all the year round in an area of 300 ha. According to the recommendations for organic farming, hay, grass silage and maize silage were self-produced. Thus, the same daily ration was fed to all the animals and the calves could eat the cows' ration. Mineral supplementation did not take place.

Clinical Examination

The owner showed us 6 calves (between 1 to 5 months old, 4 males and 2 females) that presented neurological signs. Three out of 6 calves have been left with their own dam, the other 3 presented more severe symptoms, thus they were housed in a medication area. Due to the not cooperative attitude of the animals, the calves still kept in their herds were only visually examined in the field; the neurological signs observed were ataxia, stiffness gait, intentional head tremors and muscle twitching. We found 9 more affected animals: 6 calves (same age range, 5 males and 1 female) and 3 cows. The calves showed the same signs as described above, while the adults presented mild ataxia. The remaining animals showed normal general body condition and attitude.

A complete physical examination has been performed on the 3 calves housed inside. All 3 calves showed intentional head tremors and muscle twitching; 2/3 presented severe ataxia and stiffness gait, while 1/3 calf was recumbent and unable to rise. The neurological examination showed deficits of the V and VII cranial nerves. Calves could swallow, but they were unable to grab the food. The most important clinical data were: hyperthermia (calf 1 – 39.9°C; 2 – 40.3°C; 3 – 40.8°C), tachypnea (calf 1 and 2 – 58 bpm; 3 – 72 bpm), tachycardia (calf 1 – 98 bpm; 2 – 104 bpm; 3 – over 120 bpm) and prolonged capillary refill time (CRT) (3-4'). Mild dehydration (6%) was present in all the 3 calves (Fecteau, 2015). A slightly reduced motility of the rumen has been detected in all the 3 animals. Both thorax sides presented normal auscultation and no nasal discharge was present. Joints were all normal.

Totally, 15 animals were affected, 12/15 were calves between 1-5 months of age and 3/15 were cows. Calves showed more severe signs than adults. One calf was euthanized due to poor general conditions, but necropsy was not performed, according to the owner's indication.

Macroscopic alterations of the silage, or the silos, could not be observed, while the hay presented deep alterations. Diffuse and deep whitish areas were identified in some hay bales. This aspect became as much worse as the inspection went deeper inside the bales. A total of 13 altered hay bales have been found in the storage area. The owner reported that all the animals were eating hay coming from these bales and that he did not check the forage before feeding the cows and calves. The inspection of the hay in the feeding area confirmed the presence of the same alterations.

Diagnosis and Differential Diagnosis

The main differential diagnosis were tremorgenic mycotoxicosis, Listeriosis (*Listeria monocytogenes*), Bovine Viral Diarrhea (BVD) nervous form, Bovine Herpesvirus type 1-5 (BHV1-5), and nervous ketosis.

Blood samples were collected for complete blood cell (CBC) count and biochemistry panel (total protein, urea, creatinine, total and direct bilirubin, gamma-glutamyl transpeptidase, aspartate aminotransferase, creatine kinase, and Magnesium). Urinalysis was performed for ketone bodies. Calves were also tested for infectious diseases (Listeriosis, BVD, BHV 1-5).

Multiple samples from the contaminate hay were analyzed for mycotoxins and Listeriosis, but mycotoxins were not evaluated in any biological samples. Mycotoxins were measured by using a validated HPLC/MS method.

Treatment adopted

CBC, biochemistry panel, and oligo-minerals resulted within normal ranges. All the animals were negative for the infectious agents tested. No pharmacological treatments were made.

Feed analysis showed RC at level of 345 μg/kg dry matter - DM. Ready after sampling, all the hay in feeding area and the contaminated hay bales were removed and destroyed.

All the animals, except the calf euthanized, recovered within 1 week.

Discussion

Initial clinical signs oriented to several differential diagnosis. Nervous ketosis was excluded because urinalysis came negative for ketone bodies and calves are not considered a population at risk (Fleming, 2015). Cerebellar hypoplasia caused by BVD virus induces intentional tremors and fever, but it is a condition typical of neonatal or young calves and could not explain other nervous symptoms and the neurological signs in adults (George and Metre, 2015). Tremors, fever and tachypnea might indicate BHV 1-5. However, besides increased respiratory rate, which could be explained by the hyperthermia and muscle tremors, the animals did not show other respiratory signs. The herd did not have an history of respiratory diseases, abortion or reproductive problems (Smith et al., 2015). Intentional tremors, ataxia, fever, inability to grab the food and alterations of cranial nerves might indicate Listeriosis. Also, outbreaks of listeriosis have been reported in late winter or early spring, especially after periods of very heavy rain (Boileau and Gilliam, 2017). Some reports documented encephalitic

listeriosis as multiple cases over a short period of time. However, within a bovine herd, the prevalence of encephalitic listeriosis is usually less than 5% (Boileau and Gilliam, 2017). The most common source of infection for a cow is the silage, but encephalitic listeriosis has been observed in animals consuming pasture, hay and soybean products (Boileau and Gilliam, 2017). Listeriosis was one of the most likely differential diagnosis in this case. The owner refused to perform a punction of cerebro-spinal fluid, thus Listeria was only checked in the forage and in the blood. Due to the macroscopically findings on hay, to the negative result concerning Listeriosis and to the positive result about RC in the forage, a tremorgenic mycotoxicosis was considered the most likely diagnosis and could explain both the more severe (intentional head tremors and muscle twitching, ataxia, stiffness gait, recumbency, deficit of cranial nerves, hyperthermia, tachypnea and tachycardia), and the milder symptoms (mild ataxia in adult cows) in animals fed with contaminated hay. Mild dehydration, prolonged CRT and rumen hypomotility we found in few subjects were considered consequences of the main neurological signs.

Feed analysis performed was not able to find PA in detectable concentrations. In literature, it has been reported that PA is more difficult to isolate than RC, but RC is always associated with PA in tremorgenic toxicosis caused by PA (Tiwary et al., 2009). Thus, RC is considered a valuable diagnostic marker for PA intoxication (Tiwary et al., 2009). Also, results for other potentially involved pathogens were negative. Therefore, a tremorgenic mycotoxicosis due to PA-RC from *Penicillium* contamination was the final diagnosis.

No previous problems in storing had been detected. Unusual weather conditions might have created the right circumstance for an extraordinary fungal grow and replication.

In conclusions, RC might be used in feed analysis and might represent a marker of PA allowing diagnosis of tremorgenic intoxication in bovine.

Authors' contribution

Bonelli, Turini and Sgorbini played a role in the clinical case evaluation and solving. Bonelli draft the manuscript, while Turini and Sgorbini review it.

Meucci helped in the interpretation of results concerning Roquefortine C and also revised the manuscript.

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