Slov Vet Res 2015; 52 (2): 97-102
UDC 636.2.09:636.083.14:615.9:632.958

Case Report

PERACUTE ALPHA-NAPHTHYLTHIOUREA INTOXICATION IN A COW-CALF HERD

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Summary: This article describes the intoxication of a cow-calf herd (100 animals) with alpha-naphthylthiourea (ANTU), resulting in 10 deaths. The rodenticide, which is not licensed for use in the European Union, might have been introduced into the herd via imported hay or given to the animals maliciously.

Most of the intoxicated animals presented with a peracute condition and sudden death. Clinical signs observed in affected animals included anorexia, rumen stasis, foamy discharge from mouth and nostrils, dyspnea, crackling lung sounds, and abdominal tympanic sounds during acoustic percussion and tachypnea with prominent rib movements. The affected animals were treated symptomatically with glucose, metamizole sodium, caffeine, calcium gluconate, magnesium gluconate and medicinal charcoal. In addition, non-pregnant cows were given dexamethasone. Morbidity among the herd was 13% (13/100) and the case fatality rate was 76.9% (10/13). Post-mortem examination of all cattle carcasses determined cyanotic mucous membranes, serosanguineous liquid containing gelatinous particles in the thoracic cavity and severe lung edema. Histological investigations of lung tissue samples determined a severe and fibrin-rich alveolar and interstitial edema, a mild alveolar histiocytosis and a discrete emigration of granulocytes into alveolar lumens in few locations.

A diagnosis of ANTU intoxication was made. The rodenticide was detected in tissue samples by thin layer chromatography.

To our knowledge, this is the first description of ANTU poisoning within a cattle herd. Since ANTU is still a commonly used rodenticide in many countries, ANTU intoxication should be considered in herds where a substantial number of cattle present with a progressive peracute disease with clinical signs of a white foamy discharge from the mouth and nose, tachypnea and lung edema, leading to high mortality rates.

Key words: ANTU; cattle; edema; feed; intoxication

Introduction

Alpha-naphthylthiourea (ANTU) is a colourless, odourless, crystalline powder used as an active substance in rodenticides. Synonyms include alpha-naphthyl thiocarbamide, 1-naphthyl-thiourea and N (1-naphthyl)-2 thiourea (1). ANTU was discovered by Curt Richter during World War II at the John Hopkins Hospital in Baltimore, United States (2, 3). ANTU is effective against Norway rats whereas house mice and roof rats are increasingly resistant to the compound (3). With respect to the acute toxicity of ANTU among other animal species, pet albino rats and dogs are more susceptible than cats, followed by rabbits (4). Lethal doses differ widely between species, varying from 3 mg/kg for rats, 10 mg/kg for dogs, 25 mg/kg for pigs, 30 mg/kg for horses, 50 mg/kg for cows and 75 mg/kg for cats (1, 5). ANTU

Received: 24 June 2014
Accepted for publication: 4 February 2015

is a neurotoxin which stimulates the sympathetic nervous system and causes substantial leakage of lung capillaries. The consequences of this are pleural effusion, pulmonary edema and respiratory failure (1). According to European regulation (EC) No. 1272/2008 (6), ANTU must be labelled as "fatal if swallowed" and "suspected of causing cancer" if sold within the European Union because of its highly toxic properties. Currently no specific antidote is known. Treatment is supportive and symptomatic (1).

Intoxications of cattle with ANTU may occur due to the use of the rodenticide in barns or during feed production. ANTU can be orally ingested by cattle from their environment or in the feed. However, in the European Union, ANTU is not licensed as an active substance for biocides according to EU directive 98/8/EG (7) and regulation (EU) Nr. 528/2012 (8) or for pesticides according to EU directive 91/414/EWG (9) and regulation (EG) Nr. 1107/2009 (10). This paper describes ANTU intoxication of cows from a cow-calf herd consisting of 50 Simmental cows and their Simmental Charolais crossbreed calves.

Material and methods

Case History

Animals included in this case report originated from a cow-calf herd from Carinthia in the southern part of the Austrian Alps. In addition to the cow-calf operation, the farm also had a separate barn with 80 Simmental and crossbreed finishing cattle.

The cow-calf herd was reared in a free-stall system with access to pasture, while the finishing animals were kept inside permanently. The feed ration of the finishing animals consisted of corn silage, grass silage and concentrate. The cow-calf herd was fed hay only. Drinking water for the livestock was provided by two separate wells.

The first reported incident of ANTU intoxication occurred when the farmer fed hay originating from Italy to the finishing animals (in addition to their usual silage) at the end of December 2011. Since they did not consume the hay in large amounts, he decided to feed it to the cows. The following day four cows were found dead. The imported hay was removed immediately and the carcasses were referred for post-mortem examination to the Carinthian Institute for Veterinary Disease Control.

The second incident occurred when the farmer attempted to feed the imported hay to the finishing group again 2 weeks later. As they did not eat it, he again transferred it over to the cow-calf herd. Over the following four days, 5 cows and 1 bull calf from the cow-calf herd died peracutely. Furthermore, over the course of the two incidents, 2 cases of abortion and 3 cases of retained placenta were noted. All cows and calves in the cow-calf herd were reared in the same barn, so all were exposed to the same feedstuffs. It is reasonable to assume that only the worst affected animals were presented to the veterinarian. Other animals may also have been affected, but recovered without treatment.

Clinical findings

During the first incident, 7 animals were affected and 4 of these died peracutely. The remaining 3 cows suffered from severe clinical signs of intoxication, leading to consultation with the farm veterinarian. The cows appeared lethargic, feed and water intake was decreased and rumen stasis was diagnosed. Skin turgor was slightly decreased, the mucous membranes were slightly cyanotic and scleral congestion was present. The animals were dyspneic with foamy discharge from the mouth and nostrils. The body temperature was within the reference range, while heart rate was increased. Auscultation of the lungs revealed crackling sounds and percussion of the lung fields showed hyperresonance with a tympanic sound. The clinical signs observed in affected animals were compatible with intoxication. In the second incident, 6 animals were affected, all died peracutely, no additional severely affected animals were noted. Overall, smaller cows were more likely to be affected compared to larger, more dominant animals.

Medical treatment

The three cows initially presenting with severe clinical signs of intoxication were treated with an intravenous infusion of 40 % glucose (0.5 l per cow) (Glucosteril 40 %, Fresenius Kabi, Bad Homburg, Germany) and intravenous administration of a veterinary preparation

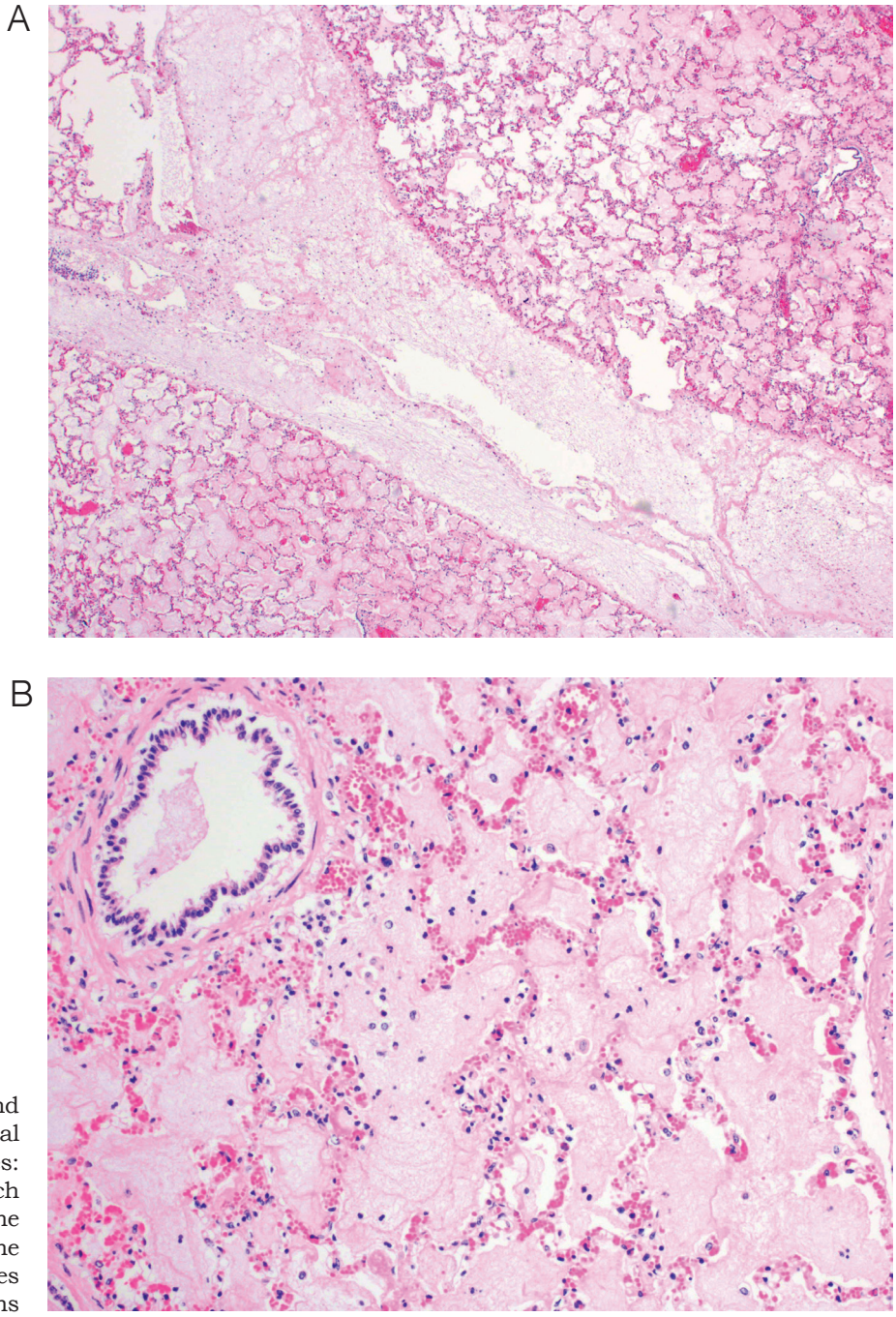

Figure 1: a (magnification x 40) and b (magnification x 400): Histological investigations of lung tissue samples: notice the severe and fibrin-rich alveolar and interstitial edema, the mild alveolar histiocytosis and the discrete emigration of granulocytes into alveolar lumens in some locations

containing metamizole sodium, caffeine, calcium gluconate and magnesium gluconate (0.25 l per cow) (Novacoc forte, Richter Pharma, Wels, Austria). Furthermore, medicinal charcoal was given orally (300 g per animal per day) (Carbopulbit, Bayer, Vienna, Austria). Additionally, non-pregnant cows were treated with an intramuscular injection of dexamethasone (0.06 mg/kg body weight) (Rapidexon, Eurovet Animal Health, Bladel, The Nederlands).

Results

Post-mortem findings

Post-mortem examination of all deceased animals was conducted at the Carinthia Institute for Veterinary Disease Control. Post-mortem examination revealed moderate cyanotic mucous membranes and thoracic cavities filled with a serosanguineous liquid containing gelatinous

particles. The lungs were severely congested and of a brownish-red color. Severe lung edema was noted and gelatinous liquor covered the lungs and extended into the interstitial tissue. Histological examination of the lungs determined severe alveolar and interstitial edema and fibrin-filled alveoli. There were remarkably few inflammatory cells present. (Figure 1a and 1b)

Laboratory investigations

Bacteriological examination failed to isolate any of the major expected pathogens such as Salmonella spp., Campylobacter spp., Pasteurella spp., Mannheimia spp. by culture or Mycoplasma spp., Mycoplasma bovis and mycoides by polymerase chain reaction (PCR). Antigen ELISA also failed to demonstrate the presence of a number of viral pathogens namely: bovine herpes virus type1, bovine virus diarrhea virus, bovine respiratory syncytial virus and bovine parainfluenza virus type 3.

Detection of ANTU in tissue samples

Liver and lung tissue from affected animals was referred to the Institute of Pharmacology, Toxicology and Pharmacy, Ludwig-Maximilians-University Munich, Germany. A thin layer chromatography revealed ANTU residues in the liver tissue (11).

Botanical and toxicological analysis of the feed

A representative sample of the imported hay from Italy was taken by the Official Federal Veterinarian and sent to the Institute of Animal Nutrition and Functional Plant Compounds, Veterinary University of Vienna, Austria. Moderate feeding quality of the hay with impeccable hygiene status was analysed. The hay sample consisted of wild oat (*Avena fatua)*, wildrye (*Elymus sp.)*, vetch (*Lathyrus sp.*), dock (*Rumex sp.*) and dog rose (*Rosa canina)*. Toxic plants were not present. Another sample of the hay was refered to the Austrian Agency for Health and Food Safety (AGES), Department of Animal Nutrition and Feedstuff to analyse the hay for tryptophan residues. Tryptophan was only determined in negligible quantity (0.0807 +/- 0.00807 mass %) by high pressure liquid chromatography (HPLC) according to regulation (EC) No. 152/2009 (12). ANTU could not be detected in hay samples.

Corn crop silage, which was fed to the youngstock on the farm, was analyzed for the occurrence of mycotoxins. Aflatoxin B1, B2, G1, G2 and dexoxynivalenol could not be detected by high pressure liquid chromatography with post-column fluorescence derivatization (HPLC-FLD) and high pressure liquid chromatography with ultra-violet detector (HPLC-UV) respectively.

Discussion

This case report describes intoxication with the rodenticide ANTU among animals from a cow-calf herd in Austria. To our knowledge, ANTU intoxication of a cattle herd has not been described previously.

The clinical features included a peracute progression, respiratory symptoms, foamy discharge from nostrils and mouth and cyanotic mucous membranes in affected animals. The mortality rate of 10 % and case fatality rate of 76.9 % demonstrate the devastating effects on the cow-calf herd. However, the exact number of poisoned animals is unknown since only the worst affected were presented to the veterinarian for examination and treatment. Post-mortem examination determined severely affected lungs, which were congested and edematous. The thoracic cavity was filled with serosanguinous fluid mixed with gelatinous particles. Numerous infectious diseases could be excluded by laboratory examinations as being the possible source of these clinical signs and level of mortality.

ANTU was identified in lung and liver samples from all examined carcasses. How the animals came into contact with the rodenticide is unclear. There may be a link to the imported hay from Italy because the farmer transferred this feed from the finishing unit to the cow-calf herd twice and on both occasions cows died the next day. Although the hay was first fed to the finishing herd, they did not consume it, perhaps due to the additionally provided corn and grass silage in their ration. As the cows in the cow-calf herd were fed solely hay, they had no choice but to eat this feedstuff, regardless of palatability and quality.

To confirm the suspicion of ANTU intoxication, samples of the imported hay were taken by the Official Federal Veterinarian and tested for residues of the rodenticide, however all samples

were negative. One possible reason for these negative results may be the timeframe between the intoxication of the cow-calf herd and the analysis of the hay four months later. Degradation of ANTU during this time could be possible. The feeding of the imported hay was prohibited by the Austrian Authorities for twelve months. After that time the farmer fed the hay to the animals without any ill effect.

Water is a common source of ruminant poisoning (13), and this was provided by two inaccessible wells on farm. There was no overt evidence that the rodenticide was introduced via the water supply, but as unused water drained off continuously this was not tested at the time of the intoxication incidents. The drinking water well was inspected by the local authorities.

Differential diagnoses considered included intoxication of the cow-calf herd with toxic plants. Brassica species, for example, contain glucosinolates which may cause similar clinical signs when fed in high quantities (14). However, no such plants could be identified in the hay fed to the herd. The cow-calf herd had access to the pasture, however, there is no vegetation growth in this region in December and January, therefore feed intake from grassland can be excluded as a possible source of toxin ingestion. Fog fever, caused by an excessive intake of D, L-tryptophan, could also be ruled out because of the very low amounts in the examined hay and the fact that the incident occurred during the winter, as acute bovine pulmonary emphysema and edema (ABPEE) is known to occur when cattle are moved from dry to lush pastures in late summer (14).

It is possible that the cow-calf herd could have ingested ANTU from their environment but, according to the farmer, ANTU was not used on the farm as a rodenticide. This finding was expected, as ANTU use is prohibited throughout the European Union, nevertheless it cannot be excluded that ANTU was given to the animals intentionally.

To conclude, this case report demonstrates evidence of ANTU poisoning among beef cattle as evidenced by rodenticide residues determined in tissue samples upon post-mortem examination. Despite extensive investigations into feed, the farm environment and management factors, the source of the intoxication remains unclear.

Acknowledgements

The authors would like to thank Georg Troyer for clinical advice as veterinarian on the farm and Clair Firth, MVM, for English language assistance.

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PERAKUTNA ZASTRUPITEV Z ALFA-NAFTILTIOUREO V ČREDI KRAV DOJILJ

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Povzetek: Ta članek opisuje zastrupitev z alfa-naftiltioureo v čredi krav dojilj (100 živali), ki povzročila 10 smrtnih primerov. Rodenticid, ki sicer ni licenciran za uporabo v Evropski uniji, je bi v čredo vnesen z uvoženim senom ali pa zlonamerno. Pri večini zastrupljenih živali smo opazili perakutno stanje in nenadno smrt. Klinični znaki pri prizadetih živalih so bili naslednji: anoreksija, zastoj vampa, penast izcedek iz ust in nosu, dispneja in prasketajoč zvok v pljučih. Pri perkusiji trebuha smo opazili značilen timpanični zvok in tahipnejo s tipičnim gibanjem reber. Prizadete živali smo simptomatsko zdravili z glukozo, metamizol natrijem, kofeinom, kalcijevim in magnezijevim glukonatom ter medicinskim ogljem. Krave, ki niso bile breje, smo tretirali tudi z deksametazonom. 13% živali v čredi je zbolelo (13/100) in med temi je bila smrtnost 76,9% (10/13). S posmrtnim patološkim pregledom goved smo ugotovili, da so bile sluznice cianotične, v prsni votlini je bila prisotna želatinasta tekočina in hud pljučni edem. S histološko preiskavo vzorcev tkiva pljuč smo ugotovili hud in s fibrinom bogat alveolski in intersticijski edem, blago alveolarno histiocitozo in kar na nekaj mestih diskretno migracijo granulocitov v alveolarni lumen. Naredili smo test na prisotnost ANTU in s tankoplastno kromatografijo odkrili rodenticid v vzorcih tkiva. Po našem mnenju je to prvi opis zastrupitve z rodenticidom ANTU pri govedu. Ker se v mnogih državah ANTU še vedno pogosto uporablja, je potrebno na tovrstno zastupitev v čredah goved pomisliti, če opazimo veliko nenadnih obolenj s kliničnimi znaki, kot je penasti izcedek iz ust in nosu, tahipneja in pljučni edem, ki pripeljejo do visoke stopnje umrljivosti.

Ključne besede: ANTU; govedo; edem; krma; zastrupitev