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Case study: Challenges in diagnosing hydrocyanic acid toxicity in cattle

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Abstract

Fourteen of 19 Corriente roping cattle became acutely ill and died after consuming hay containing johnsongrass, and then introduced into a pasture of Tifton 85 bermudagrass (*Cynodon dactylon* Pers X *C. nlemfuensis* Vanderyst). The presumptive diagnosis was hydrocyanic acid toxicity (HCN) due to consumption of the bermudagrass. Tifton 85 bermudagrass has been utilized extensively for grazing cattle in the southern United States for 20 years with no reports suggesting HCN toxicity. In this case there was lack of a thorough diagnostic workup, and there were discordant results between laboratories. This case emphasizes that producers should be aware of the potential for toxicity due to nitrates or HCN in any forage, but a complete and prompt diagnostic investigation with appropriate collection of fresh samples is imperative for a definitive diagnosis.

Key words: cyanogen, cyanide toxicity, hydrogen cyanide, Tifton 85 bermudagrass

Résumé

Quatorze vaches de race corriente sur un ensemble de 19 sont devenus gravement malades et sont mortes après avoir consommé du foin contenant du sorgho d'Alep et avoir été envoyées sur un pâturage avec du chiendent pied de poule Tifton 85 (*Cynodon dactylon* Pers X *C. nlemfuensis* Vanderyst). Le diagnostic présumé était un empoisonnement à l'acide cyanhydrique suite à la consommation de chiendent pied de poule. Le chiendent pied de poule Tifton 85 a été abondamment utilisé pour le bétail au pâturage dans le sud des États-Unis depuis plus de 20 ans sans qu'aucun rapport ne suggère l'empoisonnement à l'acide cyanhydrique. Dans ce cas-ci, il n'y a pas eu de bilan diagnostic approfondi et les résultats des différents laboratoires ne concordaient pas tous. Ce cas montre bien que les producteurs devraient être au courant du potentiel d'empoissonnement aux nitrates ou à l'acide cyanhydrique dans tout type de fourrage. Un examen diagnostic complet et rapide avec cueillette appropriée d'échantillons frais est indispensable pour obtenir un diagnostic définitif.

Introduction

On a life-cycle basis, forages are the primary source of nutrition for beef cattle. As ruminants, cattle can utilize forages as feedstuffs from land that is unsuitable for cultivation because of lack of rainfall, shallow topsoil, or erosion hazards. Although range and pasture lands are generally populated with grasses that pose no risk to livestock, certain plants may become toxic to cattle under certain growing conditions, such as nitrate accumulation or hydrogen cyanide content in plants.

The purpose of this report is to describe a case of fatal hydrocyanic acid (HCN) toxicity in beef cattle, and the challenges in establishing the definitive diagnosis.

Case History

On the afternoon and evening of May 25, 2012, 19 Corriente cattle, including steers, pregnant heifers and a bull, were each roped twice in an outdoor arena. The cattle were then briefly placed in an outdoor pen with ad libitum access to water and grass hay of unknown composition and quality. According to the trainer and veterinarian, the hay was imported from another state, was low in nutritive value, and contained johnsongrass and other grass-weed species. The hay had not been analyzed for toxins or nutritive quality prior to or after consumption.

Shortly after the cattle were offered the grass hay, the gate was opened to allow access to approximately 50 acres of Tifton 85 bermudagrass pasture that had not been grazed or used for hay since January of the same year. Approximately 1 hour after the cattle were introduced to the hay and bermudagrass pasture, the owner heard anguishing noises, and upon investigation found some cattle dead and others convulsing and spewing rumen content from their mouth.

The owner contacted his local veterinarian at approximately 8:30 the next morning. When the veterinarian arrived, there were 13 dead animals and 1 was nonresponsive; it died shortly thereafter. Ten of the dead animals were in the pen where the grass hay was located, and the remaining 4 dead animals were in the bermudagrass pasture. Nine of the dead cattle were steers, 4 were heifers in the last trimester of gestation, and 1 was the bull. Two animals were necropsied, and a tentative diagnosis of nitrate toxicity was made. Eyeballs, rumen content, liver, jejunum, serum, and forage samples from the bermudagrass pasture were collected and transported to the state diagnostic laboratory[a]. Vegetation in the hay pen was not investigated, nor were hay samples collected for laboratory testing.

Laboratory Findings

Nitrate levels were measured in ocular fluid from 2 eyeballs using a commercially available test[b], and were normal (< 20 ppm). As a result, the practitioner and toxicologist agreed nitrate toxicity could be eliminated as the cause of death, therefore forage were not tested for nitrates.

Forage samples (bermudagrass) and rumen content were tested for hydrocyanic acid (HCN) utilizing a qualitative picric acid test. Forage and rumen content were reported as +4, indicating a high level of HCN. The only other laboratory testing performed was histopathologic examination of the small intestine. A loss of villus enterocytes and loss of differential staining of villi, indistinguishable from autolysis, was reported. A field and laboratory diagnosis of HCN toxicity was established based on qualitative HCN test results on rumen content and bermudagrass samples. Other diagnostic rule-outs for causes of sudden death, such as organophosphate poisoning, acute acidosis, and acute organic arsenic toxicity, were not performed due to lack of appropriate tissue samples. The submitting veterinarian did not request these tests, as the history did not suggest exposure.

Forage samples taken 4 days later from the bermudagrass pasture were sent to 3 different laboratories to test for HCN. One laboratory used a qualitative picric acid test, 1 used a slightly modified semi-quantitative version of the picric acid test, and 1 used a quantitative distillation test. Test results varied depending on the test utilized. Results for HCN were +4 on the qualitative test, 84 ppm on the semi-quantitative test, and 28 ppm on the quantitative test, well below the reported toxic level of 200 ppm.[15] To further assess the potential for HCN toxicity in Texas, Tifton 85 bermudagrass samples were taken from pastures in Blanco, Bastrop, Washington, Burleson, Brazos, Austin, Rusk, and McLennan counties and submitted to 3 veterinary diagnostic laboratories. Results from 1 laboratory ranged from undetectable to +4 using the qualitative picric acid test. Test results of +3 or +4 when utilizing the qualitative test were not reproducible in the 2 other laboratories utilizing semi-quantitative and quantitative testing methods. Bermudagrass samples that tested +4 on the qualitative test were sent to United States Department of Agriculture-Agricultural Research Service (USDA-ARS) laboratory in Tifton, Georgia for DNA analysis, and were verified as Tifton 85 bermudagrass.

Pasture Fertilization History

Application of herbicides potentially increases risk of elevating plant cyanogenic glycosides, especially if there is reduced rainfall after application.[8,26] The pasture in this case study was treated with a pre-emergent herbicide in February; however, rainfall during January through May in Bastrop County was above normal, and much higher than in the previous 3 years. Heavy application of nitrogen fertilizer has been associated with elevated risk of HCN toxicity.[1,7,13,15,28] The dates of fertilizer application in 2012, and application rates, were as follows: on February 01, 211 lb (95.9 kg)/ac of 3-6-12 was applied, resulting in approximately 6 lb (2.8 kg)/ac of N, 13 lb (5.9 kg)/ac of P_2O_5, and 25 lb (11.4 kg)/ac of K_2O being applied to the grass. On February 08, 105 lb (47.7 kg)/ac of 0-0-60 was applied resulting in 63 lb (28.6 kg)/ac of K_2O, and on February 10, 1,720 lb (781.8 kg)/ac of limestone was applied. Later, on April 26, 235 lb (106.8 kg)/ac of 32-0-0 was applied, resulting in 75 lb (34.1 kg)/ac of N. The fertilizer application rates were considered normal for low to moderate forage production and drought recovery.

Discussion

Cyanide, prussic acid, and hydrocyanic acid (HCN) poisoning are terms used to describe toxicity in ruminants consuming plants containing cyanogenic glycosides.[7,15,28] Cyanogenic glycosides are secondary compounds thought to be produced by plants for protection against excessive defoliation by grazing animals, and predators such as insects and parasites, and may serve as a mechanism for storage of excessive metabolic compounds.[2,5,13,15,34] Increased cyanogenic glycoside levels

may result from the plants increasing the concentration of the protective glycoside or due to structural changes in the leaves.[35] Cyanogenic glycosides encompass the majority of cyano-substances in plants; however, cyanogenic lipids also exist, and both glycosides and lipids liberate HCN upon hydrolysis.[13]

Hydrogen cyanide is toxic to all animal species, but the major threat is to domestic ruminants consuming plants containing cyanogenic glycosides.[7,15,28] Rumen microbial flora have the ability to hydrolyze naturally occurring cyanogenic glycosides in plants, therefore ruminants are more susceptible than monogastric animals to HCN toxicity.[7,13,15,19,28,34] Differences in breed susceptibility to HCN toxicity in cattle have been mentioned in the literature, but information was based solely on anecdotal observations.[2,15]

Acute toxicity results when HCN combines with ferric ions in mitochondrial cytochrome oxidase, preventing electron transport in the cytochrome system and blocking oxidative phosphorylation and production of ATP. This results in the suspension of oxygen exchange and cerebellar hypoxia, the ultimate cause of death with HCN toxicity.[7,15,28] Unlike nitrate poisoning, blood is saturated with oxygen that cannot be released to the cells, resulting in bright-red venous blood.[15,28] Bright-red venous blood in acutely affected animals is often considered diagnostic, but if the animal is agonal, inhibition of respiration results in reduced oxygen intake, causing the blood to appear darker. This can complicate field diagnostics.[28]

Chronic HCN poisoning associated with metabolic, neurologic, and teratogenic conditions has occurred in humans as well as domestic and laboratory animals after consuming plants with HCN potential; however, not all of the mechanisms of toxicity are understood.[1,4,6,7,15,16,20,25,28,29,31,32,35] Studies have linked teratogenicity in hamsters and rats to consumption of plants containing cyanogenic glycosides.[1,6] An association has been shown between ewes developing goiter and subsequently delivering goitrogenic lambs when grazing pastures containing cyanogenic plants.[28] Low levels of cyanide in sorghum fodder affects the metabolic activity of animals, resulting in lowered levels of serum proteins, calcium, magnesium, and sodium. This occurrence is likely mediated through thyroid metabolism because dietary cyanide is converted to thyocyanate, which mimics iodine deficiency.[31,35]

There are over 2,500 plants, including grasses, ornamentals, commercial fruit trees, and weeds, with potential to cause HCN toxicity in ruminants;[7,15,16,28] however, the cyanogen has only been identified in approximately 200 plants.[13] The HCN potential of plants ranges from near 0 to 8,000 ppm on a dry-weight basis.[15,28] Some references report plants being potentially toxic when containing 20 mg of HCN per 100 g (200 ppm dry weight), while other sources report the same toxic level of 200 ppm on a wet basis.[3,18,28] Diagnostic laboratories contacted following the deaths of cattle in this case report toxic levels on a wet basis. Sorghums are considered toxic at levels of 500 to 750 ppm (dry weight), while other grasses are considered toxic at levels exceeding 200 ppm (dry weight).[3,4,5] Boyd et al showed that 1 g of HCN will kill a 1000 lb (454 kg) cow, and that cows are capable of detoxifying 0.5 g of HCN per hour.[5]

Variables affecting toxicity include concentration of the B-glycosidase in the plant, ingesta already present in the rumen, rumen pH, and the speed the material is consumed.[12,15,21,22,28,33] Through cyanogenesis, a cyanogenic glycoside is concentrated in the seeds, leaves, bark, and twigs of plants.[1,7,15,16,28] Cyanogenic glycosides are common to specific plants; the glycoside common in grasses and sorghum is dhurrin.[1,7] The cyanogenic glycoside associated with johnsongrass (*Sorghum halepense* (L) Pers), sudangrass (*Sorghum bicolor* (L) Moench), and bermudagrass is concentrated in the epidermis of the leaves and stems.[1,7,13,15,16,28] The enzyme B-glycosidase is contained within the plant mesophyll.[1,7,13,15,16,28]

Cyanogenic glycoside must be hydrolyzed by B-glycosidase before it can release toxic HCN.[1,7,13,15,28] When a plant is damaged, such as during freezing, cutting, drying, or crushing, B-glycosidase is released from the mesophyll and comes into contact with the cyanogenic glycoside in the epidermis.[7,13,15,16,28] Enzyme B-glycosidase hydrolyzes the cyanogenic glycoside to a hydroxynitrile aglycone and glucose.[7,13,15,16,28] There is further dissociation of the hydroxynitrile aglycone to p-hydroxybenzaldehyde and HCN.[7,13,15,16,28] Enzymatic action by B-glycosidase is not required for the dissociation of the aglycone.[28]

Most cases of acute HCN poisoning affecting domestic ruminants occur after consumption of plants containing high levels of cyanogenic glycosides.[7,13,15,16,28] Rumen pH is an important factor determining rate and amount of HCN released in the rumen after animals consume plants with HCN potential.[3,15,21,22,28] B-glycosidase is more active in a rumen environment rich in cellulose rather than starch, where the rumen pH is lower. Cattle fed grass forage or hay average an 8.45-fold higher mean rumen HCN concentration than when fed a higher concentrate (grain) diet.[1,12,13,33]

Animal tissue can be tested for HCN, with muscle and liver being the tissues of choice.[10,15,28] Liver samples must be collected within 4 hours, and muscle within 20 hours postmortem, and shipped to the laboratory in an airtight container.[10,15,28] Diagnostic testing for HCN, however, usually focuses on forage analysis, and there are 3 basic methodologies used in the laboratory diagnosis of HCN: qualitative, semi-quantitative, and quantitative. The qualitative and semi-quantitative tests rely on variations of a century-old test developed

by Guignard using picric acid.[5,30] Plant material is macerated and placed in a flask with chloroform or sulfuric acid, and a paper saturated with picric acid is placed near the stopper in the top of the flask. The flask is heated and cyanogenic glycoside is hydrolyzed to a sugar and HCN; the presence of HCN is determined by a color change in the picric acid paper.[5,30] The test is somewhat subjective as the degree of color change is related the amount of cyanide in the plant, and not quantified in ppm.[5] Instead, the degree of color change is graded on scale of +1 to +4, with +4 reported toxic for animal consumption. The semi-quantitative test is essentially a modification of the qualitative test with estimates of ppm linked to degree of color change. Currently, some laboratories conduct a quantitative test for HCN; however, this test is relatively expensive and has a longer turnaround time.

Tifton 85 is a bermudagrass hybrid (*Cynodon dactylon* Pers X *C. nlemfuensis* Vanderyst) developed by USDA-ARS.[9] This bermudagrass was the best of many F-1 hybrids between PI 290884 from South Africa and Tifton 68, a stargrass (*Cynodon nlemfuensis*) hybrid.[8] It was released in 1993 cooperatively by USDA-ARS, and the University of Georgia Coastal Plain Experiment Station in Tifton, GA. This grass has excellent potential for production of high quality hay and pasture forage, with high yield and improved animal performance compared to many other bermudagrasses.[8,16,24] The hybrid is taller and has larger stems and broader leaves than other bermudagrass varieties.[8,24] Tifton 85 has produced a 34% higher dry-matter yield with 47% higher digestible yield compared to Coastal bermudagrass.[8] Since its release, Tifton 85 bermudagrass has been utilized extensively with no reported HCN toxicities pre- or post-release.[8,24,26] Clinical signs and subjective laboratory diagnosis in this case were consistent with HCN toxicity; however, a review of literature indicated no historical problems indicative of either acute or chronic HCN toxicity with Tifton 85 bermudagrass.

The present case was lacking in the diagnostic workup that prevented a confirmatory diagnosis of HCN toxicity. The animals were given hay that contained johnsongrass prior to being allowed to enter the Tifton 85 bermudagrass pasture. Exposure to HCN usually results in animals rapidly succumbing to the toxin, and becoming recumbent in close proximity to the exposure. The majority of animals did not die the in the bermudagrass pasture; instead 10 of the 14 dead cattle were found in the hay pen, 3 near the pen and 1 animal about 80 yards (75 meters) into the bermudagrass pasture. There were no attempts to analyze hay for HCN or examine for other toxic plants in the hay pen. Necropsy pictures viewed later revealed unidentifiable grasses and weeds in the background in proximity to where the animals died and were necropsied.

Limited diagnostic samples were submitted from only 2 animals. Rumen contents were HCN positive when sampled 12 to 16 hours postmortem, prompting a diagnosis, and except for nitrates no other causes of sudden death were evaluated. Consideration was not given to cyanogenic bacteria, such as *Pseudomonas*, which are associated with rumen microflora, and can result in a false positive diagnosis in animals with advanced postmortem autolysis.[1,2]

The lethal dose (LD50) of HCN that can cause sudden-death syndrome varies among forage types and between animals. The complications associated with definitive laboratory confirmation in this case suggests quantitative tests for HCN be reassessed to provide standardized testing and reporting of toxic levels of HCN in plants and in ruminant material. The present case should stimulate more basic and translational research regarding potential cyanide toxicity from consuming forages, and include monitoring and observations of grazing livestock.

Conclusions

Tifton 85 bermudagrass was incriminated as the cause of the cattle deaths; however, the lack of a comprehensive diagnostic workup, and the discordant HCN results between 3 different diagnostic laboratories provided only presumptions and no definitive diagnosis for the death of the cattle. Tifton 85 bermudagrass has been utilized extensively for more than 2 decades throughout the southeastern US, and this case report is the first description of a possible association between Tifton 85 bermudagrass and HCN toxicity. This case received national media attention based on invalid definitive conclusions. A comprehensive investigation of any ruminant deaths or other pathologic conditions associated with any forage or feedstuff should be performed and documented.

There are risks with placing dehydrated or hungry cattle on pasture with young, immature forage growth. Knowledge of nutritive composition of hay, pastures, and good animal husbandry management protocols are useful to prevent animal losses.

Endnotes

[a]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX

[b]E.M Quant. Nitrate Test Strips, EMD Chemicals, 480 S Democrat Rd, Gibbston, NJ

[c]Prowl H_20 (pendimethalin), BASF Corporation, PO Box 13528, Triangle Park, NC

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