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Case report: Bovine ethylene glycol toxicosis

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Abstract

Acute death of 1-month-old calves on pasture that were accidentally exposed to an oil/antifreeze mix were investigated. Clinical signs were not observed and necropsy findings were non-specific. Histological diagnosis of calcium oxalate nephrosis with concurrent tubulointerstitial suppurative nephritis was supported by culture. Ethylene glycol exposure was confirmed via analytical methods.

Key words: bovine, nephrosis, ethylene glycol, toxicosis

Introduction

Ethylene glycol ($HOCH_2CH_2OH$) (EG) is primarily used as an antifreeze and a deicing agent, but is also used as a cryoprotectant, a solvent in certain industries, and an ingredient in many solutions, fluids, and stains. The most common form of ethylene glycol in cases of livestock poisoning is antifreeze, and possible sources include leaking radiators of farm vehicles, livestock access to open containers, product spillage, contamination of feed, and discarding of motor components where cattle have access. Ethylene glycol has a sweet taste leading to consumption by animals. The reported LD50 in cattle is 0.91 to 4.54 mL/lb (2 to 10 mL/kg) of 95% EG.[3] Ethylene glycol is a potent CNS depressant, and is metabolized into highly toxic metabolites that result in metabolic acidosis and acute renal failure. Metabolism of EG occurs in 2 phases beginning with the initial conversion by the enzyme alcohol dehydrogenase in the liver to glycoaldehyde, which is also a CNS depressant. Glycoaldehyde is then converted to glycolic acid via aldehyde dehydrogenase. Glycolic acid is further converted to either glyoxylic acid by glycolate oxidase or to oxalic acid by glycolate dehydrogenase. Glycolic acid and glyoxylic acid production overwhelm the metabolic enzyme systems in all organs, and this accumulation leads to a systemic metabolic acidosis. Oxalic acid combines with calcium ions in circulation, and the insoluble complex deposits in tissues, especially the renal tubules. Clinical signs reported in cattle include ataxia, anorexia, dyspnea, tachycardia, hypersalivation, recumbency, and death.[3,7] Reported necropsy findings include perirenal edema and black or tan, swollen kidneys with black, foul-smelling gastrointestinal contents.[3]

Case Description

Fresh and formalin-fixed tissues (heart, liver, and kidney) and gastrointestinal contents from a 1-month-old crossbred beef heifer calf were submitted to the Kansas State University Veterinary Diagnostic Laboratory for diagnostic investigation. The case history indicated the cow herd had been in this pasture for 2 weeks. Three calves died acutely with no known clinical signs; the deaths were spread over the course of 2 weeks. A field necropsy was performed by the referring veterinarian on the fourth calf that died, and tissues from this calf were submitted for investigation.

The deceased calves were part of a 200-head crossbred beef-cow herd in West-Central Kansas. This producer has 4 pastures and the cow herd is split among these pastures for summer grazing. All of the deaths occurred in a single pasture that contained 48 cow-calf pairs. Gross observations reported from the necropsy included abomasal distention, small intestinal distention, and enlarged kidneys that were full of fluid. The abomasum was full of plant fragments and a white, chalky material of unknown origin. Fresh and fixed heart, liver, kidney, and fresh abomasal contents were submitted for investigation. Subsequent investigation of the pasture revealed an open pan underneath a tractor in the pasture that contained what appeared to be antifreeze, oil, and water. Consultation with the owner confirmed the contents of the pan were from tractor maintenance performed 6 months prior to the deaths, and before the winter months.

Diagnostic Findings

Microscopic examination of an impression smear of the fresh kidney demonstrated elongated, flat, six-sided crystals with pointed ends, also known as "picket fence" crystals due to their resemblance to individual boards of a picket fence (Figures 1 and 2). This crystalline morphology is consistent for calcium oxalate monohydrate crystals which are frequently associated with ethylene glycol poisoning.[8] The impression smear allowed visualization of the dimensional structure of the crystals prior to formalin fixation. Microscopic examination of the kidney sections confirmed several changes including intratubular crystals and moderate to large numbers of neutrophils present within the cortical

and medullary tubular lumens. Evaluation of the sections under polarizing light confirmed the birefringent character of the intratubular crystals that were present, most often as radiating or aggregated clusters (Figures 3 and 4). Tubular epithelium surrounding the crystalline clusters was variably affected, demonstrating mild degeneration to necrosis and sloughing into the tubular lumens. The sections of heart and liver were morphologically unremarkable.

Morphologic diagnoses included tubular nephrosis with intraluminal calcium oxalate crystals and suppurative tubulointerstitial nephritis. Ethylene glycol toxicosis was the presumptive diagnosis based on the tubular nephrosis; however, the severity of the suppurative tubulointerstitial nephritis was most consistent for a concurrent ascending bacterial nephritis. Other possible causes for calcium oxalate nephrosis include ingestion of some oxalogenic plants such as kochia (*Kochia scoparia*), pigweed (*Amaranthus retroflexus*), greasewood (*Sarcobatus vermiculatus*), and *Halogeton* spp. The latter 2 species are not routinely found in Kansas, and the presence of oxalate containing plants can easily be confirmed by visual inspection of the pasture. Based on the presumptive known exposure to the antifreeze/oil mixture, further investigation to identify these plants did not occur. In addition, once the antifreeze/oil mix was removed, further cases were not reported.

Aerobic culture of the fresh kidney yielded slight growth of *Enterococcus avium* and hemolytic *Escherichia coli*. There was abundant growth of non-hemolytic *Escherichia coli*, a common urinary tract pathogen in cattle,[9] which further supports the suspicion of an ascending bacterial infection resulting in suppurative tubulointerstitial nephritis.

Gas chromatography/mass spectroscopy (GC/MS) analysis of the submitted gastrointestinal content revealed the presence of ethylene glycol. Quantitative analysis for ethylene glycol was not performed in this case based on histopathology and confirmed ingestion. In aggregate, the antifreeze/oil mixture present in only 1 pasture, the cytological and histological findings, and the GC/MS analysis confirm a diagnosis of ethylene glycol-induced nephrosis resulting in death of this calf and the presumptive cause of death in the first 3 calves.

Discussion

The toxicokinetics of ethylene glycol are well known in companion animals and humans. Ethylene glycol is rapidly absorbed and distributed into the circulation. Metabolism occurs in the liver, and the resulting metabolites are more toxic than the parent compound and are responsible for severe metabolic acidosis and acute renal failure associated with this toxicosis.[1] The terminal metabolite is oxalic acid which combines with calcium ions in circulation, and the insoluble complex is deposited in tissues, especially the renal tubules. This leads to crystal deposits and crystalluria, and is likely partially responsible for the hypocalcemia described in experimental intoxications.[4]

Clinical signs associated with ethylene glycol toxicosis include increased respiration, hypersalivation, ataxia, paraparesis, depression, recumbency, and death.[3,7] Clinicopathologic findings in experimental cases include azotemia, hypocalcemia, neutrophilia, plasma hyperosmolality, and hemolytic anemia.[3,6] Supportive care consisting of IV fluids, diuresis, calcium supplementation, and analgesics are indicated. Monitoring of BUN and creatinine will help determine prognosis for valuable animals. Treatment for these cases is generally unrewarding, as lethal renal damage has already occurred in order to cause the observed clinical signs.

Ethylene glycol toxicosis is a common occurrence in small animals, but relatively rare in livestock. The most common sources of ethylene glycol causing accidental toxicosis in North America are automotive antifreeze and windshield deicers.[5] The most recent report in cattle involved corn condensed distillers' solubles, also known as corn syrup, that contained 22.4% ethylene glycol.[2] One earlier case involved a 1-month-old calf that died secondary to ethylene glycol ingestion.[3] Crowell et al recreated the characteristic kidney lesions with oral doses of reagent grade, batch grade, and commercial antifreeze containing ethylene glycol at doses from 0.91 to 4.54 mL/lb (2 to 10 mL/kg).[3] The group also reported that pre-ruminant calves were more sensitive than ruminating cattle. The calf in the current case was estimated to weigh 150 lb (68.2 kg). Assuming the antifreeze was commercial grade (95%), a single animal would have to ingest approximately 4.5 to 23 ounces (136 to 680 mL) of undiluted ethylene glycol. The antifreeze mix used in engines is a 50/50 antifreeze/water mix further diluting the ethylene glycol. An oral dose of 9 to 46 oz (270 to 1,360 mL) of 50/50 antifreeze can cause toxicosis in a 150 lb (68.2 kg) calf. Newborn calves routinely drink 64 ounces (1,920 mL) or more in a single feeding. These calves were 4 weeks of age or older, indicating that ingestion of 1.3L of fluid for the calves in this case is well within normal physiological parameters.

Conclusion

This case report describes postmortem diagnosis of ethylene glycol toxicosis in a beef calf. The histopathological diagnosis of calcium oxalate nephrosis has traditionally been highly associated with ethylene glycol toxicosis. The visualization of picket fence calcium oxalate crystals in an impression smear of fresh kidney is abnormal, but when they are present, they are frequently associated with ethylene glycol. Identification of ethylene glycol in the gastric contents was valuable in further confirmation of the diagnosis. Rumen contents are necessary to identify ethylene glycol prior to metabolism in adult animals; the calves in this case were pre-ruminants and the abomasum still contained the parent compound. It is unknown if the bacterial nephritis is related to the oxalate nephrosis. The oxalate nephrosis could cause oliguria or anuria, leading to bacterial proliferation due to lack of physical flushing of the urinary tract. Typically, anuric

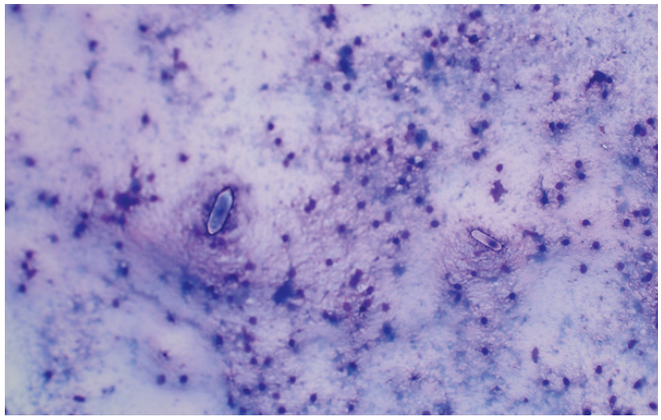

Figure 1. Impression smear of fresh bovine kidney, "picket fence" calcium oxalate monohydrate crystals 20X.

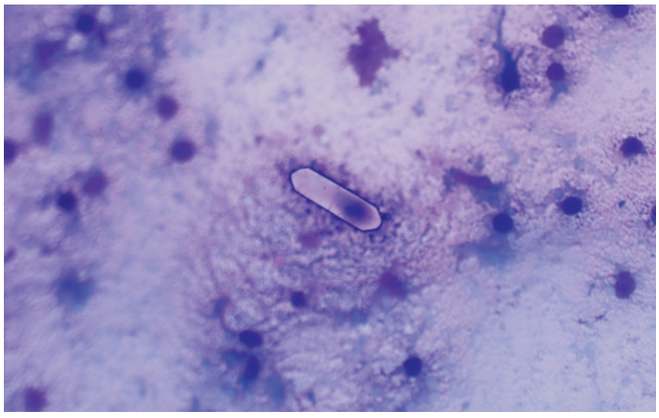

Figure 2. Impression smear of fresh bovine kidney, "picket fence" calcium oxalate monohydrate crystal 60X.

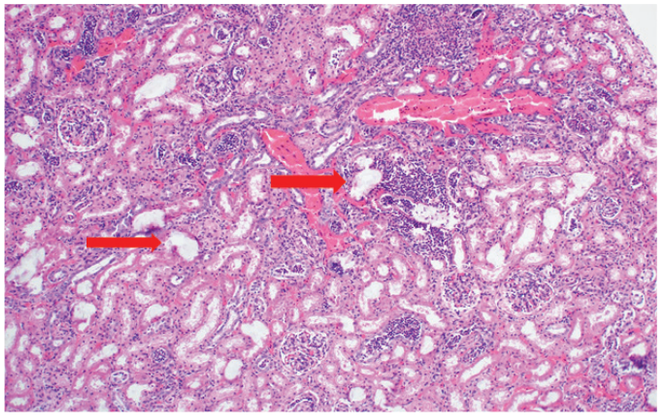

Figure 3. Bovine kidney, tubulointerstitial suppurative nephritis. H&E stain 10X. Calcium oxalate crystals (red arrows).

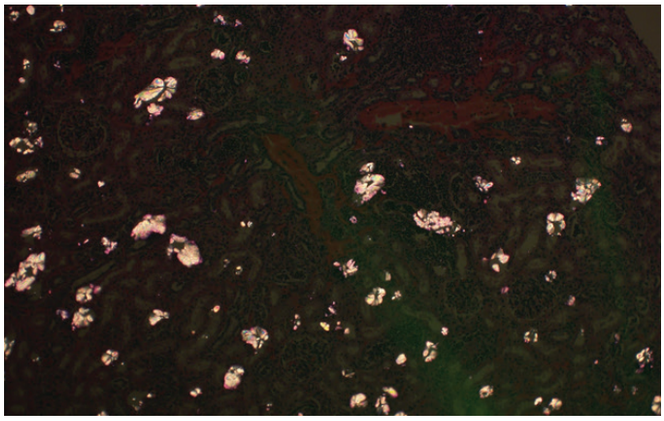

Figure 4. Same field as Figure 1 under polarizing light, calcium oxalate nephrosis 10X.

animals do not live very long, giving a small window for bacterial overgrowth. Examination of the urinary bladder of this animal would be desirable to ascertain information about the local environment of the urinary tract or a potential cystitis.

The clinical history, microscopic findings, and chemical analysis all support the diagnosis of calcium oxalate nephropathy associated with ethylene glycol ingestion. The discovered open pan of oil/antifreeze mix available to the calves made it relatively easy to identify the source of intoxication in these animals. These findings were further corroborated by the lack of additional deaths in this herd after removal of the source. This case report should remind and encourage practitioners to rely not only on molecular diagnostics when investigating cases, but to also utilize histopathology and analytical techniques to support a diagnosis when warranted. Practitioners and producers should be reminded that automobile fluids are a risk for toxic compound exposure to animals.

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References

1. Amoroso L, Cocumelli C, Bruni G, Brozzi A, Tancredi F, Grifoni G, Mastromattei A, Meoli R, Di Guarddo G, Eleni C. Ethylene glycol toxicity: A retrospective pathological study in cats. *Vet Ital* 2017;53:251-254.
2. Barigye R, Mostrom M, Dyer NW, Newell TK, Lardy GP. Ethylene glycol toxicosis in adult beef cattle fed contaminated feeds. *Can Vet J* 2008;49:1018-1020.
3. Crowell WA, Whitlock RH, Stout RC, Tyler DE. Ethylene glycol toxicosis in cattle. *Cornell Vet* 1979;69:272-279.
4. Grauer GF, Thrall MA, Henre BA, Grauer RM, Hamar DW. Early clinicopathologic findings in dogs ingesting ethylene glycol. *Am J Vet Res* 1984;45:2299-2303.
5. Jacobsen D, McMartin KE. Methanol and ethylene glycol poisonings. Mechanism of toxicity, clinical course, diagnosis and treatment. *Med Toxicol* 1986;1:309-334.

6. Singh D, Kumar M, Rao V. Experimental ethylene glycol toxicity in cow calves: Clinico-haematological and biochemical changes. *Indian J Vet Med (India)* 1994;14:68-70.
7. Singh D, Kumar M, Varshney K. Pathological changes in experimental ethylene glycol intoxication in cow calves. *Indian J Anim Sci* 1994;64:1461-1363.
8. Ukwubile CA, Targema BH, Tam LI. Prevalence of urinary calcium oxalate and other urine microscopic parameters among secondary school students in Bali, Nigeria. *ARC J Urology* 2017;2:17-21
9. Yeruham I, Elad D, Avidar Y, Goshen T, Asis E. Four-year survey of urinary tract infections in calves in Israel. *Vet Rec* 2004;154:204-206.