Chronic Copper Toxicosis in a Sheep

Clinicopathologic Conference

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History

A four year old female show sheep presented to Mississippi State University College of Veterinary Medicine's Department of Pathobiology and Population Medicine for a necropsy on 1/12/18. It had been vaccinated in the spring, prior to purchase by the current owners. It was housed with seven other sheep, one of whom died four days before. That same week there was cold weather and snow, which was unusual for the area. The six remaining sheep were reportedly losing wool due to an unspecified deficiency and had been chewing on the wood in their stall. The owners were concerned about potential deficiencies of magnesium, selenium, and vitamin E. The sheep's owners had noticed she was ill for several days prior to her death. They reported she was jaundiced, had an arched back, and agonal breathing. She ignored feed on 1/11/18 and died sometime after 9 PM that night.

Necropsy Findings

At the time of necropsy on 1/13/18, the sheep's body condition was normal at 4/9. The sclera, mucous membranes, subcutaneous and abdominal viscera were diffusely icteric. There was mild to moderate multifocal subcutaneous edema along the ventrum and ventral neck, admixed with scattered petechial hemorrhages.

The abdominal cavity contained approximately 100 ml of red-tinged watery fluid. Both kidneys were slightly enlarged and black with an indistinct corticomedullary delineation. The renal pelvises and the bladder contained a small amount of dark brown to black thick urine. The liver had a green to orange discoloration diffusely. The lungs were diffusely mottled with dark red and purple and poorly collapsed with a rubbery consistency. Throughout all the lung lobes, the airways oozed large amounts of yellow to clear foamy fluid upon cross sectioning. The

trachea contained a moderate amount of white foam distally, extending into the main stem bronchi. The rest of the necropsy was unremarkable.

Histopathologic Findings and Additional Laboratory Testing

Kidneys had acute, disseminated, severe hemoglobinuric nephrosis with tubular degeneration and necrosis. Widely disseminated throughout the cortex and medulla, up to 80% of the tubules were ectatic, and contained red to orange, granular to globular, sometimes refractile material. Fewer tubules also contained amphiphilic cellular and granular casts. There were variable degrees of epithelial degeneration and necrosis of tubular epithelial cells characterized by swelling, vacuolation, hypereosinophilia with decreased cellular detail, pyknosis or karyorrhexis. Multifocally, the swollen epithelial cells contained light brown to yellow pigment or clusters of brightly eosinophilic hyaline globules. In less affected tubules, there was disorganization of the tubules with frequent alteration and variation of the epithelial nuclear size and polarity.

The liver had subacute, disseminated, severe centrilobular degeneration and necrosis with cholestasis and ductular reaction. Diffusely, the centrilobular hepatic cords were disorganized with widened sinusoids intersecting with delicate collagenous tissue and occasional hemorrhage. The hepatocytes had moderate variation in size and shape, and were often bi- or multinucleated. There were widely disseminated bile canaliculi and prominent Kupffer cells laden with bright orange to brown pigment. The portal tracts were hypercellular with moderate numbers of lymphocytes and plump pigment-laden macrophages. In some areas portal tracts had increased oval cells, sometimes forming small caliber bile ducts. The centrilobular hepatocytes were peppered with copper-staining granules, and there were widely dispersed copper-laden macrophages throughout the section.

Lungs had acute, disseminated, severe pulmonary edema. Up to 80% of the alveolar spaces and including some bronchioles contained eosinophilic wispy fluid admixed with moderate numbers of plump alveolar macrophages. The interlobular septa were also moderately distended by the eosinophilic wispy fluid. The pulmonary vasculature was frequently dilated and engorged with blood.

The red pulp of the spleen was hypercellular and dispersed with moderate numbers of histiocytes laden with light brown globular pigment.

A liver sample was sent out for mineral analysis, and the result showed 1274 ug/g on a dry matter basis (1274 mg/kg DM) of copper in the liver (reference range provided on the report was 25-400 ug/g). The molybdenum level was 1.69 ug/g DM, just below the given reference range of 1.70-6.5 ug/g.

The diagnosis was chronic copper toxicosis.

Pathophysiology

Copper is an essential nutrient which plays a key role in producing ATP, synthesizing melanin, appropriate nervous system function, connective tissue formation and promoting growth.[2, 7, 8, 12] Ingested copper is absorbed in the intestines, bound loosely to proteins, and transported to the liver, where it binds with a protein called ceruloplasmin.[2, 6, 8, 12]

The amount of ingested copper which is absorbed depends on age and species. Newborn ruminants absorb 70-75%, which decreases to <10% in adulthood, and less than 1% in the presence of copper antagonists.[2] Lambs absorb up to 90% of ingested copper, and adult sheep absorb 1-13%.[2]

The absorption and bioavailability of copper is also strongly affected by the elements molybdenum and sulfur.[2, 3, 6] High dietary molybdenum and sulfur in the rumen form a complex

called thiomolybdate, which renders copper biologically unavailable.[2, 3, 7] The effect of thiomolybdate on the metabolism of copper is significant enough that thiomolybdate toxicity can lead to secondary copper deficiency.[3] Zinc and iron decrease the absorption of copper to a lesser extent because they compete for the binding with metallothionein.[6]

Metallothionein is a protein complex which binds with copper and prevents it from taking a toxic form. The metallothionein is then stored in lysosomes within hepatocytes. 70-90% of copper is brought into hepatocytes; from there the excess is packaged into metallothionein, and the rest is excreted via the urinary tract in small amounts,[2] redistributed to bile and excreted or reabsorbed in the intestines, or used for the formation of cerulopasmin and returned to the bloodstream.[2, 6, 12] About 80% of the copper in the bloodstream is bound within ceruloplasmin.[7, 12] It is also present in the blood in other functional forms.[7]

Excess or deficient copper is taken care of in two main ways: excretion and storage. Excretion is severely limited in ruminants; they are able to excrete less than 1% of daily ingested copper via biliary excretion, the body's main method of copper excretion.[6] Thanks to the liver storing and releasing copper to and from metallothionein as needed, overall concentration of copper in the blood still fluctuates very little, even during deficiencies and when excess copper is ingested.[2]

However, the liver has a limited capacity to store copper within metallothionein without becoming at risk for chronic copper toxicosis: that limit is about 1,000 mg Cu/kg liver.[2] Chronic copper toxicosis usually occurs when an animal ingests too much copper and too little molybdenum long term,[6] and it may occur even when copper intake is nontoxic,[3] particularly when hepatotoxins are involved. The pathophysiology of chronic copper toxicosis can be divided

into four pathways, and any single case could involve multiple pathways: simple dietary excess, insufficient molybdenum, hepatotoxins, and a phytogenously.

Simple dietary excess may occur by oversupplementation with copper (e.g. when attempting to prevent copper deficiency or manage internal parasites), access to mineral blocks or feed or milk replacer formulated for other species, high levels of palm kernel expeller in the diet, high levels of chicken litter in the diet from chickens that were supplemented with copper, pasture fertilized by manure from swine or chickens that were supplemented with copper, pasture contaminated with copper-containing fungicides, pasture contaminated with copper from industrial pollution, inappropriately formulated feed mixes, or Cu fencing or piping.[3, 6, 8, 12]

Insufficient molybdenum can cause chronic copper toxicity in a diet with normal copper levels[12] because molybdenum decreases the bioavailability of copper, as discussed previously. Copper to molybdenum ratios greater than 6:1 are more likely to result in poisoning,[6] and ratios greater than 10:1 are to be avoided in sheep feed.

Hepatotoxins leading to chronic copper toxicity, also called hepatogenous chronic copper toxicity, is the excessive retention of copper secondary to ingesting plants associated with liver damage. It generally occurs in conjunction with excess dietary copper or insufficient molybdenum. The toxin most commonly associated with this condition is pyrrolizidine alkaloid from plants, including Heliotropium, Crotalaria, Senecio, and Echium plantagineum. These plants are capable of causing hepatitis without copper metabolism abnormalities, and they can be associated with damage to other tissues, especially pulmonary tissue, depending on the plant species.[3] In the case of hepatogenous chronic copper toxicity, the plants are consumed over time in small enough amounts to cause morphologic and biochemical changes in liver cells without major impairment of liver function. Pyrrolizidine alkaloids prevent hepatocellular DNA

synthesis and mitosis, which renders the liver unable to replace necrotic hepatocytes, so surviving hepatocytes are required to take up copper released from their dying neighbors. This predisposes the affected animal to copper toxicosis. The effects are cumulative from one grazing season to the next: grazing on pyrrolizidine alkaloid rich pasture one year may seem to cause little harm, but the next grazing season the flock could break with clinical disease.[3, 8, 12]

Phytogenous chronic copper toxicosis occurs when animals ingest relatively small amounts of copper but excessive retention occurs because of specific plants with no apparent association with liver damage.[3] Trifolium subterraneum, the subterranean clover, has been associated with phytogenous copper toxicosis, and it may contain lower than normal copper levels.[3, 8] Some sources consider grazing plants with elevated copper to molybdenum ratios to fall within the phytogenous category.[8] For example, young, rapidly growing plants usually have less molybdenum than mature pastures.[8]

In all cases of chronic copper toxicosis, the accumulation of copper in the liver exceeds the ability of the metallothionein therein to store it, and copper is released acutely into the bloodstream.[7] The specific consequences can be species-specific,[12] but for the purpose of our discussion (ruminants, especially sheep), it can be divided into two phases: the accumulation or prehemolytic phase and the acute hemolytic crisis.

The prehemolytic phase can last from weeks to more than a year, and during this time the accumulation of copper in lysosomes is often accompanied by necrosis of hepatic parenchymal cells and swelling of Kupffer cells,[8] but there are no clinical signs. Hepatic storage is able to protect the body from toxicosis until storage sites become saturated.[6]

When the storage sites are saturated and an inciting event occurs, the prehemolytic phase ends. The inciting incident can be spontaneous or a stressor to the animal, which results in the

death of hepatocytes.[8] Potential stressors include diet changes,[6] shipping, hierarchic change, the administration of oxidative drugs, starvation, change of housing, extreme weather conditions, advanced pregnancy, lactation,[6, 7] and severe hepatic injury from hepatotoxins.[12]

When hepatocytes die, copper is suddenly released from hepatocellular lysosomes and blood copper levels rise, leading to clinical signs. This is the acute hemolytic phase. This phase can last hours to days[2] and often results in death. The suddenly freed copper produces reactive oxygen species which destroy mitochondria and other cellular membranes. Oxidized red blood cells spontaneously lyse intravascularly or are sequestered in the spleen and degraded. Vitamin E is also denatured, leaving the body even more vulnerable to oxidative damage. The oxidative destruction adds up to acute, severe intravascular hemolysis, which causes acute anemia, which leads to hypoxemia, which leads to further hepatocellular necrosis, especially the centrilobular and midzonal regions. The hemoglobin released from lysed red blood cells stains plasma pink and is excreted by the kidneys, causing hemoglobinuric nephrosis in the process.[2, 6, 7, 8, 12]

Hemoglobinuric nephrosis is damage caused to the kidneys when there is free hemoglobin in the blood, as a result of intravascular hemolysis. The exact mechanisms are unknown, but it has been speculated that hypoxemia and copper-induced oxidative damage play a role.[1] A 2016 study of hemoglobinuria-related kidney injury showed that hemoglobin itself also leads to oxidative damage.[4] The hemoglobin in the kidney is also what gives it the dark color and metallic sheen that are so characteristic of chronic copper toxicosis.[6]

Species and Breed Considerations

Certain species and breeds are more susceptible to chronic copper toxicosis than others. In general, the disease occurs in ruminants, but particularly sheep [3, 7, 12] due to the storage of copper being poorly regulated in sheep:[12] their hepatocytes have a higher affinity for copper, and

their biliary excretion of copper occurs at a very low rate.[9] Texel lambs and North Ronaldsay sheep have proven in studies to be more susceptible to excess accumulation than other sheep breeds.[2] Merino sheep have been found to be more resistant to chronic copper toxicosis than British breeds.[8] Young ruminants are more susceptible than adults due to their higher absorption of copper.[2, 3, 8] Goats have a higher tolerance for copper than other ruminants.[2]

Excessive but sublethal liver copper concentrations are relatively common in dairy cattle, probably due to oversupplementation.[3, 7] Acute copper toxicosis has been described in cattle as well, in adult and juvenile cattle given injections of copper disodium edetate as treatment for copper deficiency. Cattle with the acute form of copper toxicosis exhibit head pressing, ataxia, and circling, without signs of a hemolytic crisis.[8] Diagnosis of acute copper toxicosis can be made by measuring plasma copper. Plasma copper concentrations between 2.4 and 20.0 ug/mL (2.4-20 ppm) are diagnostic of acute toxicosis.[6] Acute toxicosis will not necessarily result in elevated copper levels in the liver or kidney.[8]

The Bedlington terrier is a well-known example of copper toxicosis in a dog breed. They have a genetic disease believed to be unique to their breed with high prevalence worldwide.[10] An autosomal recessive mutation in the MURR1/COMMD1 gene has been identified as the causative factor.[10, 12] This mutation leads to decreased biliary excretion of copper and subsequent accumulation in the liver. Accumulation in the liver causes ongoing necrosis of hepatocytes, chronic inflammation, and fibrosis, which eventually progresses to end-stage liver and signs of hepatic failure.[12]

Copper-associated liver disease has been identified in other dog breeds too, namely West Highland white terrier, Doberman Pinscher, Skye terrier, Dalmatian, Anatolian shepherd dog, American and English cocker spaniel, and Labrador retreiver.[5, 12] In some cases of canine

chronic hepatitis, excessive copper accumulates in hepatocytes and Kupffer cells. The role of copper in these breeds is uncertain. Copper is excreted in bile in dogs (like in ruminants), but extrahepatic cholestasis does not seem to significantly increase hepatic copper levels.[12]

Copper metabolism disorders are rarely described in cats, and more commonly in mice and rats.[5, 12] Horse appear to have high tolerance for copper, as clinical copper toxicosis was not induced experimentally when fed high levels of copper.[6]

Diagnosis

Diagnosis of chronic copper toxicosis begins with recognizing the clinical findings. Once the acute hemolytic phase begins, clinical signs develop rapidly.[6] Icterus and hemoglobinuria (dark red-to-black urine) are the classic clinical signs associated with chronic copper toxicosis. Other signs include lethargy, anorexia, pallor, tachycardia, tachypnea, abortions in pregnant animals due to hypoxemia, and death. Feces may be watery, dark, blood-tinged, or have yellowish discoloration, but these abnormal fecal findings most commonly occur in cattle, particularly when there is secondary abomasal ulceration. Further antemortem diagnostics may show hemoglobinemia, methemoglobinemia, Heinz body formation, anemia, and hemoglobinuria.[2, 6, 7, 8, 12]

Common necropsy findings include a pale to orange and friable liver, dark blue or blue-black kidneys with a metallic sheen due to entrapped hemoglobin, pale and icteric tissues, petechial and ecchymotic hemorrhages, firm lungs, possibly an enlarged and congested spleen, and a bladder filled with serosanguinous urine.[6, 8, 12]

On histology of the liver, there is hepatic necrosis, especially in the centrilobular region, pigment-laden Kupffer's cells, bile duct hyperplasia, and pericholangitis.[6, 8]

For definitive diagnosis of copper toxicosis, measurements of copper levels in the tissues are required. Blood is the most convenient sample in a live animal, but its copper concentration can be deceptive. Copper levels in serum and plasma are not elevated until just before or during a hemolytic crisis. Therefore, blood copper may be useful in diagnosing an animal that is clinically ill, but cannot be used for detecting prehemolytic chronic copper toxicosis.[6, 7, 8]

A liver biopsy is the most sensitive means of evaluating copper levels,[7] but it too has its drawbacks. Normal hepatic copper concentrations have been reported during the hemolytic crisis,[4] because during the hemolytic crisis the liver copper concentration may fall into the nontoxic range when it is released from the liver.[7, 8] Also, elevated hepatic copper concentrations have been reported in apparently healthy sheep.[11]

The copper level in kidneys may provide more diagnostic value than the liver or blood during the acute hemolytic phase.[11] If enough copper leaves the liver and circulates through the blood, kidney copper levels will rise to the toxic range.[7, 8] Therefore, it is recommended to measure copper in both the liver and the kidney if copper toxicosis is suspected.[7, 8]

Treatment

Treatment is often unsuccessful the acute hemolytic crisis has begun.[8] Supportive care for these animals includes oxygen, IV fluids, and packed red blood cells when indicated.[6] Most other treatments are directed at using sulfur and molybdenum to bind the copper in biologically unavailable forms and increase excretion.

The traditional treatment is the administration of molybdenum and sulfur in the form of ammonium molybdate or sodium molybdate and sodium thiosulphate.[6, 8] This combination has been reported to begin to decrease liver copper levels within four days of commencing treatment.[8]

Ammonion tetrathiomolybdate (molybdenum and sulfur which has already been combined into thiomolybdate) has been found to significantly decrease liver copper within six days. Administering xylazine at the same time doubles the amount of copper excreted in urine and in bile compared to giving tetrathiomolybdate alone.[6, 8]

Several other treatments have been suggested as well. Methylene blue is recommended to control methemoglobinemia.[9] Vitamin E[6] and C[9] may help protect the red blood cells from oxidative damage. Penicillamine can increase urinary copper excretion in sheep by 10-20 fold,[6, 8] but it is often cost prohibitive for livestock.[8] A single dose may be tempting, as it does increase copper excretion, but the effect is transient and insignificant for reducing total hepatic copper.[6] Trientine, a drug used to improve copper excretion in humans, has been tested in sheep with mixed results.[8]

Prevention

When determining preventative measures, sources of copper, along with molybdenum, sulfur, and iron much be considered.[2] Preventative measures for high risk animals include removal of sources of excess copper,[2, 6, 7] supplementation of copper antagonists,[2, 6, 8] feeding of species-appropriate feed and mineral mixes,[2, 6, 7] and pasture management.[3] Pasture management in the context of copper toxicosis prevention means encouraging grass growth in pastures, preventing animals from grazing lush, clover-dominant pasture in autumn (to avoid subterranean clover), preventing ingestion of hepatotoxic plants, and eradicating hepatotoxic plants from the pasture.[3] Pastures known to be copper-contaminated can be top-dressed with molybdenum phosphate at 113 g/ acre.[6]

Sheep feed should not contain more than 20 ppm copper. The ideal copper to molybdenum ratio is 6:1, and a ratio of greater than 10:1 is likely to cause toxicity. Calves should be fed no more than 50ppm copper in feed, and adult cattle no more than 100 ppm.[2]

Conclusion

When presented with a jaundiced, hemoglobinuric sheep with black kidneys on necropsy, the top differential diagnosis should be chronic copper toxicosis. Definitive diagnosis depends on copper levels in the liver and kidney which complement the clinical and histologic findings. Prevention of chronic copper toxicosis in high risk sheep depends on removing sources of excess copper, supplementing copper antagonists, and removing hepatotoxins.

References

1. Boznyski CC, Evans TJ, Kim DY, et al. Copper toxicosis with hemolysis and hemoglobinuric nephrosis in three adult Boer goats. J Vet Diagn Invest 2009; Vol 21: 395-400.
2. Committee on Nutrient Requirements of Small Ruminants. Nutrient Requirements of Small Ruminants. Washington, D.C.: The National Academies Press, 2007; 126-129.
3. Constable PD, Hinchcliff KW, Done SH, Grunberg W. Veterinary Medicine. 11th ed, Vol 1-2. St. Louis: Elsevier, 2017; 2168, 2173, 645-648, 731, 823-825.
4. Deuel JW, Schaer CA, Boretti FS, et al. Hemoglobinuria-related acute kidney injury is driven by intrarenal oxidative reactions triggering a heme toxicity response. Cell Death Dis 2016; Vol 7, Iss 1.
5. Fuentealba IC, Aburto EM. Animal models of copper-associated liver disease. Comparative Hepatology 2003; 2:5.

6. George LW. Copper toxicosis. In: Smith BP. Large Animal Internal Medicine. 4th ed. St. Louis: Mosby Elsevier, 2009; 1166-1169.
7. Herdt TH, Hoff B. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet Clin Food Anim 2011; 27: 255-283.
8. Plumlee KH. Metals and other inorganic compounds. In: In: Smith BP. Large Animal Internal Medicine. 4th ed. St. Louis: Mosby Elsevier, 2009; 1709-1712.
9. Van der Merwe D. Copper toxicity in sheep and goats. In: Proceedings. CVC in Kansas City 2009.
10. Van de Sluis B, Rothuizen J, Pearson PL, et al. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Human Molecular Genetics 2002; Vol 11, Iss 2: 165-173.
11. Villar D, Carson TL, Janke BH, et al. Retrospective study of chronic copper poisoning in sheep. An Vet (Murcia) 2002; 18: 53-60.
12. Zachary JF, McGavin MD. Pathologic Basis of Veterinary Disease. 5th ed. St. Louis: Mosby Elsevier, 2012; 44-45, 430, 446, 449, 718.