

RETROSPECTIVE STUDY OF CHRONIC COPPER POISONING IN SHEEP

Estudio retrospectivo de casos de intoxicación por cobre en ovejas

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SUMMARY

A retrospective survey was conducted on 112 cases of chronic copper poisoning in sheep diagnosed at the Iowa State University Veterinary Diagnostic Laboratory over a six year period. Animals that died with signs and lesions of a hemolytic crisis had mean (\pm SD) liver and kidney copper concentrations of 268.5 \pm 100.4 ppm and 49.9 \pm 36.2 ppm (wet weight), respectively. Kidney values were increased from normal values more consistently than liver values in animals that died with signs of a hemolytic crisis; thus, kidney is considered the tissue of choice when measurement of only one organ is requested. Analysis of 35 feed samples revealed ratios of copper:molybdenum greater than 10:1. This report also addresses recent findings on the pathogenesis and kinetics of chronic copper poisoning and the different treatment protocols recommended.

Key words: sheep, copper, toxicosis, treatment.

RESUMEN

En este trabajo se recopilan los valores de cobre en hígado y riñón de 112 casos de ovejas diagnosticadas con intoxicación crónica por cobre en el Veterinary Diagnostic Laboratory de Iowa State University durante un periodo de 6 años. Los animales que murieron con signos clínicos y lesiones de una crisis hemolítica tenían unas concentraciones medias (\pm DE) de cobre en hígado y riñón de 268.5 \pm 100.4 ppm y 49.9 \pm 36.2 ppm (peso húmedo), respectivamente. Los valores en riñón estaban aumentados con respecto a los valores normales del hígado, por lo que el riñón es el tejido de elección cuando el animal se sospecha haber muerto por intoxicación crónica de cobre. El análisis de 35 muestras de alimento procedentes de algunos de los casos diagnosticados

revelaron relaciones de cobre:molibdeno superiores a 10:1. Se hace referencia a la patogénia y toxicocinética del cobre, así como al tratamiento recomendado.

Palabras clave: oveja, cobre, toxicosis, tratamiento.

INTRODUCTION

Sheep are one of the most sensitive domestic species to excess copper intake. However, the impact of excess copper consumption by sheep varies greatly between different geographic regions depending on the copper levels in the soil. In the Midwest region of the United States where soil copper provides nutritionally adequate levels of copper in grains and forages, consumption of diets containing more than 15 ppm copper without added molybdenum predisposes sheep to chronic copper poisoning, a process that ultimately manifests itself as an acute and fulminant hemolytic crisis. The excess copper in the diet often comes from feeding sheep complete feeds contaminated with excess copper during the mixing process or from cattle or swine feeds containing elevated copper. (Osweiler et al., 1985). When copper concentrations in hepatocytes reach a point of saturation of the lysosomal system (Kumaratilake and Howell, 1989), the animal is predisposed to the characteristic hemolytic crisis in which stored copper is suddenly released into the bloodstream. Saturation of the lysosomal system is also believed to account for the necrosis of isolated hepatocytes that is commonly observed in chronic copper poisoning. The sudden release of copper by the liver usually occurs in just a few animals in the flock and is commonly associated with a stressful event such as summer heat, hauling, exhibition, breeding, shearing, lambing, or strenuous exercise. Clinical signs typically seen with the hemolytic crisis include lethargy, depression, isolation, and anorexia, and are accompanied by marked jaundice of the mucous membranes, anemia, hemoglobinuria and subsequent renal failure.

Ideally, sheep diets should contain a copper:molybdenum ratio of 6:1, and ratios in

excess of 10:1 may result in clinical copper toxicosis. Evidence of an antagonistic relationship between copper and molybdenum first came from studies in Australia (Dick and Bull, 1945). While searching for an explanation for chronic copper toxicosis, researchers incidentally observed that molybdate supplementation of cows and sheep decreased the liver storage of copper. Subsequent studies revealed that in the presence of inorganic sulfates, molybdenum formed insoluble copper thiomolybdates which are not bioavailable (Dick, 1953). Without sulfate, molybdenum had no effect and copper still accumulated in the liver. When both molybdenum and sulfate were fed to sheep, the animals developed signs of copper deficiency in spite of having normal or greater than normal blood copper concentrations, indicating that circulating copper was not available for biological function.

The half-life of copper in liver of overloaded sheep is 175 ± 91 days (Humann-Ziehanek et al., 2001); however, this is extremely variable between animals, and concentrations can remain at potentially toxic levels for more than 2 years. Because of this slow excretion rate and the low mortality in a flock, treatment to promote liver excretion is usually warranted on the rest of the flock when a diagnosis of copper poisoning is made (Humphries et al., 1988).

The purpose of this report is to provide laboratory data on confirmed cases of copper toxicosis in sheep at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) and to revise treatment protocols for chronic copper poisoning situations.

MATERIAL AND METHODS

A retrospective survey of the ISU-VDL database for cases of chronic copper poisoning

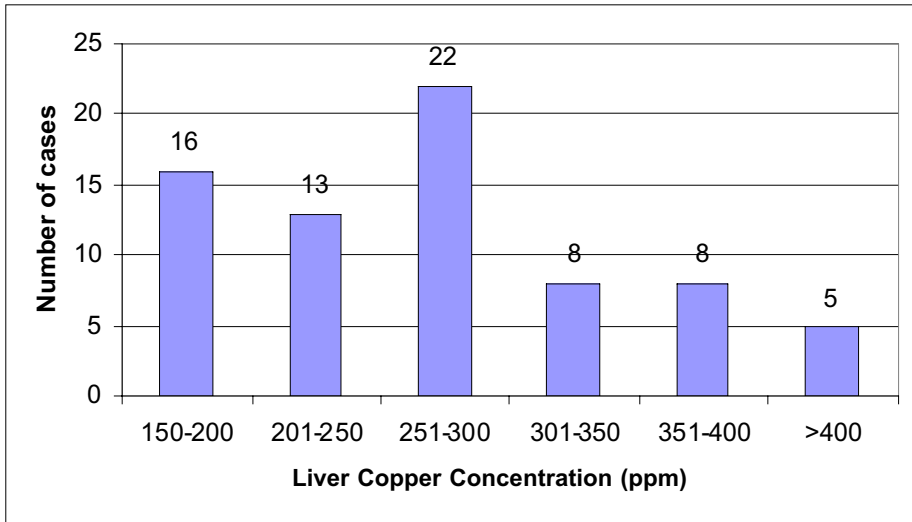


FIGURE 1. Number of cases at the different ranges of copper concentration (ppm, wet weight basis) in livers of sheep diagnosed with chronic copper poisoning.

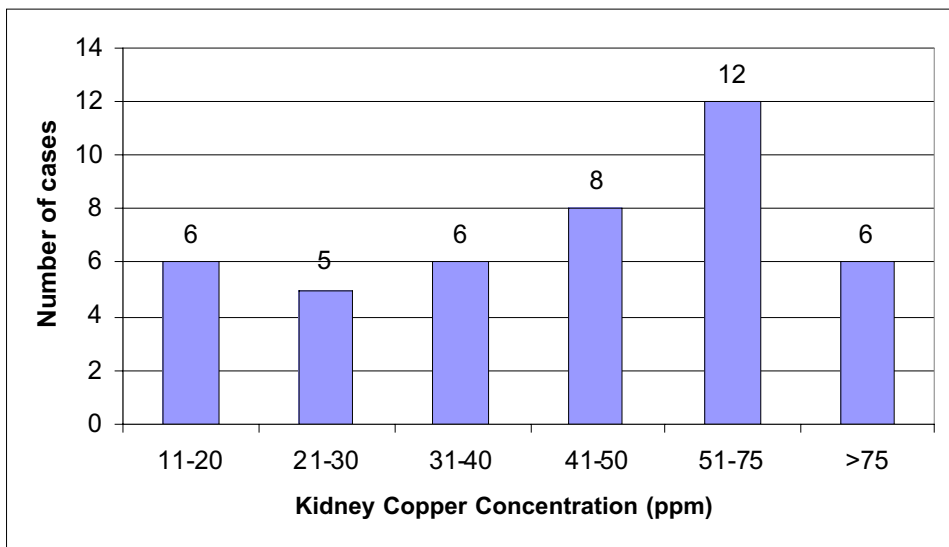


FIGURE 2. Number of cases at the different ranges of copper concentration (ppm, wet weight basis) in kidneys of sheep diagnosed with chronic copper poisoning.

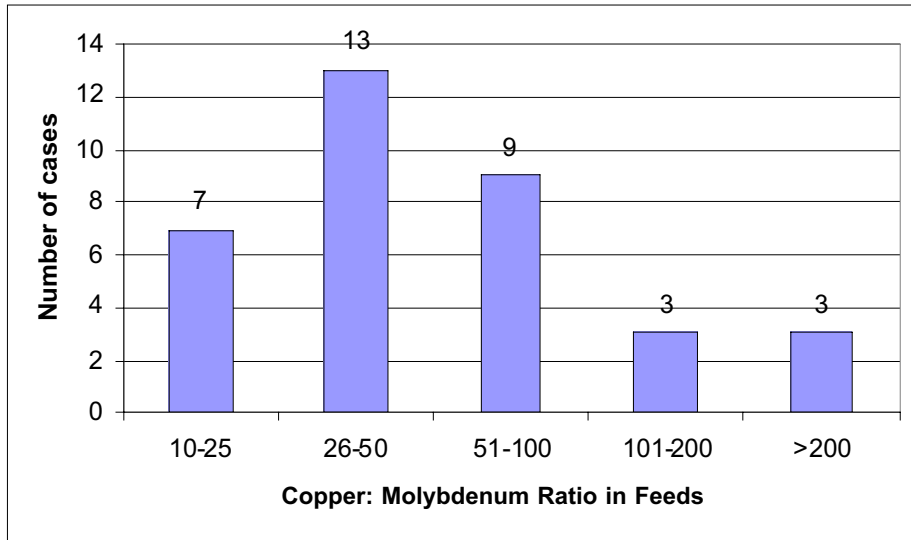


FIGURE 3. *Number of cases at each of different copper:molybdenum ratios in feeds that caused chronic copper poisoning in sheep.*

over a period of six years was conducted. A diagnosis of chronic copper poisoning was made based on the history of a hemolytic crisis as evidenced by typical gross lesions and measurement of copper levels in liver and/or kidney at toxic levels of >150 and >15 ppm (wet weight basis), respectively. In suspected cases, veterinarians usually reported gross lesions compatible with a hemolytic crisis: generalized icterus, yellowish liver and enlarged gun-metal colored kidney and bloody urine. Typically, liver and/or kidneys were initially submitted for copper analysis and when a diagnosis of copper toxicosis was made, feed was then sent for analysis. From a total of 112 cases, liver samples were analyzed in 72 cases, kidney samples in 43 cases, and feed samples in 35 cases. Copper concentrations in tissues and feed were determined by dry ash digestion, acid dissolution and analysis by flame atomic absorption spectroscopy. Molybdenum concentrations in tissues and feed were determined by dry ash digestion, chelation with ammonium pyrrolidine dithiocarbamate and extraction into methyl

isobutyl ketone. Extraction was followed with analysis by flame atomic absorption spectroscopy (Stahr, 1991).

In some cases, samples of liver and kidney were collected in 10% neutral buffered formalin and processed for routine histopathological examination.

RESULTS

The liver copper concentration ranged from 160 ppm to 550 ppm (ww) with a mean (\pm SD) of 268.5 ± 100.4 ppm in animals diagnosed with chronic copper poisoning (figure 1). The kidney concentrations ranged between 12 ppm and 220 ppm (ww), with a mean (\pm SD) of 49.9 ± 36.2 ppm (figure 2). When both liver and kidneys from the same animal were analyzed, it was typical to have concentrations of 4-10 times above the normal range for kidneys, with liver concentrations of only 1.5-2 times above normal values.

High dietary copper (>15 ppm) was found in 28 of 35 feed samples, while low (<1.0 ppm) molybdenum was found in 29 of these samples.

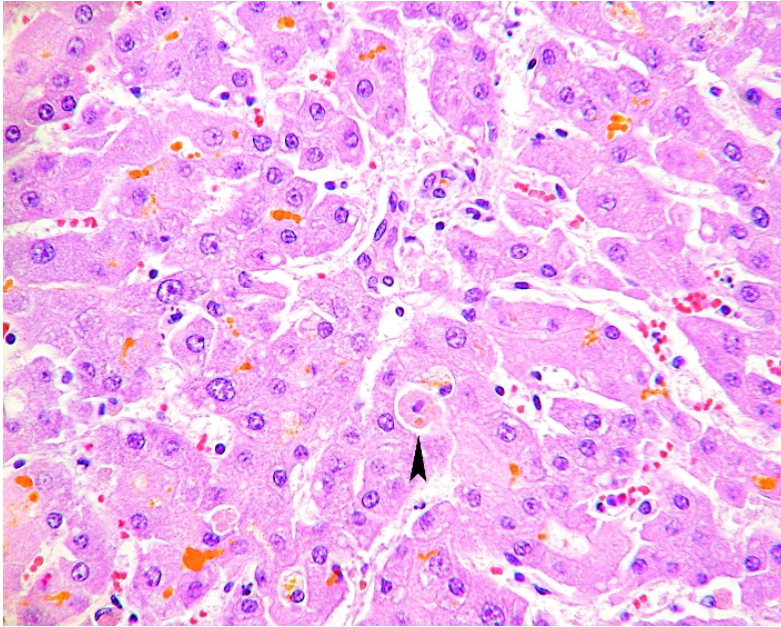


FIGURE 4. Liver from a 4-year-old Suffolk ewe that died with lesions of a hemolytic crisis. Note the bile stasis and individual hepatocyte necrosis (arrow). Liver contained 260 ppm copper (wet weight basis).

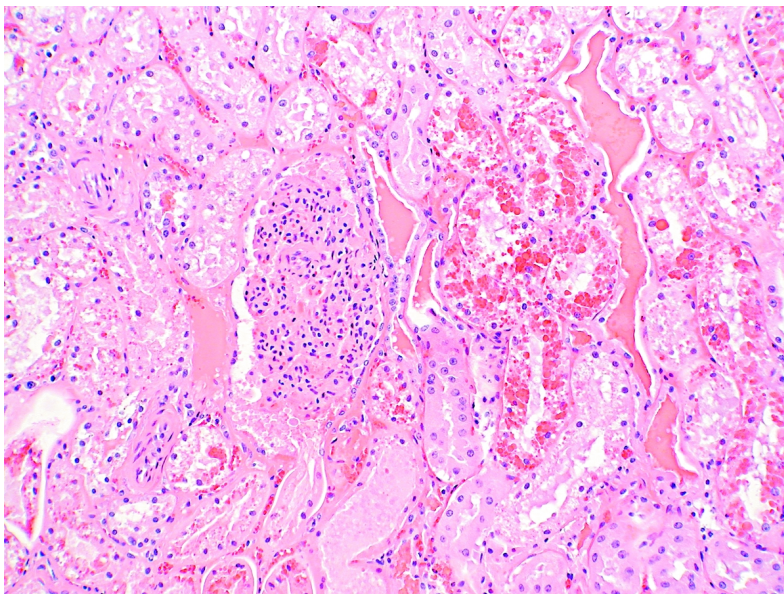


FIGURE 5. Kidney from a 4-year-old Suffolk ewe that died with lesions of a hemolytic crisis. Note the hemoglobin casts in the tubular lumens and globules within the cell cytoplasm. This kidney contained 19 ppm copper (wet weight basis).

The mean (\pm SD) concentrations of copper and molybdenum in feeds were 28.6 ± 23.7 ppm and 0.57 ± 0.37 ppm, respectively. The number of feeds with different toxic copper:molybdenum ratios is illustrated in figure 3.

In those cases in which tissues were examined microscopically, liver lesions consisted of generalized hepatocellular swelling and vacuolization. There were scattered individual necrotic hepatocytes (Figure 4) and bile accumulation, both intracellular and in canaliculi between hepatic cords. Also, mild to moderate bile duct hyperplasia was noted in portal tracts. In kidney, tubular lumens contained granular or eosinophilic material consistent with hemoglobin casts (Figure 5).

DISCUSSION

Evidence that sheep have died of copper toxicosis cannot be based solely on hepatic copper concentrations. Although our survey only addressed individual animals that died with a hemolytic crisis due to chronic copper poisoning, all animals in the flock will likely have elevated copper liver concentrations in spite of being apparently healthy. In fact, other studies have observed liver levels over 600 ppm (ww) in sheep that never showed signs of a hemolytic crisis (Humann-Ziehank et al., 2001). In their study, although animals were apparently healthy, a positive linear correlation ($r=0.872$) between plasma glutamate dehydrogenase activity and liver copper concentrations was observed. Furthermore, microscopic lesions indicative of chronic liver insult (i.e. individual hepatocyte necrosis and bile duct proliferation) were observed in their copper-loaded sheep.

Our results, together with reports of elevated liver copper concentrations but normal kidney values in apparently healthy sheep (Zantopoulos et al., 1996), suggest that copper concentrations in kidneys may provide more diagnostic value in cases of a hemolytic crisis than the liver. Normal kidney values

are 4-5 ppm (ww) and to our knowledge an elevation above 15 ppm has not been described in the literature on sheep unless the animal dies from copper poisoning. In our survey, it was common to find kidney concentrations of 50-60 ppm in animals that had liver concentrations of 200-300 ppm (ww).

Numerous studies have evaluated the predictive value of different clinical pathology parameters for diagnosing copper loading. There is inconsistency between studies as to the usefulness of liver enzymes and this is probably related to the rate of hepatocyte loss and turnover which in turn may be associated to the degree of copper overloading. Because some studies have shown that typical liver enzymes such as aspartate amino transferase (ASAT), gamma glutamyl transferase (GGT) or serum copper are only elevated immediately prior to the onset of the hemolytic crisis, they may not always be reliable diagnostic tools (Humann-Ziehank et al., 2001). In contrast, glutamate dehydrogenase appears to be consistently elevated and some authors consider this enzyme to be the best indicator for the diagnosis of chronic copper poisoning (Humann-Ziehank et al., 2001).

The cause of the copper-induced hemolytic crisis is not well understood. It is known that the antioxidant capacity (i.e. glutathione and glucose-6-phosphate dehydrogenase activity) of erythrocytes is diminished in chronically copper loaded sheep (Sansinanea et al., 1996). This may explain why lipid peroxidation rather than direct interaction of copper with the red cell membrane appears to precede hemolysis (Fernandes et al., 1988). The massive breakdown of erythrocytes results in excessive accumulation of hemosiderin and bile pigments in hepatocytes and macrophages (figure 4) that are typically observed histologically. In addition, because free hemoglobin passes into the glomerular filtrate, the tubules contain hemoglobin casts (figure 5) that are further indication of an acute intravascular hemolysis.

Occasionally, we have observed cases in which elevated copper concentrations in liver (150 ppm, ww) and kidney (50 ppm, ww) were not associated with a hemolytic crisis but with severe hepatocellular necrosis, periportal fibrosis and hyperplasia of bile ducts. In these cases, the kidney only exhibited lesions of a chronic interstitial nonsuppurative nephritis, which supports death attributed to liver disease but not kidney failure. Although such cases may not be of interest from a general population response to excess copper, they raise important questions as to the pathogenesis of copper poisoning in sheep and several arguments can be made. First, chronic copper poisoning may not always result in a fulminant hemolytic crisis as it is typically expected, instead, some animals may die from progressive liver disease. In fact, there is a breed of sheep, the North Ronaldsay, whose predisposition to present copper poisoning as a progressive liver disease has made it a proposed animal model to study non-Wilson disease in humans (Haywood et al., 2001). Secondly, it strengthens the value of the kidney as the organ of choice to diagnose copper poisoning since the elevation of copper in kidney still occurs despite the absence of a hemolytic crisis.

Several decoppering agents have been studied for their value in the treatment of copper poisoning. Zinc supplementation (Van Ryssen, 1994) and D-penicillamine (Botha et al., 1993; Humann-Ziehanck and Bickhardt, 2001) appear to be ineffective in removing copper in the bound form that occurs in the liver. The most currently accepted method is the administration of molybdenum plus sulphur with the feed, or by parenteral administration if the animal is already in the hemolytic crisis. Of the different molybdenum compounds, tetrathiomolybdate (TTM) is the only agent shown to remove copper selectively from the hepatocyte storage protein, metallothionein, without removing copper from other copper-enzymes such as ceruloplasmin or affecting the metabolism of other essential metals such as zinc or iron (Ogra and Suzuki, 1998).

The resulting copper/TTM complex is then excreted into the bile and blood, with the bile being the major route of excretion (Komatsu et al., 2000). Humphries et al. (1988) showed that subcutaneous dosages of 3.4 mg/kg b.w. ammonium TTM on three alternate days effectively reduced liver copper concentrations in loaded sheep to levels considered almost safe below 200 ppm (ww). Unfortunately, TTM is not approved for use in sheep in the U.S. We currently advise to add sodium molybdate to the feed for 3 weeks. A dose of about 100 mg sodium molybdate per head per day is recommended together with calcium sulfate (gypsum) as a source of sulfate to the feed. This dosing regimen can be attained by adding 10 pounds (4.54 kg) of a 2% sodium molybdate and 5 pounds (2.27 kg) gypsum to 1 Ton of feed. Experimental evidence has shown that this treatment promotes copper excretion via bile by removing copper from the lysosomes and cytosol of copper-loaded liver cells (Hidiroglou et al., 1984; Kumaratilake and Howell, 1989). By reducing copper content in livers, further outbreaks of copper toxicosis may be prevented in the rest of the flock. Treatment with molybdenum for more than two months in hypercuprotic sheep may induce copper deficiency (Van Ryssen, 1994) and so should be avoided.

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