Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #:**186**; Month: **April**; Year: **2022**
Answer sheet

Title: *Chronic copper poisoning in a sheep*

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Clinical History: A one-year-old female Texel sheep had anorexia and jaundice. After one day, the clinical condition progressed to lethargy, lateral recumbency, and death within hours. The sheep was part of a 20 sheep flock housed indoors and received an increasing amount of feed (specific for sheep) for the last two months as it was being prepared for a national fair.

Gross Findings: The sheep was in excellent body condition. The carcass displayed marked icterus (Fig. 1). In the subcutaneous tissue, there was multifocal petechiae and ecchymosis. The kidneys had a deep red-brown discoloration. The urinary bladder was distended and filled with dark red to black urine (Fig. 1). The liver was slightly soft and swollen, with a deep orange hue. The gallbladder was distended and filled with dark grumous bile.

Gross and Histological Images:

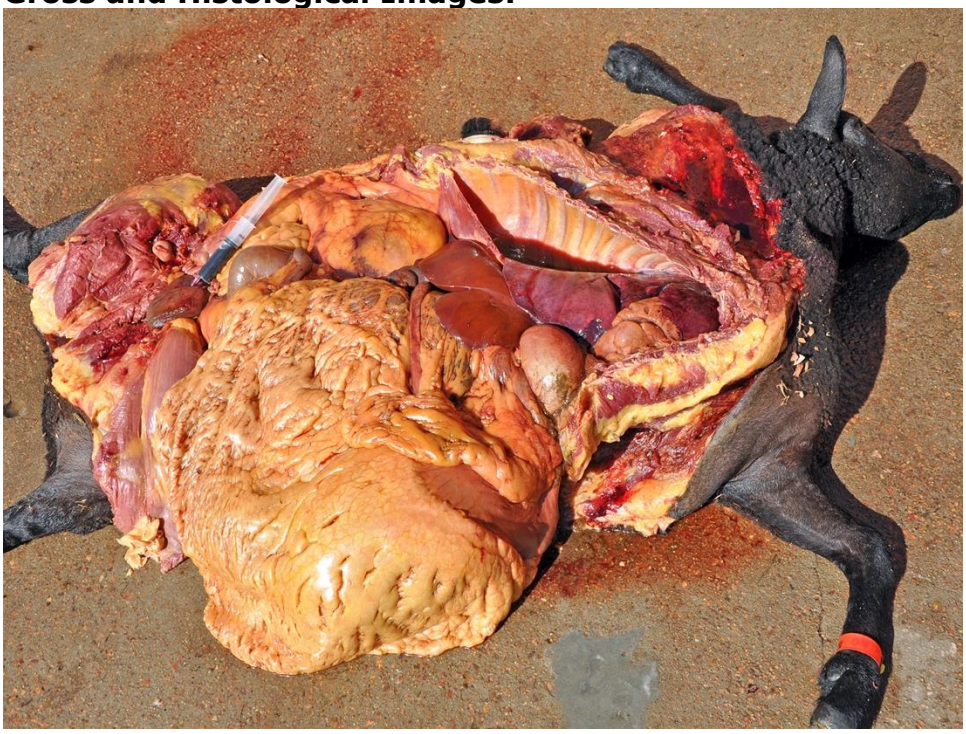

Figure 1. Subcutaneous tissue and abdominal fat are severely and diffusely yellow. The liver is diffusely deep orange. The urinary bladder is filled with dark red urine (as shown by the content within the syringe). Moderate amount of red fluid is in the thorax.

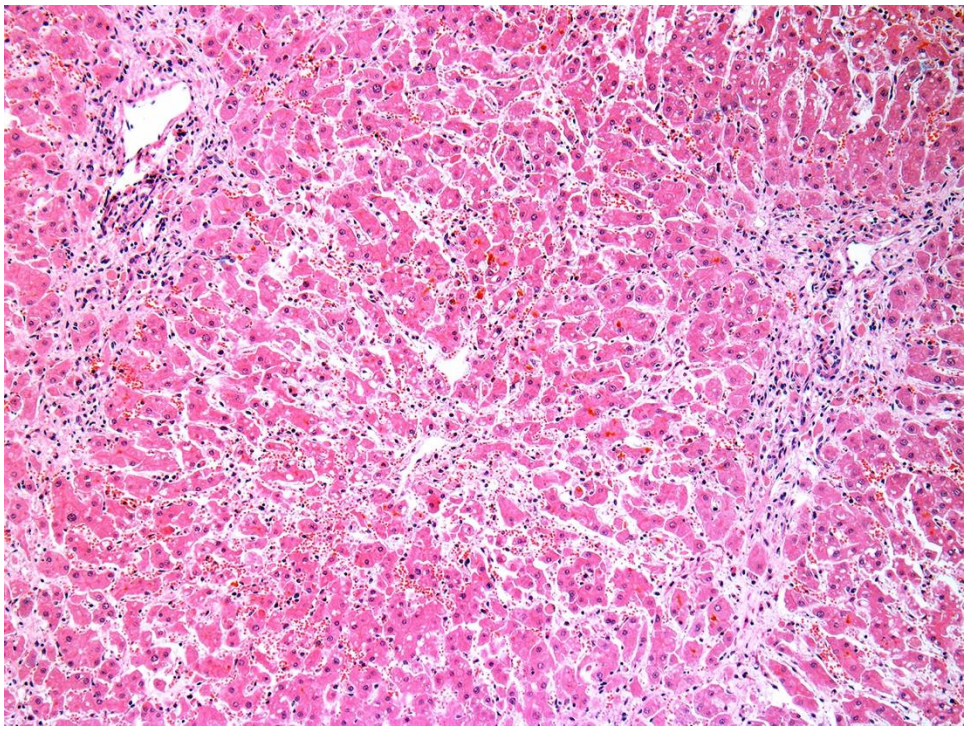

Figure 2. There is marked disorganization of hepatocyte cords, mainly in the centrilobular regions. In periportal areas there is mild fibrous tissue and mononuclear infiltrate.

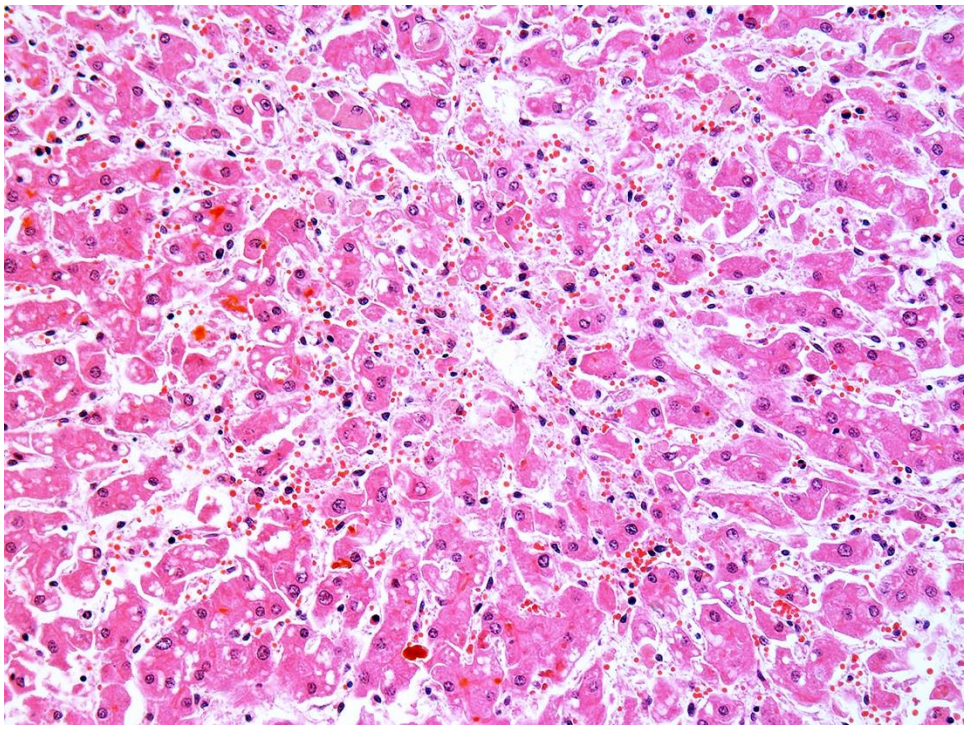

Figure 3. Liver histology. In the centrilobular areas, there is individual hepatocyte necrosis. In contrast, the remaining adjacent hepatocytes show moderate amounts of granular yellow-brown pigment deposits within biliary canaliculi and mild degenerative hepatocellular cytoplasmic changes.

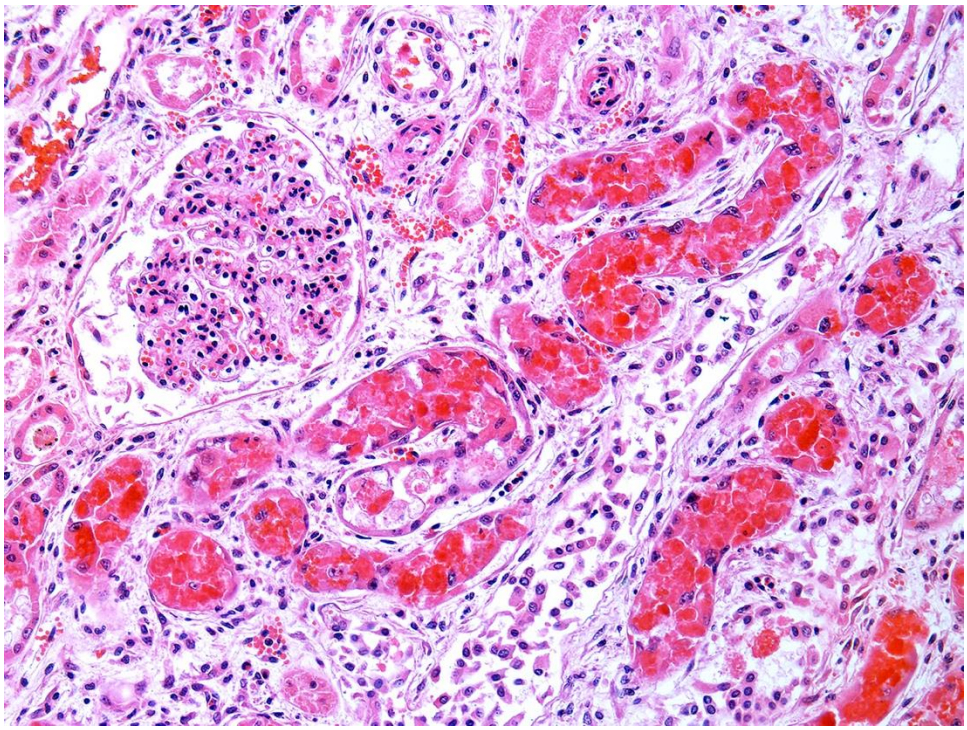

Figure 4. Renal tubular epithelial cells are severely attenuated. Renal tubules are filled with flocculent to eosinophilic dark-red granular casts.

Histological Description: Liver: Moderate disorganization of hepatocytes cords in the centrilobular region, associated with multifocal hepatocyte swelling and individual necrosis (Fig. 2). The periportal areas showed moderate proliferation of fibrovascular tissue associated with mild lymphocytes and plasma cell infiltrate. Within biliary canaliculi, there was a moderate amount of yellow-brown pigment deposits (consistent with intrahepatic cholestasis) (Fig. 3). Diffusely, a mild vacuolar degeneration of hepatocytes was observed. In the kidneys, cortical renal tubules contain flocculent to eosinophilic dark-red granular casts. Renal tubular epithelial cells are attenuated, coagulated, and pyknotic or absent (epithelial degeneration and necrosis) (Fig. 4).

Cause: Chronic copper poisoning

Pathogenesis: Elevated dietary copper intake > copper sequestration in hepatocellular lysosomes > production of reactive oxygen species > destructive lipid peroxidation > loss of lysosomal membrane integrity and damage to mitochondria > hepatocellular apoptosis > elevated free copper ions > oxidation of hemoglobin in erythrocytes > intravascular hemolysis > anemia.

Comments: Chronic copper poisoning results from the accumulation of copper in hepatic tissues over a few weeks to more than a year and can occur without any clinical manifestation (1, 4). Sheep are intoxicated due to the gradual accumulation of large amounts of copper (Cu) in the liver and its sudden release into the circulatory system, resulting in a hemolytic crisis (4). The hemolytic crisis may be precipitated by many factors, including transportation, handling, weather conditions, pregnancy, lactation, strenuous exercise, or a deteriorating plane of nutrition (3). Hepatic copper toxicosis can result from a primary metabolic defect in hepatic copper metabolism, altered hepatic biliary excretion of copper (presence of other hepatotoxins, e.g., pyrrolizidine alkaloids), or excess dietary intake of the element (1). Sheep are especially prone to copper poisoning because of the reduced biliary excretion of copper (1). The gross lesions of fatal chronic copper poisoning include the mucosa and subcutaneous tissue discolored by severe jaundice. The kidneys are deep red-brown, and the urine is deep red (consistent with hemoglobinuria). The liver is often slightly soft, swollen, and deep orange (1).

Histologically, the liver lesion varies with chronicity of exposure, from nonspecific acute centrilobular necrosis to cholangiohepatitis with periportal fibrosis (1). Our pathological findings were very similar to those described in the literature for the condition. The determination of the source of copper excess is essential for the diagnosis. Several sources are described in the literature, including feeding excessive grains to sheep, as seen in our case; feeding sheep with food compounded destined for other species (cattle); the use of grape subproducts in sheep diet (3,7).

To conclude, the diagnosis of copper poisoning is necessary to determine the copper levels in the liver of the affected sheep (4). In our case, the copper determination was 1,063 µg/g (normal concentration up to 349 µg/g) (6). Concentrations of Cu in the liver greater than 1000 mg/kg are diagnostic of copper intoxication (4,6). The diagnosis of copper poisoning and its differential diagnosis is

often based on the animal's signalment, supportive historical information, clinical signs, and gross findings at necropsy (1).

Differential diagnoses for the hemolytic crisis in sheep are scarce. In young lambs, the alpha-toxin generated by *Clostridium perfringens* type A can cause hemolysis, leading to "yellow lamb disease" (8). As copper poisoning and yellow lamb disease present similar signs and gross and microscopic findings, ancillary tests are necessary to obtain a final diagnosis. Yellow lamb disease affects only young lambs, and direct smears of the intestinal lining may reveal large numbers of gram-positive rods (8). Other intoxications related to hepatic failure and jaundice, such as *Crotalaria retusa*, *Brachiaria brizantha* and *Brachiaria decumbens* plant poisoning, were described in sheep (2,5). The differentiation between these conditions and copper poisoning is mainly obtained through anamnesis and pathological findings. In addition, intravascular hemolytic anemia-related lesions are absent in these plant intoxications.

References:

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*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. - Consider submitting an exercise! - A final document containing this material with answers and a brief discussion will be posted on the CL Davis website.

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