Diagnostic Exercise
From The Davis-Thompson Foundation*

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Answer sheet

Title: Chronic copper toxicity in a Katahdin sheep

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Clinical History: A 5-month-old, female intact, Katahdin sheep presented to Large Animal Teaching Hospital at Auburn University for a 24-hour history of abnormal neurologic status including opisthotonos, severe ataxia, and abnormal mentation. Heat stress was suspected by the owner, and unspecified medication was given. Given the lack of responsiveness and poor prognosis, the animal was humanely euthanized.

Clinical Pathology Findings: Hematology results revealed a mild anemia with evidence of strong regeneration and many ghost cells, mild to moderate leukocytosis with neutrophilia, and marked thrombocytosis. Intravascular hemolysis was suspected based on these findings. Other biochemical results were aspartate aminotransferase of 1339 U/L (normal interval range: 75-339 U/L), creatine kinase of 5340 U/L (normal interval range: 100-547 U/L), blood urea nitrogen of 86.4 mg/dL (normal interval range: 19-37 mg/ dL), creatinine of 8.6 mg/dL (normal interval range: 0.8-1.3 mg/ dL), and hemolysis index of 1113. The normal interval range is referred to Clinical Chemistry Reference Intervals in UCDAVIS veterinary medicine.

Necropsy Findings: Major gross findings are as follows: The visceral adipose tissue was diffusely pale tan to yellow (icterus). The liver was diffusely dark orange to dark brown (Fig. 1A). The gallbladder was enlarged and distended by bile. Bilaterally, the renal cortex and medulla were diffusely dark brown to black (Fig. 1B). Filling the urinary bladder was approximately 30 mL dark brown urine with numerous fine dark green to brown granules.

Gross Images:

Figure 1

Microscopic Findings:
In the sections of liver, there was a severe bridging centrilobular to midzonal hepatocellular degeneration and necrosis (Fig. 2A). In these affected areas, the hepatocytes were swollen by small, discrete and clear vacuoles (lipid). Other hepatocytes had hypereosinophilic cytoplasm and pyknotic or karyolytic nuclei. Rare hepatocytes had enlarged nuclei that were up to two to three times normal (megalocytosis) (Fig. 2B). There was minimal neutrophilic infiltrate in areas of affected hepatocytes. Multifocally, hepatocytes and Kupffer cells contained intracytoplasmic dark brown granules (hemosiderin, copper, and bile), and there were a few Rhodanine-positive granules (inset of Fig. 2B), mainly in periportal hepatocytes. Multifocally, bile canaliculi were variably expanded by dark brown linear bile plugs. Some portal triads had fibrosis, biliary hyperplasia, and scant infiltrates of lymphocytes.
In the sections of kidneys, approximately 80% of the renal tubules, with the medullary portion the most severely affected, were filled with pale eosinophilic to orange, globular, or homogenous hyalinized material (hemoglobin casts) (Fig. 2C). In the affected areas, the renal tubular epithelium was attenuated, degenerate or necrotic.
In the sections of thalamus and arbor vitae of cerebellum, the white matter was moderately spongiotic (Fig. 2D). In this affected area, there were individual, paired or clustered astrocytes with swollen nuclei, marginated chromatin, and a clear central nucleolus (Alzheimer type II cells) (inset of Fig. 2D).

Morphologic Diagnoses:
Liver: Severe acute centrilobular to midzonal hepatocellular degeneration and necrosis with intracytoplamic Rhodanine-positive granules
Kidney: Severe acute renal tubular degeneration and necrosis with hemoglobin casts.
Thalamus and arbor vitae of cerebellum: Moderate spongiosis with Alzheimer type II astrocytes.

Toxicology findings: Sections of liver lobes were submitted to the Thompson Bishop Sparks State Diagnostic Laboratory in Alabama for copper level testing. The

measured level of copper was 240 ppm, which falls in the high (100-500 ppm) range in the reference material and nears the toxic range (250-1000 ppm). The moisture of the liver is 77%.

Name of the Condition: Chronic copper toxicity.

Microscopic Images:

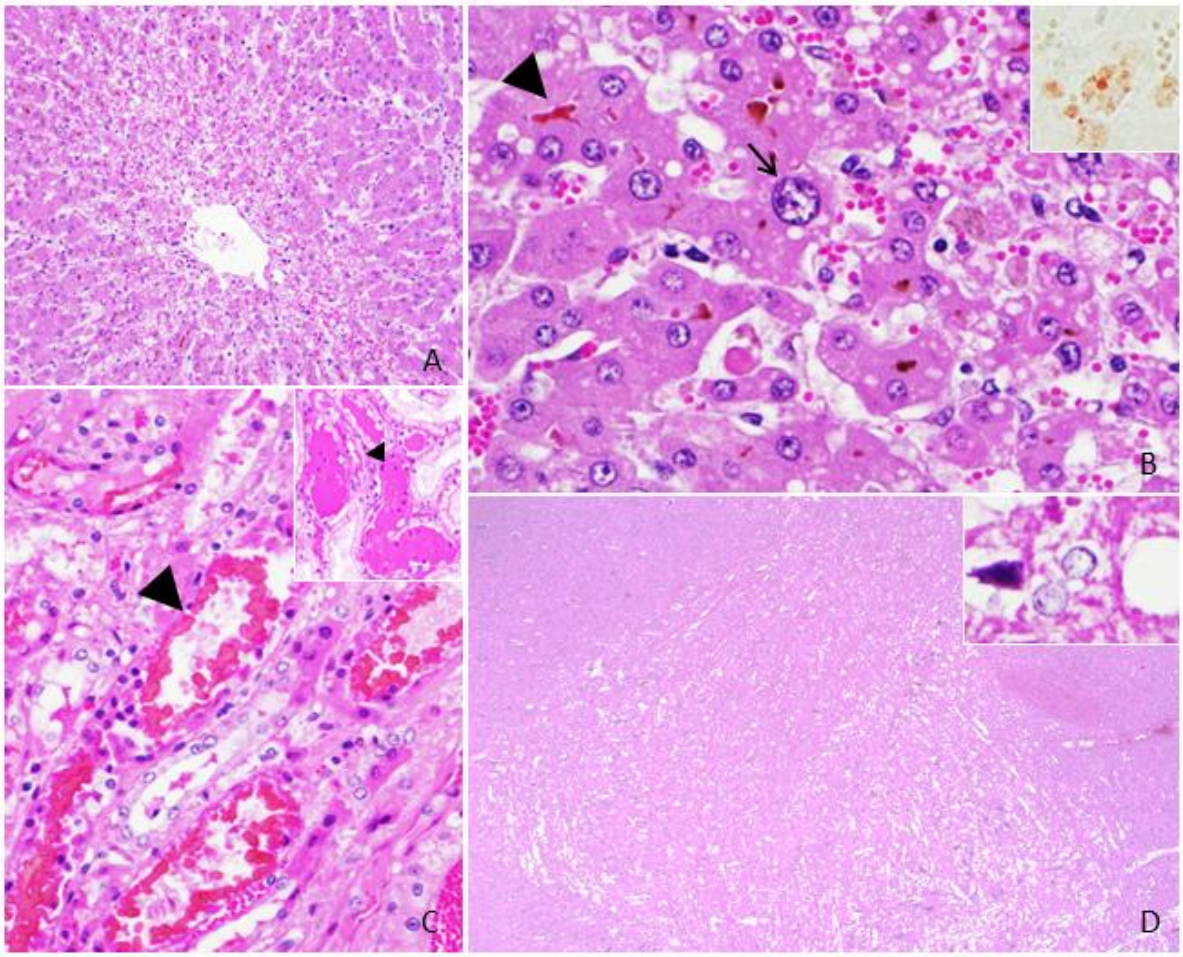

Figure 2. Sheep, chronic copper toxicity. (**A**) Liver. The centrilobular hepatocytes were affected by degeneration and necrosis. H&E, 4x. (**B**) Liver. Multiple bile canaliculi contain bile plugs (arrowhead). Rare hepatocytes were enlarged up to three times normal (arrow). H&E, 40x. Rhodanine-positive granules were in the hepatocytes (inset). H&E, 40x. (**C**) Kidney. Filling the renal tubules were pale eosinophilic homogenous (inset, arrowhead) to orange globules (arrowhead). H&E, 40x and 20x, respectively. (**D**) Thalamus. Thalamus had focally extensive spongiosis with Alzheimer type II astrocytes (inset). H&E, 4x and 40x, respectively.

Discussion: Ruminants require a balance trace element to maintain daily essential nutrient for growth and body utilization. Amongst all, copper has important roles in regulating essential biological processes. Even though the liver excretes copper, excess accumulation of copper in hepatocytes can lead to copper toxicity in multiple species (2, 3).

Sheep are recognized as an extremely sensitive species to high levels of dietary copper. Reduction of the excretion ability of copper in the biliary and urinary system as well as narrow safety ranges between the concentration of safe and toxic levels are the two main reasons for sheep being overrepresented in cases of chronic copper toxicity. Cattle and other ruminants reportedly have less sensitivity to higher copper levels, and the most common cause is the continuing ingestion of high level copper-containing diet. Regarding the monogastric animals, they seem to have a much better tolerance to high dietary copper. Hereditary predisposition to copper storage disease has been reported in certain breeds of dogs (2,7).

Two forms of non-hereditary copper toxicosis have been described, which are acute form and chronic forms (1). Acute copper toxicity has been reported following inaccurate administration of copper injections or ingestion. This acute exposure to excess copper can cause gastrointestinal irritation with mucosal erosion as well as blue-green contents. As for the chronic form, the affected sheep has a chronic copper exposure, mainly through dietary copper with over-recommended amount, but is reported to have an acute clinical presentation. In sheep, the normal level of copper in feeds is 10-20 ppm. During the copper concentration level over 1000 ppm, sheep may be clinically and hematologically normal and remain in a subclinical status. However, elevated liver-specific enzyme might be observed. If the copper accumulation is continuously rising and exceeds hepatocytes turnover rates, the high copper level will directly damage hepatocytes and lead to hepatocyte degeneration and necrosis. Free copper ion is then released from the damaged hepatocytes into the circulation where it damages erythrocyte membranes causing intravascular hemolysis. In sheep, stressors, such as pregnancy, calving, ingestion of toxic plants, or transportation, are the predisposing factors involving this hemolytic crisis event. Some of the escaping copper from liver can pass through urinary system. Therefore, the copper concentration above 1000 ppm in the blood and kidney can serve as an indicator of prior hemolytic crisis (2).

The hemolytic crisis and hemoglobinuric nephrosis are clinically manifested as weakness, anemia, and icterus, as well as elevated alanine transaminase, alkaline phosphatase, total bilirubin concentration, urea nitrogen and creatinine, high hemolytic index, and the presence of Heinz bodies (1, 5). The gross lesions include soft, swollen, yellow brown to deep orange liver, some of the cases present with atrophy and fibrosis due to the long-term liver injury that may be associated with environmental hepatoxicants (such as pyrrolizidine alkaloids). Due to the hemoglobinuria and the deposition of hemoglobin casts and oxidation to methemoglobin, the kidneys are deep red-brown to gunmetal blue to black, and the urine is deep red. Other gross changes include enlarged spleen. Few reports mentioned the gallbladder being distended by bile, although this may be secondary to inappetence.

Typical microscopic findings of chronic copper toxicity are mainly the insult of secondary intravascular hemolysis along with the direct damage to liver. Hepatocellular injury in the centrilobular regions, rhodanine-highlighted copper accumulation primarily locating at periportal or centrilobular hepatocytes, and cholestasis are the most frequently mentioned microscopic findings in cases of chronic copper toxicosis. In addition, ischemic acute renal tubular injury and luminal hemoglobin casts are the main features of secondary hemolytic crisis targeting the kidneys (3).

The tentative diagnosis of copper toxicity is based on history, clinical signs, clinical pathology results, and gross changes. The final diagnosis relies on the corresponding microscopic findings and the copper levels in liver, kidney, blood, or feed. In this present case, ALT, BUN, and creatinine had higher values comparing to the normal interval ranges. The high hemolytic index along with regenerative anemia were consistent with an intravascular hemolysis. Gross changes of liver and kidneys fitted the features of copper toxicity. The microscopic changes in the liver and kidneys indicated copper-associated liver injury with secondary hemolytic nephrosis. Samples of liver were sent for toxicology testing, and the copper levels were deemed high. The explanation for the neurologic signs reported clinically might be due to the hepatoencephalopathy triggered by liver injury (although ammonia levels were not measured to confirm hepatoencephalopathy). Therefore, a diagnosis of chronic copper toxicity was confirmed in this sheep. Although ingestion of excess copper-containing diet is considered the main cause of chronic copper toxicity in sheep, other factors including low molybdenum or toxic plants, can increase the susceptibility to develop toxicosis (9). Unfortunately, the limited previous history in this case precluded investigation of the exact source/cause.

For the purpose of comparative pathology, copper toxicosis in other species including dogs, rabbits, cats, and some captive marine animals have been reported. The rabbits had similar pathologic changes as those described in sheep, goats, and cattle. In the feline cases, fibrotic changes between the regenerative nodules were more prevalent (4, 8). However, the dogs were considered as having a hereditary copper toxicosis with a high proportion affecting breeds such as Bedlington Terrier, Doberman Pinscher, West Highland White Terrier, and Labrador Retriever (3, 6). Amongst all, the disease in Bedlington Terrier is well-studied and is proven to be an autosomal recessive disorder of copper excretion because of a mutation in the COMMD1 gene, and the typical pathologic feature is progressive liver disease or chronic hepatitis as opposed to acute hepatocellular degeneration and necrosis typically seen in sheep (2).

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