# Diagnostic Exercise

## From The Davis-Thompson Foundation*

Case #: **208**; Month: **March**; Year: **2023**

*Answer Sheet*

**Title:** Sheep, hematopoietic system, intravascular hemolytic crisis

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**Morphologic Diagnoses:** Kidney, hemoglobinuric nephrosis; Mucosal and serosal surfaces, hemoglobinuric imbibition, diffuse.

**Typical Gross Findings:** The gross findings are typical of an acute intravascular hemolytic crisis. They include mucosal and serosal hemoglobinuric imbibition, occasionally accompanied by icterus, diffusely dark-red kidneys (hemoglobinuric nephrosis) and dark-red tinged urine (hemoglobinuria). The spleen is generally enlarged, dark red and soft, and the liver might be enlarged and diffusely orange due to bilirubin accumulation.

**Typical Microscopic Findings:** Diffuse hemoglobinuric nephrosis is histologically characterized by acute tubular epithelial degeneration and necrosis (cells have hypereosinophilic cytoplasm and pyknotic nuclei, karyorrhexis or karyolysis) associated with abundant intratubular hemoglobin casts (Figure 4). Additionally, acute centrilobular necrosis might develop in the liver, mainly due to direct exposure to circulating copper, but also complicated by anemia (hypoxia) and, in some cases, shock. Additional findings in the liver might include accumulation of bilirubin in bile ducts and canaliculi (Figure 5) and, in sheep with chronic copper accumulation, infiltration of macrophages with light brown pigment, mainly in the periportal regions. The spleen is diffusely congested.

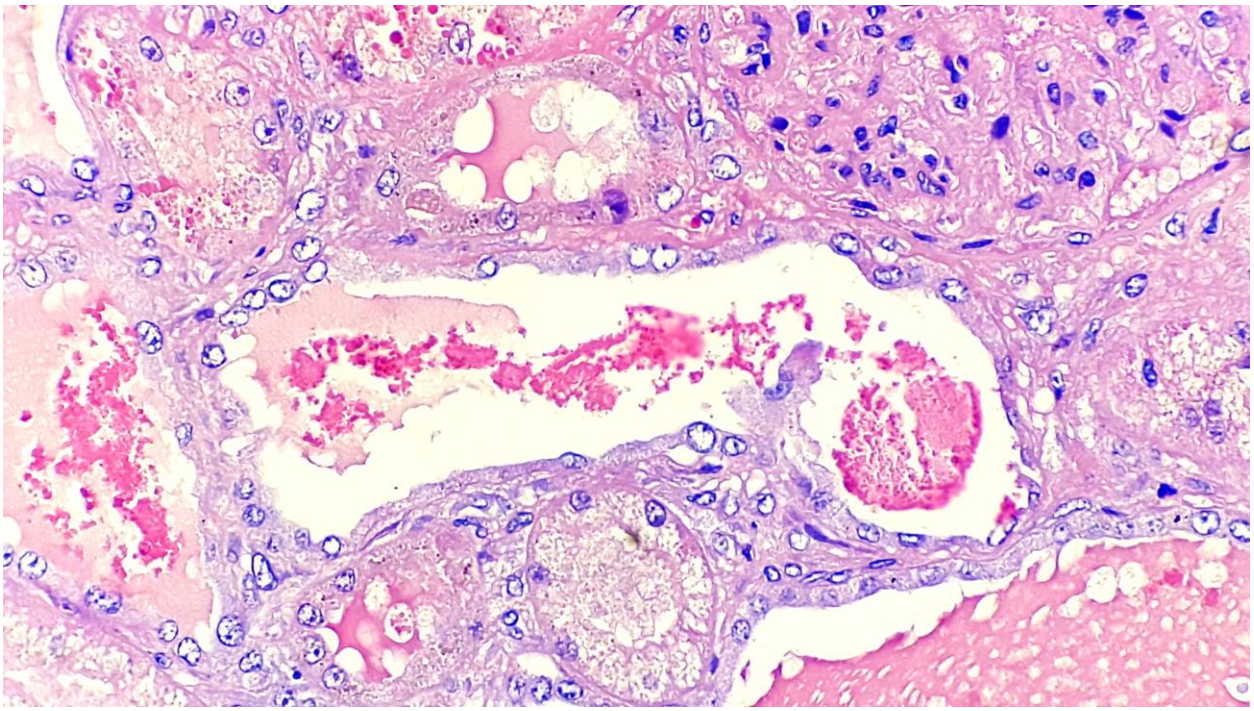

Figure 4

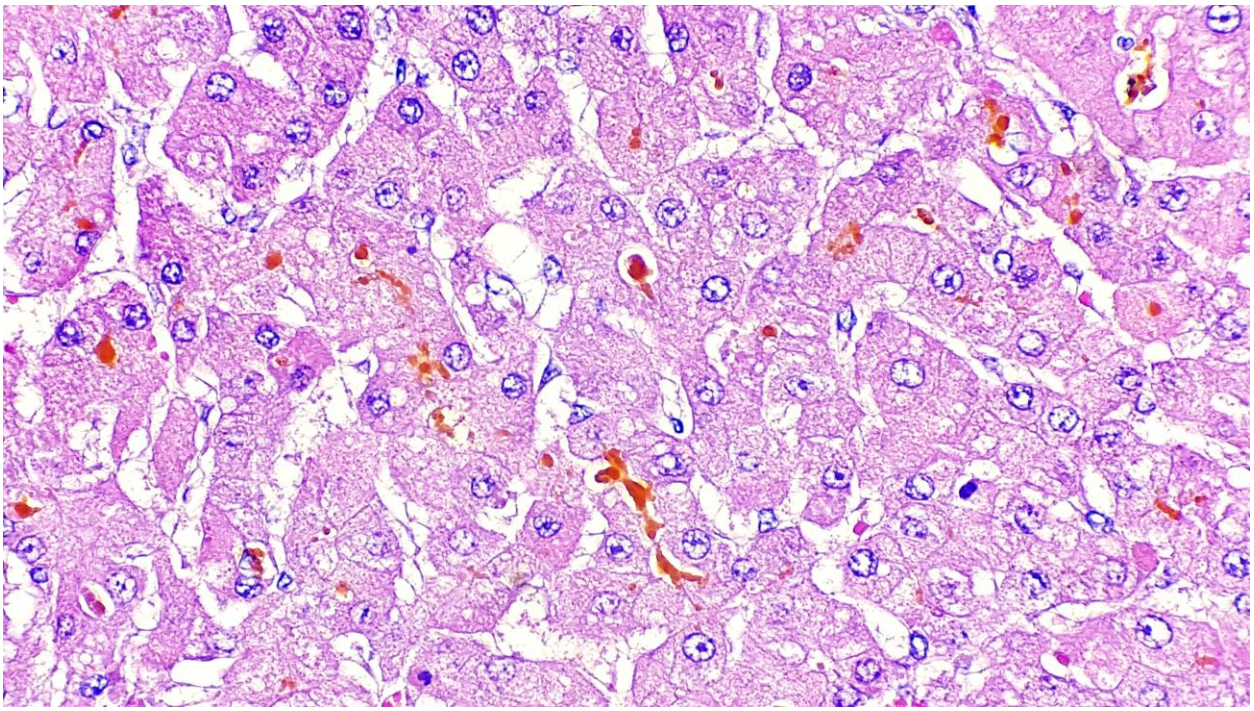

Figure 5

**Discussion:** Based on epidemiologic data, gross and histologic findings, we established a diagnosis of chronic copper intoxication in this ewe. The cause of intoxication was the ingestion of a commercial mineral supplement provided to the cattle on the property and accidently ingested by the sheep. Feed and mineral

supplements for cattle contain higher levels of copper than tolerated by sheep and are common sources of intoxication in the latter species (4). Although copper is essential for several biological processes, it is toxic at excess concentrations. In normal conditions, the dietary intake of copper is balanced by copper excretion in the bile (2, 4). When ingested in high levels, however, copper is stored in hepatocytes, culminating in continuous and repetitive cellular damage and death. Sheep have a very limited rate of copper excretion in the bile, which contributes to copper accumulation in the liver (1). Three additional factors may contribute to copper toxicosis in this species: high levels of copper intake, as seen in this case (which can be present in the water, pasture or feed); low levels of pasture molybdenum (even in the presence of normal copper concentrations), which antagonizes copper uptake; and concomitant exposure to other hepatotoxins, which in turn make the liver more susceptible and less capable of storing excessive copper (1, 2, 4). In Brazil, the main hepatotoxin responsible for this third factor are pyrrolizidine alkaloid-containing plants (mainly *Senecio*) (3, 4). In the case reported here, we believe that the high levels of copper intake were the only factor contributing to intoxication, since no hepatic lesions suggesting plant toxicosis were observed. Commercial feed containing 15-20 mg/kg of copper are already capable of producing chronic intoxication in sheep (4). The technical information in the packaging of the dietary supplement provided to the sheep in this case indicated a minimum of 650 mg of copper per kilogram. Pastures with 15-20 mg/kg of copper and low levels of molybdenum (<0.36 mg/kg) may also cause copper intoxication (4). The excess of chronically ingested copper stored in the liver may reach 1000 µg/g without any clinical signs. During this phase, copper is stored within the lysosomes of hepatocyte, causing subclinical cell damage, apoptosis, and increased mitotic rate (1,2). Macrophages containing brown granular pigment (copper-containing lipofuscins) appear in the periportal areas (2). Eventually, the liver cannot keep up with the replacement of hepatocytes that can absorb the copper released from dying cells. This leads to a sudden increase in plasma copper levels, which causes intravascular hemolysis and centrilobular necrosis as described above in the microscopic findings (1,2). Thus, although copper intoxication is chronic, the clinical presentation is acute, and begins when copper is released into the blood stream. Sick animals may die within a few hours or days. Copper poisoning is a common disease in sheep and should be included as a possible cause of hemoglobinuria and acute death (4).

**References and Recommended Literature:**

1. Brown DL, Wettere AJV, Cullen JM. Hepatobiliary System and Exocrine Pancreas. In: Zachary JF. Pathologic Basis of Veterinary Disease. 6 Elsevier; 2017. P. 440.
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