Diagnostic Exercise

From the CL Davis/SW Thompson Foundation

Case #:**227**; Month: **December**; Year: **2023**

Answer sheet

Title: Presumptive red maple leaf toxicosis

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Clinical History: A 16-year-old Quarter Horse gelding was presented for a 3-day history of lethargy, marked anemia, cyanotic oral mucous membrane, delayed jugular refill time, and dark red urine, which retained the discoloration after centrifugation. Relevant in-house laboratory findings included hematocrit of 15.3% [reference interval (RI): 32.0-48.0], mean corpuscular hemoglobin concentration (MCHC) of 61.1 g/dL (RI: 31.0-37.0), mean corpuscular volume (MCV) of 44.8 fL (RI 34.0-58.0) and a high hemolysis index (1642; RI: 0-43). Blood smear analyses revealed moderate numbers of ghost cells (Fig 1, arrows), eccentrocytes (Fig 1, arrowheads), and red blood cell agglutination within a highly hemolytic background. The urine sample was not submitted to the clin path lab for complete analysis (e.g. dipstick and sediment).

Necropsy Findings: Both kidneys had uniformly dark red cortex and medulla. The urinary bladder contained moderate amounts of dark red, thin, urine (Fig.2). Scattered throughout most of the liver parenchyma were large numbers of up to 1.5-cm diameter eosinophilic granulomas with a mineralized core (most likely parasitic granulomas, deemed incidental). Other organs were unremarkable.

Gross and Microscopic Images:

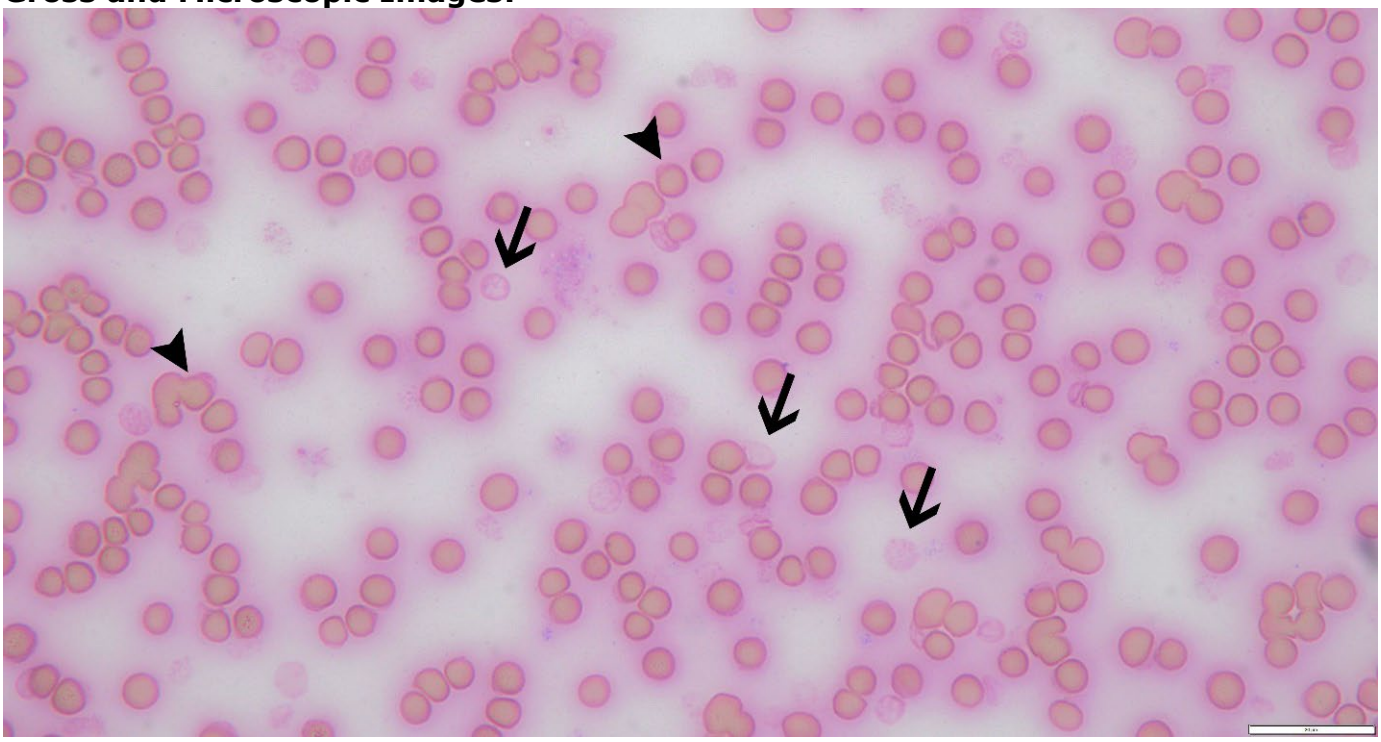

Figure 1. Blood smear cytology with some ghost cells (arrows) and eccentrocytes (arrowheads) in a highly hemolytic background.

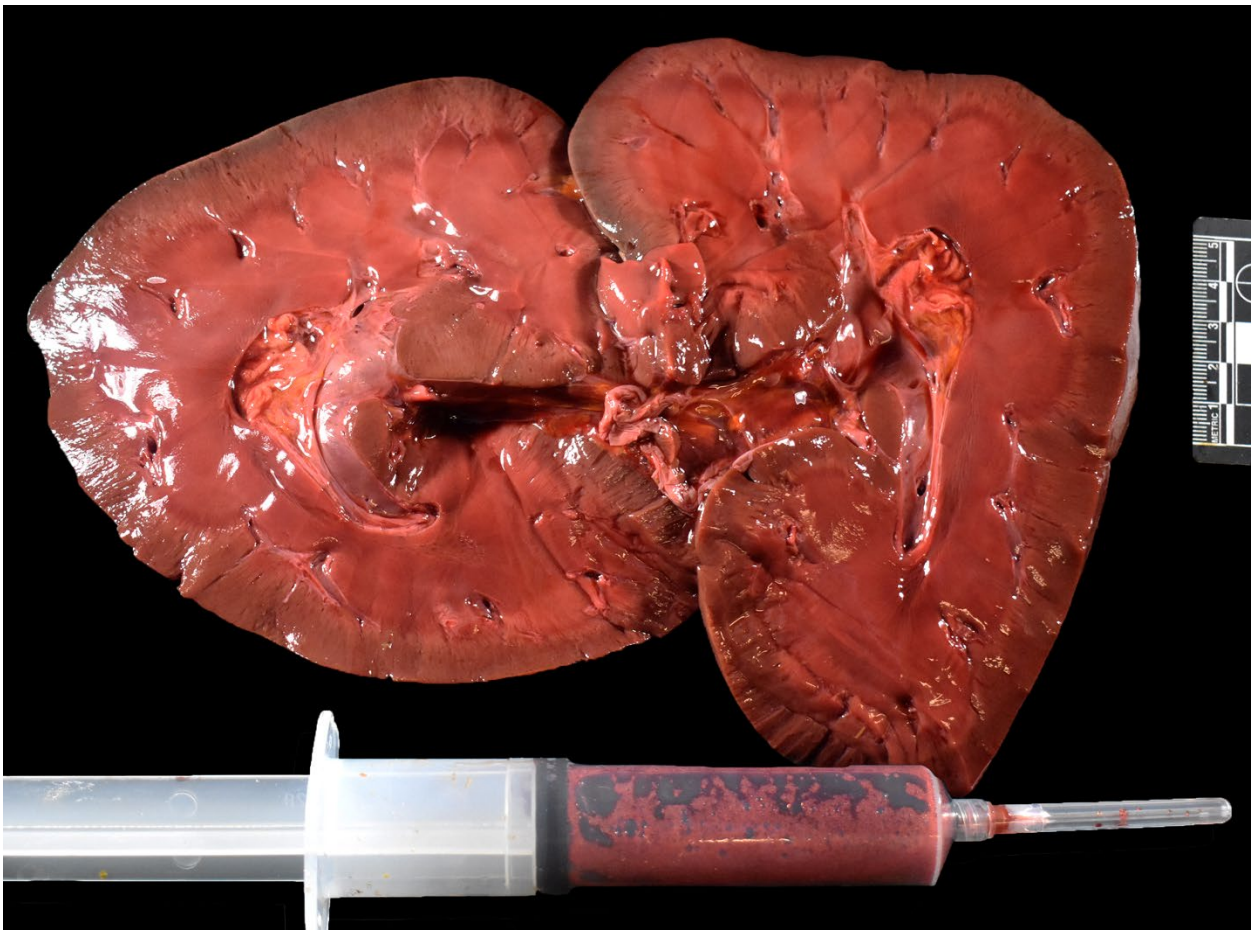

Figure 2. A transverse section of dark red kidney and hemoglobinuria.

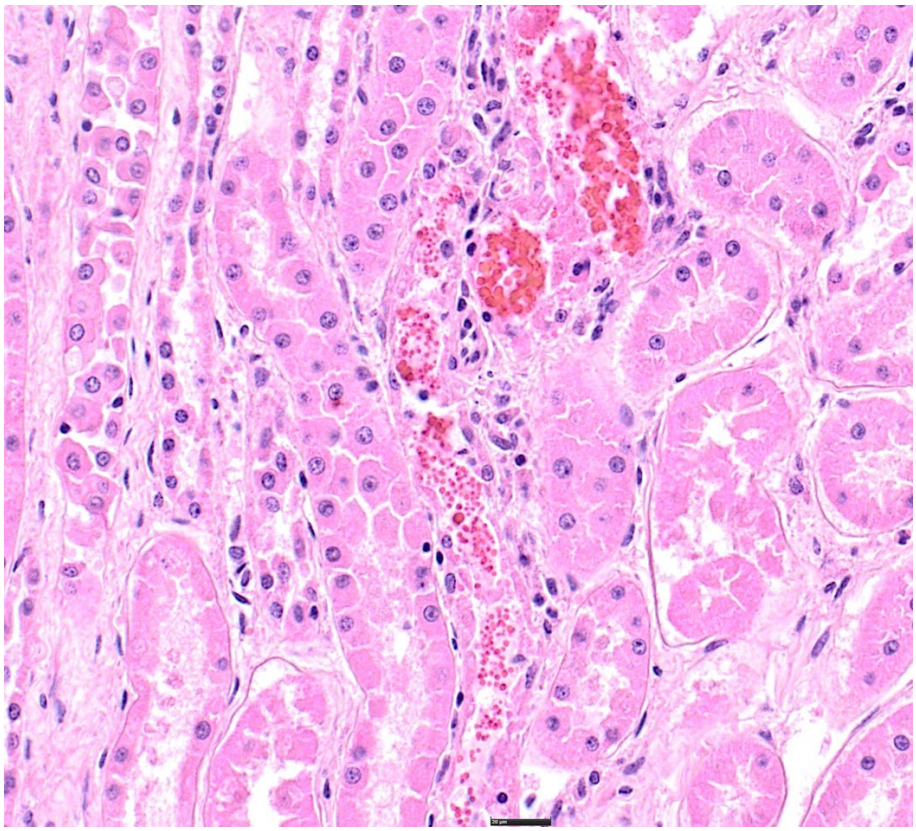

Figure 3. Renal tubules with hemoglobin casts and some necrotic lining epithelial cells.

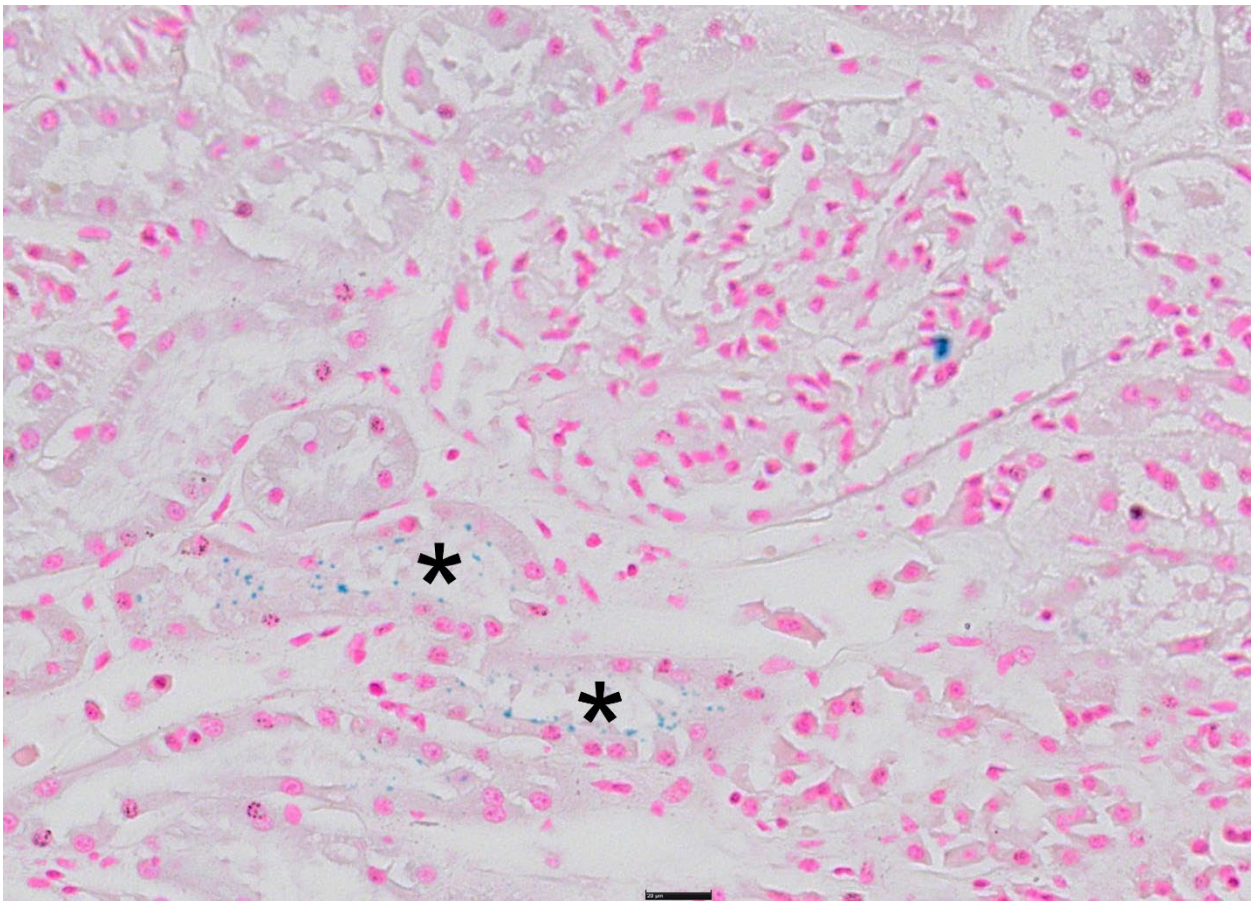

Figure 4. Prussian blue stain of kidney revealing small amounts of blue granules along the apical border of some tubular epithelial cells (asterisk), indicating iron from catabolized hemoglobin.

Follow-Up Questions:

Histological Description:
Kidney: A moderate number of cortical tubules are variably dilated, lined by attenuated epithelial cells, and filled with moderate amounts of globular to granular, bright red material (hemoglobin casts). Some tubular epithelial cells are shrunken with bright eosinophilic cytoplasm, and a pyknotic or karyolitic nucleus (necrosis). Multifocally, tubular epithelial cells contain a small amount of golden to brown cytoplasmic pigment that is highlighted with Prussian blue (consistent with iron). Similar pigment also lines the apical border of the lining epithelium.

Morphologic Diagnosis:
Kidney (cortical tubules): Epithelial necrosis with luminal hemoglobin casts, multifocal, moderate

Most likely etiology: Red maple leaf toxicosis

Comments: Red maple (*Acer rubrum*) is native to eastern United States and the intoxication is a seasonal disorder that commonly occurs during the summer and fall seasons (6,8). When the horse ingests dried or wet/wilted red maple leaves, oxidative damage to erythrocytes cell membranes and hemoglobin ensues, which will result in hemolytic anemia, methemoglobinemia, and formation of Heinz bodies and eccentrocytes (6). The toxin is still unknown but thought to be gallotannins and gallic acid that can be converted to pyrogallol in the equine gastrointestinal tract. Doses of 1.5 g/kg of dried maple leaves can be toxic for smaller equids (1,5).

Clinical signs of red maple toxicosis (RMT), which primarily affects equids, can be either from the peracute form in late fall or the hemolytic form in early fall (4,5,9). Horses with mild cases may not show any clinical signs or only mild nonspecific signs, including depression, lethargy, and inappetence. Other more common signs include pale mucous membranes, icterus, colic, urine discoloration (red or brown "chocolate"), tachycardia, tachypnea, dyspnea, fever, weakness, depression, and ataxia (2,6). Rarely, sudden death and pregnant mare abortion have also been reported with RMT.

RMT can be presumptively diagnosed based on clinicopathologic evidence of intravascular or extravascular hemolytic anemia, methemoglobinemia, and Heinz body and/or eccentrocytes formation. Intravascular and extravascular hemolysis is due to the oxidative denaturation and aggregation of hemoglobin which results in Heinz body formation. Methemoglobin is due to the oxidation of hemoglobin iron, which becomes incapable of carrying oxygen to the body (7). Chemical panels may show changes due to dehydration and renal damage, including an increase in total protein, albumin, blood urea nitrogen (BUN), and creatinine. Enzymes such as aspartate aminotransferase (AST) and creatinine kinase (CK) may also be elevated (6). Grossly, splenomegaly, renal edema, and icterus can be found in RMT horses. Microscopically, the kidneys typically have pigmentary tubular nephrosis (renal tubular necrosis with hemoglobin casts). The spleen can have erythrophagocytosis and hemosiderin accumulation (2,6).There is no specific diagnostic test for RMT, but Prussian blue staining can be used to indicate byproduct iron from hemoglobin breakdown (dark blue granules) in the tubular lumen or cytoplasm of the lining epithelium.

Differential diagnoses for hemolytic anemia in horses include immune-mediated disease, infectious agents such as equine infectious anemia, piroplasmosis, or ehrlichiosis, and toxic agents such as *Allium* spp. (onion, garlic, or chives), nitrates or nitrites, naphthalene, or *Brassica* spp. (cabbages, turnips, kale, or broccoli) (9,6). Additional causes of methemoglobinemia include nitrate poisoning, chlorate toxicosis, phenothiazine toxicosis, onion (*Allium* spp.) ingestion, congenital methemoglobinemia, flavin adenine dinucleotide (FAD) deficiency, and drugs such as phenacetin and acetanilide (2,3,5) In this case, another horse from the same property concurrently developed similar clinical signs of hemolytic anemia and, given the local epidemiology and that no infectious organisms were visualized in peripheral blood, RMT was considered most likely in this case. The gastrointestinal tract did not have suspicious digesta at post-mortem examination, but euthanasia

was performed 3 days after clinical onset, so it is possible the toxic content had been passed by the animal at the time of necropsy.

In general, dark red-to-brown urine can be indicative of hematuria, hemoglobinuria, or myoglobinuria. In cases of hematuria, the urine clears and forms a reddish sediment after centrifugation, which represents erythrocytes. Hemoglobinuria is when hemolysis happens; therefore, the urine contains free hemoglobin that does not clear after centrifugation, accompanied by a reddish or brown coloration in spun serum. Myoglobinuria is less common, but it will show when severe rhabdomyolysis is present. Free myoglobin in the dark red urine but does not discolor the serum (10). In this case, the skeletal muscle was histologically unremarkable, ruling out rhabdomyolysis and myoglobin casts.

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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 (DTF). 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 DTF website (https://davisthompsonfoundation.org/diagnostic-exercise/).

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