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Diagnostic Exercise
From The Latin Comparative Pathology Group*

Bacillary hemoglobinuria in a cow

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Clinical History:

A 436 kg, 2-year-old, pregnant Angus cross cow was presented for necropsy with a history of sudden death during calving season. Two other heifers on the property recently had late term abortions with the calves having to be pulled out from the birth canal.

Gross Findings:

The subcutaneous tissue, omentum, mesentery, perirenal adipose tissue, and intima of the major vessels were diffusely and markedly yellow (Figure 1). The right liver lobe had a large (15x11x7 cm) area of pale tan discoloration covered by a thin layer of fibrin. On the cut surface, this area was pale red and dry (Figure 2). Throughout the remaining hepatic parenchyma were irregularly shaped, serpiginous, dark green to black tracks. Some of these tracks contained adult *Fasciola hepatica* flukes in the lumen. The gallbladder was distended and filled with large amounts of dark green, thick bile. The kidneys were diffusely dark red with disseminated pinpoint, dark red foci in the cortex (Figure 3). The urinary bladder contained approximately 300 mL of dark red urine (Figure 4). The brain had a slight yellow discoloration. Approximately 300 mL of red-tinged fluid was in the thorax. Large numbers of fibrous adhesions were present between the visceral pleura, the parietal pleura, and the pericardium. Approximately 200 mL of red-tinged fluid was in the pericardial sac. There was extensive hemorrhage on the epicardium.

*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation and published in cooperation with the Brazilian Journal of Veterinary Pathology. 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: https://davisthompsonfoundation.org/diagnostic-exercise/

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Gross and Histological Images:

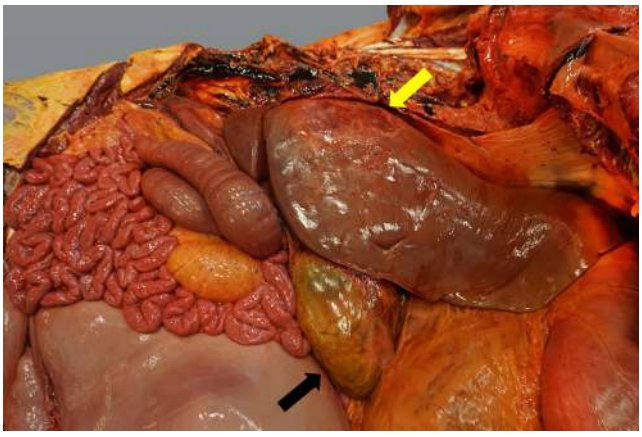


Figure 1. View of the abdominal cavity with icterus of the mesentery, omentum, and subcutaneous tissue. Note a large pale tan area in the right liver lobe (yellow arrow) and distended gallbladder (black arrow). The serosa of the small intestine is diffusely red (hemoglobin-stained from hemolysis).

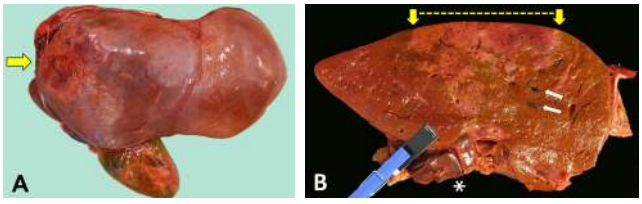


Figure 2. Liver. **A** - Diaphragmatic aspect with a large pale tan area in the right lobe, covered by a thin layer of fibrin. The gallbladder is full and distended. **B** - The cut section of the liver shows a regionally extensive pale tan and dry area (between yellow arrows). The remainder of the liver parenchyma is diffusely orange with irregularly shaped, serpiginous black tracts in the parenchyma compatible with fluke tracts (white arrows). The hepatic portal lymph node (*) is markedly enlarged and is diffusely dark brown due to accumulation of fluke pigment.



Figure 3. **A** - Dark red kidney with yellow perirenal fat. **B** - Multifocal 1 mm diameter dark red foci on the renal cortical surface.

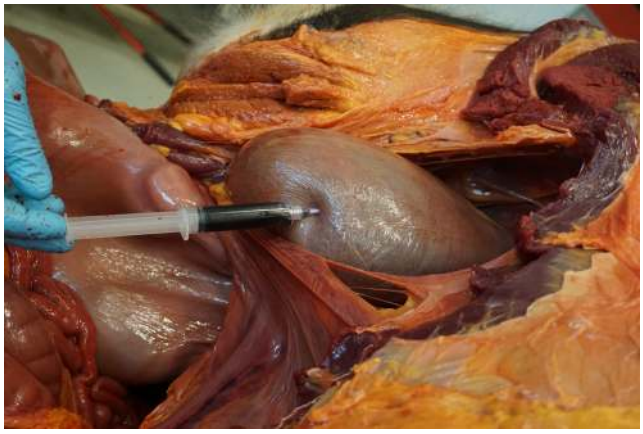


Figure 4. Dark red urine in the urinary bladder.

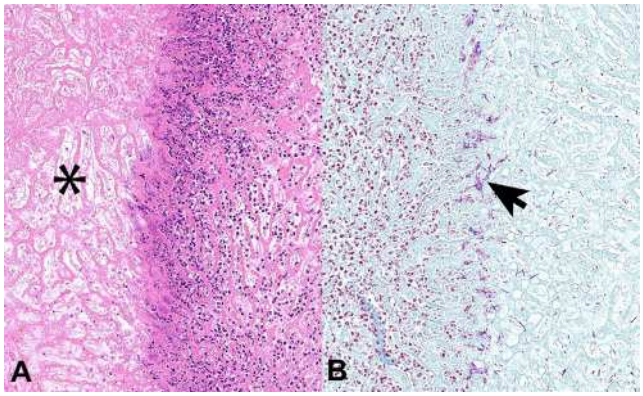


Figure 5. Liver. **A** - An area of coagulative necrosis (*) is delineated by a band of dense inflammatory infiltrate, mainly composed of degenerated neutrophils. H&E, 400x. **B** - Mirrored Gram stain image shows large numbers of gram-positive rods in the sinusoids between necrotic hepatocytes (arrow) and the leukocytic rim. Gram stain, 400x.

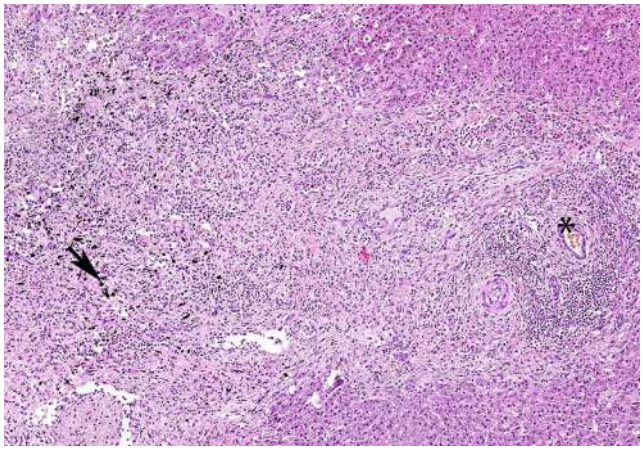


Figure 6. Liver. The portal areas are expanded by lymphohistiocytic infiltrate admixed with fibrosis, biliary ductular hyperplasia and fluke pigment (arrow). A bile duct contains bile (asterisk). H&E, 200x.

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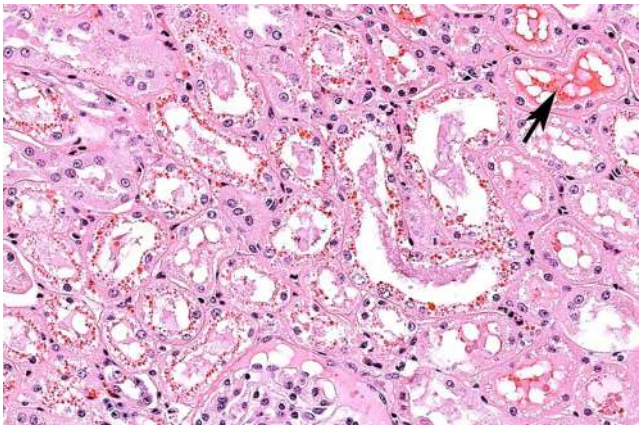


Figure 7. Renal tubular epithelial cells are attenuated with numerous flocculent red intracytoplasmic granules that occasionally fill the tubular lumina (granular casts- arrow). H&E, 400x.

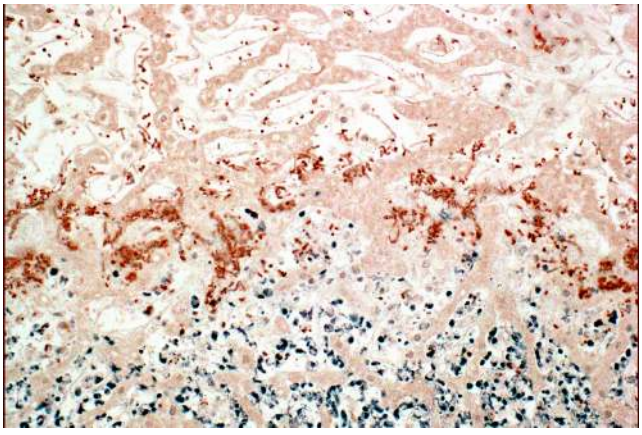


Figure 8. Liver. Immunohistochemistry for *Clostridium novyi* revealed strongly positive bacilli in the periphery of the necrotic areas. IHC, 600x.

Histological Description:

Liver: A large area of the hepatic parenchyma, contiguous with the hepatic capsule, is replaced by coagulative necrosis, characterized by hepatocytes with eosinophilic cytoplasm and karyolysis with preservation of the general hepatic architecture (acute infarct) (Figure 5A). The edges of the infarcted area are lined by a rim of degenerated neutrophils and cellular debris along with numerous gram-positive rods (Figure 5B). Throughout the remaining parenchyma, the portal areas are expanded by large amounts of connective tissue (fibrosis), irregular tortuous biliary profiles (biliary ductular reaction/hyperplasia), admixed with an inflammatory infiltrate composed of lymphocytes, histiocytes, plasma cells and fewer eosinophils and neutrophils. Clusters of free and/or histiocytic brown to black pigment are observed in the portal areas (Figure 6). The cytoplasm of the remaining hepatocytes contains small pale round vacuoles.

Kidney: Diffusely, the tubular epithelial cells are attenuated, and their cytoplasm contain numerous round red granules (hemoglobin) that occasionally fill the tubular lumina (granular casts) (Figure 7).

Morphologic diagnoses:

Liver: Severe, locally extensive, acute, hepatic necrosis with myriad intralesional gram-positive bacilli. Chronic, severe, multifocal, lymphohistiocytic and eosinophilic cholangiohepatitis with periportal fibrosis, biliary ductular hyperplasia and fluke pigment.

Kidney: Acute, moderate to severe, multifocal, tubular degeneration and necrosis with hemoglobin casts.

Ancillary testing:

Immunohistochemistry from the liver was positive for *Clostridium novyi* (Figure 8). Fluorescent antibody testing was performed in the liver for *Clostridium chauvoei*, *Clostridium novyi*, *Clostridium septicum*, and *Paeniclostridium sordellii* yielded negative results.

Disease: Bacillary hemoglobinuria or red water.

Discussion:

Bacillary hemoglobinuria (BH), also known as red water, is an infectious, but not contagious, peracute, and usually fatal disease that affects primarily cattle over one year of age that are usually introduced to new pastures. (4,5). The disease occurs endemically in many South American countries and the western United States, and it has been sporadically reported in other countries, including Canada, Australia, New Zealand, Ireland, Wales, Iran, Turkey, India, and Japan (3). It is produced by *Clostridium haemolyticum*, also known as *Clostridium novyi* type D. *C. haemolyticum* is a gram-positive, motile, and sporulated rod, which is considered one of the strictest anaerobic pathogenic clostridia. The main virulence factor is beta toxin, which is serologically indistinguishable from the beta toxin produced by *C. novyi* type B, the cause of infectious necrotic hepatitis or "black disease" in sheep (4).

The spores of *C. haemolyticum* are found in soil and may persist in the bones of cadavers for up to 2 years. When ingested by animals, they are absorbed from the intestine into the bloodstream and reach the liver via the portal circulation. The spores can survive for long periods of time in the Kupffer cells. After a hepatic damage due to various insults, mostly commonly associated with fluke migration, anaerobic conditions take place in the liver and allow for spore germination, bacterial multiplication, and production of toxins (1,2). Beta toxin causes hepatocellular and endothelial

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cell necrosis as well as erythrocyte lysis, producing the classical clinicopathologic signs of a large focus of coagulative necrosis in the liver, hemoglobinemia, anemia, icterus, and hemoglobinuria (4). In our case, the characteristic single necrotic focus in the liver was on the edge of the right lobe. In a retrospective study, the foci of hepatic necrosis were commonly seen only in the right hepatic lobe, followed by the left hepatic lobe, or both. These were usually in the central portion of the right lobe or in the ventral portion of the left lobe (3).

Migrating liver trematodes are considered the main predisposing factor for BH; however, in many cases, the initial triggering insult is not found in the liver of affected animals (3). Although adult *Fasciola* spp. was not present in gross examination of the liver nor eggs were seen in the histologic sections, the serpiginous black tracts in the liver parenchyma, enlarged dark brown hepatic lymph nodes from fluke exhaust drainage, along with cholangiohepatitis are highly suggestive of fascioliasis in this case. Other causes of liver damage that were suggested to activate spore germination include *Fusobacterium necrophorum* infection secondary to rumenitis, telangiectasia, or other various causes of hepatic hypoperfusion (3).

The presumptive clinical diagnosis of BH relies on the history of fascioliasis, hemoglobinuria and icterus, or sudden death, coupled with the typical single or sometimes multiple large infarcts in the liver (5). In this case, the gross and histologic findings coupled with the positive immunohistochemistry for *Clostridium novyi* were considered diagnostic for clostridial hepatitis, most likely bacillary hemoglobinuria. Hoewever, because the immunohistochemistry used does not distinguish between *C. hameolyticum* and *C. novyi* type B, we cannot completely rule out that the latter (responsible for necrotic hepatitis) was the agent of this lesion. In an animal found dead, differentiation from anthrax and other clostridial diseases such as blackleg may be required. In a live animal, differential diagnoses should include other diseases in which red urine is a cardinal sign such as leptospirosis, anaplasmosis, babesiosis, and chronic copper toxicosis (1).

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