Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #: 75 Month: December Year: 2016

Answer Sheet

Contributors: Renata Dalcol Mazaro,* DVM, MS candidate; Mariana Martins Flores, DMV, MS, Doctor in Veterinary Pathology; Rafael Fighera, DMV, MS, Doctor in Veterinary Pathology, Member of the Brazilian College of Animal Pathology (CBPA). *Laboratory of Veterinary Pathology, Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.

Microscopic Findings: The liver is diffusely and markedly vacuolated (vacuolar degeneration) (Figure 3). There is mild centrilobular necrosis and, in necrotic areas, there is occasional mild hemorrhage and mild inflammatory infiltrate mainly composed of plasma cells, macrophages and lymphocytes (Figure 4). Epithelial cell clusters forming ductules (biliary hyperplasia) can be seen in periportal areas (Figure 3).

Microscopic Images:

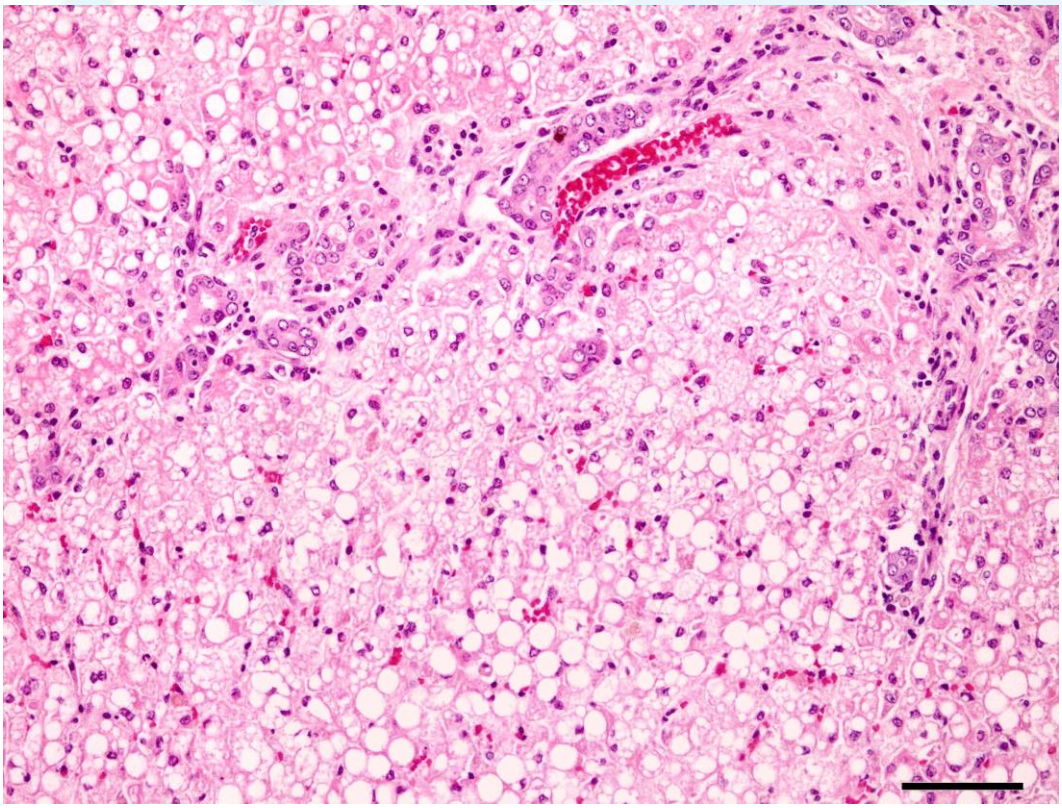

Figure 3
Hematoxylin and eosin stain [H&E]; 20X

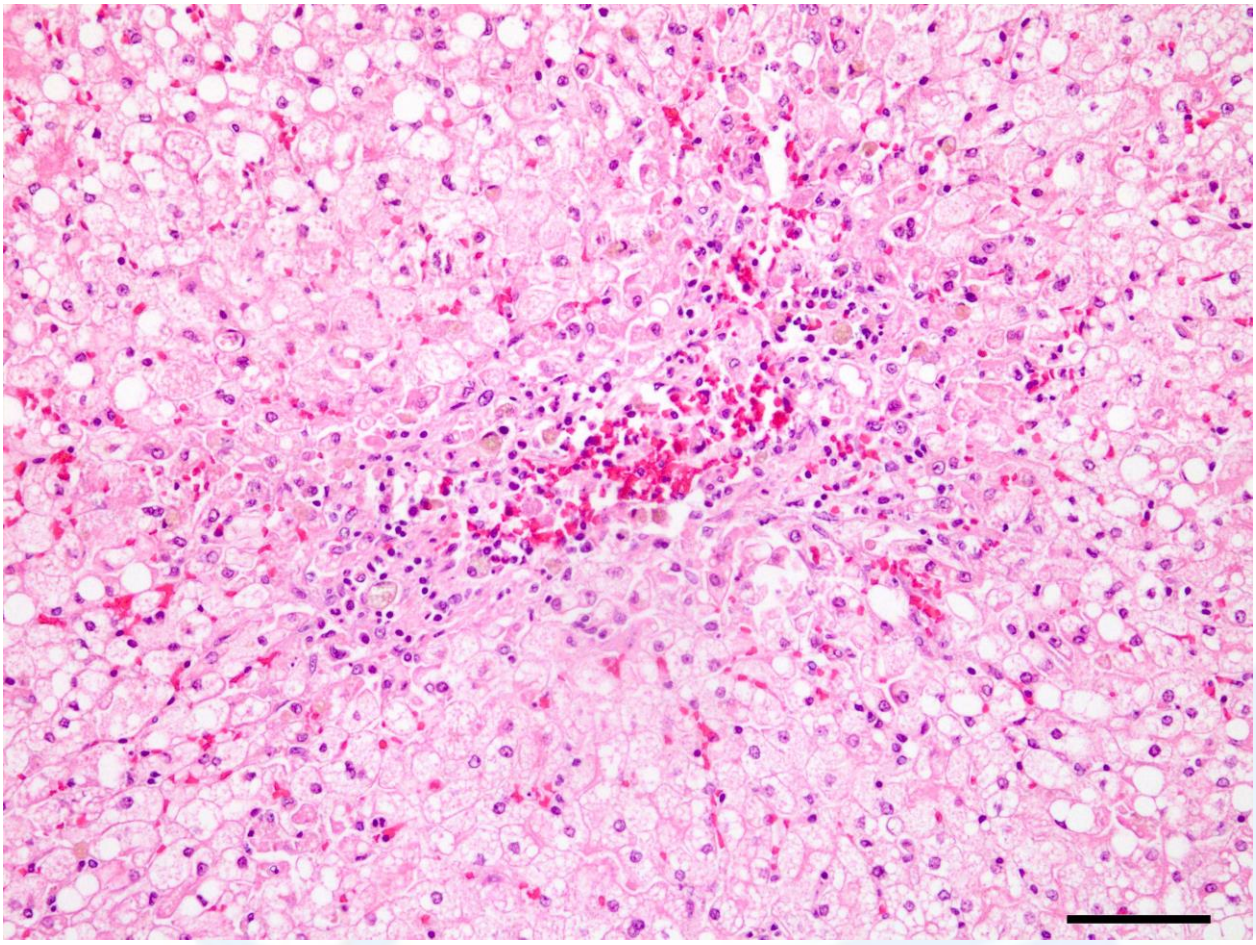

Figure 4
(H&E; 20X)

Morphologic diagnosis: Liver, acute, diffuse, severe, fatty degeneration (hepatic lipidosis); Small bowel (jejunum), acute, diffuse, severe, hemorrhage.

Etiologic diagnosis (liver): Toxic hepatopathy

Etiology: Mycotoxin (aflatoxin)

Name the condition: Acute aflatoxicosis

Typical Gross Findings: The most striking lesion of aflatoxicosis is in the liver, which is diffusely increased in size (hepatomegaly), markedly yellow, soft and friable. There is also accentuation of the lobular pattern. Other lesions include varying degrees of icterus, abdominal effusion and bleeding, particularly into the intestines.

Typical Microscopic Findings: The liver is diffusely and markedly vacuolated (vacuolar degeneration) characterized by generally one large cytoplasmic vacuole displacing the nucleus to the periphery of the hepatocytes (macrovesicular fatty degeneration) (Figure 3). There is mild

centrilobular necrosis characterized by hepatocytes with hypereosinophilic, floccular cytoplasm. The nuclei of these hepatocytes can be completely absent (karyolysis), fragmented (karyorrhexis) or a single, small, round structure with heavily condensed chromatin (pyknosis). In necrotic areas, there may be mild hemorrhage and inflammatory infiltrate mainly composed of plasma cells, macrophages and lymphocytes (Figure 4). In the cytoplasm of some hepatocytes and Kupffer cells, especially in the centrilobular area, there is accumulation of granular, brown-green pigment (hemosiderin). Epithelial cell clusters forming ductules (biliary hyperplasia) can be seen in periportal area.

Laboratory Testing: A sample from the corn flour that had been used to feed the dogs was provided by the owner and sent to a toxicology laboratory that analyzes mycotoxins (Laboratório de Análises Micotoxicológicas – LAMIC at UFSM). To determine the level of toxins present in this sample, two techniques were used: High performance liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LCMS). The levels of toxins determined by these tests were as follows: AFB1 (439 PPB), AFB2 (51 PPB), AFG1 (57 PPB) e AFG2 (7 PPB).

Discussion: Aflatoxins are di-furanocoumarins produced by fungi, especially *Aspergillus flavus*, *A. parasiticus* and *Penicillium puberulum*. There are at least 17 metabolites in this group, with aflatoxins B1 (AFB1), B2, G1 and G2 as the most important. Feed contamination by aflatoxins is influenced by temperature, humidity and substrate. Grains in virtually all production stages, including final products such as cornmeal, bread and commercial dog food, are considered the main source of these toxic fungal metabolites. Many aflatoxins have hepatotoxic, carcinogenic, mutagenic, teratogenic, or nephrotoxic effects. Aflatoxins are absorbed by the gastrointestinal tract and undergo hepatic biotransformation. After its oxidation by cytochrome p450 enzymes, AFB1 gives rise to the AFB1-8-9 metabolite (AFBO). This metabolite binds to glutathione and is then eliminated by the bile or in the urine. The disruption of cellular basal metabolism, protein synthesis, DNA repair and replication, and induction of cell necrosis reported in aflatoxicosis cases are directly induced by the AFBO and secondarily associated with the depletion or saturation of glutathione. While glutathione is still available for conjugation, low hepatotoxicity occurs; however, over time, hepatic glutathione depletion occurs faster than its formation, which leads to metabolite accumulation and, thus, exacerbation of cell injury. In cases where high AFBO doses are produced, the conjugation pathway becomes saturated, generating large amounts of accumulated toxic metabolites and resulting in severe liver damage.

The clinical course of aflatoxicosis can be acute or chronic and depends on the ingested amount of aflatoxin, the duration of exposure, the amount of food eaten, the affected animal species, and the nutritional status of each individual. Because aflatoxins are considered common contaminants, maximum concentration rates were established for raw materials consumed directly and for grains used for commercial food formulations. In Brazil, the maximum allowed concentration is 50 ppb (50 µg/kg), while in the United States, levels must be lower than or equal to 20 ppb (20 µg/kg). The most sensitive species (rabbits and chinchillas) can be fatally intoxicated with a dose as low as 1 µg/kg. Cattle, sheep and rats are much more resistant than dogs, pigs, birds, mice and fish, and this is due, in the former species, to higher levels of hepatocellular glutathione-S-transferase, the enzyme that binds a glutathione molecule to the AFBO, allowing its elimination. Dogs of any breed, gender or age can be affected; however,

females have lower biotransformation ability due to their sex hormones, and young and aged animals are more susceptible to intoxication due to their lower cytochrome p450 activity, added in aged dogs to the lower efficiency of the renal and biliary excretion systems. Clinical signs of intoxication include lethargy, vomiting, anorexia, icterus, hematochezia, melena, hematuria, diarrhea, hematemesis, ascites, pale mucous membranes, dyspnea, polydipsia, progressive weight loss, subcutaneous edema and neurological abnormalities such as altered level of consciousness, vocalization and seizures. Diagnosis is made through the association of clinical history, physical exam, gross and histopathological changes, and detection of mycotoxins either in the food or in animal tissues, secretions or excretions.

The severe hepatic lipidosis seen with aflatoxicosis is often accompanied by hemorrhage. Possible mechanisms for the hemorrhage include: A) decrease in the platelet numbers and in the levels of circulating clotting factors as a result of the increased consumption of platelets and clotting factors induced by disseminated intravascular coagulation (DIC); B) coumarin-like effect of aflatoxin B1 (AFB1); and C) AFB1-8-9-epoxide (AFBO) toxicity to hepatocytes. The DIC is triggered by factor III (tissue factor), a component of the extrinsic pathway of the coagulation cascade that is released from hepatocytes that are directly injured by the AFBO. Due to hepatic injury, factor III is metabolized more slowly, remaining in the circulation for a longer time, thus triggering the coagulation cascade via the extrinsic pathway and leading to widespread formation of microthrombi in capillaries, arterioles and venules. Another mechanism to be considered, especially for cases in which there is no significant hepatic necrosis due to the toxic effect of AFBO, is a severe and sharp decrease in the protein synthesis by the liver, which causes reduced synthesis of clotting factors. The later mechanism explains the abrupt start of bleeding signs in dogs with minimal centrilobular necrosis and severe hepatic lipidosis. As in cases of drug- or poison-induced Vitamin K deficiency or antagonism, the AFB1 coumarin-like effect, that is, its anticoagulant action, impairs the post-ribosomal carboxylation of glutamyl residues in some vitamin K-dependent clotting factors (II, VII, IX and X), thus culminating in the production of non-functional clotting factors.

In the geographic area where this case occurred (Rio Grande do Sul, Brazil), domestic dogs are frequently fed polenta, a cooked corn flour-based diet. This type of diet causes several nutritional problems by itself, for instance hyperphosphatemia, hypocalcemia and hypoproteinemia, particularly in puppies. When this flour is contaminated with aflatoxins and intoxication occurs, there are two possible presentations. In one of them, as seen in this case (acute form), the findings are typical of a toxic disease, that is, several dogs are affected (high morbidity) and die (high mortality) in a short period of time. These dogs eat high quantities of the toxin in a short period of time. In the other presentation (chronic form), the levels of aflatoxin in the corn flour are lower and the dogs are gradually intoxicated due to the cumulative effect of the toxin. These dogs develop chronic hepatic insufficiency characterized by progressive weight loss and ascites due to portal hypertension. Histologically, the liver is characterized by portal bridging fibrosis, mononuclear inflammation, bile duct hyperplasia and massive fatty degeneration. Dogs that survive for a longer time present with hepatic regenerative nodules and decreased liver size. Thus, intoxication cases associated with an “end-stage liver” are frequent. In chronic cases, the association between the hepatic lesion and its cause (the aflatoxin) is much more difficult to be established, mainly because the feed that the

dog was eating at the time of death (or for the last few days or weeks) doesn't always contain the toxin. For confirmation in these cases, determination of the aflatoxin levels in the liver or in various samples of corn flour used in the feed during the last several months prior to death should be done.

References and Recommended literature:

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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 (http://www.cldavis.org/diagnostic_exercises.html).

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