Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2011-2012

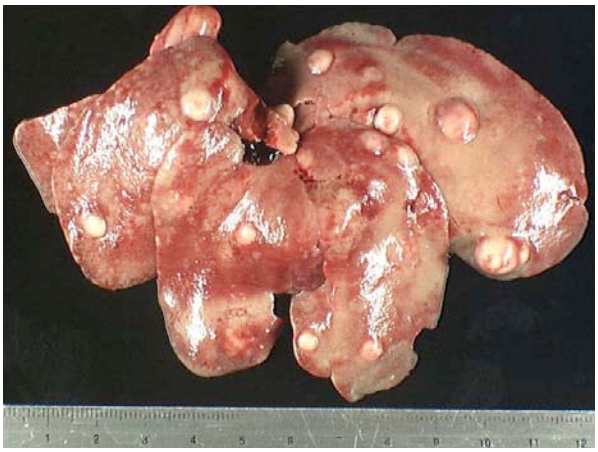
Conference 3

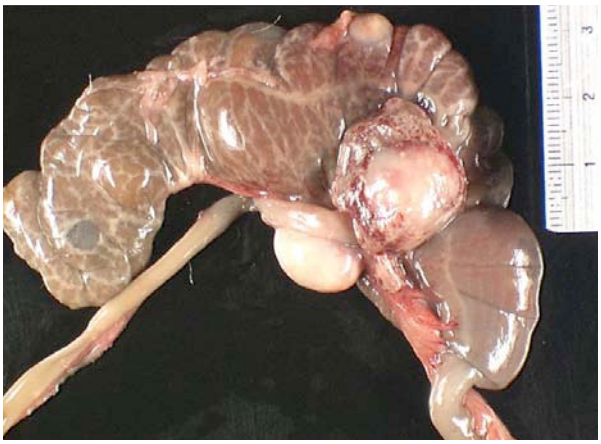
21 September 2011

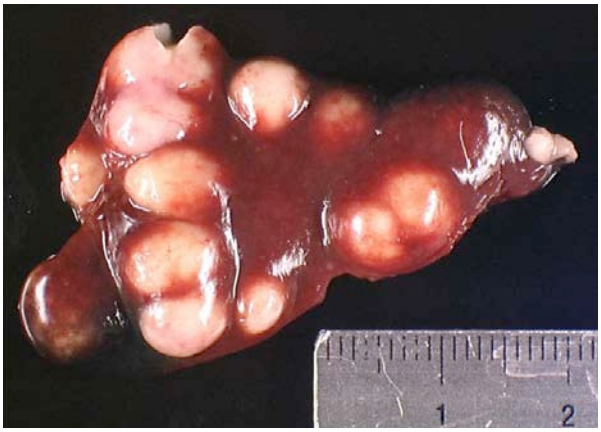
CASE I: 09.395.43 (JPC 3164204).

Signalment: 1-year-old male long-haired guinea pig (*Cavia porcellus*).

History: The patient presented at the Emergency Service of the Veterinary School of Alfort for a 10-day history of constipation, anorexia, and weight loss. An abdominal mass was found by palpation. Ultrasonography revealed multiple nodular masses in the liver, spleen and abdominal lymph nodes. Lymphoma, tuberculosis and infection by pyogenic bacteria were suspected. The guinea pig was hospitalized but died within a week.







1-1, 1-2, 1-3. Liver, ileo-caecal junction, spleen, guinea pig. Multiple, soft, white, liquid filled nodules ranging from 3 to 15 mm were found in the liver, in the spleen, and at the ileo-caecal junction as well as on the caecal serosa. Photographs courtesy of Ecole Nationale Veterinaire D'Alfort, Unité d'Histologie et d'Anatomie Pathologique, Maisons-Alfort, France

Gross Pathology: Multiple, soft, white nodules were found in the liver, in the spleen, at the ileo-caecal junction as well as on the caecal serosa. They ranged from 3 to 15 mm in diameter with a liquid center (multifocal suppurative hepatitis, splenitis, ileo-typhlitis and peritonitis).

Laboratory Results: Fine needle aspiration of an abdominal lymph node showed suppurative inflammation with high numbers of intracellular and extracellular bacteria with both coccoid and rod shapes. Repeated bacteriological analyses of samples from abdominal lymph nodes yielded mainly *Yersinia pseudotuberculosis* associated with a smaller population of *Escherichia coli.*

Histopathologic Description: Liver: Numerous, variable-sized nodules replace several portal tracts and compress the adjacent parenchyma. The largest nodules are 3 to 7 mm in diameter and are made of large colonies of coccobacilli embedded in large amounts of degenerate neutrophils, in turn surrounded by a fibrous and highly vascularized capsule rich in activated macrophages and lymphocytes (multiple hepatic abscesses). Numerous sections of biliary ducts are interspersed in the collagen bundles (biliary duct hyperplasia).

The smallest nodules are centered on colonies of coccobacilli admixed with degenerate neutrophils and necrotic, sometimes mineralized hepatocytes surrounded by activated macrophages, plasma cells, and lymphocytes. When visible, portal blood vessels are surrounded by lymphocytes and plasma cells or show invasion of their wall by macrophages and degenerate neutrophils with replacement of their media by a fibrillar eosinophilic material (fibrinoid necrosis).

In the remaining parenchyma, the cytoplasm of the hepatocytes is filled with variable-sized clear vacuoles consistent with lipid droplets (micro- and macrovacuolar steatosis). The central veins are congested. The coccobacilli do not stain with either Gram or Ziehl-Nielsen stains.

Spleen: The red and the white pulp is disrupted and compressed by multifocal, chronic, 0.5 cm-diameter abscesses and smaller pyogranulomas, with large colonies of intralesional coccobacilli. In the red pulp, siderophages are commonly seen (diffuse hemosiderosis, discrete).

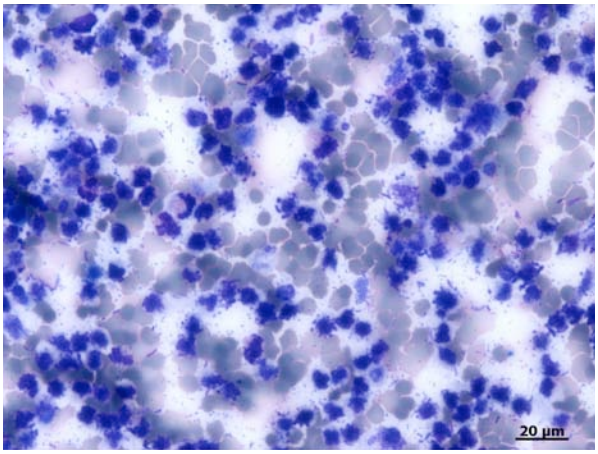
Caecum: (autolysis) (absent from the slides).

In the submucosa, with extension to the musculosa and the serosa, there is a focal accumulation of degenerate neutrophils and variably mineralized cell debris around large coccobacilli colonies, surrounded by activated macrophages and fibrous tissue. The overlying mucosa is lost (focal ulcer). The remaining mucosa displays a diffuse, discrete infiltration of the lamina propria by lymphocytes and plasma cells.

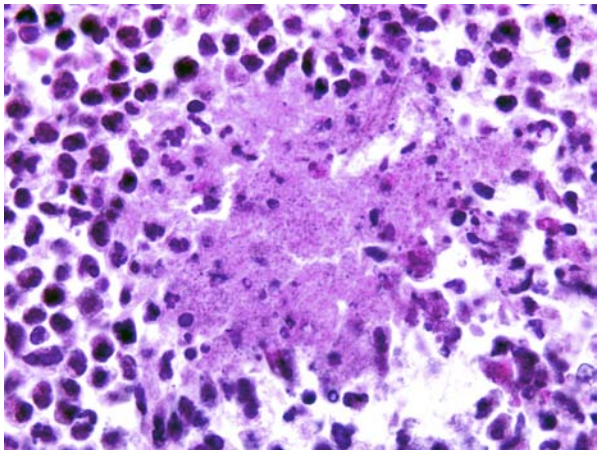
Peritoneum surrounding the caecum: (absent from the slides).

Multifocally, coccobacilli-containing abscesses are centered on blood vessels with fibrinoid necrosis of their wall. Other vessels display only intraluminal thrombi or degeneration of the wall with infiltration by macrophages and degenerate neutrophils. There is an extensive deposit of fibrin and pus in the serosa.

Caeco-colic lymph nodes: (absent from the slides).



1-4. Fine needle aspirate, abdominal lymph node, guinea pig. Suppurative inflammation with many intracellular and extracellular bacterial rods and cocci. Photograph courtesy of Ecole Nationale Veterinaire D'Alfort, Unité d'Histologie et d'Anatomie Pathologique, Maisons-Alfort, France.



1-5. Liver, guinea pig. Abscesses have a central core of degenerate neutrophils, necrotic debris and are centered on large colonies of 2-4 um bacilli, characteristic of Yersinia. (HE 1000X)

Two lymph nodes are almost entirely replaced by large abscesses surrounded by a rim of residual lymphoid tissue. There are numerous colonies of coccobacilli in the center of the abscesses. Another lymph node displays numerous macrophages in the subcapsular and medullary sinuses that are seldom binucleated with a large amount of granular cytoplasm (sinusal histiocytosis).

Contributor's Morphologic Diagnosis: Liver: Hepatitis, suppurative, multifocal, subacute, severe, with intralesional gram-negative coccobacilli colonies consistent with *Y. pseudotuberculosis.*
Steatosis, diffuse, severe.

Spleen: Splenitis, suppurative, multifocal, subacute, severe, with intralesional gram-negative coccobacilli colonies consistent with *Y. pseudotuberculosis.*

Caecum: Typhlitis, suppurative and ulcerative, transparietal, subacute, moderate with intralesional gram-negative coccobacilli colonies consistent with *Y. pseudotuberculosis.*

Peritoneum and caeco-colic lymph nodes: Peritonitis and lymphadenitis, suppurative, severe with intralesional gram-negative coccobacilli colonies consistent with *Y. pseudotuberculosis*.

Contributor's Comment: Multiple white friable foci in the liver, spleen, abdominal lymph nodes and intestines in a guinea pig may also be associated with the following bacterial diseases:
- *Salmonella* infection (*S. typhimurium, S. enteritidis, or S. dublin*): there is multifocal granulomatous to suppurative hepatitis, splenitis, lymphadenitis, and enteritis, characterized by paratyphic nodules;
- Tyzzer's disease (*Clostridium piliforme*): there is necrotizing hepatitis, ileitis and typhlitis, often with transmural involvement; the bacteria are intracellular bacilli that react positively with Warthin-Starry stain;
- *Streptococcus* infection with *S. zooepidemicus* or *S. pneumoniae*: these Gram-positive bacteria can be responsible for suppurative lymphadenitis (streptococcal lymphadenitis and pneumococcal infection), hepatitis and splenitis (streptococcal septicemia and pneumococcal infection).[7]
- Mycobacterial granulomas with Ziehl-Nielsen-positive bacteria.

Microscopically, the differential diagnosis for large plaque-like colonies of bacteria in H-E sections includes, following the « YACS » mnemonic method:
- *Yersinia* sp.
- *Actinomyces* sp., *Actinobacillus* sp., *Arcanobacter* sp.
- *Corynebacterium* sp., *Clostridium* sp.
- *Staphylococcus* sp., *Streptococcus* sp.

The morphology of the bacteria and the results of the Gram staining will further orientate the identification. In the present case, the bacteria were gram-negative cocco-bacilli; these characteristics were consistent with *Yersinia* sp.

Y. pseudotuberculosis and *Y. enterocolitica* are gram-negative coccobacilli that can survive and grow at low temperature (psychrophilia). The pathogenic strains of theses bacteria share several virulence factors, namely:
- the invasin: this adhesin binds to beta-integrins expressed on the surface of the intestinal M cells; it promotes uptake of the bacterium by these cells and triggers the production of chemotactic cytokines by epithelial cells;
- a plasmid-encoded type III secretion system that translocates *Yersinia* outer proteins (Yops) into the host cell;
- the Yops: these effector proteins allow the bacterium to remain extracellularly and evade phagocytosis and killing by neutrophils and macrophages; one mechanism can be an alteration of the actin cytoskeleton in the host cell as is the case with YopE.[4,8]

Y. pseudotuberculosis can also produce a superantigenic toxin termed *Y. pseudotuberculosis*-derived mitogen a (YPMa). Several pathogenic strains display on their chromosome a high-pathogenicity island (HPI), which carries genes of the yersiniabactin system involved in siderophore-mediated iron acquisition.[5]

Infection with *Y. pseudotuberculosis* is a zoonotic disease; yersiniosis can also occur in various domestic animals and non-human primates. Wild rodents and birds are the reservoir hosts. Transmission is fecal-oral.[2,4,6] After ingestion, *Y. pseudotuberculosis* or *Y. enterocolitica* invade Peyer's patches and lymphoid follicles. High numbers of neutrophils are recruited at the portal of entry. The lymphoid follicles and the overlying epithelium are subsequently replaced by suppurative foci. The bacterium then disseminates via lymphatics and hepatic portal veins to the draining lymph nodes and to the liver and the systemic circulation[4], as was the case in this guinea pig.

Y. pseudotuberculosis is considered to be an extracellular pathogen. It binds to the macrophages and survives attached to them. The immune response is humoral but also cellular. CD8+ T lymphocytes, considered to protect against intracellular pathogen, have been recently shown to restrict *Y. pseudotuberculosis* infection. The proposed model is that T cells could target host cells with extracellularly attached *Y. pseudotuberculosis*, thus allowing the host cells and associated bacteria to be engulfed and removed by neighboring macrophages.[3]

Yersiniosis was one of the earliest bacterial diseases recognized in guinea pigs. Several types of clinical manifestation are recognized in this species:
- a peracute septicemic form;
- an acute form with miliary, cream-colored nodules in the intestinal wall and ulceration of ileum and caecum;
- chronic infection with caseous nodules in mesenteric lymph nodes, spleen, liver and lung, emaciation, and death;
- non-fatal infection with lesions of lymph nodes of the head and neck.

Inapparent carriage in healthy animals is also reported.[6,7] The lesions of *Y. pseudotuberculosis* and *Y. enterocolitica* cannot be differentiated either grossly or microscopically. In either case, characteristic microcolonies of coccobacilli are found in microabscesses through histological examination. Confirmation by bacterial isolation is needed.[4,7]

Yersinia pestis, the cause of plague in humans and animals, is a clone *of Yersinia pseudotuberculosis* that emerged 1,500 – 20,000 years ago.[1]

JPC Diagnosis: Liver: Abscesses, multiple, with large colonies of bacilli.

Conference Comment: Since guinea pigs digest much of their diet with the aid of hind-gut fermentation, the normal cecal flora is important for physiologic digestion. The cecum is the largest part of the digestive tract usually containing up to 65% of the gastrointestinal contents.[6]

Gut flora is primarily gram-positive bacteria with anaerobic lactobacilli. Coliforms, yeasts, and clostridia may be present in small numbers. A common cause of death in *Yersinia* infection is the result of the subsequent antibiotic treatment. Certain antibiotic therapies can induce a disruption of normal gut flora which are supplanted by pathogenic bacteria such as *Clostridium dificile* and *Escherichia coli* (dysbiosis). Altered microbial ecology in the gut may produce disease and dysfunction because of the intense metabolic activity or antigenicity of the inappropriate bacterial flora to include elaboration of bacterial enzymes which degrade pancreatic enzymes, damage the intestinal brush border, deconjugate and reduce bile acids and alter the intestinal milieu in numerous ways. It is usually the subsequent bacterial antigens and exotoxins that may cause death rather than the initial infection with *Yersinia* sp.[6]

Some conference participants interpreted perceived changes in the hepatic arteries as fibrinoid vascular necrosis, noting apparent hypereosinophilia and hyalinization in the vascular walls; however, the moderator believed this to be an artifact due to perceived staining differences, resulting from the use of saffron with standard hematoxylin and eosin.

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CASE II: 09-2271-8 (JPC 3169272).

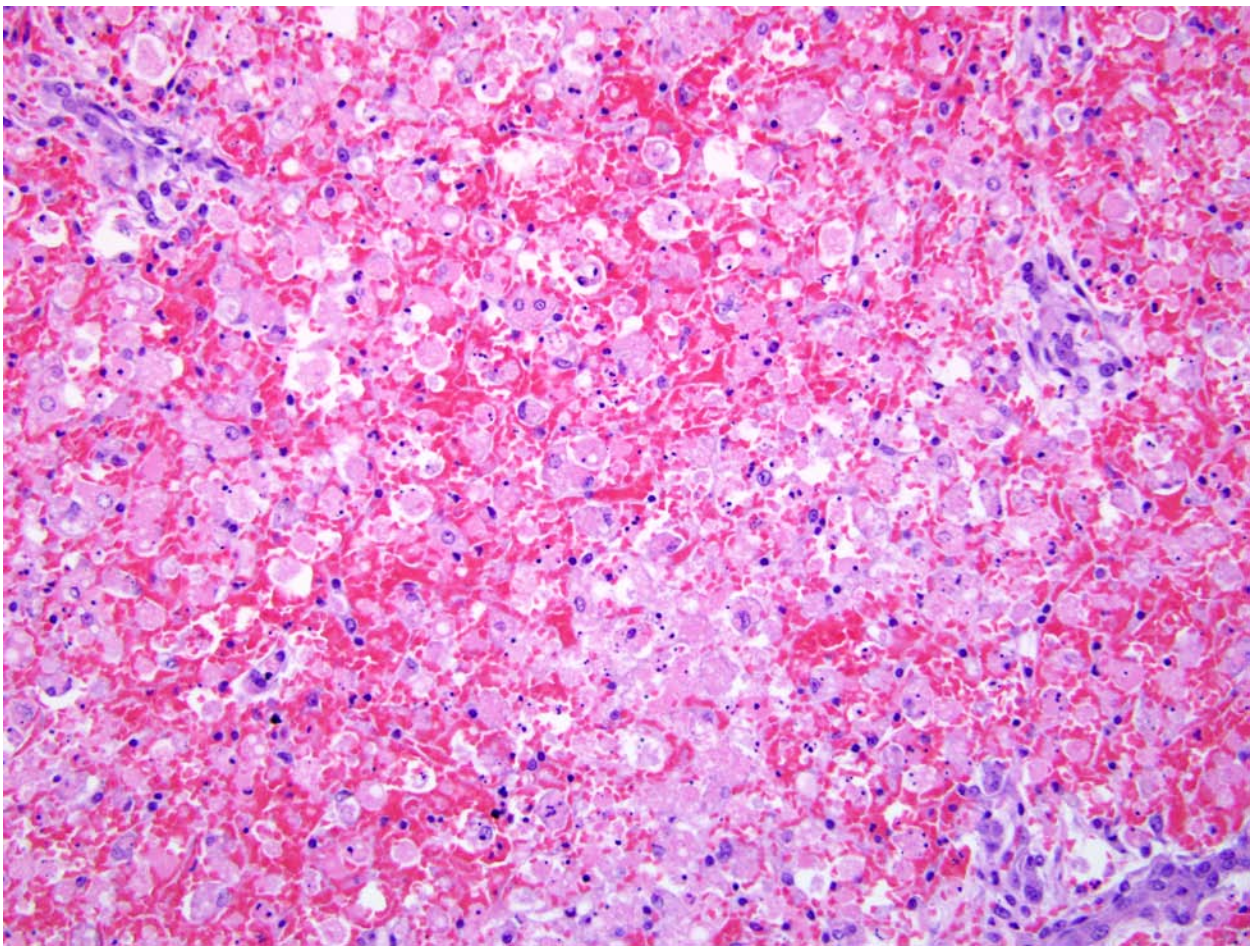
Signalment: 1-year-old (approximately) male mixed breed canine (*Canis familiaris*).

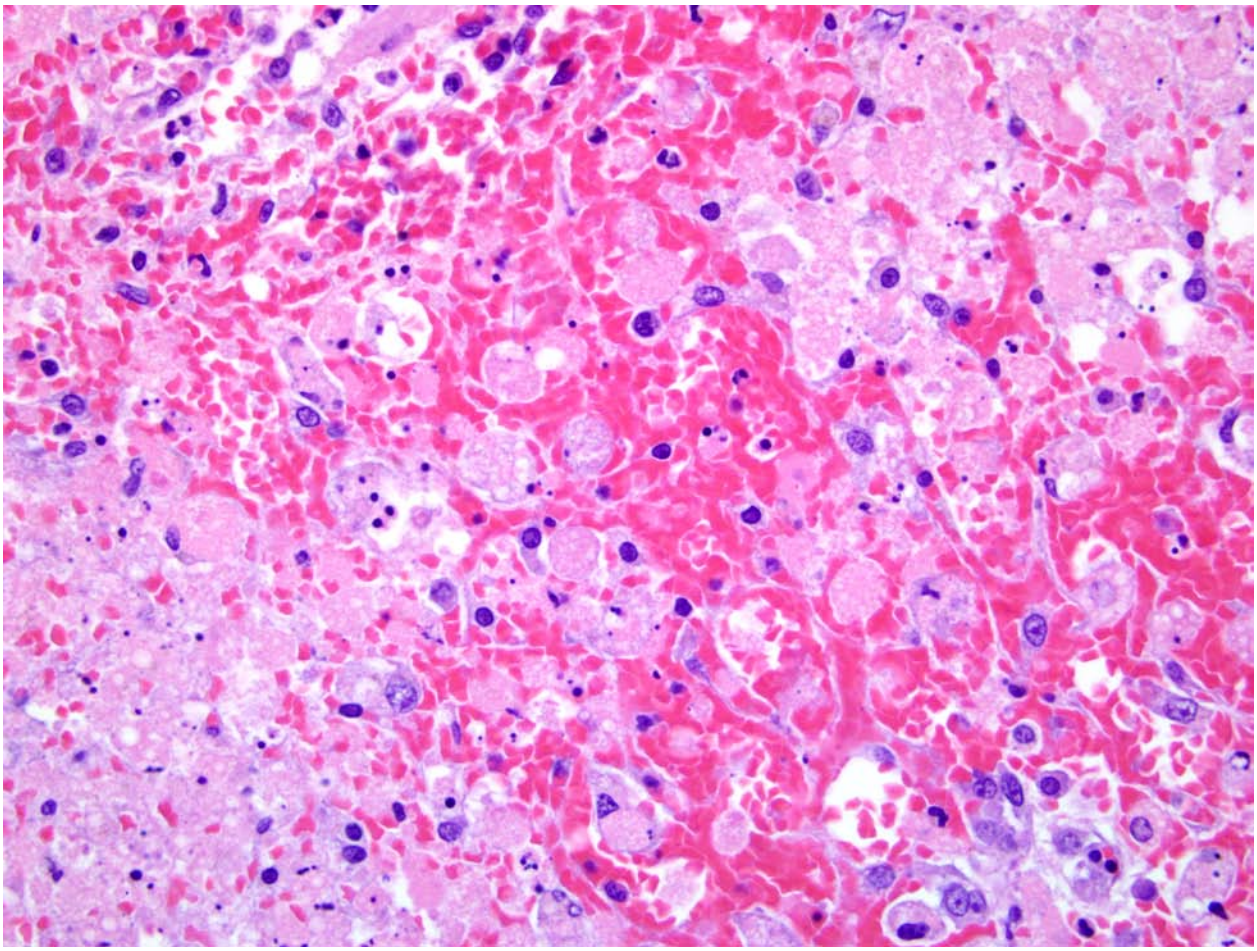
History: The dog was presented to its veterinarian with a history of consuming a white mushroom suspected to be of the *Amanita* genus. The dog began vomiting approximately 30 minutes after consumption and continued to vomit intermittently over the next 24 hours. Abdominal pain and distention, pallor, melena, and hematoma formation at venipuncture sites were noted on physical examination. The dog initially became severely lethargic then uncoordinated. Following one seizure episode the dog became obtunded. The dog remained hypoglycemic despite dextrose administration and died naturally less than 36 hours following consumption of the mushroom.

Gross Pathology: The peritoneal cavity contained approximately 600 ml of frank unclotted blood. The liver was diffusely pale and mottled with red foci throughout the parenchyma. All other organs and tissues including the heart and kidneys appeared diffusely pale. The heart had multifocal well-demarcated, slightly raised, dark red foci on the endocardial surface of the left ventricle. The serosal surface of the fundus and cardia of the stomach were dark red. The mucosal surface of the stomach appeared mildly thickened diffusely and had multifocal dark red areas. There were multifocal dark red areas on the serosal surface of the proximal duodenum. There was digested blood noted throughout the lumen of the gastrointestinal tract.

Laboratory Results: Severely elevated liver values (AST = 12,620; ALT = 7,540)
Coagulation times (PT/aPTT) were severely elevated (too high to measure)
Persistent and progressive hypoglycemia (initially 85 mg/dL, last measured value was 23 mg/dL)
Hypoproteinemia (Total protein = 2.5 g/dL; Albumin = 1.6 g/dL)
Acidemia (pH = 7.2)

2-1. Liver, dog. There is massive necrosis of hepatocytes (necrosis of hepatocytes in all parts of the hepatic lobule) with loss of sinusoidal architecture, and diffuse hemorrhage. Portal areas are in close apposition without intervening central veins. (HE 100X)





2-2. Liver, dog. Necrotic hepatocytes are disassociated, rounded up, with swollen or shrunken hypereosinophilic cytoplasm and pyknoti, or karyorrhectic nuclei. (HE 400X)

Histopathologic Description: Liver: Throughout the section there is a diffuse loss of hepatic lobular architectural organization with collapse of sinusoids in all zones, extending from central vein to portal triad. Hepatocytes are frequently individualized and either expanded with indistinct, vacuolated eosinophilic cytoplasm (hepatocellular vacuolar degeneration) or shrunken and hypereosinophilic with decreased differential nuclear staining (karyolysis) or deeply basophilic, fragmented nuclei (karyorrhexis) representing hepatocellular coagulation necrosis. Throughout the section are many large, round cells with eccentric oval nuclei and markedly vacuolated, foamy eosinophilic cytoplasm (activated macrophages). Hepatocytes in the periportal regions are separated by many erythrocytes (congestion).

Contributor's Morphologic Diagnosis: Liver: Severe panlobular acute hepatocellular coagulation necrosis.

Contributor's Comment: In the United States, *Amanita phalloides,* also known as "death cap" or "death angel" mushroom, is most commonly implicated in severe cases of mushroom hepatotoxicosis. *Amanita* species mushrooms contain a specific class of toxins referred to as amatoxins (bicyclic octapeptides).[2] Other hepatotoxic cyclopeptide-containing mushrooms include *Galerina*, and *Lepiota* species.[2] Classes of amanitin hepatotoxins include alpha, beta, gamma, and epsilon. The alpha and beta forms are the most toxic and account for over 90% of the amatoxin content of the mushroom. A 20g mushroom contains a potentially lethal dose of amatoxin (LD50 estimated at 0.5mg/kg).[3]

Amanitins bind eukaryotic DNA-dependant RNA Polymerase II and inhibit transcription and protein synthesis, resulting in cell death. Cells with a high metabolic rate are most susceptible (intestinal crypt cells, hepatocytes, proximal convoluted tubule cells). Amanitins also result in endocrine abnormalities.[4]

Reported clinical signs:
Phase 1 – Latency, the animal may appear normal for 8-12 hours following ingestion

Phase 2 – Severe GI signs due to gastroenteritis (nausea, vomiting, bloody diarrhea, abdominal pain)
Phase 3 – Apparent clinical improvement
Phase 4 – Multi-organ failure: Fulminant liver, often with coagulopathy and encephalopathy; Acute renal failure; Severe hypoglycemia, associated with the breakdown of liver glycogen.

Coma and death typically occurs 12-84 hours following ingestion of a lethal dose.[3]

JPC Diagnosis: Liver: Necrosis, massive, diffuse with biliary hyperplasia (ductal reaction type 3).

Conference Comment: The conference morphologic diagnosis parenthetically interprets the biliary hyperplasia as ductal reaction type 3. Prior to the conference, the moderator gave a wonderful presentation referencing a proposed new classification scheme by Desmet[1] for the reaction presently called biliary hyperplasia in veterinary medicine. The new classification scheme takes physiologic and embryologic responses by various cells in the liver to injury or insult into account. In summary, ductular reactions (DR) are categorized into three types based on the type of hepatic injury, the cells involved in the histologically viewed response, and their relationship to the formation of the embryonic ductal plate.

Embryonic biliary precursor cells form a periportal sheet called the ductal plate, which is progressively remodeled to generate intrahepatic bile ducts. A limited number of ductal plate cells participate in duct formation; those not involved in duct development are believed to involute by apoptosis. The ductal plate gives rise to cholangiocytes lining the intrahepatic bile ducts, including its most proximal segments. It also generates periportal hepatocytes and adult hepatic progenitor cells. During early embryogenesis, there is a single-layer ductal plate surrounding the portal vein and portal mesenchyme, followed by the formation of double layered plates. In normal development, extensive resorption of the primitive bile ducts leads to the final stage, in which a network of fine bile ducts surrounds the portal vein.

Ductular reactions generally form a structure similar to the ductal plate, and in the pathologic response, are termed mini-ductal plates. These mini-ductal plates are composed of a small central blood vessel surrounded by a small amount of mesenchyme derived from the original Disse space, and a double layer of small cholangiocyte-like cells lining a slit-like lumen with peripheral tubular dilatation.

This new system classifies DRs into type 1, in which pre-existing ducts and ductules proliferate as a result of cholestasis (and is the type most commonly referred to as "biliary hyperplasia" by veterinary pathologists); type 2A, which is ductular metaplasia induced by inflammation; type 2B, which is ductular metaplasia induced by hypoxia; and type 3, which is the activation and proliferation of liver progenitor cells (LPC) in response to massive hepatic parenchymal loss.

A central tenet which underlies these categorizations is the idea of differentiation and dedifferentiation of cells, specifically LPCs. An LPC differentiates into a mature hepatocyte by sequentially evolving from keratin 19-positive (K19+) hepatoblast, to K19+ K7+ intermediate hepatobiliary cells, then K7+ intermediate hepatocytes and finally to K7 negative mature hepatocytes. In cholangiocyte metaplasia, such as in the type 2 DRs, the mature hepatocytes dedifferentiate in a reverse fashion. Both pathways rely on Jagged1/ Notch signaling as well as a partnership with portal myofibroblasts, endothelial cells, and hepatic stellate cells, and the dedifferentiation of mature hepatocytes back to hepatoblasts recapitulates the embryonal ductal plate. It is hypothesized that the first layer of the mini-ductal plate is derived from this dedifferentiated K19+ hepatoblasts, and the second layer of the mini-ductal plate is from the activation of LPCs in the canal of Hering due to cholestasis or hypoxia.

Type 1 DR is the haphazard proliferation of pre-existing cholangiocytes due to severe cholestasis or interleukin-6 (IL-6) induction, which results in the activation of purinergic ATP receptors on cholangiocytes, which down-regulates NTPDase2 expressed on periductal portal fibroblasts that normally inhibit cholangiocyte proliferation. This physiologic response widens and elongates pre-existing ducts and ductules to accommodate the increase in toxic bile salts and reduce parenchymal damage in cholestasis or increased portal expansion by edema and inflammation. This type of structural adjustment, typically referred to as biliary hyperplasia, can be seen with the other categories of DR according to physiologic need.

Type 2A DR, which is generally periportal, is the "ductular metaplasia of hepatocytes" and formation of mini-ductal plates from LPCs, and results in the change of hepatocyte secretory polarity from horizontal as in canalicular to vertical as in cholangiocytes due to chronic cholestasis. This change in secretory polarity is due to the cytoplasmic keratin rearrangement from long-term exposure to bile salts, and also incites the dedifferentiation of mature hepatocytes to an intermediate K19+ K7+ hepatobiliary cell. This is accompanied by the activation of hepatic stellate cells located in the space of Disse into a myofibroblastic phenotype, which increases the production of connective tissue and the further induction of intermediate hepatobiliary cells

into K 7+ cholangiocytes. Both DR type 1 and 2A are reversible.

Type 2B DR generally occurs in centrilobular areas and is result of hypoxia. This reaction is microscopically similar to type 2A and is induced by hypoxia-inducible factor 1-alpha (HIF-1α). This in turn upregulates fibrotic and vasoactive mediators such as platelet-derived growth factor-A (PDGF-A), PDGFB and plasminogen activator inhibitor-1 (PAI-1). This activate HSCs and LPCs as described in DR type 2A, resulting in progressive centrolobular fibrosis and ductular metaplastic proliferation.

Finally, type 3 DR, which occurs in this case, occurs in massive parenchymal loss. This represents the traditional notion of progenitor cell-based regeneration, and results from the bipotential proliferation of remaining LPCs, resident in the remaining canals of Hering into hepatocytes and cholangiocytes. Following massive hepatic parenchymal loss, there is a massive influx of fibrous connective tissue, and this proliferation is as described in types 2A and 2B, with a tendency toward the cholangiocytic phenotype due to a preponderance of myofibroblasts.

This ductal reaction classification scheme is beginning to gain popularity in the human pathology realm, and has value due to the inherent pathogenic implication with each category. While there is a long way to go before this type of pathogenically descriptive scheme finds favor in veterinary pathology, if at all, a system like this can be very useful.

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CASE III: NTU08-968 (JPC 3138068).

Signalment: 4-year-old, male, Labrador retriever dog (*Canis familiaris*).

History: An outbreak of severe illness and death affected 181 dogs in an animal shelter starting in August 2008 and subsided in January 2009. Clinically, the dogs progressively developed signs of vomiting, anorexia, depression, icterus, ascites, melena, hematochezia or hematemesis, and eventually death. All dogs received vaccination and deworming for endo/ectoparasites. They were fed a commercialized dog food. PCR results for *Leptospira* sp., parvovirus, adenovirus, *Ehrlichia* sp., and *Babesia* sp. were all negative. Analysis for organic phosphorus and cyanide in intestinal contents was also negative. Based on the presentation and preliminary laboratory results intoxication was suspected by the clinicians.

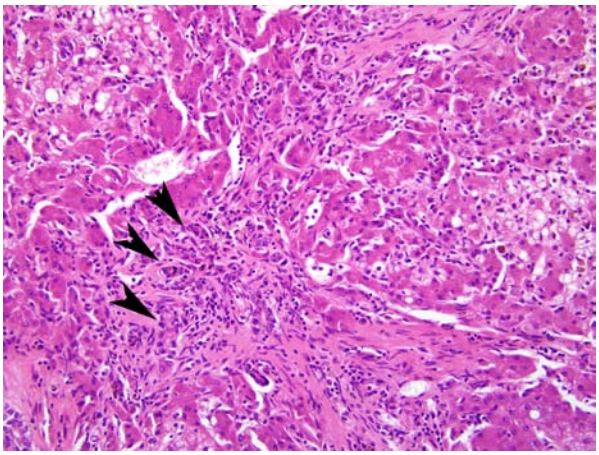
Gross Pathology: The dog was severely icteric with marked yellow discoloration on mucous membranes, skin, sclera, and adipose tissue. The abdominal cavity contained approximately 970 ml of a yellow to dark orange, translucent, watery fluid (ascites). There were a few fibrin strings adherent to the intestinal serosa. The liver was slightly enlarged, diffusely yellow-tinged and firm with locally extensive white irregular, scar-like areas. The gall bladder wall was thickened with marked submucosal edema. There were multifocal to coalescing, red foci (hemorrhage) scattered on the gastrointestinal tract, urinary bladder, pancreas, and heart. The intestines contain small to moderate amounts of dark red, tarry contents. Aside from the hemorrhage, the urinary bladder was diffusely yellow. Lungs were diffusely reddened, wet and heavy. Bronchi were filled with frothy fluid. Both kidneys were slightly enlarged, and the medulla was yellowish on cut surfaces.

Laboratory Results: Aflatoxins were detected from the commercial canine food consumed by the affected dog. The concentration of aflatoxin B1 in this commercial canine food was over 150 ppb examined by Animal Health Research Institute and TÜV Rheinland Aimex Ltd. PCR results for *Leptospira* sp., parvovirus, adenovirus, *Ehrlichia* sp., and *Babesia* sp. were all negative. Analysis of intestinal contents for organic phosphorus and cyanide were also negative.

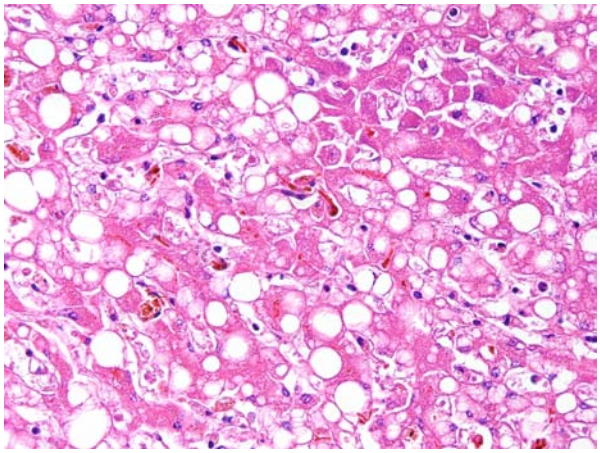
Histopathologic Description: Liver: The surface of the liver is irregular and the lobules become indistinct and irregular. The lobules are characterized by variable hepatocellular hydropic degeneration, lipidosis, and necrosis, biliary hyperplasia, and portal, periportal to bridging fibrosis. The remaining hepatocytes display cellular atypia and regeneration characterized by variably sized and shaped cells and nuclei. Scattered binucleated and multinucleated hepatocytes are also noted. Along with these changes, there is also diffuse moderate to severe inflammation characterized by locally extensive infiltration of macrophages, neutrophils, fewer plasma cells and lymphocytes accompanied with variable fibrosis. Mild to moderate bile stasis is diffusely present. Yellow to brown pigment-ladened macrophages and Kupffer cells are also frequently seen.

Contributor's Morphologic Diagnosis: Liver: Hepatocellular fatty change and regeneration, severe, with hepatitis, chronic-active, moderate; portal, periportal, and bridging fibrosis; bile duct hyperplasia; and cholestasis.

Contributor's Comment: Aflatoxins are a group of related, natural, toxic byproducts of the fungi



3-1. Liver, dog. Bridging fibrosis connects portal areas and there are numerous small biliary duct profiles (biliary hyperplasia (arrowheads). (HE 20X)



3-2. Liver, dog. Hepatocytes are swollen due to a combination of macro- and microvesicular steatosis; bile plugging (cholestasis) is prominent within these areas. (HE 400X)

Aspergillus flavus, *Aspergillus parasiticus,* and a new select *Penicillium* spp. Aflatoxin B1 is the most hepatotoxic, and also can be immunosuppressive, nephrotoxic, and carcinogenic, and cause hemolytic anemia and coagulopathies.

Aflatoxins are liposoluble and readily absorbed from the gastrointestinal tract into the portal blood. They are then transported to the liver for metabolism. Toxicosis is a result of binding of essential enzymes, which blocks DNA polymerase and ribosomal translocase and leads to the formation of DNA adducts.

It has been suggested that the carcinogenic action of aflatoxin B1 in the rat results from a capacity to bind to DNA, a characteristic similar to that of actinomycin D. However, lethal doses of actinomycin D do not produce hepatic parenchymal cell necrosis. In all species studied, the organ most affected is the liver, although other organs, particularly the kidney, show signs of damage. The distribution of the hepatic lesion is not consistent from species to species, i.e., rat and duckling, periportal; guinea pig and swine, centrilobular; dog, periportal and centrilobular; and rabbit, mid-zonal. In contrast, most other hepatotoxins, such as carbon tetrachloride, regularly induce a centrilobular lesion in both rats and guinea pigs. There is a wide range in the acute LD dose of aflatoxin B1, varying from 0.3 mg/kg for ducklings to 16 mg/kg for mature female rats. In species for which data are available, the young appear to be more susceptible than mature animals. Although the Food and Drug Administration suggests a zero tolerance for aflatoxin in food, it lists a legal limit of 20 mg/kg (ppb) in feed. For dogs, the lethal dose, 50% (LD50) value is just 500 to 1,000 mg/kg (ppb), and, 60 mg/kg (ppb) is a toxic dose.

In the present cases from the Bali shelter, at necropsy the dogs fed commercial dog feed containing aflatoxic peanut meal were jaundiced with swelling and yellowish discoloration of the liver and edema of the gall bladder identical to that seen with crude or purified aflatoxin. The causes of death are believed mainly to be due to severe hepatic damage and the subsequent secondary coagulation defect. The insufficient production of coagulation factors due to severe hepatic injury induces hemorrhages in multiple organs and tissues, including heart, gastrointestinal tract, kidney, pancreas, and adipose tissue. The order of severity and histopathology are variable between different cases, because the varying rates of metabolism between different species, ages, nutritional status and hormone levels hinder assessment of exposure in animals. Additionally, the susceptibility of individual dogs can be affected by levels of sex hormones, age, dose, and/or degree of feed refusal.

JPC Diagnosis: Liver: Macronodular hepatocellular regeneration, diffuse, severe with microvesicular steatosis, necrosis, cholestasis, bridging portal fibrosis, and biliary hyperplasia (ductular reaction type 1.).

Conference Comment: Histologic features of prominent fatty degeneration, bridging fibrosis, and severe cholestasis, marked by bile pigment within hepatocyte cytoplasm (fig) are common features of a number of hepatotoxicities, including phenobarbitol intoxication, copper toxicosis, low-grade chronic microcystin toxicity, certain homeopathic herbal mixtures and chronic aflatoxicosis as in this case. Acute aflatoxicosis, by contrast, is characterized by hemorrhage, severe fatty change, and biliary hyperplasia.

Another important histologic feature is that many portal areas have diminutive portal veins or lack them altogether. This is presumably secondary to portal hypertension from the massive dissecting fibrosis, which prohibits adequate downstream perfusion of portal veins and venules.

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CASE IV: P0802057 (JPC 3103210).

Signalment: 16-year-old bay Welsh-Pony stallion (*Equus caballus*).

History: The stallion showed reduced appetite and indolence for several weeks, accompanied by moderate to severe icterus, malodorous diarrhea and ataxic locomotion/ compulsive walking. Clinical symptoms were typical for severe hepatic failure with hepatic encephalopathy and the pony was euthanized. Other horses from the same stable, receiving the same diet, did not show any clinical signs of hepatic failure.

Gross Pathology: The stallion was moderately obese and showed intense yellow coloration of mucosal membranes and sclera as well as generalized subcutaneous edema. There was a moderate amount of free serous fluid in thorax and abdomen. The unpigmented skin between nostrils and on the upper and lower lip showed multifocal to coalescing moderate erythema with crusting, consistent with solar dermatitis. Several ulcers (2-3 cm in diameter) were present in the glandular mucosa of the stomach. The wall of the ascending colon showed severe diffuse subserosal edema. The greenish-brown liver had a diffusely thickened capsule with multifocal extensive filamentous proliferation (consistent with chronic parasitic perihepatitis), was very firm on cut surface and had an accentuated zonal/lobular pattern.

Laboratory Results: Blood parameters:

- Lymphocytes	7.7	(25-70 %)
- Granulocytes	88.7	(30-65 %)
- ALP 647		(97-269 U/l 37˚C)
- CPK 391		(<268 U/l 37˚C)
- Creatinine	197	(116-180 µmol/l)
- Total bilirubin	215	(<34 µmol/l)
- GOT 600		(22-488 U/l 37˚C)
- LDH	828	(162-412 U/l 37˚C)
- GGT 914		(16-56 U/l 37˚C)

Histopathologic Description: Liver: The architecture of the liver parenchyma is severely disturbed with marked portal fibrosis, often breaching the limiting plate and resulting in more diffuse parenchymal fibrosis, with prominent porto-portal and porto-central bridging and moderate to marked bile duct hyperplasia.

Predominantly in centrilobular regions, numerous hepatocytes show marked hepatocellular polyploidy and hepatocellular hypertrophy (megalocytosis) with marked nuclear pleomorphism often with cytoplasmic hypereosinophilia, foamy cytoplasm and multiple nuclei. Hepatocellular nuclei are often swollen, containing up to three large nucleoli and occasional mitotic figures and cytoplasmic inclusion. There are frequent intercellular bile plugs in bile canaliculi and swelling and proliferation of Kupffer cells containing bile and lipofuscin pigment. Various amounts of bile and lipofuscin pigment are also present in the cytoplasm of hepatocytes.

4-1. Sclera, horse. There is intense yellow coloration of the sclera (icterus). Photograph courtesy of University of Utrecht, Departement of Pathobiology, Utrecht, Netherlands, www.vet.uu.nl.

4-2. Skin, horse. The lightly pigmented skin of the muzzle shows multifocal to coalescing erythema with crusts (solar dermatitis). Photograph courtesy of University of Utrecht, Departement of Pathobiology, Utrecht, Netherlands, www.vet.uu.nl.

There is multifocal, predominantly centrilobular, hyperemia with absence of hepatocytes, hepatocellular swelling and eosinophilia and karyopyknosis and karyorrhexis (hepatocellular necrosis) with associated infiltration of neutrophils, macrophages and swelling and proliferation of endothelial cells.

There are diffuse, randomly scattered islands of smaller hepatocytes arranged in broadened cords of liver cells (nodular hyperplasia), with hepatocytes frequently containing intracytoplasmatic vacuoles of

varying size with sharp edges (hepatocellular lipidosis) and single apoptotic hepatocytes.

There is increased sinusoidal cellularity with infiltration of various numbers of neutrophils, histiocytes and lymphocytes.

The liver capsule shows moderate diffuse fibrosis and hypertrophic mesothelial cells and there is multifocal to coalescing mild lymphohistiocytic and neutrophilic sub-capsular infiltration.

Contributor's Morphologic Diagnosis: Hepatocellular degeneration and necrosis, centrilobular, mild, marked megalocytosis with marked portal bile duct hyperplasia, fibrosis, multifocal nodular hyperplasia and mild to moderate neutrophilic and lymphocytic inflammation (hepatitis).

Contributor's Comment: Microscopic investigation of the brain revealed subacute moderate hepatic encephalopathy, characterized by multifocal to coalescing clusters of Alzheimer type II astrocytes, predominantly in basal ganglia/ capsula interna and brainstem, multifocal mild to moderate perivascular and perineuronal edema, focally few perivascular lymphohistiocytic infiltrations and sparsely necrotizing neurons with satellitosis.

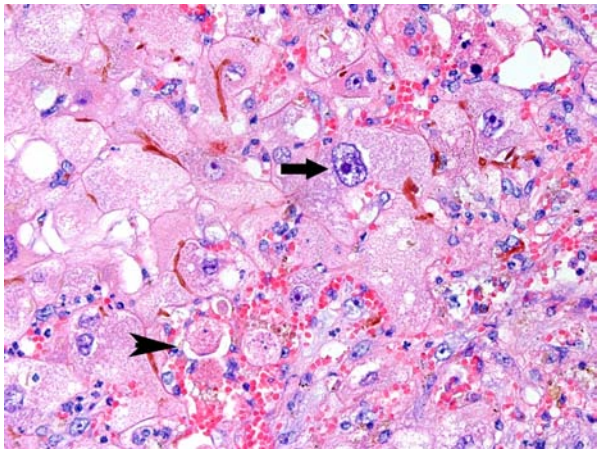
Widespread portal fibrosis, bile duct proliferation, and megalocytosis are often described as characteristic lesions observed in the liver of horses poisoned by plant-derived pyrrolizidine alkaloids (PA).[1,4,6,7] These chemicals have been found in various plant species widely distributed in the world, e.g. in genera *Senecio, Crotalaria, Heliotropium, Amsinckia, Cynoglossum, Echium, and Trichodesma.*[1,6] In The Netherlands, predominantly *Senecio* ssp. (e.g. "Jakobskruiskruid", *Senecio jacobaea*) from the *Asteraceae* family, are of increasing epidemiologic importance.[4] Ingested PAs are converted to pyrrolic esters by hepatic cytochrome p450 enzymes which mediates the N-oxidation of the esters. These esters are alkylating agents, which react with cytosolic and nuclear proteins and nucleic acids.[6] Dissociation of the alkylation products may result in the formation of new alkylating agents, which may cause cellular damage to persist after ingestion of the alkaloid has ceased.

Cytochrome p450 can also mediate a two-step hydroxylation of necine bases at C3 and C8 positions, which is followed by spontaneous dehydration to the highly reactive dehydro-pyrrolizidine (DHP). The toxic DHP metabolites are electrophilic and can bind to proteins and nucleic acids at guanine and adenine residues, and this DNA-binding activity is responsible for their genotoxicity.[6]

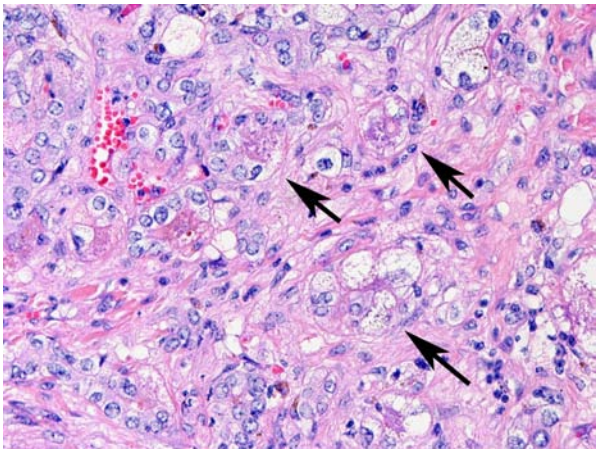
There are three common pathological expressions of PA poisoning[6]:

1. Acute periacinar zonal necrosis, occurs after ingestion of large amounts of PA (naturally occurring outbreaks are rare).
2. Hepatic atrophy with formation of regenerative nodules and characteristic pattern of hepatocellular megalocytosis, occurs after phasic (usually seasonal) repetitive exposure; this is the most common expression of field exposure to PAs.
3. Firm, atrophic livers without nodular regeneration, due to prolonged exposure.

Several studies on horses with experimental PA intoxication revealed that development of megalocytosis, as well as advanced fibrosis and bile duct proliferation, occurs in rather late/ chronic stages (up to 6 months) and/or due to prolonged low dose



4-3. Liver, horse. Megalocytes (arrows) are characteristic findings in pyrollizidine intoxication of the horse. Hepatocytes are markedly swollen by numerous intracytoplasmic fat droplets, resulting in marked bile plugging (cholesteatosis). A cluster of necrotic hepatocytes is present (arrowhead) (HE 400X)



4-4. Liver, horse. Ductular reaction, type 2A is present adjacent to portal areas. In this type of ductular reaction, hepatocytes "de-differentiate", lostiong their characteristic morphology and forming lumina (arrows).

intoxications.[4,6] Furthermore, clinical signs of toxicosis generally do not appear until weeks to months after onset of plant consumption, and as soon as marked clinical signs occur it was found that in most cases the pathological damage is irreversible.[2,4] The kinetics of conversion of alkaloids to pyrroles, and the chemical nature of these products, varies not only with plant species, but also with animal species, age and sex, and with the metabolic rate of the target cells.[4,6] For an individual PA, toxicity depends on the amount of alkaloid that can be converted to reactive metabolites, the rate of cytochrome p450-mediated generation of DHP, and the efficiency of the detoxification pathway.[6] Hence, the fact that in this case only one individual of the herd showed clinical symptoms of hepatic failure is feasible. In addition to this, estimating the onset of exposure to the toxin is very difficult.

One of the most characteristic effects of chronic exposure to toxic pyrroles is the induction of nuclear and cytoplasmic gigantism (megalocytosis). This effect is most likely due to an antimitotic effect with continued DNA synthesis.[6] Continued nucleoprotein synthesis, coupled with mitotic inhibition, probably accounts for the great increase in size of the nucleus and cytoplasm.[6] Some enlarged nuclei have cytoplasmic invaginations that can be misinterpreted as intranuclear inclusions.[6] Megalocytic cells can be up to 20 times normal size, however, many hepatocytes in an affected liver do not become megalocytic. Those that are completely inhibited do not replicate DNA at all, whereas those that are more resistant can replicate more normally and give rise to nodular populations of smaller, more normal hepatocytes. When hepatocytes are lost faster than they can be replaced, the liver can become atrophic, but the atrophy can be compensated to various degrees by megalocytosis and regenerative nodules.[6] Regenerative capacity might be, amongst others, due to species specific differences, because cattle more often show regenerative nodules than horses.[6]

Concurrently, there is usually fibroplasia and proliferation of the bile ducts. Proliferation of bile duct epithelial cells is largely explained by their unspecific propensity to respond to regenerative stimuli that prevail when liver mass is inadequate. The amount of fibroplasia varies with species and exposure, typically it is minimal in sheep, moderate in horses and may be marked in cattle.[6]

Horses are more likely to manifest signs of hepatic encephalopathy than other species, characterized by head pressing and compulsive walking, the latter of which also occurred in this patient. Although it could not be found in this case, pulmonary emphysema is described as an outstanding feature of pulmonary manifestation of certain PA intoxications in horses. The pulmonary lesions include severe vascular engorgement and edema, and diffuse fibrosis of alveolar and interlobular septa with patchy epithelialization.[6]

Other alkylating agents such as nitrosamine and aflatoxins can also result in portal fibrosis, bile duct proliferation and sometimes megalocytosis.[6,7] Due to lacking information about the quality of the patient's diet, chronic hepatic toxicosis from e.g. aflatoxins cannot be completely excluded. Although the histological lesions reveal characteristics of a chronic hepatitis, pathologists often do not include the term "hepatitis" in the morphological diagnosis of this toxic liver condition. This seems to reflect an inconsistency in the use of the term hepatitis in cases with primary liver cell necrosis due to a toxic insult resulting in an inflammatory reaction.

JPC Diagnosis: Liver: Fibrosis, portal and bridging, diffuse, moderate with hepatocellular anisocytosis and megalocytosis, necrosis, and cholestasis.

Conference Comment: Mesothelial hypertrophy was prominent in this case, and is likely the result of ascites secondary to severe hypoproteinemia. In horses, ascites is an uncommon finding as they usually manifest edema in the lower limbs. Also, the hepatocytes are frequently expanded and exhibit a "ground glass" appearance as a result of proliferation of smooth endoplastmic reticulum, likely due to induction of cytochrome P450 secondary to long term alkaloid exposure.

The moderator pointed out that in cases of hepatocellular nodular regeneration, regenerated hepatocytes develop compensatory enzymes to better accommodate repeat toxin exposure. This is recognized histologically in cases such as chronic copper toxicity, where regenerative nodules are often devoid of copper.

In this case, biliary hyperplasia can be characterized using the new scheme, as described in the previous case, as ductal reaction type 2A. The incipient metaplastic change in the bile ducts is the result of ductal formation by hepatocytes, which lose cytoplasmic eosinophilia and form lumens. Interestingly, the hepatocytes dedifferentiate to a more immature phenotype in response to chronic exposure to free bile acids secondary to severe cholestasis.[3]

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