Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #16 17 January 2024

CASE I:

Signalment:
Three-year-old male intact Lop rabbit (*Oryctolagus cuniculus*)

History:
The rabbit presented with soft stools and decreased activity. No abnormalities were found on physical examination, except for the presence of loose stools in the perianal area. Treatment with probiotics was initiated. Over the subsequent two days, the rabbit stopped producing fecal pellets and eating. It presented on the third day with no menace response, no gut sounds, and the inability to use the hind limbs. On radiographs, kidneys appeared enlarged. The rabbit died during blood sampling. CPR was attempted but was unsuccessful.

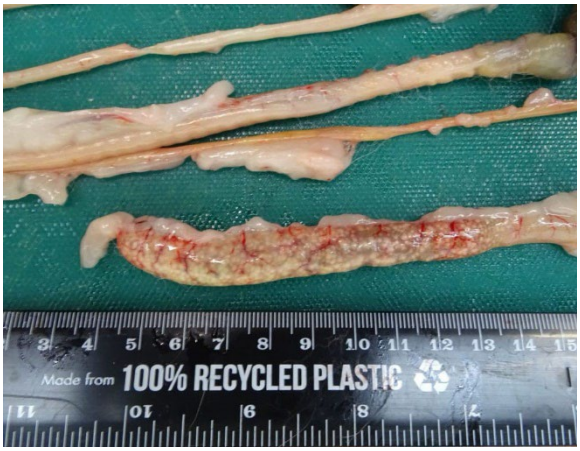

Figure 1-1. Cecum, rabbit. The cecum is distended and numerous foci of necrosis are visible through the serosa. (*Photo courtesy of:* Veterinary Pathology Service, School of Veterinary Medicine and Science, University of Nottingham, https://www.nottingham.ac.uk/vet/service-for-business/veterinary-pathology-service/companion-and-equine-pathology/index.aspx)

Gross Pathology:
Across all hepatic lobes there were multiple, well demarcated, white foci ranging from <1 to 1 mm in diameter. Affecting approximately 50-60% of the spleen, there were large multifocal to coalescing, circular, poorly delineated, well demarcated firm, cream to white nodules, which extended into the parenchyma and measured up to 9 mm in diameter, and bulged up to 3 mm from the splenic surface. The apical 10 cm of the cecal mucosa and serosa also showed numerous, multifocal to coalescing, white foci, measuring 2-3 mm in diameter, and the cecal wall was moderately thickened.

Laboratory Results:
Microbiological culture of spleen: Moderate growth of *Yersinia pseudotuberculosis*.

Microscopic Description:
Spleen: Affecting approximately 80% of the examined sections and effacing white and red pulp are large multifocal to coalescing foci of necrosuppurative inflammation characterized

by a central, hypereosinophilic area of cellular and karyorrhectic debris (lytic necrosis) admixed with viable and degenerate neutrophils surrounded by numerous macrophages, lymphocytes, and fewer plasma cells. There is an outer layer of fibrous tissue which is infiltrated by lymphocytes, plasma cells, and eosinophils, and often contains scattered to extensive areas of extravasated erythrocytes (hemorrhage) and eosinophilic, fibrillar material (fibrin). Multifocally within and often at the periphery of the areas of necrosis there are colonies, up to 100 µm in diameter, of 1-2 µm amphophilic, gram negative coccobacilli. Scattered throughout the affected and non-affected parenchyma are moderate numbers of macrophages containing intracytoplasmic, golden brown, granular pigment (hemosiderin).

Cecum: In approximately 90-95% of the examined section, the submucosa is markedly effaced and expanded by the presence of multifocal large foci of necrosuppurative inflammation which often extend into the lamina propria. These foci are characterized by a central, area of hypereosinophilic cellular and karyorrhectic debris (lytic necrosis) admixed with mostly degenerate and fewer viable neutrophils, surrounded by numerous macrophages, lymphocytes and fewer plasma cells, multifocal eosinophilic, fibrillar material (fibrin), and by an outer layer of fibrous tissue which is infiltrated by fewer numbers of lymphocytes, plasma cells, and eosinophils. Multifocally within areas of necrosis there are small numbers of scattered, 1-2 µm amphophilic, gram negative coccobacilli. The adjacent mucosal associated lymphoid tissue is multifocally compressed and the remaining submucosa is heavily infiltrated by mostly lymphocytes and fewer plasma cells and macrophages, which occasionally contain black and green, granular intracytoplasmic material. Multifocally within the lamina propria there are mildly to moderately increased numbers of lymphocytes and plasma cells.

Liver: Multifocally, adjacent to portal spaces, there are a few smaller foci of necrosuppurative inflammation with extensive lytic necrosis similar to those described in the spleen and caecum. The portal spaces are often mildly to markedly infiltrated by lymphocytes and plasma cells, which occasionally mildly extend through the limiting plate to the adjacent parenchyma. Small numbers of lymphocytes are often infiltrating the biliary epithelium. Across all examined sections, there are numerous multifocal to coalescing, midzonal to centrilobular areas where hepatocytes are markedly swollen, exhibiting a pale staining cytoplasm arranged in thin strands, or vacuolar change of the cytoplasm (hepatocellular degeneration). Rarely, there is variable occlusion of the lumen of blood vessels by fibrin thrombi with few enmeshed neutrophils and lymphocytes.

Contributor's Morphologic Diagnoses:

1. Spleen**:** Splenitis, necrosuppurative, subacute, multifocal to coalescing, severe, with intralesional colonies of coccobacilli and microabscesses
2. Cecum: Typhlitis, necrosuppurative, subacute, multifocal to coalescing, severe, with intralesional coccobacilli and microabscesses
3. Liver: Hepatitis, necrosuppurative, subacute, multifocal to coalescing, mild, with multifocal microabscesses and marked hepatocellular degeneration.

Contributor's Comment:

Yersiniosis (or pseudotuberculosis) is a disease caused by infection with gram-negative bacteria *Yersinia pseudotuberculosis* or *Yersinia enterocolitica*.[1,3,14] While susceptible,

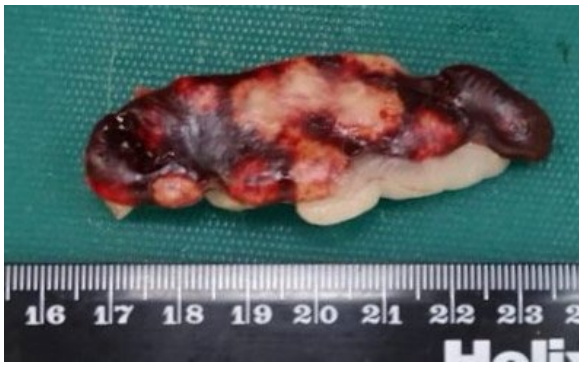

Figure 1-2. Spleen, rabbit. There are numerous foci of necrosis which are randomly scattered throughout the splenic parenchyma. (*Photo courtesy of:* Veterinary Pathology Service, School of Veterinary Medicine and Science, University of Nottingham)

domestic rabbits are not commonly affected.[1] This infection is, however, commonly seen in rodents, birds, non-human primates, ruminants, and other zoo species.[2,3,7,11,14]

Transmission is thought to occur via the fecal-oral route through ingestion of contaminated food and/or water.[1,14] Infection may result either in acute death or development of clinical signs and lesions, as is the case with ruminants and pigs.[3,14] In this case, the clinical history and length of duration of clinical signs was unknown; however, the morphology of the lesions identified would suggest a more subacute timeline of infection.

After ingestion, the bacteria colonize the intestinal epithelium M cells, resulting in the destruction of Peyer's patches and epithelium and the formation of suppurative inflammation and microabscessation in the lamina propria.[14] *Yersinia* spp. virulence requires the presence of a 70-kb virulence plasmid.[9,14] This plasmid encodes the production of Yops proteins which inhibit phagocytosis, aiding the bacterial spread to mesenteric lymph nodes and other organs.[9,14,15] The bacteria may then disseminate to the liver or systemically through the venous drainage and lymphatic system.[14] Typical post-mortem findings include necrotizing hepatitis and splenitis, fibrinonecrotizing enterocolitis, and lymphadenitis.[2,3,9,14] On histology, there may be microcolonies of gram-negative coccobacilli and formation of microabscesses.[14] It is not possible to differentiate between *Y. pseudotuberculosis* and *Y. enterocolitica* based on macroscopic and histological lesions alone.[14]

As zoonotic agents, *Y. pseudotuberculosis* and *Y. enterocolitis* can cause acute gastroenteritis and mesenteric lymphadenitis in humans.[8] In Europe, multiple strains of *Y. pseudotuberculosis* of varying pathogenicity have been identified in wild boars, and pathogenic strains of *Y. enterocolitis* have been associated with wildlife, suggesting that wildlife species may play an important role as a reservoir for human infections.[6,13] While hospitalizations and deaths secondary to these infections have been reported, it is important to differentiate yersiniosis from plague, which is caused by *Yersinia pestis* and is a World Health Organization-reportable disease with much higher fatality rates.[3,8,17]

In veterinary medicine, macroscopic findings in *Y. pestis* infections may include pulmonary, lymph node, and cutaneous/subcutaneous hemorrhages, as well as necrosuppurative lymphadenitis, necrotizing pneumonia, and multifocal abscessation with intralesional gram-negative coccobacilli with a characteristic bipolar, safety-pin-like morphology.[3] Geographic location may help include or exclude *Y. pestis* as a likely/unlikely differential.

The diagnosis of yersiniosis is typically made through identification of microabscesses with microcolonies of coccobacilli on histology and subsequent confirmation with bacterial

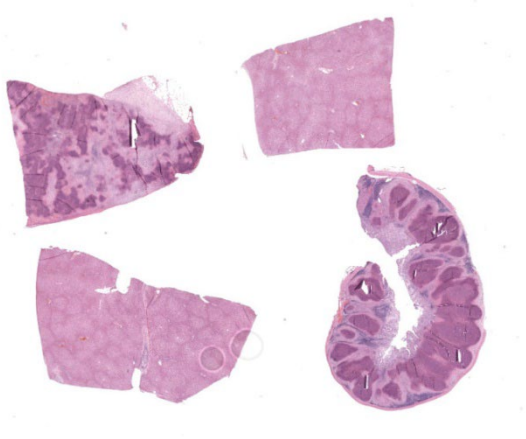

Figure 1-3. Cecum, spleen, liver, rabbit. Two sections of liver and one each of cecum and spleen are submitted for examination. (HE, 5X)

culture.[14] *Francisella tularensis* infection – tularemia - can cause small foci of caseous granulomas in the liver, spleen and lymph nodes, and could therefore be a potential differential diagnosis for the gross lesions.[4] Amongst other lesions, systemic listeriosis, caused by *Listeria monocytogenes*, can also present with hepatic necrotic foci, and could potentially be a less likely differential; however, gram-positive staining would differentiate this condition from yersiniosis.[4]

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JPC Diagnoses:
1. Cecum: Peyer's patch necrosis, diffuse, with mild, chronic typhlitis.
2. Spleen: Splenitis, necrotizing and heterophilic, chronic, diffuse, severe, with coccobacilli.
3. Liver: Vacuolar change, centrilobular and midzonal, diffuse.

JPC Comment:
The contributor provides an excellent overview of the typical course of disease in yersiniosis caused by *Y. pseudotuberculosis* and *Y. enterocolitica*. These two species are typically associated with a relatively benign gastrointestinal disease rather than the well-described, fulminant syndromes associated with *Y. pestis*, but the pathogenetic mechanisms of the enteric disease caused these species remain incompletely understood.

As the contributor notes, *Y. pseudotuberculosis* and *Y. enterocolitica* cross the mucosa through the M cells of Peyer's patches; once in the mucosa, they are engulfed by macrophages, where they survive and are trafficked to the lymph nodes and beyond.[12] A key virulence factor, shared by all three pathogenic *Yersinia* species, is a type 3 secretion system, similar to that found in *Salmonella* species, that allows the organism to inject effector proteins into the host cell cytoplasm.[12] In *Yersinia* species, these effector proteins are termed *Yersinia* outer proteins ("Yops"). Yops are injected into macrophages and neutrophils and interfere with phagocytosis and the production of reactive oxygen species, ensuring bacterial survival and dissemination.[12] Yops come in at least six different varieties – YopE, YopM, YopO, YopT, YopP, and YopH – each with its own pernicious bag of tricks aimed at frustrating the host's immune system.[5] For instance, YopP blocks pro-inflammatory NF-kappaB and MAPK-signaling pathways, and YopM is thought to block the inflammasome by binding caspase-1.[5] The end result of all of this molecular subterfuge is bacterial persistence and disease.

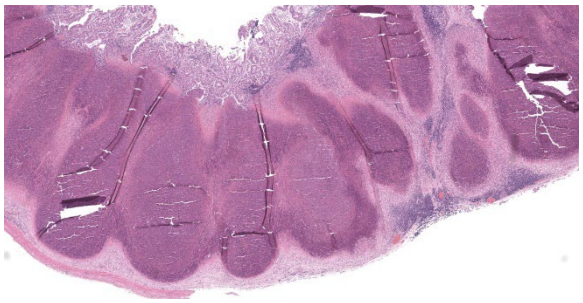

Figure 1-4. Cecum, rabbit. There is diffuse necrosis of Peyer's patches and replacement by numerous necrotic heterophils and cellular debris. (HE, 25X)

Birds and rodents are throught to be the principal reservoirs of *Yersinia pseudotuberculosis,* which causes enteric, often subclinical infection in many wild and domestic animals.[12,16] Enteric yersiniosis is relatively common in young deer and lambs, where disease is characterized by profuse watery diarrhea which can be rapidly fatal if untreated; clinically similar but less severe disease can be seen in young ruminants.[12] The septicemic form of the disease, often called pseudotuberculosis, occurs most commonly in laboratory rodents and aviary birds.[12] Finally, sporadic abortion caused by *Y. pseudotuberculosis* has been reported in cattle, sheep, and goats.[12]

Despite the canonical association of *Y. pseudotuberculosis* with enteritis, a recently reported outbreak in African lions was notable for its presentation, which was characterized by respiratory distress and pulmonary bacterial colonization without gastrointestinal lesions.[16] Also notable was the unusual pleomorphic, filamentous morphology of the bacteria, confirmed to be *Y. pseudotuberculosis* via culture.[16] This filamentous morphology has been previously described in *Y. pseudotuberculosis* infections in squirrel monkeys and is thought to be a bacterial adaptation to the use of antibiotics.[16]

Our moderator this week was Dr. Patti Pesavento, Department Chair and Professor of Pathology, Microbiology, and Immunology at the University of California Davis College of Veterinary Medicine. Dr. Pesavento began discussion of this case by noting that low-magnification evaluation is invaluable in this slide, which contains four tissues, some of which contain flashy lesions with remarkable distributions and tinctorial differences. Dr. Pesavento cautioned against getting bogged down looking at every micrometer of tissue and exhorted residents to use low magnification to spot areas of interest, take closer looks at those particular areas, and then hop back to subgross magnification for further investigation.

Conference participants found tissue identification on the section of alimentary tract to be somewhat difficult and discussed possible locations, including the vermiform appendix,

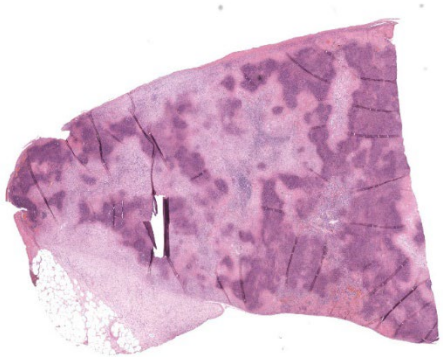

Figure 1-5. Spleen, rabbit. There is diffuse loss of the splenic architecture with multifocal to coalescing areas of lytic necrosis. There is rupture of the capsule (lower left) (HE, 25X)

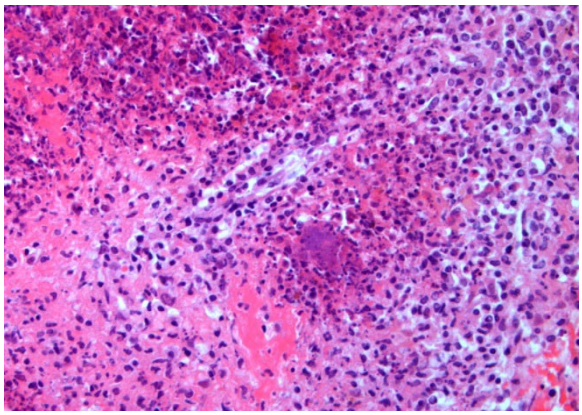

Figure 1-6. Spleen, rabbit. There are rare large colonies of coccobacilli within the spleen. (*Photo courtesy of:* Veterinary Pathology Service, School of Veterinary Medicine and Science, University of Nottingham) (HE 400X)

the cecal tonsil, the ilem, or the sacculus rotundus. Participants felt they could not be definitive based on the examined section alone due to the complete obliteration of tissue architecture both in this tissue and in the spleen. Participants also noted the presence of substantial fibrosis indicating that, despite the apparent fulminant nature of the lesions in the spleen and gastrointestinal tract, the process was likely chronic. Chronicity could also explain the lack of large cloud-like bacterial colonies which would be expected of *Yersinia* spp., the "Y" in the "STACY" mnemonic used to denote large colony-forming bacteria (the others being ***S****taphylococcus,* ***S****treptococcus* ***A****ctinomyes*, ***A****ctinobacillus,* ***C****orynebacterium*, ***C****lostridium, and* ***T****rueperella*).

Participants marveled at the liver, which had no right to look as great as it did. Given that *Y. pseudotuberculosis* enters from the gut, the liver receives the brunt of the bacterial onslaught. Dr. Pesavento noted that the liver does an admirable and largely unsung job of clearing bacteria from the portal blood and, considering the destruction noted in the spleen and gut, the relatively normal looking liver is a testament to the its effective immunologic vigilance. Some participants noted the mild periportal lymphoplasmacytic hepatitis, which is a fairly typical finding in rabbits, and thought it unrelated to the bacterial infection. Participants did appreciate a mild lipid vacuolation that seemed concentrated in the centrilobular and midzonal areas, and felt that this was the most believable lesion present in the liver.

Once participants were thoroughly unimpressed with the histologic appearance of the liver, Dr. Pesavento pulled a proverbial rabbit out of the hat, wowing the crowd with a reticulin stain. Reticulin, a connective tissue composed of type III collagen, lines the spaces of Disse. When visualized with a reticulin silver stain, the patency of these fibers can be assessed to determine the integrity of the hepatic cord and sinusoidal architecture. In this case, the seemingly bland appearance of the liver belied the fact that the entire reticulin network was completely destroyed.

Participants felt that the cecal tissue should get pride of place in the morphologic diagnosis given the striking necrosis. Participants could not convincingly identify coccobacilli in the examined cecal section, but were convinced of their presence in the spleen. The remarkable liver, despite its lack of underlying reticulin network, was felt to be largely normal in the examined section, with no areas of necrosis or identifiable bacteria. Participants felt, however, that the zonal distribution of the vacuolar change was a believable, perhaps sentinel lesion, and deserved to be included in the diagnosis.

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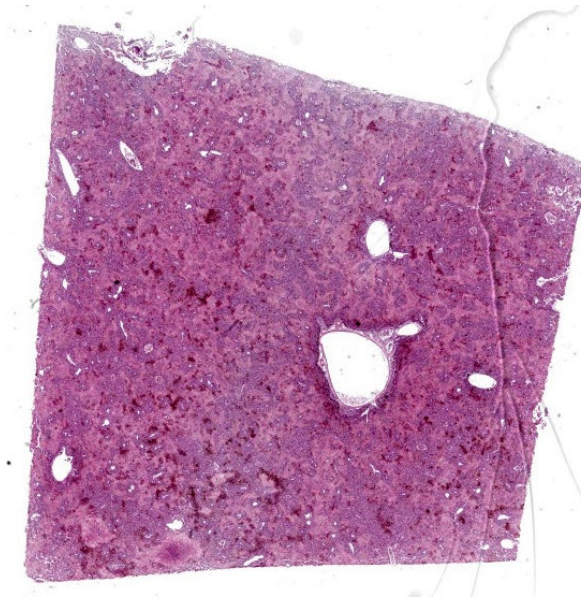

Figure 2-1. Liver, parakeet fledgling. One section of liver is submitted for examination. (HE, 5X)

CASE II:

Signalment:
2-month-old male rose-ringed parakeet (*Psittacula krameri*)

History:
Sudden death without any clinical signs of disease.

Gross Pathology:
Necropsy revealed sparse feathers in the abdomen and back areas, multifocal subcutaneous hemorrhage and hemorrhage in pectoral muscles and muscles of extremities and extensive hepatic necrosis and hemorrhage.

Laboratory Results:
PCR testing for avian polyomavirus was positive; PCR testing for psittacine beak and feather disease, avian bornavirus, herpesvirus, and *Chlamydia* was negative.

Microscopic Description:
Liver: Affecting up to 70% (this percentage somewhat varies between the slides) of the liver parenchyma there is disruption and loss of the hepatic architecture evident by either of the following: loss of differential staining and paleness of hepatocytes with karyolysis (lytic necrosis), eosinophilic cellular and karyorrhectic debris with hypereosinophilic cytoplasm and pyknosis (coagulative necrosis), and moderate numbers of extravasated erythrocytes (hemorrhage). Such changes affect mostly centrilobular and midzonal portions of the lobules and frequently coalesce (centrilobular and midzonal necrosis). Adjacent hepatocytes are frequently swollen with pale vacuolated cytoplasm (hepatocellular degeneration). Rarely such hepatocytes have mildly enlarged nuclei that contain large amphophilic to lightly eosinophilic, irregularly round inculsion bodies that completely efface the nuclear chromatin and peripheralize the nucleoli. Multifocally within the portal areas there is an increase in the number of bile duct profiles (ductular reaction). Scattered throughout the sinusoids are uncommon aggregates of hematopoietic cells with erythroid and fewer myeloid precursors (extramedullary hematopoiesis).

Contributor's Morphologic Diagnosis:
Liver: Hepatocellular necrosis and degeneration, acute, multifocal to coalescing, submassive, severe, with rare large intranuclear amphophilic viral inclusion bodies.

Contributor's Comment:
Avian polyoma virus (APV) is one of the most significant viral pathogens of caged birds. It results in substantial economic losses for aviculturalists and pet store owners each year. APV is included in the genus *Polyomavirus*, family *Papovaviridae*. Its ability to infect and

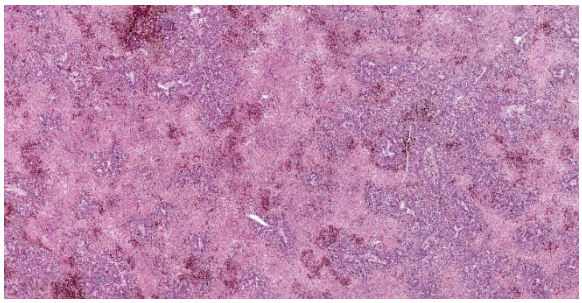

Figure 2-2. Liver, parakeet fledgling. There is a retiform pattern of pallor representing centrilobular and midzonal necrosis. (HE, 41X)

cause disease in birds is dependent on the age of the bird, the species, the immune status of the bird, and other poorly understood factors.[6]

APV was first characterized as a pathogen in young budgerigars in 1981 and was designated budgerigar fledgling disease virus.[1] Now it is called avian polyomavirus because it affects many different species of psittacine birds. To date, four polyomaviruses in birds are known, namely avian polyomavirus, goose hemorrhagic polyomavirus (GHPV), finch polyomavirus (FPyV) and crow polyomavirus (CPyV).

In fledgling and young budgerigars, APV causes acute death, abdominal distention and feather abnormalities known as "French molt." It also causes a loss of down feathers on the back and abdomen, filoplumes on the head and neck, and subcutaneous hemorrhage of nesting budgerigars. In other avian orders, including psittacine species, APV causes clinical signs similar to those observed in budgerigars, but the severity of the disease seems to be dependent on the species infected.[4] Other species of psittacine birds can be very ill at weaning with nonspecific weight loss, anorexia, partial paralysis of the gut, polyuria, and watery droppings. They have a tendency to haemorrhage easily, and may have CNS signs. Not all the birds get the disease and not all of those affected die; some (especially the older birds) recover to become asymptomatic carriers.[2]

Typical pathologic changes in psittacine birds include hydropericardium, enlarged heart, swollen liver, congested kidneys, and hemorrhage within the body cavities. Histopathology typically reveals large and slightly basophilic intranuclear inclusion bodies in various tissues, especially in the spleen, liver, and kidneys.[4]

Recent references about APV are mainly related to monitoring of free-ranging and captive birds for polyomavirus and other avian viruses. Kessler et al., 2020, demonstrated that the prevalence of polyomavirus in free-ranging *Psittacula* populations in Europe is low despite the general susceptibility of these species to this virus.[5] Rahaus and Wolff, 2005, conducted a survey to detect subclinical polyomavirus infections in captive psittacine birds in Germany and they did not detect APV in any samples.[8] However, surveys in Australia and Poland detected significant rates of infection in captive parrot populations with APV.[3,7] All of this emphasizes the need to adhere to strict biosecurity measures and regular testing for common psittacine pathogens especially in rehabilitation centers and breeding units.

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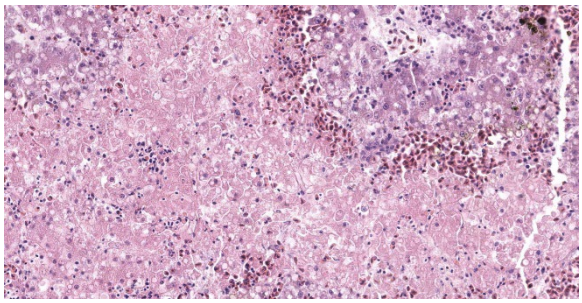

Figure 2-3. Liver, parakeet fledgling. Higher magnification of the relatively sudden transition from areas of coagulative necrosis to viable hepatocytes which are often bordered by a line of hemorrhage. (HE, 317X)

JPC Diagnosis:
Liver: Necrosis, coagulative, multifocal to coalescing, severe, with mild to moderate hepatocellular lipidosis.

JPC Comment:
The contributor provdes an excellent overview of avian polyomavirus, an important avian disease that exhibits a wide variety of case presentations ranging from subclinical disease tosudden death, depending on species susceptibility. Conference participants found this case particularly rewarding as it provide ample fodder for discussion of foundational issues in liver pathology, such as lesion distribution and the association between lesion distribution and possible etiologies.

In this liver, the distribution of the main lesion - striking, large areas of necrosis – was the main point of discussion. Dr. Pesavento began with a subgross evaluation of the distribution, which many participants felt was zonal, while others felt that the distribution, while geographic and incorporating wide swaths of tissue, was essentially random. Dr. Pesavento noted that every lobule of the liver appears to be affected, which argues against a random distribution, but agreed that the distribution of hepatic lesions in birds can generally be difficult to determine, as this case demonstrates.

The majority of participants agreed that the lesion appeared concentrated in the centrilobular and midzonal areas. Due to this zonality and the diffuse necrosis throughout all liver lobules in section, participants felt that insults expected to have a zonal distribution, such as hypoxia and toxin exposure, should be included in the differential diagnosis list. Other differentials suggested by participants included viral causes, such as psittacid herpesvirus 1, and avian polyomavirus.

Dr. Pesavento discussed the reticulin-staining in this liver, contrasting it with the rabbit liver in the previous case. In this bird, the reticulin framework appeared intact, indicating a more acute process. The intact reticulin network also suggests that the observed necrosis is affecting hepatocytes, not endothelial cells. This suggestion is bolstered by the reduced distance between the intact peri-sinusoidal reticulin networks, which is an indicator of hepatocellular loss.

Returning to the H&E slide and the proposed polyomaviral etiology, participants noted the lack of convincing polyomaviral inclusions, which are typically large, intranuclear, basophilic to amphophilic features which can substantially obscure the nucleus. Polyomaviral infections typically generate many of these dramatic inclusions and participants found their absence surprising. Participants were also perplexed by the zonal, non-random lesion distribution, which is unusual for a viral infection. The moderator noted that subclinical polyomaviral infections are relatively common, raising the possibility that this ani-

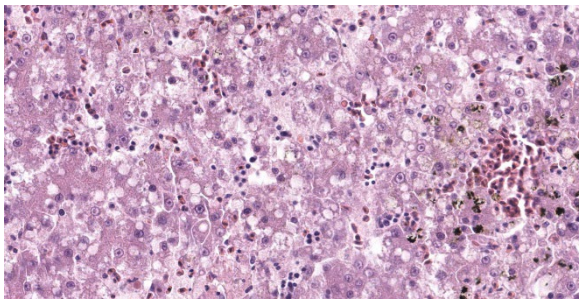

Figure 2-4. Liver, parakeet fledgling. Viable, largely periportal hepatocytes often contain cytoplasmic lipid droplets. There are hemosiderin-laden macrophages in areas of hemorrhage. (HE, 458X)

mal's lesions were not caused by polyomavirus, despite being PCR-positive for the agent.

Conference participants discussed what additional diagnostics could be used to investigate possible etiologic agents. Participants felt that Giemsa or Gram staining would be helpful to rule out septicemia, and that in situ hybridization (ISH) would be helpful to confirm the presence of polyomavirus in the liver and to determine if the virus is co-localized with areas of necrosis. Finally, participants noted that avian polyomavirus inclusions are most reliably found in the spleen, adding an H&E section of splenic tissue to the diagnostic wish list.

Despite being open to alterate etiologies for the observed lesions, conference participants largely agreed with the contributor's morphologic diagnosis. No lesion distribution was included in the JPC diagnosis as participants remained equivocal as to whether the lesions were random or zonal, and despite intense searching, no intranuclear inclusions were observed in the section examined in conference.

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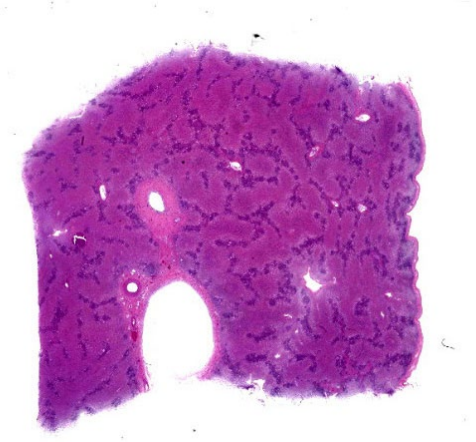

Figure 3-1. Liver, horse. A single section of liver is submitted for examination. A retiform pattern of necrosis is visible at subgross magnification. (HE, 5X)

CASE III:

Signalment:
13-year-old Appaloosa-cross mare (*Equus caballus*)

History:
The horse presented with petechiae and edema of the upper lip and appeared to be rubbing the area. The animal was treated but did not improve. The horse was found down two days later with icterus and died shortly afterwards.

Gross Pathology:
The horse was icteric. The liver was small and collapsed or flattened.

Microscopic Description:
The tissue submitted is liver. There is centrilobular to massive hepatocellular necrosis. Portal triads have bile duct hyperplasia, mild lymphoplasmacytic inflammation, and fibrosis with partial bridging between portal triads.

Contributor's Morphologic Diagnoses:
1. Massive hepatocellular necrosis
2. Mild, chronic lymphoplasmacytic hepatitis

Contributor's Comment:
This is a case of equine serum hepatitis, also known as Theiler's disease. The histologic features of this disease are unique and diagnostic. There is an acute centrilobular to massive hepatocellular necrosis and a chronic portal hepatitis. Grossly, the liver is small and collapsed and sometimes flabby or flaccid.

Equine serum hepatitis was first described by Theiler early in the 20th century. The disease was associated with the use of various biologics containing equine serum, usually vaccines, and occurred 1-3 months following the use of these products.[1,3] However, many cases occurred that had not received equine serum. The cause of the disease is now thought to be viral.[2]

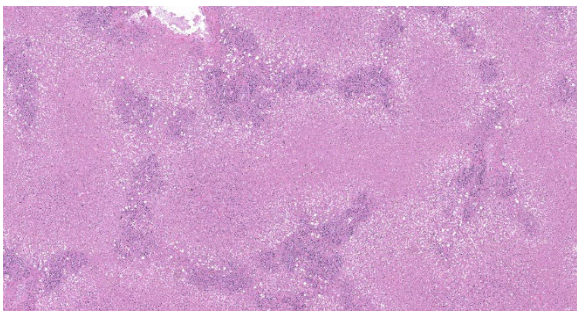

Figure 3-2. Liver, horse. There is diffuse, massive necrosis with the most profound changes in the centrilobular and midzonal areas. (HE, 41X)

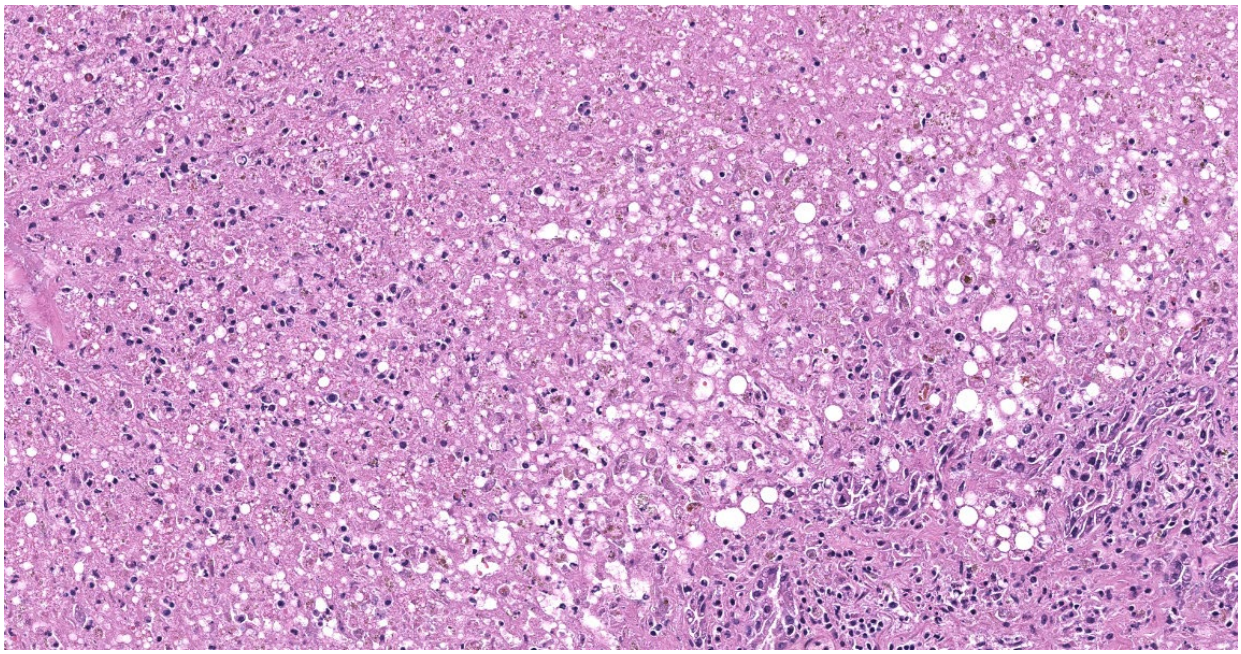

Figure 3-3. Liver, horse. There is total hepatocellular necrosis in the centrilobular and midzonal area. A portal area is present at lower right. (HE, 192X)

Contributing Institution:
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www.vetmed.vt.edu

JPC Diagnosis:
Liver: Necrosis, massive, diffuse, with stromal collapse and hepatocellular lipidosis.

JPC Comment:
Theiler's disease was first described in 1919 by South African veterinarian Sir Arnold Theiler after hundreds of horses acutely died of hepatitis after being vaccinated against African Horse Sickness.[2] Since first described, Theiler's disease has been documented worldwide in horses treated with a variety of biologics, including tetanus antitoxin, botulinum antitoxin, antiserum against *Streptococcus equi*, and equine plasma, though for the past 50 years, disease has been most commonly associated with administration of tetanus antitoxin.[2] A variety of potential causes have been investigated and proposed as the etiologic agent of Theiler's disease, most notably a pegivirus named Theiler's Disease-Associated Virus (TDAV); however, the currently accepted etiologic agent is a parvovirus, named equine parvovirus hepatitis virus (EqPV-H), which was discovered, isolated, and characterized in 2018.[1,2]

A typical case presentation of Theiler's disease involves rapidly progressive symptoms of lethargy, anorexia, and jaundice 2-3 months following the administration of blood products; clinical pathology abnormalities include elevated serum levels of liver enzymes and bilirubin.[1] Some horses may have fever and central nervous system signs such as cortical blindness, ataxia, behavior changes, or coma.[1] Histologically, Theiler's disease is characterized by massive hepatocellular necrosis, as illustrated nicely by this case. Mortality rates between 50% and 90% are reported.[1] Experimental studies of EqPV-H have revealed the virus to be endemic in the United States,

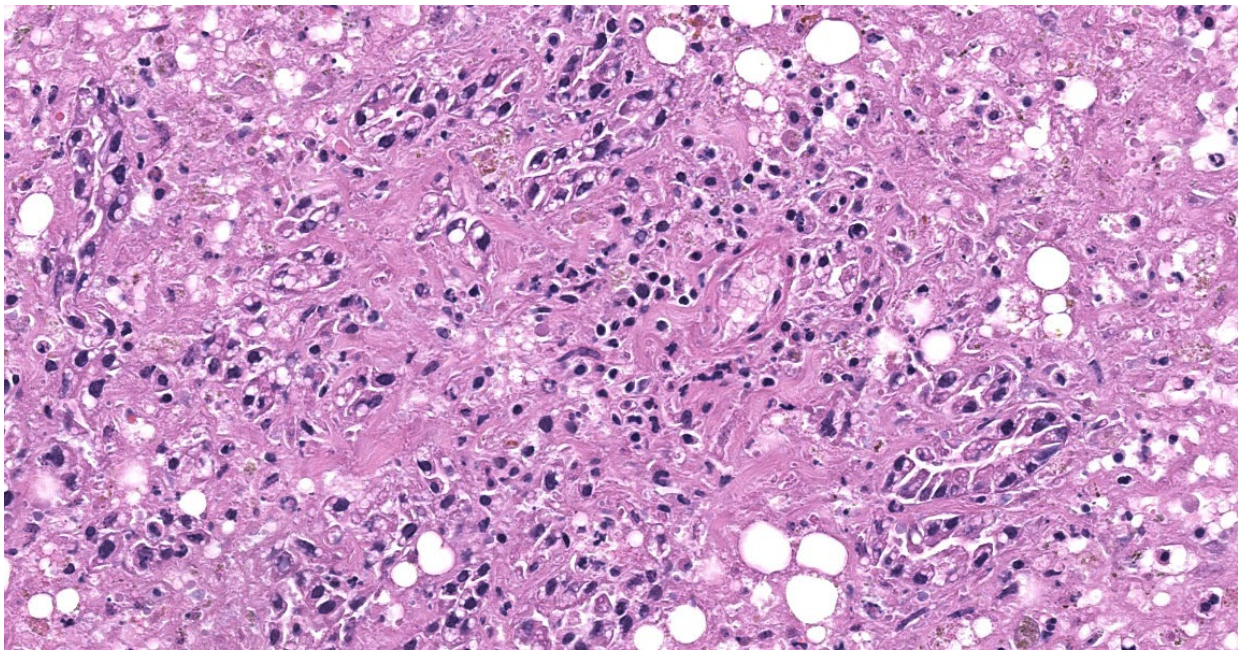

Figure 3-4. Liver, horse. Due to the massive necrosis, even normal structures in portal areas are difficult to discern. Few irregular shunken hepatocytes remain and a bile duct is evident at lower right. (HE, 397X)

parts of Europe, and China, with estimated prevalence of 2.9-17%, with up to 54% prevalence on farms with recent outbreaks of Theiler's disease.[2] The virus has been shown to be transmitted via stem cell therapy for orthopedic injuries and by oral inoculation with viremic serum; vertical transmission appears to be minimal.[2] EqPV-H is shed in oral and nasal secretion and in feces, leading to potential environmental accumulation of this hearty virus.[2] Research into effective biosecurity measures to minimize EqPV-H transmission is ongoing.

Participants felt that slide description in this case was a challenge as virtually nothing was left to describe! On subgross evaluation, however, participants noted the wrinkling of the liver capsule and the irregular distances between central veins and portal triads throughout the section, both indicators of microhepatica due to massive necrosis/stromal collapse. Dr. Pesavento contrasted this presentation with liver shunting, which typically presents with capsule wrinkling but relatively constant central vein to portal triad distances.

Even without the subgross suggestion of microhepatica, however, the massive hepatocellular necrosis evident on histological examination is characteristic of Theiler's disease, which should be included in every differential list for massive hepatic necrosis in a horse. Evaluation of a Masson trichrome illustrated some fibrosis, but participants felt that the amount of fibrosis was likely normal for a 13-year-old horse. Fibrosis is generally not a histologic feature of Theiler's disease as animals typically do not live long enough to develop it.

Participants were surprised by the reticulin stain in this case, which looked relatively normal. Similar to the previous case, the reticulin framework remained intact despite the massive necrosis, once again highlighting the hepatocellular-centric nature of this disease.

Dr. Pesavento discussed the current understanding of Theiler's disease which, in a testament to the rapid advancement of scientific knowledge, has changed and solidified since this case was originally submitted to the Wednesday Slide Conference. At that time, the current theory was that a pegivirus, termed Theiler's disease-associated virus (TDAV) was responsible for the disease; however, researchers were skeptical of this claim from the outset as pasteurization of the serum products implicated in the transmission of Theiler's disease should have incapacitated TDAV. Further research lead to the discovery EqPV-H, a parvovirus that can withstand typical pasteurization temperatures, and subsequent studies have established a strong correlation between EqPV-H and Theiler's disease.

Discussion of the morphologic diagnosis was relatively short as the hepatocellular necrosis was glaring and all-encompassing. Participants noted the periportal lymphoplasmacytic hepatitis described by the contributor, but chose to omit it from the JPC diagnosis to emphasize the necrosis-driven pathogenesis.

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CASE IV:

Signalment:
9-year-old, male castrated domestic shorthair cat (*Felis catus*)

History:
The cat presented for a 12-hour history of lethargy and inappetence. Complete blood chemistry revealed severe normocytic normochromic anemia and severe thrombocytopenia. Serum chemistry showed moderate elevation in ALT and mild azotemia. Abdominal radiographs revealed a moderate peritoneal effusion, a suspected small intestinal linear foreign body-associated mechanical obstruction, and generalized hepatomegaly. AFAST and abdominal fluid sampling and cytology confirmed a hemoabdomen of unknown origin. The patient was taken to surgery for an exploratory laparotomy.

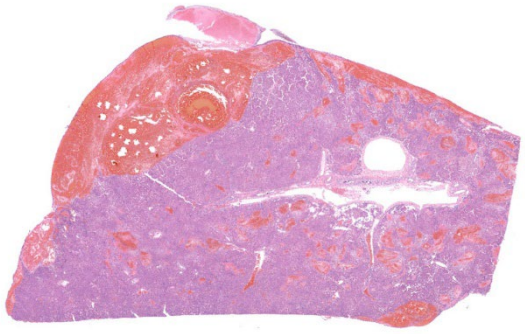

Figure 4-1. Liver, cat. The section of liver has a area of subcapsular acute hemorrhage surrounding a large dilated vessel, as well as smaller areas of dissecting hemorrhage throughout the parenchyma. (HE, 5X)

Gross Pathology:
A hepatic mass involving the left and quadrate lobes was observed during exploratory laparotomy. The remainder of the liver parenchyma was mildly friable. No other gross abnormalities were noted during the surgical procedure. The mass was excised and submitted for histopathology.

Laboratory Results:

	Result	Reference	Units
WBC	12.19	5.5-19.5	$x10^3$/ul
Neutrophil	7.07	2.5-12.5	$x10^3$/ul
Band Neutrophil	0.00	0.0-0.3	$x10^3$/ul
Lymphocyte	5.12	1.5-7.0	$x10^3$/ul
Monocyte	0.00	0.0-0.85	$x10^3$/ul
Eosinophil	0.00	0.0-0.75	$x10^3$/ul
Basophils	0.00	0.0-0.1	
RBC	3.84	4.6-10.2	$x10^6$/ul
Hemoglobin	5.5	8.5-15.3	gm/dl
Hematocrit	16.5	26.0 - 47 .0	%
MCV	43.0	38.0 – 54.0	fl
MCH	14.4	11.8-18.0	pg
MCHC	33.5	29.0-36.0	gm/dl
RDW	19.5	16.0-23.0	%
Nucleated RBC	0.00		
Platelet-Auto	52	100-518	$x10^3$/ul
MPV	11.8	9.9-16.3	fl

	Result	Reference	Units
Albumin	3.8	2.2-4.4	gm/dl
Alk Phos	12	10-90	IU/L
ALT	465	20-100	IU/L
Amylase	1124	300-1100	
T. bilirubin	0.3	0.1-0.6	mg/dl
BUN	46	10-30	mg/dl
Calcium	10.9	8.0-11.8	mg/dl
Phosphorus	3.2	3.0-6.9	mg/dl
Creatinine	2.6	0.8-2.1	mg/dl
Glucose	87	70-150	mg/dl
Sodium	159	142-164	mEq/L
Potassium	4.3	3.7-5.8	mEq/L
T. Protein	7.5	5.4-8.5	gm/dl
PTT	239	80-119	sec

Microscopic Description:
Left lateral liver lobe: Multifocally, portal tracts contain profiles of small arterioles, several of which lack portal venules. Several small arterioles extend into adjacent hepatic parenchyma, and these typically are in a bizarre orientation and have hypertrophied endothelium. Central veins are difficult to identify. The space of Disse in the centrilobular areas contains a substantial amount of eosinophilic, homogeneous, hyaline-like material. Also, there are aggregates of hyaline-like material associated with clusters of cells within the sinusoids. Often, the portal tracts are moderately expanded by infiltration of lymphocytes, plasma cells, and a few eosinophils. There are large foci of disrupted hepatic cords with sinusoids expanded by hemorrhage (telangiectasia), aforementioned hyaline-like material, and fibrin thrombi throughout the parenchyma.

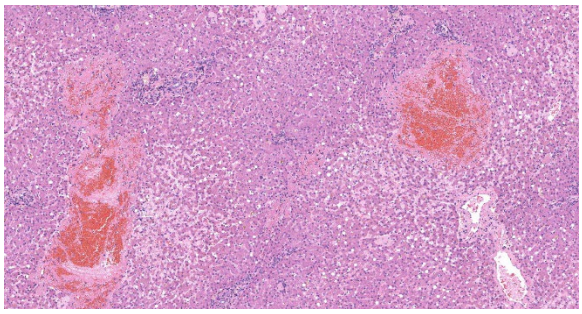

Figure 4-2. Liver, cat. Smaller areas of dissecting hemorrhage are scattered throughout the parenchyma. There is pink matrix (amyloid) within the surrounding sinusoids. (HE, 81X)

Contributor's Morphologic Diagnosis:
Left lateral liver lobe:

1. Hepatic microvascular dysplasia with portal venule hypoplasia
2. Hepatic amyloidosis, with telangiectasia, hemorrhage, and thrombosis

Contributor's Comment:
This case had morphological findings consistent with hepatic microvascular dysplasia and portal venule hypoplasia as well as amyloidosis that was confirmed by Congo Red staining. The coexistence of these two findings in this patient is considered to be unique.

Hepatic microvascular dysplasia (HMD) is an intrahepatic shunting of blood through microscopic vessels within the liver.[3] Clinical signs described in canine patients include inhibited growth, ammonium biurate crystalluria, hepatic encephalopathy, vomiting, and diarrhea.[3,7] HMD may occur as an isolated congenital malformation or may be present with a concurrent macroscopic portosystemic shunt vessel.[7] Some dogs lacking other hepatic abnormalities, such as a macroscopic congenital portosystemic shunt, may be asymptomatic.[7] Rarely, multiple acquired portosystemic shunts may arise secondary to HMD and subsequent portal hypertension.[7,12]

HMD is fairly frequently reported in small-breed dogs, particularly the Cairn Terrier and Yorkshire Terrier.[14] In Cairn Terriers an autosomal recessive pattern of inheritance has been described.[11] Cases of HMD and/or portal vein hypoplasia in the cat are infrequently reported in the literature.[12] Recently, a case of multiple acquired portosystemic shunts secondary to portal vein hypoplasia was reported in a cat.[12,14]

Histologic lesions consistent with HMD include increased arterioles in portal triad regions, ectatic pericentral vascular spaces, and mural hypertrophy of central veins.[4,7] HMD and portal vein hypoplasia are both congenital defects in the development of intrahepatic portal veins and may occur concurrently.[14] Histologically, portal vein hypoplasia is characterized by decreased numbers of portal vein branches within small portal triads.[14] If histology reveals evidence of HMD or portal vein hypoplasia, a macroscopic congenital portosystemic shunt must be ruled out by advanced imaging techniques as this abnormality results in similar histologic lesions.[7,14]

Hepatic amyloidosis is commonly found in association with generalized amyloidosis in the majority of species. Generalized, or systemic, amyloidosis is frequently associated with an overproduction of an amino-terminal fragment of the inducible acute-phase protein serum amyloid A, simply termed "amyloid A."[4] This disease occasionally occurs in a variety of species including horses, cattle, dogs, and cats, and is often a subsequent response to chronic tissue destruction or disease.[1,2,4,6,9]

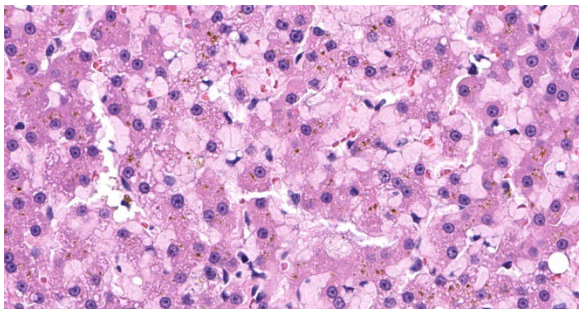

Figure 4-3. Liver, cat. Hepatic sinusoids are expanded by amyloid. There is abundant brown pigment within hepatocytes and Kupffer cells (likely hemosiderin). (HE, 605X)

Familial amyloid A amyloidosis is another potential underlying etiology of systemic amyloidosis. This disease is recognized in Chinese Shar-Pei dogs and Abyssinian and Siamese cats.[8,10] The condition bears a resemblance to familial amyloidosis in humans (familial Mediterranean fever), and affected animals exhibit episodic pyrexia, swollen hocks, renal amyloidosis, and occasional concurrent hepatic amyloidosis. [8,10]

Systemic amyloidosis in horses is most often secondary to chronic inflammation and is observed with increased frequency in horses used for the production of hyperimmunized serum.[1] Affected horses may develop icterus and other signs of hepatic dysfunction.[1] In contrast, cattle, dogs, and cats generally first exhibit clinical signs of renal dysfunction from concurrent renal amyloid deposition.[6] It is reported that cattle may rather die of the underlying primary disease.[6] Though infrequent, cats have been reported to present with spontaneous hepatic rupture, as in this case. [2,6]

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JPC Diagnoses:
1. Liver: Amyloidosis, intrasinusoidal, multifocal, marked, with parenchymal rupture and acute hemorrhage.
2. Liver: Primary portal vein hypoplasia with numerous portal arteriolar profiles.

JPC Comment:
This beautiful slide, striking at subgross, reveals even more delights upon closer examination. As the contributor nicely describes, the liver is characterized by three major pathologic processes: large accumulations of blood throughout the hepatic parenchyma, multifocal accumulations of eosinophilic hyaline material, and portal areas comprised of increased numbers of arterioles, bile ductules, and portal veins.

On subgross evaluation, the large lakes of hemorrhage are most eye-catching, and conference discussion focused initially on the character of these lesions. Participants wondered if these could represent peliosis hepatis, defined as randomly distributed, cystic blood-filled spaces in the liver.[5] This is well-reported in cats and can result either from obstruction of the portal vasculature with subsequent hepatic atrophy and dilation of the sinuisoids (referred to as telangiectasis) or from hepatocyte necrosis.[5] Telangiectasis is distinguished from hemorrhage by the presence of an endothelial lining, and participants discussed which process was predominant in this lesion, decided that the lakes of blood represented hemorrhage as they were not lined by discernable endothelium.

Discussion then turned to the eosinophilic deposits, interpreted by the contributor and conference participants as amyloid.

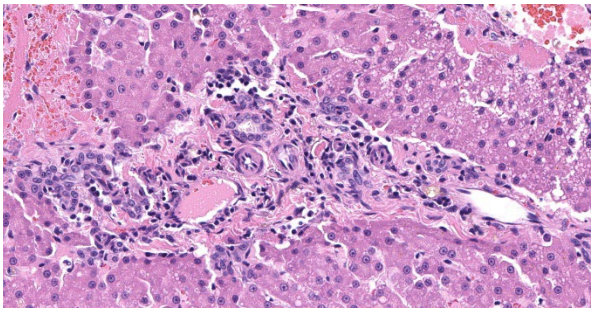

Figure 4-4. Liver, cat. There are numerous cross-section of tortuous arterioles within portal areas, including two venous profiles in this portal area. (HE, 395X)

A Congo Red performed at JPC in preparation for the conference was negative, prompting considerations of other differentials for the the eosinophilic material; however, in cats, amyloid is often best illustrated using thioflavin T staining and a post-conference thioflavin T staining performed at the University of California, Davis, was weakly positive, confirming the material as amyloid.

Discussion of the portal abnormalities quickly turned semantic, with participants discussing the terms "microvascular dysplasia" versus "portal vein hypoplasia." This distinction is challenging and likely artificial due to the stereotypical histologic response of the liver to inadequate portal vein flow.[5] With decreased portal blood flow, the portal vein and its tributaries become reduced and hepatic arteries respond to this hypoperfusion by increasing their blood flow, in the processes becoming more tortuous, proliferative, and hypertrophic.[5] Histologically, as seen in this case, this is evidenced by an increased number of arteriolar profiles in the portal tracts, sometimes with an accompanying increase in the number of biliary ductular profiles along with slight fibrosis.[5] The increased arterial hepatic flow may result in increased sinusoidal pressure and subsequent sinusoidal dilation.[5]

Primary portal vein hypoplasia is a congenital disorder of dogs and, rarely, of cats, which is characterized by the histologic findings typical of portal vein hypoperfusion discussed above.[5] The World Small Animal Veterinary Association's standardization board for canine and feline liver circulatory disorders has stated that there have been "no clinical or biochemical findings to suggest that [microvascular dysplasia] is different from primary portal vein hypoplasia" and that the authors "have decided to abandon the name microvascular dysplasia" and prefer to call this condition "primary portal vein hypoplasia," which the authors find more descriptive.[5]

Primary portal vein hypoplasia varies widely in clinical severity and morphology and overlaps histologically with other causes of hypoperfusion, including congenital portosystemic shunts, intrahepatic arterioportal fistulas, and portal vein obstruction.[5] In 30% of dogs, the vascular changes are mild with no evidence of portal fibrosis; others have moderate to marked fibrosis in the portal tracts, hypoplasia or absence of the portal veins, and a proliferation of arterioles and bile ductules, as seen in this case.[5] Importantly, this diagnosis is based on histologic examination of a liver biopsy in combination with ultrasonographic findings which exclude the presence of congenital portosystemic shunt, arteriovenous fistula, or portal vein thrombosis.[5] In this case, the portal areas contain all the classic hallmarks of hypoperfusion; however, the case history contains no record of ultrasonographic findings to exclude the other causes of hypoperfusion.

Examination of a reticulin stain illustrated complete destruction of the reticulin framwork in this case. Dr. Pesavento noted that no

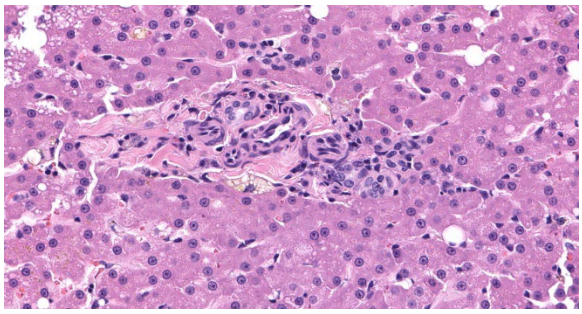

Figure 4-5. Liver, cat. Other portal areas contain numerous arteriolar profiles but are lacking venous profiles. (HE, 395X)

research currently exists on whether this is a feature of microvascular dysplasia or amyloid deposition, but the reticulin destruction in this case was striking, with small remnant fragments of reticulin extending only a short distance from central veins. The lack of a reticulin framework and the hemorrhagic presentation in this case is reminiscent of fatty liver hemorhagic syndrome in poultry, in which vacuolar swelling of hepatocytes disrupts the reticulin structure of the hepatic cords, leading to hemorrhage from the sinusoids.[13]

Given that the principal cause of death was hemorrhage, presumably due to hepatic fracture secondary to amyloidosis, participants favored placing amyloidosis front and center in the morphologic diagnosis. Despite the persistence of the term "microvascular dysplasia" in the literature, participants preferred to use the WSAVA-recommended term "primary portal vein hypoplasia" to capture the full complement of architectural derangements present in the examined portal areas.

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