



WEDNESDAY SLIDE CONFERENCE 2016-2017

Conference 2

31 August 2016

CASE I: Case #1(JPC 4085101).

Signalment: Adult female zika hybrid rabbit (*Oryctolagus cuniculus*).

History: This rabbit was part of an experimental vaccine development study. It was vaccinated twice against rabbit hemorrhagic disease virus followed by a challenge infection with rabbit hemorrhagic disease virus type 2. There were no obvious clinical findings in this rabbit following the challenge infection, and it was euthanized two weeks after the challenge infection according to the experimental plan.

Gross Pathology: The caudate lobe is mildly and diffusely shrunken with slightly rounded angles, a diffuse tan surface, and a sharp hilar demarcation line separating it from the inconspicuous remaining liver lobes. A cross section shows a markedly thickened, tan capsule; a chocolate-brown parenchyma with a pronounced tan periportal lobular pattern; and a firm texture.

Laboratory results: None

Histopathologic Description: Liver (two sections): There is diffuse disruption and



Liver rabbit. Torsion of the lobus caudatus (flipped to the left side to expose the hilar demarcation line and the undivided right hepatic lobe). (Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. <https://www.fli.de>)

collapse of the hepatic architecture with loss (atrophy) and replacement of > 95% of centrilobular and midzonal to panlobular hepatocytes by a moderately cellular, loose, reticular meshwork of pleomorphic spindloid cells (fibroblasts) and capillary sprouts. Centrilobularly-oriented parts of

the remaining periportal hepatocytes display cellular swelling due to cytoplasmic feathery vacuolation (hydropic degeneration). There is a moderate amount of binucleated hepatocytes (regeneration).

There is mild, multifocal, centrilobular sinusoidal dilatation by erythrocytes (congestive hyperemia), as well as mild multifocal accumulations of extravascular erythrocytes (hemorrhages) and a moderate, diffuse infiltration by macrophages, mostly stuffed with multiple, cytoplasmic, brown granules (hemosiderosis), and lesser and variable concentrations and combinations of lymphocytes, plasma cells, and heterophils.

There is a moderately increased amount of periportal bridging bundles of collagen fiber-rich tissue (fibrosis), a severely increased number of portal bile ducts (biliary hyperplasia), moderately dilated lymphatic vessels, and severe thickening of the hepatic capsule (fibrosis).

There is a centrally located, 500 µm in



Liver, rabbit. Cross section of the torsioned lobus caudatus showing a pronounced lobular pattern and thickened capsule due to fibrosis..(Photo courtesy of: Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Department of Experimental Animal Facilities and Biorisk Management, Südufer 10, 17493 Greifswald – Insel Riems, Germany. <https://www.fli.de>)

diameter, relatively thin-walled blood vessel (vein) which is completely occluded by a variable amount of a densely packed, hyper-eosinophilic, fibrillary to beaded mass (fibrin-rich thrombus), which is partially and/or completely (depending on the slide) replaced by fibroblasts and numerous small caliber blood vessels with hypertrophic endothelium (granulation tissue), mildly infiltrated by hemosiderin laden macrophages (hemosiderosis).

The other section of liver is unremarkable.

Contributor's Morphologic Diagnosis:

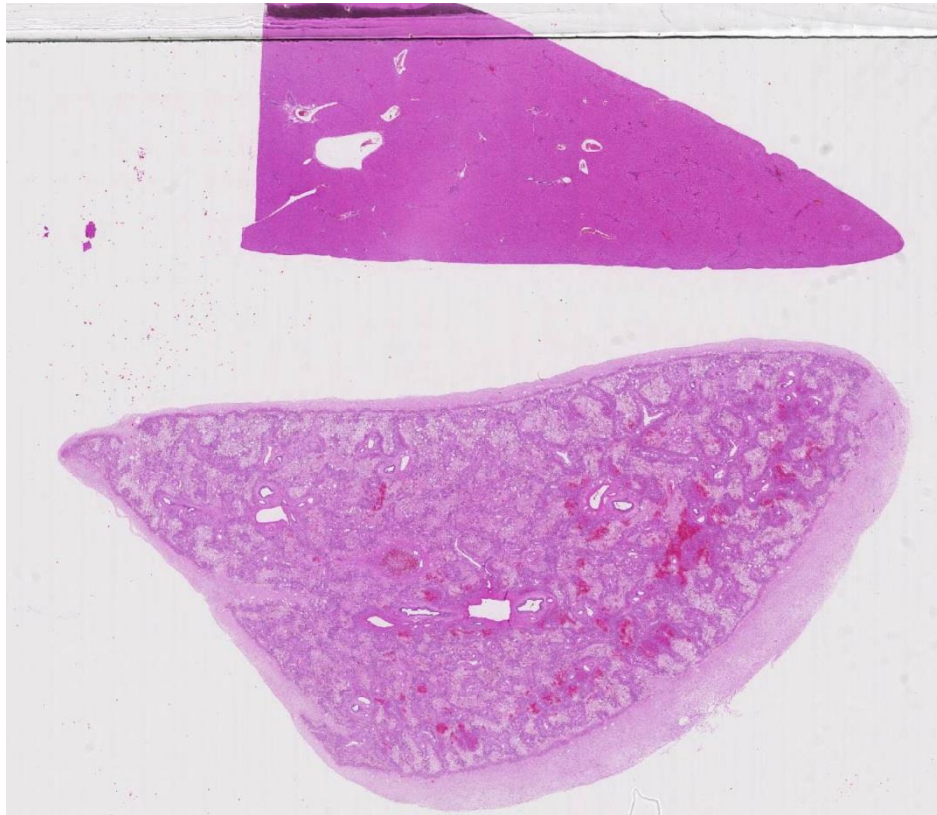
Liver. Hepatocellular degeneration and loss (atrophy), diffuse, chronic, severe with reticular replacement, periportal bridging and capsular fibrosis, bile duct hyperplasia, venous thrombosis, congestive hyperemia, hemorrhage, and hemosiderosis.

Contributor's Comment: Etiology:

The slide shows two sections from two strikingly different liver lobes from the same animal. The morphological changes in the section originating from the caudate lobe are consistent with subclinical, chronic, liver lobe torsion, whereas the architecture in the other section is unchanged. The etiology of liver lobe torsion remains enigmatic; however, a species-specific anatomical configuration with deep hepatic sulci, surgical or external trauma, congenital absence of hepatic ligaments, mass lesions within the affected lobe, and dilatation of abdominal organs are known to represent predisposing factors.^{2,7} According to a recent review, the caudate lobe is affected in 62% of cases in rabbits, whereas the left lateral lobe is most prone to torsion in most other species.⁴ The anatomic nomenclature of the liver of rabbits is controversial;¹ it generally consists of a left liver lobe, a quadrate lobe, a right liver lobe surrounding the gall bladder, a caudate lobe, and a processus papillosus. Some authors

subdivide the left and right lobe into medial and lateral parts;¹ however, there is no distinct subdivision in the present case.

Epidemiology: Liver lobe torsion occurs rarely in many species including rabbits, dogs, cats, pigs, horses, otters, rats, mice and human beings.⁴



Liver, rabbit. Subgross magnification of a normal section of liver (top) and a section from the torsed lobe (bottom). The capsule is markedly thickened with a retiform pallor representing hepatocellular loss. There is multifocal hemorrhage throughout the section. (HE, 4X)

Clinical course: Clinical sequelae of liver lobe torsion range from subclinical, to an acute crisis with lethargy, abdominal pain, jaundice, anorexia, vomiting, collapse and sudden death.^{7,8} Immediate partial hepatectomy is suggested to be the treatment of choice in symptomatic rabbits.⁶

Pathogenesis: Liver lobe torsion results in constriction of venous return leading to venous and/or arterial thrombosis, marked venous congestion, infarction, and ischemic

necrosis of the affected lobe.² Affected rabbits may develop shock, abdominal hemorrhage, and/or septic peritonitis leading to acute cardiovascular failure and death. Liver lobe torsion may be diagnosed incidentally in surviving animals. These animals will have lobular hepatocellular atrophy, fibrosis, and chronic inflammation.⁸

JPC Diagnosis:

Liver: Hepatocellular loss, centrilobular and midzonal, diffuse, severe, with marked portal bridging fibrosis, biliary hyperplasia, edema, and capsular fibrosis.

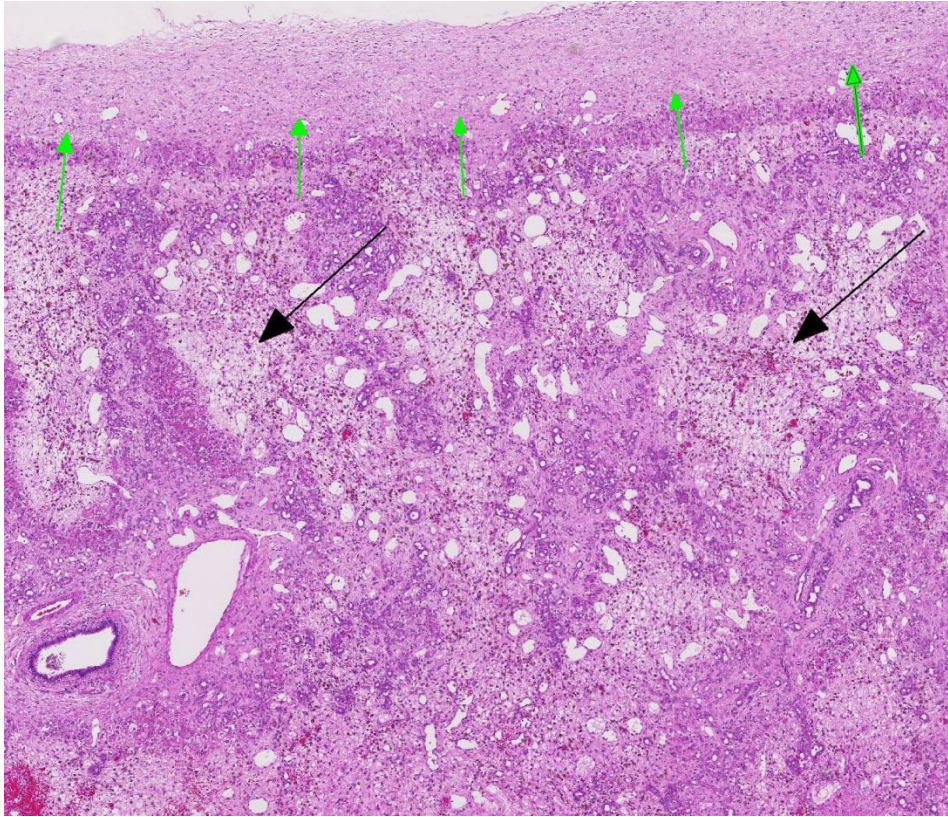
Conference

Comment:

The contributor provides a concise summary of hepatic torsion in the rabbit. This challenging case serves as a characteristic example of the liver's response to chronic severe hepatocellular injury secondary to congestion and ischemia caused by venous occlusion. In general, the liver

responds to injury in three primary manifestations: regeneration, ductular reaction, and fibrosis.^{2,5}

When hepatic injury is limited and the reticulin meshwork is intact, regeneration of mature hepatocytes is stimulated by hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor- α (TGF- α), and insulin-like growth factor (IGF).² Interleukin-6 (IL-6) secreted



Liver, rabbit. Higher magnification of previous image which the capsule is markedly expanded by fibrous connective tissue (green arrows). The pale areas represent loss of centrilobular and midzonal hepatocellular loss. Marked biliary reduplication is evident at this magnification. There is diffuse dilation of both veins and lymphatics in this section. (HE, 60X)

by TGF- α activated Kupffer cells also stimulates hepatocellular regeneration.² Once hepatic mass has been replaced, transforming growth factor- β (TGF- β) inhibits ongoing proliferation of hepatocytes. In this case, there are occasional periportal multinucleated and vacuolated hepatocytes, suggesting attempted regeneration and degeneration.²

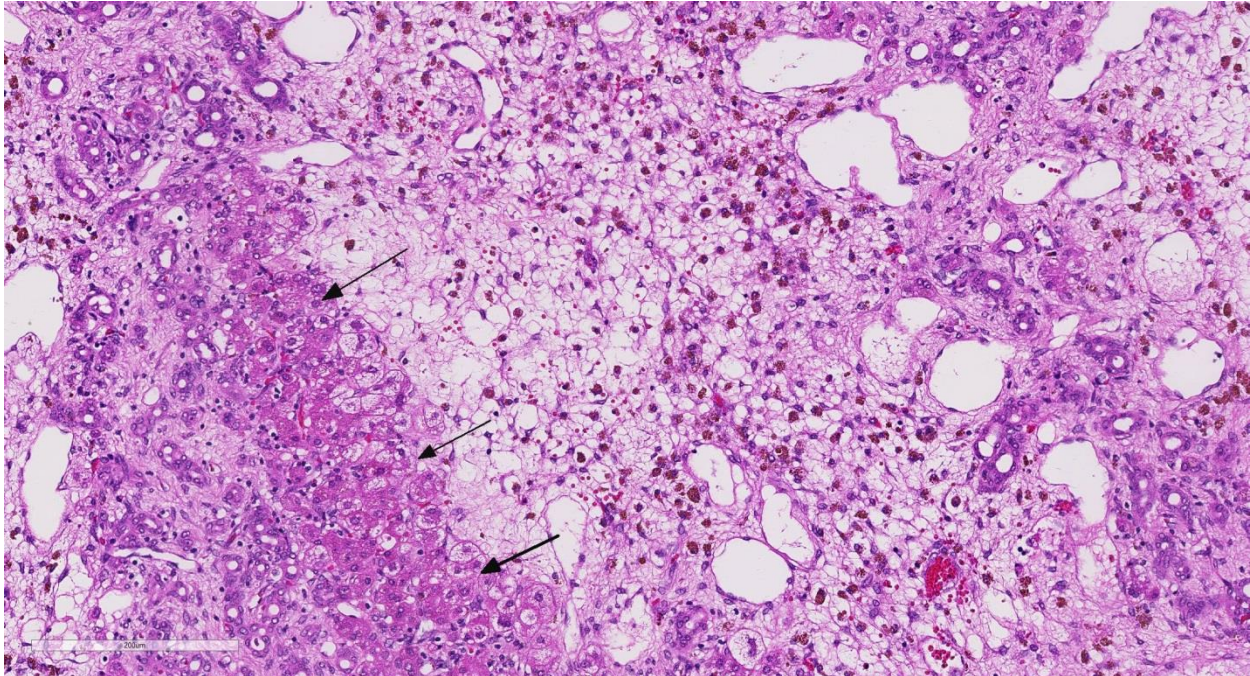
Ductular reaction is a similarly complex process. In severe liver injury with extensive loss of mature hepatocytes, there is stimulation of bipotential progenitor cells (oval cells in rodents), which normally reside in the periportal canals of Hering.^{2,3,5} The canals conduct bile from bile canaliculi to bile ducts and are partially lined by hepatocytes and biliary epithelium.^{2,5} Differentiation of bipotential progenitor cells, as

well as dedifferentiation and biliary metaplasia of mature hepatocytes, in response to severe liver damage is the basis for biliary ductular reaction patterns.^{2,5}

Conference participants briefly discussed the types of ductular reactions and a relatively new classification pattern proposed by Desmet.³ Ductular reactions are classified into type 1, in which mature bile ducts proliferate secondary to cholestasis; type 2 which is hepatocyte ductular metaplasia induced by in-

flammation, intoxication, or hypoxia; and type 3, which is the activation and proliferation of liver bipotential progenitor cells in response to massive hepatic parenchymal loss.^{2,3} In this case, the ductular reaction likely started as type 2 and likely progressed to type 3 in response to chronic venous occlusion, hypoxia, fibrosis, and diffuse severe hepatocellular loss secondary to hepatic torsion.^{2,3,5}

Finally, hepatic fibrosis also begins at the molecular level. Hypoxic hepatocytes induce hypoxia-inducible factor 1- α (HIF-1 α) and platelet derived growth factor (PDGF) to activate hepatic stellate cells within the space of Disse.² These cells then transform into myofibroblasts resulting in progressive diffuse hepatic fibrosis.² This stimulates centrilobular and periportal



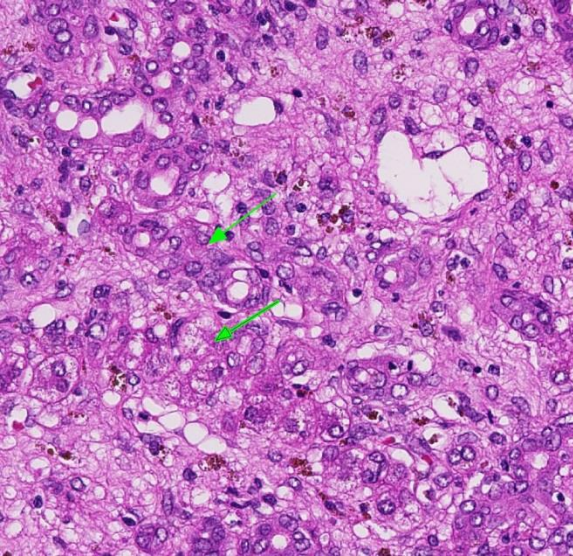
Liver, rabbit. There is diffuse loss of centrilobular and midzonal hepatocytes, with occasional sparing of periportal hepatocytes (arrows). The centrilobular and midzonal parts of the lobule are expanded by edema and contain large numbers of hemosiderin-laden macrophages. At left are large numbers of proliferating bile ductules and dilated lymphatics. Remaining hepatocytes are often swollen with abundant fat and glycogen. (HE, 260X)

hepatocytes to dedifferentiate and eventually assume a biliary ductular phenotype.³ Ongoing hypoxia and fibrosis then results in massive parenchymal loss. Remaining bipotential progenitor cells in the canals of Hering then differentiate toward the biliary duct phenotype rather than differentiating to mature hepatocytes.³ Persistence of ductular reaction further contributes to portal and periportal fibrosis through secretion of previously mentioned profibrogenic growth factors.²

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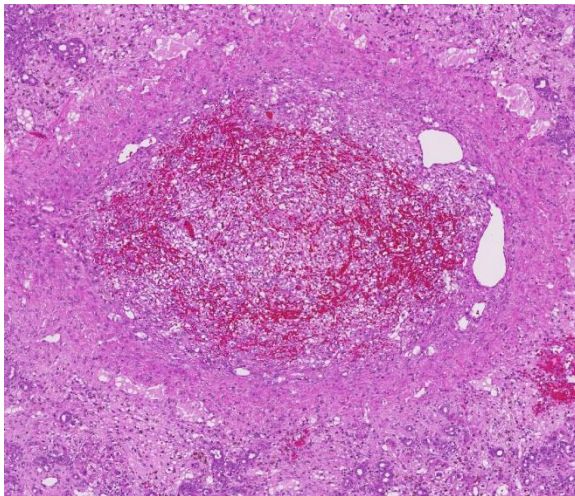
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Liver, rabbit. Hepatocytes are regenerating within areas of proliferating bile ducts (Desmet Type 2 reaction). (HE, 400X)

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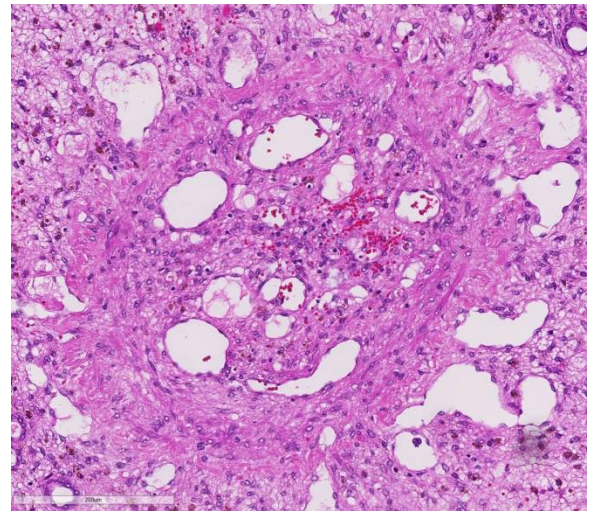


Liver, rabbit. A large branch of the portal vein is occluded by a large fibrinocellular thrombus. (HE, 40X)

reversible hepatocytic injury and hepatic amyloidosis; and
2. Hepatocellular death, hepatitis and

cirrhosis. In: *WSAVA Standards for clinical and histological diagnosis of canine and feline liver disease.* St. Louis, MO: Elsevier; 2006:78-79, 85-88.

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Liver, rabbit. The dilated lymphatics surrounding a sublobular vein impart the appearance of a "rose window" and attest to the severe edema in this section. (HE, 320X)

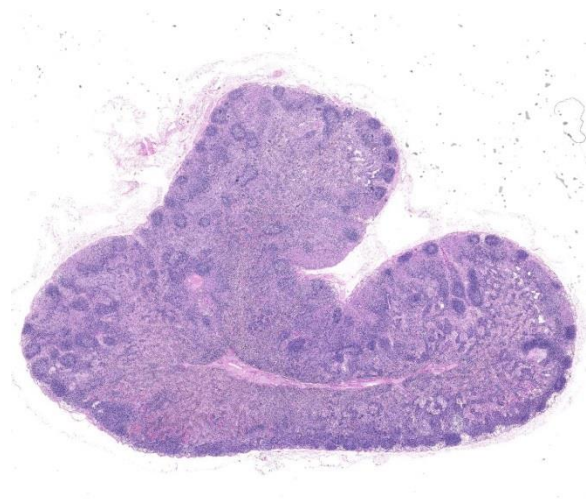
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CASE II: D16-01 (JPC 4083953).

Signalment: 1-year-old castrated male domestic shorthair (*Felis catus*).

History: The cat initially was presented to the referring veterinarian for anorexia and weight loss; upon examination, the cat had a fever, sneezing, and ocular and nasal discharge. The referring veterinarian prescribed prednisolone and doxycycline. Two weeks later, the cat was presented to the Kansas State University Veterinary Health Center. The cat was thin, and had fever, increased respiratory effort, peripheral lymphadenopathy, and ocular discharge. FIV-/FeLV and FCoV testing was negative. The cat was treated with blood transfusions, antibiotics, steroids, and oxygen supplementation, but worsened over the next two days. The cat was subsequently euthanized and sent to necropsy.



Lymph node, cat. Sinuses throughout the node are expanded by a prominent cellular infiltrate which separates and occasionally effaces lymphoid follicles (HE, 5X)

Gross Pathology: The cat was emaciated with diffuse pallor of the mucous membranes. The frontal sinuses were filled with thick yellow mucus. The submandibular, tracheobronchial, and mesenteric lymph nodes were diffusely enlarged, and the tracheobronchial lymph nodes were mottled dark-red and white. The lungs were diffusely orange-red and meaty

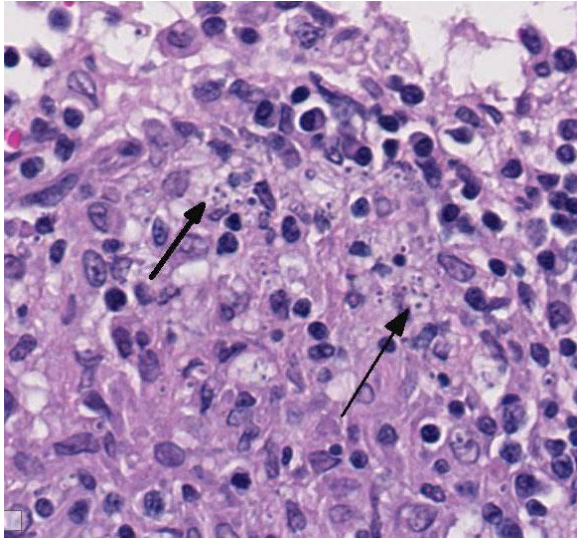
with white miliary nodules. All lung sections sank in formalin.

Laboratory results: CBC revealed minimally regenerative anemia, leukopenia consisting of neutropenia with toxic changes and increased bands, and thrombocytopenia. Serum chemistry revealed hyperglycemia, hypoalbuminemia, decreased anion gap, decreased ALT and ALP, and increased bilirubin.

A bone marrow aspirate showed high nucleated cellularity with rare precursor cells and abundant macrophages containing numerous round to oval yeast bodies (~2-4 microns in diameter) that have a thin outer halo with an eccentrically placed, crescent shaped, basophilic nucleus. The findings were consistent with histoplasmosis.

Histopathologic Description: Lymph node: The subcapsular and medullary sinuses of the lymph node are diffusely expanded by proteinaceous fluid and large numbers of macrophages. In the medullary sinuses, macrophages contain abundant hemosiderin, and there is frequent erythrophagocytosis. Occasional multinucleated giant cells are present, bearing up to 5 nuclei. Macrophages frequently contain intracytoplasmic 2-4 um diameter yeasts consisting of a 1-2 um diameter basophilic nucleus and a 1-2 um clear halo (capsule). The cortex of the lymph node contains numerous active germinal centers.

Large numbers of macrophages with intracytoplasmic yeasts are also present in the lungs (alveolar interstitium and lumina), spleen (red pulp), liver (portal tracts and sinusoids), kidney (perivascular interstitium), intestine (lamina propria and Peyer's patches), and bone marrow (diffuse), as well as circulating macrophages in the brain and heart vasculature.



Lymph node, cat. The paracortex is expanded by large numbers of epithelioid macrophages which contain numerous 2-4µm intracytoplasmic yeasts (arrows) (HE, 360X)

A GMS stain revealed abundant intracytoplasmic 2-4 µm diameter yeasts.

Contributor’s Morphologic Diagnosis

Lymph node; Granulomatous lymphadenitis, diffuse, severe, with numerous intra-histiocytic yeasts consistent with *Histoplasma capsulatum* var *capsulatum*

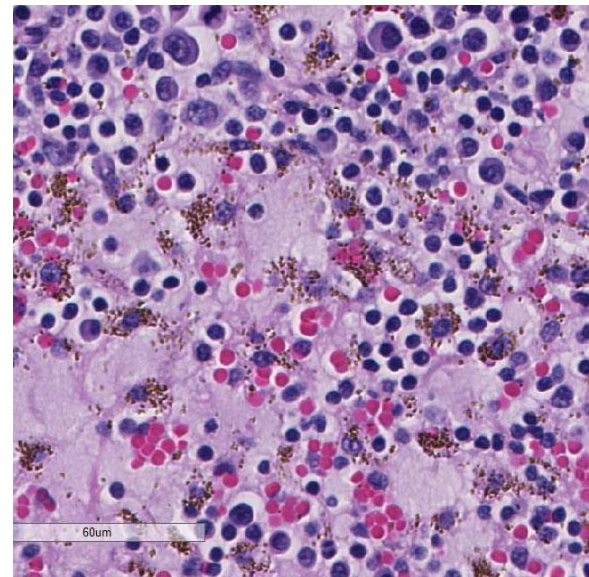
Contributor’s Comment:

Histoplasma capsulatum is a dimorphic fungus that exists in a parasitic yeast form called “*Histoplasma capsulatum*”, and an environmental mycelial form called “*Ajellomyces capsulata*”.⁷ The organism is endemic in the St. Lawrence, Ohio, and Mississippi River valleys.⁶ It is soil-borne and prefers nitrogen-rich organic matter such as bird and bat excrement.³ The disease is non-contagious and affects humans as well as a wide variety of animals.⁷ Infection is usually subclinical without signs or lesions, and can result in a latent state.³; however, clinically evident disseminated infection can occur with immunosuppression and/or heavy infectious dose, and usually results in death.³ In this case, the cat was treated with steroids, which

most likely predisposed it to the severe disseminated form of the disease.

Infection begins with ingestion or inhalation of soil contaminated with *Histoplasma* microconidia.⁴ Pulmonary or gut-associated macrophages phagocytose the microconidia, which germinate into the yeast form and replicate within phagosomes.⁷ In immunocompetent animals, Th1 cell-mediated immune responses eliminate the infection; in immunosuppressed states, the yeast-laden macrophages disseminate widely via the lymphatics and blood vessels to lymph nodes, liver, bone marrow, spleen, adrenal glands, etc.⁷

Histoplasma capsulatum evades the host’s extracellular immune defenses by residing intracellularly within macrophages, and by concealing immunostimulatory beta-glucan cell wall components with non-immunostimulatory alpha-glucans.^{5,7} The yeast is internalized via macrophage complement receptors such as CR3 and CR4 while avoiding activation of pro-inflammatory



Lymph node, cat. Medullary sinuses are expanded by edema, hemorrhage, and erythrophagocytic and hemosiderin-laden macrophages. Medullary cords contain large numbers of plasma cells, indicating reactive hyperplasia. (HE, 400X)

receptors such as TLR2 and TLR4.⁵ Once inside the phagosome, the fungus produces antioxidant enzymes including superoxide dismutase Sod3 and catalases CatB and CatP.^{5,7} It also prevents acidification of the phagosome and fusion of lysosomes by as yet unknown mechanisms.⁵ Other described virulence factors are involved with nutrient acquisition and production, including iron-scavenging hydroxamate siderophores, calcium-binding protein Cbp1, and various nucleic acid- and vitamin-synthesizing enzymes.^{5,7}

Clinical signs include fever, malaise, emaciation, diarrhea, nonregenerative anemia, and respiratory distress.²⁻⁴ Gross lesions include enlargement or thickening of affected organs – including lymphadenopathy, splenomegaly, hepatomegaly, thickened bowel walls, and interstitial pneumonia.²⁻⁴ Histologically, organ involvement is characterized by granulomatous infiltration with large numbers of mononuclear phagocytes bearing characteristic intracellular yeasts, as well as variable numbers of lymphocytes, plasma cells, and multinucleated giant cells.^{3,4} Histochemical stains such as silver stains, periodic acid-Schiff, or Giemsa stains can highlight yeast organisms. For antemortem diagnosis, cytology from rectal scrapings or fine needle aspirates from lymph nodes and internal organs can also demonstrate intrahistiocytic yeasts.^{3,4,6} Fungal culture is hazardous, however, due to infectivity of microconidia produced in the mycelial phase. Ketoconazole, itraconazole, and fluconazole alone or in combination with amphotericin B has been used to successfully treat dogs and cats.²⁻⁴ Itraconazole is the recommended treatment of choice.^{3,4}

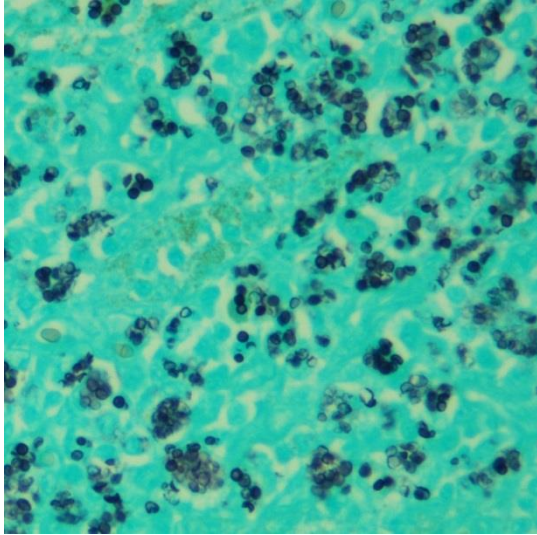
JPC Diagnoses: 1. Lymph node: Lymphadenitis, granulomatous, multifocal

to coalescing, marked, with mild reactive lymphoid hyperplasia and sinus edema.

2. Lymph node: Draining hemorrhage with hemosiderosis.

Conference Comment: The contributor provides an excellent summary of the virulence factors produced by *Histoplasma capsulatum* to evade the host immune response and produce severe disease. Post-exposure, the infected host requires both innate and adaptive immunity to neutralize the pathogen and overcome the virulence factors mentioned above.⁵ Macrophages rapidly phagocytose inhaled conidia and subsequently stimulate pro-inflammatory cytokines such as IFN- γ , TNF- α , and IL-12 associated with a Th1 cell mediated response.^{5,7} Macrophages also produce superoxide radicals, nitric oxide and other lysosomal enzymes to destroy the phagocytosed pathogen.¹ However, as mentioned by the contributor, *Histoplasma capsulatum* evades destruction within the phagolysosome and can survive, replicate, and disseminate to multiple tissues within macrophages.⁵ In this case, conference participants noted several instances of characteristic narrow-based budding of the organism within macrophages.

Conference participants also discussed dendritic cells as an important component of the innate immune response to this organism. Dendritic cells typically phagocytose and destroy the organism with higher efficacy than macrophages. In addition, they present antigens to naïve CD4+ and CD8+ lymphocytes via MHC-1 to generate a T cell mediated immune response and destroy infected cells.¹ Cell-mediated immunity (CMI) is crucial for the host to clear the organism and T cells, as the central effectors CMI, are necessary to degrade the pathogen.^{1,5,7} Severe disease occurs in immunosuppressed animals as



Liver, cat. A silver stain demonstrates the large numbers of intracytoplasmic yeasts within the cytoplasm of numerous macrophages (GMS, 400X) (Photo courtesy of:

well as hosts with a bias toward a Th2 humoral response. Humoral immune responses generally have a limited role in the clearance of intracellular pathogens.^{5,7}

The nature of the inflammation within the lymph node generated spirited discussion during the conference, as it has so many times before over the many years of the Wednesday Slide Conference. Some conference participants preferred histiocytic, rather than granulomatous lymphadenitis. Those favoring the term “histiocytic” noted the paucity of multinucleated giant cells and lack of activated fibroblasts surrounding aggregates of infected macrophages, histologic features often associated with the more typical granulomatous response. Due to variation in sections, others noted more numerous multinucleated giant cell macrophages surrounding accumulated epithelioid macrophages and preferred granulomatous lymphadenitis. Conference participants also posited that the subcapsular location of many of the aggregating macrophages may be secondary to antigen presentation within pre-existent lymphoid follicles. This would stimulate follicular lymphoid hyperplasia

and medullary plasmacytosis with Russell body-laden Mott cells, as seen in this case.

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CASE III: G11362-A661 (JPC 4085318).

Signalment: 2-month-old female Belgian warmblood foal (*Equus ferus caballus*).

History: The foal had a sudden onset of diarrhea and fever (40°C). Blood examination revealed elevated liver values and abdominal ultrasound showed edema of the colon wall. Despite treatment the foal died quickly.

Gross Pathology: The foal was admitted for necropsy and postmortem examination revealed a good nutritional condition, and moderate dehydration. Petechial bleedings were noticed on the pleura, the pericardium, the thymus, the splenic capsule and on the serosa of the intestine. The kidneys showed numerous cortical white to gray foci and congestion of the medulla. The intestines were dilated with an edematous wall and a mucoid gray content.



Kidney, foal: Grossly, the kidney surface is studded by the projections of embolic microabscesses. (Photo courtesy of:

Laboratory results: Bacteriology of the kidney: positive for *Actinobacillus equuli* subsp. *Haemolyticus*

Parasitology of the feces: positive for strongyles.

Histopathologic Description: Kidney: Randomly scattered within the cortex and occasionally extending into the medulla, there are numerous embolic microabscesses (0.3-0.40 mm in diameter) that regularly center on and efface glomeruli. These abscesses are composed of abundant necrotic debris (karyorrhexis, karyolysis, and pyknotic nuclei), admixed with many degenerate and non-degenerate neutrophils, fewer macrophages, lymphocytes and plasma cells. Multifocally within these mic-



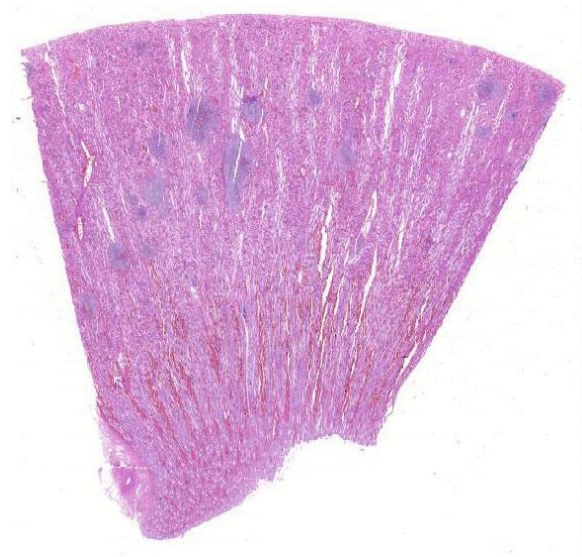
Kidney, foal: On cut section, microabscesses are present throughout the cortex, largely outlining glomeruli. (Photo courtesy of:

roabscesses, there are large colonies of basophilic coccobacilli (1x2 µm). Abscesses occasionally extend into adjacent interstitium and tubules, with degeneration and necrosis of tubular epithelium. There are multifocal areas of congestion, hemorrhage, and fibrin thrombi within vessels.

Contributor's Morphologic Diagnosis
Kidney: Acute, severe, suppurative, embolic nephritis with intralesional coccobacilli.

Contributor's Comment: Actinobacillosis, also known as sleepy foal disease, is caused by *Actinobacillus equuli*. This is a small, nonmotile, gram-negative, pleomorphic coccobacillus. Certain strains of *A. equuli*

form part of the normal flora of the gastrointestinal and respiratory tracts of horses.³⁻⁵ There is a high degree of strain variability within horse populations and within individual horses over time. It is currently unknown whether there are specific strains of *A. equuli* with greater virulence for foals and/or adult horses or



whether such strains are common in-

Kidney, foal: *On subgross examination, areas of inflammation are scattered through randomly throughout the cortex, and extend linearly along vessels into the outer medulla. (HE, 6X)*

habitants of the equine gastrointestinal and respiratory tracts.⁵

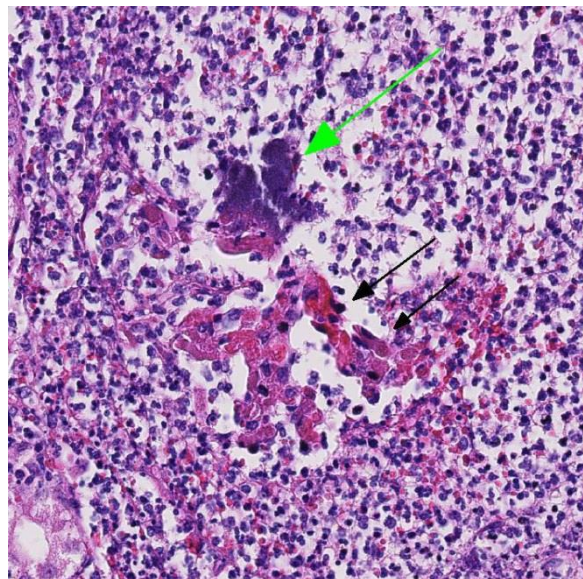
Two subspecies of *Actinobacillus equuli* have been identified: *A. equuli* subsp. *equuli*, and *A. equuli* subsp. *haemolyticus*.¹ The former appears to be pathogenic, while the latter's pathogenicity appears to be associated with its expression of a repeats-in-structural-toxin (RTX) called Aqx, which is cytotoxic for equine leukocytes.¹

Typically actinobacillosis is a disease of newborn foals and the pathogenesis of the infection remains speculative. Infection is probably acquired in utero, during

parturition, or shortly after birth as an umbilical infection.¹ Death may occur due to fulminating septicemia. In foals that survive for several days, microabscesses are seen in the kidney and other organs and a polyarthritis can be present. These microabscesses have an embolic origin and are characterized by the presence of numerous, 1-3 mm, white pinpoint foci on the cut surface throughout the renal cortex. Microscopically, glomerular capillaries contain numerous bacterial colonies intermixed with necrotic debris and extensive infiltrates of neutrophils that often obliterate the glomerulus.⁴

The lesions in our case are classic for *Actinobacillus equuli*, and the foal was also positive for strongyles. It has been postulated that migrating strongyle larvae from the intestinal tract may play a role in infection.

JPC Diagnosis: Kidney, cortex and medulla: Nephritis, embolic, suppurative,



Kidney, foal: *Glomeruli are often effaced by large numbers of degenerate neutrophils admixed with cellular debris. Here, the remnant of a glomerulus contains a large bacterial colony (green arrow) within the fragmented tuft. (black arrow) (HE, 400X)*

acute, severe, with large colonies of coccobacilli.

Conference Comment: Despite some slide variability, the histopathologic appearance of this lesion is a classic for suppurative and embolic nephritis caused by *Actinobacillus equuli*. This entity is the most common cause of suppurative and embolic nephritis in young horses.¹ In pigs, embolic nephritis is most commonly caused by *Erysipelothrix rhusiopathiae*. In cattle, *Trueperella pyogenes* from valvular endocarditis causes numerous septic emboli, which shower the renal cortex causing randomly distributed microabscesses and infarcts. *Corynebacterium pseudotuberculosis* is most common in sheep and goats, *Pasteurella multocida* in rabbits, and *Streptococcus moniliformis* in mice.^{1,4} In dogs, *Prototheca zopfii* organisms have been identified as a common cause of embolic nephritis secondary to systemic protothecosis.¹ Endotoxin expressed by gram-negative bacteria and *Streptococcus* sp. causes endothelial damage, vasculitis, and bacterial emboli.^{1,4} Most conference participants noted ectatic tubules containing necrotic and sloughed tubular epithelial cells, fibrin, hemorrhage, and proteinaceous fluid. Participants also noted occasional fibrin thrombi with colonies of coccobacilli within glomerular tufts, as well as parietal cell hyperplasia secondary to the effects of endotoxin.

This case illustrates the characteristic appearance of the large colony-forming coccobacilli, *Actinobacillus equuli*, in tissue section. In addition to discussing causes of embolic nephritis in other species, conference participants also reviewed other bacteria that form large colonies in tissue. These bacteria are difficult to distinguish from one another other on hematoxylin and

eosin stain (H&E), and require special stains or bacterial culture.¹ Gram-positive large colony forming bacteria include: *Staphylococcus*, *Streptococcus*, *Actinomyces*, and *Corynebacterium* spp.; while gram-negative large colony forming bacteria include *Yersinia* and *Actinobacillus* spp.^{1,4} Several conference members mentioned the acronym, YAACSS, as a helpful mnemonic device to remember which bacteria form large colonies in tissue section.

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CASE IV: 6M22955A (JPC 4083344).

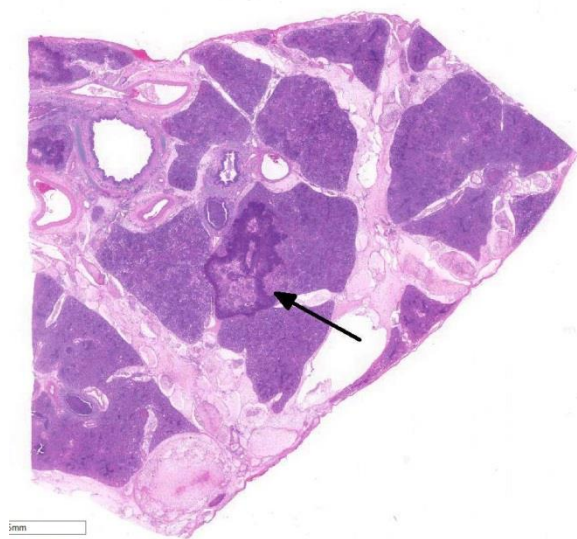
Signalment: 2-month-old male crossbreed calf (*Bos taurus*).

History: Respiratory issues in multiple 2-month old calves.

Gross Pathology: Cranioventral lung lobes were consolidated and covered in areas with fibrin.

Laboratory results: Lung: *Mannheimia haemolytica*

Immunohistochemistry: Bovine viral diarrhea virus (BVD)



Lung, calf: Subgross examination demonstrates diffuse filling of airways and alveoli with a cellular exudate and a focal area of infarction outlined by a dense band of cellular debris (arrow). The interlobular septa are expanded by edema and fibrin thrombi within lymphatics. (HE, 5X)

Animal ID	Specimen	Result	Comment
A	Assorted	Positive	marked immunolabeling

PCR - Bovine Respiratory Bacterial Panel

Animal ID	Specimen	Target Agents	Ct / Result
A, Tube #1	Lung	<i>Pasteurella multocida</i>	>40 / Negative
		<i>Mycoplasma bovis</i>	>40 / Negative
		<i>Mannheimia haemolytica</i>	19.7 / Positive
		<i>Histophilus somni</i>	16.9 / Positive

PCR - Bovine Respiratory Viral Panel

Animal ID	Specimen	Target Agents	Ct / Result
A, Tube #1	Lung	IBR	/Negative
		BCV	/Negative
		BRSV	/Negative
		BPI3	/Negative

Histopathologic Description: Lung: There are marked multifocal and extensive areas of necrosis demarcated by dense zones of neutrophils which form a subgross mosaic pattern. The necrotic regions contain large amounts of seroproteinaceous fluid, red blood cells, neutrophils and cell debris; some cells are degenerate with elongate nuclei (streaming leukocytes/oat cells). In remaining tissue, multifocal bronchi and bronchioles are dilated and contain large numbers of neutrophils and cell debris. There is diffuse thickening of the interlobular septa by edema and infiltrates of neutrophils. In one lobular area there is moderate thickening of alveolar septa by mild to moderate type II cell hyperplasia and occasional infiltrates of lymphocytes and macrophages. The pleura is thickened by edema and covered in some areas by a thick mat of fibrin and cell debris.

Contributor's Morphologic Diagnoses

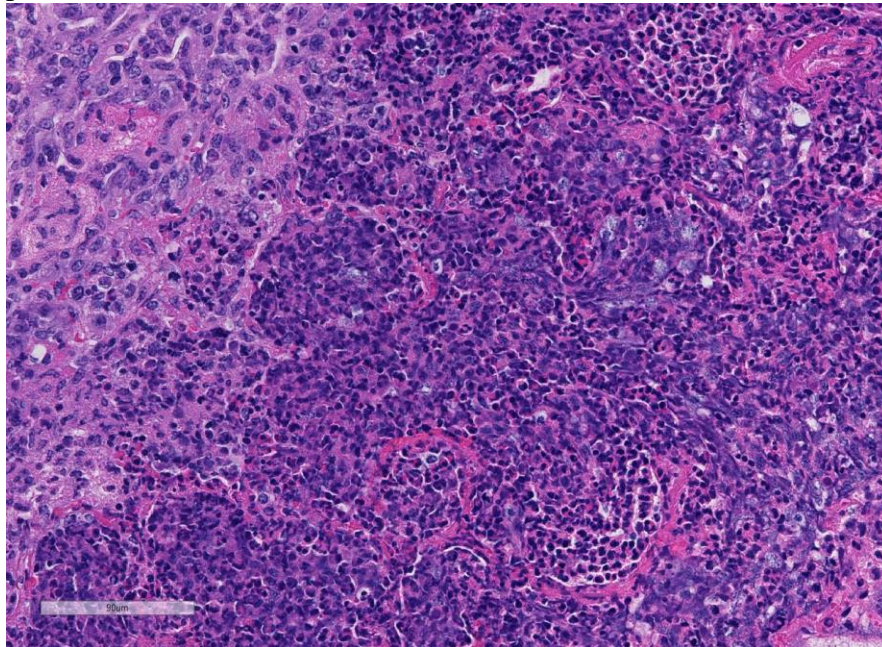
Marked multifocal and extensive acute necrotizing and fibrinosuppurative pneumonia/bronchopneumonia with fibrinous pleuritis.

Contributor's Comment:

Bovine respiratory disease (BRD) complex is multifactorial and susceptibility is related to stress, environmental and housing

conditions, management, hydration and immune status, and exposure/levels of microbial pathogens.⁵ Feedlot density has increased progressively in the United States and global demand for beef is trending upward; therefore, the incidence of BRD may continue to increase over time.⁵ Viral infections with agents such as bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPIV-3), bovine viral diarrhea virus (BVDV), bovine coronavirus (BCV), and bovine herpesvirus (BHV-1) can damage mucosal epithelia, decrease mucin production, as well as hinder innate and

cattle were BPIV-3 positive. Cattle exposed to a PI animal had an increased risk for treatment of BRD by 43%. In total, 15.9% of initial respiratory tract disease conditions were associated with exposure to a PI animal. Thus, very few cattle arrive to feedlot PI; however, those cattle are much more likely to require treatment and at place other cattle at-risk for incidence of respiratory disease.^{4,6} Of the subtypes of BVDV, BVDV1b subtype is more commonly identified than BVDV1a and BVDV2a based on a survey of BVD isolates of cattle entering feedlots.³ Experimentally, it has also been shown that exposure of steers to steers PI with BVDV enhances *M. haemolytica* disease severity.¹



Lung, calf: Alveoli are filled with degenerate neutrophils with streaming nuclei (oat cells) admixed with abundant cellular debris. (HE, 300X)

adaptive immune responses. This predisposes calves to secondary infections with bacteria, such as *Mannheimia haemolytica* and *Histophilus somni*, as in this case, as well as *Pasteurella multocida*, and *Mycoplasma bovis*.¹

In a 2005 study, the prevalence of BVDV was determined in 2000 cattle arriving to a feedlot. The prevalence of persistently infected (PI) cattle was only 0.3%, but 2.6% of chronically ill cattle, and 2.5% of dead

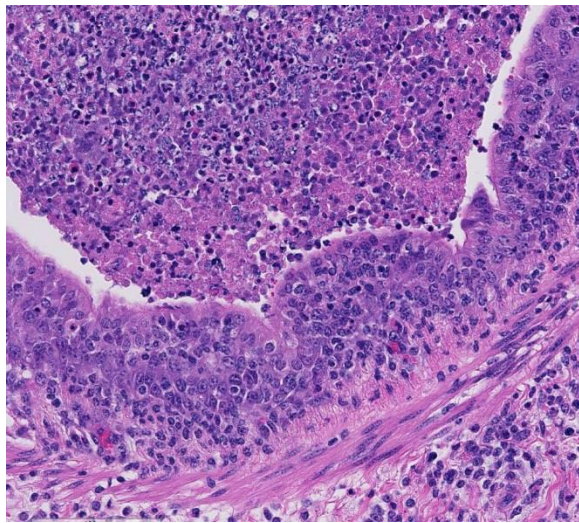
In this case, the animal had significant BVDV antigen detected by immunohistochemistry, which could alter lung and mucosal (e.g. tonsil) immunity, thus predisposing to enhanced bacterial replication/colonization. Other factors such as

stress and other viruses (e.g., BRSV, BPIV-3, coronavirus, BHV-1),

could have contributed to this predisposition along with the BVD, although lesions of RSV, BPIV-3, and BHV-1 were not seen and BHV (IBR), BCV, BRSV, and BPIV-3 were not detected by PCR.

JPC Diagnosis: Lung: Bronchopneumonia, fibrinosuppurative and necrotizing, diffuse, severe, with numerous necrotic leukocytes (oat cells)

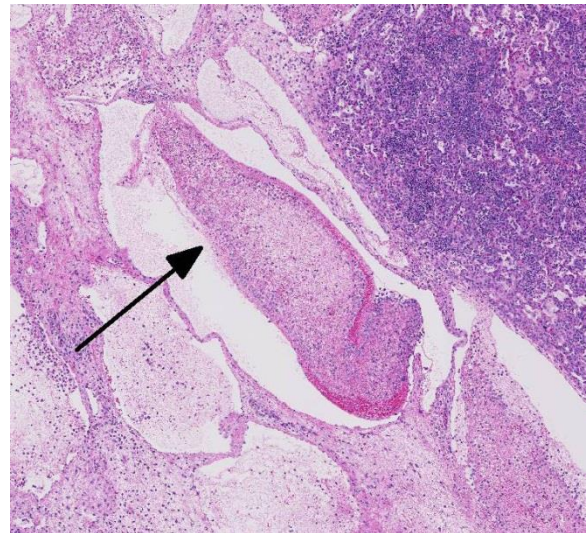
Conference Comment: This case is an excellent example of a classic presentation of the bovine respiratory disease complex (BRD). *Mannheimia haemolytica* is a gram-negative coccobacillus of the *Pasteurellaceae* family. It is typically a commensal bacterium in the nasopharynx and tonsillar crypts of immunocompetent ruminants.² Many members of this family of bacteria can produce respiratory disease and septicemia in naïve and immunosuppressed domestic animals.^{2,4,5} Other common pathogenic members of this family include: *Pasteurella multocida* of rabbits, swine, ruminants, and cats; *Bibersteinia trehalosi* and *Histophilus somni* in ruminants; *Actinobacillus pleuropneumoniae*, *A. suis*, and *Haemophilus parasuis* in swine; and *A. equuli* in horses.²



Lung, calf: Bronchioles are lined by moderately hyperplastic epithelium which is infiltrated by low numbers of neutrophils and cellular debris. The lumen contains abundant refluxed exudate from the surrounding alveoli. (HE, 300X)

There are twelve serotypes of *M. haemolytica* based on their capsular polysaccharide antigens.^{2,5} Serotypes 1 and 2 are typically isolated from the upper respiratory tract of cattle as part of the normal commensal flora;² however, in a

naïve or stressed animal, serotype 1 is often isolated from pneumonic lungs. As mentioned by the contributor, stress and cold weather causes the proliferation of commensal bacteria in the upper respiratory tract, overwhelming pulmonary defenses. In addition, co-infection with the respiratory viruses mentioned above can reduce mucociliary escalator clearance and impair the function of alveolar macrophages.²

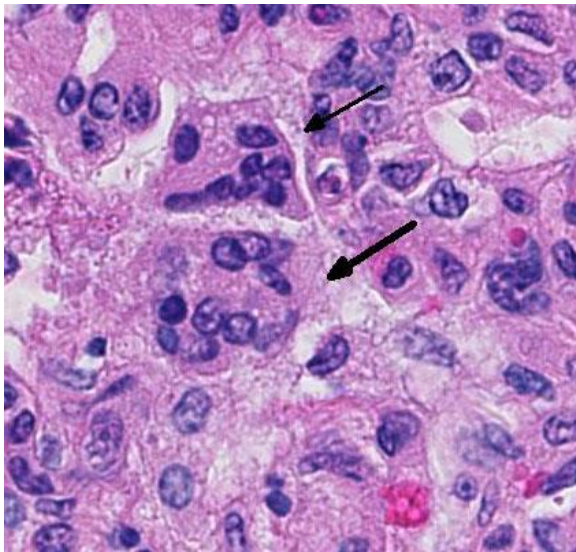


Lung, calf: Interlobular septa are expanded by abundant fibrin, edema, and moderate numbers of infiltrating neutrophils. Interlobular septa often contain fibrinocellular thrombi. (ARROW) (HE, 60X)

Conference participants briefly discussed the various virulence factors for *M. haemolytica* and how they contribute to the classic histopathologic appearance of this case. Virulence factors include: leukotoxin, lipopolysaccharide, capsular polysaccharide, transferring-binding proteins A and B, O-sialoglycoprotease, neuraminidase, IgG1-specific protease, outer membrane proteins, adhesins and fimbriae.² These virulence factors allow *M. haemolytica* to resist host clearance, avoid host defenses, and impair leukocyte function. The result is massive recruitment of neutrophils into alveoli, as seen in this case.^{2,5} Neutrophils are ineffective at killing bacteria and cause damage to capillary endothelial cells,

resulting in hemorrhage, fibrin and edema in alveolar, interstitial, and interlobular spaces.²

Leukotoxin, a type of repeats in toxin (RTX), is an important exotoxin produced by *M. haemolytica*. It induces leukocyte recruitment at low concentrations and lysis of leukocytes and platelets in high concentrations.² Leukotoxin binds to CD18 on the leukocyte surface, forming a pore in the cell membrane. Interestingly, BoHV-1 causes the up-regulation of CD18 on the surface of neutrophils which makes these cells more susceptible to leukotoxin.² Lytic and necrotic leukocytes exhibit a streaming pattern of basophilic chromatin and are commonly referred to as “oat cells.”^{1,2,5,6}



Lung, calf: Rare multinucleate viral syncytia (arrows) are present within some sections. (HE, 400X)

Most conference participants noted multifocal fibrin thrombi present throughout pulmonary parenchyma. Additionally, in many slides, there was a sharply demarcated area of tinctorial change with loss of differential staining surrounded by a band of degenerate neutrophils- interpreted as an infarct. Focal areas of coagulation necrosis and infarction are characteristic for *M.*

haemolytica.² These are secondary to thrombosis as well as direct damage to the lung by leukocyte secretion of pro-inflammatory IL-8, oxygen radicals, and nitric oxide synthetase. Activated alveolar macrophages also express tissue factor, which promotes the formation of fibrin thrombi.²

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Self-Assessment - WSC 2016-2017 Conference 2

1. Which of the following is not true concerning liver lobe torsion?
 - a. The caudate lobe is affected in 62% of cases in rabbits, whereas the left lateral lobe is most prone to torsion in most other species.
 - b. When hepatic injury is limited and the reticulin meshwork is intact, regeneration of mature hepatocytes is stimulated by hep-atocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor- α (TGF- α), and insulin-like growth factor (IGF).
 - c. Once hepatic mass has been replaced, tran-sforming growth factor- β (TGF- β) inhibits ongoing proliferation of hepatocytes.
 - d. Hypoxic hepatocytes transform into myofibroblasts resulting in progressive diffuse hepatic fibrosis.

2. Which of the following is true about *Histoplasma capsulatum*?
 - a. It survives within macrophages.
 - b. It is the only dimorphic fungus that does not have a mycelial phase in the environment.
 - c. It is endemic to the San Joaquin Valley.
 - d. Clearance of the organism is largely the result of an effective humoral response.

3. Which of the following does not produce large colonies in tissue?
 - a. *Yersina enterocolitica*
 - b. *Actinobacillus equuli*
 - c. *Klebsiella pneumonia*
 - d. *Corynebacterium kutscheri*

4. The severity of Bovine Respiratory Disease (BRD) is worsened by concurrent infection with bovine pestivirus.
 - a. True
 - b. False

5. Which of the following viruses is not considered part of the syndrome of Bovine Respiratory Disease?
 - a. Bovine herpesvirus-1
 - b. Bovine coronavirus
 - c. Bovine retrovirus
 - d. Bovine
 - e. parainfluenza virus-3