## WEDNESDAY SLIDE CONFERENCE 2012-2013

# Conference 10

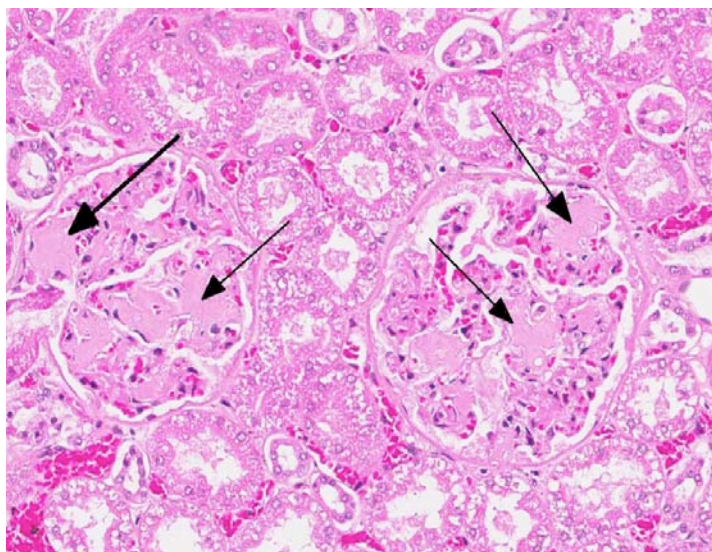**2 January 2013**

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**CASE I:** S08-0943 (JPC 3136037).

**Signalment:** 3.5-year-old, neutered male, Shar Pei *(Canis lupus familiaris).*

**History:** The animal was sent to the Institute of Veterinary Pathology from the small animal clinic of the University of Zurich without any anamnesis



*1-1. Kidney, dog: Diffusely, glomerular tufts are segmentally to globally expanded by variable amounts of amorphous, finely fibrillar to waxy, lightly eosinophilic material (amyloid) (arrows). (HE 172X)*

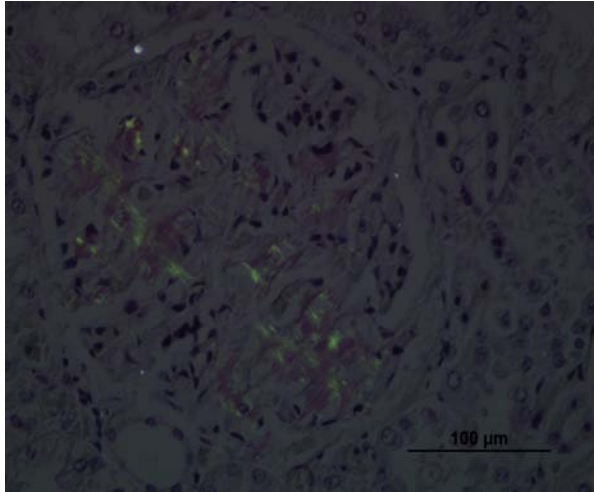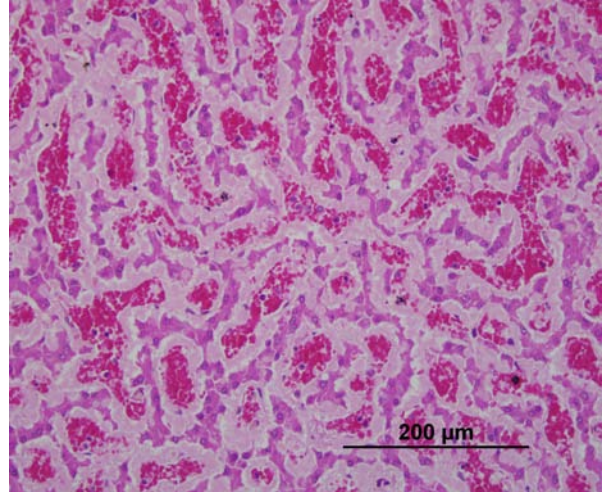but with the question of liver amyloidosis and proteinuria.

**Gross Pathologic Findings:** Liver was moderately enlarged, friable and soft. Kidneys were slightly enlarged, pale, increased in consistency and had a finely granular cortical cut surface.

**Histopathologic Description:** Liver: An abundant amount of amorphous, eosinophilic, homogeneous, extracellular material interpreted as amyloid is deposited diffusely in the space of Disse and encroaches on adjacent hepatic parenchymal cells and sinusoids. There is multifocal deformity, pressure atrophy and disappearance of hepatocytes, causing total replacement of large areas of liver parenchyma. There is a small amount of the same material in the vessel walls of few portal areas.

Kidney: In all glomeruli, there is a segmental to diffuse, moderate to abundant amount of amorphous, eosinophilic, homogeneous, extracellular material in the mesangial area and in the subendothelium of glomerular capillaries.

*1-2. Kidney, dog: Glomerular amyloid shows apple-green birefringence when transilluminated with polarized light. (Congo Red 400X)*



*1-3. Liver, dog: Diffusely, sinusoids are expanded by abundant amyloid within the subendothelial space, which compresses hepatocytes and distorts hepatic plate architecture. (HE 360X)*

The depositions gradually develop until the glomeruli, when entirely involved, are enlarged and appear as hypocellular, eosinophilic, homogeneous spheres in which the capillaries are obliterated. With Congo red, amyloid is stained a light orange-red. In the medulla, not visible on the slide, the tubules are dilated and contain a striking amount of homogeneous, pink, hyaline casts interpreted as protein (proteinuria).

**Contributor's Morphologic Diagnosis:** 1. Liver: Amyloidosis, diffuse, severe.
2. Kidney, glomeruli: Amyloidosis, severe.

**Contributor's Comment:** Amyloidosis of Shar-Pei dogs, also known as Shar-Pei fever, hock fever, swollen hock syndrome, and familial renal amyloidosis of Chinese Shar-Pei dogs, is a well-recognized syndrome of unknown etiology. Clinical signs include lethargy, anorexia, recurrent fever, joint swelling and pain, and/or cellulitis over affected joints.[1] Renal and hepatic involvement are described in 3 reports from the early 1990s documenting amyloidosis in a total of 17 Chinese Shar-Pei dogs having common ancestry.[2-4] The disorder is postulated to have autosomal recessive inheritance, and affected dogs typically manifest recurrent bouts of fever before developing renal amyloidosis.[1] Histopathologic findings of amyloidosis in this case were similar to those in previous reports. In Shar-Pei dogs, hepatic amyloid deposition is commonly observed within the space of Disse, vessel walls, and perivascular areas. In the kidney, both glomerular and interstitial medullary

deposition have been reported.[1] Additionally, the dog in this case had a small amount of amyloid deposition in the spleen.

Amyloidosis is a group of disorders in which amyloid is deposited in the walls of small blood vessels and extracellularly in a variety of sites, particularly in renal glomeruli. All amyloid fibrils have a β-pleated sheet structure.[5] By light microscopy and standard hematoxylin and eosin staining, amyloid appears as an amorphous, eosinophilic, hyaline, extracellular substance.[6] The histologic diagnosis is usually confirmed with special stains. Amyloid is stained a light orange-red with Congo red, and then exhibits green birefringence in polarized light.[5]

Amyloidosis is seen in a number of presentations:
1. Immunoglobulin-derived (primary, or AL) amyloidosis is the most common form in humans, but it is uncommon in domestic animals. AL amyloid is produced from immunoglobulin light chains in plasma cell dyscrasias as a product of monoclonal B-cell proliferation.[5]
2. Reactive systemic (secondary or AA) amyloidosis is the most common form in domestic animals. AA amyloid is derived from serum protein AA, an acute-phase reactant apoprotein product of hepatocytes predominantly. Serum protein AA is produced in excess as a result of chronic antigenic stimulation, such as occurs in persistent infectious, inflammatory or neoplastic (nonimmunocyte dyscrasias) conditions.[5,7]

3. Familial amyloidosis is a systemic form of AA amyloidosis that is hereditary in some breeds of dogs and cats.[7]   AA amyloidosis occurs in Beagles, Shar-Pei dogs, gray Collies, English Foxhounds, Abyssinian cats and Siamese and Oriental cats.
4. Apolipoprotein AI-derived amyloidosis affects the pulmonary vessels of old dogs.
5. Islet amyloid polypeptide-derived amyloidosis is common in the pancreatic islets of cats with non-insulin-dependent diabetes mellitus.[5]

Both AA and AL amyloidosis can occur in either systemic or localized forms.   The cause of amyloid fibril formation and deposition is obscure, but the nidus theory postulates that amyloid fibrils serve as templates for fibril growth and as scaffolding for fibril polymerization. Amyloid-enhancing factor may be involved.[5]

**JPC Diagnosis:**   1. Liver, space of Disse: Amyloidosis, diffuse, severe, with cholestasis and hepatocyte atrophy and loss.
2. Kidney, glomeruli: Amyloidosis, segmental to global, diffuse, marked, with scattered tubular degeneration and necrosis.

**Conference Comment**:  In addition to the points discussed by the contributor in their excellent summary, conference participants discussed several properties of amyloid, including anatomic locations of deposition in various species, clinicopathologic findings in amyloidosis and characteristics of amyloid on electron microscopy. The location of amyloid deposits varies based on the pathogenesis and the species affected. Overall, the kidney is the most common site for amyloid deposition in many domestic species, with the glomeruli being most frequently affected in most domestic species, except the cat where medullary interstitial deposition of amyloid is more common.  The space of Disse in the liver is also a common site for amyloid deposition in birds, cattle, horses, dogs and cats.  The spleen is a frequent site in reactive systemic amyloidosis, with amyloid deposits occurring in the periarteriolar lymphoid sheaths and red pulp. Organs affected in localized amyloidosis include the skin and nasal septum and turbinates in horses and pancreatic islets of Langerhans in cats.[6]

Clinocopathologic consequences of renal amyloidosis include the nephrotic syndrome, which is characterized by the tetrad of hypercholesterolemia, proteinuria, hypoproteinemia, and generalized edema. Additionally, a urine-protein-to-urine-creatinine ratio (UP/UC) greater than 18 is associated with renal amyloidosis.   Ultrastructually, amyloid fibrils are non-branching, 7.5 to 10 nm in diameter, and lack the periodicity which is characteristic of fibrin and collagen diameter tubules by electron microscopy.[7]

There was some slide variation, with some slides having only the section of kidney.

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## CASE II: 48646 (JPC 4019427).

**Signalment:** 7-year-old, male castrated, Russian Blue cat *(Felis catus)*.

**History:** The cat had a history of weight loss for approximately one year, and left tarsal arthrodesis surgery five months prior. Clinical workup at the time of surgery revealed sternal lymphadenopathy, thick-walled intestines, mild anemia, thrombocytopenia and neutropenia. FIV/FeLV tests were negative.
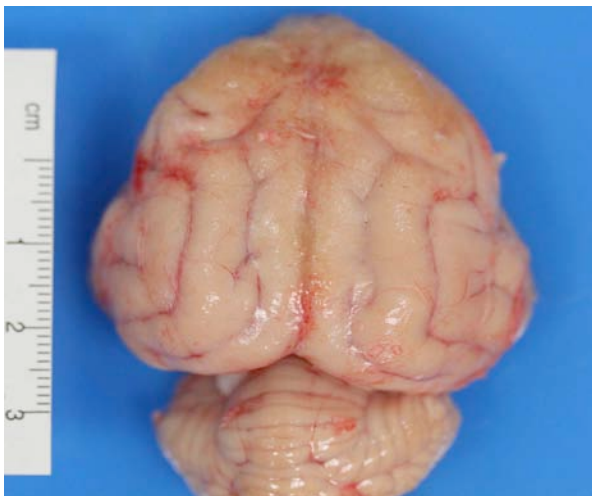
More recent clinical presentation was for an ulcerated lip mass which had been noted for eight weeks. The owners also described that the cat had been wandering around the house, stuck in small spaces, was incoordinated, stumbling and vocalizing, with a decreased appetite. On physical exam, there were multifocal neurologic signs including decreased menace bilaterally, positional nystagmus and inappropriate mentation. Additional findings included left retinal detachment, heart murmur and thickened intestines with palpation.

**Gross Pathology:** On the lower left lip, there is a 1.0 x 0.5 x 0.5 cm ulcerated, firm red nodule. The left tarsal joint is fused (tarsal arthrodesis). Internally, subcutaneous and visceral fat stores are markedly reduced, with mild yellow discoloration of the adipose tissue (icterus).
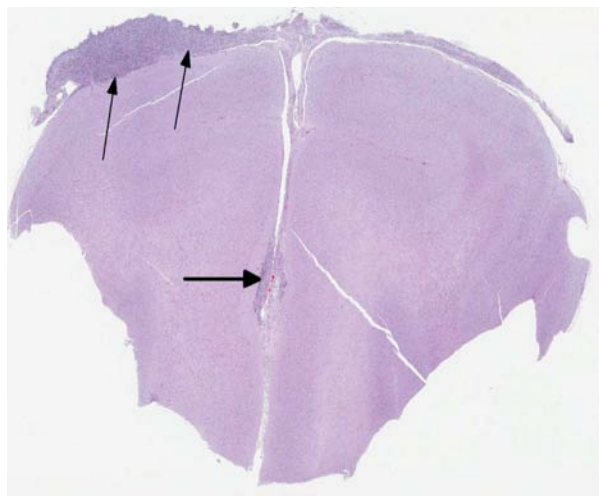
The vermis of the cerebellum is focally compressed and flattened against the underlying brainstem at the foramen magnum (herniation). The leptomeninges of the rostral and dorsal cerebrum are thickened by granular, irregular, yellow to tan material (meningitis). When the fixed brain is sectioned, there is marked leptomeningeal expansion by similar granular material, with adherence of the right and left hemispheres of the rostral cerebrum at the longitudinal cerebral fissure.

**Laboratory Results:** Normocytic, normochromic, nonregenerative anemia, mildly elevated ALT and AST, mild hypoalbuminemia and hyperglobulinemia.
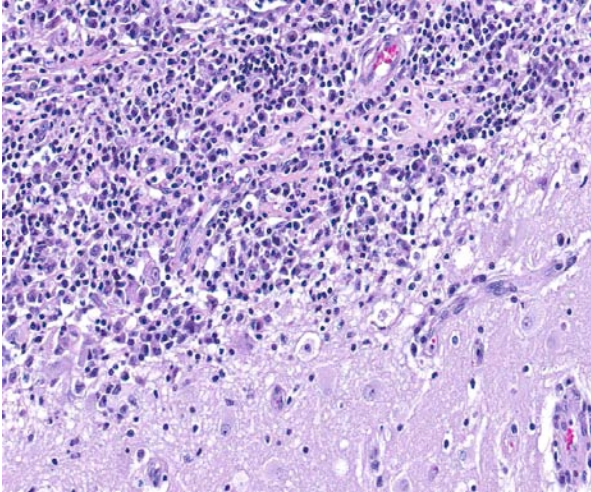
Cytology: Aspirate of mass from the right lower lip region: All slides are examined and are found to be similar; the specimen is of moderate cellularity and contains moderate numbers of red blood cells with low to moderate numbers of nondegenerate neutrophils and activated macrophages. Rare tissue cells are identified and these are uniform fibrocytes showing no evidence of dysplasia or atypia. On several of the slides there are extremely low numbers of intracellular yeast structures; these are 1-3 microns in diameter, round to oval with a variably thick capsule and are primarily found within the cytoplasm of very few of the macrophages. The yeast organisms most resemble *Histoplasma* sp.
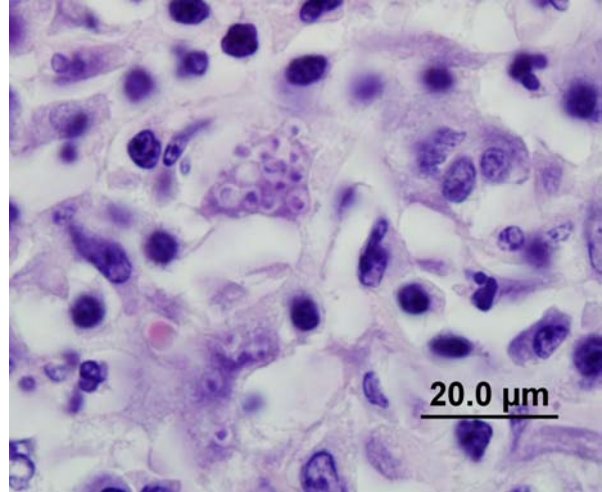


2-1. Cerebral cortex, telencephalon, cat: The leptomeninges of the rostral and dorsal cerebrum are thickened by granular, irregular, yellow to tan material. (Photograph courtesy of Animal Medical Center, http://www.amcny.org)
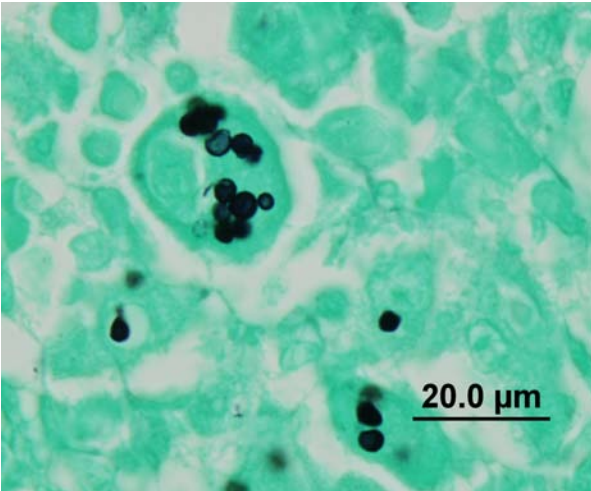


2-2. Cerebral cortex, telencephalon, cat: Diffusely, the meninges are markedly expanded by a dense cellular infiltrate. (HE 5X)

*2-3. Cerebral cortex, telencephalon, cat: The meningeal infiltrate is composed of numerous macrophages and neutrophils with fewer numbers of lymphocytes and plasma cells. The inflammatory infiltrate occasionally extends into and replaces the superficial cerebral cortex. (HE 400X)*



*2-4. Cerebral cortex, telencephalon, cat: Macrophages and multinucleated macrophage giant cells contain one or numerous (up to 20) 2-4 m diameter, round to oval intracytoplasmic yeast. These intracytoplasmic structures are characterized by a central, 1-2 m, eosinophilic nucleus which is surrounded by clear space. (HE 1000X)*



*2-5. The somewhat irregular shape of Histoplasma capsulatum is well demonstrated by silver stains.*

Microscopic interpretation: Mild to moderate mixed (neutrophilic and histiocytic) inflammatory infiltrate associated with intracellular yeast organisms.

Comment: Although the numbers of yeast structures are quite low, they provide unequivocal evidence of a primary fungal etiology and are most characteristic of *Histoplasma capsulatum*.

**Histopathologic Description:** One section of rostral cerebrum is examined. The leptomeninges, including those along the longitudinal cerebral fissure, are markedly expanded up to 3 mm by large populations of inflammatory cells which extend into the brain parenchyma. Inflammatory populations consist of macrophages, neutrophils, lymphocytes and plasma cells. Multinucleate cells and occasional Mott cells are present. Multifocally, macrophages and multinucleate cells contain one or numerous (up to 20) 2-4 μm diameter, round to oval intracytoplasmic yeast characterized by a central, 1-2 μm, eosinophilic nucleus which is surrounded by clear space. Inflammatory cells similar to those described above are observed within Virchow-Robin spaces and infiltrate the adjacent neural parenchyma. Macrophages commonly contain enlarged nuclei with marginated chromatin and eosinophilic material (presumed reactive change). Small vessels in the regions of infiltration exhibit endothelial hypertrophy and there is regional rarefaction of the neuropil (edema). Gliosis is abundant, consisting of astrogliosis and numerous gemistocytic astrocytes. Fewer microglial cells are present, which exhibit rod cell morphology. Round, eosinophilic structures (spheroids) are also dispersed throughout the infiltrated brain parenchyma.

*Histoplasma* immunohistochemistry (provided by the University of Connecticut Veterinary Medical Diagnostic Laboratory): Brain: Multifocally throughout the meninges, within macrophages, there are numerous positive staining, intracellular, spherical, 3-4 μm diameter organisms (*Histoplasma capsulatum*).

Similar, positive staining organisms are identified in the previous bone biopsy, lungs, adrenal glands and eye.

**Contributor's Morphologic Diagnosis:** Brain (rostral cerebrum): Severe, chronic, pyogranulomatous, lymphoplasmacytic meningoencephalitis with intralesional, intracytoplasmic fungal yeast (consistent with *Histoplasma capsulatum*), regional edema, spheroids and gliosis with gemistocytic astrocytosis.

**Contributor's Comment:** *Histoplasma capsulatum* is a dimorphic, soil-borne fungus, existing in the environment as a mycelial form, and in the host as a yeast.[2,3] Infections with this fungus are prevalent in the Midwest and southern United States, and regions along the Ohio, Missouri and Mississippi Rivers.[2,7,9] The organism grows best in soil containing nitrogen-rich organic matter, including bird and bat excrement.[3] Infection typically develops via inhalation or ingestion, and most animals clear the infection without developing clinical signs of disease.[1,2,6] In dogs and cats, macrophages phagocytize the organisms and can distribute them to other organ systems. Clinical signs are typically nonspecific and include weight loss, lymphadenopathy, lethargy, fever, and respiratory signs as well as cutaneous nodules, ocular disease, diarrhea, and lameness.[1-3,6] Histoplasmosis is the second most common fungal disease reported in cats after cryptococcosis.[1,3]

A retrospective study of 22 cases of feline histoplasmosis found that amongst three categories (disseminated, pulmonary and gastrointestinal), disseminated was the most common manifestation, occurring in 68% of cases. The most frequent sites of infection were listed as the lungs, lymph nodes, liver, spleen, kidney, adrenal glands, eyes, bone marrow and the gastrointestinal tract.[1] Disseminated infection with involvement of the brain is rarely reported in cats and dogs.[4,7,9] In this case, the brain was the most severely affected organ, and the cat re-presented for its neurologic signs. Immunohistochemistry for *Histoplasma* was performed at the University of Connecticut Veterinary Medical Diagnostic Laboratory. The combination of H&E, silver stains and IHC confirmed *Histoplasma* organisms in the brain, eye, lungs, adrenal glands, bone marrow at site of previous surgery (taken at the time of surgery) and the ulcerated lip lesion. Demonstrable organisms in the ulcerated lip lesion correlated with the cytologic results. In one report, a review of cases from the authors' institution revealed that several cats with oral histoplasmosis presented with focal or multifocal exophytic or ulcerative lesions within the oral cavity, with no apparent systemic involvement.[6] Osseous lesions with associated soft tissue swelling or joint effusion and lameness have been described with feline histoplasmosis.[3] *Histoplasma* organisms were immunohistochemically identified in the bone marrow biopsy obtained 5 months before euthanasia, indicating a long time frame of infection. Although the intestines were palpably thickened, neither granulomatous enteritis nor intralesional organisms were identified at postmortem examination; however, the intestines displayed mucosal fibrosis, which may indicate previous infection.

The cat in this case had a history of being indoor-only for 3 years, and prior to this had intermittent access to the outdoors in rural Pennsylvania. Other reports of histoplasmosis in indoor only cats without travel to endemic regions suggest that household dust or potting soil are possible sources of infection.[3] Some studies found a high prevalence of FeLV in cats with the disseminated form of histoplasmosis, whereas others found a low prevalence.[1,4] In our case, the cat was negative for both FIV and FeLV. Typical clinicopathologic changes in cats with disseminated histoplasmosis include normochromic, normocytic, nonregenerative anemia, hypoalbuminemia, and thrombocytopenia, with variable leukocyte counts.[3,4] Nonregenerative anemia is suspected to result from chronic inflammatory disease, *Histoplasma* infection of the bone marrow, and intestinal blood loss in gastrointestinal disease. Some cats have been reported to have hyperproteinemia, hyperglobulinemia, mild hyperglycemia or hyperbilirubinemia, and elevations of alanine aminotransferase activities. Hypercalcemia has been reported in several cats and is likely due to granulomatous disease.[3] In our case, mild elevations of ALT and AST may be explained by concurrent chronic cholangiohepatitis.

**JPC Diagnosis:** Cerebrum: Meningoencephalitis, pyogranulomatous, multifocal, moderate with intrahistiocytic yeast.

**Conference Comment:** The contributor provided a very good summary of histoplasmosis caused by *Histoplasma capsulatum* var. *capsulatum* in dogs and cats. Conference participants reviewed two other variants of *H. capsulatum*: *H. capsulatum* var. *farciminosum* (also referred to as *H. farciminosum*) and *H. capsulatum* var. *duboisii.* *H. farciminosum* causes equine epizootic lymphangitis in horses and mules, characterized by ulcerated discharging cutaneous nodules located along thickened lymphatic vessels, and regional lymphadenopathy, resembling farcy. *H. capsulatum* var. *duboisii* causes African histoplasmosis in humans and nonhuman primates.[5,8]

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**CASE III:** 12-9677 (JPC 4019858).

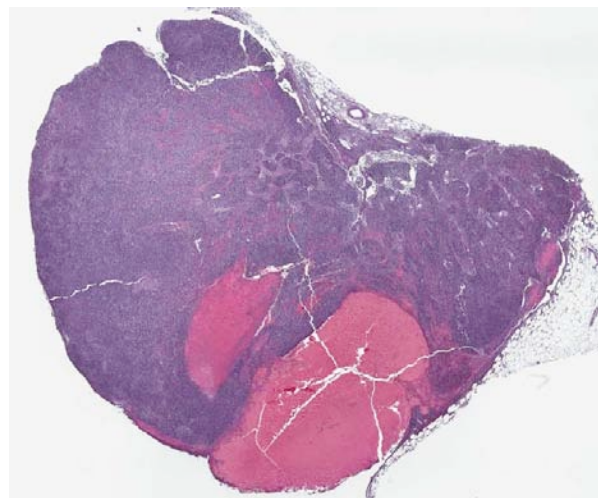**Signalment:** 9-year-old, male, neutered, Golden Retriever mix dog (*Canis familiaris*).

**History:** One year prior to presentation, the dog had developed epistaxis and a right nasal mass that was diagnosed as nasal adenocarcinoma following biopsy and histopathologic examination. The epistaxis responded well to treatment with tramadol, firocoxib, and Chinese herbs. In March of 2012, the dog presented for a 3-day history of progressive anorexia, diarrhea, vomiting, and lethargy. The dog was salmon fishing 10 days prior to admission and was found with a fish carcass. The owners elected empirical therapy and additional medications prescribed were meropitant and metronidazole. The dog re-presented two days later due to lack of improvement and development of dark colored diarrhea. On physical exam he was dull and panting, with a temperature of 99.8 degrees Celsius and a heart rate of 150 bpm. The right mandibular lymph node was moderately enlarged and there was dark brown fecal staining on fur around perineum. Due to the lack of response to the medications and continuing anorexia, the owner's elected euthanasia and necropsy.

**Gross Pathology:** Mucous membranes and abdominal fat were pale with a yellow tinge. There was an irregular, thin, undulating mass in the right ethmoid concha measuring 2 x 3 x 2 cm. On impression smear of the mass there were clusters of epithelia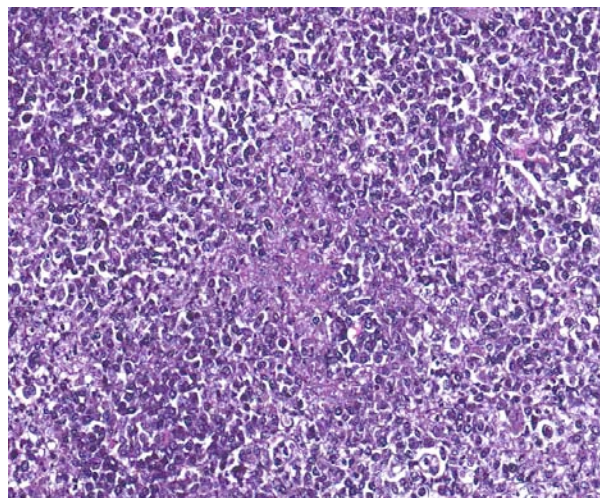l cells with anisokaryosis and prominent nucleoli and also macrophages with Neorickettsial organisms found intracytoplasmically. There was lymphadenopathy characterized by an enlarged right mandibular lymph node (2 x 1 x 2 cm), enlarged tracheobronchial lymph nodes (0.5 x .05 x 0.5 cm to 1 x 2 x 1 cm) with multifocal hemorrhages, and enlarged mesenteric lymph nodes (1 x 1 x 1 cm to 2.5 x 1 x 1.5 cm) also with multifocal hemorrhages. On impression smears of the mesenteric and tracheobronchial lymph nodes there were large numbers of organisms consistent with *Neorickettsia* within macrophages. The spleen was moderately enlarged with a vaguely cobblestone surface and was meaty on cut section.

**Laboratory Results:** Mildly increased ALP 298 U/L (RI: 10-84); CBC: Normal; U/A: Unremarkable; PCR: Lymph node and feces, positive for Neorickettsial spp real time PCR assay (still in validation process).
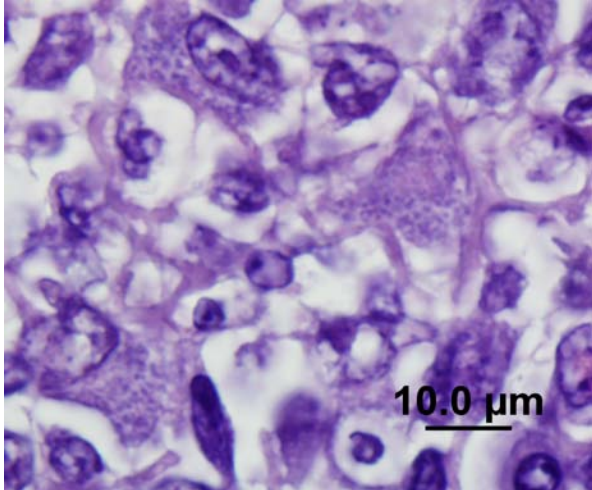
**Histopathologic Description:** The lymph node is hyperplastic and hemorrhagic. The medulla is edematous often with large foamy macrophages filled with red blood cells (erythrophagocytosis). Macrophages are also often characterized by large, swollen, pale basophilic cytoplasm with numerous fine blue organisms. There are multifocal areas of necrosis characterized by leukocytes with pyknotic and karyorrhectic nuclei, cellular debris, occasional neutrophils and flocculent amphophilic debris. There are multifocal areas of hemorrhage. The cortex is
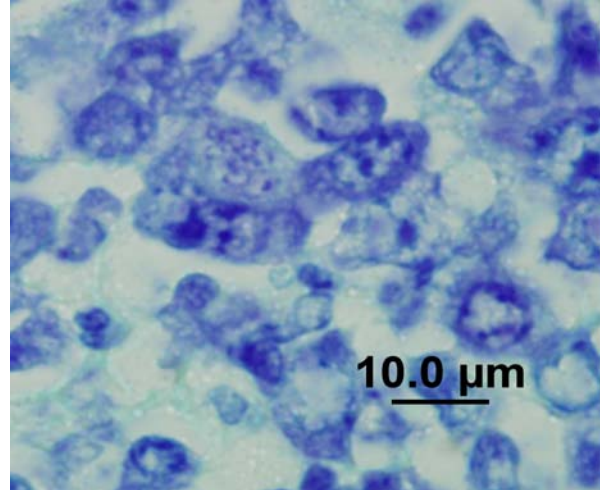


*3-1. Lymph node, dog: Marked hyperplasia largely effaces normal lymph node architecture. (HE 7X)*



*3-2. Lymph node, dog: Cortical lymphoid follicles are often replaced by necrotic debris. (HE 200X)*

3-3. Lymph node, dog: Throughout the lymph node, macrophages occasionally contain numerous small intracytoplasmic coccobacilli (rickettsia). (HE 1000X)



3-4. Lymph node, dog: Coccobacilli can also be demonstrated with Giemsa stains. (HE 1000X)

packed with large histiocytes and fewer lymphocytes and plasma cells. Sinuses often contain macrophages displaying erythrophagocytosis. Subcapsular sinuses are congested often with erythrophagocytic macrophages and deposition of fibrillar to amorphous eosinophilic material deposits consistent with fibrin.

**Contributor's Morphologic Diagnosis:** Lymph node: granulomatous lymphadenitis with hemorrhage and intralesional rickettsial organisms (salmon poisoning- *Neorickettsia helminthoeca*).

**Contributor's Comment:** Salmon poisoning disease (SPD) was first described in 1814 and occurs in northwest coastal regions including California, Oregon, Washington, and southern British Columbia.[10] Recently, SPD has been reported in Brazilian dogs confirmed with IHC and PCR for *N. helminthoeca*.[5,6] The disease is caused by *Neorickettsia helminthoeca* and the definitive host includes foxes, coyotes, and dogs; captive bears have also been reported to be highly susceptible to clinical disease after ingestion of trematode infected fish.[2,3,7] *N. helminthoeca* require three hosts for life cycle completion.[6] The life cycle includes a trematode vector, *Oxytrema silicula,* considered important in the geographic distribution of the disease, and a fish.[6] Miracidia of *Nanophyetus salmincola* penetrate the fresh water snail, *Oyxtrema silicula,* the cercariae then infect the second intermediate host by skin penetration or ingestion of infected snails or free cercariae.[1,4] The second intermediate host is most often salmon or trout.[1] The cercariae develop into metacercariae and become encysted in tissue of the salmon including eyes, kidneys, liver, intestine, fins and musculature.[1] Salmon in enzootic area are often heavily infected with the metacercariae.[1] Infection in dogs occur when uncooked fish is ingested and the metacercariae mature into flukes in the intestine.[1] The fluke does not cause the clinical signs but eggs appear in the feces of dogs five to ten days after infection and are a method of diagnosis.[1] The neorickettsial organisms, present in all stages of the fluke life cycle, spread via macrophages to visceral tissues of the dog causing disease.[1,8] The pathogenesis is still unclear in some ways, but the fluke attaches deep in the intestinal mucosa causing an inflammatory response and release of *N. helminthoeca* by an unknown mechanism.[6] The *N. helminthoeca* are taken up by intestinal macrophages and are disseminated through the lymph systems to lymph nodes where they proliferate with subsequent hypertrophy of the lymph nodes due to influx of macrophages and edema.[6] Enteritis is caused by the inflammation of the Peyer's patches and intestinal lymphoid tissue.[6] The organism is found in circulation of infected dogs 8-12 days after infection.[3] Mortality rate is about 90% in untreated cases and death occurs at least 18 days after ingestion of metacercariae.[1,6] Dogs are immune to reinfection but only with the same strain. Subsequent infection of recovered dogs has been reported with alternate strains resulting in disease.[8,10] A similar but sometimes less severe disease has been described in the Elokomin River of

Washington called Elokomin fluke fever.[6] This disease has a wider host range and the causative agent is thought to be another strain of *N. helminothoeca*.[6,10]

Pathologic findings include enlarged mesenteric lymph nodes. Other nodes are usually less affected. Peyer's patches are hypertrophic and the entire intestinal tract may be hemorrhagic.[6] Histopathologic findings are usually in the intestine and mesenteric lymph nodes with lymphoid tissue depletion and proliferation of lympho-histiocytic cells with neorickettsial bodies.[6] Giemsa or Machiavello's stains demonstrate organisms within macrophages.[6] Non-suppurative leptomeningitis and centrilobular fatty degeneration in the liver have also been described.[6] In this case there was a non-suppurative leptomeningitis and multifocal areas of hepatocellular vacuolation suggestive of lipid in the liver. The lymph nodes examined, including tracheobronchial and mesenteric, had obscured architecture with high cellularity and multifocal areas of necrosis with infiltration by large foamy macrophages containing neorickettsial organisms. There were similar findings in the spleen.

Diagnosis is based on a combination of fecal sedimentation and flotation, abdominal ultrasound, lymph node aspirate and PCR.[10] Identification of operculated trematode eggs via direct smear or washing-sedimentation in feces of infected dogs 5-8 days post infection or identification of the adult fluke within the intestine are methods of diagnosis.[6] Trematode infection may also occur in dogs recovered from salmon poisoning disease and during recovery.[6] Microscopic identification of neorickettsial bodies within macrophages may be performed on lymph node aspirates.[6] Definitive diagnosis includes isolation and culture of *N. helminthoeca*, serology, and PCR.[6] In this case, PCR was performed on lymph node impression smears, serum, and feces and was positive in both lymph node aspirate and fecal sample but the serum was negative. Giemsa and Rickettsia-Pierce VanderKamp staining identified organisms in the macrophages in several tissues including the adenocarcinoma, multiple lymph nodes, spleen, intestine, and liver. In endemic areas this disease should be considered in dogs with clinical gastrointestinal signs such as anorexia, vomiting, and diarrhea as recognition of the disease and prompt treatment

with a tetracycline type antibiotic is important for patient survival. Although it was known that the dog had been near salmon prior to development of clinical signs the owners were certain that the dog did not ingest any salmon. This case illustrates the importance of treatment with a tetracycline family antibiotic in dogs developing clinical signs of gastroenteritis when salmon exposure is known or suspected.

**JPC Diagnosis:** Lymph node, mesenteric: Lymphadenitis, necrotizing, diffuse, moderate to severe with hemorrhage and intrahistiocytic coccobacilli.

**Conference Comment:** The contributor provides a thorough and current review of salmon poisoning disease in dogs. Conference participants discussed the pathogenesis and temporal qualities of the histopathologic lesions in the submitted lymph node. The multifocal preponderance of plasma cells and the expanded paracortex are evidence of a reactive lymph node; however, the most significant feature is the marked necrosis, characterized by the loss of normal follicular architecture and replacement by necrotic debris admixed with fibrin, hemorrhage, numerous macrophages and fewer neutrophils, as well as multifocal, widespread lymphocytolysis. Participants hypothesized that this sample represents a necrotizing lymphadenitis due to *N. helminthoeca* infection in a lymph node with pre-existing reactive hyperplasia. Additionally, the large amount of subcapsular and sinus hemorrhage was speculated to be due to draining from hemorrhagic enteritis, a feature commonly seen in this disease.

The genus *Neorickettsia,* along with three other genera (*Anaplasma, Ehrlichia,* and *Aegyptianella*) comprise the family *Anaplasmatacea,* within the order *Rickettsiales.* *Anaplasmatacea* organisms are minute, non-motile Gram-negative obligate intracellular bacteria that lack cell walls and infect cells of hemopoietic origin. Generally, they cause arthropod-borne systemic disease in animals as well as humans. *Anaplasmatacea* species of importance in veterinary medicine are summarized in the included table.[9]

**Contributing Institution:** Oregon State University Veterinary Diagnostic Laboratory
http://vetmed.oregonstate.edu/diagnostic

| Pathogen | Host | Vector | Target cells | Disease |
|---|---|---|---|---|
| *Aegyptianella pullorum* | Poultry | Ticks | Erythrocytes | Aegyptianellosis |
| *Anaplasma bovis* | Cattle | Ticks | Monocytes, macrophages | Bovine anaplasmosis |
| *Anaplasma marginale* | Ruminants | Ticks | Erythrocytes | Anaplasmosis |
| *Anaplasma ovis* | Sheep, goats | Ticks | Erythrocytes | Anaplasmosis |
| *Anaplasma phagocytophilum*<br><br>*\*Formerly Ehrlichia equi and Ehrlichia phagocytophila* | Ruminants, horses, humans | Ticks | Granulocytes | Tick-borne fever, equine and human granulocytic ehrlichiosis |
| *Anaplasma platys* | Dogs | Ticks? | Platelets | Canine cyclic thrombocytopenia |
| *Ehrlichia canis* | Dgos | Ticks | Monocytes, macrophages | Canine monocytic ehrlichiosis |
| *Ehrlichia ewingii* | Dogs | Ticks | Granulocytes | Canine granulocytic ehrlichiosis |
| *Ehrlicia* (formerly Cowdria) *ruminantium* | Ruminants | Ticks | Granulocytes | Heartwater |
| *Ehrlichia ondiri* | Cattle | Ticks? | Granulocytes, monocytes | Bovine petechial fever |
| *Ehrlichia ovina* | Sheep | Ticks | Monocytes, macrophages | Ovine ehrlichiosis |
| *Neorickettsia elokominica* | Dogs, bears, raccoons | Flukes | Monocytes, macrophages, lymphoid cells | Elokomin fluke fever |
| *Neorickettsia helminthoeca* | Dogs, bears | Flukes | Monocytes, macrophages, lymphoid cells | Salmon poisoning disease |
| *Nerickettsia risticii* | Horses | Flukes | Monocytes, ntestinal epithelial cells, mast cells | Potomac horse fever |

Table adapted from *Veterinary Microbiology and Microbial Disease,* 2nd ed, 2011.

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<u>**CASE IV:**</u>  N335/11  (JPC 4007441).

**Signalment:**  1-year-old male rabbit, *Oryctolagus cuniculus*.
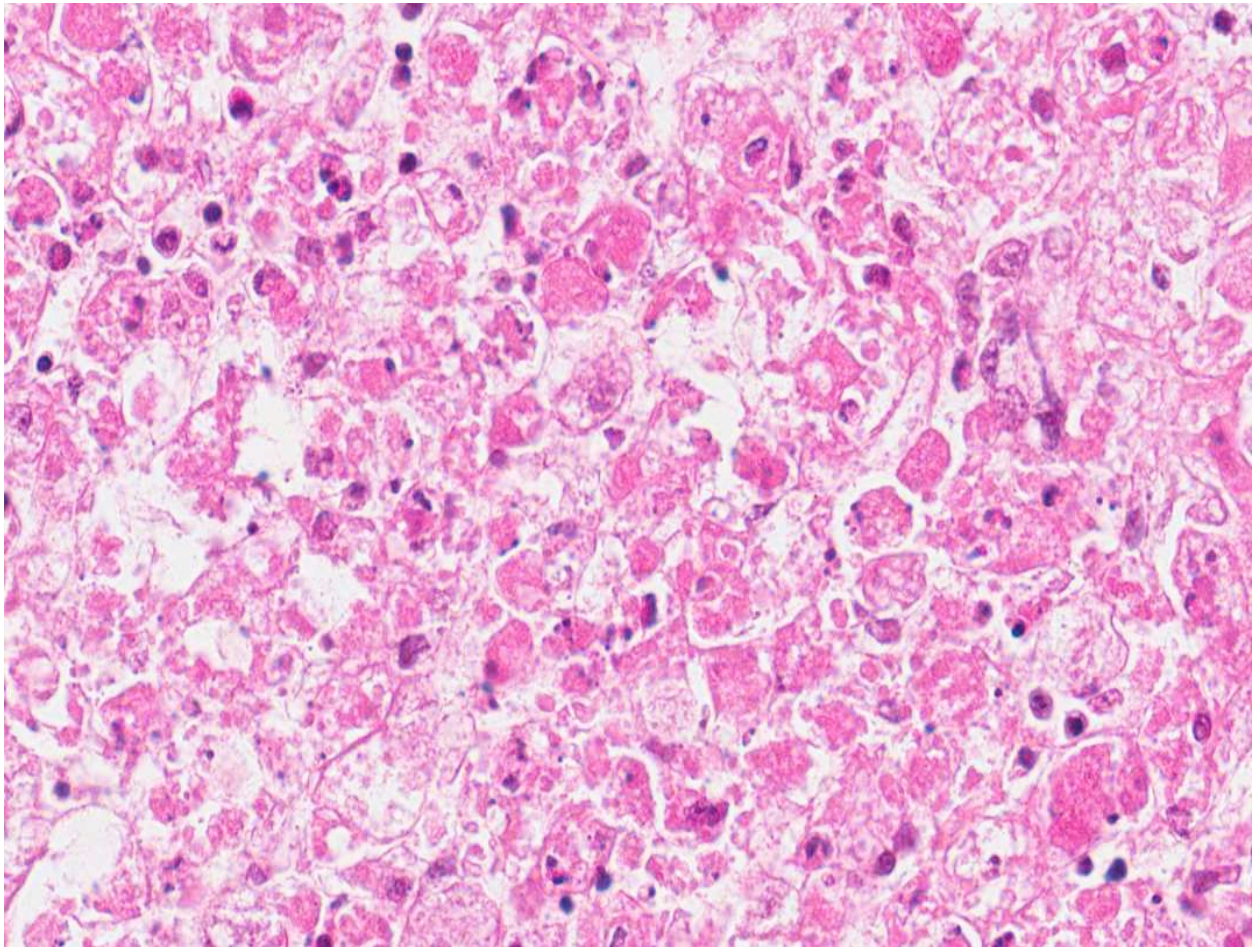
**History:**  Found dead without premonitory signs.

**Gross Pathology:**  Poor body condition score (2/5).  Bilateral diffuse lung congestion. Diffusely pale, enlarged liver containing multiple randomly distributed < 1mm diameter, ovoid pale yellow lesions.
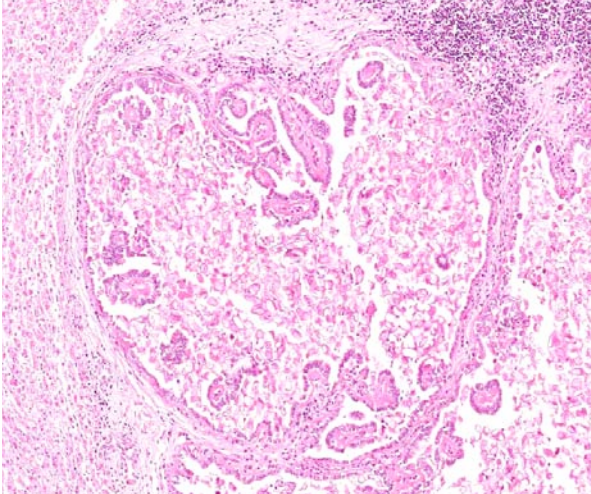
**Histopathologic Description:**  Liver: Diffuse hepatocyte necrosis with the cells exhibiting hyper-eosinophilic, frequently shrunken and fragmented cytoplasmic outline and karyopyknosis/lysis.  Entire hepatic acini are involved including cells of the limiting plates (massive necrosis), with associated light scatterings of heterophils (peracute event).  Bile ducts are severely distended with florid papillomatous hyperplasia of the lining epithelium and in some cases by infiltrates of macrophages, multinucleate macrophage giant cells with surrounding mantles of admixed lymphocytes and plasma cells in the fibrotic portal stroma. Eosinophilic refractile debris along with myriad ovoid to oblong structures with well-defined cytoplasmic boundaries and contained crescent-shaped structures with eosinophilic cytoplasm and a single nucleus (unsporulated coccidial oocysts) and smaller, ovoid structures with basophilic granular content (macrogametes) noted in duct lumens.
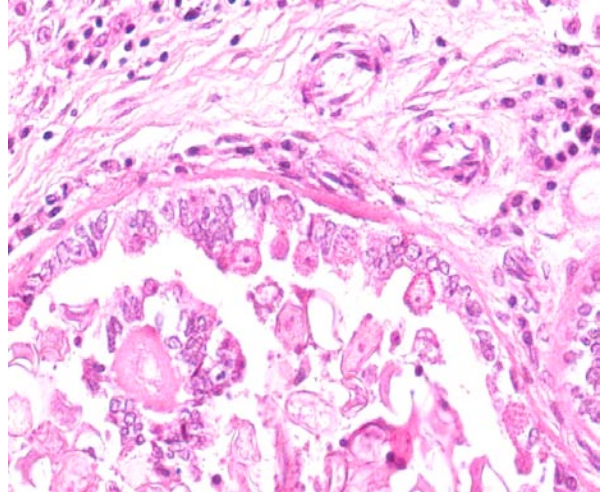
**Contributor's Morphologic Diagnosis:**  Liver: 1. Hepatocyte necrosis, peracute, diffuse (massive), severe, consistent with a diagnosis of viral haemorrhagic disease of rabbits.
2. Bile duct hyperplasia and granulomatous cholangitis, chronic, severe with intra-luminal oocysts/macrogametes consistent with a diagnosis of hepatic coccidiosis.



*4-1. Liver, rabbit: There is diffuse massive necrosis (necrosis in all areas of the hepatic lobule) with loss of hepatic plate architecture. (HE 400X)*

4-2. Liver, rabbit: Liver, rabbit: Multifocally, bile ducts are markedly ectatic, and filled with numerous coccidial oocysts and fronds of proliferating epithelium. (HE 16X)



4-3. Liver, rabbit: Biliary epithelial cells often contain macro- and microgametes of Eimeria steidae. (HE 400X)

**Contributor's Comment:** This case was unusual in that the rabbit presented with a severe, peracute necrotising hepatitis, consistent with a diagnosis of viral haemorrhagic disease of rabbits (VHD), superimposed on more longstanding lesions of hepatic coccidiosis. The most consistent pathological findings in VHD are severe necrotising hepatitis and disseminated intravascular coagulation (DIC).[2,5,13,14,17] Complete hepatic acini including the hepatocytes of the limiting plates are frequently affected in this case justifying the designation massive necrosis. In previous reports hepatocyte necrosis is described as confined to the peripheral zones of lobules.[5] Such extensive peracute hepatic necrosis in rabbits found dead without premonitory signs is considered pathognomonic for VHD.

VHD is an acute, highly fatal disease of European wild and domestic rabbits, first reported from the People's Republic of China in 1984.[5,10] The causative agent is a calicivirus.[10,13] The virus spreads via oral, nasal or parenteral transmission and rapidly replicates in the liver of adult rabbits resulting in death within 48 - 72 hours.[9,13] Virus-induced hepatocyte death is due to apoptosis and in experimentally induced disease, in-situ hybridization identified viral replication in both hepatocytes and macrophages, suggesting infected macrophages contribute to viral dissemination.[2,7,14] Naturally occurring VHD is rarely seen in rabbits less than two months of age, possibly due to differences in the leucocyte response to hepatocyte infection between adults and juveniles.

The lymphocytic rather than heterophilic response observed in the more resistant younger rabbits possibly reflects a protective host response to viral antigens on the hepatocyte surface.[4]

Hepatic coccidiosis in rabbits occurs worldwide and is caused by infection with *Eimeria stiedae*.[6,16] The coccidial oocysts are highly resistant and remain viable in soil and on fomites for long periods. Rabbits become infected by ingesting sporulated oocysts which are broken down in the duodenum, releasing sporozoites that penetrate the intestinal mucosa and travel to the liver either via the blood stream or within macrophages in the lymphatic system. In the liver the sporozoites invade the bile duct epithelium. Following developmental and reproductive phases oocysts are produced and are passed via the bile ducts into the intestines. Oocysts become infective one to four days after they are shed in the faeces.[6,16] The environment can become heavily contaminated in intensive conditions and wild rabbits can be a potential source of infection to domestic rabbits allowed access to grass grazed by wild rabbits.[6]

Infection of bile duct epithelial cells results in hyperplasia and inflammation with large numbers of ellipsoid oocysts in the walls and lumen of the bile ducts.[12] Destruction and regeneration of the bile duct epithelium results in significant cystic enlargement, papillomatous hyperplasia and duct reduplication. There is usually accompanying infiltration of plasma cells, lymphocytes, and occasional epithelioid cells.[12,15] Some enlarged bile ducts rupture initiating a granulomatous

response and accompanying portal fibrosis is often prominent.[12] The oocysts may obstruct biliary outflow resulting in a distended bile duct and ischaemic necrosis can occur in the surrounding liver parenchyma due to compression of adjacent blood vessels by the bile duct enlargement.[12] In rabbits which survive such infection, fibrous tissue can replace much of the normal liver parenchyma.[16]

While there may be no clinical signs in mild infections, heavy infections can result in abdominal enlargement and ascites, with jaundice occurring in advanced stages of the disease. These signs reflect blockage of the bile ducts and interference with hepatic function.[12] Serum biochemistry may reveal elevated alkaline phosphatase, ALT and total bilirubin.[6]

**JPC Diagnosis:** 1. Liver: Necrosis, massive, diffuse.
2. Liver: Cholangiohepatitis, proliferative and lymphoplasmacytic, diffuse, moderate, with coccidial oocysts and gametocytes.

**Conference Comment:** Viral hemorrhagic disease virus (also referred to as rabbit hemorrhagic disease virus, RHDV) along with the closely-related and highly pathogenic European brown hair syndrome virus (EBHSV) make up the *Lagovirus* genus of the family *Caliciviridae*.[8] RHDV causes severe acute periportal to mid-zonal hepatic necrosis resulting in disseminated intravascular coagulopathy in rabbits in the *Oryctolagus* genus; whereas EBHSV causes similar disease in members of the *Lepus* genus. Recently there has been a report of an outbreak of a novel virus, designated Michigan rabbit calicivirus (MRCV), in a privately-owned New Zealand White rabbitry in Michigan. This outbreak was associated with a case fatality rate of 32.5%, with clinical signs including vulvar hemorrhage, epistaxis, ataxia, diarrhea, opisthotonos, ocular discharge, vocalization, hepatic necrosis and death. Experimental infection resulted in subclinical disease, and viral RNA sequencing and capsid amino acid sequencing indicated this calicivirus is distinct from other know lagoviruses.[3] It was suggested that MRCV represents a new variant of the nonpathogenic RCV-like group that includes RCV, Ashington and Lambay.[1] Further research is needed to fully elucidate the phylogenetic relationships of these viruses.[3]

Caliciviruses are non-enveloped, icosahedral, single stranded RNA viruses with characteristic 32 cup-shaped depressions on their surface. Other caliciviruses of veterinary importance include: vesicular exanthema of swine virus, San Miguel sea lion virus, feline calicivirus, and murine norovirus. Other caliciviruses in the genera *Norovirus* and *Sapovirus* have been reported to cause disease in cattle, pigs, wildlife, and non-human primates.[8]

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