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Department of Veterinary Pathology
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Conference Moderator: MAJ Dana Scott
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Falls Church, VA 22041

CASE I – F01 (AFIP 2784498)

Signalment: Juvenile, rainbow trout (*Oncorhynchus mykiss*)

History: Fish are bred in ponds with supply from a local stream. In September several fish died and were sent for histopathologic examination.

Gross Pathology: External signs: Darkened body (melanosis), bilateral exophthalmos, abdominal distension and anemic gills.

Internal signs: Renal swelling, more prominent posteriorly, with multiple beige to brown firm nodules (up to 1 cm in diameter).

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Granulomatous nephritis, multifocal, severe with multiple protozoan parasites.

Contributor's Comment: Proliferative kidney disease (PKD) is a disease of salmonids (rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, Atlantic and Pacific salmon, grayling *Thymallus thymallus*, arctic charr *Salvelinus alpinus*). It mainly affects juvenile fish and typically develops seasonally at water temperature of 15 °C or higher. In rainbow trout, morbidity may be 100% while mortality is usually below 30-50%, the latter being temperature dependant. The precise identity of the parasite is still under investigation, but there is some evidence that the proliferative kidney disease organism unknown (PKX) is an extrasporogonic developmental stage of a myxosporean belonging to *Tetracapsula bryosalmonae n.sp.* Extrasporogonic as well as sporogonic stages of the parasite can be stained in tissue sections using monoclonal antibodies or GS-1 lectin and

standard immunostaining techniques. Bryozoans are recognised as a further host in the life cycle of *Tetracapsula bryosalmonae* n.sp.

AFIP Diagnosis: Kidney: Nephritis, granulomatous, multifocal and coalescing, moderate, with intratubular and interstitial myxosporidians, rainbow trout (*Oncorhynchus mykiss*), piscine.

Conference Comment: The causative organism of proliferative kidney disease (PKD) is a myxozoan in the family Saccosporidae, genus *Tetracapsula*. Recent literature reports the species as *bryosalmonae* (Canning, 1999) and *renicola* (Kent, 2000). Myxozoa, characterized by spores of multicellular origin and two or more polar filaments, parasitize poikilotherms and annelids. The host range of the PKD organism is broad and includes many *Oncorhynchus* sp., *Salmo* sp., Grayling, and Pike. Hematopoietic hyperplasia and damage to vascular endothelium characterize the initial stages of infection. With lesion progression, a severe diffuse granulomatous response develops composed primarily of macrophages with fewer lymphocytes. Macrophages appear epithelial, whorl around parasites, and gradually replace hematopoietic tissue resulting in a significant anemia with hematocrits reported as low as 11% (normal - 40%). Hemolysis, as suggested by increased iron deposits in the spleen, and hematopoietic hypoplasia secondary to chronic disease, contribute to the low hematocrit.

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CASE II – 980846-19 (AFIP 2717908)

Signalment: 24-year-old, female, rhesus macaque (*Macaca mulatta*)

History: This Rhesus macaque was euthanized following a long-term clinical history of prolonged recovery from anesthesia and heart disease. Several hours were required to recover following treatment with standard doses of Telazol (normal recovery time is less than 30 minutes), and a grade IV-V holosystolic murmur was present at time of euthanasia.

Anesthesia (Ketamine)-associated arrhythmias were first noted when this monkey was approximately 11 years old, and a systolic murmur was first reported when the monkey was 12 years old.

Gross Pathology: At necropsy, the left adrenal gland contained a 2 x 1 x 0.5 cm dark red mass which was partially surrounded by a discontinuous thin margin of yellow-tan adrenal cortical tissue. The heart was enlarged to at least twice normal size, had thin ventricular walls and dilated chambers, and severe left atrioventricular valvular insufficiency. Both the left and right side ventricular walls contained coalescing bands of intermixed fibrosis and pale myocardium.

Laboratory Results: Tissue sections stained by argyrophil methods (Sevier-Munger) showed numerous dense brown to black cytoplasmic granules in the tumor cells. By immunohistochemistry, the tumor cells were intensely immunoreactive for chromogranin A and neuron-specific enolase and S-100 and synaptophysin. Tumor cells were essentially negative for GFAP, NFP and vimentin.

Contributor's Morphologic Diagnosis: Adrenal gland: pheochromocytoma.

Contributor's Comment: Examination of H&E stained sections showed this adrenal tumor to have an atypical histologic appearance. Unlike most pheochromocytomas, in which tumor cells form small lobules separated by fine connective tissue septa and capillaries, this tumor consisted of myriad blood filled

spaces that appeared to be lined by polyhedral to low cuboidal tumor cells. The neoplastic cells had moderate amounts of finely granular, pale eosinophilic cytoplasm, and ovoid nucleus, an inconspicuous nucleolus, and were supported by a well-developed reticulin network. In most areas, endothelial cells did not appear to separate the two to four cells-thick layers of tumor cells from the blood elements. The expansile tumor mass had completely replaced normal adrenal medullary tissues and had reduced the normal adrenal cortical tissue to a thin incomplete marginal band. Mitoses were very rare and invasion of blood vessels and surrounding tissues was absent.

Ultrastructurally, many of the round to ovoid tumor cells were seen to contain abundant electron-dense cytoplasmic granules and arrays of rough endoplasmic reticulum. Tumor cells were also seen to be separated from the vascular lumens by a very thin but complete endothelial cell lining. The positive argyrophilic reaction of cytoplasmic granules in the tumor cells, the positive immunoreactivity for chromogranin A and NSE, and the ultrastructural appearance of electron-dense cytoplasmic granules are consistent with a diagnosis of pheochromocytoma and suggest that the cardiomyopathy in this monkey could have been caused by excessive catecholamine release from a functional pheochromocytoma.

In H&E stained sections of the heart (not included in this submission), there was extensive myocardial fibrosis and some acute myocytolytic areas. This pattern is typical of prolonged and decompensated stress cardiomyopathy which is caused by catecholamines cardiotoxicity.

AFIP Diagnosis: Adrenal gland: Pheochromocytoma, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: Pheochromocytomas are neuroendocrine neoplasms derived from chromaffin cells of the adrenal medulla. They are infrequently functional and are most commonly reported in dogs, cattle, and rats. Histologically, more typical pheochromocytomas are composed of small round to polygonal cells with finely granular cytoplasm and are arranged in small nesting or alveolar patterns (Zellballen). Considerable nuclear pleomorphism can be present. A diagnosis of malignancy is often based on invasion of the capsule, invasion of adjacent structures (e.g. caudal vena cava), or metastasis.

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CASE III – PA-3767 (AFIP 2787399)

Signalment: Adult, male, Cynomolgus monkey (*Macaca fascicularis*)

History: Two monkeys had unexplainably died per-acutely during routine bronchoscopic evaluation of the right lung. An additional animal (the case submitted here) was found moribund and dyspneic the following day (~ 12-16 hours after a similar bronchoscopy) and died during examination.

Gross Pathology: At necropsy, the right lobes were reported to be congested and somewhat edematous. The lung block was insufflated with formalin and submitted. Upon receipt and pre-trimming examination of the wet (fixed) tissue, a marked firmness was present within the hilar portions of the right diaphragmatic, right anterior and to a lesser extent, right middle lung lobes. On cut surface, circumscribed and somewhat distinctly margined areas of consolidation and pallor were noted in the hilar regions of these lobes, with remaining lobar tissue appearing congested and mildly edematous. Several cystic/cavitary areas 2 – 5 mm in diameter were seen throughout right lung tissue.

Laboratory Results: None.

Contributor's Morphologic Diagnosis:

1. Fibrinous and proteinaceous alveolar fluid accumulation, focally extensive to diffuse, marked to severe, with associated patchy foci of neutrophilic inflammation and alveolar wall degeneration
2. Early hyaline membrane formation, multifocal, mild (some sections)
3. Anthracotic pigment, multifocal, primarily peri-vascular, mild

(Histomorphological changes consistent with acute alveolar damage were not observed in any areas of left lung tissue examined.)

Overall Diagnostic Impression: Consistent with acute lung injury

Contributor's Comment: The microscopic findings are consistent with an acute lung injury, and the alveolar damage present resulted in the development of an early phase of a condition analogous to adult respiratory distress syndrome (ARDS) as described in humans and experimental animal models.

ARDS is a common endpoint associated with multifactorial causes – all of which injure the integrity of the barrier associated with fluid and protein flow in the lung. There are two broad categories of mechanisms of acute lung injury: those that are indirect (i.e. requiring the participation of intermediary mechanisms such as host defenses) and those that are direct (i.e. injury probably occurs as a result of contact between an offending substance and lung tissue). The list of initiating factors of ARDS in people includes a wide variety of infectious causes, aspiration, trauma, hemodynamic disturbances, including shock, numerous drugs, inhaled toxins, certain metabolic, neurologic or obstetrical/gynecologic disorders and other miscellaneous causes including electrical injury. Pulmonary morphological changes depend more on the severity and duration of injury than cause. Exudative, proliferative and fibrotic changes usually appear in sequence.

In this case, the loss of two additional animals per-acutely during benign bronchoscopy the previous day and a variety of other exclusionary factors, focused primary attention on possible electrical malfunction of the endoscopy equipment. Evaluation of the scope and power source by hospital equipment engineers revealed electrical abnormalities consistent with significant shock potential.

Arrhythmias and death due to electrical malfunction during endoscopy have been reported in people. We believe that the two animals, which expired per-acutely during bronchoscopic evaluation the previous day, probably died due to an electrically induced fatal arrhythmia. In the animal submitted for this conference, no post-manipulation electrocardiographic data was available and microscopic evaluation of heart tissue, including conduction system did not reveal morphological abnormalities. The potential of cardiac conduction disturbances as a contributing factor in this animal's death was not excluded.

AFIP Diagnosis: Lung: Edema and fibrin deposition, alveolar and perivascular, diffuse, severe, with fibrin thrombi, multifocal septal necrosis, and neutrophilic inflammation, cynomolgus monkey (*Macaca fascicularis*), nonhuman primate.

Conference Comment: Acute (Adult) respiratory distress syndrome (ARDS) is the result of diffuse damage to the capillary endothelium and alveolar epithelium with failure of normal fluid barriers. Although the exact pathogenesis is unclear, the initial condition is characterized by an inappropriate inflammatory response and mediated by the complex interaction of numerous chemical mediators. Neutrophils and macrophages, and to a lesser extent epithelial cells and fibroblasts, secrete various proteases, eicosanoids, toxic oxygen products, and cytokines (IL-1, IL-6, IL-

8, and TNF α). As one of many functions, IL-1 and TNF α activate endothelial cells. Activation results in cytoskeletal reorganization and endothelial gap formation; rapid redistribution of P-selectin (present in Weibel-Palade bodies) to the cell surface; increased expression of E-selectin and immunoglobulins (ICAM-1 and VCAM-1); and secretion of cytokines (IL-1, IL-6, IL-8).

Additional factors involved in the pathogenesis of ARDS include LTB₄ and C5A which are chemotactic for neutrophils; macrophage inhibiting factor, an extrapulmonary factor produced by the pituitary gland, which increases expression of IL-8 and TNF α ; and antiinflammatory mediators such as soluble TNF receptors, IL-1 receptor antagonists, IL-8 autoantibodies, IL-10, and IL-11. Recently, the role of the neutrophil in the pathogenesis of ARDS has been questioned as ARDS may develop in severely neutropenic human patients or in neutrophil independent animal models.

Resolution is mediated through removal of protein from the alveoli by diffusion or active removal by endothelial cells (pinocytosis) or macrophages (phagocytosis); reepithelialization via type II pneumocyte hyperplasia; and clearance of inflammatory cells (neutrophils) by apoptosis.

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CASE IV – S99-1265 (AFIP 2790942)

Signalment: 7-years-old, female, Alsatian-mix (*Canis lupus familiaris*), canine

History: The animal suddenly showed a severely reduced general body condition with fever (39.9 °C). Polydipsia and vomiting were observed. It was treated symptomatically with antibiotics, antiemetics, corticosteroids and intravenous infusions without success and died the next day with seizures. The dog used to go swimming in a lake.

Gross Pathology: The subcutaneous fatty tissue and mucosal surfaces of the carcass showed a light yellow color (generalized icterus and anemia). The body lymph nodes were generally enlarged with dark red discoloration. The small intestine was filled with dark red to black, pasty contents. Petechial to ecchymotic hemorrhages were found on serosal surfaces, in the subcutis, stomach, urinary bladder, and heart. The lung showed massive, diffuse bleeding. Kidneys were slightly swollen and dark red.

Laboratory Results:

Urea (cranial eye chamber fluid): > 56 mmol/l (-> uremia, severe)

Fluorescent serology (kidney swabs): negative for *Leptospira icterohaemorrhagiae*

Bacterial cultures (liver, kidney, and spleen): negative (positive for antibiotics)

- Contributor's Morphologic Diagnoses:**
1. Liver: Hepatocellular dissociation, severe, diffuse, with intrahepatocellular and intrasinusoidal spirilliform bacteria
 2. Intrahepatic cholestasis, moderate
 3. Kidney: Tubulonephrosis, acute, severe, diffuse, numerous microthrombi in interstitial vessels and glomeruli (DIC), intratubular proteinaceous casts, fibrinoid degeneration of vessel walls and severe interstitial and glomerular hemorrhages with intratubular and intraepithelial spirilliform bacteria
 4. Kidney: Nephritis, acute, interstitial, purulent, moderate to severe

Contributor's Comment: Silver stained sections (Steiner's method) of liver and kidney revealed a moderate number of irregularly distributed, approximately 5-20 um long, thin, spiralled, silver-positive, intracellular (hepatocellular, resp. intraepithelial) and extracellular (sinusoidal, resp. intratubular) bacterial organisms with a morphology compatible with *Leptospira* sp.

This animal was the first of a series of seven histologically confirmed (plus six suspected) cases of canine leptospirosis investigated in our institute in the spring and early summer of 1999. Among these were not only young animals but also adult vaccinated individuals. Serologic testing of some of these cases showed high titers for leptospira serovars, which are not included in commercially available vaccines (e.g. 1:3200 *L. bratislava*, 1:1600 *L. bataviae*).

The kidney swabs used for indirect immunofluorescence for *Leptospira icterohaemorrhagiae* were negative in all cases. In 4 cases frozen tissue samples have been sent to the Texas Veterinary Medical Diagnostic Laboratory System (TVMDLS, laboratory of Dr. Loyd and W. Sneed) where a PCR for *Leptospira* sp. was performed. But even these results were all negative.

Nevertheless the final diagnosis leptospirosis was given, based on the detection of silver-positive spirochetal bacteria in kidney and/or liver which showed typical histological alterations. In literature the type of nephritis is mostly described as interstitial and lymphoplasmacellular. In contrast to this, all our cases showed a rather severe purulent interstitial nephritis, severe DIC, and altered vessel walls of the glomeruli and the interstitium.

AFIP Diagnoses: 1. Kidney: Nephritis, tubulointerstitial, necrotizing and hemorrhagic, acute, diffuse, severe, with fibrinoid vasculitis and fibrin thrombi, Alsatian-mix (*Canis lupus familiaris*), canine.
2. Liver: Hepatocellular dissociation, diffuse, severe, with degeneration, hemorrhage, and frequent multinucleate hepatocytes.

Conference Comment: Upon infection leptospiral organisms multiply rapidly within the bloodstream and quickly disseminate to multiple tissues where further replication takes place. Severity of lesions is both host and serovar dependent with serovars canicola and grippotyphosa associated with renal lesions and minimal hepatic involvement while icterohaemorrhagiae and pomona serovars result in more significant hepatic lesions. In general, younger animals are more severely affected with more significant hepatic lesions regardless of the specific serovar. Infection is normally cleared by humoral response except within the kidney where organisms persist for weeks to months within tubular epithelium.

Prepatent periods, intermittent shedding, low organism numbers, fastidious growth requirements, and serovar cross-reactivity can make specific serovar diagnosis troublesome.

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