The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CONFERENCE 1
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Armed Forces Institute of Pathology
Washington, DC 20306-6000

CASE I – N97-238 (AFIP 2593295)

Signalment: 1 month-old, Arabian, male, equine

History: This foal presented with a one week history of lethargy, uveitis, bloody diarrhea and multiple synovial effusions. Owner elected euthanasia due to poor response to treatment and poor prognosis.

Gross Pathology: The mucosa of the colon was red and contained multiple discrete areas of ulcerations with indurated margins. The colonic ulcers ranged from 0.2 to 1 cm in diameter. There were multiple well-described yellow nodules ranging from 0.2 to 1 cm in diameter randomly distributed in the liver parenchyma. These nodules exuded a pale viscous fluid. Small abscessations were present in the lung. The retropharyngeal, tracheobronchial and mesenteric lymph nodes were enlarged.

Laboratory Results: Aerobic culture of liver and mesenteric lymph nodes resulted in 100% very heavy growth of *Rhodococcus equi.* The samples were negative for *Salmonella spp.*

Fibrinogen: 600 mg/dl
Plasma protein: 4.5 g/dl
RBC 5.4 M/ul
WBC 46.9 ul with shift to the left

Intracellular bacteria were noted within neutrophils.

Contributor's Morphologic Diagnosis: Hepatitis, granulomatous, chronic, multifocal, severe, liver with intralesional intracellular and extracellular Gram-positive bacterial rods. Etiology: *Rhodococcus equi*

Contributor's Comment: The pulmonary form of *Rhodococcus equi* is generally more common than the intestinal form. Both forms may occur together and may spread to

draining lymph nodes. It is uncommon for more widespread dissemination to occur, resulting in hepatic abcsessations, as in this case. Hypopyon has been reported in systemic *Rhodococcus equi* infection. The uveitis, as in this case, may be a less severe manifestation. *Rhodococcus* has been isolated from other domestic animal species as well. On occasion it affects human beings.

AFIP Diagnosis: Liver: Hepatitis, pyogranulomatous, multifocal and random, moderate, with numerous intrahistiocytic coccobacilli, Arabian, equine.

Conference Comment: A normal inhabitant of soil, herbivore and avian feces, and the equine intestinal tract, *Rhodococcus equi* causes significant worldwide morbidity and mortality in foals aged one to three months. Natural infection with *R. equi* is acquired via inhalation or ingestion, with subsequent bacteremia and hematogenous spread within macrophages. In foals, it most commonly results in severe pyogranulomatous bronchopneumonia with tracheobronchial pyogranulomatous lymphadenitis; and less frequently ulcerative enterocolitis, and rarely osteomyelitis, and hypopyon. In this case, the pyogranulomatous hepatitis strongly suggests hematogenous spread of *R. equi* to the liver via the portal circulation.

R. equi is a Gram-positive, pleomorphic, facultative, intracellular coccobacillus. The virulence of this bacterium is strongly associated with a plasmid that encodes a 15-17 kD virulence associated protein, VapA. VapA generates a profound humoral immune response. There is blockage of phagosome – lysosome fusion and premature lysosome degranulation within macrophages. Virulence proteins enable the bacterium to multiply and survive. Cytokine, enzyme, and bacterial toxin release result in widespread caseous necrosis and additional recruitment of infected macrophages to the affected area.

R. equi can also infect cattle, sheep, goats, pigs, and rarely dogs and cats. It is zoonotic, especially in the immunocompromised.

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CASE II – PA3473 (AFIP 2839311)

Signalment: 9.5 year-old female Rhesus macaque (*Macaca mulatta*)

History: This adult obese breeder female had given birth to a large (774 gram) infant 11 days earlier. The mother and baby were transferred from an individual housing cage unit to a gang pen with 5 other animals 3 days previously. She underwent rapid weight loss, became weak, lethargic and was euthanized in a moribund condition after failure to respond to supportive therapy.

Gross Pathology: The esophageal mucosa was uniformly thickened and rugous in appearance, with multiple, confluent linear ulcers and extensive dark bloody fluid. The liver was enlarged, pale and friable. Kidneys were uniformly pale.

Laboratory Results: Urinalysis: +++ glucose, ++ ketones

Clinical chemistries:
Blood glucose was 269 mg/dl. There was also a modest elevation in BUN, creatinine and mild hyponatremia and hypocalcemia.

Pharyngeal culture yielded a heavy growth of *Candida albicans*.
Herpes B cultures (oral, conjunctival and vaginal) were negative.

Contributor's Morphologic Diagnoses:
1. Esophagus: Esophagitis, necrotizing & ulcerative, with superficial yeast and pseudohyphal structures consistent with *Candida* (organisms are not plentiful in some slides)
2. Liver: Vacuolar (fatty) change, macrovacuolar, hepatocytes, patchy-diffuse, moderate
3. Pancreas: Islet cell amyloidosis, diffuse, severe

Contributor's Comment: The rapid weight loss experienced by this obese, post-parturient macaque may well have been associated with a poorly controlled diabetic state. Obese rhesus monkeys that lose weight rapidly regardless of the specific inciting cause can progress into a clinical-pathologic condition known as fatal fasting syndrome.

The stress associated with the housing change and post-parturient status, exacerbated by an uncontrolled diabetic state appeared to initiate this significant weight loss and trigger fat mobilization, leading to this fatal metabolic disorder. The morphological changes seen in this case, although not as severe as sometimes present in this syndrome, are quite typical; including the presence of vacuolar change in renal tubular epithelium (not submitted). Hepatic fatty change is also described in diabetes and it is possible that some of the lipid accumulation in this case may be associated with the presence of this underlying condition. Both processes may be related to the same underlying metabolic process – i.e. altered carbohydrate metabolism and increased fat mobilization.

The diabetic condition present in this monkey is likely related to the severe accumulation of amyloid within pancreatic islets. The principal constituent of pancreatic amyloid is amylin, a 37 amino acid polypeptide which is islet cell derived. In other macaques (*M. nigra*), the quantity of islet amyloid accumulation correlates directly with impaired insulin secretion, glucose clearance and the severity of the diabetic state. This correlation is not confirmed in cats and humans, in which similar islet cell pathology is commonly recognized.

Severe necrotizing and ulcerative esophagitis secondary to *Candida albicans* was also present in the case. Diabetic patients have a higher carriage rate for numerous organisms, including *Candida*, particularly when metabolic control is unsatisfactory. Additionally, there is a general increased susceptibility to infection in diabetics, which, although multifactorial, may be related to a large extent to the hyperglycemic impairment of chemotaxis, adherence, phagocytosis and intracellular killing of microorganisms in leukocytes.

AFIP Diagnoses: 1. Pancreas, islets of Langerhans: Amyloidosis, diffuse, moderate, Rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Pancreas, exocrine: Degeneration and atrophy, diffuse, mild to moderate, with epithelial cell vacuolation.
3. Liver: Vacuolar degeneration, lipid type, diffuse, moderate.
4. Esophagus: Esophagitis, necrotizing and ulcerative, acute, diffuse, moderate, with fungal pseudohyphae and rare yeasts, etiology consistent with *Candida sp.*

Conference Comment: Fatal fasting syndrome is most commonly seen in obese, female, mature macaques with a history of sudden anorexia. Clinicopathologically, there can be elevated BUN and creatinine, and anemia. Suggested control measures for this syndrome include weight management and reduction of stress.

Pancreatic islets of Langerhans include alpha, beta, delta, F (pancreatic polypeptide), and enterochromaffin cells. Blood glucose concentration is maintained in narrow homeostatic limits by the antagonistic actions of insulin and glucagon. Insulin is secreted by beta cells in response to hyperglycemia. The primary targets of insulin are hepatocytes, adipocytes, and myocytes. Insulin acts on carbohydrates to facilitate glycolysis; on lipids to stimulate lipogenesis; and on proteins to form ATP, DNA and RNA. Insulin acts to decrease lipolysis, proteolysis, ketogenesis, and gluconeogenesis, which lower blood glucose. Glucagon is released from alpha cells in response to hypoglycemia. Glucagon acts to increase glycogenolysis, gluconeogenesis, and lipolysis, which increase blood glucose.

Other pathological changes that can occur with diabetes mellitus include cataract formation, retinopathy, renal vasculopathy, pancreatic acini ectasia, pancreatic necrosis, renal lipidosis, and microvascular disease.

Amyloid deposition in the islets begins in the pericapillary region, which results in the gradual replacement and loss of islet cells, with subsequent insulin decrease. When islet amyloid exceeds 50-60%, diabetes mellitus can be recognized, and the severity is related to the amount of islet amyloid. Stains that demonstrate the dichroism of amyloid include Congo red, toluidine blue and sulfated alcian blue.

Conference participants experienced difficulty in identifying the esophagus because of the paucity of mucosa. A Gomori's methenamine silver nitrate stain verified the presence of *Candida* organisms.

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CASE III – 01-14264 (AFIP 2841368)

Signalment: 10 year-old, female mixed breed dog

History: Mass palpable in the jugular furrow was removed surgically. There were no associated clinical signs. The dog was clinically normal 6 months after surgical removal of the mass.

Gross Pathology: The mass was described by the referring veterinarian as 10 cm x 5 cm and egg-shaped.

Laboratory Results: None

Contributor's Morphologic Diagnosis: Thyroid C cell carcinoma

Contributor's Comment: Sections of thyroid gland were mostly composed of cells arranged in solid packets separated by delicate fibrovascular stroma. Thyroid tissue was reduced to a few compressed follicles at one margin. Cells comprising the mass were medium sized, polygonal, with distinct cellular borders and abundant, palely amphophilic, granular cytoplasm. Nuclei were relatively uniform, round, medium sized, with coarsely stippled chromatin, and up to two small basophilic nucleoli. Mitoses averaged two per 400x field. Many tumor cell lobules had central, small lakes of erythrocytes. Tumor cells formed sheets infiltrating between collagen fibers of the capsule. Rarely, cells within the tumor were lining follicles containing eosinophilic colloid. A few blood vessels had subendothelial aggregates of tumor cells. One section had parathyroid tissue.

Blocks were submitted to Prairie Diagnostic Services in Saskatoon, Saskatchewan, Canada for immunohistochemistry. Tumor cells were variably positive for calcitonin. Thyroglobulin positive cells at the periphery of the tumor were felt to be entrapped non-neoplastic follicular cells (see accompanying photomicrograph).

Tumors of thyroid C cells (also called parafollicular cells or ultimobranchial derivatives) are most commonly seen in aged bulls and horses and in some strains of laboratory rats. They are considered rare in dogs and other species. The term 'medullary carcinoma' has been applied to the tumors in humans and in dogs. Tumors in bulls are most properly called ultimobranchial tumors as they are complex tumors composed of both neoplastic C cells and primitive ultimobranchial elements; they are often associated with multiple endocrine neoplasms and elevated circulating calcitonin levels.

In dogs, most C cell tumors are solitary and non-functional. Although they are less common than follicular cell carcinomas, it is important to differentiate them from solid forms of the more aggressively malignant follicular tumors. Thyroid C cells (and C cell tumors) in dogs are positive by immunohistochemistry for calcitonin, calcitonin gene-related peptide and chromogranin A, whereas follicular cell tumors are routinely positive for thyroglobulin. In this case, differential staining for calcitonin and thyroglobulin allowed a diagnosis of C cell carcinoma. Although there was histologic

evidence of vascular invasion, short term follow up on this dog suggested a long clinical course, as has been reported previously for canine medullary carcinoma.

AFIP Diagnoses: 1. Thyroid gland: C cell carcinoma, mixed breed, canine.
2. Parathyroid gland: Hyperplasia, nodular, multifocal, mild.

Conference Comment: Canine thyroid gland neoplasms occur most often in old dogs, and the vast majority detected clinically are follicular cell carcinoma; C cell carcinoma is rare. The most frequent clinical sign associated with C cell carcinoma in dogs is a palpable ventral cervical mass. Affected dogs are usually euthyroid; however, hypothyroidism and rarely hyperthyroidism can occur. Serum calcium level is usually normal; however, hypocalcemia has been reported. Hypocalcemia can result in hypertrophy and hyperplasia of parathyroid gland chief cells. Features used to differentiate adenoma from carcinoma include: size of neoplasm, atypia, frequency of mitoses, and extracapsular invasion. The contributor has provided a concise summary of the immunohistochemical staining characteristics of C cell and follicular cell carcinomas.

The primary differential diagnosis in this case is thyroid follicular cell carcinoma, solid type. The neuroendocrine pattern and immunohistochemistry results provided by the contributor support the diagnosis of C cell carcinoma.
Additionally, immunohistochemistry performed in our laboratory revealed chromagranin and neuron specific enolase in neoplastic cells. The Churukian-Schenk method also demonstrated neurosecretory granules in tumor cells and pre-existing C cells. These results provide additional support for the diagnosis.

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CASE IV – 89-5450-3 (AFIP 2839000)

Signalment: 3 month-old Holstein calf

History: Animal found dead.

Gross Pathology: Severe fibrinonecrotic bronchopneumonia affecting 50% of the lungs.

Laboratory Results: *Mannheimia haemolytica* was isolated in pure culture from the pneumonic lesions. Respiratory syncytial virus and BVD virus were also demonstrated.

Contributor's Morphologic Diagnosis: Fibrinonecrotic bronchopneumonia

Contributor's Comment: In this lung, there is a fibrinous alveolitis with many clusters of degenerate inflammatory cells appearing as round cells with pyknotic nuclei and cells with elongated or streaming nuclei (oat cells). There are focal areas of coagulation necrosis with many bacterial colonies; these areas are marginated by densely packed degenerate leukocytes. Several interlobular and subpleural lymphatics are thrombosed. Lesions of bronchiolar necrosis are also present. The characteristic histologic feature of fibrinous pneumonias caused by *Mannheimia haemolytica* is the presence in the fibrinous exudate of clusters of degenerate inflammatory cells with elongated or streaming nuclei (oat cells). These cells are not a feature of the fibrinous pneumonias caused by *Haemophilus somnus* in which degenerate leukocytes appear as round cells with pyknotic nuclei. These oat cells seem to result from the effect on inflammatory cells of a leukotoxin produced by *M. haemolytica*. This exotoxin is specifically lethal to leukocytes and platelets of ruminants by the formation of cell membrane pores. Similar oat cells are found in pneumonic lesions caused by *Actinobaccillus pleuropneumonia* in pigs; this bacterium produces Apx I, Apx II and Apx III toxins lethal for pig alveolar macrophages. Similar lesions are caused by strains of *Actinobacillus suis* producing Apx I and Apx II toxins. The degenerate round cells with pyknotic nuclei found in these pneumonias are probably degenerate alveolar macrophages by the effect of other bacterial toxins such as endotoxins. We see these degenerate round cells in acute pneumonias of other species caused by Gram-negative bacteria such as *E. coli* in dogs.

AFIP Diagnosis: Lung: Pneumonia, fibrinous and necrotizing, suppurative, diffuse, severe, with oat cells, colonies of coccobacilli, marked interlobular edema, and lymphatic fibrin thrombi, Holstein, bovine.

Conference Comment: A normal inhabitant of the bovine nasal cavity, *Mannheimia haemolytica* is a Gram-negative bacillus that causes sporadic, severe, respiratory disease in young cattle. Bovine pulmonary mannheimiosis occurs secondary to environmental stress and is predisposed by viral infections, including parainfluenza 3 virus, bovine herpesvirus-1, and bovine respiratory syncytial virus. There are four virulence factors associated with *M. haemolytica*: leukotoxin, endotoxin (lipopolysaccharide), fimbriae, and polysaccharide capsule. The endotoxin modifies bovine leukocyte function, activates macrophages for release of TNFα, IL-1β, and IL-8, which amplify the inflammatory response, and is toxic to endothelium. The leukotoxin is

a heat-labile protein exotoxin that, as the contributor stated, destroys leukocytes through formation of a membrane pore. Fimbriae aid in upper respiratory tract colonization of the bacteria, and a capsule inhibits complement, phagocytosis, and intracellular killing of the organism. *M. haemolytica* also causes disease in lambs, kids, and rarely pigs.

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