The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 1

5 September 2007

Moderator:

Dr. Michelle Fleetwood, DVM, Diplomate ACVP

CASE I – 07-15544 (AFIP 3066312).

Signalment: 13-year-old, castrated, male, Quarter horse (*Equus caballus*)

History: There were multiple subcutaneous masses in the lateral aspect of both right and left proximal forearms. These lesions appeared within the past 6 months. Lesions are not apparently painful and there is no associated lameness or other clinical signs. The horse is heterozygous for the hyperkalemic periodic paralysis mutation. A portion of each lesion was excised and submitted for histopathology. No association with underlying skeletal muscle was detected at surgery.

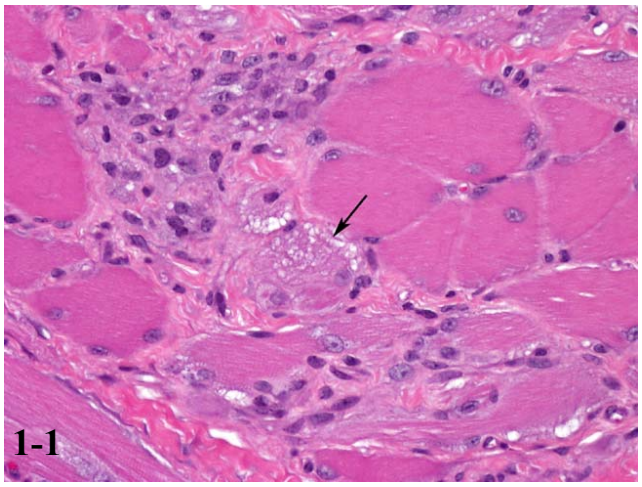
Gross Pathology: Discrete firm pale tan nodular masses with normal overlying haired skin

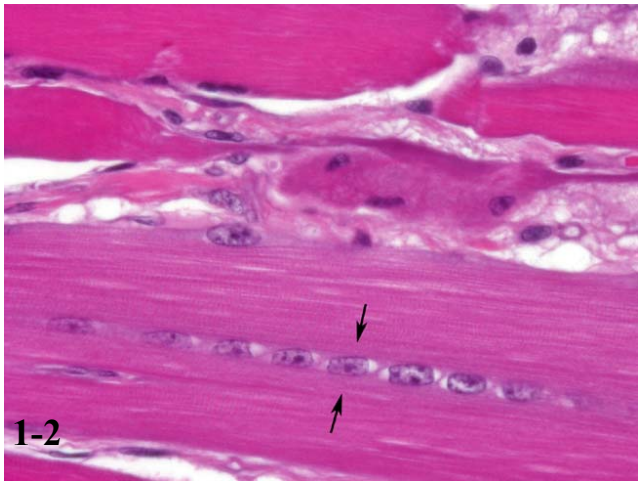
Laboratory Results: *Blastomyces dermatitidis* was isolated from a lung swab prior to necropsy.

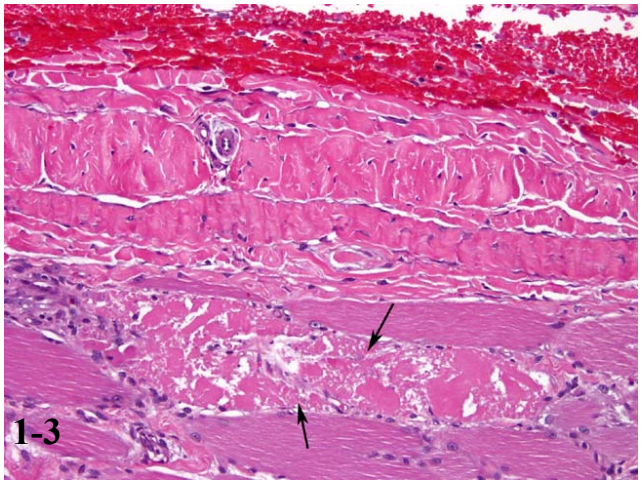
Histopathologic Description: Two wedge samples, one from the right foreleg and one from the left foreleg, were submitted and representative sections were submitted for histopathology. Both lesions are similar and are composed of haired skin with underlying cutaneous skeletal muscle (presumed cutaneous omobrachialis, although the site appears slightly more distal than anatomy texts describe for insertion of this muscle in the horse). Architecture of the skeletal muscle is markedly distorted to effaced, with expansion to form irregular nodular masses. Myofibers within the masses exhibit varying degrees of the following changes:

- Disarray of orientation, with some fibers in transverse section, others in longitudinal section, and still others in oblique section
- Severe chronic myopathic change, including marked variation in fiber size with fiber hypertrophy and also rounded to angular atrophy, endomysial and perimysial fibrosis, internal nuclei, fiber splitting, and subsarcolemmal pale zones containing pale pink to gray finely granular material (sarcoplasmic masses)
- **Degenerative (fig, 1-1)** and **regenerative (fig. 1-2)** changes, including segmental coagulation **necrosis (fig. 1-3)** – often with macrophage infiltration – and vacuolar degeneration. Small diameter, slightly basophilic fiber segments with prominent euchromatic nuclei – often in clusters or short chains - are indicative of myofiber regeneration
- Multifocal, typically mild to moderate, interstitial infiltrates of lymphocytes

Masson's trichrome stain confirms the presence of endomysial and perimysial fibrosis. Sarcoplasmic masses and vacuoles do not stain with either trichrome stain or Periodic acid-Schiff stain for glycogen, and no abnormal gly-







cogen aggregates are present.

Contributor's Morpho logic Diagn osis: Skeletal muscle, cutaneous omobrachialis: Pseudotumor consistent with focal myositis.

Contributor's Comment: The term muscle pseudotumor encompasses a group of benign non-neoplastic processes causing mass lesions within skeletal muscle.[1-5] The muscle pseudotumors recognized to date in animals are myositis ossificans, musculoaponeurotic fibromatosis ("desmoid tumor"), and fibrotic myopathy in horses, and myositis ossificans and a lesion simply termed muscle pseudotumor in dogs. The latter lesion is characterized by profound myopathic changes, interstitial connective tissue infiltration, mild to moderate myofiber necrosis and regeneration, and a variable degree of inflammation, most often lymphocytic.[2] These features are also typical of the muscle pseudotumor reported as focal myositis in people.[1,3-5] Diagnosis of any muscle pseudotumor relies on a clinical history of a nodular mass within skeletal muscle, with no other neuromuscular or systemic disease signs, as in the absence of this history a diagnosis of muscular dystrophy, chronic denervation atrophy, or chronic myositis is possible.

Muscle pseudotumors in people occur most often in limb muscle, although other sites are possible. Patients describe these lesions as either non-painful or as being associated with mild discomfort or dull pain.[1,3-5] Although trauma has been proposed as a cause, careful case studies of affected people have not detected a history of prior trauma to the area.[1,3-5] Subclinical muscle tearing has been speculated to be a possible cause.[3] Evidence of peripheral nerve damage has been detected within some muscle pseudotumors in people, but is not common and is thought to be a secondary event rather than a primary cause.[4] There is no apparent age or gender predisposition in people.[1,3-5]

In people, muscle pseudotumors must be differentiated from localized initial forms of polymyositis.[5] No such association has been identified in animals. This horse was otherwise clinically normal, and the history of being heterozygous for hyperkalemic periodic paralysis was not considered to be related to the development of these le-

1-1 Skeletal muscle, Quarter horse. Myofiber degeneration, characterized by swollen, pale, vacuolated sarcoplasm (arrows). (H&E 400X)

1-2 Skeletal muscle, Quarter horse. Myofiber regeneration, characterized by basophilic sarcoplasm with large frequently rowed, internalized nuclei (arrows). (H&E 600X)

1-3 Skeletal muscle, Quarter horse. Myofiber necrosis, characterized by hypereosinophilic sarcoplasm with loss of cross striations, fragmentation and pyknotic, karyolytic or karyorrhectic nuclei (arrows). (H&E 200X)

sions. It is curious that this case occurred bilaterally, in what appears to be the distal cutaneous omobrachialis muscle, in a lateral location that is less likely to be traumatized than cranial areas. Similar to case studies of focal myositis in people, there was no history of trauma to this area.

Muscle pseudotumors in animals have not been described as being associated with pain. Locations include within limb muscle,[2] as in this case, but these lesions have also been seen in scapular[2] and laryngeal muscle (unpublished observations). In pseudotumors of dogs and horses that this contributor has studied, lymphocytic inflammation is extremely variable and often not prominent. A similar situation is described in people with focal myositis.[1,3] An additional characteristic histopathologic finding in focal myositis-like muscle pseudotumors in animals, apparently not described in human cases, is prominent disarray of myofiber arrangement, with the finding of transverse, longitudinal, and obliquely arranged myofibers within the same section.[2]

Surgical excision of these lesions is curative in people and also in animals. Progression beyond the initial growth phase, which can be rapid, is not described.[1,3-5] In this current case only portions of the lesions had been excised at the time of this submission. Follow up is planned in order to determine future behavior.

AFIP Diagnosis: Haired skin and skeletal muscle, cutaneous omobrachialis (per contributor): Myocyte degeneration, necrosis and loss, hypertrophy, and regeneration, focally exensive, moderate, with myofiber disarray, fibrosis, and mild chronic-active myositis, Quarter horse (*Equus caballus*), equine.

Conference Comment: The contributor provides a thorough review of muscle pseudotumors in dogs and horses. Not much is known about this idiopathic condition, and without knowledge of clinical history, or gross images, it is a difficult diagnosis to make. It is thought that focal myositis, myositis ossificans, and musculoaponeurotic fibromatosis (desmoid tumor) arise from an abnormal response to muscle trauma, while fibrotic myopathy results from a denervation injury. There was a small amount of variability in the amount of fibrosis and inflammation among slides. Several slides contained areas with a high mitotic rate, which were interpreted as areas of intense regeneration. No infectious organisms were seen on special stains performed at AFIP [Brown & Brenn (B&B), Brown & Hopps (B&H), Gomori's methenamine silver (GMS), Periodic acid-Schiff (PAS), Ziehl-Neelsen (ZN)].

This case presents great examples of the histologic changes in skeletal muscle response to injury. Degenerating muscle is swollen with pale vacuolated sarcoplasm. Necrotic muscle fibers are shrunken and hypereosinophilic, with a loss of cross-striations, and may be fragmented. Regenerative muscle has basophilic sarcoplasm with multiple centralized and linearly-arranged nuclei (nuclear rowing). They are often surrounded by an increased number of satellite cells. Other common changes include atrophy, hypertrophy and fibrosis. The myofiber disarray is a characteristic lesion of focal myositis/muscle pseudotumor in horses and dogs, and along with the clinical history, helps distinguish it from other causes of skeletal muscle degeneration and necrosis.

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References:

1. Colding-Jørgensen E, Laursen H, Lauritzen M: Focal myositis of the thigh: report of two cases. Acta Neurol Scand 88:289-292, 1993
2. Cooper BJ, Valentine BA: Muscle pseudotumors. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed, pp. 359-361. Iowa State Press, Ames, IA, 2002
3. Heffner RR, Armbrustmacher VW, Earle KM: Focal myositis. Cancer 40:301-306, 1977
4. Heffner RR, Barron SA: Denervating changes in focal myositis, a benign inflammatory pseudotumor. Arch Pathol Lab Med 104:261-264, 1980
5. Vercelli-Retta J, Ardao G, De Cabrera M: Focal myositis and its differential diagnosis. a case report and review of the literature. Ann Pathol 8:54-56, 1988

CASE II - 07-533 (AFIP 3067221).

Signalment: 12-yr-old male, castrated, West Highland white terrier (*Canis familiaris*), dog

History: Starting in November 2006, the patient developed periodic episodes of coughing fits (dry, hacking, non-productive). Coughing episodes increased over several weeks. In January 2007, the owners noticed the dog had increased respiratory rate and effort. The dog was started on Clavamox® but the respiratory problems continued with no improvement. Two days prior to admis-

sion (1/9/2007), the owner reported that the dog had respiratory distress with an abdominal component, and lethargy.

On presentation, the patient's mucus membranes were cyanotic, pulse = 162, respiratory rate = 60 – 80, and crackles were ausculted bilaterally. No murmur was heard, but heart sounds were difficult to hear over the crackles. The dog was placed in an oxygen cage and heart rate decreased to 120 and mucous membranes were pink. Jugular pulses were increased. Cough could not be elicited on tracheal palpation. Respiratory rate and effort remained increased while in the oxygen cage.

Only one lateral thoracic radiograph was able to be obtained before the dog became very distressed and was placed back in the oxygen cage. The radiograph showed mild to moderate right sided cardiomegaly and diffuse interstitial to alveolar lung pattern, more pronounced dorsocaudally.

A brief echocardiogram, with the dog standing in the oxygen cage, revealed extremely enlarged right ventricle with thickened free wall.

Physical exam, radiographic and echocardiographic studies were all consistent with pulmonary fibrosis and pulmonary hypertension.

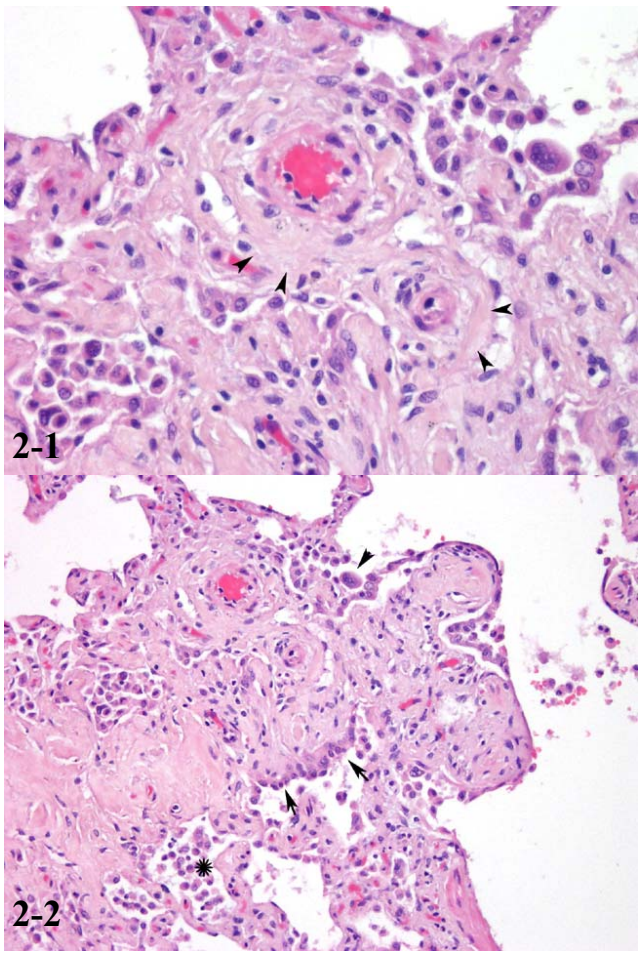
Laboratory Results: Complete blood count and chemistry profile were fairly unremarkable with the following abnormalities:

Leukocytosis (23.5000 x103/ul; reference range 4.900 – 16.900 x103/ul) with mature neutrophilia (19.270 x103/ul; reference range 2.800 – 11.500 x103/ul). No abnormalities were noted on the differential. Alkaline Phosphatase was markedly increased (1484 U/L; reference range 12 – 121 U/L)

Gross Pa thology: The lungs did not collapse when negative pressure was released. All lung lobes were diffusely dark pink, firm, and were meaty and dark red on cut section. The capsular surfaces of both kidneys were pitted and irregular with a tightly adhered capsule and multifocal <1mm diameter cortical cysts. There were bilateral mature cataracts.

Histopathologic D escription: Lungs – There is diffuse thickening of the alveolar septae with fibroblasts and homogenous eosinophilic fibrillar material (collagen). Occasionally, septae are **dramatically thickened (fig. 2-1)** up to 5 times. Partially or completely filling the alveolae are numerous **macrophages (f ig. 2-2)** with light pink vacuolated cytoplasm with occasional **multinucleate cells (fig. 2-2)**. There is marked **type II pneumocyte hyperplasia (fig. 2-2)**. Occasionally there is light purple mineralized material within the alveoli. Some sections contain a thick trabecula of dense collagen lined by hypertrophied type II pneumocytes.

Masson's trichrome stain shows moderate diffuse staining of the alveolar septae. There is multifocal to diffuse staining of cells within the alveolar septae for smooth muscle actin (myofibroblasts). There is negative staining for Collagen type I. (Reliable immunostains for collagen III, and IV were unavailable). Many intraalveolar cells stain positive for cytokeratin (pneumocytes).



2-1 Lung, West Highland white terrier dog. Diffuse pulmonary interstitial fibrosis (arrowheads). (H&E 400X)
2-2 Lung, West Highland white terrier dog. Pulmonary interstitial fibrosis with type 2 pneumocyte hyperplasia (arrows), numerous alveolar macrophages (star) and occasional multinucleated giant cells (arrowhead). (H&E 200X)

Contributor's Morpho logic Diagn osis: Lung – Marked, diffuse, chronic, interstitial fibrosis with type II pneumocyte hyperplasia.

Contributor's Comment : Idiopathic interstitial lung disease is a complicated and poorly understood disease process that, in the dog, has been described mostly in the terrier breeds with the West Highland white terrier having the highest incidence.[1] The clinical signs consist of coughing, dyspnea, exercise intolerance, and cyanosis. The signs develop slowly, and affected dogs deteriorate progressively over months.[2] Inspiratory crackles are a common physical exam finding and the main radiographic changes consist of mild to severe increased interstitial pattern and right sided cardiomegally. Bronchoscopic findings are often normal or show mild airway mucoid reaction.[2] Usually there are no hematologic or serum biochemical abnormalities.

Histopathologic findings consistently show generalized thickening of the interstitium by variable amounts of eosinophilic extracellular matrix. The process can range from diffuse to multifocal or regional. The most severe cases have multifocal areas of type II pneumocyte hyperplasia. There are often variable amounts of inflammatory cells (lymphocytes, plasma cells, macrophages.) Masson's trichrome stains the extracellular matrix expanding the alveolar septae as collagen.[1] Immunohistochemistry reveals that there can be a mixture of type I and type III collagen depending on the severity and chronicity of the disease.[1] Ultrastructurally, the extracellular matrix consists of numerous bundles of electron dense fibrils aligned parallel to one another. Individual fibrils have even spaced band periodicities (collagen).[1]

Differentials for idiopathic interstitial lung disease include, chronic bronchiolitis, neoplasia, and infectious diseases.[5] Idiopathic interstitial lung disease is of unknown etiology. Infectious processes, drug reactions, exposure to toxins or dust, and connective tissue disorders have been hypothesized as potential etiologies. Diagnosis, treatment, and determining an underlying etiology is difficult because by the time clinical signs are seen, there is usually irreversible loss of pulmonary function (fibrosis), and the inciting cause may no longer be present.

In human medicine there are a group of idiopathic pneumonias with similar features of shortness of breath, radiographic evidence of diffuse pulmonary infiltrates and varying degrees of inflammation, and fibrosis. The terminology in human medicine for these diseases has changed. Previously, many form of idiopathic interstitial pneumonia were termed "idiopathic pulmonary fibrosis", which is now reserved for a specific type also known as "usual interstitial pneumonia" or "cryptogenic pulmonary fibrosis".[3] This disease in humans has some similarities as the disease seen in West highland White terriers but technically the same. Other types of idiopathic interstitial pneumonias besides usual interstitial pneumonia, include, acute interstitial pneumonia, non-specific interstitial pneumonia, cryptogenic organizing pneumonia, and desquamative interstitial pneumonia-respiratory bronchiolitis interstitial lung disease.[3]

AFIP Diagn osis: Lung: Fibrosis, interstitial, diffuse, marked, with type II pneumocyte hyperplasia, and intraalveolar macrophages and multinucleated giant cells, West Highland white terrier (*Canis familiaris*), canine.

Conference Com ment: The contributor provides an excellent review of interstitial lung disease of the West Highland white terrier. Idiopathic Pulmonary Fibrosis also occurs in middle-age to older cats. Adult horses develop nodules of interstitial pulmonary fibrosis (Equine multinodular pulmonary fibrosis).

Additional causes of pulmonary fibrosis were discussed. Anything that damages type I pneumocytes or alveolar endothelium may lead to pulmonary fibrosis. Causes of alveolar damage include irradiation, septicemia, thermal injury, vomit aspiration, toxic gases (e.g., oxygen toxicity) and toxins (e.g., paraquat).

Other conditions with an increased prevalence in West Highland White Terriers include craniomandibular osteopathy, polycystic liver and kidney disease, hyperplastic dermatosis, and chronic hepatitis and cirrhosis.

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References:

1. Norris AJ, Naydan DK, Wilson DW: Interstitial lung disease in West Highland white terriers. Vet Pathol 42:35-41, 2005
2. Corcoran BM, Cobb M, Martin MWS, Dukes-McEwan J, French A, Fuentes VL: Chronic pulmonary disease in West Highland White Terriers. Vet Rec 144:611-616, 1999
3. Gross TJ, Hunninghake GW: Idiopathic pulmonary fibrosis. N Engl J Med 345:517-525, 2001
4. Lobetti RG, Milner R, Lane E: Chronic idiopathic pulmonary fibrosis in five dogs. J Am Anim Hosp Assoc

37:119-127, 2001
5. Webb JA, Armstrong J: Chronic idiopathic pulmonary fibrosis in a West Highland white terrier. Can Vet J 43:703-705, 2002

CASE III – 05-4349 (AFIP 3064906).

Signalment: Female, weaner pig

History: This pig is from a group of weanling pigs purchased by an FFA group. All had loose stools and fair body condition. Swine dysentery was suspected.

Gross Pa thology: On gross necropsy, the submitting veterinarian noted swollen mesenteric lymph nodes and liquid gut contents; small and large intestines were purple.

Laboratory Results: Dark field examination of colonic scrapings were negative for spirochetes. Moderate numbers of *Campylobacter coli* were isolated from the intestines. PCR for *Lawsonia intracellularis* was not performed in this case.

Histopathologic D escription: In a section of ileum there is a diffuse, proliferative and necrotizing inflammatory lesion. Intestinal glands are long and lined by tall, amphophilic cells with a high mitotic rate. Goblet cells are decreased in number. Peyer's patch lymphoid follicles are necrotic and proliferative glands are herniated into those spaces. Histiocytes replace follicular centers and surround necrotic foci. Glands in these and other areas are often dilated and filled with necrotic debris. The lamina propria is mildly expanded by lymphocytes and plasma cells.

Steiner's silver technique shows numerous short, curved rods within the apical portions of glandular epithelial cells.

Contributor's Morpho logic Diagn osis: Proliferative ileitis (porcine proliferative enteropathy)

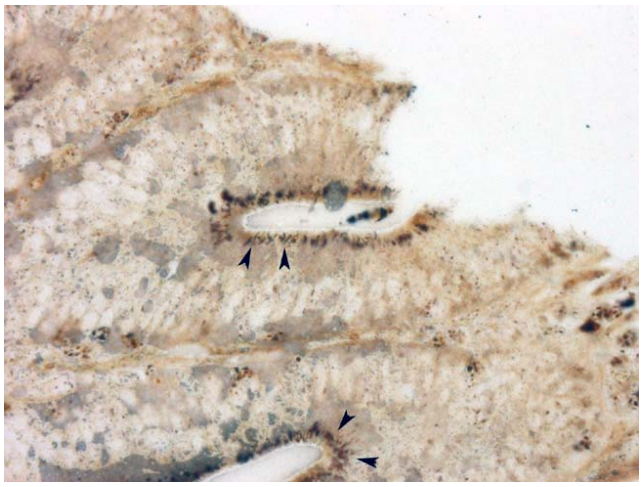
Contributor's Comment: Porcine proliferative enteropathy (PE) is a collection of syndromes all caused by infection by the obligate intracellular organism *Lawsonia intracellularis*.[1] The organism is prevalent in swine worldwide and is shed by infected pigs for weeks. Clinical disease is seen most commonly in feeder pigs. Signs vary from mild, subclinical disease with decreased weight gain and unthriftiness to severe diarrhea, cachexia and death or to death from acute intestinal hemorrhage. Morbidity and mortality vary with the different syndromes.

As an obligate, intracellular pathogen, pathogenesis of *L. intracellularis* related disease requires active uptake by intestinal epithelial cells. Localization of lesions to the ileum may be related to uptake of organisms by epithelium associated with Peyer's patches. Organism are initially taken up in membrane-bound vesicles and later released into the cytoplasm where they multiply. Cell division is required for bacterial proliferation. The mechanism by which *L. intracellularis* stimulates proliferation and dedifferentiation of ileal epithelial cells are poorly understood. Studies have shown, however, that the organism suppresses the inflammatory response by decreasing both B cell and T cell numbers, while macrophage numbers increase.[4]

Gross lesions are characteristic of the various forms of the disease. **Proliferative ileitis** , also called intestinal adenomatosis, is characterized by ridge-like thickening of terminal portion of the ileum, occasionally extending cranially or caudally to involve the cecum and proximal spiral colon. The marked thickening can be observed from the serosal surface as accentuation of the normal reticular pattern of the ileum. **Necrotic enteritis** is characterized by coagulative necrosis of the adenomatous mucosa, likely the result of anaerobic bacterial proliferation. Chronic infection, ulceration and stricture may result in a lesion called **regional ileitis** , characterized by severe hypertrophy of the muscular layers of the ileum. **Proliferative hem orrhagic enter opathy** may occur when extensive necrosis and ulceration causes massive hemorrhage into the lumen of the ileum. Grossly, the typical adenomatosis lesion is overlain by clotted blood and fibrin. Animals affected by this form of the disease may die acutely from exanguination.

In all forms of the disease, the histologic features are similar. The characteristic morphology is that of marked hyperplasia of intestinal crypt epithelium with loss of goblet cells and minimal inflammation. Mitotic activity is high and glands become crowded, branched and dilated by accumulation of necrotic debris. Hyperplastic glands may protrude into the lymphoid follicles of the submucosa (a prominent feature in this case).

Differential diagnoses for diarrhea and weight loss in feeder pigs include swine dysentery and salmonellosis, both of which have distinct gross and histologic lesions centered mainly on the cecum and colon. Porcine circovirus-2, the agent of postweaning multisystemic wast-



3-1 I leum, pig. Multifocally, obscuring the apical surface of enterocytes are myriad argyrophilic, short curved bacteria (arrowheads). (Warthin-Starry 600X)

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ing disease (PMWR), reportedly can cause similar histologic lesions in the absence of co-infection with *L. intracellularis*.[2] That intestinal lesion is characterized by a necrotic, proliferative enteritis with marked replacement of Peyer's patches by histiocytes and multinucleate giant cells, which can also be a feature of PE. However, characteristic botryoid cytoplasmic inclusions of PCV-2 infection should help differentiate the 2 diseases. No PCV-2 inclusions were seen in this case.

Although primarily a disease of pigs, *L. intracellularis* can infect many species, most notably young horses, causing a similar proliferative enteropathy. The organism has also been investigated as an agent of inflammatory bowel disease in human beings.[6]

AFIP Dia gnosis: Ileum: Ileitis, proliferative, diffuse, marked, with villar atrophy and fusion, lymphoid necrosis, crypt herniation and crypt abscesses, pig (*Sus scrofa*), porcine.

Conference Com ment: *Lawsonia intracellularis* has been identified as the causative agent of a proliferative enteropathy in a number of species. It primarily affects the ileum in horses, sheep, ostriches, guinea pigs, pigs, rabbits and hamsters; the cloaca in emus; and the colon in ferrets, foxes and rats. The numerous **short curved rods (fig. 3- 1)** can be visualized with a silver stain (e.g. Warthin-Starry) and are located in the apical portion of the intestinal epithelial cells.

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References:

1. Brown CC, Baker DC, Barker K: The alimentary system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp 206-209. Saunders Elsevier, Edinburgh, 2007.
2. Jensen TK, Vigre H, Svensmark B, Bille-Hansen V: Distinction between porcine circovirus type 2 enteritis and porcine proliferative enteropathy caused by *Lawsonia intracellularis.* J Comp Path 135:176-182, 2006
3. Lawson GHK, Gebhart CJ: Proliferative enteropathy. J Comp Pathol 122:77-100, 2000
4. MacIntyre N, Smith DGE, Shaw DJ, Thomson JR, Rhind SM: Immunopathogenesis of experimentally induced proliferative enteropathy in pigs. Vet Pathol 40:421-432, 2003
5. McOrist S, Gebhart CJ: Proliferative enteropathies. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, pp. 727-737. Blackwell Publishing, Ames, IA, 2006
6. Mickalski CW, Francesco Di Mola F, Kummel K, Wendt M, Koniger JS, Giese T, Giese NA, Friess H: Human inflammatory bowel disease does not associate with *Lawsonia intracellularis* infection. BMC Microbiol 6:81-88, 2006
7. Straw BE, Dewey CE, Wilson MR: Differential diagnosis of disease. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, pp. 727-737. Blackwell Publishing, Ames, IA, 2006

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CASE IV - NADC WCS 02 (AFIP 2841700).

Signalment: 45-day-old, female, crossbred, Caesarian-derived, colostrum-deprived (CDCD), domestic swine (*Sus scrofa domestica*)

History: This pig was experimentally inoculated with 10^3 $TCID_{50}$ porcine circovirus type 2 (PCV 2) at 21 days of age. The pig was anorexic and icteric days 20-24 post inoculation. The pig was also febrile (rectal temperature > 40.0°C) for seven days prior to euthanasia and necropsy on day 24 post inoculation.

Gross P athology: There was marked, generalized lymphadenopathy. Icterus was observed in the skin, sclera, subcutaneous tissue, pericardium, urine, and periosteal tissues. The liver was markedly enlarged and had a mot-

tled yellow, tan, and red color pattern. There were multifocal white foci throughout the parenchyma of the kidney.

Laboratory Results: PCR on fresh tissues for PCV2 was positive from multiple tissues; PCR on fresh tissues for porcine parvovirus was negative.

Virus isolation for PCV2 was positive from multiple tissues; virus isolation for PRRS virus was negative.

In situ hybridization for PCV was positive from multiple tissues including the liver.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, diffuse, subacute, lymphohistiocytic, necrotizing, severe, with occasional intracytoplasmic botryoid inclusion bodies.

Contributor's Comment: These slides contain sections of liver in which there is diffuse alteration of the normal hepatic architecture. There is marked separation of hepatic cords due to distension of the sinusoids by clear space, erythrocytes, and low to moderate numbers of inflammatory cells. Hepatocytomegaly is a pronounced feature, and binucleated hepatocytes are commonly observed. Single-cell necrosis characterized by pyknosis, karyorrhectic debris, and Councilman bodies are a common feature. Foci of lymphocytes and macrophages can be seen haphazardly arranged throughout the sections. Neutrophils can occasionally be observed within the sinusoids. Intrahepatocellular bile pigment can frequently be observed. Some sections contain hepatocytes with intracytoplasmic, amphophilic to basophilic inclusion bodies. Multiple, variably sized inclusion bodies arranged in clusters (botryoid) can often be seen within a single cell. Less frequently basophilic intranuclear inclusion bodies are present.

Porcine circoviruses are members of the family *Circoviridae* which contain the smallest viruses known to infect animals. *Circoviridae* contain the genera circovirus (porcine circoviruses, pigeon circovirus, and psittacine beak and feather disease virus) and gyrovirus (chicken anemia virus). The human transfusion-transmitted virus (TTV) has been proposed to be grouped within the family *Circoviridae.*

Porcine circoviruses are icosahedral, nonenveloped, and contain a single-stranded, circular DNA genome of approximately 1,760 bases, and measure 17-20 nm in diameter. Porcine circoviruses have been sub-grouped into two types based on genomic differences. Porcine circovirus type 1 was first recognized as a contaminant of the PK-15 cell culture line and has not been proven to cause clinical disease in swine. Porcine circovirus type 2 has been associated with outbreaks of postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS). PCV1 and PCV2 are antigenically similar but can be segregated by serologic tests.

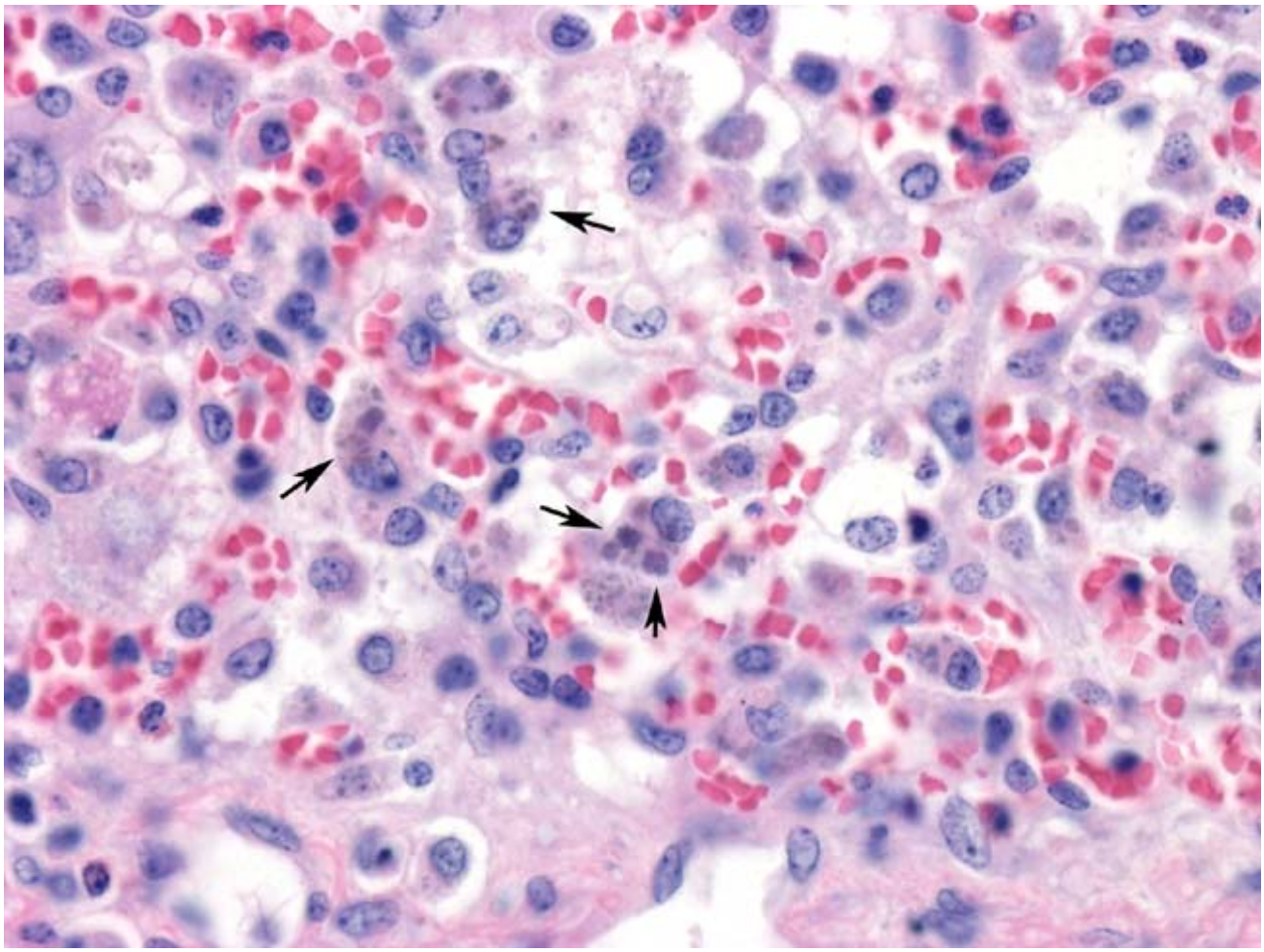
PMWS was first recognized in high-health status swine herds in western Canada in 1991 and has since been reported world-wide. PMWS is a low morbidity syndrome characterized by weight loss, failure to grow, diarrhea, dyspnea, and jaundice. Common gross lesions include generalized lymphadenopathy, hepatomegaly, gastric ulceration, nephritis, and interstitial pneumonia. Microscopically, there is disseminated depletion of lymphoid follicles, lymphohistiocytic inflammation in multiple tissues, interstitial nephritis, hepatitis, and bronchointerstitial pneumonia. The pathognomonic intracytoplasmic botryoid clusters of amphophilic to basophilic, variably sized inclusion bodies can be found within numerous cell types, particularly macrophages, depending on the stage of infection.

The lesions of PMWS have been reproduced with PCV2 alone and in combination with other viral agents, including porcine parvovirus and PRRS virus. This sample comes from experimental reproduction of PMWS in CDCD pigs with PCV2 alone.

AFIP Diagnosis: 1. Liver: Hepatitis, necrotizing and lymphohistiocytic, diffuse, severe, with karyomegaly and few basophilic botryoid intracytoplasmic inclusions, CDCD pig (*Sus scrofa*), porcine.
2. Gallbladder: Cholecystitis, neutrophilic, diffuse, mild, with pericholecystic edema.

Conference Comment: PMWS develops most often in pigs 5-12 weeks old and has a morbidity rate of approximately 5-10%. Although PCV2 alone can induce PMWS, PCV2 will result in more severe disease during a co-infection with either porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV). Activation of the immune response increases replication of PCV2. The role of PCV2 in other diseases of swine is controversial because PCV2 can be isolated from healthy pigs. The isolation of PCV2 alone does not result in a diagnosis of PMWS; the diagnosis also requires the consistent gross and clinical signs.

The primary gross lesion of PMWS is generalized lymphadenopathy. Other gross findings may include hepatomegaly, gastric ulceration, nephritis and interstitial pneumonia. The histologic lesions of PMWS include



4-1 Liver, pig. Multifocally, expanding hepatocyte cytoplasm there are basophilic to amphophilic botryoid inclusions (arrows). (H&E 600X)

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lymphohistiocytic inflammation in multiple organs with **basophilic intracytoplasmic botryoid inclusions (fig. 4-1)**, lymphoid depletion, and granulomatous interstitial pneumonia. Porcine dermatopathy and nephropathy syndrome (PDNS) is primarily associated with PCV2, but has also been associated with PRRSV, *Pasteurella multocida,* and *Streptococcus* sp.

Gross lesions of PDNS include red papules over the hindquarters, perineum and ears, and enlarged edematous kidneys with petechiae. Histological lesions include vasculitis, hemorrhage, necrosis, and acute exudative glomerulonephritis.

Conference attendees discussed differentiating PRRS from PMWS. Lymphocytes are the predominant inflammatory cell in cases of PRRS, whereas macrophages dominate in PMWS. The intracytoplasmic basophilic inclusion bodies are specific to a diagnosis of PCV2 infection.

PCV2 is a nonenveloped, icosohedral, DNA virus that forms paracrystalline arrays. Conference participants discussed other viruses that form paracrystalline arrays on EM. A useful mnemonic device used by AFIP residents at the AFIP is 'PICA' for Polyomavirus, Picornavirus, Iridovirus, Circovirus, and Adenovirus.

Not all sections contained gallbladder.

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References:
1. Allan GM, Ellis JA: Porcine circoviruses: a review. J Vet Diagn Invest 12:3-14, 2000
2. Allan GM, McNeilly F, Kennedy S, Daft B, Clarke EG, Ellis JA, Haines DM, Meehan BM, Adair BM: Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. J Vet Diagn Invest 10:3-10, 1998
3. Allan GM, Phenix KV, Todd D, McNulty MS: Some biological and physico-chemical properties of porcine circovirus. J Vet Med B. 41:17-26, 1994
4. Bolin SR, Stoffregen WC, Nayar GPS, Hamel AL: Postweaning multisystemic wasting syndrome induced after experimental inoculation of cesarean-derived, colostrum-deprived piglets with type 2 porcine circovirus. J Vet Diagn Invest 13:185-194, 2001
5. Ellis J, Krakowka S, Lairmore M, Haines D, Bratanich A, Clark E, Allan A, Konoby C, Hassard L, Meehan B, Martin K, Harding J, Kennedy S, McNeilly F: Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. J Vet Diagn Invest 11:3-14, 1999
6. Harding JCS, Clark EG, Strokappe JH, Wilson PI, Ellis JA: Postweaning multisystemic wasting syndrome: epidemiology and clinical presentation. Swine Health and Production 6:249-254, 1998
7. Kennedy S, Moffett D, McNeilly F, Meehan B, Ellis J, Krakowka S, Allan GM: Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. J Comp Pathol 122:9-24, 2000
8. McNeilly F, Kennedy S, Moffett D, Meehan BM, Foster JC, Clarke EG, Ellis,JA, Haines DM, Adair BM, Allan GM: A comparison of in situ hybridization and immunohistochemistry for the detection of a new porcine circovirus in formalin-fixed tissues from pigs with postweaning multisystemic wasting syndrome (PMWS). J Virol Methods 80:123-128, 1999
9. Rosell C, Segales J, Plana-Furan J, Balasch M, Rodriguez-Arrioja GM, Kennedy S, Allan GM, McNeilly F, Latimer KS, Domingo M: Pathological, immunohistochemical, and in-situ hybridization studies of natural cases of postweaning multisystemic wasting syndrome (PMWS) in pigs. J Comp Path 120:59-78, 1999
10. Segalés J, Allan GM, Domingo M: Porcine circovirus diseases. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, pp. 727-737. Blackwell Publishing, Ames, IA, 2006
11. Tischer I, Gelderblom H, Vettermann W, Koch MA: A very small porcine virus with circular single-stranded DNA. Nature 295:64-66, 1982

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 2

12 September 2007

Moderator:

Dr. Sarah Hale, DVM, Diplomate ACVP

CASE I – PA5-60150 (AFIP 2985164).

Signalment: 2-month-old, filly, Quarter Horse, Equine, *Equus caballus*

History: The filly had a body temperature of 103°F and harsh lung sounds. Ultrasound examination showed "comet tails" on pleural surface. The animal was treated using azithromycin and banamine. The animal improved slightly and naxcel was included in the treatment. Despite the treatment, the animal was found dead in the stall. Prior to this episode, the filly was given two injections of hyperimmune plasma to prevent *Rhodococcus equi* infection.

Gross Pathology: A 2-month-old,quarter horse filly was submitted for necropsy. The foal was in good body condition with adequate deposits of fat stores present. Hydration appeared adequate. The lungs were diffusely reddened, firm, and sank in formalin. There was a single 7 cm nodular area of caseation within the right cranioventral lung lobe. The tracheobronchial lymph nodes were markedly enlarged and contained a thick creamy exudate. The distal third of the trachea was hemorrhagic and contained linear streaks of ulceration/erosion. The omentum and mesentery were hemorrhagic. There was a large bilobed abscess, approximately 15 cm in diameter, within the mesentery by the ceco-colic junction. The center of the abscess was filled with pasty white necrotic material. A similar abscess was present within the mesentery adjacent to the jejunum. This abscess was adhered to the wall of the jejunum, and the overlying mucosa was focally ulcerated. There was a focal irregular area of hyperkeratosis in the nonglandular portion of the stomach next to the margo plicatus. There was an area of subcutaneous hemorrhage in the dorsal lumbar area.

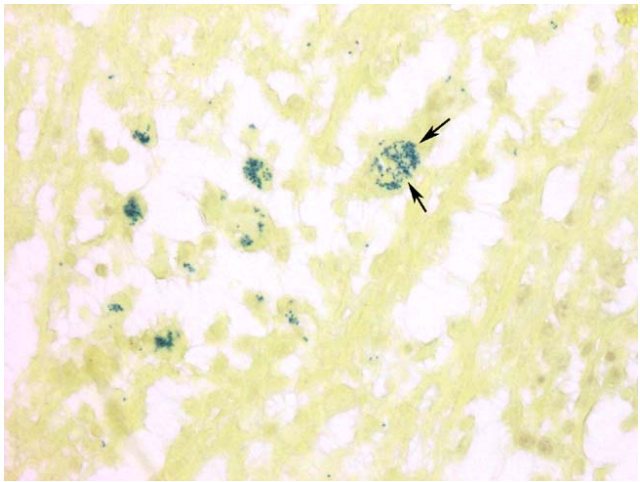
Laboratory Results:
1. Bacteriology: Lung abscess, lymph node swab yielded *Rhodococcus equi*
2. Fluorescent Antibody Tests: Negative for Adenovirus, EHV 1 & EIV
3. Virus isolation from lung: Negative

Histopathologic Description: A section of consolidated lung was examined, and is characterized by filling of alveoli with fibrin, macrophages, and occasional neutrophils. Occasional type II cell hyperplasia is present. Scattered alveoli contain multinucleated giant cells. Small numbers of plasma cells are present within some alveoli and within the thickened alveolar septae. The alveolar exudate occasionally is necrotic. Bronchioles and bronchi are generally devoid of inflammatory cells. A section of lung from the right cranioventral lung lobe contains large areas of abscessation. These foci contain sheets of degenerating neutrophils admixed with fewer

macrophages and replace normal pulmonary parenchyma. Many of the macrophages contain **intracytoplasmic bacteria consistent with *Rhodococcus* (fig. 1-1)**. There is mild fibroplasia around these areas, and the adjacent alveoli contain multinucleated giant cells, macrophages, lymphocytes, and plasma cells. There is acute hemorrhage within the deep lamina propria of the trachea. There is diffuse congestion, and the overlying mucosa is focally ulcerated. Ulcerated foci are covered with degenerated neutrophils and fibrin, and the base is infiltrated with neutrophils, macrophages, and occasional multinucleated giant cells.

Contributor's Morphologic Diagnosis: 1. Severe diffuse histiocytic bronchointerstitial pneumonia with focal abscessation with intracellular bacteria
2. Acute ulcerative tracheitis with hemorrhage and pyogranulomatous inflammation

Contributor's Comment: The pulmonary lesions present in the caudoventral portion of the lungs were typical in distribution and gross and microscopic appearance to lesions caused by *Rhodococcus equi*. The mesenteric lymph node involvement is also common in this disease. The diffuse inflammatory changes present in the rest of the lung actually predominated in this case, and have not been typically associated with this disease. These changes have been described in a group of foals with *Rhodococcus* infection.[3] An underlying viral etiology was suspected in these foals, but attempts to demonstrate a viral component were generally unrewarding. It may be that the bacteria are inducing a hypersensitivity type response in areas of the lung not colonized by bacteria. The reason this response occurs in particular groups of animals is unknown.



1-1 Lung, Quarter horse. Intrahistiocytic gram positive bacteria, consistent with Rhodococcus sp.(arrows).

Twenty-three foals, between 1 and 7 months old, with signs of acute respiratory distress, were examined at the Veterinary Medical Teaching Hospital (VMTH), University of California, Davis, between 1984 and 1989. Characteristic features included sudden onset of severe respiratory distress and tachypnea, cyanosis unresponsive to nasal oxygen, pyrexia, hypoxemia, hypercapneic respiratory acidosis, poor response to treatment, and histopathologic lesions of bronchiolitis and bronchointerstitial pneumonia. Seven of the 23 foals were normal before the onset of respiratory distress, 3 foals were found dead, and 13 foals were being treated for respiratory tract infections at the time of presentation. Laboratory data obtained for 13 horses showed increased plasma fibrinogen concentration (630.7 +/- 193 mg/dL), leukocytosis (18,607 +/- 7,784/microL), and neutrophilia (13,737 +/- 8,211/microL). Thoracic radiographs showed a diffuse increase in interstitial and bronchointerstitial pulmonary opacity and, in 5 foals, an alveolar pulmonary pattern of increased density was also seen. In 3 foals, heavy interstitial infiltration proceeded to a coalescing nodular radiographic appearance. Microbiological culture of tracheobronchial aspirates (TBA) from 9 foals yielded bacterial growth, but no one bacterial species was consistently isolated. Microbiological culture of postmortem specimens of the lung from 6 foals yielded growth of bacteria that included *Escherichia coli*, *Enterobacter* spp., *Proteus mirabilis*, *Klebsiella pneumoniae*, *Rhodococcus equi*, or beta-hemolytic *Streptococcus* spp. Tracheobronchial aspirates from 4 foals and lung samples collected from a further 4 foals at necropsy yielded no bacterial growth. Cultures were not taken from two foals premortem or postmortem. Virologic examination of TBA, lung tissue, or pooled organ tissue from 12 foals was negative. Viral culture of TBA from 1 foal showed cytopathic effects and positive immunofluorescence for equine herpes virus type II (EHV-II). In addition to the 3 foals that were found dead, 11 foals died or were euthanatized. Pathologic lesions were limited to the lungs in 50% of the foals; the remainder also had bowel lesions suggestive of hypoxic injury. The predominant histopathologic pulmonary lesions included bronchiolitis, bronchiolar and alveolar epithelial hyperplasia, and necrosis. Many bronchioles were filled with mucoid and fibrinocellular exudate. The peribronchiolar interstitium and adjacent alveolar spaces were also infiltrated with inflammatory cells and contained proteinaceous edema fluid. Type II cell hyperplasia and hyaline membrane formation were observed in the majority of foals and in 2 foals alveolar multinucleate giant cells were also present.[3]

Later, another foal from the same farm was submitted for necropsy. The second foal had similar gross and histopa-

thologic lesions indicating an endemic infection.[2]

AFIP Diagnosis: 1. Lung: Pneumonia, interstitial, necrotizing, histiocytic, lymphoplasmacytic, and neutrophilic, diffuse, marked, with fibrin and hyaline membranes, Quarter horse (*Equus caballus*), equine.
2. Lung: Pneumonia, pyogranulomatous, focally extensive, severe, with intrahistiocytic coccobacilli.

Conference Comment: *Rhodococcus equi* is a facultative, intracellular, Gram-positive bacteria that is present in soil and feces and is often enzootic on farms.[4] Two classic forms of the disease are suppurative to pyogranulomatous bronchopneumonia and ulcerative enterocolitis. Approximately half of the foals affected with the respiratory form have concurrent intestinal lesions. Intestinal lesions without the respiratory form is not common.[4] The lymph nodes, joints, bones, genital tract, and other organs may also be involved.[4] There are sporadic reports in other species, including cattle, goats, pigs, dogs, cats, and immunocompromised humans.

Rhodococcus equi appears to be easily killed by neutrophils but not macrophages. Upon entry through either inhalation or ingestion the bacteria are phagocytosed by either alveolar or intestinal macrophages. Several proposed virulence factors encoded by plasmids allow survival within macrophages. Vap A, Vap B, and Vap C, as well as glycolipids, capsular polysaccharides, and "equi factors" (cholesterol oxidase and choline phosphohydrolase) contribute to the virulence of certain *Rhodococcus equi* strains.[1] They prevent lysosomal fusion and/or result in premature lysosomal degranulation, survival of the bacteria, and death of the macrophage.[4]

Diffuse interstitial pneumonia is not a classic lesion of *Rhodococcus equi* pneumonia and is likely due to a separate disease process. The findings of necrotizing interstitial pneumonia with hyaline membrane formation are suggestive of the acute phase of diffuse alveolar damage (DAD). DAD results from diffuse injury to type I pneumocytes with subsequent hyaline membranes formation, type II pneumocyte proliferation and interstitial fibrosis.[3] These histologic lesions are non-specific, and identification of an etiologic agent is often difficult. Causes of DAD include, but are not limited to, thermal injury, toxic gases, septicemia, ingested toxins (paraquat, kerosene, *Brassica*, and perilla mint), endotoxemia, acute hypersensitivity reactions, ventilator-induced injury, and chronic left heart failure.[3]

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http://www.vet.uga.edu/vpp/index.html

References:
1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 226. Elsevier Limited, St. Louis, MO, 2007
2. Chaffin MK, Cohen ND, Martens RJ, Edwards RF, Nevill M: Foal-related risk factors associated with development of *Rhodococcus equi* pneumonia on farms with endemic infection. J Am Vet Med Assoc 223:1791-1799, 2003
3. Lakritz J, Wilson WD, Berry CR, Schrenzel MD, Carlson GP, Madigan JE: Bronchointerstitial pneumonia and respiratory distress in young horses: clinical, clinicopathologic, radiographic, and pathological findings in 23 cases (1984-1989). J Vet Intern Med 7:277-288, 1993
4. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, ed. McGavin MD, Zachary JF, 4th ed, pp.519-520. Mosby, Inc., St. Louis, MO, 2007

CASE II – 4029-07 (AFIP 3065685).

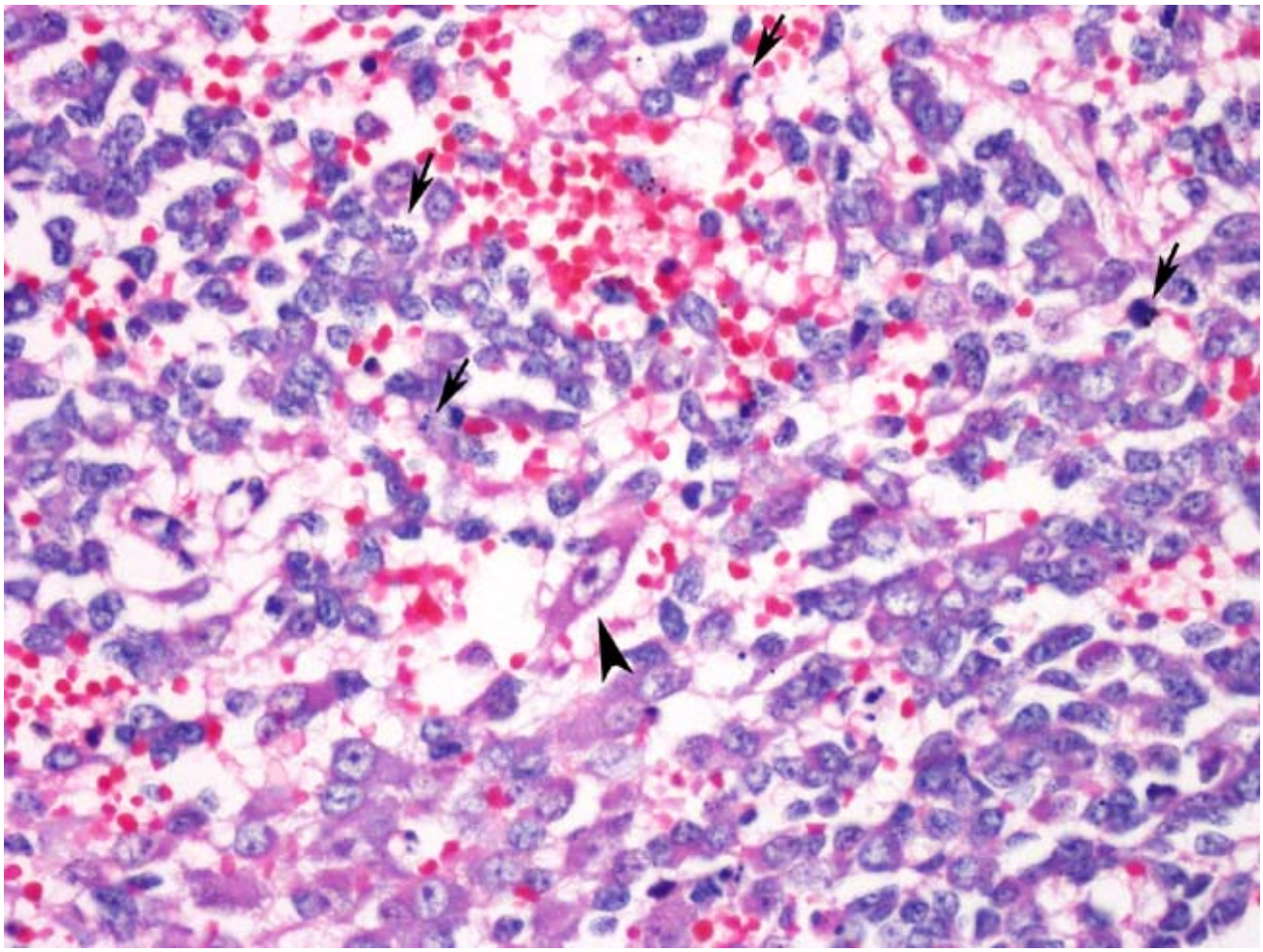
Signalment: 13-month-old, neutered male, mixed breed dog, *Canis familiaris*

History: This dog was presented for lethargy and progressive emaciation of several weeks duration. Fluid therapy, antibiotics, antifungals, and anti-inflammatory treatments were unsuccessful. The animal became recumbent and painful and was euthanized.

Gross Pathology: The animal is severely emaciated, evidenced by lack of internal body fat and severe muscle wasting. There is a very large, firm, gray-white and mottled red mass in the mediastinum measuring 30x9x15cm that compresses the lungs caudoventrally. The mass surrounds the esophagus, trachea, and great vessels but does not appear to compress their lumens. The spleen is meaty and markedly enlarged (30x10x3cm). The liver contains numerous gray-white solid and sometimes fluid-filled vesicles scattered diffusely.

Laboratory Results: Complete blood count and chemistry profile were fairly unremarkable with the following abnormalities:

CBC results from one week prior to euthanasia include



2-1 Spleen and liver, dog. Multifocally, the neuroblastoma contains cells which resemble ganglion cells (arrowhead and lower left). The small neoplastic cells consistent with neuroblasts have a high mitotic rate (arrows). (H&E 400X)

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the following abnormalities (normal values with reference range in brackets)

Hematocrit	17.5%	[37.0 – 55.0]
Hemoglobin	6.2 g/dL	[12.0 – 18.0]
Platelets	118 x10^9/L	[175 – 500]

Serum chemistry profile taken at the same time was normal except for a slightly low creatinine (0.4 mg/dL [0.5 – 1.8]).

AGID testing for aspergillosis, blastomycosis, coccidiodomycosis, and histoplasmosis was negative. Blood parasite analysis was negative. Bacterial culture of the liver taken at necropsy was negative.

Reticulocyte counts were 8.1% one week prior to euthanasia.

Histopathologic D escription: Sections of the tumor mass, spleen, and liver are submitted.

Sections of the tumor mass consist of sheets of small, polygonal to stellate cells with scant cytoplasm and dense, central nuclei within a fibrovascular stroma. Islands of large, polygonal cells resembling neurons are scattered irregularly within lobules. Similar foci are seen in the liver, but the spleen is filled primarily with the small cells.

The small cells observed in all sections are consistent with **neuroblasts (fig. 2-1),** whereas the larger neuron-like cells resemble ganglion cells. The histologic features are consistent with ganglioneuroblastoma, a rare tumor arising from the sympathetic ganglia. This one was malignant and sent metastases to the liver and

spleen. Metastases were not found in the lung.

Contributor's Morphologic Diagnosis: Ganglioneuroblastoma with hepatic and splenic metastasis

Contributor's Comment: Neuroblastomas are rare tumors that originate from the sympathetic nervous system ganglia and have been reported in a number of domestic animal species, including dogs, cats, pigs, horses, and cattle.[2] Ganglioneuroblastoma is differentiated from the more primitive neuroblastoma histologically by the presence of a mixed population of cells including large ganglion-like cells, small neuroblastic cells, and Schwannian stroma in varying proportions. Ganglioneuroblastoma has been reported as solitary lesions in the canine olfactory epithelium[5], brain[4], oral mucosa[6], and thorax.[7]

Clinical signs will vary depending on the location of the tumor. In all of the previously cited cases, clinical signs were limited to the effects of the space occupying mass on the local tissue. This dog was presented for non-specific lethargy, pain, and recumbency that was most likely due to severe compression of the heart and lungs. The cause of the anemia and thrombocytopenia in this case was not identified.

Immunohistochemical markers used in previous studies[4-7] demonstrate consistent staining of ganglion-like cells with neurofilament protein (NFP), but variable patterns of staining with markers such as S100, synaptophysin, GFAP, and NSE. Case reports are few therefore a pattern of immunoreactivity is not clearly established for the dog. Studies of human neuroblastic tumors[8] report variable staining for these and other markers.

AFIP Diagnosis: Spleen; liver; and mediastinal mass (per contributor): Neuroblastoma with multifocal poorly differentiated ganglion cells, dog (*Canis familiaris*), canine.

Conference Comment: Neuroblastic tumors include neuroblastoma, ganglioneuroma, and ganglioneuroblastoma. Neuroblastomas may occur in both the PNS and CNS. They are most commonly located in the adrenal medulla or in the sympathetic ganglia. Neuroblastomas in the PNS are derived from neuroectodermal cells of the neural crest and show varying degrees of differentiation toward postmitotic neuroblasts. Ganglioneuromas arise from primitive neuroepithelial cells but further differentiate towards neoplastic neurons. Those tumors exhibiting histologic features of both well-differentiated neurons and neuroblastic cells are called ganglioneuroblastomas.[2] Ganglioneuroblastomas are thought to originate from the cranial and spinal ganglia or sympathetic ganglia of the autonomic nervous system. They consist of ganglion cells, Schwann cells, and nerve fibers in variable levels of differentiation.[2]

In this case, the predominant cell type is neuroblastic. There are scattered areas containing poorly differentiated ganglion cells that lack Nissl substance. Following the conference the case was reviewed in consultation with pathologists in the AFIP Department of Soft Tissue Pathology. Their diagnosis, based on the human classification of the International Neuroblastoma Pathology Committee (INPC), was neuroblastoma (Schwannian stroma-poor), differentiating subtype, with ganglion cells. The designation "Schwannian stroma-poor" indicates neuroblastic cells forming groups and nests without or with limited Schwannian proliferation. In the "differentiating subtype", Schwannian stromal development containing mature and maturing ganglion cells comprise less that 50% of the neoplasm. We made our diagnosis based on the predominance of neuroblastic cells and absence of mature ganglion cells.

Contributor: Arkansas Livestock & Poultry Commission, #1 Natural Resources Drive, Little Rock AR 72205 www.arlpc.org

References:

1. Doss JC, Ditmyer H, Godken M, Hennings LJ: Metastatic ganglioneuroblastoma in a dog, submitted as an abstract for the 2007 AAVLD meeting.
2. Koestner A, Higgins RJ: Tumors of the endocrine system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 715-716, 736-737. Iowa State University Press, Ames, IA, 2002
3. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Histological classification of tumors of the nervous system of domestic animals. In: WHO International Histological Classification of Tumors of Domestic Animals, ed. Schulman FY, pp. 13–38. Armed Forces Institute of Pathology, Washington, DC, 1999.
4. Kuwamura M, Kotera T, Yamate J, Kotani T, Aoki M, Hori A: Cerebral ganglioneuroblastoma in a golden retriever dog. Vet Pathol 41:282-284, 2004
5. Mattix ME, Mattix RJ, Williams BH, Ribas JL, Wilhelmsen CL: Olfactory ganglioneuroblastoma in a dog, a light, ultrastructural, and immunohistochemical study. Vet Pathol 31:262–265, 1994
6. Nakamura K, Ochiai K, Kadosawa T, Kimura T, Umemura T: Canine ganglioneuroblastoma in the oral mucosa. J Comp Pathol 130:205-208, 2004
7. Schulz KS, Steele KE, Saunders GK, Smith MM, Moon ML: Thoracic ganglioneuroblastoma in a dog. Vet

Pathol 31:716-718, 1994
8. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roaki B: Terminology and morphologic criteria of neuroblastic tumors. Cancer 86:349-363, 1999
9. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 948-949. Elsevier, St. Louis, MO, 2007

CASE III – 5983-02 (AFIP 2841678).

Signalment: 4-year-old, Tennessee walking horse (*Equus caballus*), gelding

History: The horse was examined in late March for severe lethargy that rapidly progressed to recumbency later that same day. The animal was euthanized late that evening based on the poor prognosis. The horse had been purchased the previous day and transported to the farm in Tennessee from Kentucky. A killed tetanus Eastern/ Western encephalitis flu vaccine was administered approximately 5 days before the purchase. The owner was unaware of any previous vaccinations having been given to the horse.

Gross Pathology: There were no significant gross findings.

Laboratory Results: Rabies virus examination was negative utilizing fluorescent antibody methods. Eastern equine encephalitis virus was isolated in mice and cell culture from the brain. The sample was also negative for West Nile virus and positive for Eastern equine encephalitis viral RNA utilizing reverse transcriptase polymerase chain reaction testing.

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis, suppurative, subacute, severe, Tennessee walking horse, equine

Contributor's Comment: Multiple sections of brain from varying sites were submitted and feature a widespread meningoencephalitis with extensive perivascular cuffing consisting of neutrophils and mononuclear cells. Multiple suppurative foci were also relatively common within portions of gray matter with scattered neuronal degeneration and necrosis being evident. Intense inflammatory foci are sometimes associated with necrosis of neuropil. A few neutrophils and mononuclear cells are present within pia-arachnoid spaces.

Eastern equine encephalitis is an alphavirus in the togavirus family that causes encephalitis in both humans and horses. The reservoir host is wild birds, where virus replicates to sufficiently high titers to facilitate vector transmission of the disease. Mosquitoes serve as the biological vector for Eastern equine encephalitis. In contrast to birds, horses and humans are "dead-end" hosts since a sufficient viremia to allow transmission does not occur. Infected horses often present with fever, anorexia, and lethargy that ultimately progresses to a range of neurological signs that include paresis, seizures, paralysis, and death. Mortality due to Eastern equine encephalitis is quite high, often approaching 90%.

Eastern equine encephalitis is sporadically seen in Tennessee, primarily in western portion of the state during the months of August and September. The horse in this case was euthanized in late March due to the infection, and defies a simple explanation since the biological vector would not yet be available. Iatrogenic transmission has been suspected in another recent case of EEE and administration of a "killed" vaccine several days prior to onset of clinical signs warrants consideration in this case. Additionally, the rapid clinical progression and the severity of inflammation seen in the brain could reflect introduction of a much larger inoculum than would be seen in association with normal vector-borne disease.

AFIP Diagnosis: Brain: Meningoencephalitis, necrotizing, neutrophilic, lymphoplasmacytic, and histiocytic, diffuse, moderate, Tennessee walking horse (*Equus caballus*), equine.

Conference Comment: The contributor gives an excellent review of the Eastern equine encephalitis (EEE) virus. Other members of the Togaviridae family include Alphaviruses such as western equine encephalomyelitis (WEE), Venezuelan equine encephalomyelitis (VEE), Highlands J, and Semliki forest viruses, and Flaviviruses including Cache Valley virus, St. Louis encephalitis, and Japanese B encephalitis viruses.[3]

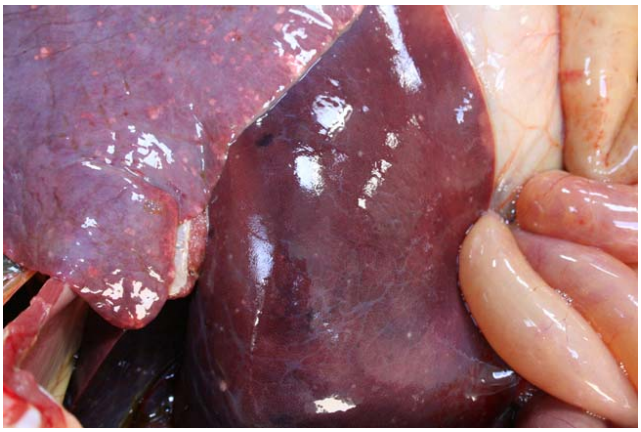
EEE, WEE, and VEE are caused by related but distinct alphaviruses. EEE and VEE are lethal in approximately 90% of cases, whereas WEE is less virulent with approximately 40% mortality in the horse. In endemically infected areas, EEE and WEE are maintained by a wild bird-mosquito (reservoir-vector) cycle, particularly in swampy or tropical areas. Avian reservoirs maintain sufficient viremia to permit infection of mosquitoes. The infection of domestic animals and humans occurs with the movement of virus from swampy areas carried by reservoirs, vectors, or both. *Culiseta* sp. and *Culex* sp. of

mosquitoes are most important in maintaining endemic infections.

Contributor: C. C. Kord Animal Disease Laboratory, Tennessee Department of Agriculture, Nashville, TN 37204

References:

1. Del Piero F, Wilkins PA, Dubovi EJ, Biolatti B, Cantile C: Clinical, pathological, immunohistochemical, and virological findings of Eastern equine encephalomyelitis in two horses. Vet Pathol 38:451-456, 2001
2. Franklin RP, Kinde H, Jay MT, Kramer LD, Green EN, Chiles RE, Ostlund E, Husted S, Smith J, Parker MD: Eastern equine encephalomyelitis virus infection in a horse from California. Emerg Inf Dis 8:283-288, 2002
3. George, LW: Diseases producing cortical signs. In: Large Animal Internal Medicine, ed. Smith, BP, 2nd ed., pp. 1018-1021. Mosby, St. Louis, MO, 1996
4. Hahn CN, Mayhew IG, Mackay RJ: Disease of multiple or unknown sites. In: Equine Medicine and Surgery, eds. Colahan PT, Mayhew IG, Merritt AM, Moore JN, 5th ed., vol. 1, pp. 884-888. Mosby, St. Louis, MO, 1999
5. Maxie MG, Youssef S: Nervous system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 4th ed., vol. 2, pp. 423-424. Elsevier Limited, St. Louis, MO, 2007
6. Rakich, PM, Latimer KS: Cytology. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., p. 322. Blackwell Publishing, Ames, IA, 2003
7. Summers BA, Cummings JF, de Lahunta A: Inflammatory diseases of the central nervous system, In: Veterinary Neuropathology, eds. Summers BA, Cummings JF, de Lahunta A, pp.144-146. Mosby, St. Louis, MO, 1995



4-1 Liver and lung, horse. Random pinpoint foci of necrosis. Photograph courtesy of the University of Pennsylvania, School of Veterinary Medicine Laboratory of Pathology & Toxicology, Philadelphia, PA 19104-787 http://www.vet.upenn.edu/departments/pathobiology/pathology/

CASE IV - N0713427A (AFIP 3065826).

Signalment: Two-day-old, male, Thoroughbred, *Equus caballus*, equine.

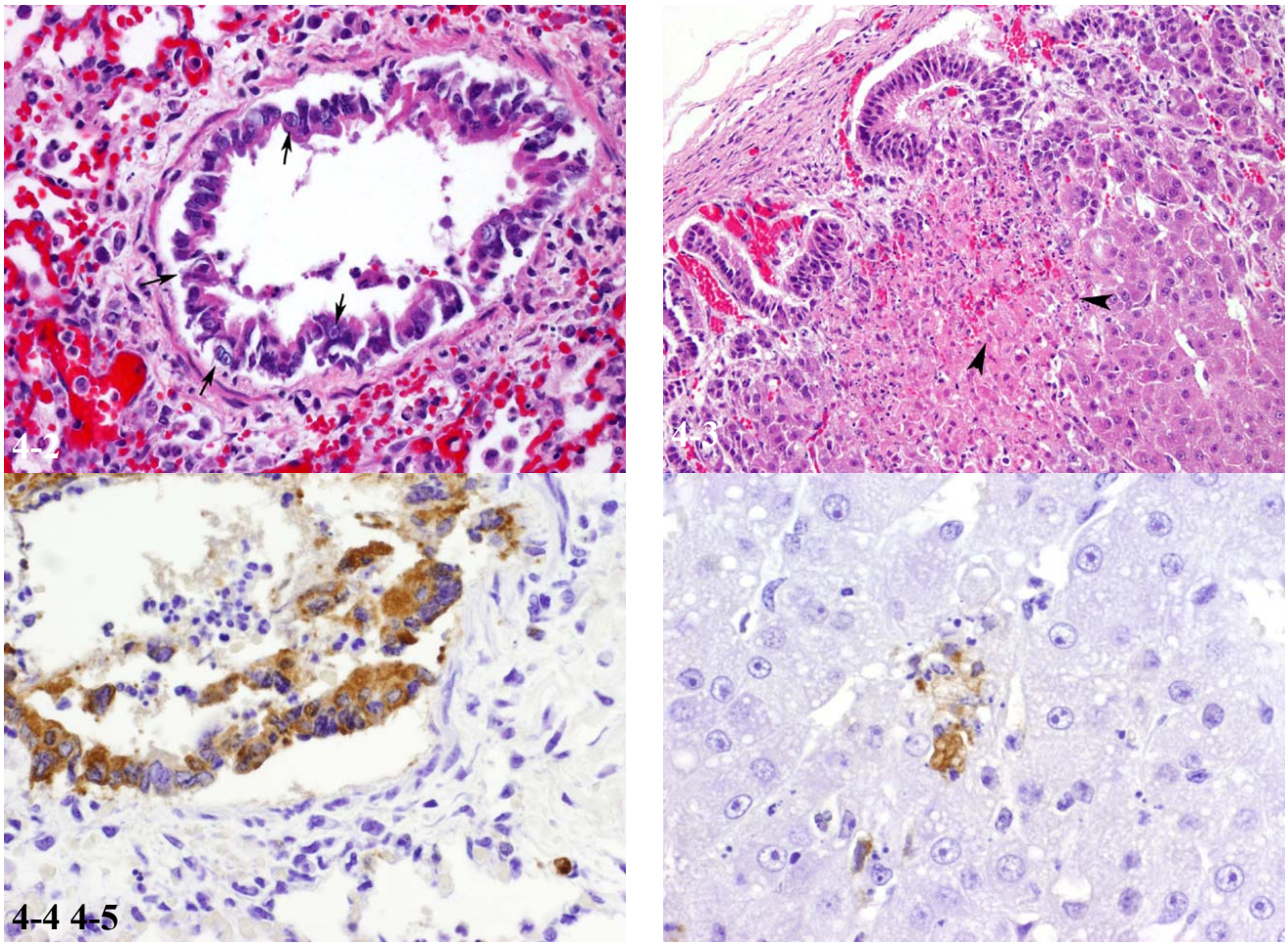
History: The colt presented at 9 hours of age with a history of premature placental separation at birth. Severe respiratory disease developed whilst the colt was hospitalized and worsened despite mechanical ventilation.

Gross Pathology: The lungs are heavy and edematous, and mottled red purple. The most cranioventral portions have numerous air filled pockets under the pleura. Scattered on the pleural surface and also on cut section are multiple white, less than 3mm diameter. The pericardium is expanded by edema. The capsular surface and parenchyma of the liver contain randomly disseminated pinpoint to less that 3mm diameter white-gray **foci** (**fig. 4-1**). The cortex of the adrenal glands contains scattered hemorrhagic foci.

Laboratory Results: Virus isolation performed on lung, liver, kidney, spleen and thymus was positive for EHV-1 and negative for EVA. FA performed for EHV-1 antigens on lung and liver was also positive, and EVA FA was negative. Aerobic culture of the lung produced no growth. Leptospira was not detected in the lung, liver, spleen and kidney by FA.

Histopathologic Description: Within the lung, there is extensive necrosis of the respiratory epithelium, predominantly affecting bronchioles but also bronchi and terminal airways. Sloughed cells admixed with necrotic debris and inflammatory cells accumulate within the lumens. Both necrotic cells and viable epithelium contain eosinophilic nuclear inclusion bodies that peripheralize the chromatin (**Cowdry type A**) (**fig. 4-2**), and there is formation of epithelial syncytia. Extending into alveoli (which are often necrotic) are accumulations of fibrin, neutrophils and macrophages. Type II pneumocytes are hyperplastic. Interlobular septa are edematous and contain an infiltrate of macrophages and neutrophils.

Scattered randomly within the adrenal cortex are areas of



4-2 Lung, horse. Many nuclei of the bronchiolar epithelium contain an eosinophilic inclusion body which is surrounded by a clear halo and peripheralizes the chromatin (arrows). (H&E 600X)
4-3 Adrenal cortex, horse. Necrosis and hemorrhage (arrowhead). (H&E 200X)
4-4 Lung, horse. Pulmonary epithelial cells are immunohistochemically positive for equine herpesvirus type 1 antigen. Photomicrograph courtesy of the University of Pennsylvania, School of Veterinary Medicine Laboratory of Pathology & Toxicology, Philadelphia, PA 19104-787, http://www.vet.upenn.edu/departments/pathobiology/pathology/
4-5 Adrenal gland, horse. Leukocytes within the adrenal gland are immunohistochemically positive for equine herpesvirus type 1 antigen. Photomicrograph courtesy of the University of Pennsylvania, School of Veterinary Medicine Laboratory of Pathology & Toxicology, Philadelphia, PA 19104-787, http://www.vet.upenn.edu/departments/pathobiology/pathology/

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congestion, hemorrhage and **necrosis** (**fig. 4-3**). Immediately adjacent to the cytoclastic debris are cells that contain eosinophilic nuclear inclusion bodies that peripheralize the chromatin.

Equine herpesvirus 1 (EHV-1) antigen was detected by immunohistochemisty within the nucleus and cytoplasm of several epithelial cells and leukocytes in the **lung** (**fig. 4-4**) and **adrenal gland** (**fig. 4-5**). Appropriate positive and negative controls were used and examined and worked accordingly.

Contributor's Morphologic Diagnosis: 1. Lung: Bronchointerstitial pneumonia, necrotizing, acute, diffuse, severe with eosinophilic nuclear inclusion bodies and epithelial syncytia.
2. Adrenal gland: Adrenalitis, necrotizing, acute, multifocal, moderate with eosinophilic nuclear inclusion bodies.

Contributor's Comment: Equine herpes virus 1 is an alphaherpes virus, responsible for causing abortion, perinatal foal mortality, respiratory disease and neurologic disease in horses.[7] Due to its direct effect on breeding and performance, and also through interference with horse movement, EHV-1 is of major economic and welfare importance in horse related industries throughout the world.[11] Pregnant mares exposed to infection abort three weeks to four months after exposure to infection. Abortion occurs anytime after five months gestation, but more commonly from nine months to term. Foals may be born alive, as in this case, but death occurs within a few days.[4,11]

The pathogenesis of EHV-1 abortion is not fully elucidated.[11] Virus is translocated from the maternal circulation to the uterus and placenta. Uterine lesions consist of vasculitis in the small arterioles of the endometrium.[11] In some cases, abortion can occur without fetal lesions or virus spread to the fetus, presumably from widespread virus-related thrombosis and infarction leading to premature placental separation and expulsion of the fetus.[14] Placental lesions in these cases consist of chorionic necrosis and fibrinoid vascular necrosis of chorionic blood vessels with fibrin thrombi.[13,14] EHV-1 has been detected in endometrial and chorionic endothelial cells in experimental and spontaneous cases of abortion by ISH and immunohistochemistry.[12,13,15]

More commonly virus spreads to the fetus. In addition to placental endothelial cells, DNA ISH also has identified EHV-1 in necrotic debris associated within infarcted microcotyledons, debris within endometrial glands and also trophoblasts, suggesting trophoblast infection results from diffusion of virus from sites of endometrial infarction and also from emptying of debris from infected glands directly onto the surface of trophoblasts.[12]

EHV-1 infection of the fetus results in well described and documented lesions. Grossly, the aborted fetus is usually fresh with subcutaneous edema and petechiae of the mucous membranes. The lungs are edematous and the trachea may contain a fibrinous cast. The liver contains miliary white foci of necrosis. The spleen may contain prominent lymphoid follicles.[2,9] Histologic lesions consist of necrosis and eosinophilic intranuclear inclusion bodies in parenchymal organs, especially the liver and adrenal glands, with minimal inflammatory cell infiltrate, lymphocytolysis in the thymus and bronchointerstitial pneumonia.[2,6] Syncytia formation in EHV-1 infection, as seen in this case, is rarely described. Previous reports include syncytia in the lungs of aborted fetuses[6] and in experimental neurologic disease.[3]

AFIP Diagnosis: 1. Lung: Pneumonia, bronchointerstitial, necrotizing, acute, multifocal, moderate, with fibrin, edema, syncytia, and eosinophilic intranuclear inclusion bodies, Thoroughbred (*Equus caballus*).
2. Adrenal gland, cortex: Necrosis, multifocal, with rare eosinophilic intranuclear inclusion bodies.

Conference Comment: The contributor includes an excellent review of EHV-1 associated abortions. EHV-1 is transmitted primarily through the respiratory system. Following an initial replication in the upper respiratory mucosal epithelium, the virus is transmitted throughout the body via mononuclear cells, primarily T-lymphocytes. Horses are latently infected for life.

There are three types of Equine Alphaherpes viruses:

EHV-1: Equine viral abortion, myeloencephalopathy, respiratory disease
EHV-3: Equine coital exanthema
EHV-4: Rhinopneumonitis virus

EHV-1 and EHV-4 both can cause abortion, although it occurs more often with EHV-1. EHV-1 and EHV-4 both can cause respiratory disease, although it is more common with EHV-4.

Slide variation includes some slides with syncytia in the adrenal cortex.

Contributor: University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology & Toxicology, Philadelphia, PA 19104-7871
http://www.vet.upenn.edu/departments/pathobiology/pathology/

References:

1. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 629. Elsevier Limited, St. Louis, MO, 2007
2. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, ed. Mc Gavin MD, Zachary JF, 4th ed., pp1298-1299. Mosby Elsevier, St Louis, Missouri, 2007
3. Jackson TA, Osburn BI, Cordy DR, Kendrick JW: Equine herpesvirus 1 infection of horses: studies on the experimentally induced neurologic disease. Am J Vet Res 38: 709-719, 1977
4. LeBlanc, MM: Abortion. In: Equine Medicine and Surgery, eds. Colahan PT, Mayhew IG, Merritt AM, Moore JN, 5th ed., vol. 2, pp1205-1206. Mosby St Louis,

Missouri 1999
5. Maxie MG, Youssef S: Nervous system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 4th ed., vol. 1, pp. 431-431. Elsevier Limited, St. Louis, MO, 2007
6. Mukaiya R, Kimura T, Ochiai K, Wada R, Umemura T: Demonstration of equine herpesvirus-1 gene expression in the placental trophoblasts of naturally aborted equine fetuses. J com Path 123:119-125, 2000
7. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Veterinary Virology, pp 301-303. Academic Press, San Diego, California, 1999
8. Paradis MR: Equine respiratory viruses. In: Large Animal Internal Medicine, ed. Smith BP, 2nd ed., pp. 587-588, Mosby, St. Louis, MO, 1996
9. Rooney JR, Robertson JL: Equine Pathology, pp 246-248. Iowa State University Press, Ames, Iowa, 1996
10. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 532-533, Elsevier Limited, St. Louis, MO, 2007
11. Slater J: Equine herpesviruses. In: Equine Infectious Diseases, eds. Sellon DC, Long MT, 1st ed., pp 134-145. Saunders Elsevier, St Louis, Missouri, 2007
12. Smith KC, Borchers K: A study of the pathogenesis of equid herpesvirus-1 (EHV-1) abortion by DNA in-situ hybridization. J comp Path 125:304-310, 2001
13. Smith KC, Mumford, JA, Lakhani K: A comparison of equine herpesvirus-1 (EHV-1) vascular lesions in the early versus late pregnant equine uterus. J Comp Path 114:231-247, 1996
14. Smith KC, Whitwell KE, Blunden AS, Bestbier ME, Scase TJ, Geraghty RJ, Nugnet J, Davis-Poynter NJ, Cardwell JM: Equine herpesvirus-1 abortion: atypical cases with lesions largely or wholly restricted to the placenta. Equine Vet J 36:79-82, 2004
15. Szeredi L, Aupperle H, Steiger K: Detection of equine herpesvirus-1 in the fetal membranes of aborted equine fetuses by immunohistochemical and in-situ hybridization techniques. J Comp Path 129:147-153, 2003

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 3

19 September 2007

Moderator:

Dr. Matthew Starost, DVM, PhD, Diplomate ACVP

CASE I – CRL#1 (AFIP 2936452).

Signalment: Female, approximately 6 months of age, *nu/+* mouse, congenic with BALB/c mouse (*Mus musculus*)

History: Routine submission from gnotobiotic colony for colony health monitoring

Gross Pathology: Firm anterior ventral cervical mass

Contributor's Morpho logic Di agnosis: Myoepithelioma, submandibular salivary gland

Contributor's Comment: Salivary gland tumors are generally rare in mice. An exception is myoepitheliomas in female BALB/c mice, where an incidence of 16.1/100,000 was reported in a large breeding colony at The Jackson Laboratories and 36/5090 in control groups in chronic studies.[1,5] The lower incidence at Jax probably reflects the fact that breeding mice are rarely kept past 6-8 months of age, with most animals leaving the colony at 4-6 weeks of age. Myoepitheliomas are rarely observed in male BALB/c mice or in mice of other strains.

Myoepitheliomas in mice can vary from solid to having numerous large cavities resulting from necrosis of tumor cells. This tumor was more solid than most we see, with only small cystic areas embedded in a tumor consisting of sheets and swirls of epithelioid to spindle cells. This histologic pattern and location are considered sufficient for diagnosis, with the key differentials being a complex adenoma and carcinosarcoma. Tumors with extensive squamous differentiation can also be confused with squamous cell carcinomas.

Although not apparent in most of these sections, mouse myoepitheliomas are invasive. They can also metastasize to the lungs, although this is seen infrequently in the diagnostic health monitoring laboratory setting, where subgeriatric mice are killed for other reasons or when tumors first become apparent.

AFIP Diagn osis: Submandibular salivary gland: Myoepithelioma, mouse (*Mus musculus*), rodent.

Conference Com ment: The myoepithelial cell is a modified epithelial cell that is located between the epithelial cell and the basement membrane. They contain long cytoplasmic processes that contract upon sympathetic or parasympathetic stimulation. Although myoepitheliomas can occur in any tissue, they most commonly arise from the **submaxillary** and **parotid s alivary glands** , mammary tissue, and sweat glands.[3] Neoplastic cells are positive for cytokeratin, actin, calponin and myosin.[2] Salivary myoepithelial neoplasms are rare in domestic animal

species.[2]

Contributor: Charles River Laboratories, Pathology Department, 251 Ballardvale St., Wilmington, MA 01887 www.criver.com

References:

1. Botts S, Jokinen M, Gaillard ET, Elwell MR, Mann PC: Salivary, harderian, and lacrimal glands. In: Pathology of the Mouse, ed. Maronpot RR, p. 59. Cache River Press, Vienna, IL, 1999
2. Head KW, Else RW, Dubielzig RR: Tumors of the alimentary tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 413, 415. Blackwell Publishing, Ames, IA, 2002
3. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 120-121. Blackwell Publishing, Ames, IA, 2007
4. Savera AT, Zarbo RJ: Defining the role of myoepithelium in salivary gland neoplasia. Adv Anat Pathol 11:69-85, 2004
5. Seely JC: Salivary glands. In: Pathobiology of the Aging Mouse, eds. Mohr U, Dungworth DL, Capen CC, Carlton WC, Sundberg JP, Ward JM, pp. 261-265. ILSI Press, Washington, DC, 1996

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CASE II – 05-999 (AFIP 3031552).

Signalment: Male, intact, INS-GAS mouse (*Mus musculus*) on an FVB background, adult, approximately 35-weeks-old

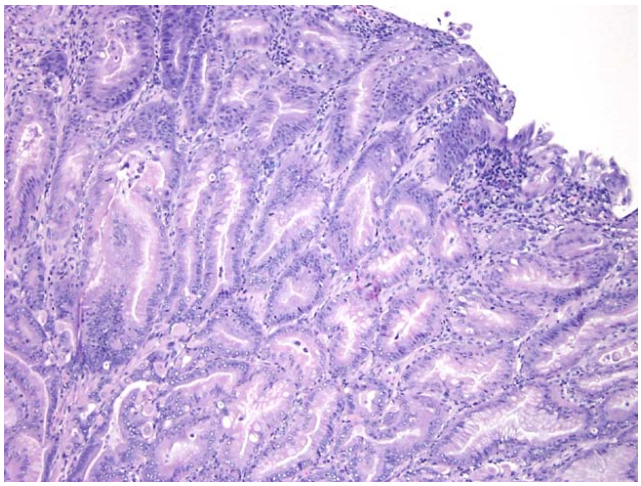
History: These three mice were experimentally infected with *Helicobacter pylori* (SS1) strain at 6 weeks old.

Laboratory Results: Serology was negative for a standard panel of murine infectious pathogens.

Gross Pathology: All mice exhibited similar changes to the stomach; only the fundus exhibited diffusely thickened gastric folds. One mouse had gastric hemorrhage.

Contributor's Morphologic Diagnosis:
Stomach. Gastric intraepithelial neoplasia (GIN)

Contributor's Comment: Only one of the three mice is on each given slide, with 3 sections from one mouse. All mice exhibited similar histologic changes: gastric mucosal and submucosa inflammation, epithelial **hyperplasia, metaplasia, and dysplasia (fig. 2-1)**. Dysplastic changes supportive of a diagnosis of GIN include severe loss of gland organization and columnar orientation, intraglandular folding, papillary and ductular projections, elongated branched and tortuous glands, and pseudostratification up to several cells deep. Moderate to marked cellular pleomorphism, cellular atypia, visible mitoses, and occasional bizarre mitotic figures are present. Within dysplastic foci are single apoptotic cells characterized by diffusely granular to hyaline eosinophilic cytoplasm, karryorhexis, and nuclear pyknosis.



2-1 Stomach, INS-GAS mouse. The gastric mucosa is thickened and gastric glands are frequently tortuous, elongated, lack normal orientation and are lined by piled up epithelium. (H&E 100X)

Phagocytosed apoptotic cells or their remnants were common in cytoplasmic vacuoles in neighboring epithelial cells. Transition zones from normal to hyperplastic to dysplastic epithelium were usually discernible and occurred in a wave of decreasing severity aborally from the cardia such that lesions were invariably most severe adjacent to the cardia. There is severe chief cell atrophy and loss of up to 50-70% of parietal cells. Parietal cells, sometimes in large numbers, exhibit small vacuolar to foamy metaplasia. Frequently there is cystic dilation of glands, often with crypt abscesses, surface epithelial "tattering" as well as small surface erosions. There are multifocal areas of mineralization within dilated glands.

The insulin gastrin (INS-GAS) mouse is a transgenic mouse bearing the transgene that overexpresses amidated gastrin, the main biologically active form of gastrin. The transgene targets expression of human gastrin to the pancreatic islets. In adult mammals, gastrin is expressed mainly in the antral G cells of the stomach where it con-

trols the acid secretion and stimulates mucosal proliferation. The INS-GAS mice spontaneously develop gastric cancer, but this requires the virtual lifetime of the animal (1-2 years). However, when male INS-GAS mice are infected with *H. pylori*, they uniformly developed atrophy, intestinal metaplasia, and dysplasia by 6 weeks and carcinoma by 24 weeks.

Gastric carcinoma is the most common of malignant tumors of the stomach in people. It is the second most common tumor in the world. It exhibits a male-to-female ratio of about 2:1. The major factors thought to affect the genesis of gastric cancer include environmental factors, host factors and genetic factors. One environmental factor, infection by *H. pylori*, present in most cases of intestinal-type carcinoma. Chronic infection with *H. pylori* generally increases the risk for developing gastric carcinoma by five-to six-fold. The bacterial infection causes chronic gastritis, followed by atrophy, intestinal metaplasia, dysplasia and carcinoma. One host determinant that may influence the development of gastric cancer is gastrin. Hypergastrinemia occurs early in the course of human *H. pylori* infection, precedes the development of atrophic gastritis, and often resolves after eradication. In addition, *in vitro* gastrin stimulates gastric epithelial cell proliferation.

In addition to host factors, bacterial determinants also contribute to gastric carcinogenesis. One virulence-associated *H. pylori* constituent is the *CagA* (cytotoxin associated gene A) pathogenicity island, which is present in approximately 60% of United States strains, and carriage of a *Cag*+/- strain augments the risk for atrophic gastritis and distal gastric adenocarcinoma compared with that incurred by *Cag* -/- strains. Several *cag* genes encode products that possess homology to components of type IV secretion systems, and, following *H. pylori* adherence to epithelial cells in vitro, the product of the terminal gene in the island (*CagA*) is translocated into the host cell, in which it undergoes Src-dependent phosphorylation and activates a phosphatase (SHP-2), leading to cellular morphologic changes. Loss of *CagE* temporally retards but does not abrogate pathologic progression.

All experiments were approved by the Committee on Animal care at Massachusetts Institute of Technology.

AFIP Di agnosis: 1. Stomach, glandular: Epithelial hyperplasia and dysplasia, diffuse, marked, with lymphoplasmacytic and neutrophilic gastritis (gastrointestinal intraepithelial neoplasia), INS-GAS mouse (*Mus musculus*), rodent.
2. Duodenum; pancreas: No significant lesions.

Conference Comment: Gastrointestinal intraepithelial neoplasia (GIN) is a term used to represent putative preinvasive neoplastic lesions not grossly visible. GIN is synonymous with atypical hyperplasia, atypia, microadenoma, carcinoma in situ, and dysplasia.[1]

Conference participants reviewed the primary cell types and functions of cells located within the stomach, such as parietal cells, chief cells, G cells, D cells, enterochromaffin-like cells, and mucous neck cells.

In the INS-GAS mouse, human heptadecapeptide gastrin is produced by the islet β cells and secreted into circulation. This gastrin release in turn causes an initial stimulation of gastric acid secretion (2-3 months of age), increasing the number of parietal and enterochromaffin-like cells. However, mice older than 5 months have a decline in acid secretion until 20 months of age when there is virtually no secretion at all, which coincides with decreasing numbers of parietal and enterochromaffin-like cells.[8]
Helicobacter spp. are Gram-negative spirochetes, approximately 3μm in length with 4-6 flagellae. Several species may contain ureases, catalases, oxidases, prote-

Helicobacter sp. in various animal species:

Mouse[5]	*H. bilis*, *H. hepaticus*, *H. muridarum, H. rodentium, H. typhlonius, H. ganmani, H. rappini, H. mastomyrinus, H. muricola* (Korean wild mice)
Rat[5]	*H. bilis*, *H. muridarum, H. rodentium, H. trogontum, H. typhlonius*
Ferret[2]	*H. mustelae*
Hamster[5,6]	*H. aurati*, *H. cineadi, H. mesocricetorum, H. cholecystus*
Gerbil[5]	*H. hepaticus, H. bilis*
Dog[2]	*H. felis*, *H. heilmannii*
Cat[2]	*H. felis, H. pylori*, *H. heilmannii*
Pig[2]	*H. heilmannii*

ases, and phospholipases. *Helicobacter* spp. have been implicated in gastric lymphoma and carcinoma in ferrets and humans. *Helicobacter hepaticus* can cause an acute focal, non-suppurative necrotizing hepatitis in mice.

In this case, despite experimental inoculation, no *Helicobacter* sp. were noted with Warthin-Starry or Steiner's stains performed at AFIP.

Contributor: Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA 02139.

References:

1. Boivin GP, Washington K, Yang K, Ward JM, Pretlow TP, Russell R, Besselsen DG, Godfrey VL, Doetschman T, Dove WF, Pitot HC, Halberg RB, Itzkowitz SH, Groden J, Coffey RJ: Pathology of mouse models of intestinal cancer: consensus report and recommendations. Gastroenterology 124:762-777, 2003
2. Dubois A: Animal models of *Helicobacter* infection. Lab Anim Sci 48:596-603, 1998
3. Fox JG, Wang TC, Rogers AB, Poutahidis T, Ge Z, Taylor N, Dangler CA, Israel DA, Krishna U, Gaus K, Peek RM: Host and microbial constituents influence *Helicobacter pylori*-induced cancer in a murine model of hypergastrinemia. Gastroenterology 124:1879-1890, 2003
4. Liu C, Crawford JM:The gastrointestinal tract. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 822-824. Elsevier Saunders, Philadelphia, PA, 1999
5. Owen RJ: *Helicobacter* – species classification and identification. Br Med Bull 54:17-30, 1998
6. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 58-61, 140, 190, 211. Blackwell Publishing, Ames, IA, 2007
7. Rogers AB, Taylor NS, Whary MT, Stefanich ED, Wang TC, Fox, JG: *Helicobacter pylori* but not high salt induces gastric intraepithelial neoplasia in B6129 mice. Cancer Res 65:10709-10715, 2005
8. Wang TC, Dangler CA, Chen D, Goldenring JR, Koh T, Raychodhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG: Synergistic interaction between hypergastrinemia and *Helicobacter* infection in a mouse model of gastric cancer. Gastroenterology 118:36-47, 2000
9. Wang TC, Koh TJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Dockray GJ: Processing and proliferative effects of human progastrin in transgenic mice. J Clin Invest 98:1918-1929, 1996

CASE III – CP03-0248 (AFIP 2938291).

Signalment: 6-month-old, male, Ptc +/- Background strain: C56BL/6;129SJ, mouse

History: The mouse was hunched, lethargic and had a rough hair coat.

Gross Pathology: A soft tissue mass was present in the cranial vault. The mass was attached to the cerebellum and compressed the adjacent brain parenchyma.

Contributor's Morphologic Diagnosis: Brain: Medulloblastoma

Contributor's Comment: A densely cellular mass is present in the cranium. The mass arises from the cerebellum and compresses the entire brain. The cells are arranged in densely packed sheets with occasional rosette formation. Neoplastic cells have elongated carrot shaped nuclei with dense chromatin and moderate pale eosinophilic cytoplasm with indistinct cell borders. Anisokaryosis and anisocytosis are minimal. Mitotic figures are rare.

Patched (Ptc) controls growth and pattern formation in early neural development and adult cerebellum. Ptc gene encodes a Sonic hedgehog (Shh) receptor and a tumor suppressor protein. Shh binds to Ptc, activates smoothened which leads to over expression of Gli-1 and some Wnt and TGF-ß gene families. Without Hh signaling, Ptc represses transcription of these target genes and itself. [2-4] Absence of Ptc causes derepression of target genes. Hedgehog (Hh) protein induces a high level of Ptc transcription through inhibition of Ptc function. While many aspects of signaling remain obscure it is clear that balance between Hh protein and Ptc is critical for normal development. Ptc expression is reduced by up to 50% in Ptc heterozygous mice. This causes ectopic expression of Shh target genes and uncontrolled cell proliferation. In Ptc heterozyogous mice, medulloblastomas have been reported as early as 5 weeks in 8.3% of mice.[3] Tumor incidence increases with age in Ptc heterozygous mice with an incidence of about 30% at six months of age. Ptc mutation has been associated with basal cell carcinoma, fibroma, medulloblastoma and rhabdomyosarcoma in man.[4]

Medulloblastomas arise from primitive neuroectodermal cells. Some cells may express neurofilament protein or synaptophysisn. However, the majority of the cells will be undifferentiated. During fetal development, cerebellar granular cells develop in the external granular layer then migrate past Purkinje cells to form the granule cell layer.

Remnants of the fetal external granular layer in the form of proliferative rests are thought to be the source of medulloblastoma cells.[5] Medulloblastomas occur with some frequency in young cattle and dogs and sporadically in pigs and cats.[7]

AFIP Diagnosis: Cerebellum: Medulloblastoma, mouse (*Mus musculus*), rodent.

Conference Comment: The contributor gives an excellent explanation of the functions of Ptc in cell growth regulation. Activation of the hedgehog pathway is shown to influence the growth of several neoplasms including medulloblastomas. Hedgehog effectors Gli1 and *Bcl*II are increased in areas of decreased apoptosis within medulloblastomas.[1]

Medulloblastomas are a subset of the primitive neuroectodermal tumors (PNETs). Medulloblastomas are derived from a germinal neuroepithelial cell and presumably arise from the matrix cells of the external granular layer.[6] Typical light microscopic findings can include palisading of neoplastic cells and rosette formation, polygonal to elongate ("carrot shaped") nuclei, and frequent mitoses. Immunohistochemical reactivity for various neural markers can vary according to the degree of differentiation.

In this case, there is extension into the inner ear in some sections.

Contributor: St. Jude Children's Research Hospital, Department of Pathology, Memphis, TN, 38105-2794

References:

1, Bar EE, Chaudhry A, Farah MH, Eberhart CG: Hedgehog signaling promotes medulloblastoma survival via *Bcl*II. Am J Pathol 170:347-355, 2007

2. Dahmane N, Sanchez P, Gitton Y, Palma V, Sun T, Beyna M, Weiner H, Ruiz i Altaba A: The sonic hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. Development 128:5201-5212, 2001

3. Goodrich LV, Milenković L, Higgins KM, Scott MP: Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 277:1109-1113, 1997

4. Hahn H, Wojnowski L, Specht K, Kappler R, Calzada-Wack J, Potter D, Zimmer A, Müller U, Samson E, Quintanilla-Martinez L, Zimmer A: *Patched* target Igf2 is indispensable for the formation of medulloblastoma and rhabdomyosarcoma. J Biol Chem 275:28341-28344, 2000

5. Kim JYH, Nelson AL, Algon SA, Graves O, Sturla LM, Goumnerova LC, Rowitch DH, Segal RA, Pomeroy SL: Medulloblastoma tumorigenesis diverges from cerebellar granule cell differentiation in *patched* heterozygous mice. Developmental Biology 263:50-66, 2003

6. Koestner A, Higgins RJ: Tumors of the nervous system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., p. 715. Blackwell Publishing, Ames, IA, 2002

7. Summers BA, Cummings JF, de Lahunta A: Tumors of the central nervous system. In: Veterinary Neuropathology, 1st ed., pp. 378-379. Mosby-Year Book Inc, St. Louis, MO, 1995

8. Wetmore C, Eberhart DE, Curran T: Loss of *p53* but not *ARF* accelerates medulloblastoma in mice heterozygous for *patched*. Cancer Res 61:513-516, 2001

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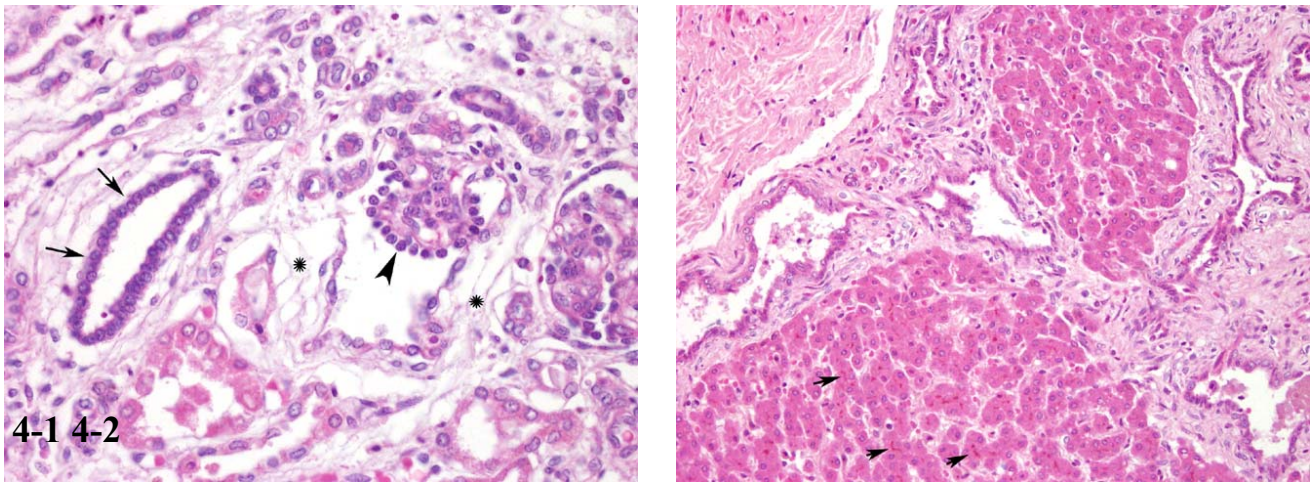
CASE IV - 02A499 (AFIP 3065784).

Signalment: Newborn, female, pigtailed macaque (*Macaca nemestrina*)

History: This animal belongs to the breeding colony from Tulane National Primate Research Center (TNPRC). This newborn macaque was found dead.

Gross Pathology: This macaque was in poor body condition, emaciated and dehydrated. One kidney was pale, firm, extremely enlarged (3 X the normal size) and slightly irregular.

Histopathologic Description: The submitted specimen consists of a section of kidney and liver. Grossly the kidney is surrounded by a thick capsule of connective tissue with numerous incomplete radial bands of connective tissue producing an abnormal lobar organization. Throughout the kidney renal corpuscles are small **(fetal glomeruli) (fig. 4-1)** predominantly in the outer cortex. There is expansion of both the cortical and medullary interstitium by abundant mesenchymal tissue. In the medulla there are multiple immature ductular structures lined by low columnar to flattened epithelial cells with prominent and hyperchromatic **nuclei** surrounded by poorly differentiated (immature) **mesenchymal tissue (fig. 4-1)**. There are also multiple cysts containing granular and fibrillar eosinophilic exudation lined by the flattened epithelial cells, islands of undifferentiated mesenchyme (cartilage matrix), and immature collecting ducts lined by psudoestratified columnar epithelial cells representing persistent metanephric ducts. The histological examination of the liver revealed hyperplasia of the intrahepatic bile ducts with multiple cystic or saccular dilata-



4-1 Kidney, pigtailed macaque. Renal dysplasia characterized by primitive mesenchyme (stars), fetal glomeruli (arrowhead) and primitive tubules (arrows). (H&E 400X)
4-2 Liver, pigtailed macaque. Portal areas are markedly expanded by increased numbers of bile duct profiles and abundant fibrous connective tissue. Hepatocytic cytoplasm and bile canaliculi often contain amber-brown globular pigment interpreted as bile (arrows) (H&E 200X)

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tions lined by low cuboidal epithelial cells and overgrowth of **portal connective tissue (fig. 4-2)**.

Contributor's Morpho logic Di agnosis: 1. Kidney: Cystic renal dysplasia
2. Liver: Bile duct ectasia and hyperplasia associated with portal fibrosis

Contributor's Comment: Cystic diseases of the kidney are a heterogeneous group comprising hereditary, developmental but nonhereditary, and acquired disorders. A useful classification of renal cysts is as follows:[2]

1. Cystic renal dysplasia
2. Polycystic kidney disease
    a. Autosomal-dominant (adult) polycystic disease
    b. Autosomal-recessive (childhood) polycystic disease
3. Medullary cystic disease
    a. Medullary sponge kidney
    b. Nephronophthisis
4. Acquired (dialysis-associated) cystic disease
5. Localized (simple) renal cysts
6. Renal cysts in hereditary malformation syndromes (e.g., tuberous sclerosis)
7. Glomerulocystic disease
8. Extraparenchymal renal cysts (pyelocalyceal cysts, hilar lymphangitic cysts)

Renal dysplasia is defined as disorganized development of renal parenchyma due to abnormal differentiation. This disease refers to a developmental disorder of renal parenchyma due to imperfect inductive interaction between the mesonephric duct and the matanephric blastema.[2]

In human beings renal dysplasia can be unilateral or bilateral and is almost always cystic. The abnormalities in the collecting system are common. In gross appearance, the kidney is usually enlarged, extremely irregular, and multicystic. The cysts vary in size from microscopic structures to some that are several centimeters in diameter.[11]

In dog the histologic features used to diagnose renal dysplasia include the prescence of 1) fetal or immature glomeruli, 2) fetal or immature tubules, 3) persistent metanephric ducts surrounded by primitive mesenchyma, 4) bone or cartilage in the parenchyma, and 5) anomalous presence of interstitial fibrous connective tissue.[6,13] Renal dysplasia has been reported in many breeds, such as the Golden Retriever, Labrador Retriever, Shih Tzu, Bull Mastiff, Boxer, and Finish Harrier [1,6,7,9,10,13-17] and human patients.[11]

In Rhesus macaque (*Macaca mulatta*) adult polycystic kidney disease and infantile polycystic kidney disease has been reported, and in Cynomolgus monkey (*Macaca fascicularis*) a case of spontaneous congenital polycystic kidney has been also described.[3,8,18]

In this case, the histological examination of the liver showed hyperplasia with cystic and saccular dilatations

of the intraepithelial bile ducts lined by low cuboidal epithelial cells associated with overgrowth of portal connective tissue.

In human being abnormalities of the biliary tree are an heterogeneous group of congenital lesions (von Meyemburg complexes, polycystic liver disease, congenital hepatic fibrosis, and Caroli disease) in which the primary abnormality is altered architecture of the intrahepatic biliary tree.[4]

The precursor of the intrahepatic biliary tree is a double-layered sleeve of cells know as the ductal plate (DP). The DP first arises from hepatocyte precursors surrounding hilar portal vein vessels, and more peripheral regions of the DP then develop sequentially. During the remainder of gestation, a process of DP remodeling occurs in which small areas of the double layer separate to form tubules, which join to form the intrahepatic biliary tree, while the remaining regions of the DP involute. Hepatic fibropolycystic diseases are thought to originate from failures in this process and are known collectively as DP malformations. However depending on the size of the bile ducts affected and the time during organogenesis, different syndromes, with marked overlap, can be differentiated. Caroli disease is a subcategory of these diseases, characterized by multiple cystic and segmental saccular dilations of the larger intrahepatic bile ducts.[4] Caroli syndrome involves malformations of smaller bile ducts and congenital hepatic fibrosis, marked by portal tract enlargement with irregular and broad bands of collagenous tissue, in which variable numbers of abnormally shape bile ducts are embedded.[4,5] Caroli syndrome is often associated with autosomal recessive polycystic kidney disease (ARPKD), and both the hepatic and renal processes reflect developmental process in the context of different organs. [4,5]

The present report described a congenital cystic renal dysplasia associated with bile ducts ectasia and prominent portal fibrosis in a new born Pigtailed macaque (*Macaca nemestrina*), a combination not previously reported in macaques.

The finding in our case of renal and bile duct dysplasia mimics previously reported cases in human and rat of Caroli syndrome.

AFIP Diagnosis: 1. Kidney: Renal dysplasia, characterized by fetal glomeruli, primitive mesenchyme, immature tubules, tubular ectasia and cysts, and interstitial and capsular fibrosis, with minimal lymphoplasmacytic interstitial nephritis and mineralization, pigtailed macaque (*Macaca nemestrina*), primate.

2. Liver: Biliary duct hyperplasia, diffuse, marked, with biliary duct ectasia, portal fibrosis, and cholestasis.

Conference Comment: The contributor gives a good review of the different forms of cystic kidney diseases with emphasis on renal dysplasia. There is debate in the literature on what features are necessary for the diagnosis of renal dysplasia. Although cartilaginous or osseous metaplasia is described in the human literature, it is rarely present in dysplastic kidneys of animals.[11]

Renal dysplasia has been associated with fetal infections of feline panleukopenia virus, canine herpesvirus, and bovine viral diarrhea virus, as well as hypovitaminosis A during gestation in swine.[11] There is not an apparent connection between the renal dysplasia and the biliary duct hyperplasia with congenital hepatic fibrosis (Caroli Syndrome) in the present case.

It is possible that the small cysts seen in this case are due to autosomal recessive polycystic kidney disease (ARPKD). It is important to make the distinction that although ARPKD is associated with Caroli syndrome, it is not a defining feature of the disease process.

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References:

1. Abraham LA, Beck C, Slocombe RF: Renal dysplasia and urinary tract infection in a bull mastiff puppy. Aust Vet J 81:336-339, 2003
2. Alpers CE: The kidney. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 955-1021. Elsevier Saunders, Philadelphia, PA, 1999
3. Baskin GB, Roberts JA, McAfee RD: Infantile polycystic renal disease in a rhesus monkey (*Macaca mulatta*). Lab Anim Sci 31:181-183, 1981
4. Crowford JM: The liver and biliary tract. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 877-937. Elsevier Saunders, Philadelphia, PA, 1999
5. Desmet VJ: Pathogenesis of ductal plate malformation. J Gastroenterol Hepatol 19:S356-S360, 2004
6. DiBartola SP, Chew DJ, Boyce JT: Juvenile renal disease in related standard poodles. J Am Vet Med Assoc 183:693-696, 1983
7. Hoppe A, Karlstam E: Renal dysplasia in boxers and Finnish harriers. J Small Anim Pract 41:422-426, 2000
8. Kessler MJ, Roberts JA, London WT: Adult polycystic kidney disease in a rhesus monkey (*Macaca mulatta).*

J Med Primatol 13:147-152, 1984
9. Kerlin RL, Van Winkle TJ: Renal dysplasia in golden retrievers. Vet Pathol 32:327-329, 1995
10. Lobetti RG, Pearson J, Jimenez M: Renal dysplasia in a Rhodesian ridgeback dog. J Small Anim Pract 37:552-555, 1996
11. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 439-443. Elsevier Limited, St. Louis, MO, 2007
12. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 625-627. Elsevier, St. Louis, MO, 2007
13. Picut CA, Lewis RM: Microscopic features of canine renal dysplasia. Vet Pathol 24:156-163, 1987
14. Miyamoto T, Wakizaka S, Matsuyama S, Baba E, Ohashi F, Kuwamura M, Yamate J, Kotani T: A control of a golden retriever with renal dysplasia. J Vet Med Sci 59:939-942, 1997
15. Morita T, Michimae Y, Sawada M, Uemura T, Araki Y, Haruna A, Shimada A: Renal dysplasia with unilateral renal agenesis in a dog. J Comp Pathol 133:64-67, 2005
16. Ohara K, Kobayashi Y, Tsuchiya N, Furuoka H, Matsui T: Renal dysplasia in a Shih Tzu dog in Japan. J Vet Med Sci 63:1127-1130, 2001
17. Schulze C, Meyer HP, Blok AL, Schipper K, van den Ingh TS: Renal dysplasia in three young adult Dutch kooiker dogs. Vet Q 20:146-148, 1998
18. Sakakibara I, Honjo S: Spontaneously occurring congenital polycystic kidney in a cynomolgus monkey (*Macaca fascicularis*). J Med Primatol 19:501-506, 1990

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 4

3 October 2007

Moderator:

Shelley Honnold, DVM, Diplomate ACVP

CASE I – 07L-1736 (AFIP 3066074).

Signalment: 6-week-old, female, Bulldog, canine

History: This animal is from a litter of 5 puppies which had recurrent diarrhea and chest problems from birth. Three siblings had died in a period of 9 days. Death was described as sudden, occasionally preceded by rigidity and vocalisation. The mother was fully vaccinated.

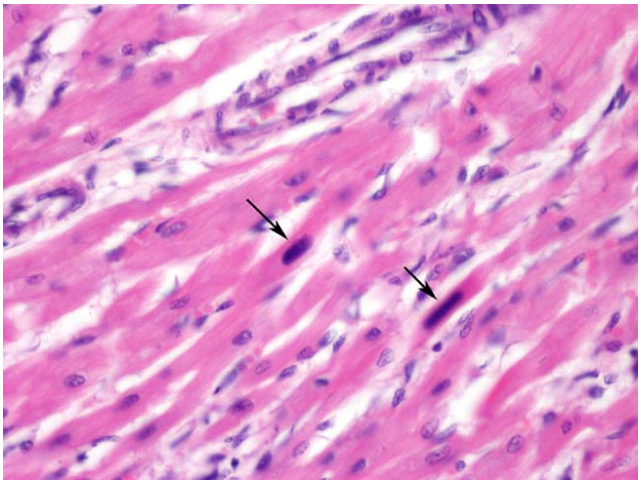
Gross Pa thology: The heart was diffusely pale and moderately enlarged and a mild pericardial effusion was observed. The lungs were markedly and diffusely oedematous and the liver was severely congested.

Laboratory Results: Immunohistochemistry for canine parvovirus was performed on sections of myocardium, lung, stomach, small intestine, colon, liver, pancreas, gall bladder, spleen, thymus, and bone marrow. Positive immunolabelling was observed in the intranuclear inclusion bodies of the cardiac myocytes.

Histopathologic D escription: Conference slides vary and include sections of left ventricle, septum and right ventricle but similar histological changes are consistently seen throughout.

There is moderate to severe, multifocal to coalescing, interstitial, predominantly lymphocytic and histiocytic infiltration with occasional scattered plasma cells and neutrophils, together with mild to moderate interstitial fibrosis. The myocardial fibers exhibit moderate diffuse anisocytosis and multifocal loss of striation, with cytoplasmic eosinophilia and occasional fragmentation. Multifocally, single, large (up to approximately 20x40 µm), homogeneous and occasionally stippled, basophilic,

1-1 Heart, dog. Cardiomyocyte nuclei contain basophilic inclusion bodies which obscure and peripheralize chromatin (arrows). (H&E 400X)



mostly elongated, **intranuclear inclusion bodies (fig. 1-1)** are observed in myofibers. In the cells containing inclusion bodies, the nuclear chromatin is clumped at the nuclear membrane.

Contributor's Morphologic Diagnosis: Heart, myocardium: Myocarditis, diffuse, moderate, lymphohistiocytic, necrotizing, subacute to chronic, with intranuclear inclusion bodies, Bulldog, canine.

Contributor's Comment: Canine Parvovirus (CPV) is a non-enveloped single-stranded DNA virus grouped informally in the Feline Parvovirus subgroup of the genus Parvovirus (*Parvoviridae* family).[6,16]

Parvoviridae are amongst the smallest DNA viruses, the virion being 18 to 26 nm in diameter, and are composed entirely of protein and DNA.[11] The lack of fatty components, typically present in viral envelopes, makes these viruses stable under diverse environment conditions and difficult to eliminate through disinfection. They can however, be inactivated by bleach, formalin and sunlight.[7]

The host range is one of the widest, the subfamily Parvovirinae infecting vertebrates, including humans and the subfamily Densovirinae infecting insects.

CPVs infect domestic dogs and several species of wild canids, including coyotes, bush dogs, gray wolves, raccoon dogs and manned wolves. Presently two autonomous parvoviruses are known to infect dogs: CPV type 1 and CPV type 2.

CPV type 1 was initially named Minute Virus of Canines and was first identified in 1970.[1] It was proven to cause enteritis and diarrhoea in neonatal canines. It appears to be highly related to the Bovine Parvovirus[14] and is antigenically unrelated to CPV type 2. To date, only few cases have been reported.

On the other hand, CPV type 2 appeared as a pandemic disease and presented characteristics of an epidemic infection affecting dogs of all ages. Although there is no specific data to support this, we can assume from the low mortality rate, that death was mostly restricted to canines less than 4 months old.[3] It was first detected in 1978 but is estimated to have emerged up to 10 years before.[10]

CPV type 2 is thought to result from mutations of feline panleukopenia parvovirus (FPV), with which it shares more than 98% of the DNA sequence[4] or from a closely related carnivore parvovirus.[12]

CPV type 2 was found to be unable to infect cats although it was able to replicate *in vitro* in feline cells.[13] As opposed to this, FPV was found able to replicate *in vivo* in the canine thymus but unable to replicate *in vitro* in canine cultured cells.

The CPV type 2 strain was soon replaced, worldwide, by two new lineages: CPV2a, identified in 1980, that later gave rise to CPV2b, identified in 1984, both with the added ability to replicate in cats and produce clinical signs in experimentally infected cats.[12,18] In Italy, Vietnam and Spain, a third type, CPV2c has been identified.[10] This antigenic drift is accompanied by successive replacement of prevalent strains by newer serotypes.[13] This is exemplified by the gradual disappearance of CPV2b from the dog population in Italy[6,10] and the replacement of CPV2a by CPV2b in the UK.[5]

The mutations occur at the level of the VP1/VP2 gene (encoding capsid proteins) and the new serotypes have an improved binding ability to its receptor, the canine transferrin receptor.[17]

CPVs require host cells to be in the S-phase to replicate as they are unable to induce it. They replicate within the nuclei of infected cells and are highly dependent on the cellular function.[11]

Pathology and clinical signs are dependent on the time of infection and which cells are in a highly mitotic rate at that particular moment within the host. Infection occurs oronasally and disease develops after 3 to 10 days of incubation.[6] After faeco-oral infection, the virus is taken up by the epithelium over the tonsils and Peyer's patches. In 1 to 2 days after experimental inoculation, the virus can be found in the mesenteric lymph nodes. Further dissemination of virus particles into other central or peripheral lymphoid tissues occurs via infected lymphoblasts. Following the lysis of infected cell, viruses are released and contribute to elevate the viremia which is only terminated if neutralising antibodies appear, typically 5 to 7 days post infection. Moderate pyrexia usually occurs.[2]

If the infection takes place up to two weeks postnatally and the puppies do not have sufficient neutralising antibodies, nonsuppurative myocarditis is the most common condition. On the other hand, if infection occurs later than these two weeks, due to the fast replication of the epithelia of the small intestines and bone marrow granulopoiesis, hemorrhagic gastroenteritis with lymphoid depletion is the pathological picture. The two forms of the disease rarely occur at the same time in an individual or group of animals. When nonsuppurative myocarditis

occurs, it is usually detectable between the third and eighth week of life, but can be asymptomatic until animals are six months old. Puppies frequently succumb to sudden death but can also present symptoms of congestive heart failure due to myocardial scarring or conduction failure. Grossly, the main findings are cardiomegaly and lesions in the myocardium, more pronounced in the left atrium and left ventricle. Pericardial and pleural effusions, ascites and hepatomegaly can also be observed. In the myocardium lesions are pale and streaky and often accompanied by multifocal petechial.[8] Microscopically, single, homogeneous, basophilic or amphophilic, roundish to elongated, intranuclear, Feulgen-positive inclusion bodies are observed.[2, 8] The chromatin is clustered at the nuclear membrane. Inclusion bodies are more frequent in late incubation, before extensive exfoliation (in the intestinal form) or infected cell lysis. In animals 4 to 7 weeks old, separation of the thin myocytes by extracellular oedema, histiocytes, fibroblasts and fibrous tissue is seen. Myocytes appear granulated and with fragmented cytoplasm. In animals 6 to 9 weeks old, inflammation is more severe and mainly lymphoplasmacytic. In juvenile animals, 14 to 24 months old, inflammation is milder, histiocytes and fibroblasts are more frequent, and fibrosis more extensive.

AFIP Diagn osis: Heart: Myocarditis, lymphohistiocytic, chronic, multifocal, moderate, with necrosis and loss and basophilic intranuclear inclusion bodies, Bulldog (*Canis familiaris*), canine.

Conference Comment: The contributor gives an excellent review of canine parvovirus type 2. Canine parvovirus requires actively dividing cells for replication. Lesions of canine parvovirus, such as those of intestinal crypt cells and lymphoid cells, reflect this dependence on actively dividing cells (radiomimetic).[9] The viral genome does not include DNA polymerase, so the virus depends on DNA polymerase expressed during the S phase of the cell cycle for transcription of viral DNA.[16]

Intranuclear inclusions are usually observed late in the incubation phase and prior to the lysis or exfoliation of the cells. Therefore it is possible that intranuclear inclusions may not be seen in samples submitted for histopathology.[2]

Other potential causes of myocarditis in canines include:[8,19]
Viral: Morbillivirus (canine distemper)
Parasitic: *Neospora caninum, Trypanasoma cruzi, Toxoplasma gondii*
Ricketsial: *Rickettsia rickettsii, Ehrlichia canis, Bartonella elizabethae*
Fungal/Algae like: *Prototheca* sp.
Spirocheatal: *Borrelia burgdorferi*

Other parvoviruses in animals include porcine parvovirus (SMEDI); feline parvovirus (feline panleukopenia); rat parvovirus (Kilham rat virus); minute virus of mice; goose parvovirus; and two genetically and antigenically distinct parvoviruses in mink (mink enteritis virus, which causes similar lesions as feline parvovirus, and Aleutian mink disease virus, which causes immune complex glomerulonephritis and arteritis).[16]

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References:

1. Binn LN, Lazar EC, Eddy GA, Kajima M: Recovery and characterization of a minute virus of canines. Infect Immun 1:503-508, 1970
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 177-178. Elsevier Limited, St. Louis, MO, 2007
3. Carmichael L: An annotated historical account of canine parvovirus. J Vet Med B Infect Dis Vet Public Health 52:303-311, 2005
4. Chang S, Sgro J, Parrish C: Multiple aminoacids in the capsid structure of canine parvovirus coordinately determine the canine host range and specific antigenic and hemagglutination properties. J Virol 66:6858-6867, 1992
5. Davies M: Canine parvovirus strains in the UK. Vet Rec 160:416, 2007
6. Decaro N, Martella V, Elia G, Desario C, Campolo M, Lorusso E, Colaianni ML, Lorussi A, Buonavoglia C: Tissue distribution of the antigenic variants of canine parvovirus type 2 in dogs. Vet Microbiol 121:39-44, 2007
7. Ettinger SJ, Feldman EC: Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, 6th ed., pp. 646-647. Elsevier Saunders, St. Louis, MO, 2005
8. Fox PR, Sisson D, Moïse NS: Textbook of Canine and Feline Cardiology: Principles and Clinical Practice, 2nd ed., pp. 832-833. Saunders, Philadelphia, PA, 1999
9. Gelberg, HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 378-379. Elsevier, St. Louis, MO, 2007

CASE II – 1 (AFIP 3065544).

Signalment: Juvenile (approximately 8-12 months), male intact, DSH, *Felis catus*, cat

History: This was a free living cat from a cattery of the city of Milan. The cat was found dead and sent for a full necropsy.

Gross Pathology: Elevated numbers of fleas were found in the hair coat. The cat was anemic. All central metacarpal and metatarsal pads were **swollen** (**figs**.**2-1** and **2-2**) and softened. The chin had grossly visible comedoes consistent with feline acne**.**

In the abdominal cavity renal lipidosis and hyperemia of most organs was evident.

In the thoracic cavity severe pulmonary edema and severe constrictive cardiomyopathy were present.

Histopathologic D escription: The normal microanatomical structures of the dermis and the underlying adipose tissue are variably effaced to completely obscured by a perivascular to diffuse infiltrate of mature plasma cells. Variably abundant **Mott cells (fig. 2- 3)** with Russell bodies and rare binucleated plasma cells can be seen. Occasional mature small lymphocytes, neutrophils and macrophages are also present.

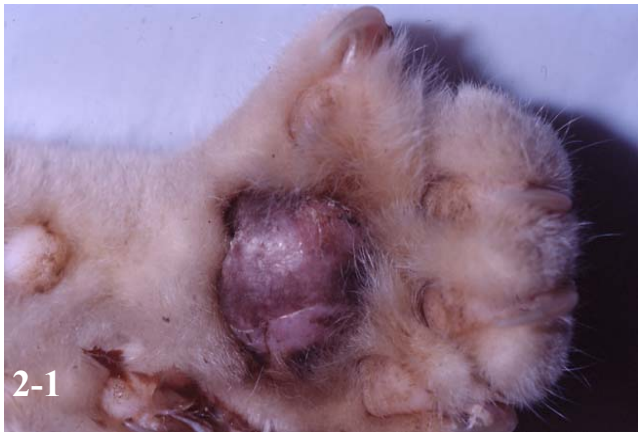
The epidermis is hyperkeratotic, irregularly hyperplastic and occasionally characterized by infiltration of plasma cells. Superficial erosion or serocellular crusting characterizes some of the sections.

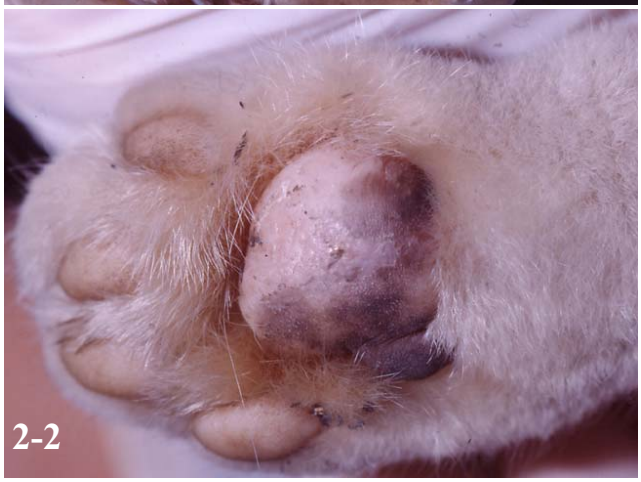
Additional microscopic findings:
Hepatic lipidosis, diffuse membranous glomerulonephritis, renal tubular hyaline casts and diffuse tubular lipidosis.

Contributor's Morphologic Diagnosis: Severe multifocal to diffuse chronic lymphoplasmacytic pododermatitis.

Contributor's Comment: Feline plasma cell pododermatitis is a rare disease of unknown pathogenesis. It is clinically characterized by soft and spongy swelling of multiple foot pads. Breed, age, or sex predilections have not been noted.[6] The central metatarsal and metacarpal pads are most consistently involved. The lesion is generally painless but lameness may develop secondary to ulceration and extensive hemorrhage.[6,11]

The cause and pathogenesis of feline plasmacytic pododermatitis are still unknown however, the marked plasma cell infiltrate, consistent hypergammaglobulinemia and response to immunosuppressive (i. e. glucocorticoids) or immunomodulating (i. e. tetracyclines) therapy suggest an immune dysfunction.[7,11] In some cats concurrent plasmacytic stomatitis, renal amyloidosis or immune-mediated glomerulonephritis have been reported. In

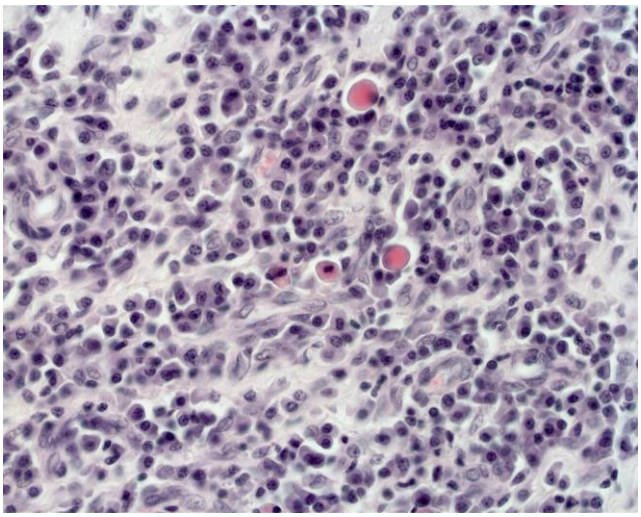




2-1 Footpad, cat. The metacarpal pad is diffusely swollen.
2-2 Footpad, cat. The metacarpal pad is diffusely swollen.

Photographs courtesy of the Dipartimento di Patologia Animale, Igiene e Sanita' Pubblica Veterinaria, Sezione di Anatomia Patologica e Patologia Aviare, Facolta' di Medicina Veterinaria, Milano, Italy
http://www.anapatvet.unimi.it

some cases, spontaneous remission of the lesions occurs, whereas in others there is a seasonal exacerbation of the disease. Recurrence in warm weather may support an allergic origin. Doxycycline monohydrate has been reported to produce partial or complete clinical remission in more than half of the cases.[1] The response of feline plasma cell pododermatitis to doxycycline is possibly due to its immunomodulatory effects, but since doxycycline has also antibacterial activity an infectious etiology has also been suggested. In a recent study, no infectious agent has been demonstrated by anti-BCG immunohistochemistry and by PCR assays for *Bartonella* spp., *Ehrlichia* spp., *Anaplasma phagocytophilum*, *Chlamydophila felis*, *Mycoplasma* spp., *Toxoplasma gondii*, and Feline herpesvirus 1 (FHV-1), further supporting the non-infectious hypothesis.[2] A possible link between feline plasma cell pododermatitis and concurrent feline immunodeficient virus (FIV) infection was suggested in one study, but the role of the virus remains controversial. Plasma cell pododermatitis has been also treated with wide surgical excision of the affected footpads, suggesting that other factors are probably involved.[4]



2-3 Footpad, cat. Infiltrate of numerous plasma cells. Note scattered Mott cells, which contain eosinophilic, globular Russell bodies. (H&E 400X)

Histologically, lesions are characterized by large numbers of plasma cells, occasionally in a predominantly perivascular pattern. The dermis and often the underlying adipose tissue of the pawpads are diffusely infiltrated with plasma cells. Russell body-containing plasma cells (Mott cells) are often conspicuous. Binucleated plasma cells and mitoses have also been described.[4] The epidermis is acanthotic with variable erosion, ulceration, and exudation. Neutrophilic infiltration is variable, but neutrophils can be up to 50% of the infiltrate and their presence is not dependent upon ulceration. Eosinophils are rare. Edema of the dermis, deep perivascular tissue, or interlobular septa of the fat pad may be seen. Blood vessels are often prominently dilated and congested, and hemorrhage may be present.[6]

AFIP Diagnosis: Footpad: Pododermatitis, plasmacytic, chronic, diffuse, marked with fibrosis, domestic short hair (*Felis catus*), feline.

Conference Com ment: There is slide variation with multifocal erosions and/or ulcerations in some sections but not in others, as well as variable amounts of fibrosis.

Mott cells are plasma cells that contain large eosinophilic, amorphous globules (Russell bodies) within their cytoplasm. These Russell bodies are composed of immunoglobulin (γ globulin).[8] Characteristic features of plasma cells on a transmission electron micrograph include an eccentric nucleus, alternating areas of electron dense heterochromatin, and electron lucent euchromatin forming a "cart-wheel" pattern, a prominent Golgi apparatus (the "perinuclear hof" seen in light microscopic section), abundant rough endoplasmic reticulum (cytoplasmic basophilia in light microscopic section), and few mitochondria.[9]

Conference participants discussed the concurrent findings of plasmacytic stomatitis, immune-mediated glomerulonephritis, and renal amyloidosis that are occasionally seen in animals with plasmacytic pododermatitis. AA amyloid (reactive systemic amyloidosis) is the most common form found in animals, where AL amyloid (Immunoglobulin-derived amyloidosis) is the most common form in humans.[3] In cats, amyloid in the kidney is most commonly deposited in the interstitial spaces of the medulla with relative sparing of the glomerulus. In most other animals, amyloid is primarily deposited in the glomerulus.[3] However, in familial amyloidosis, the amyloid is primarily deposited in the renal medullary interstitium in the Shar Pei dog and the glomerulus in the Abyssinian cat, while it is primarily deposited in the liver in Siamese cats.[10]

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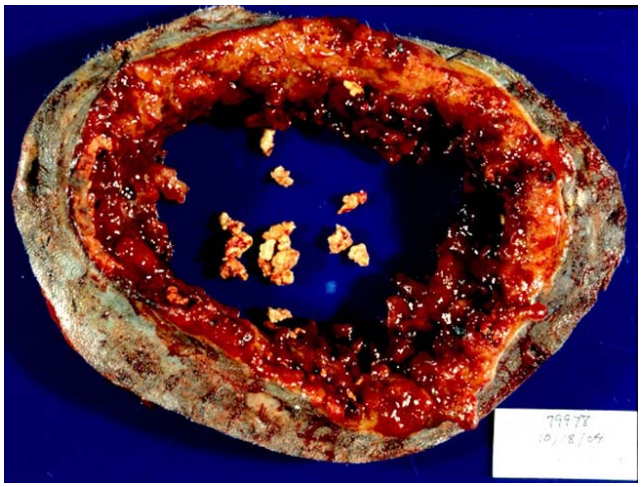
References:
1. Bettenay SV, Mueller RS, Dow K, Friend S: Feline plasmacytic pododermatitis: a prospective study of a novel treatment using systemic doxycycline. Vet Rec 152:564-566, 2003
2. Bettenay SV, Lappin MR, Mueller RS: An immunohistochemical and polymerase chain reaction evaluation of feline plasmacytic pododermatitis. Vet Pathol 44:80-83, 2007
3. Charles JA: Pancreas. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 463-465. Elsevier Limited, St. Louis, MO, 2007
4. Dias Pereira P, Faustino AM: Feline plasma cell pododermatitis: a study of 8 cases. Vet Dermatol 14:333-337, 2003
5. Ginn PE, Mansell JEKL, Rakich PM: Skin and appendages. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 661-663. Elsevier Limited, St. Louis, MO, 2007
6. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Plasma cell pododermatitis. In: Skin Disease of the Dog and the Cat, 2nd ed., pp. 363-364, Blackwell Science, Ames, IA, 2005
7. Gruffydd-Jones TJ, Orr CM, Lucke VM: Foot pad swelling and ulceration in cats: a report of five cases. J Small Anim Pract 21:381-389, 1980
8. Myers RK, McGavin MD: Cellular and tissue responses to injury. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 43. Elsevier, St. Louis, MO, 2007
9. Porter KR, Bonneville MA: Fine Structure of Cells and Tissues, 3rd ed., pp. 2-5, Lea & Febiger, Philadelphia, PA, 1968
10. Snyder PW: Diseases of immunity. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 247-249. Elsevier, St. Louis, MO, 2007
11. Taylor JE, Scmeitzel LP: Plasma cell pododermatitis with chronic footpad haemorrhage in two cats. J Am Vet Med Assoc 197:375-377, 1990



3-1 Skin and subcutis, horse. There is a large crateriform area containing small, firm granular nodules associated with tissue necrosis and eosinophilic inflammation (kunkers). Photograph courtesy of the College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061 www.vetmed.vt.edu

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CASE III – 04-2071 (AFIP 3063516).

Signalment: 5-year-old, female, Quarter horse

History: Large (15 cm diameter), ulcerative skin lesion on ventral midline

Gross Pathology: This 15 cm diameter, ulcerated, crater-like mass in the skin has a 3 cm thick fibrous wall extending through the subcutis. Within the ulcerated surface, 1 mm yellow granules are seen (**kunkers**) (**fig. 3-1**)

Laboratory Results: *Pythium insidiosum* was cultured from the cutaneous wound and the presence of *P. insidiosum* DNA was confirmed by PCR

Histopathologic Description: The mass is mostly fibrovascular connective tissue containing a diffuse inflammation of eosinophils, lymphocytes and plasma cells. Distinct foci of **coagulative necrosis** (**fig. 3-2**) are present circumscribed by a thin rim of macrophages. Within the necrotic foci are **hyphae** (**fig. 3-3**) that are negatively stained and show only a clear space outlined by the hyphal wall. The hyphae are 10 microns in width with non-parallel sides and rare septation. A **Gomori's methenamine silver** (**fig.3-4**) stain outlines the hyphal wall.

Contributor's Morphologic Diagnosis: Skin: chronic, ulcerative and fibrotic dermatitis with multifocal caseous granulomas and intralesional hyphae

Contributor's Comment: The gross appearance, location of the lesion, and the hyphae within the lesion are most consistent with an infection by *Pythium insidiosum*. The organism was cultured from the lesion and confirmed with PCR. Pythium is a water-born organism classified as an Oomycte in the kingdom Protista. The organism requires a water environment with decaying

vegetation to maintain its life cycle and temperatures between 30°C and 40°C for reproduction. Infective zoospores are produced and invade damaged plant or animal tissue. Animals acquire the infection with prolonged exposure to freestanding water containing the Pythium organism.

Cutaneous disease in horses is the most common infection seen in animals, but infection is reported in many other animal species and in other locations, such as the intestinal and respiratory tracts.[1] Cutaneous lesions usually occur on the distal extremities or ventrum, areas most likely to be in contact with stagnant water.

The zoospores produce an ulcerative lesion that rapidly expands to a large mass of granulation tissue containing draining sinus tracts. Distinct yellow to tan masses, 2-10 mm in diameter, form within the sinus tracts and are known as "kunkers". Kunkers represent areas of tissue necrosis containing hyphae and necrotic eosinophilic inflammation.

AFIP Diagnosis: Haired skin: Dermatitis and panniculitis, pyogranulomatous and eosinophilic, focally extensive, severe, with ulceration, vasculitis, and few hyphal structures, Quarter Horse (*Equus caballus*), equine.

Conference Comment: There is significant slide variation with some sections having prominent vasculitis with fibrin thrombi.

Pythium insidiosum and *Lagenidium* sp. are aquatic dimorphic water molds of the kingdom Protista. *Lagenidium* sp. have only been described in dogs.[4] The infective stage of pythium is a biflagellate zoospore that is attracted chemotactically to injured tissue. Upon contact with host tissue the zoospores lose their flagellae and form germ tubes to allow penetration and invasion of tissue.[7]

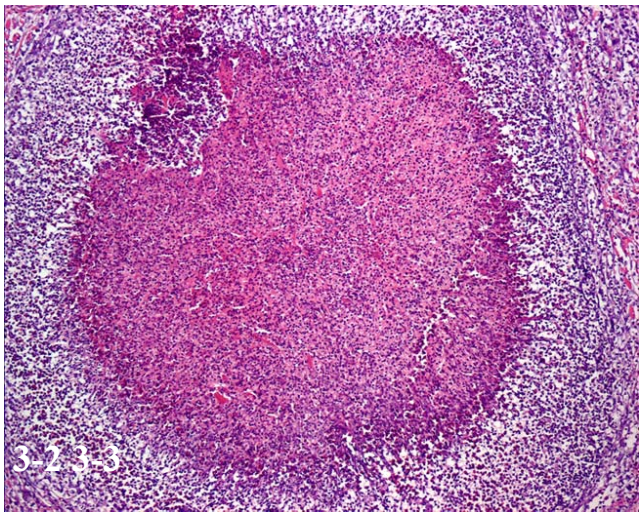
Histologic features of pythiosis are granulomatous inflammation with foci of liquefactive necrosis and extensive fibrosis with eosinophilic coagula. Circulating monocytes are recruited from the circulation by several chemotactic stimuli such as C5a, TGF-α, and platelet derived growth factor. The tissue macrophages are then activated primarily by IFN-γ, or by endotoxin.[5]

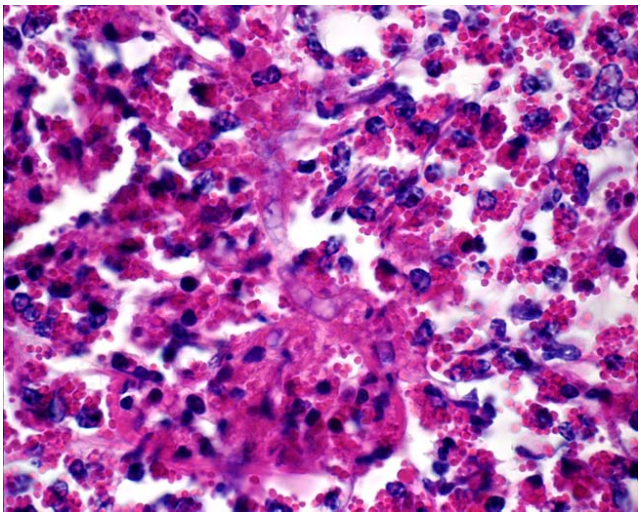
Pythium sp. stain well with GMS but do not stain well with PAS.[7] Fungal hyphae or hyphal-like structures are distinctive between various entities; the key diagnostic features are provided in table 1.[6]

Eosinophils are a prominent feature of equine pythiosis. Residents discussed the four primary differentials for eosinophilic and granulomatous dermatitis in a horse:

3-2 Subcutis, horse. Eosinophilic granuloma. (H&E)
3-3 Subcutis, horse. Multifocally, the granulomatous inflammation is admixed with the outlines of hyphal structures with non-parallel walls and rare septation. (H&E)

Photomicrographs courtesy of the College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061 www.vetmed.vt.edu





3-4 Subcutis, horse. The lesion contains few Gomori's Methenamine Silver (GMS) positive hyphae which are approximately 10 micrometers in width, with non-parallel walls and rare septation. (GMS 400X)

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1. Pythiosis – Hyphal-like structures, negative images on H&E that are highlighted with GMS stain, necrotizing vasculitis
2. Habronema – Nematode larvae within eosinophilic granulomas
3. Mast cell tumors – monomorphic population of mast cells surrounding eosinophilic granulomas
4. Eosinophilic collegenolytic granulomas – Usually centered on collagen bundles

Contributor: College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061
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References:

1. Chaffin MK, Schumacher J, McMullan WC: Cutaneous pythiosis in the horse. Vet Clin N Amer:Eq Prac 11: 91-103, 1995
2. Ginn PE, Mansell JEKL, Rakich PM: Skin and appendages. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., pp 704-707. Elsevier Ltd, Philadelphia, PA 2007
3. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin diseases of the dog and cat, 2nd ed., pp. 303-309. Blackwell Science, Oxford, UK, 2005
4. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1196. Elsevier, St. Louis, MO, 2007
5. Kumar V, Abbas AK, Fausto N: Acute and chronic inflammation. In: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 877-937. Elsevier Saunders, Philadelphia, PA, 1999
6. Rippon JW: Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes, pp. 569, 638, 653, 687, 739. W.B. Saunders Company, Philadelphia, PA, 1988
7. Scott DW, Miller WH: Equine Dermatolgy, pp. 287-293. Elsevier Science, St. Louis, MO, 2003

Table 1. Hyphae and hyphal-like structures in tissue sections.

Species	Hyphael type	Septae	Width	Branching	Walls	Misc
Aspergillus sp	Hyphae, +/- fruiting bodies	Regularly septate	2.5-4.5 μm	Acute angle, dichotomous	Parallel walls	Conidia not seen in tissue
Candida sp.	Hyphae, pseudo-hyphae & budding yeast	septate	3-6 μm	Irregular branching		
Pseudallescheria boydii	Hyphae with conidiophores	septate	5 μm	Less acute, highly branching, intertwined		15-20μm intercalary chlamydoconidia (swollen cells)
Pythium insidiosum	Hyphal like structures	Rarely septate	2-9 μm	Irregularly branching	Non-parallel	Kunkers or leeches
Zygomycetes	May occur as 5-30μm chlamydoconidia	Few septae	3-25 μm	Non-dichotomous irregular branching	Non-parallel	*Mucor* sp., *Basidiobolus* sp., *Rhizopus* sp.

CASE IV - Case 1 (AFIP 3027389).

Signalment: Feline, Domestic shorthair cat, 5 years, neutered male

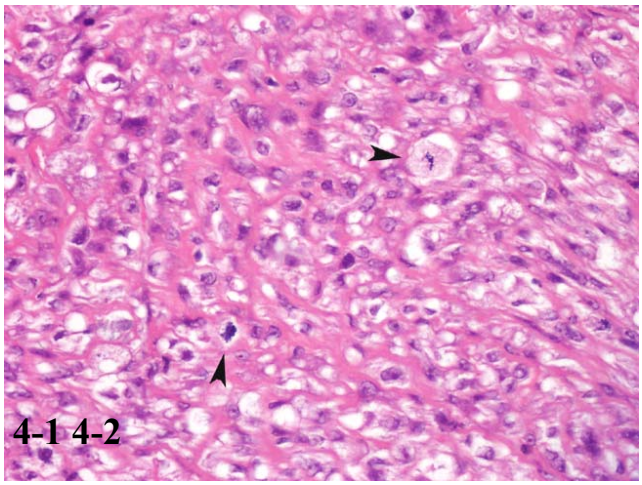
History: The cat had a subcutaneous, mobile, non-painful mass located in the interscapular region, which was surgically excised and submitted for histopathological evaluation. It was reported that the cat had been previously vaccinated in this area including administration of a rabies vaccine.

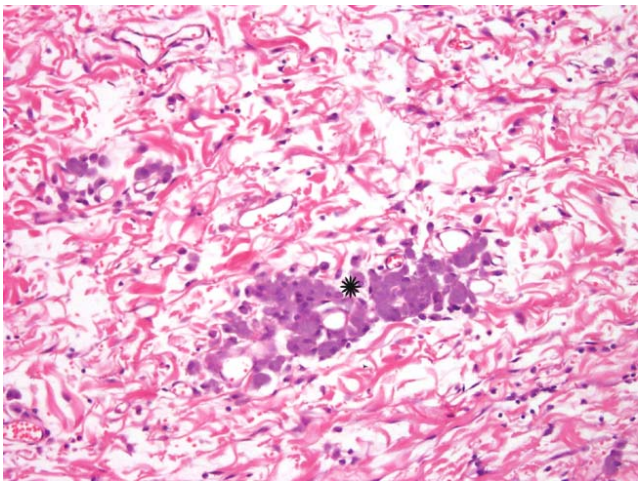
Histopathologic Description: Haired skin; The subcutis contained a well-demarcated, unencapsulated mesenchymal tumor composed of interlacing streams and bundles of neoplastic spindle cells, separated by fibrovascular stroma. The neoplastic cells had indistinct borders and a scant amount of pale eosinophilic cytoplasm. Most neoplastic cells contained a single, ovoid to elongated, vesicular nucleus with one to several, prominent basophilic nucleoli. Anisocytosis, anisokaryosis and cellular pleomorphism were moderate. **Mitoses (fig. 4-1)** ranged from 2-6 per high power dry field. Scattered multinucleated neoplastic cells were observed. Multifocal nodular aggregates of lymphocytes and fewer plasma cells were present at the tumour periphery, located mainly perivascularly, and also in the adjacent subcutis. Clustered **macrophages (fig. 4-2)**, which contained intracytoplasmic bluish-grey granular material (interpreted as possible phagocytosed vaccine adjuvant) were present in the tumour, at the tumor margin and within the deeper subcutis. In some sections, the tumor contained poorly defined, variably-sized irregularly-shaped cystic cavities. The cystic cavities contained eosinophilic material, cellular debris, foamy macrophages, mildly basophilic fluid and/ or red blood cells.

Contributor's Mor phologic Di agnosis: Haired skin, subcutis; Fibrosarcoma with nodular lymphocytic and histiocytic infiltrates and presence of bluish-grey material within intralesional histiocytes (suspected vaccine-associated sarcoma)

Contributor's Comm ent: The tumor was diagnosed as a vaccine-associated fibrosarcoma based on the reported location in the interscapular region, the history of previous vaccine injections in this area together with the typical microscopic features characteristic of this type of fibrosarcoma. Microscopic features of vaccine-associated fibrosarcomas include subcutaneous location, peripheral lymphocytic aggregates, intralesional large histiocytes with intracytoplasmic bluish-grey material and areas of micro-cavitation.[2,8]

Feline vaccine-associated sarcomas (FVAS), also known as post-vaccine and vaccine-induced sarcomas, are most commonly associated with the administration of killed feline leukemia (FeLV) and rabies vaccines.[11,15] The tumors are located in the subcutis of the common vaccination sites e.g. dorsal neck, interscapular region, dorsolateral thorax, hindleg and dorsal lumbar region.[3,7,8] The most common type of vaccine-associated sarcoma is the fibrosarcoma[8], which was observed in this case. Other





4-1 4-2

4-1 Subcutis, cat. The neoplastic cells have a high mitotic rate (arrows). (H&E 400X)
4-2 Subcutis, cat. Infiltrating macrophages often contain intracytoplasmic, amphophilic granular material interpreted to be vaccine material (star). (H&E 200X)

sarcomas which have been reported in a similar setting include rhabdomyosarcomas[8], osteosarcomas[8], chondrosarcomas[8], malignant fibrous histiocytomas[8] and myofibroblastic sarcomas.[5]

Fibrosarcomas are composed of spindle cells arranged in interlacing bundles, are located in the dermis and/or subcutis and are often circumscribed, but can have infiltrative margins. In vaccine-associated fibrosarcomas, spindle cells are admixed with histiocytoid cells and scattered multinucleated giant cells.[8,16] Microscopic features differentiating feline vaccine-associated fibrosarcomas from non-vaccine associated fibrosarcomas include prominent inflammation (peripheral lymphocytic aggregates)[2,3,8], presence of large histiocytic cells with intracytoplasmic bluish-grey material[1,2,7] and areas of cavitation.[2] In comparison to nonvaccine-associated fibrosarcomas, vaccine-associated fibrosarcomas are considered to have a higher degree of cellular pleomorphism and more inflammation.[3] The bluish-grey intrahistiocytic material contained aluminum by electron probe x-ray microanalysis.[7] Aluminium hydroxide is a common adjuvant used in killed feline leukemia (FeLV) and rabies vaccines. In feline VAS, a positive correlation was observed between increased number of neoplastic multinucleated giant cells and higher tumor grade, whereas the tumor grade was not influenced by the degree of inflammation.[2]

The average age of cats with vaccine-associated fibrosarcoma is younger (with a median age of 8 years) than the age of cats affected by non-vaccine associated fibrosarcomas (with a median age of 11 years).[3] Feline vaccine-associated fibrosarcomas are locally invasive and aggressive with a high rate of local recurrence and more rare metastases to draining lymph nodes[10], mediastinum [20] and lungs.[1,20]

Although the exact pathogenesis of vaccine-associated sarcomas is unknown, an association between persistent antigenic stimulation, chronic inflammation and/or wound healing and neoplastic transformation of primitive mesenchymal cells resulting in soft tissue sarcomas is suspected.[15] After subcutaneous injection of rabies vaccine, focal granulomatous injection-site reactions often with central necrosis, peripheral lymphocytic aggregates and presence of globular grey-blue material within the cytoplasm of some macrophages have been reported.[6] The growth factors FGF-b and TGFα, which are involved in wound healing and neoplastic transformation of mesenchymal cells, are expressed by tumor cells in feline VAS.[19] Based on ultrastructural[16] and immunohistological[8] studies, myofibroblasts which are involved in wound healing, have been identified in feline VAS.

Platelet-derived growth factor (PDGF) plays a role in the migration and proliferation of fibroblasts.[13] By using VAS cell lines, it was shown that neoplastic cells of VAS express the Platelet-derived growth factor receptor (PDGFR- β) and that inhibition of this receptor inhibited cell growth.[12] Mutations of the tumor suppressor gene p53, which are genomic alterations involved in tumorigenesis[14], have also been identified in feline VAS.[18] In addition, over-expression[19] and altered expression[10] of the P53 protein, which is most commonly caused by mutation of its gene, have been detected in feline VAS.

Feline vaccine-associated sarcomas and feline post-traumatic ocular sarcomas[4,23] are similar in morphology and pathogenesis: both tumors can be composed of a spectrum of sarcomas suspected to arise within a background of persistent inflammation or wound healing.[4, 15]

Sarcomas associated with vaccination sites have also been reported in ferrets[17] and dogs.[21] Microscopic features of these tumors are similar in all species.

AFIP Diagnosis: Haired skin, subcutis: Fibrosarcoma, domestic short hair (*Felis catus*), feline.

Conference Comment: The contributor provides a thorough overview of feline vaccine-associated sarcomas. Conference participants discussed the cell cycle as well as the four classes of regulating genes (growth promoting genes, growth inhibiting tumor suppressor genes, genes that regulate apoptosis, and genes involved in DNA repair) and how defects in any of these genes may lead to uncontrolled cellular proliferation and tumorigenesis.

Sarcomas in cats have also been associated with subcutaneous administration of long acting drugs and with the presence of foreign materials, such as non-absorbable suture or microchips. Ocular sarcoma may develop following ocular trauma.[22]

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References:

1. Briscoe C, Lipscomb T, McKinney L: Pulmonary metastasis of a feline vaccination-site fibrosarcoma. J Vet Diagn Invest 10:79-82, 1998
2. Couto SS, Griffey SM, Duarte PC, Madewell BR: Feline vaccine-associated fibrosarcoma: morphologic distinctions. Vet Pathol 39: 33-41, 2002
3. Doddy FD, Glickman LT, Glickman NW, Janovitz EB:

Feline fibrosarcomas at vaccination sites and non-vaccination sites. J Comp Path 114: 165-174, 1996
4. Dubielzieg RR, Everitt J, Shadduck JA, Albert DM: Clinical and morphological features of posttraumatic ocular sarcomas in cats. Vet Pathol 27: 62-65, 1990
5. Dubielzieg RR, Hawkins KL, Miller, PE: Myofibroblastic sarcoma originating at the site of rabies vaccination in a cat. J Vet Diagn Invest 5: 637-638, 1993
6. Hendrick MJ, Dunagan CA: Focal necrotizing granulomatous panniculitis associated with subcutaneous injection of rabies vaccine in cats and dogs: 10 cases (1988-1989). J Am Vet Med Assoc 198: 304-305, 1991
7. Hendrick MJ, Goldschmidt MH, Shofer FS, Wang Y, Somlyo AP: Postvaccinal sarcomas in the cat; epidemiology and electron probe microanalytical identification of aluminum. Cancer Res 52: 5391-5394, 1992
8. Hendrick MJ, Brooks JJ: Postvaccinal sarcomas in the cat: histology and immunohistochemistry. Vet Pathol 31: 126-129, 1994
9. Hershey AE, Sorenmo KU, Hendrick MJ, Shofer, FS: Prognosis for presumed feline-associated sarcoma after excision: 61 cases (1986-1996). J Am Vet Med Assoc 1: 58-61, 2000
10. Hershey AE, Dubielzig RR, Padilla ML, Helfand SC: Aberrant p53 expression in feline vaccine-asssociated sarcomas and correlation with prognosis. Vet Pathol 42: 805-811, 2005
11. Kass PH, Barnes WG, Sprangler WL, Chomel, BB, Culbertson MR: Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats. J Am Vet Med Assoc 203: 396-405, 1992
12. Katayama R, Huelsmeyer MK, Marr AK, Kurzman ID, Thamm DH, Vail DM: Imatinib mesylate inhibits platelet-derived growth factor activity and increases chemosensitivity in feline vaccine-associated sarcoma. Cancer Chemother Pharmacol 54: 25-33, 2004
13. Kumar V, Abul A, Faustro N: Tissue renewal and repair: regeneration, healing and fibrosis. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abul A, Faustro N, 7th ed., pp. 87-118. Elsevier Inc, Philadelphia, Pennsylvania , 2005
14. Kumar,V, Abul, A, Faustro, N: Neoplasia. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abul A, Faustro N, 7th ed., pp.269-342. Elsevier Inc, Philadelphia, Pennsylvania , 2005
15. Macy DW, Hendrick MJ: The potential role of inflammation in the development of postvaccinal sarcomas in cats. Review in Vet Clin North Am Small Anim Prac 26: 103-109, 1996
16. Madewell BR, Griffey SM, McEntee MC, Leppert VJ, Munn RJ: Feline-associated fibrosarcoma: an ultrastructural study of 20 tumors (1996-1999). Vet Pathol 38:196-202, 2001
17. Munday JS, Stedman NL, Richey LJ: Histology and immunohistochemistry of seven ferret vaccination-site fibrosarcomas. Vet Pathol 40: 288-293, 2003
18. Nambiar PR, Jackson ML, Ellis, JA, Chelack BJ, Kidney BA, Haines DM: Immunohistochemical detection of tumor suppressor gene p53 protein in feline injection site-associated sarcomas. Vet Pathol 38: 236-238, 2001
19. Nieto A, Sanchez MA, Martinez E, Rollan E: Immunohistochemical expression of p53, fibroblast growth factor-b, and transforming growth factor-α in feline Vaccine-associated sarcomas. Vet Pathol 40: 651-658, 2003
20. Rudmann DG, Van Alstine WG, Doddy F, Sandusky GE, Barkdull T, Janovitz EB: Pulmonary and mediastinal metastases of a vaccine-site sarcoma in a cat. Vet Pathol 33: 466-469, 1996
21. Vascellari M, Melchiotti E, Bozza MA, Mutinelli F: Fibrosarcomas at presumed sites of injection in dogs: characteristics and comparison with non-vaccination site fibrosarcomas and feline post-vaccinal fibrosarcomas. J Vet Med 50: 286-291, 2003
22. Vascellari M, Melchiotti E, Mutinelli F: Fibrosarcoma with typical features of postinjection sarcoma at site of microchip implant in a dog: histologic and immunohistochemical study. Vet Pathol 43:545-548, 2006
23. Zeiss CJ, Johnson EM, Dubielzig RR: Feline intraocular tumors may arise from transformation of lens epithelium. Vet Pathol 40: 355-362, 2003

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 5

17 October 2007

Moderator:

Marc E. Mattix, DVM, MSS, Diplomate ACVP

CASE I – 06-29523 (AFIP 3066303).

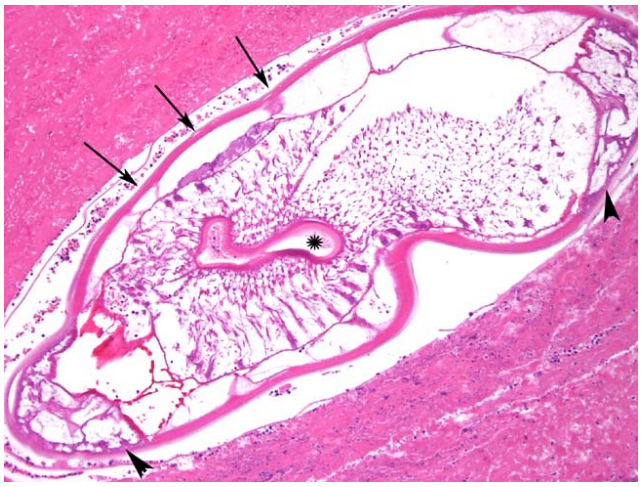
Signalment: 14-year-old, intact female, Thoroughbred, equine (*Equus caballus*)

History: The horse had a two day history of colic. Exploratory surgery revealed a 12 cm in diameter mass cranial to the left kidney which was not surgically resectable. Euthanasia was elected after a rapid decline in health post surgically, that was unresponsive to medical management.

Gross Pathology: 10 cm of the cranial mesenteric artery, immediately distal to the ostium, was dilated 3 cm and thickened (6 mm). The vessel was partially occluded by a 3.5 cm long red to purple, friable coagulum (thrombus) that was tenaciously adhered to the intimal surface. The intima was diffusely rough and granular.

Laboratory Results:
Clinical pathology abnormalities at surgery:
Neutrophils=16.1X10^3/ul (5.5-12.0)
Lymphocytes=0.79X10^3/ul (1.5-5.0)
Total protein = 8.1 g/dL (5.5-7.5)
Serum globulin = 4.9 g/dL (2.6-4.0)
Sodium = 133 mEq/L (137-148)
Chloride = 90 mEq/L (98-110)
Potassium = 1.9 mEq/L (2.9-5.3)
Sodium/Potassium ratio = 70 (28-36)
Glucose = 133 mg/dL (71-100)
Alk phos = 262 U/L (45-239)
Total bilirubin=2.7 mg/dL (0.6-2.6)
CPK = 1507 U/L (120-350)
SDH = 15.9 U/L (0.2-7.0)
All other values were within normal limits.

Histopathologic Description: The arterial lumen is occluded by an eosinophilic, amorphous coagulum (thrombus) containing alternating layers of free erythrocytes, intact and degenerate neutrophils, and necrotic debris (lines of Zahn) and multiple 1-2 mm cross sections of nematodes. The **nematodes (fig. 1-1)** have a bright eosinophilic, thick, smooth, cuticle with lateral cords and platymyarian musculature surrounding a central digestive tract. The thrombus adheres to and blends in with the vessel wall. The endothelium is mostly absent and the internal elastic lamina is disrupted, fragmented, and coiled. The tunica intima is diffusely thickened by proliferative immature fibrous connective tissue which also penetrates the tunica media and extends to and expands the adventitia, with separation and individualization of smooth muscle fibers. The intima is diffusely infiltrated by many neutrophils and relatively fewer eosinophils extending in from the lumen in declining numbers to the subjacent tunica media. The deep tunica media and adventitia is punctuated by variably sized aggregates of



1-1 Artery, Thoroughbred horse. Cross section of adult nematode characterized by a thin cuticle, coelomyarian-platymyarian musculature (arrows), prominent lateral cords (arrowheads), a pseudocoelom, and central digestive tract (star). (H&E 100X)

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lymphocytes and plasma cells admixed with foamy and hemosiderin-laden macrophages and rare clusters of neutrophils.

Contributor's Morphologic Diagnosis: Cranial mesenteric artery: Arteritis, chronic, severe, suppurative and lymphoplasmacytic.
Cranial mesenteric artery: Thrombus, acute, with intralesional nematodes.

Contributor's Comment: *Strongylus vulgaris* is one of three species of the genus Strongylus which occur in the horse and is considered to be the most damaging to the host.[6] The life cycle involves the ingestion of third-stage larvae which penetrate the mucosa and submucosa of the small and large intestines. Seven days after ingestion most of the larvae have molted to become fourth-stage larvae which then penetrate the submucosal intestinal arterioles and migrate along the intima, eventually reaching the mesenteric artery. Migrations during the fourth stage of development lead to the gross lesions which range from tortuous intimal tracts to thrombotic lesions, often referred to as "verminous aneurisms", and arteritis.[6] The small bulging tracks containing larvae, and the associated endothelial damage serves as a nidus for the development of thrombi. The arteritis and fibrosis of the arterial wall is attributed to both the disruption of the internal elastic lamina and the inflammatory response induced by the larvae. Larvae are generally found in intimal thrombi of the artery and rarely in the tunica media and adventitia.[7] Research has shown that the curvature of the vessels, not the direction of blood flow, influences migration patterns and larva prefer to migrate longitudinally along vessels[1], which accounts for the localization of the larvae in the mesenteric artery. Migration into the aorta is very infrequent, presumably because the cranial mesenteric artery branches at a right angle from the aorta. The larvae molt to the fifth stage after 3-4 months and return to the cecum and colon, where they develop into adults in two months and begin reproduction.

S. vulgaris is thought to cause colic via thromboembolic obstruction of the cranial mesenteric artery (with secondary infarction of the bowel), reduced blood flow to the branches off the cranial mesenteric artery, interference with innervation due to pressure on abdominal autonomic plexuses, or disruption of ileal motility by toxic products generated from degenerating larvae.[3]

The prevalence of cranial mesenteric arteritis due to *S. vulgaris* in horses has ranged from 80% in 1937 to 98% in 1991[7] with a dramatic decline to 6% in the late 1990's[5]. The drastic decrease in incidence has been attributed to the instigation of effective anthelmentic programs.

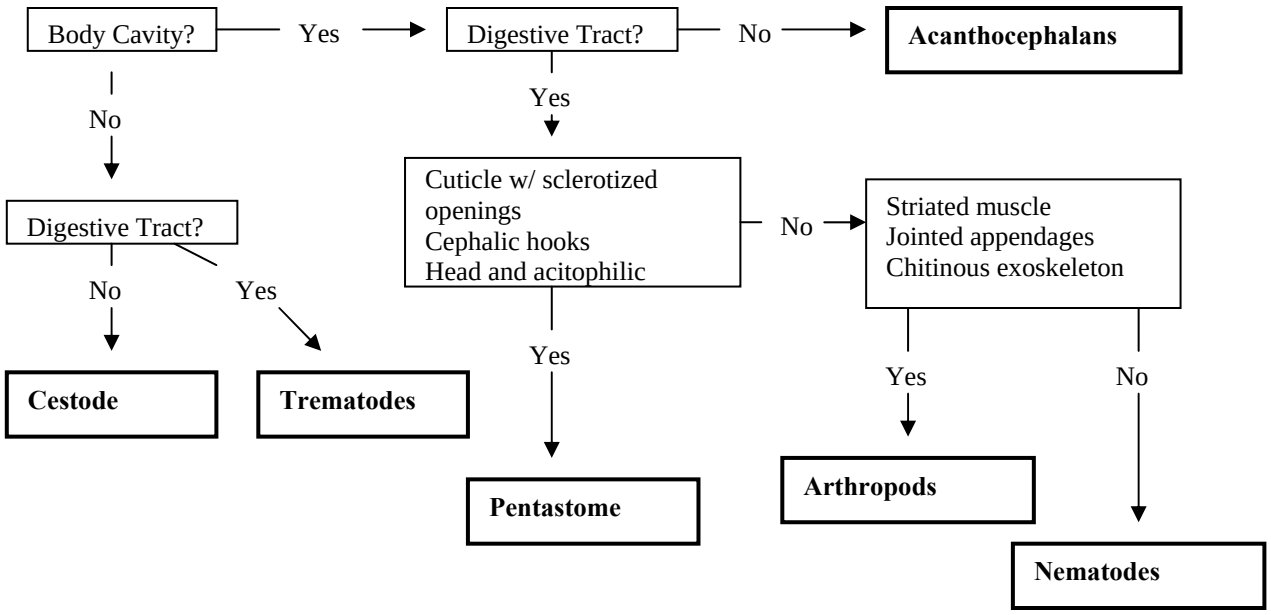
AFIP Diagnosis: Artery: Arteritis, chronic-active, multifocal to coalescing, moderate with marked diffuse transmural fibrosis, mural fibrin thrombus and intraluminal larval strongyles, Thoroughbred (*Equus caballus*), equine.

Conference Comment: *Strongylus vulgaris* is the only large strongyle that is known to undergo portions of its development within the equine arterial system.[6] The other two large strongyles that are known to commonly affect horses are *S. edentates* and *S. equines*. *S. edentates* normally migrates via the portal system to the liver, molts to L_4 within the liver parenchyma, and then returns to the cecum via hepatic ligaments. *S. equines* migrates through the peritoneal cavity to the liver then the pancreas and re-enters the cecum and right ventral colon via direct penetration.[2]

Identification of organisms as nematodes is determined by evaluating specific structures. The accompanying flow chart aids in categorization of metazoan parasites **(fig. 1-2).**

Other vascular parasites include:

- Blood flukes of mammals and birds – *Schistosoma* sp., *Heterobilharzia* sp., *Orientobilharzia* sp.
- *Onchocerca* sp. – within the walls of the aorta of cattle, buffalo and goats



1-2 Key to categorization of parasites in tissue section.

- *Dirofilaria immitis* – heartworm of dogs, cats, sea lions, muskrats

Brugia sp. – tropical parasite of dogs and cats

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http://www.cvm.uiuc.edu/path

References:

1. Aref S: A random walk model for the migration of *Strongylus vulgaris* in the intestinal arteries of the horse. The Cornell Veterinarian 72:64-75, 1982
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 247-248. Elsevier Limited, St. Louis, MO, 2007
3. Drudge JH, Lyons ET: Large strongyles. recent advances. Vet Clin North Am Equine Pract 2:263-280, 1986
4. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 356-357. Elsevier, St. Louis, MO, 2007
5. Lyons ET, Swerczek TW, Tolliver SC, Bair HD, Drudge JH, Ennis LE: Prevalence of selected species of internal parasites in equids at necropsy in central kentucky (1995-1999). Vet Parasitol 92:51-62, 2000
6. Maxie, MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 89-91. Elsevier Limited, St. Louis, MO, 2007
7. Morgan SJ, Stromberg PC, Storts RW, Sowa BA, Lay JC: Histology and morphometry of *Strongylus vulgaris*-mediated equine mesenteric arteritis. J Comp Pathol 104:89-99, 1991

CASE II – 07L21FF (AFIP 3065937).

Signalment: 19-month-old, male, American foxhound, *Canis familiaris*, dog.

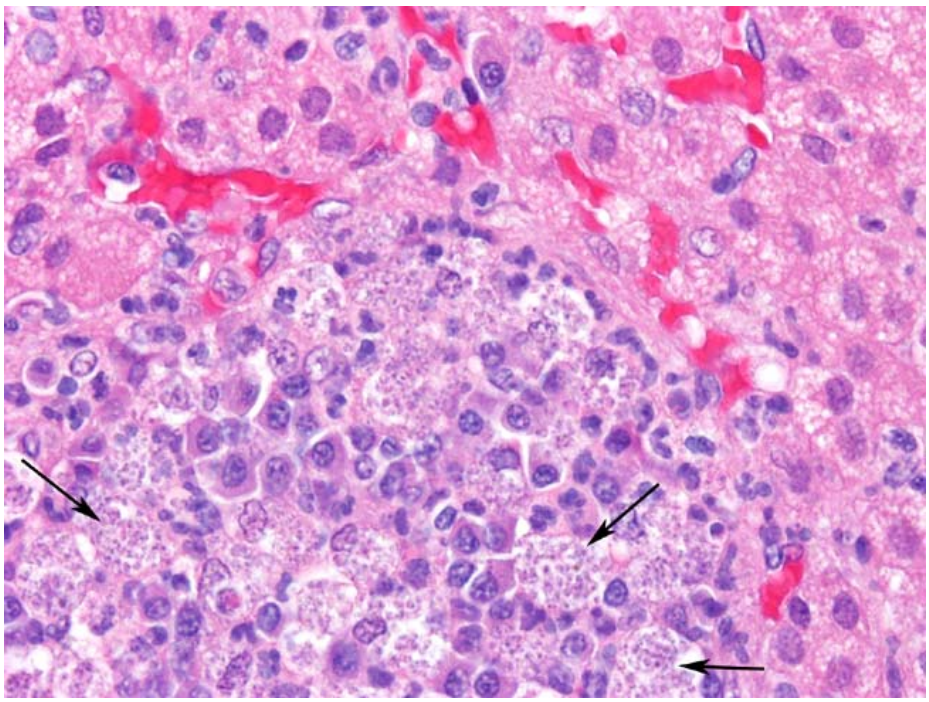
History: This male American foxhound dog along with a sibling was donated to Iowa State University because both were seropositive for *Leishmania* spp. This animal was born in August of 2005 to a *Leishmania* positive bitch, and both siblings became serologically positive for *Leishmania* in January of 2007. Following seroconversion the dog became anemic, thrombocytopenic and leukopenic. Upon presentation, the dog exhibited epistaxis and was progressively losing weight.

Gross P athology: The animal was thin to emaciated with minimal adipose tissue in body cavities and subcutaneous tissues. The liver was diffusely and markedly enlarged (1.65kg), pale, and firm with a diffuse finely granular texture. The spleen was diffusely and markedly enlarged (38g) and pale with finely granular capsular surface texture. All lymph nodes, including peripheral, mesenteric and mediastinal nodes were markedly enlarged. Bilaterally the kidneys were moderately enlarged and diffusely pale, and there was little peri-renal adipose tissue.

Laboratory Results: A complete blood count revealed a non-regenerative anemia and thrombocytopenia, while a chemistry panel showed elevation in alkaline phosphatase and alanine transferase as well as hypoproteinemia. On urinalysis there was 4+ protein in the urine. Spleen and bone marrow samples were culture positive for *Leishmania* by the Centers for Disease Control and Prevention (CDC). Real-time PCR on whole blood performed at ISU and the CDC were positive for *Leishmania infantum*.

Histopathologic D escription: Kidney: Multifocal glomeruli have thickened Bowman's capsules and approximately 60-70% of glomeruli have markedly thickened and prominent capillary loops with numerous synechiae. Multiple glomeruli are shrunken and hypocellular (sclerosis). Within the interstitium, there are multifocal to coalescing accumulations of inflammatory cells, primarily lymphocytes, plasma cells and macrophages, with moderate numbers of macrophages containing one or more 1-2 µm round to oval basophilic organisms. Cytologically these organisms are ovoid, 1-3 µm in diameter, with a round, basophilic nucleus and a rod-shaped kinetoplast.

2-1 Adrenal gland, American foxhound. Expanding the adrenal cortex, there are aggregates of lymphocytes, plasma cells and macrophages. Macrophages frequently contain high numbers of protozoa which are ovoid, 1-3 µm in diameter, with a round, basophilic nucleus and a rod-shaped kinetoplast (arrows). (H&E 600X)



Adrenal glands: Multifocally throughout the adrenal cortex, there are multiple foci of lymphocytes, plasma cell, and macrophages. Many of the macrophages contain numerous small intracellular **organisms (fig. 2-1)** as described above.

Contributor's Morphol ogic Diagnosis:

1. Kidney:

a. Glomerulonephritis, membranous, severe, chronic, diffuse, with multifocal glomerulosclerosis.

b. Interstitial nephritis, lymphoplasmacytic and granulomatous, severe, chronic, multifocal to coalescing, with intrahistiocytic organisms consistent with *Leishmania* species.

2. Adrenal gland: Adrenalitis, granulomatous and lymphoplasmacytic, moderate, chronic, multifocal with intra-histiocytic organisms consistent with *Leishma-*

nia species.

Contributor's Comme nt: The changes in the kidney and adrenal gland are consistent with disseminated visceral leishmaniasis. Parasites were also present within macrophages in the liver, spleen, lymph nodes, pancreas and bone marrow (not submitted for evaluation). *Leishmania infantum* is a protozoal parasite that causes visceral leishmaniasis. Natural hosts include rodents, small mammals, dogs, and humans, although infection is usually accidental.[4] Leishmaniasis is transmitted to the host by the sandfly bite after which the promastigote form of the parasite is phagocytosed by macrophages.[4] Once within the host cell the parasite transforms into amastigotes and multiples, eventually leading to systemic spread of the parasite. Parasite control requires the induction of a T_H1 immune response characterized by production of interferon gamma and interleukin 12 that function to activate infected macrophages to kill the intracellular pathogen.[4] Visceral leishmaniasis is characterized by fever, weight loss, hepatomegaly, splenomegaly, skin lesions and epistaxis.[4] Histologically there are focal granulomas with intra-histiocytic organisms in affected organs as well as lymphofollicular hyperplasia within the spleen and lymph nodes.[6] Membranous glomerulonephritis is a common finding in both canine and human patients with visceral leishmaniasis and is secondary to antigen-antibody complex formation and subsequent deposition within the mesangium of the glomerulus.[1]

Although endemic in southern Central and South America, the Middle East, Central Asia and Africa, this disease is also present in the United States and sporadic cases have been reported, usually travelers returning from an endemic area.[5] In the year 2000, a foxhound kennel in New York reported four foxhounds to be infected with *L. infantum*.[3] The sandfly vector is present within the United States, although at this time it has not be determined if sandfly transmission of *Leishmania* occurs in this country. Other mechanisms have been postulated in transmission of canine visceral leishmaniasis and include vector-independent modes such as breeding and direct contact. There may also be a genetic or breed susceptibility to infection, as numerous foxhounds have tested positive and infection appears to be widespread within this breed in the United States, indicating a possible public health threat.[2]

AFIP Diagnosis: 1. Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, subacute, marked with multifocal to coalescing lymphoplasmacytic interstitial nephritis, protein casts, and intrahistiocytic amastigotes, etiology consistent with *Leishmania* sp., American foxhound (*Canis familiaris*), canine.

2. Adrenal gland: Adrenalitis, histiocytic, neutrophilic, and plasmacytic, multifocal, moderate, with intrahistiocytic amastigotes, etiology consistent with *Leishmania* sp.

Conference Comment: *Leishmania* are protozoan parasites of the family Trypanosomidae, order Kinetoplastida.[5] They survive within the cytoplasm of mammalian macrophages as amastigotes (leishmanial form) that are 2.0µm in diameter with a vesicular nucleus, no flagella and a small basophilic kinetoplast.[6]

There are three forms of Leishmaniasis:[6]
1. Cutaneous (oriental sore) *L. tropica* – Mediteranean sea
2. Mucocutaneous (espundia) *L. braziliensis* – Central America
3. Visceral (kala-azar) *L. donovani* – Europe, Africa and Asia

The primary insect vectors for *Leishmania* sp. include the phlebotomine sand flies (*Lutzomyia* sp. and *Phlebotomus* sp.). Of the fourteen *Lutzomyia* sp. in North America, three are known to be capable of transmitting *Leishmania mexicana* (cutaneous leishmaniasis in Mexico and Texas).[3] Other forms of transmission that have been implicated include mechanical transfer through ticks, shared needles, sexual contact, and bite wounds, as well as transmammary and transplacental transmission.[5]

Upon phagocytosis by macrophages, the organism survives within the phagolysosome despite the activated proteinases and the low environmental pH (4.5-5.0).[4] Studies of the cutaneous form of leishmaniasis in mice caused by *L. major* indicate immunity depends on an IL-12 driven CD4+, T_H1-type response with production of IFN gamma. A CD4+, T_H2-type response with production of IL-4 and IL-10 results in susceptibility.[3]

The initial case of visceral Leishmaniasis in a foxhound in North America occurred in 1980.[5] Since that time, visceral leishmaniasis caused by the *Leishmania donovani* complex (*L. donovani*, *L. infantum*, *L. chagasi*) has been identified in 21 states in the U.S. and 2 Canadian provinces.[5]

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References:
1. Costa FAL, Goto H, Saldanha LCB, Silva SMMS,

Sinhorini IL, Silva TC, Guerra JL: Histopathologic patterns of nephropathy in naturally acquired canine visceral leishmaniasis. Vet Path 40:677-684, 2003
2. Duprey ZH, Steurer FJ, Rooney JA, Kirchhoff LV, Jackson JE, Rowton ED, Schantz, PM: Canine visceral leishmaniasis, United States and Canada, 2000-2003. Emerg Infect Dis 12:440-446, 2006
3. Gaskin AA, Jackson J, Birkenheuer A, Tomlinson L, Gramiccia M, Levy M, Steurer F, Kollmar E, Hegarty BC, Ahn A, Breitschwerdt EB: Visceral leishmaniasis in a New York foxhound kennel. J Vet Intern Med 16:34-44, 2002
4. Roberts LJ, Handman E, Foote SJ: Science, medicine, and the future: leishmaniasis. BMJ 321:801-804, 2000
5. Schantz PM, Steurer FJ, Duprey AH, Kurpel KP, Barr SC, Jackson JE, Breitschwerdt EB, Levy MG, Fox JC: Autochthonous visceral leishmaniasis in dogs in North America. J Am Vet Med Assoc 226:1316-1322, 2005
6. Valli VEO: Hematopoietic system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 302-304. Elsevier Limited, St. Louis, MO, 2007

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CASE III – 06/8597611 Boyd V10112 (AFIP 3069488).

Signalment: 2-year-old, female, Jersey, *Bos taurus*, bovine

History: Three of 100 mature Jersey cattle died with no observable clinical signs. They were female dry cattle at pasture. The clinical differential diagnosis was Clostridial disease, botulism or anthrax. The clinician opened the carcass and quickly discovered an enlarged spleen. A 2 by 3 inch sample of spleen was removed and the clinician made his own smears and submitted the spleen sample and smears to the laboratory as a possible case of anthrax.

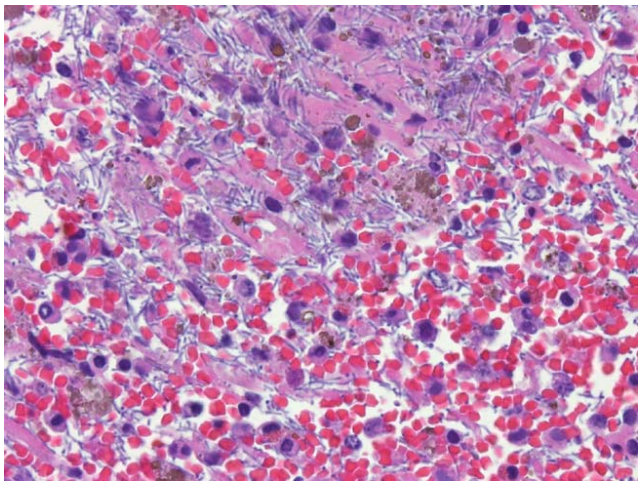
Gross Pathology: The cow had an enlarged and swollen spleen that oozed thick dark blood from the cut surface.

Laboratory Results: A smear from the spleen was prepared and stained with polychrome methylene blue. There were numerous large square ended bacilli with a pink capsule. Blood from the spleen was cultured on sheep blood agar plates and incubated aerobically. Within 12 hours of incubation, there were large numbers of small dry irregularly shaped ground glass colonies. The bacterial colonies were sensitive to penicillin. These findings are consistent with *Bacillus anthracis.*

Histopathologic Description:
The spleen was enlarged and congested with large numbers of extracellular erythrocytes filling the red pulp with a marked reduction in amount of the white pulp and small lymphoid follicles. Widely dispersed throughout the spleen were myriads of extracellular bacteria. The **bacteria (fig. 3- 1)** were large and uniform in size bacilli, approximately 3um long and 1um wide. There were large numbers of widely dispersed macrophages that were filled with pigment, confirmed as hemosiderin. There was disruption of the parenchyma, due to autolysis of the tissue.

Contributor's Morp hologic D iagnosis: Acute diffuse splenic haemorrhage with numerous extracellular bacilli.

Contributor's Comm ent: Anthrax is a peracute, acute or subacute highly contagious disease of domestic animals and humans caused by the bacterium *Bacillus anthracis*.[1] Necropsy of animals infected with *Bacillus anthracis* is not recommended because exposure to air allows the bacteria to sporulate, resulting in extremely resistant anthrax spores that contaminate the environment for years.[2] The recommended means of diagnosis is collection of a peripheral blood smear without opening the carcass. Peripheral blood smears in anthrax cases have large numbers of Gram positive rod shaped bacteria, with square ends and a pink capsule stained by methylene blue, Giemsa or Schaeffer and Fulton's malachite green technique. The bacteria need to be differentiated from Clostridial bacteria, that have rounded corners and no



3-1 Spleen, Jersey, cow. Diffusely the splenic parenchyma is disrupted by high numbers of large bacilli measuring approximately 3 -7 um long and 1um wide. (H&E 600X)

capsule. The presence of *Bacillus anthracis* in a blood smear can be confirmed by microbiological culture or PCR. The USA Naval Medical Research Center developed diagnostic tests for anthrax are being trialed for their suitability, in Australian conditions, for the 'pen-side' diagnosis of anthrax in livestock.[4]

Anthrax is a disease syndrome recognized for centuries and a pathogen that is widely distributed around the world. In 1823 anthrax was the first disease of humans and animals shown to be caused by a micro-organism.[1] Anthrax occurs sporadically in Australia affecting sheep, cattle, infrequently pigs and rarely goats and horses.[5] It is largely confined to the "anthrax belt" which extends through the middle of the Australian states of New South Wales and into northern and central Victoria.[4,5] This laboratory in Victoria would typically diagnose 2 or 3 cases of anthrax per year. In January and February 2007, there was an unusual outbreak of anthrax in central Victoria with this laboratory diagnosing 37 positive anthrax cases, on eight farms from approximately 300 submissions from the surveillance area. The last significant outbreak of anthrax in Victoria was between January and March 1997, when anthrax was diagnosed on 83 properties with 202 cattle and 4 sheep confirmed to have died of anthrax.[6] In Australia, effective control of anthrax infection is achieved by vaccination of in contact farms and livestock.

Ruminants are typically infected with anthrax by ingestion of spores that germinate in the intestinal tract to form encapsulating vegetative cells that replicate and spread to the regional lymph nodes and then disseminate systemically.[2] Infection may also occur by cutaneous abrasion and insect bites.[1] Extremely rarely it is possible, in cattle, to initiate an infection by inhaling spores while grazing dry dusty contaminated sites.[1] *Bacillus anthracis* produces exotoxins termed lethal toxin and edema toxin. The toxins and the capsule of the bacteria inhibit phagocytosis, increase capillary endothelial permeability and delay clotting.[1] Animal species vary in their susceptibility to anthrax infection. Species easily infected with anthrax include cattle, goats, sheep, monkey, mouse, guinea pigs, horses and chimpanzees. Species resistant to anthrax but once infection is established, are highly susceptible to effects of the exotoxins include dog, pig and NIH black and Fisher rats.[1] Humans can be infected with anthrax by inhalation, ingestion or cutaneous abrasions. Human cases of anthrax are rare in Australia and there have been only four cases in the last ten years; all have been the cutaneous form and most of the cases have been in farmers or rendering plant workers.[3]

AFIP Dia gnosis: Spleen: Congestion, acute, diffuse, severe, with lymphocytolysis, and myriad bacilli, Jersey (*Bos taurus*), bovine.

Conference Comment: The Centers for Disease Control and Prevention classifies anthrax as a Category A agent of bioterrorism. Category A agents have the potential to pose a threat against public health, spread across a large area or need public awareness, and need a great deal of planning to protect the public's health. Despite this potential, humans are relatively resistant to natural infection.

Infection of both humans and animals can occur through ingestion, percutaneously, or more rarely through inhalation of anthrax spores. Under certain conditions, spores have been known to remain viable in the soil up to 200 ±50 years. Germination of spores occurs between 20^{o}-40^{o} C and in conditions of greater than 80% relative humidity. Upon ingestion of spores, the organisms quickly germinate to the encapsulated toxin-producing vegetative form. The capsule is a poly-D-glutamate capsule that inhibits phagocytosis.[1]

Lethal toxin inhibits mitogen-activated protein kinase-kinase and results in terminal shock through the release of tumor necrosis factor (TNF) and interleukin-1 (IL-1). Edema factor results in altered intracellular water and ion concentrations through the abnormal production of c-AMP. Edema factor has also been implicated in preventing mobilization and activation of leukocytes. The presence of the capsule and two toxins effectively results in prevention of phagocytosis, increased capillary endothelial permeability and decreased blood clotting ability.[1]

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References:

1. De Vos V, Turnbill PCB: Anthrax. In: Infectious Diseases of Livestock, eds. Coetzer JAW, Tustin RC, 2nd ed., pp. 1788-1818. Oxford University Press South Africa, Cape Town, South Africa, 2004
2. Fry MM, McGavin MD. Bone marrow, blood cells and lymphatic system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 811. Elsevier, St. Louis, MO, 2007
3. Kolbe A, Yuen MG, Doyle BK: A case of human cutaneous anthrax. Med J Aust 185:281-282, 2006
4. Muller JD, Wilks CR, O'Riley KJ, Condron RJ, Bull R, Mateezun A: Specificity of an immunochromatographic test for anthrax. Aust Vet J 82:220-222, 2004
5. Snedden HR, Albiston HE: Bacterial diseases. In:

Diseases of Domestic Animals in Australia Part 5, vol. 1, pp. 12-26. Commonwealth Government Printer, Canberra, Australia,1965
6. Turner AJ, Galvin JW, Rubira RJ, Miller GT: Anthrax explodes in an Australian summer. J Appl Microbiol 87:196-199, 1999

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CASE IV - 05-801 (AFIP 2987052).

Signalment: 1-year-old, intact, female, Rocky Mountain goat (*Oreamnos americanus*)

History: This goat exhibited 3 months of progressive respiratory difficulty that was unresponsive to antibiotic therapy, leading to euthanasia. She was one of a small group (approximately 25) of captive bred Rocky Mountain goats at a private facility. This animal and several others were raised on raw goat's milk obtained from a local dairy goat farm. At the time the animals were raised, the dairy goat farm was believed to be free of caprine arthritis encephalitis virus (CAEV), but CAEV was subsequently confirmed on the premises. A 2-year-old mountain goat that had received the raw goat's milk from the same dairy was euthanized approximately 1 month later due to progressive weight loss, dyspnea, and recent onset of left sided spastic hemiparesis. Pulmonary lesions were similar in this second animal, and in addition there was a locally extensive unilateral nonsuppurative inflammatory and demyelinating lesion within the cranial cervical spinal cord.

Gross Pathology: Postmortem was performed approximately 24 hours following euthanasia. The animal was in thin body condition. The lungs failed to collapse and were diffusely dark red, firm, and meaty. Pulmonary lymph nodes were moderately enlarged. There were no other significant gross findings.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.)**:**
Escherichia coli isolated from lung and pulmonary lymph node. No mycoplasma isolated from lung or pulmonary lymph node. Fluorescent antibody testing of lung and pulmonary lymph node was negative for IBR and BVD viruses.

Histopathologic Description: Slides from two different blocks are submitted. Both exhibit similar changes. There is severe diffuse interstitial fibrosis with prominent perivascular and peribronchiolar lymphoid aggregates. There is diffuse type II pneumocyte hyperplasia, although many cells have sloughed due to postmortem artifact. Alveoli contain protein, often in aggregates, and many prominent macrophages. Smooth muscle associated with terminal bronchioles is hyperplastic. One section contains a locally extensive zone of intrabronchial necrotic debris.

Contributor's Morphologic Diagnosis: Lung: Severe diffuse chronic interstitial pneumonia with lymphoid hyperplasia consistent with Caprine Arthritis Encephalitis Virus (CAEV) infection

Contributor's Comment: The history of progressive dyspnea associated with weight loss and the histopathologic lesions within the lung are characteristic of pulmonary disease due to CAEV infection. It should be noted, however, that the severe interstitial fibrosis in this case is somewhat unusual.[1] Secondary infection by *E. coli* is suspected.

CAEV is one of a family of small ruminant lentiviruses (SRLV) that cause chronic inflammatory disease in goats (genus *Capra*, subfamily Caprinae, family Bovidae) and sheep (genus *Ovis*, subfamily Caprinae, family Bovidae). Viral integration into host DNA causes persistent infection, primarily of monocytes, macrophages, and dendritic cells.[2,4] Mammary gland involvement is common, and infection is most often due to ingestion of milk from infected dams. Infection from direct contact is also possible, but is less common. Experimental infection of Mouflon-domestic sheep hybrids by CAEV has been reported.[3] Recent phylogenetic studies have identified multiple subgroups of SRLV. One group, SRLV subtype A4, has been found to be directly transmissible and interchangeable between goats and sheep.[5]

This is the first known instance of disease compatible with CAEV occurring in a Rocky Mountain goat (genus *Oreamnos*, subfamily Caprinae, family Bovidae). No involvement of joints or mammary gland were identified in these two cases. Neurologic disease due to CAEV is most common in goats 2-4 months of age, but sporadic cases occur in adults.[6] Although immunohistochemical confirmation of CAEV was still in progress at the time of submission, various factors strongly suggest CAEV as the cause of infection in this and the second affected Rocky Mountain goat. The histopathologic lesion of diffuse interstitial pneumonia with type II hyperplasia and lymphoid hyperplasia are characteristic of pulmonary lentivirus infection.[1] Although infection by another member of the SRLV family cannot be ruled out in this case based on findings to date, the history of ingestion of raw goat's milk from a CAEV positive herd, and the

characteristic locally extensive demyelinating myelitis in the second goat (as opposed to the more diffuse and strongly periventricular spinal cord lesions of maidi-visna virus)[6] strongly suggest CAEV infection in this captive bred Mountain goat.

AFIP Diagnosis: Lung: Pneumonia, interstitial, chronic, diffuse, moderate, with marked interstitial fibrosis, lymphoid hyperplasia, and type II pneumocyte hyperplasia, Rocky Mountain goat (*Oreamnos americanus*), caprine.

Conference Comment: Slide variability included multifocal areas of acute neutrophilic alveolitis likely due to secondary bacterial infection. However, no organisms were seen.

Small ruminant lentiviruses (SRL), in the family Retroviridae, include the closely related maedi-visna virus (ovine progressive pneumina) and caprine arthritis encephalitis Virus. The viral gene of lentiviruses is a single-stranded RNA and encodes for various genes, including:[1]

- gag – Group specific neucleocapsid and matrix glycoproteins (detected by antibody based tests)
- pol – Reverse transcriptase
- env – Surface glycoprotein, mediates receptor binding and entry into the cell (target for neutralizing antibodies)

Infection with CAEV results in two main manifestations of the disease: slowly progressive arthritis in adult goats and more acute neurologic disease in kids 2-4 months old.[1] The arthritic lesions tend to localize within the carpus, but the tarsus, fetlock, stifle, and atlanto-occipital joint can be affected as well. Neurologic signs are variable and include encephalitis, progressive ataxia and weakness. Pneumonia occurs less frequently but can be the main presenting feature or occur in combination with the joint or neurologic lesions. The distinctive pulmonary lesion includes alveoli filled with densely eosinophilic fluid, type II pneumocyte hyperplasia, and alveolar septa thickened by lymphocytes. Type II pneumocyte hyperplasia is not a prominent feature in the pneumonia of ovine progressive pneumonia.[1]

In contrast to other lentiviruses in animals (including the various species specific immunodeficiency viruses of simians, humans, felines, and bovines), the SRLs do not cause immunosuppression as a primary feature. However, secondary bacterial infection by *Pasteurella multocida* or *Arcanobacterium pyogenes*, as well as parasitic infection by *Dictyocaulus* sp. or *Protostrongylus* sp., can commonly be seen in association with SRL infection.[1]

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References:

1. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 618-620. Elsevier Limited, St. Louis, MO, 2007
2. Guiguen F, Mselli-Lakhal L, Durand J, Du J, Favier C, Fornazero C, Grezel D, Balleydier S, Hausmann E, Chebloune Y: Experimetnal infection of Mouflon-domestic sheep hybrids with caprine arthritis-encephalitis virus. Am J Vet Res 61:456-461, 2000
3. Hötzel I, Cheevers WP: Host range of small-ruminant lentivirus cytopathic variants determined with a selectable caprine arthritis-encephalitis virus pseudotype system. J Virol 75:7384-7391, 2001
4. Mdurvwa EG, Ogunbiyi PO, Gakou HS, Reddy PG: Pathogenic mechanisms of caprine arthritis-encephalitis virus. Vet Res Commun 18:483-490, 1994
5. Shah C, Huder JB, Boni J, Schonmann M, Muhlherr J, Lutz H, Schupbach J: Direct evidence for natural transmission of small-ruminant lentiviruses of subtype A4 from goats to sheep and vice versa. Virol 78:7518-7522, 2004
6. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, pp. 128-132. Mosby, St. Louis, MO, 1995

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 6

24 October 2007

Moderator:

Dr. Anthony Confer, DVM, PhD, Diplomate ACVP

CASE I – 03032021 (AFIP 2890683).

Signalment: Tissues from a 2.5-year-old, Hereford cow (*Bos taurus*)

History: Patient presented with a 6-8 month duration of upper respiratory noise and a 2 week history of bloody nasal discharge. Physical examination reveals normal temperature and pulse, increased respiration, inspiratory stridor with multiple 0.5-2.0cm in diameter, tan firm sessile to polypoid masses present in both nares.

Gross Pathology: The patient is in excellent nutritional condition. Significant gross findings were limited to the nasal mucosa. The nasal mucosa of the bilateral nasal passages were expanded by multifocal and coalescing, raised, 0.5cm-2.0cm in diameter, granular to smooth, firm nodules that are present beginning 3cm caudal to the opening of the nares extending 15cm back into the nasal passage.

Laboratory Results: Fungal culture of gross lesions: *Pseudoallescheria boydii*
Bacterial culture of gross lesions: Small number of contaminants (*Streptococcus sp*., *Bacillus sp*.)
BVD immunohistochemistry (ear notch): negative

Contributor's Morphologic Diagnosis: Granulomatous rhinitis, multifocal, severe with intralesional fungal organisms identified at culture as *Pseudoallescheria boydii*.

Contributor's Comment: In March to April of 2003, several individual cattle from geographically isolated herds across the state of Oklahoma presented with nearly identical clinical signs and gross lesions consistent with nasal granulomas. Cultures from the lesions revealed either *Pseudoallescheria boydii* or *Bipolaris sp*. Interestingly, similar fungal organisms were incidentally present within an ear notch skin sample (granulomatous dermatitis) obtained for BVD immunohistochemistry in this patient.

Although uncommon, *Pseudoallescheria boydii* typically causes localized infections in cutaneous and subcutaneous connective tissues. Within lesions, the organism is often arranged as densely entangled hyphae (2-5µm) and swollen cells (15-25 µm) that can be grossly evident as tissue grains or granules. Within the nasal mucosa of this cow, the organisms were disseminated, and even when visualized with silver stains, did not form entangled hyphae. In fact, hyphae were inconspicuous compared to the variably-sized spherical swollen cells.

Other than the cutaneous and subcutaneous mycetomas, *Pseudoallescheria boydii* has also been implicated in bovine abortions.

AFIP Diagnosis: Nasal mucosa: Rhinitis, eosinophilic and granulomatous, diffuse, severe, with numerous fungal conidia and few hyphae, Hereford cow (*Bos Taurus*), bovine.

Conference Comment : This case, as published in the November 2007 issue of Veterinary Pathology, gives a good overview of *Pseudoallescheria boydii*.[9]

P. boydii are 5-8µm septate hyphae that form 6-30µm terminal round conidia with a discrete outer wall. They may exhibit narrow- or broad-based budding. GMS is preferred over PAS for demonstrating the hyphae and conidia. The case presented in conference is unusual in that it consists of numerous 6-30µm round, occasionally budding conidia, with relatively few hyphae. In some slides, the conidia are light brown in H&E sections.

P. boydii are ubiquitous within the environment. However, infections by this fungus are extremely rare and primarily reported in immunocompromised patients. In this case, there was no evidence the cow was immunocompromised. In animals, *P. boydii* primarily causes trauma-induced eumycotic mycetomas. It has rarely been associated with equine and bovine abortions, pneumonia in a calf, granulomatous rhinitis and onychomycosis in the horse, and eumycotic mycetoma and keratomycosis in the dog and horse. Unlike in dogs, nasal infections of cattle with *P. boydii* do not typically invade the underlying bone.

Gross differentials for rhinitis in cattle include atopic rhinitis, neoplasia (e.g. lymphoma, squamous cell carcinoma), foreign body, actinobacilloisis, actinomycosis, and other fungal diseases (e.g. rhinosporidiosis, aspergillosis and phycomycosis). *P. boydii* differs from *Aspergillus* sp. and *Fusarium* sp. by an absence of both angioinvasion and dichotomous branching.

Treatment of *P. boydii* is difficult and requires antifungal-susceptibility testing since the organism exhibits some level of inherent resistance to most antifungal agents.

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References:

1. Chandler FW, Watts JC: Pathologic Diagnosis of Fungal Infections, pp. 75-79, 251-256. American Society of Clinical Pathologists, Chicago, IL, 1987
2. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1194-1195. Mosby Elsevier, St. Louis, Missouri, 2007
3. Knudtson WU, Kirkbride CA: Fungi associated with bovine abortion in the northern plains states (USA). J Vet Diagn Invest 4:181-185, 1992
4. Kwon-Chung KJ, Bennett JE: Pseudallescheriasis and Scedosporium infection. In: Medical Mycology, pp. 678-694. Lea & Febiger, Malvern, PA, 1992
5. Larone DH: Medically Important Fungi: A Guide to Identification, 4th ed., pp. 196-197. ASM Press, Washington, DC, 2002
6. Rippon JW: Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes, 3rd ed., pp. 651-677. W. B. Saunders Company, Philadelphia, PA, 1988
7. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 508-509. Elsevier Limited, St. Louis, MO, 2007
8. Schwartz DA: Pseudallescheriasis and Scedosporiasis. In: Pathology of Infectious Diseases, eds. Connor DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE, vol. 2, pp. 1073-1079. Appleton & Lange, Stamford, CT, 1997

CASE II – UFSM-2 (AFIP 2897020).

Signalment: 6-month-old, female, Charolais, bovine

History: This calf belonged to a group of 210 beef calves with ages varying from 6-8 months. They were weaned at 3 months of age and placed in a pasture of 4 hectacres. This pasture was very wet and had accumulated water in several places. At one end of the pasture there was a feed trough where the calves had *ad libitum* access to corn silage and to concentrate (wheat bran) at the proportion of 1%/bw/day. During mid-fall of 2003 (3 months after being introduced onto the 4 ha.) 20 calves developed a respiratory disease with clinical signs including cough, rectal temperature of 40ºC-41ºC, serous nasal discharge, tachypnea, dyspnea, labored breathing through the mouth, and anorexia with weight loss. Four calves died after a clinical course of disease of 5-8 days. The remaining 16 sick calves were treated with Doramectin 1%, and recovered slowly.

Gross Pathology: The carcass was thin and dehydrated. The lungs had large, red-gray, firm, slightly depressed, patchy areas of consolidation, mainly in the dorsocaudal

lobes. Alternating with the consolidated depressed areas there were pink-white bulging, emphysematous areas of the lung parenchyma. The lumen of the trachea and major bronchi contained large tangles of numerous slender, white, 5-8 cm, nematodes mixed with a mucinous, foamy exudate. These parasites had morphology compatible with *Dictyocaulus* spp. The areas of consolidation could be appreciated on the cut surface of the lungs. At the cut surface multiple 1 mm in diameter, yellow foci could be observed randomly distributed in the lung parenchyma, and the parasites could be seen in the luminae of bronchi. There was an excess of 50 ml of yellow turbid fluid in the pericardial sac. Moderate dilatation was observed in the right ventricle of the heart, and a slight nutmeg pattern was seen in the liver. Large numbers of *Haemonchus contortus* were found in the abomasum and moderate numbers of *Oesophagostumum* spp. were found in the large intestine.

Laboratory Results: The parasite found in the airways was identified as the trichostrongilid nematode, *Dictyocaulus viviparous*

Contributor's Morphologic Diagnosis:
1) Interstitial pneumonia, proliferative, with nematodes and larvae consistent with *Dictyocaulus viviparus*, Charolais, bovine.
2) Bronchitis and bronchiolitis, chronic, suppurative, with epithelial hyperplasia and broncholitis obliterans, Charolais, bovine.
3) Heart, dilatation of the right ventricale (*Cor pulmonmale*), Charolais, bovine (slides not included).
4) Nutmeg liver, Charolais, bovine (slides not included).

Etiologic diagnosis: Parasitic bronchitis and pneumonia

Etiology: *Dictyocaulus viviparus*

Contributor's Comment : The lesions present in the submitted slides are consistent with a patent infection of *Dictyocaulus viviparus* in cattle. Except for the aberrant migration of *Ascaris suum* larvae in the bovine lung, *D. viviparous* is the only lung worm in this animal species.[8] Bovine dictyocaulosis occurs worldwide but is seen more frequently in areas of high rainfall and intense irrigation.[7] In the southern hemisphere it occurs mainly after weaning in calves less than one-year-old, during the fall (as was the case of the present outbreak) or winter and even in the first months of spring.[7] The adult *D. viviparus* may reach 8 cm in length, and is a nematode which has a direct life cycle.[3] The female worms lay eggs in the trachea and bronchi of affected animals. The eggs hatch rapidly and first stage larvae are coughed up, swallowed, and shed in the feces. In the pasture, depending on the climate, the larvae can develop within 7 days into infective third stage larvae. Upon being ingested with the grass by cattle, they penetrate the small-intestinal wall and gain access to the mesenteric lymph nodes were they molt to the L4 stage; these are taken by blood and lymph to the lungs, where they locate in the pulmonary capillaries of the ventral portions of the caudal lobes. [3,8]

Approximately seven days after ingestion, penetration of the alveoli occurs, and from there the larvae reach the bronchioles where the final molt (L5) takes place; during further development the young adults move up to the bronchi. The prepatent period is 3-4 weeks.

The lesions produced by *D. viviparus* depend on the susceptibility of the host and on the number of invading larvae. There are two main manifestations of clinical disease caused by *D. viviparus.* The primary infection occurs in calves younger than 1-year-old, and even older cattle previously unexposed to *D. viviparus*, that come into contact with heavily parasitized pastures for the first time. And, this was the case for the calves of this report. The second manifestation is referred to as **reinfection syndrome**. This condition occurs 14-16 days after immune, adult cattle that have been infected previously with *D. viviparus*, are placed on heavily contaminated pasture. Clinical signs include respiratory distress, marked coughing, increased respiratory rate, projectile diarrhea, dramatic milk yield drop, and harsh respiratory sounds. This second manifestation being the function of an allergic reaction.[1,3]

The primary infection can be subdivided into a **penetration phase** (1-7 days), a **prepatent phase** (approximately 7 to 25 days after infection), a **patent phase** (approximately 25 to 55 days after infection), and a **postpatent phase** (approximately 55 to 90 days after infection). The penetration phase is usually not associated with clinical signs.[3] As the larvae reach the alveoli in the prepatent phase there is coughing, increased respiratory rate, but death is infrequent unless complications occur. In this phase no adult worm can be found in the airways; even though *D. viviparus* larvae can be seen in the smears of expectorated mucous, they are not detectable in the feces. In the patent phase clinical signs are marked and include coughing, increased respiratory rate, labored breathing, decreased intake of food and water, loss of condition, harsh respiratory sounds, crackling sounds in the lung, and subcutaneous emphysema. Deaths are frequent in this phase and lesions observed at necropsy include bronchitis, bronchiolitis, parasitic pneumonia with consolidation and collapse of the lung lobes, and the presence of hyaline membranes. Secondary bacterial bronchopneumonia is seen in some cases.[1,7] As is the

case in this report, adult worms, larvae, and eggs are observed in the airways. Many larvae are passed in the feces and can be detected by the Baermann technique. Recovery occurs in the late patent phase, with gradual waning of the clinical signs leading to a recovery over several months time. However, deaths may occur in 25% of cases due to complications such as sudden exacerbation of dyspnea at days 45-60, after secondary bacterial infection.[1,8] Lesions in these fatal cases include pulmonary edema, hyaline membranes, alveolar epithelial hyperplasia, and interstititial emphysema.

In the case reported here, *Cor pulmonale* was observed to be associated with hydropericardium and nutmeg liver. This was interpreted as being caused by impediment of blood transit through the lung, and thus congestive heart failure.

The diagnosis of this case was straightforward since the epidemiology, clinical signs, typical lesions, and the presence of large characteristic worms permitted a definite diagnosis. All things considered, if an animal with the above discussed symptoms was examined superficially, without necropsy and discovery of nematodes, ARDS would be a definite differential.

AFIP Diagnosis: 1. Lung: Bronchitis and bronchiolitis, chronic, multifocal to coalescing, moderate, with multifocal bronchiolitis obliterans, adult and larval nematodes and ova, etiology consistent with *Dictyocaulus viviparus*, Charolais (*Bos taurus*), bovine.
2. Lung: Pneumonia, interstitial, acute, diffuse, severe with fibrin.
3. Lung: Bronchopneumonia, suppurative, multifocal, marked.

Conference Comment: The contributor gives an excellent overview of the life cycle of *Dictyocaulus viviparus*, and the various stages of infection.
Reinfection is necessary to maintain immunity as a de-

Lungworms of selected domestic and wild mammals:

- *Aelurostrongylus abstrusus* – cats; catarrhal bronchiolitis, submucosal gland hyperplasia, granulomatous alveolitis, alveolar fibrosis
- *Eucoleus aerophilus (Capillaria aerophila)* – dogs, cats, foxes; dogs and cats usually have very mild infection
- *Crenosoma vulpis* – foxes, occasionally dogs; eosinophilic catarrhal bronchitis and bronchiolitis
- *Filaroides hirthi*, *Andersonstrongylus milksi (Angiostrongylus milksi, F. milksi)* – dogs, mink; pyogranulomatous, eosinophilic pneumonia
- *Oslerus* (*Filaroides*) *osleri* – wild canids; single/multiple 1-10mm diameter, firm, gray-pink, sessile or polypoid, submucosal nodules in trachea and bronchi, usually at tracheal bifurcation
- *Angiostrongylus vasorum* – dogs, foxes; inhabits pulmonary artery and right ventricle
- *Dictyocaulus filaria* – sheep and goats; catarrhal and eosinophilic bronchitis and bronchiolitis
- *Dictyocaulus viviparus* – cattle; pneumonia, bronchitis, pulmonary edema and emphysema
- *Dictyocaulus arnfieldi* – horses, donkeys; obstructive or eosinophilic bronchitis, edema, atelectasis
- *Muellerius capillaris* – sheep and goats; small subpleural nodules; alveolar fibrosis +/- granulomatous inflammation
- *Protostrongylus rufescens* – sheep and goats; lambs and kids; adults live in bronchioles; results in pulmonary nodules and eosinophilic bronchiolitis.
- *Metastrongylus apri* – pigs; growth retardation, bronchitis, catarrhal inflammation

crease in the immune response is seen in as little as 100 days following infection. A hypersensitivity reaction seen in animals with the reinfection syndrome caused by *D. viviparus*, can have clinical signs and lesions indistinguishable from acute bovine pulmonary edema (ABPE).[8]

In the case presented at this conference there appears to be a more acute interstitial component underlying the verminous pneumonia with features of acute respiratory distress syndrome (ARDS). In addition, the multifocal suppurative bronchopneumonia may be the result of a secondary bacterial infection, which is not uncommon in these cases.

Bronchiolitis obliterans is a lesion of chronic bronchiolar damage that consists of either fibrous polyps occluding the bronchiolar lumen or intraluminal aggregates of inflammatory cells that obstruct the airways.[2] It can occur following a variety of pneumonias caused by agents such as bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPIV-3), infectious bovine rhinotracheitis (IBD), *D. viviparus*, bacteria, toxic gases, and hypersensitivity reactions.[2]

Histologic features of metastrongyles include a body cavity, intestine lined by few multinucleated cells with microvilli, accessory hypodermal chords, coelomyarian musculature, and, in females, a uterus with larvae or embryonated eggs.[4]

We are grateful to Dr. Chris Gardiner, AFIP consultant in veterinary parasitology, for his review and comments on this interesting case.

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References:

1. Breeze RG: Parasitic bronchitis and pneumonia. Vet Clin North Am (Food Anim Pract) 1:277-287, 1985
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 559-561, 615-653. Elsevier Limited, St. Louis, MO, 2007
3. Eysker M: Dictyocaulosis in cattle. Com Cont Ed Pract Vet 16:669-675, 1994
4. Gardiner CH, Poynton SL. An Atlas of Metazoan Parasites in Animal Tissues. Armed Forces Institute of Pathology, Washington, D.C., 2006
5. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 522-548. Elsevier, St. Louis, MO, 2007
6. Panuska C: Lungworms of ruminants. Vet Clin Food Anim 22:583-593, 2006
7. Ruas JL: Pneumonia verminótica [Parasitic pneumonia]. In: Doenças de Ruminantes e Eqüinos [Diseases of Ruminants and Horses], ed. Riet-Correa, F, Schild AL, Méndez MC, Lemos RAA, 2 ed, vol. 1, pp. 85-89, Varella, São Paulo, Brazil, 2001 [Text in Portuguese]
8. Smith JA, Grooms D: The interstitial pneumonias. In: Large Animal Medicine, ed. Smith BP, 3rd ed., pp. 571-581, Mosby Inc., St. Louis, MO, 2002

CASE III – 02B5194 (AFIP 2839228).

Signalment: A 10-year-old, male, Labrador retriever

History: The dog had recently become ataxic, non-responsive to the owner, uncomfortable when lying down, and had watery diarrhea for several days before presentation. Radiographs revealed diffuse calcification throughout the small intestine, enlarged liver, and diffuse spondylosis of the thoracic and lumbar vertebral columns. An ultrasound revealed multiple hypoechoic nodular masses in the liver and diffuse hyperechogenicity of the submucosa of the small intestine. Because of the grave prognosis, owner elected euthanasia. Biopsy specimens of liver, spleen, and small intestine were collected.

Gross Pathology: Received for biopsy was a 10 cm segment of small intestine. The wall was firm and thickened and had multiple, 1-3 mm, white, raised foci on the serosal surface. The entire circumference of the submucosa was prominent, gritty and white.

Laboratory Results: CBC and serum chemistry panel revealed anemia, elevated liver enzymes (AST, ALP, ALT, and total bilirubin), hypoalbuminemia, hyperglobulinemia, and hypocholesterolemia.

Contributor's Morphologic Diagnosis: Small intestine: Enteritis, granulomatous, eosinophilic, transmural, chronic, severe, with large numbers of intralesional, mineralized and unmineralized trematode eggs (*Heterobilharzia americana*)

Contributor's Comme nt: *Heterobilharzia americana* (Digenea: Schistosomatidae) is a blood trematode that causes canine schistosomiasis in North America. Natural *H. americana* infection has been reported in bobcat, armadillo, beaver, dogs, coyote, a captive born Brazilian tapir, mountain lion, mink, nutria, opossums, raccoons,

red wolves, swamp rabbits, white-tailed deer, etc. Geographically the natural infection is essentially limited to the southern Atlantic Coast states (Florida, Georgia, North Carolina, South Carolina) and Gulf Coast states (Texas, Louisiana, Mississippi), although it has been reported in Kansas.

Members of this family, Schistosomatidae, are the only trematodes that live in the blood stream of warm-blooded hosts. They are dioecious, the male bearing the female in the ventral gynaecophoric canal. There are no metacercaria. The cercariae become fork-tailed and penetrate directly through the skin of the host. In the eggs there are no opercula. The intermediate hosts are snails of the genera *Fossaria cubensis (Lymnaea cubensis)* and *Pseudosuccinea columella*. The adult worms reside in the mesenteric veins. The eggs laid in the mesenteric veins produce enzymes to erode through submucosa and mucosa of the intestine to reach the intestinal lumen. Some of the eggs are carried by the venous flow to the liver, spleen, and other organs such as the lungs and brain. Once the eggs containing mature miracidia leave the host, they must reach water of low osmotic pressure in order to hatch. The miracidia swim actively until they find snails of the right species. They bore into them and become mother sporocysts that produce daughter sporocysts that in turn produce cercariae. A single miracidium can produce several thousand cercariae. They leave the snail, swim in the water, and enter the host by penetrating through the skin and into the lymphatics.

Clinical signs of canine *H. americana* infection include dermatitis due to skin penetration, coughing, chronic intermittent mucoid to hemorrhagic diarrhea, and anorexia. Significant clinical pathology findings include anemia, hyperglobulinemia, hypoalbuminenia, eosinophilia, and hypercalcemia in some cases. No hypercalcemia or eosinophilia was noted in this particular case, however. Although the pathogenesis of hypercalcemia in schistosomiasis is not fully understood, a recent report described hypercalcemia with elevated parathyroid hormone-related protein (PTHrP) in canine schistosomiasis. Fecal floatation is usually ineffective in the diagnosis of *H. americana* infection. Saline sedimentation or a miracidia hatch is necessary to diagnose the infection. Incidentally, the liver (not submitted) of the current case had eosinophilic granulomatous hepatitis with numerous intralesional trematode eggs.

AFIP Diagnosis: Small intestine: Enteritis, granulomatous, submucosal, circumferential, multifocal to coalescing and multifocally transmural, severe, with myriad schistosome eggs, Labrador retriever (*Canis familiaris*), canine.

Conference Comment: *Heterobilharzia americana* and *Schistomatium douthitti* are the two species of schistosomes that infect mammals in the United States of America.[7] Although typically limited to the southern Atlantic and Gulf of Mexico coastal states, *H. americana* infection in Kansas has been reported, presumably linked to the importation of infected raccoons into the state during the mid-20th century.[7]

The most tissue damage occurs during oviposition and extrusion of the eggs through the tissue. The ideal movement of eggs to the outside world includes penetration of the mesenteric vessels, lamina propria and exit into the intestinal lumen, through secretions produced by the miracidium as well as through mechanical disruption.[6] When the eggs migrate in the wrong direction or get swept into the portal or systemic circulation, they can induce a granulomatous reaction in a variety of organs (lymph node, liver, lungs, etc.), depending on where they lodge.[6]

The eggs within tissue usually invoke a hypersensitivity reaction, resulting in a granulomatous response that is followed by degeneration or mineralization of the schistosome eggs and eventual fibrosis. [6] The mineralization of the schistosome eggs in this case is unusual in its extent.

Although hypercalcemia was not seen in this case, it has been associated with chronic granulomatous inflammation.[8] Activated macrophages produce calcitriol that is not regulated by parathyroid hormone, calcitriol, or calcium levels.[8] Causes of hypercalcemia are listed below:

- Neoplasia (lymphoma, multiple myeloma, adenocarcinoma of the apocrine gland of the anal sac, tumors metastatic to bone)
- Primary hyperparathyroidism (hyperplasia, adenoma, adenocarcinoma) - elevated levels of circulating parathormone cause increased intestinal absorption of calcium and phosphorus as well as increased renal activation of vitamin D
- Granulomatous inflammation (canine blastomycosis, bovine paratuberculosis, schistosomiasis)
- Hypoadrenocorticism (increased tubular resorption of calcium)
- Osteolytic lesions of bone
- Immobilization
- Metabolic acidosis
- Renal failure in horses (rarely canine renal failure associated with familial disease)

Contributor: Louisiana State University, School of Veterinary Medicine, Baton Rouge, Louisiana, 70803

References:

1. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 437-439. Elsevier, St. Louis, MO, 2007
2. Fradkin JM, Braniecki AM, Craig TM, Ramiro-Ibanez F, Rogers KS, Zoran DL: Elevated parathyroid hormone-related protein and hypercalcemia in two dogs with schistosomiasis. J Am Anim Hosp Assoc 37:349-355, 2001
3. Goff WL, Ronald NC: Certain aspects of the biology and life cycle of *Heterobilharzia americana* in East Central Texas. Am J Vet Res 42:1775-1777, 1981
4. Krotoski WA, Job CK, Cogswell FB, Malek EA: Enzootic schistosomiasis in a Louisiana armadillo. Am J Trop Med Hyg 33:269-272, 1984
5. Malek EA, Short RB, Teehan WH, Jama A: Differential susceptibility of snail hosts to *Heterobilharzia americana* from Texas and Louisiana. J Parasit, 73:872-873, 1987
6. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 95-97. Elsevier Limited, St. Louis, MO, 2007
7. McKown RD, Veatch JK, Fox LB: New locality record for *Heterobilharzia americana*. J Wildl Dis 27:156-160, 1991
8. Rohrer CR, Phillips LA, Ford SL, Ginn PE: Hypercalcemia in a dog: a challenging case. J Am Anim Hosp Assoc 36:20-25, 2000

CASE IV - 1-777-05 (AFIP 2977999).

Signalment: 3-day-old, male, beef calf, black Angus

History: Diarrhea affecting multiple calves at 3-7 days of age. Calves are unresponsive to treatment and die 1-2 days after onset of clinical signs.

Gross Pa thology: Segmental, dark red, small intestine with semifluid bloody content

Laboratory Results:
Serum IgG(1) >2000 mg%
E coli K99 negative
Gram stains of intestinal contents reveals moderate bacilli
Clostridium perfringens cultured anaerobically. *C. perfringens* PCR genotyping:

Alpha	Beta	Epsilon	Iota	Beta2	Enterotoxin
POS	POS	NEG	NEG	NEG	NEG

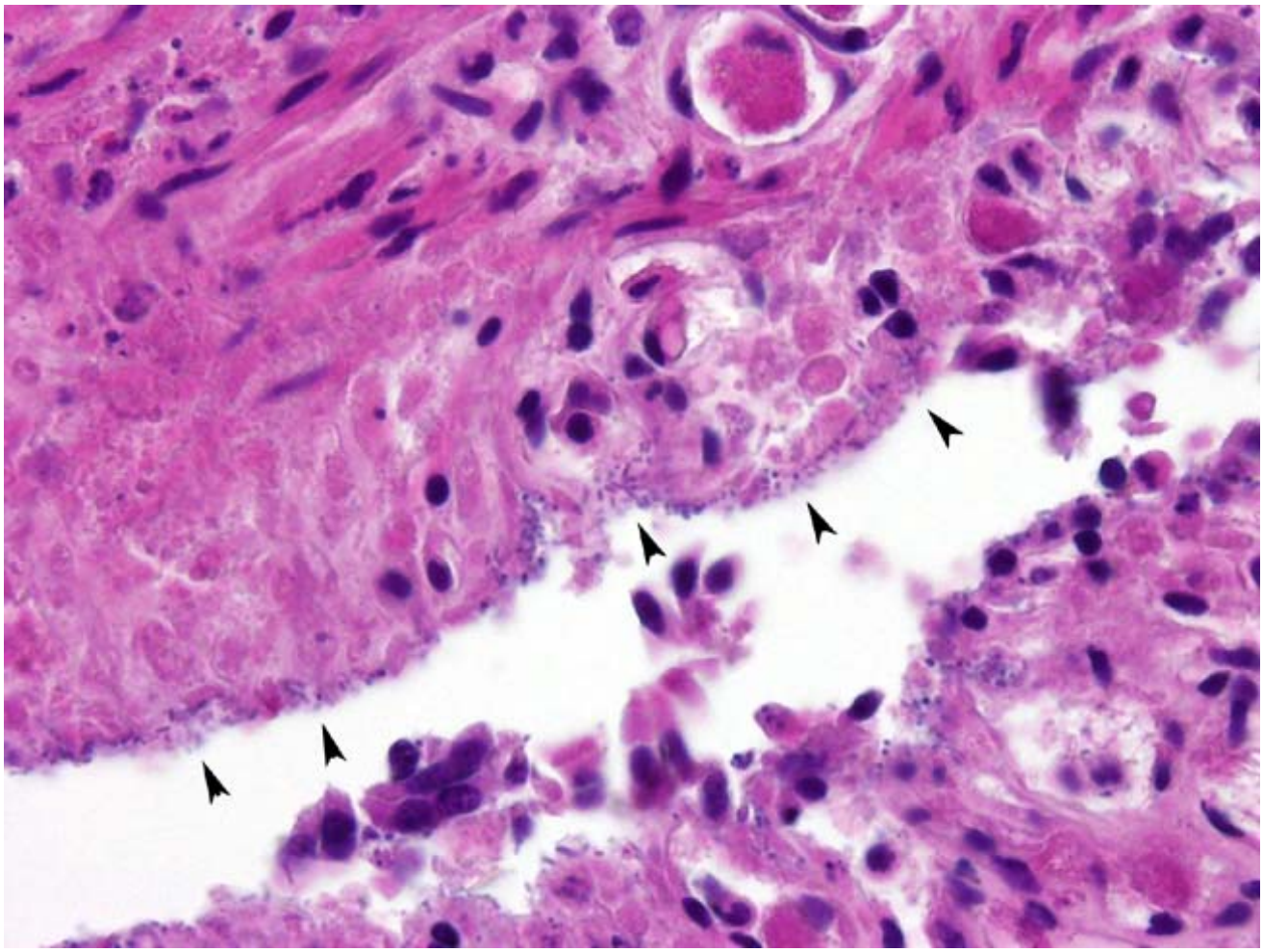
Contributor's Mor phologic Diagn osis: Small Intestine: Enteritis, necrotizing, acute, segmental, marked, with bacilli

Contributor's Comment: This is a typical case of neonatal hemorrhagic enterotoxemia (necrotic enteritis) due to *Clostridium perfringens* type C, as determined by PCR genotyping. This is a disease of neonatal calves, lambs, foals and piglets.[3] *Clostridium perfringens* types A and C are increasingly common in poultry, due to reduced use of antibiotic growth promoters.[4] Clinical signs include hemorrhagic diarrhea, dehydration, anemia and weakness. Although sporadic in occurrence, this disease has a high morbidity and mortality despite treatment. Disease is primarily due to elaboration of cytotoxic beta-toxin. This toxin is readily degraded by trypsin. The trypsin inhibitors in colostrum, effective in facilitating absorption of intact maternal immunoglobulins, also inhibit the degradation of beta-toxin in neonates.[5] Death can be due to dehydration and fluid/acid-base derangement or due to secondary gram-negative septicemia/endotoxemia. This case presented with a mild interstitial pneumonia suggestive of bacteremia/endotoxemia and had adequate passive transfer of maternal antibody, both typical ancillary findings. Vaccination for *C. perfringens* type C and D is readily available and widely used.

AFIP Dia gnosis: Small intestine, villi: Necrosis, diffuse, with fibrin thrombi, and myriad mixed bacilli, Angus (*Bos taurus*), bovine.

Conference Comment: The five types of *Clostridium perfringens* are differentiated by their production of one or more of the four types of antigenic exotoxins.[1] Diagnosis depends on demonstration of the toxin with the presence of hemorrhagic and necrotizing enteritis.[3] Bacterial **colonization (fig. 4- 1)** alone will not produce disease or determine a diagnosis. Disease production is dependent on toxin type and the toxin's effect on tissue, either through local toxin inducing necrotizing effects, secretory effects of locally acting enterotoxins, or systemic effects of absorbed (entero)toxins.[1]

Alpha toxin is a lecithinase (phospholipase) that damages cell membranes causing necrosis or lysis of erythrocytes, platelets, leukocytes, and endothelial cells. Beta toxin is a trypsin labile, pore forming toxin that causes necrosis, decreases mobility of intestinal villi, and enhances bacterial attachment to the villi. The Epsilon toxin is produced



4-1 Small intestine, Black Angus calf. The necrotic, denuded villi are lined by a dense layer of bacilli that are 3-7 um long by 1-2 um wide (arrowheads). (H&E 600X)

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as a prototoxin and activated by enzymatic digestion (i.e, by trypsin in the intestine), and causes necrosis. Iota toxin increases capillary permeability and is also produced as a prototoxin that is activated by proteolytic enzymes.[1]

Another *Clostridium perfringens* toxin identified as ß2 has been described in recent literature. Despite its name, ß2 toxin is unrelated to the Beta toxin.[1] The gene cpb2 codes for ß2-toxin, but not all cpb2 positive strains of *C. perfringens* produce the ß2 toxin *in vitro*.[6] It has been implicated in enteric disease in swine and typhlocolitis in horses.[1]

Contributor: Montana Veterinary Diagnostic Laboratory, Bozeman, MT, 59715

www.state.mt.us/liv/lab/index.asp

References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 213-221. Elsevier Limited, St. Louis, MO, 2007

2. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 358-367. Elsevier, St. Louis, MO, 2007

3. Michelsen PGE: Diseases caused by *Clostridium perfringens* toxins (enterotoxemia; yellow lamb disease; lamb dysentery; necrotic enteritis). In: Large Animal Internal Medicine, ed. Smith BP, 3rd ed., Mosby Inc., St. Louis, MO, 2002

4. Van Immerseel F, de Buck J, Pasmans F, Huyghebaert G, Haesebrouck F, Ducatelle R: *Clostridium perfringens*

in poultry: an emerging threat for animal and public health. Avian Pathol 33:537-549, 2004
5. Radostits OM, Gay CC, Blood DC, Hinchcliff KW: Diseases caused by *Clostridium* sp. In: Veterinary Medicine, 9th Ed., pp.770-773. WB Saunders Co., London, 2000
6. Schotte U, Truyen U, Neubauer H: Significance of ß2-toxigenic *Clostridium perfringens* infections in animals and their predisposing factors-a review. J Vet Med 51:423-426, 2004
7. Jones TC, Hunt RD, King NW: Diseases caused by bacteria. In: Veterinary Pathology, 6th ed., pp.420-422. Williams and Wilkins, Baltimore, MD, 1997

Clostridium perfringens – Types, toxins and diseases

Type	Toxin				Diseases
	Alpha	Beta	Epsilon	Iota	
A	+ +	-	-	-	• Gas gangrene • Food Borne Illness humans • Necrotic enteritis - Chickens • Gastroenteritis - Ferrets • Yellow lamb disease - enterotoxemia, western US • Colitis X in horses - unproven association
B	+	+ +	+	-	• Lamb dysentery • Hemorrhagic enteritis - calves, foals, guinea pigs - UK, S. Africa, Middle East
C	+	+ +	-	-	• Enterotoxic hemorrhagic enteritis - neonatal lambs, goats, cattle, pigs • Struck - Adult sheep, UK
D	+	-	+ +	-	• Overeating disease/ pulpy kidney - Sheep, cattle, goats • Focal symmetric en cephalomalacia - Sheep
E	+	-	-	+ +	• Enterotoxemia – calves, lambs. guinea pigs, rabbits

Table adapted from Brown et al.[1] & Jones et al.[7]

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 7

31 October 2007

Moderator:

Dr. Wayne Anderson, DVM, PhD

CASE I – D6-29 (AFIP 3031647).

Signalment: Female intact Crl:CFW(SW) mouse (*Mus musculus*), approximately 3.5-months-old

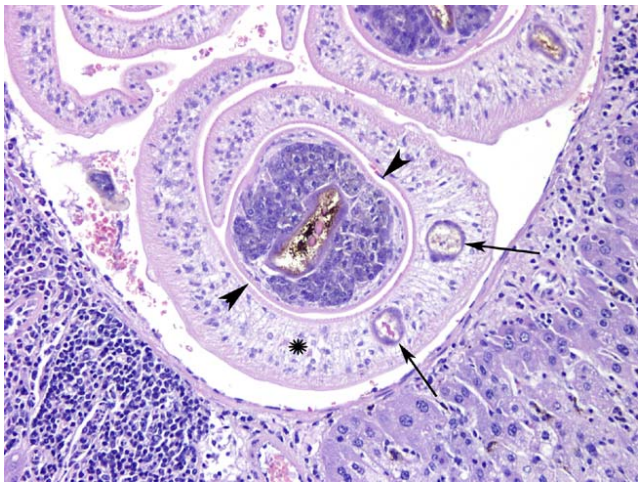
History: This mouse was in a group of mice exposed percutaneously via the tail to an average of 167 *Schistosoma mansoni* cercariae on 3/29/06 at the Biomedical Research Institute NIAID Schistosomiasis Resource Center (http://www.schisto-resource.org), which is a repository that provides schistosome parasites, snail vectors, and infected mammals for researchers. The mice were shipped to our animal facility 5 days following infection. Over a 3-day period 6.5 weeks post infection, several mice were found dead and were necropsied.

Gross Pathology: The mouse had moderate autolysis. The liver was brown and mottled with numerous yellow-tan irregularly shaped foci up to 3 mm diameter extending throughout the parenchyma.

Histopathologic Description: In sections of liver, dense accumulations of lymphocytes, eosinophils, epithelioid macrophages, and neutrophils with fewer multinucleated giant cells and plasma cells surround parasite ova or intravascular adult trematodes in periportal tissue or are randomly scattered in the parenchyma. The ova range up to approximately 110 µm by 50 µm, are nonoperculated, have a thick yellow to brown shell occasionally with a sharp spiny process (lateral spine) protruding from one end of the eggs, and contain a miracidium. A dark yellow-brown granular pigment is present in occasional macrophages surrounding the eggs. Fibroblast proliferation is associated with the inflammation in some areas. Hepatic venules are expanded by adult trematodes parasites filled with parenchyma and lacking a body cavity. The **trematodes (fig. 1-1)** are paired, with a smaller trematode (female) present within coiled larger trematodes (male). The caeca of the parasites contain yellow-brown granular pigment and red blood cells. The larger (male) parasites have surface undulations or protuberances, interpreted to be tegmental tuberculations. There are multiple areas of coagulative hepatocyte necrosis scattered throughout the liver. Most hepatocytes are moderately swollen by clear discrete vacuoles or lacy vacuolar change.

Contributor's Morphologic Diagnosis: Hepatitis, chronic, granulomatous, periportal to random, circumoval, with multifocal hepatic necrosis associated with intralesional schistosome eggs and intravascular adult schistosomes, liver

Contributor's Comment: Schistosomiasis is a disease affecting over 200 million people[7] and is caused by trematodes, most commonly *Schistosoma mansoni*, *Schisto-*



1-1 Liver, Crl:CFW(SW) mouse. Adult trematodes are paired, with the smaller female (Arrowheads) within a gynecophoric canal of the coiled, larger male. The parasites are composed of a tegument with underlying cortical muscle layer, and a spongy, solid body (star) within which are paired caecae (arrows) containing yellow-brown granular pigment and red blood cells. (H&E 200X)

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soma haematobium, and *Schistosoma japonicum*. Freshwater snails (*Biomphalaria* for *S. mansoni*; *Bulinus* for *S. haematobium*, and *Oncomelania* for *S. japonicum*) serve as intermediate hosts.[3] Treatment programs help reduce morbidity, but the prolonged control efforts often cannot be sustained due to lack of resources in affected countries, and so vaccines can hopefully be developed to reduce the intensity of infection and disease severity. Schistosome research includes vaccine development, regulation of T_H1 and T_H2 responses, immunopathology of granulomas and fibrosis, and eosinophil function.[5]

Life cycle[3]: Only the cercarcial stage released by freshwater snails is infectious to humans. A schistosome miracidium hatched from an egg in the water penetrates a snail and multiplies into many cercariae, which escape back into the water. The cercariae penetrate the skin of the definitive host and then develop into schistosomules, which migrate into blood vessels and travel to the lungs. After a week, the schistosomules migrate to the liver and hepatic portal venules, where sexual maturity and pairing occurs. The adults travel to mesenteric venules, where *S. mansoni* eggs penetrate the vessel walls and pass through the wall of the intestine into the feces.

Human Disease[3]: In humans, symptoms of acute infection include fever, cough, asthma, hives, and diarrhea with marked eosinophilia. Hepatosplenomegaly and lymphadenopathy can occur. Eggs are passed in feces, but many embryonated eggs remain in body tissues. Eggs released in mesenteric venules can be carried in the intrahepatic portal system where they lodge in the hepatic sinusoids and provoke granulomas. After the initial reaction to the eggs released by the parasites, immune downregulation can decrease these signs. In chronic infections, granulomas (eosinophils, plasma cells, lymphocytes, macrophages, and giant cells) occur around eggs, often with subsequent fibrosis. Symmers' pipestem fibrosis is seen grossly as tracts of portal fibrosis resembling white clay pipestems throughout the tissue, which contributes to portal hypertension and often occurs in patients that lack a downregulation of the immune response. Adult schistosomes in veins do not evoke a host response, but there can be significant reaction to dead worms following treatment or late stages of infection. Glomerulonephritis can also occur, probably due to immune complexes. Colonic polyps containing many schistosome eggs and adults can occur with *S. mansoni* and *S. haematobium* infections. Bilharziomas in the intestinal serosa and mesentery containing fibrous and inflammation around masses of eggs can develop. Cardiopulmonary changes can include pulmonary arteritis and cor pulmonale. Schistosome eggs can also enter the meninges and spinal cord causing meningitis and myelitis. Dermatitis and rashes can occur to the schistosomules. *S. haematobium* causes urogenital schistosomiasis.

Mouse Model of Schistosomiasis[7]: The mouse is a widely used model of experimental schistosomiasis. Mice are usually infected percutaneously through the tail or abdomen by exposure to cercaria in water or can be injected subcutaneously or intraperitoneally with cercaria.[5] Infected mice develop hepatic granulomas and immunoregulatory responses similar to humans. Somular antigens that cross-react with schistosomal egg antigens (SEA) induce T-lymphocyte activation and $T_{M/E}$ cell expansion. Hepatic granulomas develop as a delayed-type hypersensitivity (DTH) response by SEA-reactive $CD4^+$ ($\alpha\beta^+$) MHC-II-dependent T cells. Other cells present in the granulomas included $CD8^+$ T cells, B cells, eosinophils, mast cells, NK cells, basophils, macrophages, neutrophils, $\gamma\delta^+$, and fibrocytes. Mice develop granulomas in the liver, colon, and Peyer's patches, which begin to decrease in size about 8 weeks post infection due to downregulation of the immune response. Hepatic granuloma size was controlled by both T_H1 and T_H2 responses: T_H1 cytokines (IL-2, IFN-γ), T_H1 cytokine receptor (IFN-γ), T_H2 cytokines (IL-4, IL-5, IL-10, IL-13, TNF-α), and a T_H2 cytokine receptor (IL-4α). Fibrosis was associated with IL-4 and TGF-β, and was independent of the regulation of the hepatic granulomas.

Parasite description[3,4]: Schistosomes are unique from other trematodes infecting humans in that they live in blood vessels, they have separate sexes (most trematodes are hermaphroditic), the eggs are nonoperculated, and the metacercariae are not encysted. Anatomical features of mature schistosomes include an oral and ventral sucker at the anterior end, the lack of a body cavity, a brown granular schistosomal pigment often found in the parasite's cecum, tegmental tuberculations in *S. mansoni* males, and a gynecophoral canal in males which holds the female. *S. mansoni* eggs are 114-175 µm by 45-68 µm and have a lateral spine.

AFIP Diagn osis: Liver: Hepatitis, granulomatous and eosinophilic, random and portal, moderate, with trematode eggs and intravascular trematodes etiology consistent with *Schistosoma mansoni*, Crl:CFW(SW) mouse (*Mus musculus*), rodent.

Conference Comment: The contributor gives an excellent review of schistosomiasis in animals and humans. *Schistosoma mansoni* is a member of the family Schistosomatidae, which are the blood flukes of mammals and birds. The three genera that make up this family include *Schistosoma*, *Heterobilharzia* and *Orientobilharzia*. Schistosomatidae of veterinary importance include *Heterobilharzia americana* in mammals of the southern USA, as well as *Orientobilharzia turkestanicum, O. dattai* and *O. bomfordi* in Asia. Other Schistosomatidae are separated into four groups depending on egg morphology and intermediate snail hosts.[6]

1. ***S. haematobium* group**
   S. bovis – southern Europe, tropical Africa and Asia; portal and mesenteric veins (ruminants, horses, camels, pigs)
   S. mattheei – central and southern Africa; stomach, urogenital, portal and mesenteric veins (ruminants)
   S. curassoni – west Africa (ruminants)
   S. leiperi – central Africa (artiodactyls)
2. ***S. mansoni* group** – central Africa
   S. rodhaini (dogs, carnivores)
3. ***S. indicum* group** – India and southeast Asia
   S. spindale – mesenteric veins (ruminents, horses, dogs)
   S. nasale – nasal mucosal veins (cattle, goats, horses)
   S. indicum – portal and mesenteric veins (herbivores)
   S. incognitum – (swine, dogs)
4. ***S. japonicum* group** – Far East
   S. japonicum – (human, domestic animals)
   S. mekongi (dogs, humans)

Mixed infections may occur. *S. bovis* and *S. japonicum* are the most pathogenic in cattle and sheep.

Morphological characteristics of trematodes include a digestive tract and no body cavity.[4] Schistosome eggs contain a miricidium, a lateral spine, and have no operculum.[3]

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. We are grateful to Dr. Gardiner for his comments and advice on this interesting case.

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References:

1. Anderson WI, Smiler KL, Leopard DM, Bell RM: Hepatic granulomas in a female Cynomolgus monkey (*Macaca fascicularis*). Lab Animal 24:17-19, 1995
2. Anderson WI, King JM, Uhl EM, Hornbuckle WE, Tennant BC: Pathology of experimental *Schistosoma mansoni* infection in the Eastern Woodchuck (*Marmota monax*). Vet Pathol 28:245-247, 1991
3. Cheever A, Neafie RC: Schistosomiasis. In: Pathology of Infectious Diseases, ed. Meyers WM, vol.1, pp. 23-47. Armed Forces Institute of Pathology and American Registry of Pathology, 2000
4. Gardiner CH, Poynton SL: Morphological characteristics of trematodes in tissue section. In: An Atlas of Metazoan Parasites in Animal Tissues. pp. 46-49. Armed Forces Institute of Pathology, Washington, D.C., 2006
5. Lewis F: Schistosomiasis. In: Current Protocols in Immunology, pp. 19.1.1-19.1.28. John Wiley & Sons, Inc., New York, NY, 1998
6. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 95-97. Elsevier Limited, St. Louis, MO, 2007
7. Stavitsky AB: Regulation of granulomatous inflammation in experimental models of schistosomiasis. Infect Immun 72:1-12, 2004

CASE II – CASE 1 (AFIP 3066021).

Signalment: Adult, male, Wistar Hannover rat (*Rattus norvegicus*)

History: An approximately 2-year-old, male, sentinel rat was found dead with no premonitory clinical signs.

Gross Pathology: Gross findings included prominent, dark-red mesenteric vasculature. Additional findings included approximately 3 ml of hemorrhagic fluid in the abdomen, a deformed spleen that was constricted in the middle, an 2.0 cm diameter, thin walled cyst filled with clear fluid and extending from the pancreatic region, and a 1.0 cm diameter clear cyst protruding from the right kidney.

Histopathologic Description: Large and medium sized muscular mesenteric and pancreatic arteries are primarily affected. Some arteries have acute lesions of intimal and medial fibrinoid necrosis with thrombosis and luminal dilatation, destruction of the elastic laminae, mural hemorrhage, and segmental to global transmural infiltration of neutrophils, eosinophils, and mononuclear cells. Inflammatory infiltrates multifocally extend from the adventitia into the periarterial tissues. Other arteries have chronic lesions of irregular mural thickening due to fibrosis (sometimes causing narrowing of the lumen), mononuclear cell infiltrates, and organizing / recanalizing thrombi. Mural mineralization is also multifocally present within the medial layers of some arteries.

Other lesions on the slide include a dilated cystic structure adjacent to the pancreas interpreted to be a dilated lymph vessel as well as mesenteric lymph nodes with sinuosidal erythrocytosis and phagocyte hemosiderosis.

Contributor's Morphologic Diagnosis: 1. Mesenteric and pancreatic arteries; Polyarteritis, necrotizing, multifocal, with mixed cell inflammation, fibroplasia, mural hemorrhage, and occlusive thrombi, rat
2. Mesenteric arteries, media; Mineralization, moderate, multifocal
3. Lymph nodes (multiple); Erythrocytosis, sinusoidal, moderate, multifocal, with phagocyte hemosiderosis
4. Abdomen, peripancreatic; Dilated lymph vessel, focal

Contributor's Comment: The signalment, anatomical locations, gross appearance, and histologic characteristics of this case are consistent with those of polyarteritis nodosa (PAN). PAN is a progressive degenerative, inflammatory, and necrotizing disease that most commonly affects small to large arteries of the mesentery, pancreas, kidney, and testis.[7] The aorta, arterioles, and smaller caliber vessels are typically spared.[2] As observed in this case, the presence of acute, healing, and old lesions within a single animal is highly characteristic of PAN.[12] Clinically, polyarteritis nodosa tends to occur in aged rats (reports vary between 500 and 900 days). Although polyarteritis nodosa is most often an incidental finding in aged rats, it can be fatal if, as is likely in this case, severely thrombosed mesenteric arteries rupture leading to fatal hemorrhaging into the abdominal cavity.[2]

PAN is typically considered to be an immune-mediated disease,[9,12] but the disease has also been associated in some studies with corticosteroid administration, estrogen treatment, exposure to chemical carcinogens, and hypersensitivity.[2] PAN has a high incidence in spontaneous hypertensive rat strains as well as in rats with late stage chronic nephropathy (which was present in this case).[7,9] The multifocal, moderate mineralization of the arterial media seen in this animals may also be secondary to chronic renal disease.[7]

Other related lesions observed microscopically in this animal included a focally extensive area of splenic coagulative necrosis with abundant intralesional thrombi, inflammation, hemorrhage, and hemosiderosis. This likely represents an infarct associated with the thrombi initiated by necrotizing polyarteritis.

AFIP Diagnosis: 1. Pancreas and mesentery: Arteritis and periarteritis, proliferative and necrotizing, chronic, multifocal, severe, with multifocal mineralization and thrombosis, Wistar Hannover rat (*Rattus norvegicus*), rodent.
2. Pancreas, exocrine: Atrophy, multifocal, mild.
3. Lymph node: Draining hemorrhage, chronic, with sinusoidal ectasia.

Conference Comment: The characteristic histologic lesion of polyarteritis nodosa (PAN) is segmental fibrinoid degeneration and thickening of the tunica media of affected arteries with an inflammatory infiltrate composed mainly of mononuclear cells with fewer neutrophils.[9] The size of the vessel lumen are markedly variable, with potential thrombosis with or without recanalization.[9] Both acute and chronic inflammatory processes may occur within the same individual.

In rats, lesions occur most commonly in medium-sized arteries of the mesentery, pancreas, pancreatic-duodenal arteries, and testis of male, Sprague-Dawley and spontaneous hypertensive rat (SHR) strains.[9] Microscopic lesions may occur in most organs except for the lungs.[9] In mice, most lesions occur within small and medium-sized arteries of the tongue, pancreas, heart, kidneys, mesentery, urinary bladder, uterus, testes, and gastrointestinal tract of MRL and NZB mice.[8,9]

Changes within the vessel walls can be highlighted with special stains. The modified Movat's pentachrome method stains elastic laminae black, collagen and reticular fibers yellow, ground substance and mucin blue, fibrin

intense red, and muscle fibers red.[11] With the Movat's pentachrome method, the quantity of intimal proliferation is readily apparent as are disruptions of the elastic laminae. Other stains such as Masson's trichrome and smooth muscle actin aid in differentiating increased amounts of intimal connective tissue from smooth muscle hyperplasia.[11]

In dogs, lesions similar to polyarteritis nodosa occur as a syndrome of unknown etiology termed juvenile polyarteritis syndrome or "beagle pain syndrome".[13] An immune-mediated etiology is suspected.

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References:

1. Bishop SP: Animal models of vasculitis. Toxicol Pathol 17:109-117, 1989
2. Carlton WW, Engelhardt JA: Polyarteritis, rat. In: Monographs on Pathology of Laboratory Animals, eds. Jones TC, Mohr U, Hunt RD, vol. 9, pp. 71-76. Springer-Verlag, Berlin, 1991
3. Chamanza R, Parry NMA, Rogerson P, Nicol JR, Bradley AE: Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. Toxicol Pathol 34:357-363, 2006
4. Cohen JK, Cai LQ, Zhu YS, La Perle KM: Pancreaticoduodenal arterial rupture and hemoabdomen in ACI/SegHsd rats with polyarteritis nodosa. Comp Med 57:370-376, 2007
5. Kempner W, Peschel E, Black-Schaffer B: Effect of diet on experimental hypertension and on the development of polyarteritis nodosa in rats. Circ Res 3:73-78, 1955
6. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 69-73. Elsevier Limited, St. Louis, MO, 2007
7. Mohr U, Dungworth DL, Capen CC: Cardiovascular system. In: Pathobiology of the Aging Rat, vol. 1, pp. 306-309. ILSI press, Washington, D.C, 1996
8. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., p. 105. Blackwell Publishing, Ames, IA, 2007
9. Percy DH, Barthold SW: Rat. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., p. 164. Blackwell Publishing, Ames, IA, 2007
10. Porter, BF, Frost P, Hubbard GB: Polyarteritis nodosa in a Cynomolgus macaque (*Macaca fascicularis*). Vet Pathol 40:570-573, 2003
11. Prophet EB, Mills B, Arrington JB, Sobin LH: Laboratory Methods in Histotechnology, pp 128-130. American Registry of Pathology, Washington DC, 1994
12. Schoen FJ: Blood vessels. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 539-540. Elsevier Saunders, Philadelphia, PA, 1999
13. Son WC: Idiopathic canine polyarteritis in control beagle dogs from toxicity studies. J Vet Sci 5:147-150, 2004
14. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 606-607. Elsevier, St. Louis, MO, 2007

CASE III – A N02-189, AN 02-326, AN02-395, A N04-734 (AFIP 3069049).

Signalment:
AN-02-189-6: 3.5 Mo, male, B6, 129 hybrid, *Mus musculus*, mouse
AN-02-326-6: 5 Mo, male, B6, 129 hybrid, *Mus Musculus*, mouse
AN-02-395-8: 3 Mo, female, B6, 129 hybrid, *Mus musculus*, mouse
AN-04-734-8: 6 Mo, male, B6, 129 hybrid, *Mus musculus*, mouse

History: All four mice are homozygous mice for the p53 gene. The mice were on studies to access the influence of p53 on tumor development. All four mice were having labored breath and were submitted for necropsy.

Gross Pathology: A large pale gray mass filled the anterior thoracic cavity. One or more red nodules were present in various locations of the heart.

Laboratory Results: Lymphoma cells express CD3 and TdT.
Vascular tumors had variable expression of CD31, CD34 and VEGRF-2.

Histopathologic D escription: There are one or more nodules in the ventricular and/or septal wall of the heart and they consist of blood filled vascular channels of varying size. The vascular channels are lined by cells with spindle, round or oval nuclei with thin attenuated cytoplasm. The nuclei vary in size, have finely stippled to open chromatin and one or more small nucleoli. The tumors have a high mitotic rate, and an occasional atypical mitosis. In focal areas at the periphery of the nodules

the tumor cells infiltrate the adjacent myocardium or epicardium.

The tumors have multifocal CD31 expression, diffuse CD34 expression and diffuse VEGFR-2 expression in the cells lining the vascular channels. The cytological morphology and IHC profile are considered to be consistent with a hemangiosarcoma. In the lung, sections of some of the AN02-326 slides there are large tumor cells in a small artery and in the lymphomatous infiltrate surrounding the artery. The size of the nucleus of these cells suggests the cells may be metastatic hemangiosarcoma cells. Since it was not possible to confirm this with IHC, it is also possible the cells are lymphoma cells. In a few slides of AN-02-395 there are metastatic foci of tumor cells in the mediastinal fat. In one of the foci tumor is altering the integrity of a blood vessel.

A lymphoma is present in three of the cases associated with this submission. In case AN02-189 the lymphoma extensively involves the mediastinum, one of the mediastinal lymph nodes and the lung focally. In case AN02-326 the lymphoma involves both thymic lobes and does not extend outside of the thymic lobes. In case AN02-395 the lymphoma involves both thymic lobes, breaches the thymic capsule and extends into the adjacent mediastinal fat. The lymphoma associated with all three cases has a high mitotic rate, mild to moderate apoptosis and a starry sky appearance due to tingible body macrophages. The lymphoma cells have nuclei that are predominantly smaller than or equal to that of macrophage nuclei. The lymphoma cells have nuclei that are round, angular or irregular in shape and scanty cytoplasm. Predominantly, the nuclei have fine chromatin and inconspicuous or small nucleoli. However, a few cells have a large nucleus with medium size amphophilic nucleoli. The lymphoma cells of all three cases express CD3 and TdT with strong intensity. The cytological morphology and IHC profile of the lymphomas in all three cases are consistent with a T-cell lymphoblastic lymphoma.

Contributor's Morphologic Diagnosis:

AN-02-189 1. Heart, ventricular wall left: Hemangio sarcoma.
2. Mediastinum: T-lymphoblastic lymphoma.

AN-02-326 1. Heart; ventricular wall right left septum: Hemangiosarcoma multiple.
2. Thymus: T-lymphoblastic lymphoma, bilateral noninvasive nodule with invasion of the epicardium.

AN-02-395 1. Heart; ventricular, septum right: Hemangiosarcoma.
2. Mediastinum, fat: Hemangiosarcoma, multifocal, metastatic.

AN02-734 1. Heart; ventricular wall: Hemangiosarcoma, invasive.
2. Mediastinal, fat: Steatites.

Contributor's Comment: It is reported in the literature[1-2,4-6] that the majority of p53-/- mice die by six months of age due to their tumor load. Lymphomas, sarcomas and carcinomas occur in varying proportions depending on whether the mice are p53-/- or p53+/-.[2,4] Lymphomas are more common than sarcomas in p53-/- mice. In contrast, sarcomas are more common than lymphomas in p53+/- mice. The sarcomas consist of a variety of lineages with hemangiosarcomas and osteosarcomas being the most common.[2,4] Compared to wild type mice, hemangiosarcomas occur at a high frequency in both p53-/- and p53+/- mice. The incidence of hemangiosarcoma is 20-23% in p53-/- mice and 4-6% in p53+/- mice.[1,2,4] Except for one report, the incidences of hemangiosarcomas occurring in the heart of p53-deleted mice is not indicated and in that report a hemangiosarcoma was also present the perirenal fat.[1] The fact that a p53 deficient mouse may have a hemangiosarcoma in multiple tissues may account for the lack of tissue delineation of the hemangiosarcomas in these reports. In the contributor's experience hemangiosarcomas often occur in variety of organs including the heart in mice with a p53 deletion. The problem arises in determining whether the hemangiosarcomas are metastases of a monocentric tumor or whether the hemangiosarcomas in the various tissues are primary tumors of multicentric origin. Except for small metastatic foci in AN-02-326 and AN-02-395, the heart was the only organ identified at necropsy and histiological examination of multiple tissues to have a hemangiosarcoma in the 4 cases associated with this submission. Therefore, it was felt the hemangiosarcoma in these cases represent examples of a primary hemangiosarcoma arising within the heart.

Lymphoma occurs in 60-70% of p53-/- mice and approximately 30% of p53+/- mice.[1-2,4-6] Approximately, 75% of the lymphomas in these mice are of T-lymphocytic lineage with the vast majority being of the lymphoblastic type. The lymphomas associated with 3 of the cases associated with this submission are a T-cell lymphoblast lymphoma typical of those that develop in p53-/- mice. The primary focus of the submission was to illustrate an example of primary hemangiosarcomas that occur in p53-/- mice. Strain background can affect the types and frequency of tumors in genetic manipulated mice. However, the incidence of lymphomas and hemangiosarcomas are similar in p53-/- mice of the 129 background and p53-/- mice of the C57Bl/6,129 hybrid back-

Stage	Cyclin-CDK Complex	Inhibitors
G1	Cyclin D/ cdk 4	P16INK4a & p21
G1—>S	Cyclin E/ cdk 2	P27
S—>G2—>M	Cyclin A/ cdk 2	
M	Cyclin B/ cdk 1	

ground.[2,5]

AFIP Di agnosis: 1. Heart, ventricle: Hemangiosarcoma, B6,129 Hybrid mouse (*Mus musculus*), rodent.
2. Mediastinum; lymph node; thymus; lung: Lymphoma.

Conference Comment: The p53 gene encodes a transcriptional regulatory protein that is involved in regulation of the cell cycle and apoptosis following DNA damage.[2] In the normal cell cycle, progression from one stage to the next is controlled by certain cyclin and CDK complexes. The activity of the cyclin and CDK complexes are tightly controlled by CDK inhibitors of two main classes: Cip/Kip (p21 & p27), and INK4/ARF (p16INK4a & p14ARF). Transcriptional activation of the Cip/Kip inhibitor p21 is controlled by p53.[7]

Loss of functional p53 gene is linked to Li-Fraumeni syndrome in people, and results in an inherited predisposition to cancer development. Genetically engineered p53 knockout or deficient mice have greatly increased incidence of numerous neoplasms including osteosarcoma, soft-tissue sarcomas, and lymphomas.[2]

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References:

1. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Butel JS, Bradley A: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. Nature 356:215-221, 1992
2. Donehower LA, Harvey M, Vogel H, McArthur MJ, Montgomery CA, Park SH, Thompson T, Ford RJ, Bradley A: Effects of genetic background on tumorigenesis in p53 deficient mice. Mol Carcinog 14:16-22, 1995
3. Fry MM, McGavin MD: Bone marrow, blood cells, and lymphatic system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 798-802. Elsevier, St. Louis, MO, 2007
4. Harvey M, McArthur MJ, Montgomery CA, Butel JS, Bradley A, Donehower LA: Spontaneous and carcinogen-induced tumorigenesis in p53 deficient mice. Nat Genet 5:225-229, 1993
5. Harvey M, McArthur MJ, Montgomery CA, Bradley A, Donehower LA: Genetic background alters the spectrum of tumors that develop in p53-deficient mice. FASEB J 7:938-943, 1993
6. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA: Tumor spectrum analysis in p53-mutant mice. Curr Biol 4:1-7, 1994
7. Kumar V, Abbas, AK, Fausto N: Robbins and Cotran Pathologic Basis of Disease, eds., 7th ed., pp. 289-292. Elsevier Saunders, Philadelphia, PA, 1999
8. Mahler JF, Flagler ND, Malarkey DE, Mann PC, Haseman JK, Eastin W: Spontaneous and chemically induced proliferative lesions in Tg.AC transgenic and p53-heterozygous mice. Toxicol Pathol 26:501-511, 1998
9. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, p. 209. Elsevier Limited, St. Louis, MO, 2007
10. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 48, 114-115, 170-170, 182-185, 205, Backwell Publishing, Ames, IA, 2007
11. Petruska JM, Frank DW, Freeman GB, Evans EW, MacDonald JS: Toxicity and carcinogenicity studies of chlorpromazine hydrochloride and *p*-Cresidine in the *p53* heterozygous mouse model. Toxicol Pathol 30:696-704, 2002
12. Storer RD, French JE, Haseman J, Hajian G, LeGrand EK, Long GG, Mixson, LA, Ochoa R, Sagartz JE, Soper KA: p53+/- Hemizygous knockout mouse: overview of available data. Toxicol Pathol 29:30-50, 2001
13. Usui T, Mutai M, Hisada S, Takoaka M, Soper KA, McCullough B, Alden C: CB6F1-rasH2 mouse: overview of available data. Toxicol Pathol 29:90-108, 2001
14. Valli VEO: Hematopoietic system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 209-210. Elsevier Limited, St. Louis, MO, 2007
15. Van Vleet JF, Ferrans VJ: Cardiovascular system. In:

Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 593-597. Elsevier, St. Louis, MO, 2007

CASE IV - V07 - 00474 (AFIP 3067193).

Signalment: 3-year-old, female, New Zealand white rabbit, *Oryctolagus cuniculus*

History: The rabbit was not eating and drinking for three days; diet consisted of pellets. No other history provided.

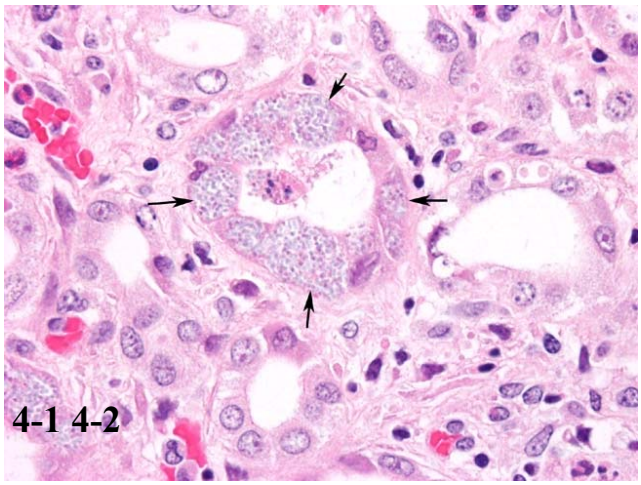
Gross Pa thology: The rabbit was in poor body condition with mild postmortem autolysis. The lungs were moderately congested and edematous. No other gross lesions were present.

Laboratory Results: Aerobic culture from the lung identified rare coagulase negative *Staphylococcus* sp. Fecal flotation was positive for *Eimeria* sp. oocysts.

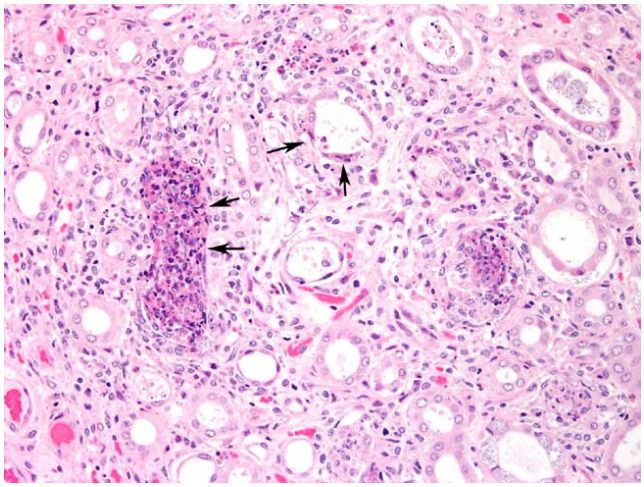
Histopathologic D escription: Numerous cortical and medullary tubules are dilated and are at least partially lined by swollen renal tubular epithelial cells that contain a cytoplasmic 10-15 micron diameter **cyst (fig. 4-1)** filled with myriad 3-5 micron diameter round to oval protozoal tachyzoites. Many of the affected renal tubular epithelial cells are **degenerate and necrotic (fig. 4-2)** with the tubular lumen filled with protozoa, cellular debris, and protein. The tubules with necrotic epithelium are lined by flattened and regenerative epithelial cells. Moderate numbers of the affected tubules are markedly dilated by numerous neutrophils and smaller numbers of macrophages, many of which contain cytoplasmic protozoa. The tubules filled with neutrophils are surrounded by macrophages, neutrophils, lymphocytes, and plasma cells. The renal pelvis contains a few perivascular foci of lymphocytes and plasma cells. The transitional epithelium of the renal pelvis is mildly hyperplastic.

Contributor's Morp hologic Di agnosis: Kidney: Nephritis, tubulointerstitial, multifocal, moderate with tubular dilatation, tubular epithelial degeneration and necrosis with intralesional protozoa, New Zealand white rabbit, lagomorph, etiology consistent with *Encephalitozoon cuniculi*;

Kidney: Pyelitis, lymphoplasmacytic, perivascular mild, with mild transitional epithelial hyperplasia



4-1 4-2



4-1 Kidney, New Zealand White rabbit. Tubular epithelial cells are greatly expanded by protozoal cysts (arrows) characterized by a 1 µm cyst wall bounding myriad 3-5 micron diameter round to oval tachyzoites. (H&E 600X)

4-2 Kidney, New Zealand White rabbit. Multifocally, there is tubular epithelium attenuation, degeneration and necrosis (arrows), and the lumina are filled with necrotic debris (cellular casts). (H&E 200X)

Contributor's Comme nt: Encephalitozoonosis is caused by *E cuniculi*. *E. cuniculi* and other microsporidial parasites have been regaining interest as opportunistic pathogens for individuals with a compromised immune systems.

E. cuniculi was first described as the etiologic agent of "infectious motor paralysis" in 1922 and was once thought to be a confounding disease affecting mainly laboratory rabbits.

E. cuniculi is a member of the phylum Microspora that includes single-celled, spore- forming, obligate intracellular parasites with a direct life cycle. *E. cuniculi* is the most extensively studied microsporidium that can infect a large variety of mammals.[8]

Microsporidia are traditionally considered protozoal organisms although novel genetic and molecular evidence suggest closer phylogenetic relationship to fungi. These findings include the presence of a particular mitochondrial heat shock protein that is more closely related to that of fungi as well as the composition of α- and β-tubulin resemble more fungal tubulins. The organism contains chitin and trehalose that are also typical fungal components (reviewed in [9]).

E. cuniculi has three strains: strain I was found in rabbits and humans, strain II in rodents and blue foxes, and strain III in dogs and humans. Identification of microsporidia species in humans and animals suggest possible zoonotic transmission. In humans, mostly immunocompromised (such as HIV positive patients with AIDS) individuals are infected. Though strain III of *E. cuniculi* is found in humans and dogs, no direct evidence suggests that dogs can transmit the disease to humans.[1]

The organism contains a coiled polar filament in the mature spore stage. The sporoplasm following extrusion from the spore coat becomes capable of invading a susceptible host cell. Upon entry multiplication occurs in association with a cytoplasmic vacuole. Sporoblasts develop into mature spores and the ruptured host cell releases the organisms into the surroundings that can then infect other cells.[7] The organism does not contain mitochondria and peroxisomes. Intervening DNA sequences (introns) are rare in microsporidial DNA suggesting that the parasite is highly adapted to survive in their hosts.[9]

Infection in mammals most often occurs by ingestion or inhalation of contaminated urine or feces shed by infected hosts. Also, infection by transplacental transmission and traumatic inoculation has also been described.[1] In the large domestic rabbits the infection is usually subclinical and renal lesions are frequently found incidentally. Occasional nervous signs with mortality can occur in young, heavily infected New Zealand rabbits. Dwarf rabbits are especially susceptible. Infection in mice, guinea pigs, nonhuman primates, carnivores and other mammals have also been reviewed.[9] In neonatal dogs and blue foxes severe symptoms may develop.[3] A serological study in the UK among pet rabbits tested as either companion of infected rabbits or part of a health screen have shown 14 of 38 asymptomatic rabbits to be seropositive. 87 rabbits showing neurological, renal or ocular signs suggestive of encehalitozoonosis were also tested.[2]

For rabbits, the usual source of infection is spores shed in the urine from actively infected rabbits but they are also readily infected experimentally by oral or respiratory routes. The spores pass to the systemic circulation via infected mononuclear cells. Target organs initially are those with high blood flow such as lung, liver and kidney. At 1 month after oral inoculation moderate to marked lesions can be demonstrated primarily in lung, liver, kidney, and not in the CNS. At 3 months postinoculation, moderate to severe lesions are present in the kidney and minimal changes in heart, lung, liver, and also in the brain. Serum titers can be detected at 3-4 weeks and become high at 6-9 weeks. Spores are shed at 1 month in the urine up to 2 months. Shedding terminates at 3 months.[7]

The parasite is able to infect a large variety of cell types such as neurons, epithelial cells of ependyma and choroid plexus, renal tubular epithelium, endothelium and macrophages.[3] Lesions in the kidney occur as focal, irregular, depressed, 1-100 mm diameter areas. In severe cases lesions may extend into the underlying cortex. Granulomatous lesions are evident in the interstitium of the lung, kidney and liver at 1 month postinoculation. In the lung, focal to diffuse interstitial pneumonitis with mononuclear cell infiltration may occur. Hepatic lesions are characterized by focal granulomatous inflammatory response with periportal lymphocytic infiltration. Focal lymphocytic infiltrates may also occur in the myocardium. Early lesions in the kidney display focal to segmental granulomatous interstitial nephritis with degenerated epithelial cells and mononuclear cell infiltration. Lesions minimally involve glomeruli. Spores may be present in epithelial cells, macrophages, inflammatory foci, or free within collecting tubules. At 1-2 months postinoculation organisms are already present in the kidneys. At a later stage, in renal lesions interstitial fibrosis, collapse of parenchyma, mononuclear cell infiltrations are typical. The organism is eliminated from the kidneys by this time.

In the CNS, lesions do not occur until after the 1 month

of exposure. Changes are focal nonsuppurative, granulomatous, meningoencephalomyelitis with astrogliosis and perivascular lymphocytic infiltration. Astroglial cells and granulomatous inflammatory foci contain organisms. Lesions can be detected in the absence of organisms. *E. cuniculi* infection has been also associated with cataractous changes. Organisms can be identified within the affected lens stroma. These cases are likely caused by intrauterine infections.[7] In the nervous system, the lesions are wide spread nonsuppurative meningoencephalomyelitis and the severity varies unpredictably in the different parts suggesting random localization of the organism and irregular distribution of inflammatory vascular changes. Small vessels are surrounded by focal gliosis and microscopic granulomas. Mononuclear cells form cuffs around larger vessels that show segmental fibrinoid change involving the adventitia and perivascular space and eventually appear similar to epithelial cells. Astrocytosis occurs in the surrounding parenchyma. The vascular lesions in the meninges, in the acute infection resemble polyarteritis nodosa and in the chronic disease become dominated by sclerotic changes with persisting perivascular cuffing and granulomatous reactions. Surviving puppies develop progressive renal disease.[4]

Staining properties of the organism and the nature of the inflammatory response can differentiate it from other infections such as *Toxoplasma gondii*. Gram positive stains mark organisms as 1.5-2.5 µm in size. Carbon fuchsin stains them purple. In addition, serology tests are available such as modified India ink immunoreaction test as well as indirect immunofuorescent microscopy and a dot ELISA test. An intradermal skin test has been used also to detect infected rabbits.[7] Electron microscopy can reveal the organisms in parasitophorous vacuoles as well as the distinctive polar filaments.[3]

AFIP Di agnosis: Kidney: Nephritis, tubulointerstitial, necrotizing, chronic-active, multifocal, moderate, with myriad microsporidia, etiology consistent with *Encephalitozoon cuniculi*, New Zealand white rabbit (*Oryctolagus cuniculus*), lagomorph.

Conference Comment: The contributor gives an excellent review of encephalitozoonosis. The numerous organisms present in this case, is very unusual. Infections are usually subclinical with very few organisms which are difficult to find.[4] Histologic changes are seen most commonly in the brain and kidney and are usually the result of a segmental vasculitis caused by parasitism of vascular endothelium.[4] In this case a mixture of chronic and acute inflammatory lesions are noted associated with the tubules.

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References:

1. Didier PJ, Snowden K, Alvarez X, Didier ES: Microsporidiosis. In: Infectious Diseases of the Dog and Cat, ed. Green CE, 3rd ed., pp. 711 - 716. Saunders, St. Louis, MO, 2006
2. Harcourt-Brown FM, Holloway HK: *Encephalitozoon cuniculi* in pet rabbits. Vet Rec 152:427-431, 2003
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th Ed., pp. 575 - 578. Lippincott Williams & Wilkins, Baltimore, Maryland, 1997
4. Maxie MG, Youssef S: Nervous system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 433-435. Elsevier Limited, St. Louis, MO, 2007
5. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 479. Elsevier Limited, St. Louis, MO, 2007
6. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 662-664. Elsevier, St. Louis, MO, 2007
7. Percy DH, Barthold SW: Rabbit. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 290-294. Blackwell Publishing, Ames, IA, 2007
8. Szabo JR, Shadduck JA: Experiemental encephalitozoonosis in neonatal dogs. Vet Pathol 24:99-108, 1987
9. Wasson K, Peper RL: Mammalian microsporidiosis. Vet Pathol 37: 13-28, 2000
10. Wilcock BP: Eye, eyelids, conjunctiva and orbit. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1396-1397. Elsevier, St. Louis, MO, 2007
11. Zeman DH, Baskin GB: Encephalitozoonosis in squirrel monkeys (*Saimiri sciureus*). Vet Pathol 22:24-31, 1985

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 8

7 November 2007

Moderator:

Dr. Victoria Hoffman, DVM, DACVP

CASE I – NOVARTIS CASE 2 (AFIP 3066019).

Signalment: Adult, male, CD-1 mouse (*Mus muscularis*)

History: An approximately 2-year-old, male, sentinel mouse was found dead with no premonitory clinical signs.

Gross P athology: Gross findings included numerous white, firm masses up to 0.8 cm in diameter in the lung. Additional findings included numerous red masses up to 0.5 cm in diameter in all lobes of the liver and approximately 2 ml of blood in the abdominal cavity.

Histopathologic D escription: The lung tumor was peripherally-located, had a glandular and papillary pattern, and was nonencapsulated and expansile resulting in compression of adjacent tissues. Glandular structures were lined with rows of cuboidal to columnar cells that enclosed central lumen and had only slight cellular atypia. Some areas had solid sheets of cells interspersed by cholesterol clefts. The tumor was multicentric (only observed in some submitted sections), with intrabronchiolar growth.

Areas of lung adjacent to the tumor had multifocal to coalescing inflammatory infiltrates composed of numerous large eosinophilic macrophages and multinucleate cells admixed with eosinophils, neutrophils, and lymphocytes within alveolar and bronchiolar spaces and associated with intra- and extracellular eosinophilic, acicular crystals.

Contributor's Morphologic Diagnosis: 1. Lung; Bronchioloalveolar adenocarcinoma, multicentric, mouse
2.Lung; Eosinophilic crystalline pneumonia, moderate, multifocal, mouse

Contributor's Comme nt: This animal had numerous spontaneous neoplasms including multicentric bronchioloalveolar carcinoma and hepatic hemangiosarcoma (the latter not submitted). Bleeding into the abdominal cavity from the hepatic tumors was the cause of death.

Eosinophilic crystalline pneumonia (ECP, formerly referred to as acidophilic macrophage pneumonia[3]) can occur spontaneously or in association with other pulmonary lesions such as infectious processes or tumors.[2] ECP can be subclinical to fatal and tends to increase in incidence with age and is more prevalent in specific strains of mice (highest incidence in 129S4/SvJae).[2]

The characteristic crystals in ECP were previously believed to be Charcot-Leyden crystals, a protein found in eosinophils and basophils.[3] Now it is known, however,

that the crystals are composed of Ym1 protein, also referred to as T-lymphocyte-derived eosinophil chemotactic factor.[1] Ym1 is secreted by activated macrophages and is homologous to chitinase.[2] Its normal function is not well-defined, but is believed to be involved in host immune defense, eosinophil recruitment, and cell-cell and cell-matrix interactions consistent with tissue repair.[2] Macrophages activated by type 2 cytokines produce large amounts of Ym1.[2]

In addition to resulting in extravasation of eosinophils and recruitment of T cells, Ym1 crystals likely contribute to lung inflammation through mechanical damage and enzymatic degradation.[1]

AFIP Diagn osis: 1. Lung: Adenocarcinoma, CD-1 mouse (*Mus muscularis*), rodent.
2. Lung: Intraalveolar histiocytosis, multifocal, moderate, with abundant intracytoplasmic eosinophilic crystals (eosinophilic crystalline pneumonia).

Conference Comment : Eosinophilic crystalline pneumonia is a common background lesion of C57BL/6 background mice. In one report there was an 87% incidence with an overrepresentation of females in 129S4/SvJae mice.[2] It can be a spontaneous lesion, or associated with pulmonary adenomas, lymphoproliferative disease, allergic pulmonary disease and parasitic or fungal infections.[2]

There are four types of Ym proteins (Ym1, Ym2, Ym3, and Ym4). Although these proteins are members of the chitinase family of proteins, they do not possess any of the chitinase enzymatic activity.[2] Ym1 and Ym2 have approximately 95% of the same sequence identity but are expressed in different tissues. Ym1 is expressed in the lung and spleen, but not in the stomach, while Ym2 is expressed in the stomach, but not the lung or spleen.[2]

Lesions associated with eosinophilic crystalline pneumonia can range from subclinical to severe and fulminating. Three patterns of lung lesions predominate.[2] The first consists of diffuse interstitial inflammatory infiltrates of macrophages, multinucleate cells, eosinophils, lymphocytes, occasional neutrophils, with moderate to severe lymphoplasmactyic perivascular and peribronchiolar cuffing. The second consists of little to no crystals, with macrophage infiltrates localized to regions of a lung tumor. The third consists of focal to multifocal infiltrates localized around bronchioles with large rectangular crystals in the airways and minimal macrophage infiltrates.[2] The case presented in this conference appeared consistent with the second pattern of distribution and was unusual in that it had little to no interstitial reaction despite numerous intraalveolar macrophages with abundant eosinophilic crystals.

Pulmonary adenomas and adenocarcinomas are among the most common primary pulmonary neoplasms in mice. A-strain mice are particularly susceptible, and their development in this strain is usually associated with activation of K-*ras* within these tumors.[4] Other less common primary lung tumors within mice are squamous cell carcinoma, papilloma, neuroendocrine carcinomas, and adenosquamous carcinomas.[4]

The cell of origin for pulmonary adenomas and adenocarcinomas are thought to be either type II pneumocytes or Clara cells.[4] Ultrastructural features of Clara cell differentiation include apical cytoplasmic accumulation of smooth endoplasmic reticulum, which can be admixed with lamellar surfactant granule formation.

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References:

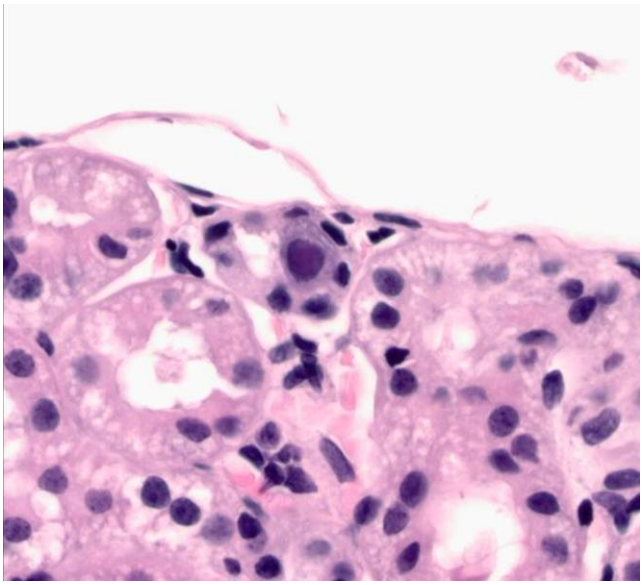
1. Guo L, Johnson RS, Schuh JCL: Biochemical characterization of endogenously formed eosinophilic crystals in the lungs of mice. J Biol Chem 275:8032-8037, 2000
2. Hoenerhoff MJ, Starost MF, Ward JM: Eosinophilic crystalline pneumonia as a major cause of death in 129S4/SvJae mice. Vet Pathol 43:682-688, 2006
3. Murray AB, Luz A: Acidophilic macrophage pneumonia in laboratory mice. Vet Pathol 27:274-281, 1990
4. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 117-118. Blackwell Publishing, Ames, IA, 2007
5. Wilson DW, Dungworth DL: Tumors of the respiratory tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 385-389. Blackwell Publishing, Ames, IA, 2002

CASE II – WIL-416051 (AFIP 3067175).

Signalment: Male, 2-year-old, Cynomolgus monkey (*Macaca fascicularis*)

History: This monkey was euthanized at the end of a 14 day toxicity study. There were no clinical observations.

Histopathologic D escription: This is a section of kidney characterized by widespread, multifocal, peritubular



2-1. Kidney, Cynomologus monkey. Rarely, tubular epithelial cells contain 5-7 µm intranuclear viral inclusion bodies. (H&E 400X)

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infiltrates of predominantly lymphocytes and plasma cells. Adjacent tubules are often lined by epithelial cells with enlarged hyperchromatic nuclei or epithelial cells that are in various stages of degeneration and sloughing. Lymphoplasmacytic infiltrates are also present within or obliterate tubules and tubular basement membranes are breached. Rarely, large, eosinophilic, intranuclear **inclusion bo dies (2 -1)** are present within tubular epithelial cells.

Contributor's Morpho logic Diagn osis: Kidney: Interstitial nephritis, lymphoplasmacytic, multifocal, moderate with epithelial intranuclear inclusions.

Contributor's Comment: The histopathology and presence of intranuclear inclusions are consistent with a viral etiology, in particular polyoma virus. Several simian polyoma viruses have been described and compared to the JC and BK polyoma viruses in humans.[1] Polyoma viruses can cause latent infections in healthy hosts, but clinical disease occurs in immunocompromised hosts. Polyoma virus infection is a noted cause of severe nephritis and renal rejections in immunosuppressed renal transplant recipients. In clinically affected hosts, the virus can also cause progressive multifocal leukoencephalopathy. However, renal polyoma virus infections are often typically mild and self-limiting.

In this study, several monkeys in vehicle-treated groups as well as test-article treated groups had interstitial nephritis. Several female monkeys in this study also had mononuclear infiltrates (inflammation) of the smooth muscle of the gastrointestinal tract as evidence of infection, with no relationship to dose of test articles. Additionally, there were no clinical chemistry changes associated with renal disease or infection. Thus, we interpreted these findings to be incidental to the study.

AFIP Diagn osis: Kidney: Nephritis, interstitial, lymphoplasmacytic, multifocal to coalescing, moderate, with multifocal tubular epithelial karyomegaly and rare intranuclear inclusion bodies, Cynomolgus monkey (*Macaca fascicularis*), primate.

Conference Comment: Polyoma viruses and papilloma viruses are double stranded DNA viruses belonging to the Papovaviridae family of viruses. Several polyoma viruses, including SV40, simian agent 12, polyoma virus papionis-2 and lymphotropic papovavirus, infect old world primates. Closely related polyoma viruses in humans include BK polyoma virus and JC polyoma virus.

All polyoma viruses share two regulatory proteins, known as the large T and small T antigen, which can serve as primers in PCR identification.[1] Clinically overt disease due to polyomaviruses are commonly associated with immunosuppression, often as a result of either infection with Simian Immunodeficiency Virus or the use of immunosuppressive drugs.

Cynomolgus Polyoma Virus, antigenically and genomically related to Simian Virus 40, has been reported to cause renal dysfunction and tubulointerstitial nephritis in immunosuppressed Cynomolgus monkeys. Intranuclear inclusions within karyomegalic (2-3X normal) tubular epithelial cells were a consistent finding within affected kidneys.[1]

The differential diagnosis for these inclusions includes cytomegalovirus with characteristic large, dense, intranuclear inclusions often surrounded by a halo (owl's eye cells), and adenovirus with very large intranuclear inclusions that are deeply basophilic, "smudgy", and not surrounded by a clear halo.

Contributor: Millennium Pharmaceuticals, Inc., 35 Landsdowne Street, Cambridge, MA 02139

Reference:

1. van Gorder MA, Pelle PD, Henson JW, Sachs DH, Cosimi AB, Colvin RB: Cynomolgus polyoma virus infection: a new member of the polyoma virus family

causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed Cynomolgus monkeys. Am J Pathol 154:1273-1284, 1999

CASE III – MK0610249 (AFIP 3069155).

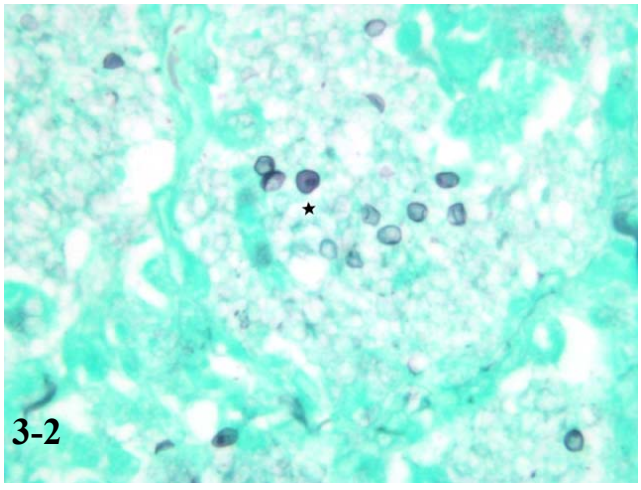
Signalment: Young, adult, Rhesus macaque (*Macaca mulatta*)

History: The presenting animal was inoculated with simian influenza virus, SIVmac239, and simian human immunodeficiency virus, SHIVDH12 (MD14YE) on June 1, 2005. Eighteen weeks post inoculation, only SIVmac239 was detectable in the blood. Throughout the 64-week course of the study, CD4+ T cell levels consistently declined, ranging from 26% to <3% of cells/µL of blood. CD8+ T cell levels fluctuated, ranging as high as 73% of cells/µL of blood, then dropped severely between weeks 62 and 64 to 26% of cells/µL of blood. On August 22, 2006, week 63, the animal presented with a rapid respiration rate. Chest radiographs were obtained and results indicated diffuse pneumonia. Total white blood cell count was 13,000 cells/µL of blood; lymphocyte count was 1248 cells/µL, monocyte count was 1040 cells/µL, and eosinophil count was 1287 cells/µL. The animal was euthanized.

Gross Pa thology: Upon opening the chest cavity, the lungs did not collapse. The lungs were grayish-pink to light brown and were mild to moderately **consolidated (3-1)**. Gross appearance of the lungs was consistent with diffuse pneumonia. The trachea contained a small amount of frothy white fluid. The spleen was mildly enlarged uniformly. No other abnormalities were noted in the other major organs or tissues.

Histopathologic D escription: The lung exhibited diffuse alveolitis with alveoli containing foamy eosinophilic fluid with an admixture of small to moderate numbers of neutrophils, alveolar macrophages, multi-nucleated histiocytic giant cells, and numerous extracellular fungal organisms consistent with *Pneumocystis carinii*. Centrally to peripherally located within the fungal organisms was a small nucleus of the cystic and trophic forms of Pneumocystis. There was mild to moderate type II pneumocyte hyperplasia and within the septae, there was a mild mononuclear infiltrate comprised mainly of lymphocytes and a smaller number of plasma cells. Gomori methenamine silver **(GMS) (3-2)** stains of the lung sections and imprint smears were positive for the cystic forms of *Pneumocystis carinii*.

Cytology: Lung imprints were prepared using a cut margin of lung stained with a modified Wright-Giemsa stain. Cytologic examination revealed red blood cells, alveolar macrophages, type II pneumocytes, neutrophils, and nu-



3-1. Lung, Rhesus macaque. The lungs are diffusely consolidated and pale. Photograph courtesy of the National Institute of Health, Office of Research Services, Bethesda, Maryland

3-2. Lung, Rhesus macaque. GMS positive, 4-6 um fungal cysts within alveoli. (star). (Gomori Methenamine Silver 400X)

merous round to oval extracellular organisms measuring approximately 4 µm in diameter. Nuclei were evident within the organisms and were consistent with the cystic and trophozoite forms of *Pneumocystis carinii*.

Contributor's Morphologic Diagnoses: Lung: Alveolitis, diffuse, acute with numerous fungal organisms consistent with *Pneumocystis carinii*.

Contributor's Comment: *Pneumocystis carinii* was once mis-identified as a protozoan, but was re-classified as a fungal organism based on mitochondrial genomic gene sequences, amino acid sequences of peptides and proteins, and comparative analysis of the 16 S ribosomal RNA.[5] Transmission of the fungus can be from host to host or from the environment.[15] Although *Pneumocystis* typically causes pneumonia in immunocompromised individuals, asymptomatic hosts may be carriers.[9,13,14,15] Immunocompetent hosts tend to develop transient *Pneumocystis* infections and can transmit infection to susceptible hosts by an airborne route.[6] *Pneumocystis* pneumonia is the most prevalent opportunistic infection in immunocompromised patients infected with human immunodeficiency virus (HIV) and is particularly difficult to study because the organism cannot be cultured outside the host lung; therefore, animal models continue to be the best source of information on *Pneumocystis*.[4,14] There are 5 host-specific species of *Pneumocystis*: *Pneumocystis carinii, Pneumocystis jiroveccii, Pneumocystis murina, Pneumocystis oryctolagi*, and *Pneumocystis wakefieldiae*. *Pneumocystis carinii* species infects SIV positive macaques and the *Pneumocystis jiroveccii* species infects HIV positive humans.[1,7,12] Wild born and laboratory bred macaques have similar susceptibilities to *Pneumocystis carinii*, however, wild born macaques exhibit higher levels of antibody titers to the fungus.[8] The first report of infection in nonhuman primates included two aged owl monkeys and two young chimpanzees with myeloproliferative neoplasia.[3] Lungs affected with severe *Pneumocystis* pneumonia typically exhibit alveolar type-II cell hypertrophy and intra-alveolar foamy eosinophilic material and variable neutrophilic lung inflammation that may result in diffuse alveolar damage, impaired gas exchange, and respiratory failure.[6,14] The *Pneumocystis* fungi attach to type-I alveolar epithelial cells and, although the organisms do not alter the metabolic, barrier, or structural functions of the alveolar cells, they are able to proliferate due to impaired cell-mediated immunity. Infection also leads to changes in pulmonary surfactant production initiated by the inhibition of phosphatidylcholine from type II alveolar cells.[6] *Pneumocystis* organisms exist as cystic and haploid trophozoite forms throughout their life-cycle, but during infection, the trophozoite form is more abundant than the cystic form.[14] In the transmission electron micrographs of a thin section of *Pneumocystis* infected lung from this macaque, cyst and trophozoite forms are evident with trophozoites predominating. Free ribosomes and glycogen are present in both forms. Cysts are typically spherical in shape, contain up to eight intracystic bodies from which trophozoites originate, and have a mean diameter of 5-8 µm. The mean diameter of an intracystic body is 1-2 µm.[5] Each intracystic body contains components characteristic of eukaryotic cells including a centrally located nucleus, endoplasmic reticulum, and other organelles. The cyst wall is two layers thick, measuring approximately 50 nm.[5] Trophozoites are generally grouped into an interdigitating cluster, are individually pleomorphic, and are closely associated with pneumocytes. There are very few organelles, including a nucleus with a centrally to peripherally placed nucleolus. Free ribosomes predominate within the infective form. A 20-30 nm thick, non-homogenous coat surrounds the entire trophozoite, including the interdigitations, and is a characteristic feature of the trophozoite form. The coat may help anchor the trophozoite to other trophozoites or alveolar epithelium or it may be involved in nutrient uptake.[5] Trophozoites adhere to the alveolar epithelium to establish infection and to initiate a mitogen activated protein kinase signaling cascade for mating and proliferation of the fungus.[14] CD4+ T lymphocytes are integral in the defense against *Pneumocystis*.[6] By recruiting and activating macrophages and monocytes to attack the invading organism, CD4+ cells behave as memory cells to initiate the host's immune response.[14] Continuously declining CD4+ levels in this animal to <3% of cells/µL of blood allowed the *Pneumocystis* organisms to extensively proliferate and exert their adverse effects. SIV positive monkeys infected with *Pneumocystis* are unable to recruit CD4+ cells to the lung and develop an inflammatory response characterized by an increased neutrophil and CD8+ cell infiltration.[4] Not all of the imposed alveolar damage is due to the adherence of *Pneumocystis* organism to the type-I alveolar epithelial cells. TNF-a, an inflammatory cytokine that regulates the immune response by recruiting neutrophils, lymphocytes, and monocytes to an area of infection, is also a contributing factor in damage to the alveoli. While TNF-a helps mount an immune response, it also releases oxidants, cationic proteins, and proteases responsible for subsequent damage to pulmonary tissue.[14] Trimethoprim-sulfamethoxazole combined with corticosteroid therapy to suppress lung inflammation is the preferred and most effective course of treatment for *Pneumocystis* and is usually begun when the CD4+ cell count is less than 200 per cubic millimeter, a state of a heightened risk of infection.[14] Adverse effects of the drug are common, however, and use of this prophylaxis has rapidly increased the multidrug resistance of infection bacterial pathogens found in human immunodefi-

ciency virus-infected animals.[10,14]

AFIP Dia gnosis: 1. Lung: Pneumonia, interstitial, histiocytic and neutrophilic, chronic, diffuse, moderate, with type II pneumocyte hyperplasia, multinucleate giant cells, and myriad intraalveolar fungi, etiology consistent with *Pneumocystis carinii*, Rhesus macaque (*Macaca mulatta*), primate.
2. Cytological specimen, impression smear, lung: Numerous epithelial cells, macrophages with vacuolated cytoplasm, few neutrophils, and myriad 3-5um round cysts containing punctate organisms (trophic bodies) on a blue, granular, proteinaceous background.

Conference Comment: The contributor gives an excellent overview of *Pneumocystis carinii*. *Pneumocystis carinii* has also been described in pigs, foals, dogs, and other domestic animals with underlying immunosuppression.[2] The characteristic histologic finding is an eosinophilic foamy or flocculent material within alveoli with numerous intra- and extracellular fungal bodies. The organism can be more readily identified with periodic acid-Schiff (PAS) procedure or Gomori methenamine silver (GMS) stain.[2]

Contributing Institution: National Institutes of Health, Division of Veterinary Resources, Office of Research Services, Bethesda, MD

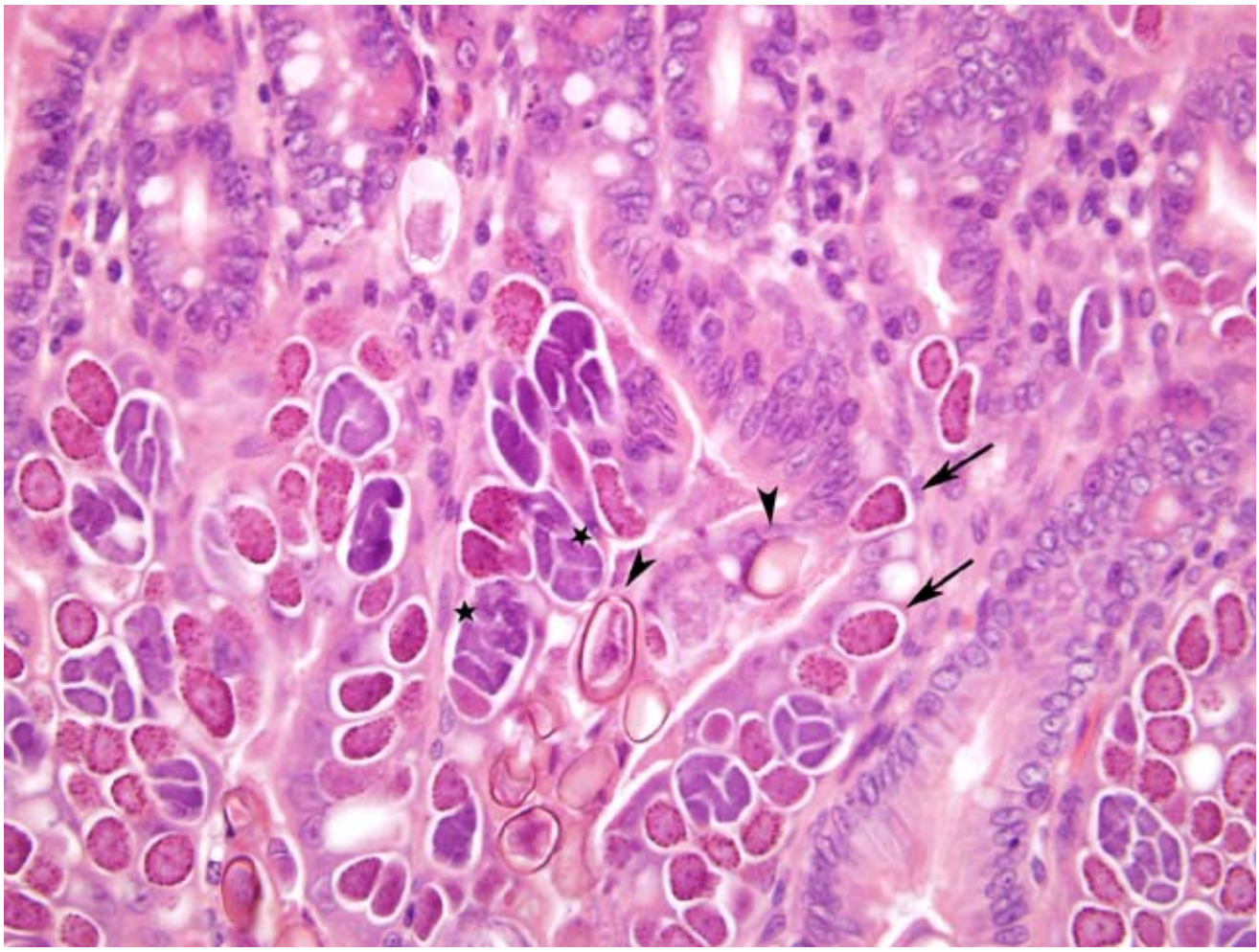
References:
1. Board KF, Sangita P, Lebedeva I, Capuano S, Trichel AM, Murphey-Corb M, Rajakumar PA, Flynn JL, Haidaris CG, Norris KA: Experimental *Pneumocystis carinii* pneumonia in simian immunodeficiency virus-infected Rhesus macaques. J Infect Dis 187:576-588, 2003
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 593. Elsevier Limited, St. Louis, MO, 2007
3. Chandler FW, McClure HM, Campbell WG, Watts JC: Pulmonary pneumocystosis in nonhuman primates. Arch Pathol Lab Med 100:163-167, 1976
4. Croix DA, Board K, Capuano S, Murphey-Corb M, Haidaris CG, Flynn JL, Reinhart T, Norris KA: Alterations in T lymphocyte profiles of bronchoalveolar lavage fluid from SIV- and *Pneumocystis carinii*-coinfected Rhesus macaques. AIDS Res Hum Retroviruses 18:391-401, 2002
5. de Souza W, Benchimol M: Basic biology of *Pneumocystis carinii*-a mini review. Mem Inst Oswaldo Cruz 100:903-908, 2005
6. Dei-Cas E: *Pneumocystis* infections: the iceberg? Med Mycol 38:23-32, 2000
7. DeManche C, Berthelemy M, Petit T, Polack B, Wakefield AE, Dei-Cas E, Guillot J: Phylogeny of *Pneumocystis carinii* from 18 primate species confirms host specificity and suggests coevolution. J Clin Microbiol 39:2126-2133, 2001
8. Fujita M, Furuta T, Kojima S, Kurata T, Yoshikawa Y: Survey for *Pneumocystis carinii* infection of wild-born and laboratory-bred monkeys by indirect immunofluorescences and cyst-staining methods. Jpn J Med Sci Biol 49:113-120, 1996
9. Furuta T, Fujita M, Mukai R, Sakakibara I, Sata T, Miki K, Hayami M, Kojima S, Yoshikawa Y: Severe pulmonary pneumocystosis in simian acquired immunodeficiency syndrome induced by simian immunodeficiency virus: its characterization by the polymerase-chain-reaction method and failure of experimental transmission to immunodeficiency animals. Parasitol Res 79:624-628, 1993
10. Huovinen P: Resistance to trimethoprim-sulfamethoxazole. Clin Infect Dis 32:1608-1614, 2001
11. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 471, 536, 546. Elsevier, St. Louis, MO, 2007
12. Medrano FJ, Montes-Cano M, Conde M, de la Horra C, Respaldiza N, Gasch A, Perez-Lozano MJ, Varela JM, Calderon EJ: *Pneumocystis jirovecii* in general population. Emerg Infect Dis 11:245-250, 2005
13. Stahl J, Sage MR: Radiological-pathological correlation: Alveolar pattern. Australas Radiol 45:74-97, 2001
14. Thomas CF, Limper AH: *Pneumocystis* pneumonia. N Engl J Med 350:2487-98, 2004
15. Vogel P, Miller CJ, Lowenstine LL, Lackner AA: Evidence of horizontal transmission of *Pneumocystis carinii* pneumonia in simian immunodeficiency virus-infected Rhesus macaques. J Infect Dis 168:836-843, 1993

CASE IV - CRL 1 (AFIP 3065936).

Signalment: 8-week-old, male, NZW rabbit, *Oryctolagus cuniculus*

History: Submitted for routine colony surveillance. No clinical signs reported

Histopathologic D escription: Small intestine: More than 95% of epithelium is distorted by intracellular coccidia, including all villi and some crypts. Remaining crypts are compressed. The lamina propria has a slight infiltrate of lymphocytes, plasma cells, macrophages and



4-1 Small intestine, New Zealand White Rabbit. Many epithelial cells are expanded and distorted by intracellular coccidia of various developmental and reproductive stages. Oocysts are surrounded by a refractile isotropic wall (arrowheads). Microgametes (stars) and macrogametes (arrows) represent the two "genders" involved in gametogeny. (H&E 400X)

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heterophils. Parasitized mucosal cells have marginalized nuclei and contain one of **three phases (4-1)**. Some cells contain nonsporulated oocysts, also abundant in the lumen. Oocysts are approximately 30x15 to 30x20 microns, with a thick, dark, refractile, isotropic wall. A micropyle is visible on a few oocysts. Other epithelial cells contain macrogametocytes or microgametocytes in various stages of development. In general, the less developed forms are present deeper in the crypts. All coccidial forms appear limited to the lumen and the epithelium.

Contributor's Morpho logic Di agnosis: Coccidiosis diffuse, severe, with severe loss of absorptive epithelium and mild, subacute enteritis.

Contributor's Comment: *Eimeria magna* was detected by fecal centrifugation and concentration on this rabbit. *Eimeria perforans* was detected in another rabbit from this cohort, so all likely had a mixed infection.

Despite the striking degree of mucosal involvement, no diarrhea was reported, and no evidence of it was noted at necropsy. Nonetheless, *E. magna* is reported to be of moderate pathogenicity.[4]

Intestinal coccidiosis continues to be common in rabbits, probably due to multiple factors, including the frequency of inapparent infections, the massive number of oocysts shed by infected rabbits, and the resistance of oocysts to many disinfectants. More than 12 species of intestinal coccidia have been reported in rabbits,[6] most of which live in the small intestine. Definitive diagnosis requires

examination of sporulated oocysts. In our experience, oocysts will sporulate in fecal samples left at room temperature (presumably as in the wild) or in the refrigerator for several days, but sporulation is more reliably accomplished by incubation at room temperature for 1-5 days in a potassium dichromate solution. Sporulated oocysts are readily speciated based on size, appearance, and the appearance, if present, of the micropyle and residual body.

The life cycle of *E. magna* is typical of *Eimeria* spp. All *Eimeria* are host-specific and have a direct life cycle.[2] Oocysts are not infective until sporulation, so ingestion of cecotroph feces does not result in autoinfection. Ingestion of sporulated oocysts (sporocysts) results in release (excystation) of sporozoites. These invade enterocytes, round up and form trophozoites, and multiply asexually by schizogony (merogony), forming schizonts (meronts) that may contain more than 100,000 merozoites. Merozoites escape the host cell, resulting in death of that cell. Each merozoite can then invade another host cell for the next generation. The number of asexual generations is characteristic of each *Eimeria* sp., *E. magna* has been variously reported as having four or five[3,5] asexual generations prior to gametogony. In gametogony, the final generation merozoites form either macrogametocytes (female) or microgametocytes (male). Macrogametocytes have a single nucleus, and numerous peripheral PAS-positive granules. Microgametocytes are multinucleate. Each nucleus becomes incorporated into a small biflagellate sperm-like microgametocye. After fertilization by the microgametocyte, the macrogametocyte develops into an oocyst. It has been estimated that one oocyst of *E. magna* can produce more than 25,000,000 oocysts in a susceptible host.[4] Given that feces from asymptomatic rabbits may contain more than 400,000 oocysts/gram, the potential for massive infection is apparent. Clinical disease is thought to result primarily from loss of functional mucosa and loss of mucosal barrier integrity as cells are lost.

We personally find it interesting that such massive infection did not cause significant debility (note the normal mesenteric fat) or diarrhea. The general sparing of the crypts may indicate that the rabbit retains sufficient epithelial replacement capacity, although no increase in mitotic rate was noted. Sparing of the crypts and the observation of generally less developed forms deeper along the villus also suggest two evolutionary adaptations of this parasite: first, that sparing the crypts is advantageous to the replication of the parasite as the host is not rapidly killed; and second, that invasion of cells near the crypt or along the side of the villus may be preferable as those cells are less likely to be shed prior to completion of a particular phase in the life cycle.

AFIP Dia gnosis: Small intestine, mucosa: Coccidial macrogametes, microgamonts, and oocysts, intraepithelial and intraluminal, myriad, New Zealand white rabbit (*Oryctolagus cuniculus*), lagomorph.

Conference Comment: The Family Eimeriidae includes *Eimeria* and *Isospora*. Coccidia of domestic animals are relatively host and tissue specific. A table listing the common *Eimeria* and *Isospora* species of animals and the tissues in which they are found has been included for quick reference (see next page)**.**

Conference participants briefly reviewed the coccidian life cycle. Oocysts are shed in feces and sporulate. The oocysts of each species are morphologically distinct, but share similar features. The oocysts of *Eimeria* have four sporocysts, each with two sporozoites, with a total of eight sporozoites in each oocyst. The oocysts of *Isopora* have two sporocysts, each with four sporozoites, with a total of eight sporozoites in each oocyst. Ingested sporozoites excyst in the intestine and invade epithelial cells where they round up and form trophozoites. Asexual replication or schizogeny follows forming schizonts containing merozoites. The schizonts rupture, releasing the merozoites, which infect other epithelial cells and continue to replicate. Merozoites eventually form sexual stages (male-microgamete, female-macrogamete) which unite to form oocysts.[2]

Conference attendees also reviewed the ultrastructural features of apicomplexans, specifically *Toxoplasma*: parasitophorous vacuole, rhoptries, micronemes, apical conoid, apicoplasts, and dense granules.

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. We are grateful to Dr. Gardiner for his comments and advice on this interesting case.

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References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 261-269. Elsevier Limited, St. Louis, MO, 2007
2. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protoozan Parasites in Animal Tissues, 2nd ed., pp. 20-21, Armed Forces Institute of Pathology, Washington, D.C., 1998
3. Pakandl M, Eid AN, Licois D, Coudert P: *Eimeria magna* Pérard,1925: study of the endogenous develop-

***Eimeria* and *Isospora* of Animals**		
Geese & ducks	*E. truncata*	Kidney
Sandhill whooping cranes	*E. reichenowi*	Disseminated
Parrots	*E. psittaculae*	Intestine
Chicken	*E. acervulina*	Duodenum
Chicken	*E. necatrix*	Mid-intestine
Chicken	*E. tenella*	Ceca
Cattle	*E. bovis*	Small intestine, cecum, colon
Sheep	*E. ashata*	Small intestine
	E. bakuensis	Small intestine
	E. ovinoidalis	Ileum, large intestine
Goats	*E. Christenseni*	Small intestine
	E. arlongi	Small intestine
	E. ninakohlyakimovea	Large intestine
Horses	*E. leukarti*	Small intestine
Swine	*I. suis*	Intestine
	E. debliecki	
	E. porci	
	E. scabra	
Dogs	*I. canis*	Ileum, cecum occasionally
Cats	*I. felis*	Small intestine, colon occasionally
Mice	*E. falciformis*	Colon
Rabbit	*E. stiedae*	Bile ducts
	E. intestinalis	Ileum, cecum
	E. flavescens	Ileum, cecum
Guinea pig	*E. caviae*	Large intestine
Ferret	*E. furonis*	Gallbladder, bile duct

ment of parental and precocious strains. Vet Parasitol 65:213-222, 1996

4. Percy DH, Barthold SW: Rabbit. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 287-290. Blackwell Publishing, Ames, IA, 2007

5. Ryley JF, Robinson TE: Life cycle studies with *Eimeria magna* Pérard, 1925. Z Parasitenkd 50:257-275, 1976

6. Schoeb TR, Cartner SC, Baker RA, Gerrity LW: Parasites of rabbits. In: Flynn's Parasites of Laboratory Animals, ed. Baker DG, pp. 451-499. Blackwell Publishing, Ames, IA, 2007

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 9

28 November 2007

Moderator:

Lance Batey, DVM, DACVP

CASE I – 18079-05 (AFIP 3031126).

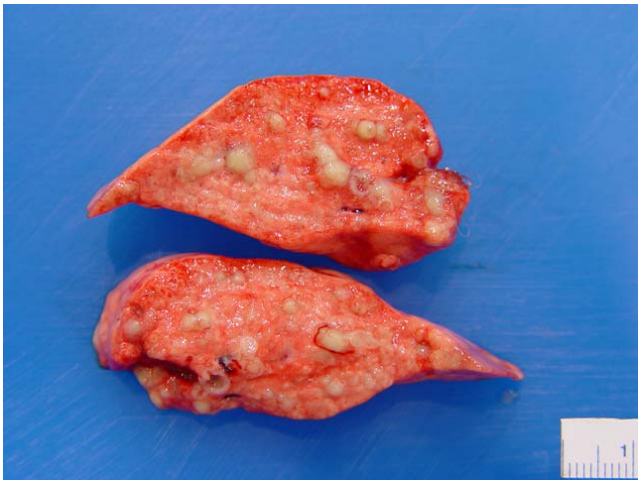
Signalment: Red fox (*Vulpes vulpes*), juvenile, female

History: This red fox was found alive at a golf course in late July. It was showing signs of illness: weak, heaving, breathing heavily, not scared of humans. This was the second fox in one week from the same golf course with similar signs. The animal was shot in the head with a .22-caliber rifle and submitted for necropsy.

Gross Pathology: This female red fox was considered to be a young of the year (approximately 3-4-months-old), based on its small size. It was in poor body condition (no fat in subcutis, only a small amount of fat around the base of the heart and in the mesentery). Both lungs were very emphysematous. The mucosa of the caudal region of the trachea was covered by a small to moderate amount of creamy whitish material and contained a few slightly raised plaques, about 2-3 mm in diameter. Almost the entire bronchial tree of both lungs was filled with creamy yellow material suggestive of **pus (1 -1)**. The stomach was empty. The large intestine contained a moderate amount of fecal material.

Laboratory Results: *Streptococcus* species (group G), was isolated in large numbers from a bronchial swab.

Histopathologic Description: Lesions were confined to the respiratory tract and, in the lungs, were centered around the bronchial tree. Several cross and oblique sec-



1-1. Lung, red fox. On cut section, the bonchi and bronchioles are multifocally expanded and occluded by abundant purulent material. Photograph courtesy of the Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Canada

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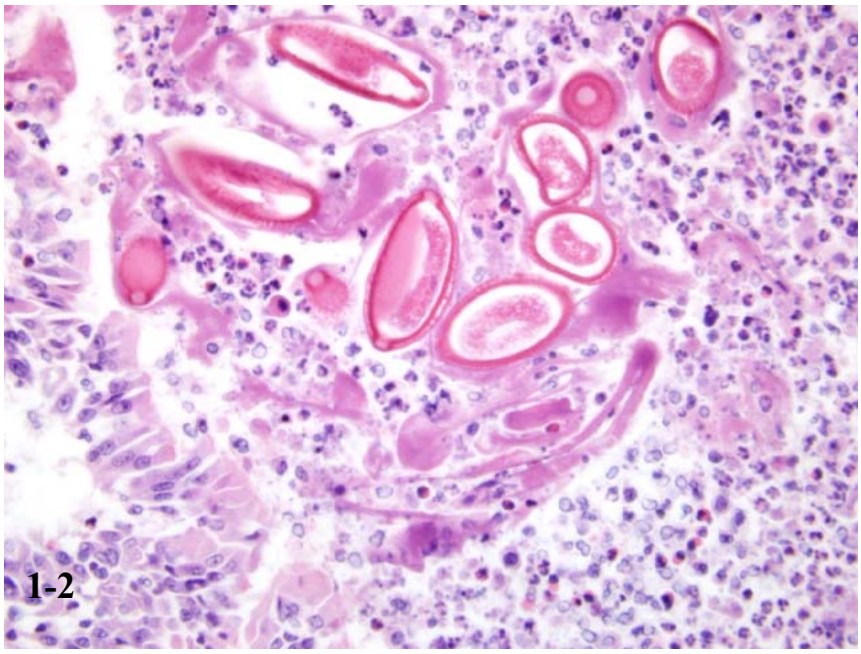
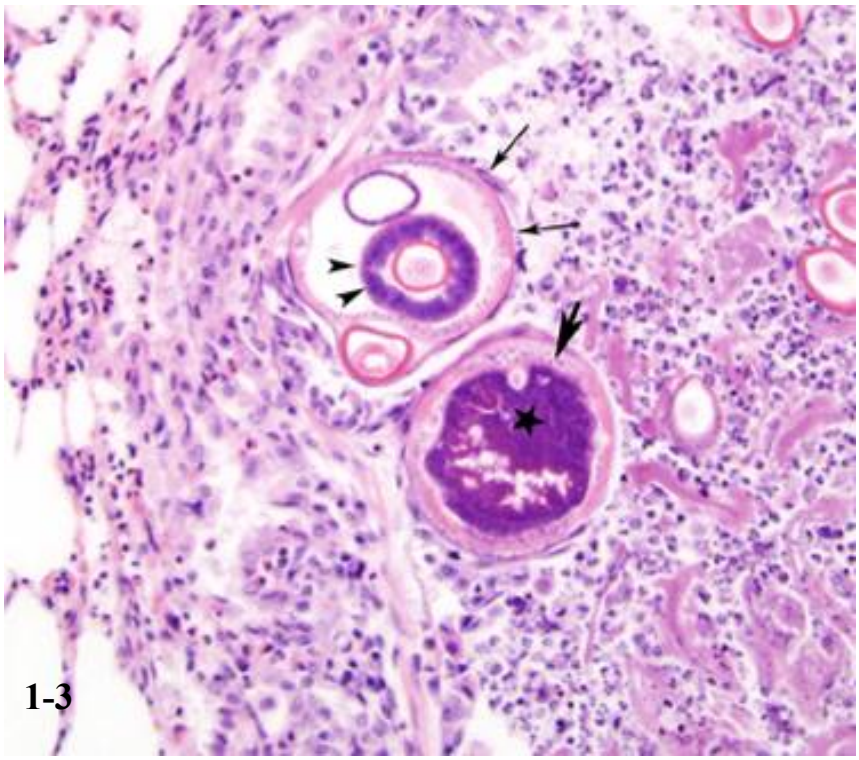
tions of small nematodes, mixed with numerous **ova (1-2)** (often with an operculum evident at both ends), large numbers of macrophages and some neutrophils, rested on the mucosal epithelium of small and large bronchi or were embedded within it. Much of this epithelium was markedly hyperplastic, particularly in the larger bronchi, although areas of epithelial loss were also evident. The submucosa of the larger intrapulmonary bronchi was infiltrated by numerous plasma cells and some eosinophils. Many ova, several of them degenerated, could be found within bronchioles and alveoli where they were associated with a pyogranulomatous reaction, including multinucleated giant cells that had phagocytized some of these ova. Lesions similar to those within the lungs were in the main bronchi and in the caudal region of the trachea, together with fibrosis, necrosis and fibrin accumulation (tissues not included). The pulmonary nematodes were identified in tissue sections as ***Eucoleus a erophilus*** **(1-3)** (formerly *Capillaria aerophila*).[3]





Contributor's Morphologic Diagnosis: Severe chronic pyogranulomatous verminous bronchopneumonia

Contributor's Comment: This young red fox had a severe pulmonary parasitic infection, likely complicated by a secondary bacterial infection. The nematode *Eucoleus aerophilus* belongs to the superfamily Trichinelloidea, whose members typically parasitize epithelial surfaces of vertebrates.[4] *Eucoleus* aerophilus has been reported at a low prevalence in most fecal surveys of dogs and cats and is enzootic in wild foxes in many parts of the world.[4,9] In the three Maritime provinces of Canada (Prince

1-2. Lung, red fox. Oval metazoan ova with a thick iridescent wall which is often bi-operculate, admixed with necrotic debris, pyogranulomatous and eosinophilic inflammation, fibrin and edema. (H&E 400X)

1-3. Lung, red fox. Frequently, expanding the lumenae of the bronchiolar tree, admixed with necrotic debris, pyogranulomatous and eosinophilic inflammation, fibrin and edema are cross sections of adult Eucoleus aerophilus, which are characterized by a thin cuticle (small arrows), hypodermal or bacillary band (large arrows), and occasionally a stichosome (star) or a single genital tract (arrowheads). (H&E 400X)

Edward Island, New Brunswick, Nova Scotia), more than 65% of wild red foxes that have been examined were infected by this parasite.[9] The life cycle of *E. aerophilus* is mainly direct through the fecal-oral route, although earthworms that have ingested the ova with soil can also act as facultative intermediate or, more likely, paratenic hosts.[4]

Clinical signs in infected dogs and cats are generally characterized by a low-grade chronic cough. The disease among foxes raised in earthen runs on fur farms, however, used to be much more severe, with poor growth, decreased fur quality, and substantial mortality from bronchopneumonia, particularly among young animals.[7] Control of the disease was achieved by shifting the animals to raised cages with wire bottoms. In free-living wild red fox, the degree of infection may vary among animals but is probably generally higher in young-of-the-year because of their immature immune system. It is conceivable that, every year, a number of young fox die from pneumonia caused by this parasite, either because their immune system is particularly inefficient or because they are exposed to a very large number of eggs. In this case, secondary infection by *Streptococcus* species group G had likely contributed to the animal's death. Most bacteria of this group isolated from animals are *S. canis*. This bacterium can be isolated from mucous membranes of asymptomatic domestic carnivores, but it can also cause opportunistic infections, including suppurative bronchopneumonia and septicemia.[10]

The nematode *Crenosoma vulpis* is another common pulmonary parasite in the red fox population of the Canadian Maritime provinces and often occurs concurrently with *E. aerophilus*.[9] This parasite may also be a common cause of respiratory disease in domestic dogs presented with clinical signs of chronic cough.[1] *Crenosoma vulpis* has an indirect life cycle, using snails and slugs as intermediate hosts.[11] *Eucoleus aerophilus* is longer but more slender than *C. vulpis* (Table 1).[8] Whereas *E. aerophilus* is oviparous, *C. vulpis* is ovoviviparous, and it also tends to inhabit deeper regions of the bronchial tree.[9] Adults and first stage larvae of *Crenosoma vulpis* were not identified in the lungs of this fox.

Other potential nematode parasites of the respiratory system of red fox in this country include *Oslerus (Filaroides) osleri*, *Dirofilaria immitis*, and *Angyostrongylus vasorum*. Infection by *O. osleri* is characterized by the formation of discrete nodules typically found near the bifurcation of the main bronchi, whereas both *D. immitis* and *A. vasorum* are parasites of the pulmonary arterial tree rather than of the airways.[3] Moreover, the North American distribution of *A. vasorum* is currently confined to the Atlantic Canadian province of Newfoundland.[2]

AFIP Diagnosis: Lung: Bronchopneumonia, pyogranulomatous and eosinophilic, multifocal, severe, with bronchiolar epithelial hyperplasia, aphasmids and eggs, etiology consistent with *Eucoleus aerophilus*, red fox (*Vulpes vulpes*), canine.

Conference Comment: The capillarids are a large group of parasites that have been divided into numerous different genera on more than one occasion. The former *Capillaria* affecting dogs and cats is now primarily divided into three genera:[3] *Eucoleus*, which is found in the airways; *Aonchotheca*, which is found in the intestinal tract; and *Pearsonema*, which is found in the urinary bladder. Other genera of veterinary importance include *Calodium*, which is found in the liver of rats and other mammals.[3] The division into these genera is not universally accepted as some researchers prefer the previous genus name *Capillaria*.[6]

The life cycle of *Eucoleus* (*Capillaria*) *aerophilus* can be either primarily direct or less commonly indirect involving an earthworm as an intermediate paratenic host. Eggs are deposited in the pseudostratified ciliated epithelium, work their way up the respiratory tree, are swallowed, and are then passed out with the feces.[4]

Physical characteristics of *E. aerophilus* are similar to

Table 1: Comparison of the dimensions of adult specimens of Eucoleus aerophilus and Crenosoma vulpis.[8]

	Length		Diameter	
	Male	female	male	female
E. aerophilus	15-25 mm	20-40 mm	60-100 µ	100-180 µ
C. vulpis	3.5-8 mm	12-16 mm	280-320 µ	300-480 µ

those of other aphasmid nematodes.[5] Aphasmids lack a pair of sensory papillae on their caudal end. They lack the prominent lateral cords seen in phasmid nematodes. They have a hypodermal band and one genital tract in the female.[5]

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http://www.upei.ca/~avc/index.html

References:

1. Bihr T, Conboy G: Lungworm (*Crenosoma vulpis*) infection in dogs on Prince Edward Island. Can Vet J 40:555-559, 1999
2. Bourque A, Whitney H, Conboy G: *Angiostrongylus vasorum* infection in a coyote (*Canis latrans*) from Newfoundland and Labrador, Canada. J Wildl Dis 41:816-819, 2005
3. Bowman DD, Lynn RC, Eberhard ML: Georgis' Parasitology for Veterinarians, 8th ed., pp. 190-196, 216-222, 229-233. Elsevier Science, St. Louis, MO, 2003
4. Campbell BG: *Trichuris* and other Trichinelloid nematodes of dogs and cats in the United States. Comp Cont Educ Pract Vet 13:769-778, 1991
5. Gardiner CH, Poynton SL: Morphological characteristics of trematodes in tissue section. In: An Atlas of Metazoan Parasites in Animal Tissues. pp. 40-42. Armed Forces Institute of Pathology, Washington, D.C., 2006
6. Hamier AN, Rupprechtt CE: A retrospective histopathologicla survey of capillariasis in raccoons from the eastern United States. J Parasitol 84:180-181, 1998
7. Hanson KB: Tests of the efficacy of single treatments with tracheal brushes in the mechanical removal of lungworms from foxes. J Am Vet Med Assoc 82:12-33, 1933
8. Levine ND: Nematode Parasites of Domestic Animals and of Man, pp. 292, 543. Burgess Publishing Company, Minneapolis, MN, 1968
9. Nevárez A, López A, Conboy G, Ireland W, Sims D: Distribution of *Crenosoma vulpis* and *Eucoleus aerophilus* in the lung of free-ranging red foxes (*Vulpes vulpes*). J Vet Diagn Invest 17:486-489, 2005
10. Songer JG, Post KW: Veterinary Microbiology: Bacterial and Fungal Agents of Animal Disease, pp. 43-53. Elsevier Inc., St. Louis, MO, 2005
11. Soulsby EJL: Helminths, Arthropods and Protozoa of Domesticated Animals, 7th ed., pp. 282-283, 340-341. Lea & Febiger, Philadelphia, PA, 1982

CASE II – NIAH-No.1 (AFIP 3065665).

Signalment: 9-week-old, female, Leghorn chicken, avian

History: In March 2003, extensive swelling or bulbous protrusions of the integument were noticed in the layer chickens of approximately 11 weeks of age, in a commercial flock. The affected chickens had almost no clinical signs other than the tumors and ate, drank, and walked normally. Approximately 80 of 27,000 (0.3%) chickens in the affected flock were slaughtered because of the tumors. Similar tumors had also occurred in the chickens of another farm originating from the same lot of breeder hens.[15] Both affected flocks had been vaccinated by the wing-web method for fowl pox and Marek's diseases and were sprayed with the infectious bronchitis vaccine at hatching.

Gross Pathologic Findings: The tumors were primarily observed in the head or wing, had a maximum diameter of 10 cm, were usually solitary, soft to firm but not hard, and creamy white to a dull red in color and grew slowly. The overlying skin was wounded in some cases, resulting in ulceration.

Laboratory Results: RT-PCR analyses were performed on the tumor tissues of two affected chickens with a primer pair targeted at the gp85 region of the env gene of subgroups A to E avian leukosis/sarcoma virus (ALSV). Tumor samples were PCR positive. Nucleotide sequence analyses indicated that these viruses belonged to subgroup A of ALSV and had 97.5% nucleotide sequence homology with myeloblastosis-associated virus type 1-like strain 1 of Canadian isolate.[17]

Fifteen of 20 serum samples obtained from 19 weeks of age clinically normal chickens from the affected layer farm were strongly reactive against subgroup A of ALSV, and eight of them were also strongly reactive against subgroup B by an enzyme-linked immunosorbent assay (ELISA).[19]

Histopathologic Description: The tumor was unencapsulated and composed of loose areas with abundant mucinous matrix. Stellate or spindle shaped cells were predominant, whereas mitotic figures were uncommon. Short collagen fibers, which are stained blue by the azan stain and Masson trichrome stain, were observed in the matrix. The mucinous matrix was stained positively with alcian blue pH 2.5 and colloidal iron and showed metachromasia by toluidine blue staining: it was negative with alcian blue pH 1.0, high iron diamine, and periodic acid-Schiff reaction. The positive reactions disappeared after

digestion with hyaluronidase. These results demonstrate that the matrix contained hyaluronic acid.

Tumors were specifically stained by a rabbit anti-ALSV serum and two mouse monoclonal antibodies against subgroup A ALSV, and the inner bulb of Herbst corpuscles was strongly stained. Normal cutaneous and subcutaneous tissues of the affected chickens were negative.

Electron microscopy revealed viral particles in the tumor that were 74-97 nm in diameter, had a core (34-46 nm in diameter) and envelopes, and sometimes showed the budding process. Their ultrastructural characteristics were identical with those of type C retroviruses.[8]

Contributor's Morphologic Diagnosis: Skin: Myxoma, Leghorn, chicken, avian

Contributor's Comm ent: Generally, neoplastic diseases other than Marek's disease or lymphoid leukosis are sporadic; however, in the present case, subcutaneous tumors were observed in many layer chickens in a flock. A congenital or genetic disease was suspected at first because a similar disease had occurred in the chickens of another farm originating from the same lot of breeder hens. The results of immunohistochemistry, electron microscopy, RT-PCR analysis, and ELISA indicate that the neoplastic diseases in the present case are associated with subgroup A of ALSV infection. It seems that the virus is exogenous because the ALSV antigen positive area is localized and, in the tumor, it corresponds with the C-type viral particles observed area. Eight of 20 chickens from the affected farm had an antibody to subgroup B; however, it might be a cross-reaction caused by group-specific antigens of ALSV. We conclude that 17 samples had antibodies against subgroup A ALSV. The existence of matrix-inclusion bodies containing ribonucleic acid in the myocardium is further evidence in favor of this viral infection.[3] It is possible that the virus is transmitted vertically from hen to progeny through the egg or horizontally from bird to bird at the breeding site. Chickens inoculated in the wing web with avian sarcoma virus develop sarcomas at the site of inoculation.[5] Epizootic outbreaks of solid tumors have been reported, and the physical transmission of ALSV among newly hatched chickens during the vaccination procedure has been suggested.[6] However, in our case, tumors were observed not only at the site of inoculation, but also in other areas. Further studies are required to elucidate the epizootiology of our case.

The tumor contained large amounts of mucin and involved histologic lesions similar to those previously reported in cases of myxoma in chickens[2, 16] and was therefore diagnosed as myxoma. Myxoma is composed of embryonal connective tissues.[9] Myxoma in the subcutis, spleen, kidney, ovary, and mesentery in chickens has been previously reported.[1, 16, 20] Replication-defective avian retroviruses and Rous sarcoma virus affect mesenchymal cells and cause sarcomas.[12] Various tumors have been reported in chickens inoculated with specific strains of ALSV; for example, strain F-1A of subgroup A has been associated with lymphoid leukosis, erythroblastosis, fibrosarcoma, and hemangioma in inoculated chickens.[7]

Today, it seems that leukosis-free flocks have been established, most commercial flocks consist of genetically resistant lines, the eradication of horizontally transmitted viruses has been accomplished,[12] and, accordingly, there has been a sharp reduction in the incidence of diseases associated with ALSV infection with the exception of subgroup J ALSV.[14,18] However, in our unpublished data, layer flocks frequently have ALSV antigens in Japan, and we are concerned about an outbreak of diseases associated with ALSV. The present epizootic outbreak of neoplastic disease is therefore unusual and worthy of study.

AFIP Diagn osis: Feathered skin: Myxoma, leghorn chicken (*Gallus domesticus*), avian.

Conference Comment : The contributor gives a good overview of the ALSV-induced myxoma in this flock of commercial chickens.

Viruses of the avian leukosis/sarcoma virus (ALSV) group are members of the *Alpharetrovirus* genus of the family Retroviridae. Other species within this genus include the Rous sarcoma virus and other replication defective viruses that carry various oncogenes. ALSVs are divided into 6 subgroups, A-E and J, based on differences in their viral envelope. Viral replication requires a reverse transcriptase that synthesizes a DNA provirus of the RNA virus. This DNA provirus is then integrated into the host cell genome where viral RNAs are transcribed, which are then translated into precursor and mature viral proteins.

In addition to subgroups, strains of ALSV are generally classified according to the predominant neoplasm they produce, such as lymphoid leukosis virus (LLV), avian erythroblastosis virus (AEV), avian myeloblastosis virus (AMV), and avian sarcoma virus (ASV). Although the oncogenic spectrum of the strains are usually characteristic, they can overlap and are affected by viral origin, dose, and route of inoculation, as well as by host age, genotype, and sex.

Retrovirus of Animals[12]

Alpharetrovirus	Avian leukosis viruses, avian carcinoma viruses, avian sarcoma viruses, Rous sarcoma virus, duck spleen necrosis virus
Betaretrovirus	Mouse mammary tumor virus, Jaagsiekte
Gammaretrovirus	Feline leukemia virus, feline sarcoma virus, porcine type C virus, many murine leukemia viruses, many murine sarcoma viruses
Deltaretrovirus	Bovine leukemia virus, human and simian T lymphotropic viruses
Epsilonretrovirus	Walleye dermal sarcoma virus, walleye epidermal hyperplasia viruses
Lentivirus	Human immunodeficiency virus, simian immunodeficiency viruses, maedi/visna virus, caprine arthritis-encephalitis virus, feline immunodeficiency virus, equine infectious anemia virus, bovine immunodeficiency virus
Spumavirus	Bovine, feline, simian, and human foamy viruses

Gross and histologic differential diagnosis for neoplasms and lesions that may be caused by ALSV strains include:

- Lymphoid, erythroid, and myeloid infiltrates

- Marek's disease, lymphoid leukosis virus, and reticuloendotheliosis virus have very similar gross and histologic lesions. They may be differentiated via PCR or serology
- Erythroblastosis – the liver and bone marrow are usually cherry red
- Myeloblastosis – the liver is usually pale red and the bone marrow is whitish, grossly the lesions are similar to lymphoid leukosis

- Myelocytomatosis: Distinctive character and location, is usually nodular and multiple, occurs on the surface of bone in association with the periosteum and near cartilage

- Hemangioma: Wounds, bleeding from feather follicles, hemorrhages, and sarcomas

- Renal tumors: Renal enlargement caused by hematomata, lymphoid leukosis, or accumulation of urates

- Osteopetrosis: Other osteopathies such as rickets, and osteoporosis

Connective tissue tumors: Granulomas, tuberculosis, pullorum disease

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References:

1. Campbell JE, Appleby EC: Tumours in young chickens bred for rapid body growth (broiler chickens): a study of 351 cases. J Pathol Bacteriol 92:77-90, 1966
2. Fadly AM, Payne LN: Leukosis/sarcoma group. In: Diseases of Poultry, eds. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, 11th ed., pp. 465-505. Iowa State Press, Ames, IA, 2003
3. Gilka F, Spencer JL: Viral matrix inclusion bodies in myocardium of lymphoid leukosis virus-infected chickens. Am J Vet Res 46:1953-1960, 1985
4. Ginn PE, Mansell JEKL, Rakich PM: Skin and appendages. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, p. 762. Elsevier Limited, St. Louis, MO, 2007
5. Halpern MS, Ewert DL, Flores LJ, Fujita DJ, Aldrich CE, Mason WS: Sarcoma growth in 15I5372 chickens infected with avian sarcoma viruses of subgroup B or G. Virology 134:472-476, 1984
6. Henderson BE, Gardner MB, Charman HP, Johnson EY, Rucio T, Sarma P, Alena B, Huebner RJ: Investigation of an increase of solid tumors in chickens vaccinated against Marek's disease. Avian Dis 18:58-66, 1974
7. Hihara H, Yamamoto H, Arai K, Yoshino T, Shimizu T: Oncogenicity of three strains of avian leukosis virus in white leghorn chicks of BK line. Natl Inst Anim Health Q (Tokyo) 20:87-95, 1980
8. Jawetz E, Melnick JL, Adelberg EA: Tumor viruses. In: Review of Medical Microbiology, 16th ed., pp. 488-498. Maruzen Asia Ltd., Singapore, 1984
9. Jones TC, Hunt RD, King NW: The skin and its appendages. In: Veterinary Pathology, 6th ed., pp 817-871.

Williams and Wilkins, Baltimore, 1997
10. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, p. 51. Elsevier Limited, St. Louis, MO, 2007
11. Goldschmidt MH, Hendrick MJ: Tumors of the skin and soft tissues. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 91-92. Blackwell Publishing, Ames, IA, 2002
12. Murphy FA, Gibbus EPJ, Horzinek MC, Studdert MJ: Retroviridae. In: Veterinary Virology, 3rd ed., pp. 363-389, Academic Press, San Diego, 1999
13. Nakamura K, Higahi T, Yamada M, Imai K, Yamamoto Y: Basophilic intracytoplasmic viral matrix inclusions distributed widely in layer hens affected with avian-leukosis-virus-associated tumours. Avian Pathol 36:53-58, 2007
14. Nakamura K, Ogiso M, Tsukamoto K, Hamazaki N, Hihara H, Yuasa N: Lesions of bone and subcutaneous tumors in chickens 945 bone marrow in myeloid leukosis occurring naturally in adult broiler breeders. Avian Dis 44:215-221, 2000
15. Ono M, Tsukamoto K, Tanimura N, Haritani M, Kimura KM, Suzuki G, Okuda Y, Sato S: An epizootic of subcutaneous tumors associated with subgroup A avian leukosis/sarcoma virus in young layer chickens. Avian Dis 48:940-6, 2004
16. Reece RL: Some observations on naturally occurring neoplasms of domestic fowls in the State of Victoria, Australia (1977-87). Avian Pathol 25:407-447, 1996
17. Spencer JL, Benkel B, Chan M, Nadin-Davis S: Evidence for virus closely related to avian myeloblastosis-associated virus type 1 in a commercial stock of chickens. Avian Pathol 32:383-390, 2003
18. Takami, S, Goryo M, Masegi T, Okada K: Histopathological characteristics of spindle-cell proliferative disease in broiler chickens and its experimental reproduction in specific pathogen-free chickens. J Vet Med Sci 66:231-235, 2004
19. Tsukamoto K, Kono Y, Arai K, Kitahara H, Takahashi K: An enzyme-linked immunosorbent assay for detection of antibodies to exogenous avian leukosis virus. Avian Dis 29:1118-1129, 1985
20. West JL: An avian splenic myxoma. Avian Dis 18:139-141, 1974

CASE III – 01-5058, 01-5257, 01-5262 (AFIP 3069576).

Signalment: Three young adult (3-year-old), male, squirrel monkeys (*Saimiri sciureus*)

History: Over the course of 8 days, three monkeys presented to the veterinary service center with severe respiratory distress. The animals were sedated for examination and blood collection. Auscultation revealed severe inspiratory strider but normal sounding lung fields. The larynx appeared abnormal in all three animals but was difficult to completely visualize. One animal died following intubation and the other 2 were euthanized the following day due to poor prognosis.

Gross Pa thology: Lesions were remarkably similar in all three animals. There was unilateral firm thickening of the left side of the larynx in 2 animals and bilateral swelling in 1. Lungs were normal.

LABORATORY RESULTS (clinical pathology, microbiology, PCR, ELISA, etc.)**:**
CBC abnormal findings – elevated WBC (11.7-13.3 K/ml, normal ref range 7.9 +/- 2.8 SD) due to increases in neutrophil counts were noted in all 3 animals.

Clinical chemistry abnormal findings – elevated CPK in all three animals (2928, 5038, and 7752 IU/L, normal CPK ref ranges 562 +/- 1379.8 IU/L).

Cultures were obtained from the laryngeal lesions from 2 of the 3 animals. A pure culture of *Bordetella bronchiseptica* was obtained from both.

Histopathologic D escription: Tissues from all three monkeys contained similar lesions. The normal histoarchitecture of the larynx is markedly altered by a necrotizing inflammatory process that expands the submuscosa and dissects between laryngeal muscle fibers. The inflammation, which consists almost exclusively of viable and degenerate neutrophils within a background of granular eosinophilic and basophilic matrix (fibrinous exudate, necrobiosis, and mucinous degeneration), widely separates and isolates muscle fibers between the intrinsic and extrinsic laryngeal cartilages. Myofiber necrosis in this region is prominent, while extrinsic laryngeal musculature is less severely affected. Multifocally, the overlying mucosa is ulcerated, partially covered by suppurative exudate, and contains expanded pockets of degenerate neutrophils and fibrin. Mucous glands are also disrupted and entrapped within the inflammation. A Gram stain revealed very low numbers of Gram negative coccobacilli, a few of which exhibited filamentous morphology.

Contributor's Morpho logic Diag noses: Larynx: Laryngitis, necrosuppurative, severe, transmural, chronic, with necrotizing myositis and intralesional Gram nega-

tive bacteria.

Contributor's Comme nt: *Bordetella bronchiseptica* can colonize and cause disease in a wide range of mammals and is associated with acute tracheobronchitis in dogs (kennel cough) and cats, atrophic rhinitis in swine, snuffles in rabbits and experimentally can produce acute pneumonias in rats.[1] Members of the *Bordetella* genus include *B. bronchiseptica*, *B. pertussis*, *B. parapertussis*$_{hu}$ and *B. parapertussis*$_{ov}$ and all possess several virulence factors including filamentous hemagglutinin (FHA), fimbriae, pertactin, LPS, dermonecrotic toxin (DNT), tracheal cytotoxin (TCT), and others, but only *B. pertussis* has pertussis toxin.[3] These factors contribute to the organisms' ability to colonize respiratory epithelium, but may also contribute to cellular damage and immune regulation. These squirrel monkey cases represent a severe and very interesting manifestation of infection with this agent. Although isolation of this organism from the laryngeal lesions of these three squirrel monkeys does not definitively prove that it was the inciting cause, the lesions are consistent with those that could be produced by a highly pathogenic organism possessing such potent virulence factors.

It is also interesting that all three squirrel monkeys presented with such similar lesions within a relatively short period of time. These monkeys were housed off site in a structure with large garage-like doors that could be opened in warm weather, yet when closed, still had space above and below that would allow access to birds, insects, rodents, and possibly wind blown sticks and leaves. The similarity in age, gender (all young males) and housing of these three animals suggest that an environmental and/or behavioral component may have contributed to their susceptibility. Because many mammals can carry *B. bronchiseptica* in their upper respiratory tracts yet remain asymptomatic, we cannot definitively prove that this organism was the cause of the severe laryngeal lesions. However, pure cultures directly isolated from the lesions of all three animals are supportive that this organism was directly responsible.

Other causes of laryngitis or laryngeal lesions in animals include oral necrobacillosis (calf diphtheria) due to *Fusobacterium necrophorum*, or laryngeal ulcers often seen in feed lot cattle.[2]

AFIP Diagnosis: Larynx: Laryngitis, necrosuppurative, subacute, focally extensive, severe, with multifocal muscle degeneration, necrosis, hemorrhage, and ulceration, squirrel monkey (*Saimiri sciureus*), primate.

Conference Com ment: *Bordetella* spp. are aerobic, non-fermentative, gram-negative coccobacilli. There are six identified species with three of veterinary importance (see table below).

B. bronchiseptica has several virulence factors that promote colonization and that enable the bacterium to escape destruction in the host. Attachment virulence factors include fimbriae, and two non-fimbrial outer membrane proteins (filamentous hemagglutinin and pertactin). Replication is enhanced by production of hydroxamate siderophores and binding proteins that mobilize iron from transferrin, lactoferrin, and heme. Factors that allow escape from destruction include:[1]

1. Adenylate cyclase toxin/hemolysin (also called cyclosin)
    a. Hemolysin binds to the host cell and facilitates entry of the adenylate cyclase domain.
    b. Adenylate cyclase toxin causes an increase of cAMP intracellularly, which inhibits the respiratory burst of macrophages and prevents phagocytic activity of heterophils.

2. Dermonecrotic toxin (DNT) - Intracellular bacterial toxin released upon lysis of the bacteria; inhibits the Na/K ATPase pump and causes vasoconstriction

3. Lipopolysaccharide - Pyrogenic and mitogenic; causes macrophage chemotaxis and activation; induction of tumor necrosis factor production

4. Tracheal cytotoxin – stimulates nitric oxide production and interferes with mucociliary function

5. Type III secretion products – undefined products; inactivate transcription factor NF-κB and modulate effects on

Bordetella spp. of veterinary importance:[1]

B. bronchiseptica	Infectious tracheobronchitis (kennel cough) in dogs; atrophic rhinitis in pigs
B. avium	Coryza in turkeys
B. hinzii	Commensal in respiratory tract of chickens; opportunistic infections in humans

host immune response

B. bronchiseptica infections are often seen in conjunction with other bacterial or viral coinfections. It is generally considered the primary cause of kennel cough in dogs, but canine parainfluenza virus 2, canine adenovirus 2, canine distemper virus, and *Mycoplasma* spp. have been known to have predisposing roles.[2] Atrophic rhinitis complex generally includes *B. bronchiseptica*, *Pasteurella multocida*, *Haemophilus parasuis*, and viral infections including porcine cytomegalovirus.[4] *B. bronchiseptica* actively promotes colonization of the nasal cavity by *P. multocida* which in turn produces cytotoxins that inhibit osteoblastic activity and promote osteoclastic reabsorption.[4]

Some sections submitted by the contributor included an adjacent lymph node with multifocal sinus histiocytosis and erythrophagocytosis, interpreted as draining hemorrhage.

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References:

1. Bemis DA, Shek WR, Clifford CB: *Bordetella bronchiseptica* infection of rats and mice. Comp Med 53:11-20, 2003
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 638-639. Elsevier Limited, St. Louis, MO, 2007
3. Cotter PA, DiRita VJ: Bacterial virulence gene regulation: an evolutionary perspective. Annu Rev Microbiol 54:519-565, 2000
4. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 481-483, 491, 542. Elsevier, St. Louis, MO, 2007
5. Mann PB, Wolfe D, Latz E, Golenbock D, Preston A, Harvill ET: Comparative Toll-like receptor 4-mediated innate host defense to *Bordetella* infection. Infect Immun 73:8144-8152, 2005
6. Pilione MR, Harvill ET: The *Bordetella bronchiseptica* type III secretion system inhibits gamma interferon production that is required for efficient antibody-mediated bacterial clearance. Infect Immun 74:1043-1049, 2006

CASE IV - Case 1 (AFIP 3073874).

Signalment: 38-week-old, male, Sprague Dawley rat, *Rattus norvegicus*

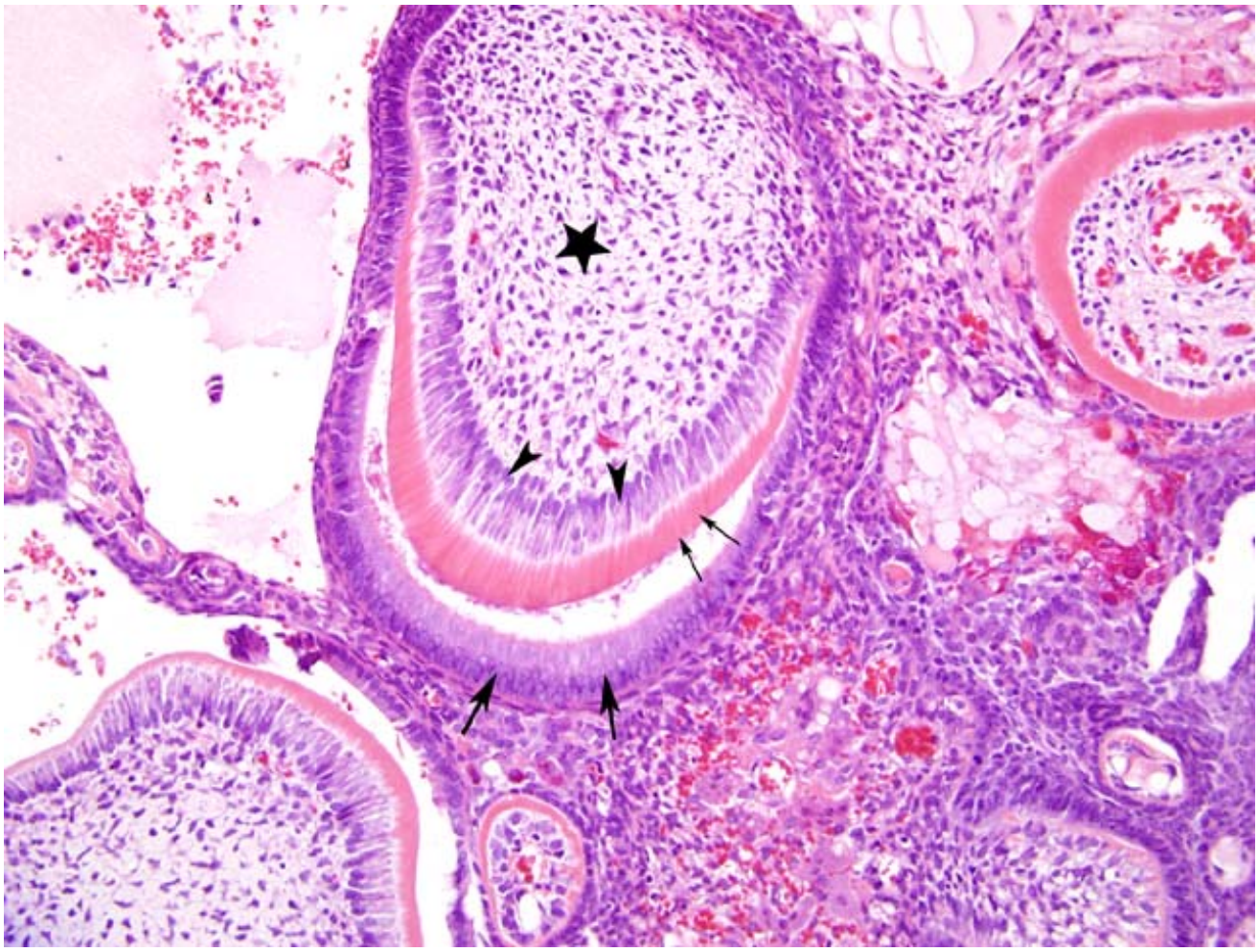
History: The rat was euthanized at week 38 of a chronic toxicity study due to a large mass involving the left maxillary region.

Gross Pathologic Findings: A red, firm mass was noted that involved the entire left maxillary region. It was 35 X 25 X 20 mm. The mass appeared the same on the cut surface.

Histopathologic D escription: The normal bony architecture of the maxilla is largely replaced by a poorly circumscribed **mass (4 -1)** that is predominantly composed of epithelial and mesenchymal elements that frequently form variably-sized and shaped tooth-like structures. The epithelial elements of the mass include ameloblasts and odontoblasts that are generally well-differentiated; these cells often palisade along eosinophilic extracellular material with fine, tubular cavities (dentin) or lesser amounts of a more basophilic, hyaline material (enamel). Spindled to stellate mesenchymal cells with an accompanying vascular component (dental pulp) are often present centrally within the abortive tooth-like structures. Multifocally within the mass, odontogenic cells occasionally form cords and nests that are not associated with dental hard substance; these structures sometimes have central cavitations that contain degenerating cells and necrotic debris. There are multifocal areas of necrosis, inflammation, hemorrhage, pigment, and new bone formation within or at the periphery of the mass.

Contributor's Morphologic Diagnosis: Odontoma

Contributor's Comment: An odontoma is a dental neoplasm (or hamartoma) in which cellular maturation and differentiation have progressed to the stage of development of both enamel and dentin. Two types are recognized: complex odontoma and compound odontoma. Complex odontomas contain dental pulp, mesenchymal cells, and hard tissue elements, but there is poor differentiation of the cellular components such that the mass has little resemblance to normal tooth architecture. Compound odontomas have a higher degree of cellular differentiation, and their hard tissue elements resemble abnormally shaped tooth-like structures (denticles). The hard tissue generally appears as dentin (an acellular, smooth, eosinophilic material) with smaller amounts of cementum (resembling bone). Enamel may be completely removed upon decalcification, in which case it will appear as a clear space or cleft in close apposition to the dentin. In-



4-1 Maxilla, Sprague-Dawley rat. The neoplasm is composed of both mesenchymal and epithelial components which recapitulates dental structures. Note the mesenchymal population of spindle to stellate cells resembling dental pulp (star), the regimented layer of odontoblasts (arrowheads) which are bordered by dentin (small arrows) and the peripheral layer of ameloblasts (large arrows). (H&E 200X)

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completely decalcified specimens may have a small amount of enamel that stains basophilic with hematoxylin and eosin.[9] Complex odontomas are rare in all species but are most commonly seen in young horses and young dogs. Compound odontomas generally present as mass-like lesions of the jaw of young canines.[7] Both types of odontomas have been described in the rat.[9,3] In those species such as the rat where incisors grow continuously throughout the animal's lifetime, odontoma must be distinguished from dysplasia or a malformation that is congenital or secondary to malocclusion or fracture of the incisor tooth.[3]

Odontomas and other odontogenic tumors have been experimentally induced in rats by carcinogens such as methylnitrosourea and related compounds.[4]

AFIP Diagnosis: Bone, maxilla: Compound odontoma, Sprague-Dawley rat (*Rattus norvegicus*), rodent.

Conference Comment : Currently there is controversy on the classification of odontomas as either neoplasms[7] or non-neoplastic hamartomas.[6] Odontomas are tumors in which there is a combination of both odontogenic epithelial components and dental matrix structures such as dentin and enamel. The inductive theory of odontogenesis states that the ameloblastic epithelium promotes the surrounding mesenchymal cells to become odontoblasts. These odontoblasts produce dentin, which is necessary for the ameloblasts to form enamel. Neoplasms

composed of only epithelium without hard tissues are termed ameloblastomas.[8]

Odontomas can be classified into various types based on their components and organization:

- Complex odontoma[2] – Contains well differentiated dental tissues, including dentin, enamel matrix, odontogenic epithelium, and cementum (horses and rodents) that do not form tooth-like structures
- Compound odontoma[2] – Contains cords of odontogenic epithelium, with intermittent complete odontogenesis forming tooth-like structures (denticles). Occasionally there is bone matrix formation surrounding or adjacent to the denticles.
- Odontoameloblastoma[2] – Contain areas of ameloblastic epithelium that are separate from other areas of complex or compound odontomas.
- Ameloblastic fibro-odontoma[2] – Contain both dental epithelial tissues (resembling dental lamina) and mesenchymal tissues (resembling dental pulp) that are associated with enamel and dentin.
- Dentinoma[3] – Contain odontoblasts producing a calcified dentin tissue with no evidence of enamel formation.

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References:

1. Boy SC, Steenkamp G: Odontoma-like tumours of squirrel elodont incisors-elodontomas. J Comp Pathol 135:56-61, 2006
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 26-27. Elsevier Limited, St. Louis, MO, 2007
3. Brown HG, Hardisty JF: Oral cavity, esophagus, and stomach. In: Pathology of the Fischer Rat, eds. Boorman GA, Eustis SL, Elwell MR, Montgomery CA, Jr., MacKenzie WF, pp. 18-19. Academic Press, San Diego, CA, 1990
4. Cullen JM, Ruebner BH, Hsieh DP, Burkes EJ: Odontogenic tumors in Fischer rats. J Oral Pathol 16:469-473, 1987
5. Gelbery HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 314. Elsevier, St. Louis, MO, 2007
6. Head KW, Cullen JM, Dubielzig RR, Else RW, Misdorp W, Patnaik AK, Tateyama S, van der Gaag I: Histological classification of tumors of the alimentary system of domestic animals. In: World Health Organization Histological Classification of Tumors of Domestic Animals, 2nd series, volume X, p. 52, Armed Forces Institute of Pathology, Washington DC, 2003
7. Head KW, Else RW, Dubielzig RR: Tumors of the alimentary tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 406-407, Iowa State Press, Ames, IA, 2002
8. Jang DD, Kim CK, Ahn B, Kang JS, Nam KT, Kim DJ, Han DU, Jung K, Chung HK, Ha SK, Choi C, Cho WS, Kim K, Chae C: Spontaneous complex odontoma in a Sprague-Dawley rat. J Vet Med Sci 64:289-291, 2002
9. Long PH, Leininger JR, Nold JB, Lieuallen WG: Proliferative lesions of bone, cartilage, tooth, and synovium in rats. MST-2 In: Guides for Toxicologic Pathology, STP/ARP/AFIP, Washington, D.C., 1993

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 10

5 December 2007

Moderator:

Dr. Don Schlafer, DVM, DACVP, DCVM, DACT, PhD

CASE I – A02-388X (AFIP 3038528).

Signalment: 31-year-old, female, Rhesus macaque (*Macaca mulatta*)

History: Used in an aging study examining neuropathological and behavioral changes related to age. Euthanized at end of study.

Gross Pathology: The right ovary is enlarged (5 x 6 cm) with multiple, large multiloculated cysts, the largest measuring approximately 3.5 to 4cm in diameter. On cut section, the cysts are demarcated by thin walls and contain an orange to red, proteinaceous material. Vagina, cervix and uterus are normal. The left ovary is twice enlarged and contains a solid mass.

Histopathologic Description: The right ovary contains two distinct tumors (a cystadenoma and a granulosa cell tumor) that replace the normal ovarian tissue. The cystadenoma is composed of numerous large cysts filled with eosinophilic homogenous material (proteinaceous fluid), lined by flat cuboidal epithelium with small basophilic nuclei, minimal eosinophilic cytoplasm, separated by and growing along dense collagenous connective tissue. The neoplastic cells form papillary projections into the cysts and in the surrounding interstitium. Mitoses are rare. The granulosa cell tumor is composed of a population of neoplastic cells arranged in stratified layers of polygonal cells with small basophilic nuclei and scant cytoplasm lining spaces with flocculent amphophilic material and growing along a fibrovascular stroma. This population of cells occasionally forms rings of pallisading cells at the center of which is deeply eosinophilic material **(Call-Exner bodies) (fig. 1-1)**. Nests and packets of these cells infiltrate the thin rim of dense ovarian stromal tissue at the periphery. Primary follicles are rare consistent with the monkey's age. Deeply basophilic irregular material (mineral) is multifocally present. Mitoses are rare.

Contributor's Morphologic Diagnosis: Ovary: Papillary serous cystadenoma and Granulosa cell tumor, macrofollicular (coincident in the same ovary)

Contributor's Comment: Ovarian masses can be separated into cysts and neoplasms. Cysts within the ovary are identified by dilatations not involving gonadal or stromal tissue. Serous inclusion cysts have been reported in the bitch and cystic rete tubules have been reported in the bitch and queen. Cysts involving the gonadal stroma are typically cycle dependent and include: cystic graffian follicles, follicular cysts, anovulatory luteinized cysts, and cystic corpus luteum. These are frequently associated with cows and sows.[1] In humans, cystic follicles are so frequent they can be considered physiologic originating from graffian follicles.[2]

Ovarian neoplasms account for 6% of all cancers in the female and are the fifth most common form of cancer in women in the United States (excluding skin cancer). Due to the difficult rate of early detection, these neoplasms are responsible for almost half of the deaths from cancer of the female genital tract. There are numerous types of ovarian tumors, both benign and malignant. About 80% are benign, and these occur mostly in young women between the ages of 20 and 45 years. The malignant tumors are more common in older women between the ages of 40 and 65 years.[2]

Ovarian neoplasms are generally broken into four categories—germ cell, sex cord stromal, surface epithelial, and mesenchymal. A summary of diagnostic criteria can be found in the *Histological Classification of Tumors of the Genital System of Domestic Animals, AFIP Second Series, Volume VI.*[3]

We classified this ovary as having a component of a granulosa cell tumor **(fig. 1-1)** based on its morphologic appearance of cells containing spherical-to-oval, hyperchromatic nuclei, distinct nucleoli, and scant eosinophilic cytoplasm. The Call-Exner bodies supported this diagnosis. The other cystic component of this ovary is composed of infoldings and papillary projections of subsurface small cuboidal epithelium, scant connective tissue stroma, and rare mitoses consistent with papillary serous cystadenoma. Immunohistochemistry for the cystic tissue was strongly positive for cytokeratin, confirming its **epithelial origin (fig. 1-4)**. The granulosa cell component was positive for **inhibin (fig. 1-2)**.

Dual ovarian tumors in non-human primates have not been reported.[5] Dual tumors in ovaries have been reported in humans, but are generally rare.[6,7] The pathogenesis for dual tumors is unknown, but postulated theories include: collision neoplasm, in which two tumors develop spontaneously; a heterologous differentiation within a granulosa cell tumor; or a teratomatous neoplasm with a bidirectional differentiation. Recently, a

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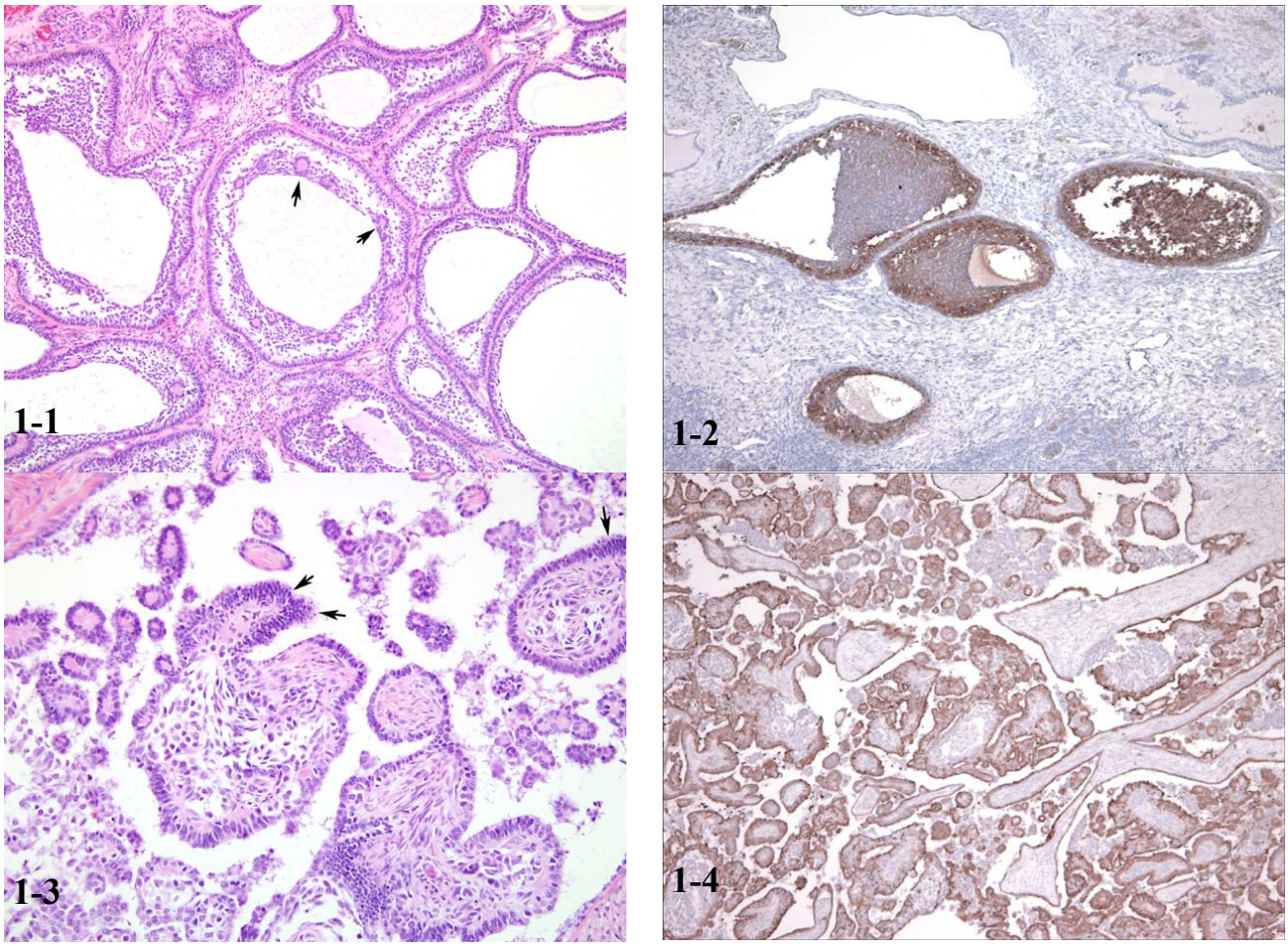
Table 1-1. Key ovarian neoplasms documented in veterinary species. (Courtesy New England Primate Research Center, Harvard Medical School, Division of Comparative Pathology, Southborough, MA, 01772)

Equine	Granulosa cell tumor (almost always unilateral, slow growing, and benign; elevated inhibin in 90%) Cystadenoma—most common tumor of surface epithelium
Bovine	Granulosa cell tumor
Feline	Malignant granulosa cell tumor
Canine	Papillary cystadenocarcinoma, malignant granulosa cell tumor; malignant teratoma
Murine	typically irradiation induced, all types; spontaneous cystadenoma and granulosa cell tumors have been reported.
Rat	Osborne-Mende strain 330, 33% of rats > 18 months develop granulosa cell tumors; Sprague Dawley predisposed to a variety of histological subtypes
NHP	Granulosa cell tumor, teratoma, and cystadenocarcinoma have been reported in baboons, recently choriocarcinomas have been reported in macaques
Ferret	Sex cord stromal resulting in alopecia (Comp Med 2003)
Fish	Ovarian carcinoma in a koi carp (Aus Vet J, 2006)
Poultry	Adenocarcinoma of turkeys and chickens in intensive-laying conditions. Cornell has C strain genetically predisposed to epithelial cancer
Snake	Granulosa cell tumor (especially garter snakes)
Lizard	Teratomas (especially iguanas)



1-1. Ovary, Rhesus macaque. The granulosa cell tumor is composed of stratified trabeculae of polygonal cells as well as rings of pallisading neoplastic cells surrounding small cores of eosinophilic, acellular, homogeneous material (Call-Exner bodies) (arows) (H&E 200X)
1-2. Ovary, Rhesus macaque. The neoplastic cells of the granulosa cell tumor are multifocally immuno-positivite for inhibin. (Inhibin 40X)
Photomicrograph courtesy of the New England Primate Research Center, Harvard Medical School, Division of Comparative Pathology, Southborough, MA 01772
1-3. Ovary, Rhesus macaque. The cystadenocarcinoma displays features of low grade malignancy to include karyotypic polymorphism as well as piling of the neoplastic cells (arrows) (H&E 200X)
1-4. Ovary, Rhesus macaque. Cystadenocarcinoma. The epithelial component is immunoreactive for cytokeratin (cytokeratin 40X). Photomicrograph courtesy of the New England Primate Research Center, Harvard Medical School, Division of Comparative Pathology, Southborough, MA 01772

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case of mucinous cystadenoma and granulosa cell tumor was reported in a 57-year-old woman.[7] The immunohistochemical profile of this tumor demonstrated diffuse positive CK7 and focal weak CK20 within the mucinous component. The granulosa cell component was strongly alpha-inhibin positive and diffuse calretinin positive while being negative for epithelial membrane antigen (EMA) and anticytokeratin antibody AE1/3.

Mixed ovarian tumors in the veterinary literature are also extremely rare. A brief literature review shows key ovarian neoplasms documented in veterinary species (Table 1-1):

For more information, the reader is directed to previous ovarian neoplasm submissions to AFIP as well as the

following websites:

- http://radiology.uchc.edu/eAtlas/nav/msOvary.htm
- http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=16309432&dopt=Abstract

AFIP Diagnosis: 1. Ovary: Papillary serous cystadenocarcinoma, Rhesus macaque (*Macaca mulatta*), primate. 2. Ovary: Granulosa cell tumor.

Conference Comment: Granulosa cell tumors are the most common ovarian tumor in large animals. They are generally benign in the cow and horse but are often malignant in dogs and cats.[1] Sex cord-stromal tumors may be hormonally active and produce varying amounts of progesterone, estrogen, testosterone, and inhibin.[4] Anestrus, nymphomania, or stallion-like behavior are often seen in the mare, while the bitch may develop prolonged estrus or pyometra.

Epithelial tumors of the ovary generally arise from the surface epithelium, rete ovarii, and from the subsurface epithelial structures (SES) of the bitch. The bitch is unique in that the canine is the only domestic animal to contain SES, resulting in tumors of the ovary being common only in the bitch. This case was studied in consultation with pathologists in the Department of Gynecologic and Breast Pathology of the Armed Forces Institute of Pathology who agree that the epithelial portion of the neoplasm exhibited areas of low-grade carcinoma **(fig. 1-3).** Multifocally neoplastic epithelial cells exhibited moderate cellular atypia and pile up to 5 cell layers thick. Features of malignancy without evidence of metastasis or vascular invasion include a larger size, necrosis, hemorrhage, cellular atypia, piling up of neoplastic cells, increased mitotic index, and stromal invasion.[4]

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References:

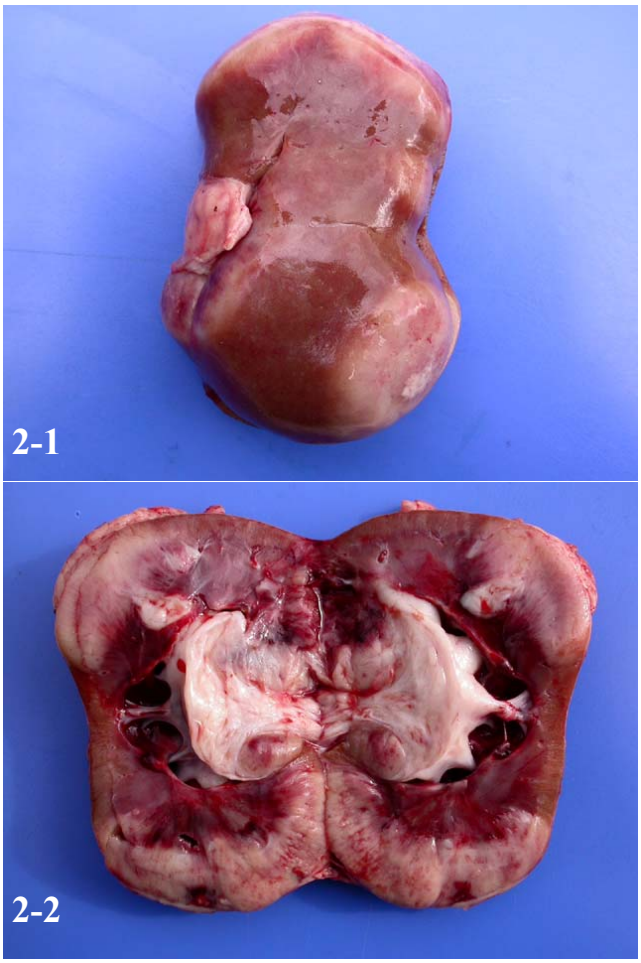
1. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1280-1281. Elsevier, St. Louis, MO, 2007
2. Crum CP: The female genital tract, ovarian tumors. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 1093-1104. Elsevier Saunders, Philadelphia, PA, 2005
3. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielson S: Histological Classification of Tumors of the Genital System of Domestic Animals, vol. 4, pp. 24-25, 56-63. Armed Forces Institute of Pathology, Washington, D.C., 1998
4. Maclachlan NJ, Kennedy PC: Tumors of the genital systems. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 547-557. Blackwell Publishing, Ames, IA, 2002
5. Moore CM, Hubbard GB, Leland MM, Dunn BG, Best RG: Spontaneous ovarian tumors in twelve baboons: a review of ovarian neoplasms in non-human primates. J Med Primatol 32:48-56, 2003
6. Moid FY, Jones RV: Granulosa cell tumor and mucinous cystadenoma arising in a mature cystic teratoma of the ovary: a unique case report and review of literature.

Classification of ovarian tumors[3]

Sex cord-stromal (gonadostromal) tumors	• Granulosa cell tumor (granulose-theca cell tumor) • Thecoma (theca cell tumor) • Interstitial cell tumor (luteoma, lipid cell tumor, steroid cell tumor)
Germ cell tumors	• Dysgerminoma • Teratoma • Embryonal carcinoma
Epithelial tumors	• Papillary adenoma, papillary cystadenoma • Papillary adenocarcinoma • Rete adenoma
Mesenchymal tumors	• Hemangioma • Leiomyoma

2-1. Kidney, Labrador Retriever. Grossly the kidney is enlarged with multiple, randomly distributed masses elevating the capsule.
2-2. Kidney, Labrador Retriever. On cut surface, the renal parenchyma is expanded and disrupted by 3 coalescing masses replacing the cortex, medulla, pelvis and ureter.

Photographs courtesy of Claudio S.L. Barros of the Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, 97105-900, RS, Brazil



Ann Diag Path 8:96-101, 2004
7. McKenna M, Kenny B, Dorman G, McCluggage WG: Combined adult granulosa cell tumor and mucinous cystadenoma of the ovary: granulosa cell tumor with heterologous mucinous elements. Inter J Gyne Path 24:224-227, 2005
8. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 450-453. Elsevier Limited, St. Louis, MO, 2007

CASE II – UFSM-2 (AFIP 2992242).

Signalment: 5-year-old, male, Labrador retriever, canine

History: On June 5, 2004 the dog was presented with a serosanguineous discharge form the prepuce. It was reported by the owner that these signs had started a week ago. On physical examination, a large mass (10 cm in diameter) could be palpated in the prepuce. The owner reported that the growth was noticed two years ago. Extrusion of the penis revealed an 8x10 cm lobulated and verrucous mass involving the caudal aspects of the penile shaft. On cytologic examination of samples obtained of fine needle aspiration the mass, a diagnosis of transmissible venereal tumor (TVT) was made. The dog was placed on chemotherapy (vincristine, 4 weekly injections of 0.5 mg/m^2 body weight). The tumor had regressed somewhat but an enlargement was noticed in the inguinal lymph node one month after the start of the therapy. The penis was amputated and the lymph node excised. At the histopathological examination TVT was confirmed as the primary tumor and metastatic TVT was diagnosed in the lymph node. The dog was sent home with no further treatment. Two months later it returned to the Veterinary Teaching Hospital for check-up when "a mass in the abdominal cavity" was palpated. An ultrasound of the abdominal cavity revealed an enlarged left kidney which was excised and sent to the pathology laboratory. The dog's condition seemed to have somewhat improved and it was sent home again. A couple of weeks after surgery the dog started to progressively loose weight and after two months it was euthanatized. The owner did not permit a necropsy.

Gross Pathologic Findings: The surgical specimen was that of an enlarged kidney (12.0x7.0x5.5 cm) which presented large multifocal white masses protruding from the **capsular surface (fig. 2-1)**. At cut surface the pelvis and calices were marked dilated and large amounts of clear fluid oozed from the pelvis. Three large homogenous white **coalescing masses (fig. 2-2)** involved cortex, medulla pelvis and ureter. In some places sparse hemorrhagic and necrotic foci could be observed in the neoplastic mass.

Laboratory Results: Cytology performed in sample collected by fine needle aspiration of the penile tumor revealed clusters of round cells with high nuleus: cyto-

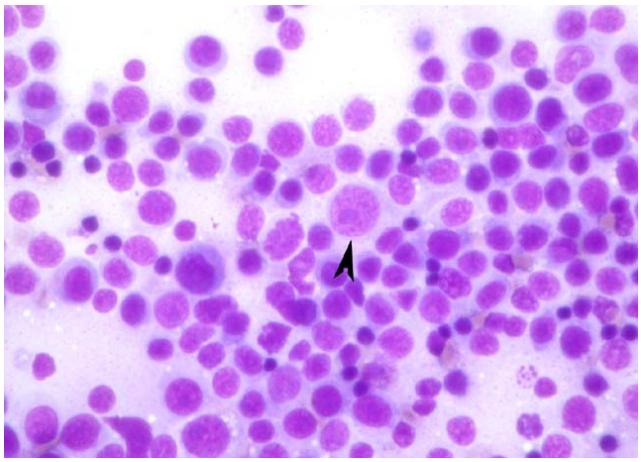
plasm ratio and moderate anisocytosis. The nucleus of these cells was round or oval and formed by loose chromatin with one irregularly shaped conspicuous nucleolus. The cytoplasm of these cells was scant, slightly basophilic, and occasionally revealed multiple small **vacuoles (fig. 2-3)**. There were multiples cells in mitosis, occasionally binucleated cells and moderate numbers of small lymphocytes.

Contributor's Morphologic Diagnosis: Kidney, metastatic transmissible venereal tumor, 5-year-old, male, Labrador retriever, canine

Contributor's Comment: Canine transmissible venereal tumor (TVT), also referred to as Sticker tumor, infectious sarcoma, venereal granuloma, canine condyloma, transmissible lymphosarcoma, transmissible tumor of reticular cells, transmissible histiocytoma and hemoblastoma was described in the 19th century and reportedly is one of the major canine diseases in underdeveloped countries, mainly in those with temperate, tropical or subtropical climates.[13] The tumor affects exclusively dogs and is considered always potentially malign;[12] the cell of origin of the tumor is still unknown, although iummunohistochemical studies support an histiocytic lineage.[9] Attempts on viral isolation from TVT tissues have consistently failed in the past; more recently viral particles have been isolated from TVT fragments but the inoculation of theses particles in dogs failed to reproduced the disease;[8] despite of these negative findings, the viral etiology is favored by some researchers.

In one study, the neutering of bitches affected by the neoplasm resulted in rapid regression of TVT suggesting that the tumor is somewhat hormone-dependant. Additionally, TVTs reportedly tend to be benign in males and frequently metastatizing in females.[5,8,12] While the number of chromosomes in somatic canine cells is 76, the number of chromosomes in the cells of TVT is consistently 58. Such a disparity led, in the past, to the belief that the neoplasm had been acquired from another animal species. The analysis of the MHC molecules on cells of TVT from dogs of different geographic origins revealed that all have the same surface antigens.[8]

The transmission of TVT occurs by allogenic transplantation of viable neoplastic cells from an affected dog to a susceptible one, normally during copulation; however other means of transmission are possible and include licking, biting, and scratching.[5,8,12] The neoplasms affect the external genitalia (penis and vagina) and the skin adjacent to these areas.[5,8,13] Less commonly affected sites include nasal cavity, eyes, lips and other skin regions.[8,12]



2-3. Kidney, Labrador Retriever. Cytological preparation, fine needle aspirate. Neoplastic cells have a small amount of palely basophilic cytoplasm which is microvacuolated. (arrowhead). Photomicrograph courtesy of Claudio S.L. Barros of the Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, 97105-900, RS, Brazil

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Dogs of both sexes and all ages are affected but the disease is more prevalent in sexually active dog (average age 4-5-year-old) living in areas with large populations of stray dogs. [12,13]

Gross aspects of TVT are variable but most are either firm or friable verrucous papillary or nodular masses protruding from the surface of penis or vulva.[5] The tumors can be small single nodules or multilobulated masses up to 15 cm in diameter.[5] The surface of these neoplasms are smooth, granular and commonly ulcerated from where bleeding is frequent.[8]

Histologically, TVTs consist of round to oval cells which are closely similar to macrophages and are arranged in ribbons or pallisades; their nuclei are large, round, centrally located in the cell displaying clusters of chromatin and a single prominent, centrally located nucleolus. The cytoplasm of TVT cells is moderate in amount, faintly basophilic and vacuolated. Mitotic index is high and the tumor parenchyma is infiltrated by variable numbers of lymphocytes, plasm cells and macrophages. In regressing TVTs, inflammation, necrosis and fibrosis are frequently seen. [5,8]

The prognosis for TVTs is guarded as these tumors are self limiting in most of the cases,[8] but not uncommonly metastasize to regional lymphnodes, spleen, liver, kidney, peritoneum, lungs and central nervous system.[8,12] Furthermore, surgical excision only results in recurrence

which in some reports is close to 60% of the cases.[12] In a study carried out in a dog colony, the neoplasm was transmitted through 40 generations in a total number of 564 dogs, 68% of which developed the disease and 87% had spontaneous regression of the tumor within 180 days. When dogs are submitted to specific chemotherapy, the prognosis is good in the majority of cases. Dogs recovering from this neoplasm acquire cellular and humoral immunity.[1]

In the case of this report, necropsy was not allowed and thus was not possible to determine the route of metastasis. It is not possible to ascertain if metastasis foci were present in other organs but the surgeon reported only the tumor in the left kidney. The presence of neoplastic tissue in the pelvis and ureter raises the possibility that dissemination of the tumor might have occurred by ascending the urinary tract.

AFIP Diagnosis: Kidney: Transmissible venereal tumor, metastatic, Labrador retriever (*Canis familiaris*), canine.

Conference Comment: The contributor gives an excellent description of canine transmissible venereal tumors (TVT). Currently the TVT is the only known naturally transmitted neoplasm. However, it has been proposed that the recently described Devil facial tumor disease may be transmitted in a similar manner.[7]

It is not known how the tumor evades the host immune system. Class I and II major histocompatibility antigens are not expressed by the tumor cells until regression occurs.[11] Cells of TVTs secrete TGF-β1 and IL-6 which suppress the expression of major histocompatibility antigens.[14]

The histologic appearance of the neoplasm can vary greatly depending on the stage of growth or regression. During regression, TVT cells express major histocompatibility complex class II antigens, and therefore the neoplasms are often infiltrated by inflammatory cells, particularly T lymphocytes. The effect of vincristine administration on the cytomorphology and level of regression in this tumor is difficult to assess.

Contributor: Claudio S.L. Barros of the Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, 97105-900, RS, Brazil

References:

1. Armed Forces Institute of Pathology: Conference 26, Case I. In: 26th Wednesday Slide Conference 2000-2001, pp. 183-185. AFIP, Washington, D.C., 2001 http://www.afip.org/vetpath/WSC/wsc00/00wsc26.htm
2. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1306-1307. Elsevier, St. Louis, MO, 2007
3. Foster RA: Male reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1346-1347. Elsevier, St. Louis, MO, 2007
4. Foster RA, Ladds PW: Male genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 618-619. Elsevier Limited, St. Louis, MO, 2007
5. Goldschmidt MH, Hendrick MJ: Tumors of the skin and soft tissues. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 115-117, Iowa State Press, Ames, IA, 2002
6. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielsen S: Histological classification of tumors of the genital system of domestic animals. In: World Health Organization Histological Classification of Tumors of Domestic Animals, 2nd series, volume IV, p. 23-38, 69, Armed Forces Institute of Pathology, Washington DC, 1998
7. Loh R, Hayes D, Mahjoor A, O'Hara A, Pyecroft S, Raidal S: The immunohistochemical characterization of devil facial tumor disease (DFTD) in the Tasmanian devil (*Sarcophilus harrisii*). Vet Pathol 43:896-903, 2006
8. MacEwen EG: Transmissible venereal tumor. In: Small Animal Clinical Oncology, eds. Wintrow SJ, Macewen EG, 3rd ed., pp. 651-656. Saunders, Philadelphia, PA, 2001
9. Mozos E, Méndez A, Gómez-Villamandos JC, de las Mulas JM, Pérez J: Immunohistochemical characterization of canine transmissible venereal tumor. Vet Pathol 33:257-263, 1996
10. Park MS, Kim Y, Kang MS, Oh SY, Cho DY, Shin NS, Kim DY: Disseminated transmissible venereal tumor in a dog. J Vet Diagn Invest 18:130-133, 2006
11. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 547-548. Elsevier Limited, St. Louis, MO, 2007
12. Susaneck S: Canine transmissible venereal tumor. In: Veterinary Oncology Secrets, ed. Rosenthal RC, pp. 207-209. Hanley & Belfus, Philadelphia, PA, 2001
13. van Heerden J: Problemas dos animais de pequeno porte nos países em desenvolvimento. In: Tratado de Medicina Interna Veterinária: Moléstias do Cão e do Gato, ed. Ettinger SJ, 3rd ed., pp. 225-234. Manole, São Paulo, SP, 1992
14. von Holdt BM, Ostrander EA: The singular history of a canine transmissible tumor. Cell 126:445-447, 2006

CASE III – MK0605183 (AFIP 3069157).

Signalment: 16-year-old, female, Rhesus macaque (*Macaca mulatta*)

History: The animal had been infected with Simian immunodeficiency virus (SIV) and was being treated with Depo Provera for endometriosis. The animal had a history of several episodes of dehydration despite adequate access to water. Previous bouts of dehydration had responded to fluid therapy. On the day of presentation, the animal was found down in the cage, hypothermic, dehydrated and weak with a distended abdomen. Intravenous fluid therapy, including IV dextrose, was initiated prior to the collection of blood.

Laboratory results: The serum chemical findings were consistent with chronic renal disease (see values table 3-1). The animal was euthanized after failing to respond to IV fluid therapy.

Gross Pathology: The animal was in lean body condition with adequate hydration. Multifocally within the abdomen, the omentum was attached loosely to the peritoneal wall. In between the loops of intestine and on the capsular surface of the kidneys, there were multifocal, small, white, fibrous adhesions. Within the caudal abdomen, surrounding and invading the body of the uterus and effacing the ovaries, and compressing the colon and bladder was a 3 x 3.5 x 6 cm^3 cystic mass. The mass contained approximately 10ml of clear fluid and had an irregularly thickened, lobular inner surface. The lungs were congested with moderate edema and the kidneys were pale. No other significant lesions were noted in the heart, liver, spleen, gastrointestinal tract, or brain.

Histopathologic Description: Submitted sections were from the uterus, ovary, and oviduct. Multifocally, the serosal surfaces of these organs were irregularly expanded by a thick band of homogenous, eosinophilic material with numerous, widely spaced, 15 – 25µm polygonal cells and few glands lined by cuboidal epithelium. The polygonal cells had distinct cell borders with ample, fibrillar cytoplasm. Nuclei were round – oval, with reticular chromatin and a single nucleolus [decidualized stromal cells]. No mitotic figures were seen within the polygonal cells. In some sections, there were small lymphoid aggregates in the stroma. Some glands contained cellular and necrotic debris, but RBCs and hemosiderophages were not a common finding. Few sections contained small foci of mineralization. On some slides, the endometrium was expanded by similar decidualized stromal cells and ample eosinophilic stroma.

Special stains/Immunohistochemistry: The eosinophilic matrix was not birefringent with Congo red staining and few fibrils were seen with trichome stain. The polygonal cells failed to stain with macrophage [HAM56], muscle [Desmin], and epithelium [AE1/AE3] markers.

Contributor's Morphologic Diagnoses: Caudal abdominal mass: Endometriosis with decidualized stromal cells.

Contributor's Comment: AFIP confirmed the diagnosis of endometriosis with decidualized stromal cells. In addition to the uterus and ovaries, endometriosis affected the intestines, kidneys, colon, and bladder in this animal. Although endometriosis is not an unusual lesion in macaques, the presence of the large polygonal decidualized stromal cells with abundant eosinophilic, homogenous stroma was not a change we had commonly encountered. The endometriotic stromal cells we have seen in the past have been small, spindloid, contained little to no cytoplasm, had spindle-shaped nuclei and were not widely separated by stroma.

Endometriosis, the presence of endometrial tissue outside the uterus, is a progressive disease that occurs in women and old world primates, primarily during their reproductive years. [5,10,12,17] Clinical signs associated with endometriosis include dysmenorrhea, dyspareunia, pelvic pain, interference with intestinal and urinary bladder function and reduced fertility.[10] In rhesus, there may be similar signs with irregular vaginal bleeding or heavy menses and signs of pain include lying down in the cage, anorexia, grimacing, decreased grooming, restlessness, and vocalization.[5,10]

On gross examination or laparotomy, endometriosis may have a varied appearance but is most easily recognized by red/brown [chocolate] cysts on the serosal surface of pelvic and abdominal organs. The cysts are endometriotic glands containing viable and degenerate RBCs and hemosiderophages.[5,15] Endometriotic lesions may also exist as clear cysts and variably-sized adhesions between organs.[15] In women with endometriosis, the organs most likely affected (in descending order) are: ovaries, uterine ligaments, rectovaginal septum, pelvic peritoneum, as well as laparotomy scars.[4] Endometriosis may also occur outside of the peritoneum and, rarely, in men.[12]

Endometriosis is definitively diagnosed by laparoscopic surgery to biopsy/remove suspected lesions with histologic examination of the samples. [11] The diagnosis of endometriosis is based upon the presence of endometrial

Table 3-1. Laboratory results, case 3.

Analyte	Value	Normal	Units
BUN	175↑	5 - 25	mg/dL
Creatinine	4.4↑	0.5 - 1.1	mg/dL
Total protein	4.7↓	6 - 8.5	g/dL
Sodium	119↓	145 - 152	mmol/L
Chloride	92↓	105 - 115	mmol/L
Potassium	7.3↑	3 - 4.5	mmol/L
Calcium	1.92↓	2.1 - 2.55	mg/dL
Phosphorous	>12↑	3 - 4.5	mg/dL
Glucose	470↑	60 - 120	mg/dL
WBC	20.8↑	5 – 13.5	K/μL
Polys - calc	18.7 ↑	1.6 – 7.4	K/μL
HCT	34	33 - 45	%

glands and stroma outside the uterus.[4,12] In women, stromal changes that may be associated with endometriosis are fibrosis, numerous small vessels, aggregates of foamy and pigmented [hemosiderin] macrophages, smooth muscle metaplasia, and myxoid change.[3] Progestin treatment, which this macaque received, can cause decidual change in the endometrial stromal cells so that stromal cells appear large and polygonal rather than small and spindeloid. The appearance of endometriotic glands can range from normal-appearing glands lined by cuboidal epithelial cells to glands lined by flattened epithelium that may be mistaken for ectatic vessels.[3] Hormone treatment, pregnancy, and menopause will change the appearance of both the stroma and the glands. For suspected cases of endometriosis, the immunohistochemical marker CD10, which stains both normal and endometriotic stromal cells, can be used to assist in making the diagnosis.[3]

Historically, endometriosis has been thought to develop and progress via one of three theoretical pathways:

1. Regurgitation/implantation: Retrograde menstruation, the backflow of uterine contents, including epithelial cells, and debris through the fallopian tubes into the peritoneal cavity[10], may cause endometriosis.[4]
2. Metaplasia: The peritoneum of the pelvis, which arises from the same embryonic coelomic epithelium as the endometrium, undergoes metaplastic change to develop into endometriosis.[4,8,12]
3. Vascular or lymphatic dissemination: Endometriotic tissue may be transported through pelvic veins and lymphatics to cause endometriosis in tissues distant from the uterus such as lungs and lymph nodes.[4]

The current understanding is that the first step in the pathogenesis of endometriosis is the regurgitation of endometrial contents into the peritoneal cavity.[2] However, retrograde menstruation occurs in 76-90% of women, most of who never develop endometriosis.[5] How the ectopic endometrial tissue develops into endometriosis is still being defined. Although the disease estrogen-dependent,[5] the progression is likely multifactorial in-

volving hormonal, immune, genetic and environmental influences.[10] Significant factors associated with the development of endometriosis are: kinship with affected individuals; abdominal surgeries such as cesarean sections, fetal instrumentation, ovarian follicle aspirations and embryo transfers; age; and with environmental factors such as exposure to dioxin, polychlorinated biphenyls (PCBs) and ionizing radiation.[1,17] Additional risk factors for the development of endometriosis in humans are short menstrual cycle length, prolonged menses, low parity number and increased serum estrogen.[15]

Recent work using gene expression analysis to evaluate the uterine endometrium of women with endometriosis and those without the disease has identified numerous genes that are both up and down regulated in comparison to non affected women. In addition, the uterine endometrium of women with moderate/severe endometriosis is less sensitive to the effects of progesterone. In normal endometrium, progesterone has an antiproliferative effect and, after ovulation, leads to the onset of secretory phase and deciduization of stroma. In the endometrium of affected women, progesterone resistance leads to enhanced cellular survival, as well as decreased regulation of DNA synthesis and cellular mitosis.[2]

Nonprimate and primate animal models have been developed to study the genesis and progression of endometriosis. Endometriosis can be induced in rodents and rabbits by implanting autologous or human uterine tissue within the peritoneum. These models have been helpful in the study of early events involved in the attachment of endometrial stroma and glands after implantation into the abdomen. In addition, the influence of immune and inflammatory components in the development of endometriosis can be more easily studied in a sequential manner in smaller animal models. However, rodents and rabbits lack a true menstrual cycle and the endometriotic lesions they develop differ from those seen in women.[15]

Endometriosis occurs spontaneously and can be induced in primates.[15] In colonies of captive macaques, spontaneous endometriosis has been seen in up to 25% of the population.[10,17] Rhesus macaques have been most often studied and are a good model for human endometriosis in that their menstrual cycle is approximately 28 days long, with a 4 day menstrual bleeding period, they tend to develop the disease during their reproductive years, and have disease that progresses with time. In addition, the clinical signs, gross and histologic lesions in the macaques are similar to those seen in women.[10,17]

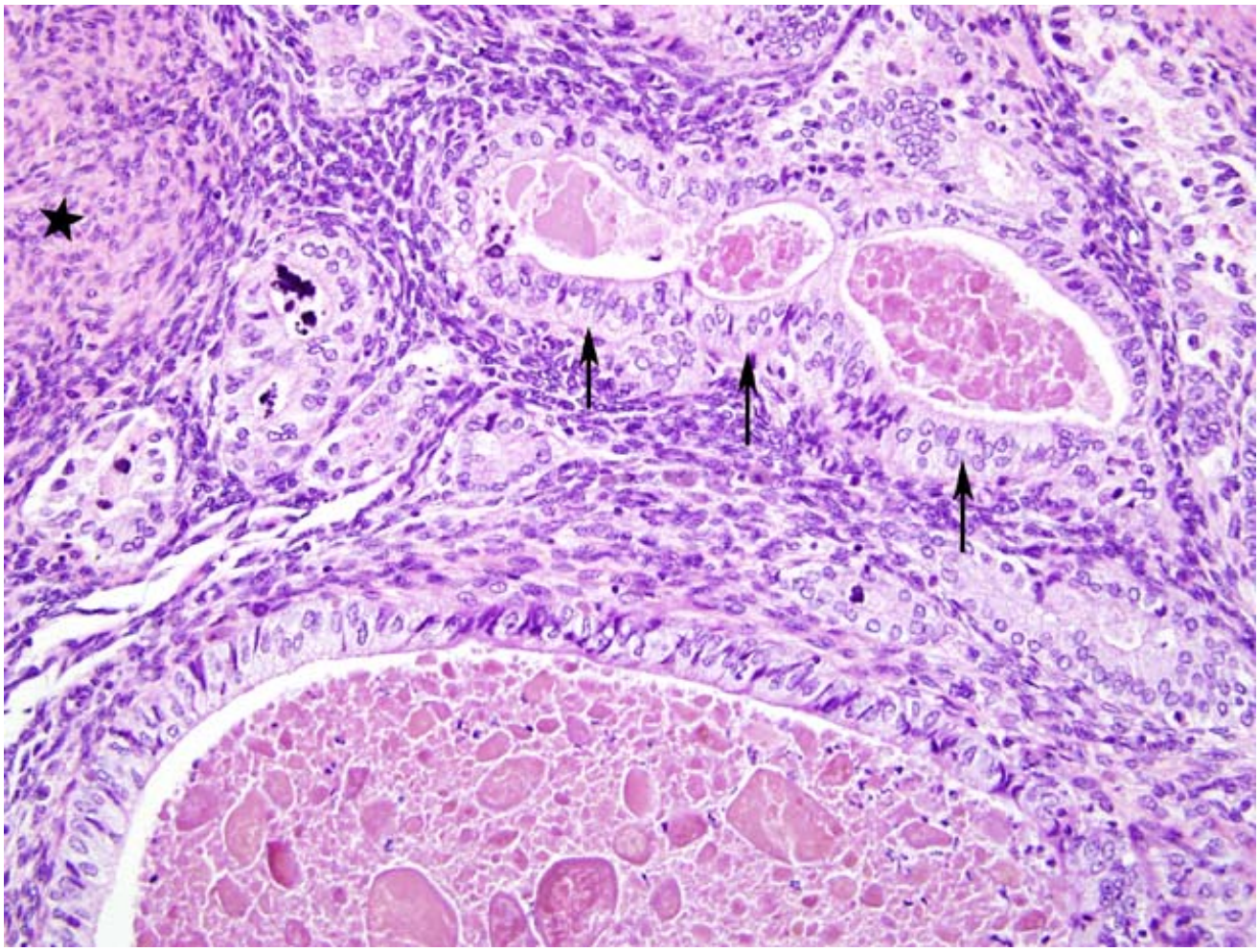
The treatment goals for endometriosis are to decrease pain, decrease the size of endometriotic lesions, remove associated obstructions, and restore fertility.[11] Treatment may involve surgical removal of endometrial explants within the abdomen or medical treatment to cause the ectopic tissue to atrophy or both. Unfortunately, clinical signs may recur after surgery or after hormonal treatment is discontinued.[12,11] Medical therapies target the hypothalamic-pituitary-gonadal axis, by selective modulation of estrogenic and progestogenic pathways, by inhibiting angiogenesis, or by modulating inflammatory and immunological responses.[5,11] Depo Provera, a commonly used progestin-based treatment, will cause endometrial tissue to atrophy, but also may reduce bone density. Other hormone treatments include gonadotropin-releasing hormone agonists, progestogens, androgenic agonists, combined oral contraceptive therapy, and antiprogestogens may also lead to osteoporosis.[11] More recent therapies include selective progesterone-receptor modulators (SPRMs), selective estrogen-receptor modulators (SERMs) which reduce estrogen but have a bone-sparing effect, immunomodulatory drugs, angiogenesis inhibitors which may interfere with implantation and vascularization of ectopic implants, aromatase inhibitors (AIs) which decrease non-ovarian sources of estrogen, and statins which reduce the growth of endometrial stroma *in vitro*.[5]

Renal changes included multifocal, moderate chronic infarcts with mild chronic interstitial nephritis as well as moderate acute tubular necrosis with tubular proteinosis. Adverse renal effects from Depo Provera treatment have not been reported.[13] Clinical veterinarians did not find a cause for the episodes of dehydration and no other animals in colony developed unexplained dehydration. A specific agent for the renal changes was not identified. No opportunistic infections or changes associated with SIV infection were seen in the other tissues of this animal.

AFIP Diagnosis: Ovary and uterus: Endometriosis, with decidualized stromal cells, Rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: The contributor gives an excellent overview of endometriosis.

Endometriosis is defined as **endometrial glands or stroma (fig. 3-1)** explanted to abnormal locations within and outside the uterus.[3] The explanted tissue responds to hormonal stimulation similar to normal endometrium.[4] It has been reported in humans, old world monkeys, and apes.[15] Key histologic features include endometrial glands, endometrial stroma, and pigment-containing histiocytes. These features may vary depending on hormonal stimulation. In longstanding cases of endometrio-



3-1. Ovary and uterus, Rhesus macaque. Endometrial glands (arrows) and stroma (star) expanding the ovary and abnormal locations within the uterine wall.. (H&E 200X)

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sis, the endometrial glands or endometrial stroma may be obscured by fibrosis or an infiltrate of histiocytes that contain hemosiderin, ceroid, or lipofuscin.[3]

Adenomyosis is endometrial stroma and/or glands within the myometrium of the uterine wall and has been reported in humans and domestic animals.[4,14]

Contributing Institution: Division of Veterinary Resources, National Institutes of Health, Bethesda, MD

References:

1. Barrier BF, Bates GW, Leland MM, Leach DA, Robinson RD, Propst AM: Efficacy of anti-tumor necrosis factor therapy in the treatment of spontaneous endometriosis in baboons. Fertil Steril 81:775-779, 2004
2. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA, Giudice LC: Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology 148:3814–3326, 2007
3. Clement PB: The pathology of endometriosis: A survey of the many faces of a common disease emphasizing diagnostic pitfalls and unusual and newly appreciated aspects. Adv Anat Pathol 14:241-260, 2007
4. Crum CP: The female genital tract. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 1083-1084. Elsevier Saunders, Philadelphia, PA, 2005

5. Hompes PGA, Mijatovic V: Endometriosis: The way forward. Gynecol Endocrinol 23:5-12, 2007
6. Kang J, Chapdelaine P, Laberge PY, Fortier M: Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells. Hum Reprod 21:592-599, 2005
7. Klemmt PAB, Carver JG, Kennedy SH, Koninckx PR, Mardon HJ: Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. Fertil Steril 85:564-572, 2006
8. Lane B, Oxberry W, Mazella J, Tseng L: Decidualization of human endometrial stromal cells in vitro: effects of progestin and relaxin on the ultrastructure and production of decidual secretory proteins. Hum Reprod 9:259-266, 1994
9. Mantena SR, Kannan A, Cheon YP, Li Q, Johnson PF, Bagchi IC, Bagchi MK: C/EBPβ is a critical mediator of steroid hormone-regulated cell proliferation and differentiation in the uterine epithelium and stroma. Proc Natl Acad Sci USA 103:1870-1875, 2006
10. Mattison JA, Ottinger MA, Powell D, Longo DL, Ingram DK: Endometriosis: Clinical monitoring and treatment procedures in Rhesus monkeys. J Med Primatol, doi:10.1111/j.1600-0684.2006.00208.x, 2007
11. Mihalyi A, Simsa P, Mutinda KC, Meuleman C, Mwenda JM, D'Hooghe TM: Emerging drugs in endometriosis. Expert Opin Emerg Drugs 11:503-524, 2006
12. Olive DL, Schwartz LB: Endometriosis. N Engl J Med 328:1759-1769, 1993
13. Physicians' Desk Reference. 61st ed., Thompson PDR, Montvale, NJ, 2007
14. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 464-465. Elsevier Limited, St. Louis, MO, 2007
15. Story L, Kennedy S: Animal studies in endometriosis: A review. ILAR J 45:132-138, 2004
16. Yang S, Fang Z, Suzuki T, Sasano H, Zhou J, Gurates B, Tamura M, Ferrer K, Bulun S: Regulation of Aromatase P450 expression in endometriotic and endometrial stromal cells by CCAAT/enhancer binding proteins (C/EBPs): decreased C/EBPβ in endometriosis is associated with overexpression of aromatase. J Clin Endocrinol Metab 87:2336-2345, 2002
17. Zondervan KT, Weeks DE, Colman R, Cardon LR, Hadfield R, Schleffler J, Trainor AG, Coe CL, Kemnitz JW, Kennedy SH: Familial aggregation of endometriosis in a large pedigree of Rhesus macaques. Hum Reprod 19:448-455, 2004

CASE IV - Case 1 (AFIP 3066305).

Signalment: Heart tissue and fetal membranes are from a male, mixed-breed goat fetus, with a crown to rump measurement of 28cm and weighing 911g.

History: A group of pregnant does at various stages of gestation were co-mingled with three, BVDV (Type 2) persistently infected heifers.

Gross Pathologic Findings: Examination of fetal tissues revealed no significant gross lesions.

Laboratory Results:
Tests performed on fetal tissues
Microbiology:
Bacterial cultures, negative
Fluorescent Antibody
BVDV, positive
Immunohistochemistry
BVDV, positive
Polymerase chain reaction (PCR)
BVDV, positive (typed as Type 2)
Chlamydia, negative
Bluetongue virus (BTV), negative
Virus Isolation
Negative on tissues from this case submission; however, BVDV was isolated from 2 other aborted fetuses with similar histological lesions in this study.

Histopathologic Description: From tissues submitted: Placenta: Creating a thin band along the superficial chorionic stroma, there are scattered necrotic cells admixed with abundant cellular debris. The overlying trophoblastic epithelium is often absent within the more affected regions. The vessels deep to the more affected areas are similarly peppered with nuclear debris, which occasionally obscures the endothelium and muscular layers of the vessel wall. In the deeper stroma, there is a mild, multifocal infiltrate of individually scattered mononuclear cells.

Heart: The heart exhibits a mild infiltrate of mononuclear inflammatory cells forming scattered infiltrates within the epicardium and forming perivascular cuffs within the myocardium.

Contributor's Morphologic Diagnosis: 1. Placenta: Marked, acute, multifocal to coalescing, necrotizing placentitis and vasculitis
2. Heart: Mild, multifocal, non-suppurative epicarditis and perivascular myocarditis

Contributor's Comment: Based upon the histological lesions and ancillary testing (FA, IHC and PCR), the abortion syndrome in these goats is blamed on BVDV infection.

Although BVDV most commonly infects cattle, the virus can also be found in other domesticated and wild ruminants.[1] It was hypothesized that these species may serve as a reservoir for the disease.[1] Seroprevalence to BVDV has also been recognized in many other domesticated and wild ruminant species, the geography of which continues to expand.[1,6,7,12]

Experimental and natural intraspecies transmission of ruminant pestiviruses has been confirmed.[8] This is also supported by the observation that higher seroprevalence rates of BVDV in goats occurs in regions/countries where goats are more likely to be co-mingled with cattle or wild ruminant species.[7] Goats are thus infected by exposure to other persistently infected species.

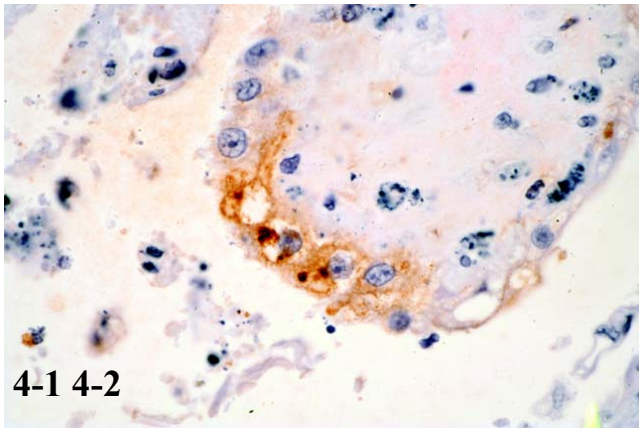
The reproductive consequences of BVDV infection in cattle have been reviewed.[5] BVDV disease in goats, reproductive failures or otherwise, have been less characterized. Experimental infection of adult goats with BVDV results in seroconversion with formation of neutralizing antibodies that persisted for up to 4 years.[9] Infection of kids similarly showed development of neutralizing antibodies accompanied by an impaired growth rate, but otherwise, no significant clinical symptoms.[10] Field outbreaks and experimental inoculation of BVDV in goats has produced reproductive failures.[8] These have been characterized by barreness, abortions, stillbirths or births of weak kids and birth of kids exhibiting clinical signs similar to border disease accompanied by histological lesions in the CNS.[8,11] Also, kids born to does experimentally infected with BVDV during gestation were highly contagious for goats and other susceptible ruminant species.[10]

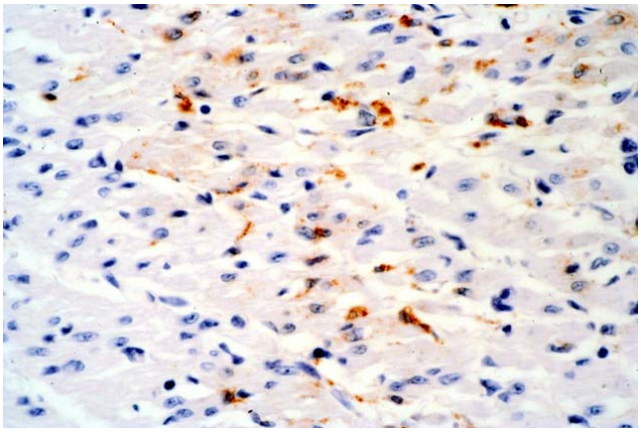
BVDV should be considered as a cause of abortion in goats or perinatal deaths in goats with or without CNS disease. This is especially true in geographic regions where goats and cattle (persistently infected BVDV cattle) are in close proximity.

AFIP Diagnosis: Placenta: Placentitis, necrotizing, multifocal, moderate, goat (*Capra hircus*), caprine.

Conference Comment: Diffuse autolysis obscured many histologic features and complicated the assessment of necrosis in this case. Pathologic lesions of BVD infection in fetal tissues are not considered characteristic and are rarely seen due to fetal autolysis.[13] Although the Bovine Viral Diarrhea Virus was not isolated from this particular animal, a PCR, **immunohistochemistry (figs. 4-1 and 4-2)**, and fluorescent antibody were all positive, making diagnosis highly probable. Convincing vasculitis was not present in the slides reviewed during the conference, but this is possibly due to slide variation.

BVDV is a RNA virus of the Pestivirus genus in the family Flaviviridae.[2] BVDV is known to naturally and subclinically infect pigs, sheep, goats, and several wild Afri-





4-1 Placenta, goat. Multifocally placental trophoblasts are immunoreactive for BVDV.
4-2 Heart, goat. Multifocally within the myocardium, cells are immunoreactive for BVDV.

Photomicrographs courtesy of the Department of Veterinary Pathobiology and the Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, OK

can ruminants.[6] Other pestiviruses in animals include Porcine Pestivirus (classical swine fever virus/hog cholera virus) in swine, and Ovine Pestivirus (Border disease virus) in sheep.[13]

BVDV infection may result in three main disease syndromes: embryonal/fetal disease (transplacental infection), mucosal disease (infection in immunotolerant animal), or bovine viral diarrhea (infection in immunocompetent animal).[13]

The outcomes of transplacental infection with BVDV are presented in table 4–1 below.

Mucosal disease results when an immunotolerant cow is infected with a cytopathic strain of BVDV either from an exogenous source, or through a mutation of the endogenous non-cytopathic strain.[13]

Postnatal infection with BVDV in an immunocompetent animal predominantly result in enteritis primarily of the ileum and proximal colon. Multifocal erosions may occur in oral and esophageal areas.[13]

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References:

1. Baker JC: Bovine viral diarrhea virus: a review. J Am Vet Med Assoc 190:1449-1458, 1987
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 140-147. Elsevier Limited, St. Louis, MO, 2007
3. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1299. Elsevier, St. Louis, MO, 2007
4. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 370-372. Elsevier, St. Louis, MO, 2007
5. Grooms DL: Reproductive consequences of infection with bovine viral diarrhea virus. Vet Clin North Am Food An Pract 20:5-19, 2004.
6. Kim I-J, Hyun B-H, Shin J-H, Lee K-K, Lee K-W, Cho K-O, Kang M-I: Identification of Bovine Viral Diarrhea Virus type 2 in Korean native goat (*Capra hircus*). Virus Res 121:103-106, 2006.
7. Krametter-Froetscher R, Loitsch A, Kohler H, Schleiner A, Schiefer P, Moestl K, Golja F, Baumgartner W: Prevalence of Antibodies to Pestiviruses in goats in Austria. J Vet Med 53:48-50, 2006.
8. Loken T: Ruminant pestivirus infections in animals other than cattle and sheep. Vet Clin North Am Food An Pract 11:597-614,1995.
9. Loken T, Krogsrud J, Bjerkas I: Outbreaks of border disease in goats induced by a pestivirus-contaminated orf

Table 4 –1. Outcomes of transplacental infections with BVDV[13]

Cytopathic Strain of BVDV	Prior to 100 days of gestation	embryo and fetuses resorption or expulsion days to months following infection.
	100 -150 days of gestation	teratogenic effects on fetal organs that may result in microencephaly, cerebellar hypoplasia, hydraencephaly, hydrocephalus, microphthalmia, thymic aplasia, hypotrichosis, alopecia, brachygnathism, growth retardation, and pulmonary hypoplasia
Non-Cytopathic Strain of BVDV	Prior to 100-125 days of gestation	Immunotolerance results in birth of persistently infected calf
	After 150 days of gestation	Fetuses mount relatively normal immune response and are born with circulating antibodies

vaccine, with virus transmission to sheep and cattle. J Comp Pathol 104:195-209 , 1991.
10. Loken T, Bjerkas I, Larsen HJ: Experimental pestivirus infections in newborn goat kids. J Comp Pathol 103: 277-288, 1990.
11. Loken T, Bjerkas I: Experimental pestivirus infections in pregnant goats. J Comp Pathol 105: 123-140, 1991.
12. Mishra N, Dubey R, Rajukumar K, Tosh C, Tiwari A, Pitale SS, Pradhan HK. Genetic and antigenic characterization of bovine viral diarrhea virus type 2 isolated from Indian goats (Capra hircus). Vet Microbiol. 2007, *In press*.
13. Potgieter LND: Bovine viral diarrhoea and mucosal disease. In: Infectious Diseases of Livestock. eds. Coetzer JAW, Tustin RC, 2nd ed., vol. 2, pp. 946-963. Oxford University Press, Cape Town, South Africa, 2004
14. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 480-483. Elsevier Limited, St. Louis, MO, 2007

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 11

12 December 2007

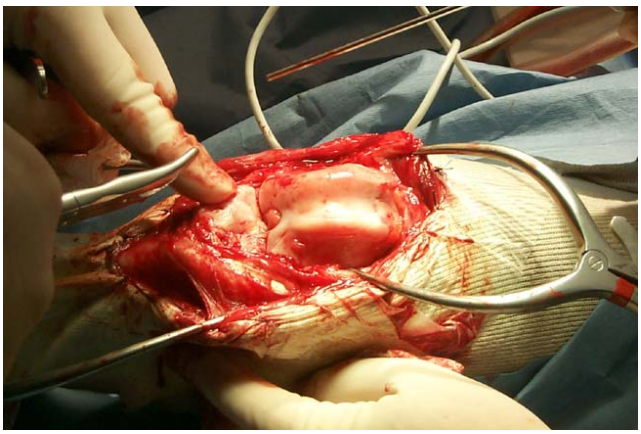
Moderator:

Dr. Steven Weisbrode, DVM, DACVP

CASE I – 306729 (AFIP 2840709).

Signalment: 1-year-old, female, boxer, canine

History: The dog presented to a referral hospital for chronic (greater than one month duration) grade II out of VI lameness on the left hind limb with a short stride and mild muscle atrophy. Physical exam revealed pain on manipulation of the left stifle, moderate left stifle thickening, and mild decreased range of motion. Radiographs revealed a fluffy proliferation in the region of the left stifle fat pad, and thickening with slight mineralization of the medial aspect of the joint. A percutaneous bone core biopsy was performed and was consistent with multifocal osteocartilaginous metaplasia. Due to the dog's age at the time of the initial biopsy (9 months), it was recommended that surgery to remove the lesional tissue be postponed until symphyseal closure occurred. The dog returned for arthrotomy 5 months later. At surgery, moderate **degenerative join t di sease (fig. 1 -1)** was noted with numerous osteophytes on the medial trochlear ridge. A bony mass was present that incorporated the medial joint capsule from the level of the proximal trochlea to the tibial plateau and from the medial collateral ligament to the patellar tendon. The mass was seen to be contiguous with the patellar fat pad and both the cranial medial and lateral menisci. The joint capsule was removed with the mass (excision was complete grossly).



1-1. Left stifle, boxer. The medial trochlear ridge is moderately proliferative and expanded by osteophytes. Photograph courtesy of Dr. Brian Huss, Vescone (Waltham, MA) and the Angell Memorial Animal Hospital, Pathology Department, 350 S. Huntington Ave., Boston, MA 02130

Gross P athology: Tissues submitted for histologic evaluation consisted of a 5.5 x 3.5 x 2.0 cm and a 2.0 x 1.5 x 1.2 cm piece of hard, nodular, white-gray tissue lacking orienting anatomic features.

Laboratory Results: Aerobic culture of the joint at the time of initial core biopsy was negative.

Contributor's Morphologic Diagnosis: Synovial osteochondromatosis

Contributor's Comment: The submitted specimen consists of sections of joint capsule that include the synovial membrane and fibrous layer. There is a poorly delineated, expansile, multinodular mass comprised of broad trabeculae of woven and lamellar bone that are lined in many areas by a single layer of osteoblasts. In most areas, these trabeculae arise from foci of collagenous tissue via endochondral ossification. Hematopoietic cells and adipose tissue are present within the intertrabecular spaces.

Synovial osteochondromatosis, or synovial chondrometaplasia, is a proliferative disorder of undifferentiated stem cells of the synovium. The proposed pathogenesis is the transformation of fibroblast-like cells under the influence of extracellular chondroid matrix material into chrondroblastic cells. It is these transformed cells that are believed to give rise to the characteristic cartilaginous nodules. These nodules may grow and project out form the synovium on delicate vascular pedicles, or as seen in this case, may form a more broad-based nodular mass. When their base is narrow, they often break off and form loose bodies within the joint. The chondrocytes of the loose body are nourished by the synovial fluid and thus will continue to form more cartilage matrix and increase in size. This will often result in degenerative joint disease due to physical damage to the adjacent joint capsule and articular cartilage. If the nodules remain attached to the synovium (as was seen in this case) they will often undergo endochondral ossification with the formation of broad trabeculae of bone. In dogs, the disease is usually seen in medium to large breeds, and has been described in the scapulohumeral, coxofemoral, talocrural, and stifle joints. There is usually no history of trauma or primary degenerative joint disease (such as osteochondritis dissecans).

In humans, osteochondromatosis is uncommon and most often occurs in the stifle joint of middle aged males. The condition in humans has been classified into primary and secondary forms. The primary form is described as the spontaneous formation of intrasynovial nodules in an otherwise normal joint. It is usually confined to one joint, most commonly a larger joint (e.g. knee, hip, shoulder, elbow, and ankle). Histologically, the primary form has been described as foci of chondrometaplasia that contain chondrocytes with cellular atypia. The recurrence rate with this form is considered high. Secondary synovial osteochondromatosis is a similar condition that follows traumatic, degenerative, or inflammatory joint diseases. In this condition, detached fragments of cartilage or subchondral bone become implanted within the synovium and incite the formation of chondrometaplastic nodules. With the secondary form, there is little to no cytological atypia, and the condition usually responds favorably to surgical intervention, provided that the initial joint disease is not allowed to progress.

In the past, an attempt has been made to apply the abovementioned classification to canine patients with osteochondromatosis, but this has proven to be difficult, as many lesions in the dog do not fit all the criteria of either classification. In the present case, there was no appreciable cellular atypia seen and there was evidence of degenerative joint disease. This would be most consistent with a classification of secondary osteochondromatosis. However, the patient had no history of trauma, and the lesion was confined to one stifle joint. This is more typical of the primary form. A lack of cellular atypia argues for the secondary form, but cellular atypia may not be seen in all primary cases. The presence of degenerative joint disease is not in of itself an adequate criterion for the secondary form, as the formation of osteochondromatous nodules can lead to degenerative changes within the adjacent synovium. Regardless of the classification scheme used, the prognosis for dogs with this condition appears to depend on the degree of degenerative joint disease noted at the time of surgery, as well as the ability to perform total synovectomy with removal of any loose bodies. Recurrence with incomplete removal of the affected synovium and/or loose bodes has been reported. Complete synovectomy may be impossible, however, and temporary relief has been reported with loose body removal alone. The dog in this report was doing well (no lameness reported) 2 months post-surgery.

Differential diagnosis should include severe degenerative joint disease, osteochondral fractures secondary to trauma, osteochondritis dissecans, and neoplasia, particularly chondrosarcoma.

AFIP Diagnosis: Joint capsule (per contributor): Osteochondral metaplasia (osteochondromatosis), diffuse, marked, Boxer (*Canis familiaris*), canine.

Conference Comment : The contributor gives a good review on a poorly characterized, rare condition in animals. We essentially agree with the contributor's diagnosis of osteochondromatosis, although we prefer the terminology of osteochondral metaplasia to describe the morphologic lesion.

Osteochondral metaplasia can occur within any synovial lined structure, such as a joint, tendon sheath, or bursa.[4]

Ectopic ossification of these structures requires a vascular supply, and the presence of detached osseous bodies (joint mice) implies a previous attachment to the synovial surface.

The underlying cause of osteochondral metaplasia in animals is not known. Due to the relatively limited responses of the joint, it can often be difficult to distinguish osteochondral metaplasia from other disease processes that result in intra-articular joint bodies such as osteochondrosis or chip fractures.[4]

There is some variability in the slides. Most slides exhibit lamellar bone formation, and only a few slides exhibiting both lamellar and chondroid bone formation. Lamellar bone is formed by endochondral ossification with a sharp line of demarcation between cartilage and bone.[5] Chondroid bone is formed directly from fibrocartilage, with blending of the cartilage and bone.

Contributor: Dr. Brian Huss, Vescone (Waltham, MA) Angell Memorial Animal Hospital, Pathology Department, 350 S. Huntington Ave., Boston, MA 02130

References:

1. Edinger ET, Manley PA: Arthrodesis of the shoulder for synovial osteochondromatosis. J Small Anim Pract 39:397-400, 1998
2. Flo GL, Stickle RL, Dunstan RW: Synovial chondrometaplasia in five dogs. J Am Vet Med Assoc 191:1417-22, 1987
3. Gregory SP, Pearson GR: Synovial osteochondromatosis in a Labrador retriever bitch. J Small Anim Pract 31:580-583, 1990
4. Newell SM, Roberts RE, Baskett A: Presumptive tenosynovial osteochondromatosis in a horse. Vet Radiol 37:112-115, 1996
5. Thompson K: Bones and joints. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 136-145. Elsevier Limited, St. Louis, MO, 2007

CASE II – 07-1271 (AFIP 3066003).

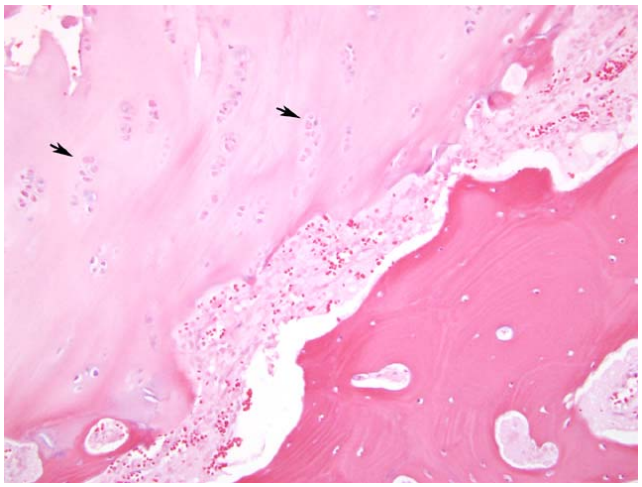
Signalment: Two-year-old, male castrate, Main Coon cat.

History: Femoral neck fracture. Femoral head osteotomy performed. Radiographically the femoral neck appeared less dense and slightly lytic?

Histopathologic Description: The femoral head has a normal shape and the articular cartilage is microscopically normal. There is variable lack of differential staining in marrow, bone lining cells and osteocytes interpreted to be artifacts of decalcification. The marrow is mostly fatty with limited hematopoiesis and areas of acute hemorrhage. At the deep specimen margin there is bone debris within the marrow spaces that is presumed to reflect method of surgical removal since no sawing was done to the specimen once received. There are shredded fragments of hyaline cartilage present on the deep margin in what would be the location of the growth plate. Compared with a normal active growth plate, the cartilage is hypocellular and chondrocytes are present in **irregular groups (fig. 2-1)** rather than columns.

Contributor's Morphologic Diagnosis: Femoral head: Dysplasia and fracture of physis

Contributor's Comment: This particular case of atraumatic fracture of the femoral capital epiphysis (in a cat over one year of age) was selected to submit to the Wednesday Slide Conference because, in our experience, it is typical of the appearance of such specimens. While the most important lesions are in the growth plate, little growth plate is present on many of these specimens received for histopathologic evaluation. Only fragments of the physis remain on the femoral head and these fragments have variable hypocellularity and disorganization. The remainder of the femoral head has no significant lesions. Subtle marrow or bone lining-cell changes are not possible to detect in this specimen due to over-decalcification. Most important in considering the diagnosis of physeal dysplasia at this site in the cat is the PRESENCE of a growth plate relative to the age of the cat. The microscopic appearance of the plate might actually be non-specific and reflect that although the plate has not closed, it is not contributing to longitudinal growth. On average, the growth plate at the femoral neck in cats closes at 40 weeks.[10] Castration delays this by about 6 weeks but this delay is NOT associated with increased length of the bone (reported for the radius).[11] Therefore, although it is open longer, the growth plate is not significantly adding to longitudinal growth. The age of presentation of cats with physeal fractures appears to have changed from earlier to more recent literature. Reports of physeal fractures of the femoral neck in cats in 1993 and 1996 have no cat older than 12 months of age affected.[3,9] Publications in 2001[2], 2002[6], 2004[5] and 2006[8] report femoral capital physeal fractures mostly in cats older than 12 months (one paper restricted itself to cats over 12 months of age)[6] and one cat in these reports was 4 years



2-1. Femoral head, Maine Coon cat. Multifocally, surrounded by abundant cartilaginous matrix are few irregular clusters of chondrocytes (arrows). (H&E 200X)

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old and still had several open physes.[8] Most of these cats are castrated over-weight males and have other growth plates open well beyond the age expected for normal closure. One study reported the contralateral physis open in 13 of 18 cats with slipped physes of the femoral head.[6] Many of these cases of fracture of the physis of the femoral head in cats older than 12 months appear not to be associated with trauma. This is similar to the condition in the pig for the physis of the femoral head which is considered a form of osteochondrosis (epiphysiolysis).[4] In the pig however, the lesions develop mostly between ages 6-18 months. At 18 months, skeletal maturity is reported to be reached.[4]

The classification of this lesion as a dysplasia in cats, appears to be appropriate but the disorganization and hypocellarity seem more likely to be secondary to failure to properly close than a primary chondrodysplasia of the growth plate. The conclusion that the growth plates WERE normal during growth is supported by the fact that the cats appear to have reached normal skeletal growth with normal appearing skeletons within normal time. Since the signals for longitudinal growth have apparently appropriately ceased in these cats but the signals for closure have either not been recognized or sent, it is understandable that the chondrocytes remaining in the non-functional growth plate would not have their normal arrangement and density. Likely the persistence of the plate and not its abnormal arrangement and density of chondrocytes is predisposing it to slip with minimal trauma in these heavy fully grown cats.

AFIP Diagnosis: Femoral head: Dysplasia and fracture of physis, Maine Coon (*felis domesticus*), feline.

Conference Comment: Feline physeal dysplasia is characterized by the observation of irregular clusters of chondrocytes that are separated by abundant matrix on both the epiphyseal and the metaphyseal side of the physeal cartilage cleavage site.[1,2] This is in contrast to a traumatic fracture, in which the chondrocytes retain their linear arrangement on both sides of the fracture site.[2]

Although the underlying cause of feline physeal dysplasia is not known, it has been associated with various factors including genetics, nutrition, obesity, endocrine imbalances, and other factors.[1] Due to causing a delay in physeal closure, neutering has been previously considered associated with feline physeal dysplasia[6], although in more recent literature this observation has been challenged.[8] It is not known if the association with obesity is due to the increased stresses placed on the physis from the additional weight, causing failure under conditions of minimal trauma, or if there is an underlying endocrine abnormality that results in both obesity as well as a weakened physis.[2]

Epiphysiolysis in pigs is a manifestation of osteochondrosis, which has many clinical manifestations. The growth plate in affected pigs is usually characterized by a focal failure of endochondral ossification in which retained cartilage extends into the metaphysis.[2,12] The chondrocytes of the cartilage core usually maintain their normal alignment. This differs from feline physeal dysplasia where the entire physis is usually affected, and the physis consists of irregular clusters of chondrocytes that have lost their normal alignment.[2]

The Salter-Harris classification system has been used to classify fractures of the growth plate in animals. In this system, fractures are divided into five types based on their location **(table 2-1).**

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http://vet.osu.edu/biosciences.htm

References:

1. Burke J: Physeal dysplasia with slipped capital femoral epiphysis in a cat. Can Vet J 44:238-239, 2003
2. Craig LE: Physeal displasia with slipped capital femoral epiphysis in 13 cats. Vet Pathol 38:92-97, 2001
3. Culvenor JA, Black AP, Lorkin KF, Bradley WA: Repair of femoral capital physeal injuries in cats – 14 cases. Vet Comp Orthop Traumatol 9:182-185, 1996
4. Dewey CE: Disease of the nervous and locomotor

Table 2-1. Salter-Harris classification system[13]

Type 1	Fracture through the physis without involvement of the epiphysis or metaphysis
Type 2	Fracture involving the metaphysis and extending into the physis
Type 3	Fracture involving the epiphysis and extending into the physis
Type 4	Fracture involving the epiphysis and metaphysis going through the physis
Type 5	Compressive fracture of the physis, crushing the growth plate

systems. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 92-95. Blackwell Publishing, Ames, IA, 2006
5. Fischer HR, Norton J, Kobluk CN, Reed AL, Rooks RL, Borostyankoi F: Surgical reduction and stabilization for repair of femoral capital physeal fractures in cats: 13 cases (1998-2002). J Am Vet Med Assoc 224:1478-1482, 2004
6. McNicholas Jr. WT, Wilkens BE, Blevins WE, Snyder PW, McCabe GP, Applewhite AA, Laverty PH, Breur GJ: Spontaeous femoral capital physeal fractures in adult cats: 26 cases (1996-2001). J Am Vet Med Assoc 221:1731-1736, 2002
7. Moores AP, Owen MR, Fews D, Coe RJ, Brown PJ, Butterworth SJ: Slipped capital femoral epiphysis in dogs. J Small Anim Pract 45:602-608, 2004
8. Newton AL, Craig LE: Multicentric physeal dysplasia in two cats. Vet Pathol 43:388-390, 2006
9. Peréz-Aparicio FJ, Fjeld TO: Femoral neck fractures and capital epiphyseal separations in cats. J Small Anim Pract 34:445-449, 1993
10. Smith RN: Fusion of ossification centers in the cat. J Small Anim Pract 10:523-530, 1969
11. Stubbs WP, Bloomberg MS, Scruggs SL, Shille VM, Lane TJ: Effects of prepubertal gonadectomy on physical and behavioral development in cats. J Am Vet Med Assoc 209:1864-1871, 1996
12. Thompson K: Bones and joints. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 136-145. Elsevier Limited, St. Louis, MO, 2007
13. Baillière's Comprehensive Veterinary Dictionary, Baillière Tindall, Philadelphia, PA, 1988

CASE III – 335-07 (AFIP 3074807).

Signalment: Five (four male, one female) young guinea pigs, *Cavia porcellus*

History: Five young guinea pigs from a pet shop developed fever, lethargy, joint swelling and reluctance to move over a period of a few weeks. Their diet had been a pelleted food formulated by the shop owner. Comparable signs had been observed in other guinea pigs in the colony in the preceding summer but in the intervening period all guinea pigs had appeared healthy.

Gross Pathology: Four male and one female guinea pigs were necropsied. All had subcutaneous and intramuscular hemorrhage involving the proximal hindlimbs and hemarthrosis of the stifle joints. In some animals, there was also hemorrhage into the elbow, carpal and tarsal joints. All other organs were grossly normal.

Histopathologic Description: There was both loose and organizing fibrin within the stifle joint and the synovium contained acute hemorrhage and edema, dark brown pigment consistent with hemosiderin, and moderate fibrosis. There was prominent synovial hyperplasia. The periosteum was lifted from the bone by edema and hemorrhage and there was hemosiderin present in the edematous tissue. The perimysium and fascial tissues also contain hemorrhage and severe **edema (fig. 3-1)** and a moderate degree of fibrosis. There was hemorrhage separating myofibers, and degeneration of myofibers.

There were no identifiable osteoclasts on the periosteal surface of the diaphysis. The endosteal surface hosted an adequate population of osteoblasts. There was replacement of the terminal plate and cancellous bone of the epiphysis with loose connective tissue containing residual osteoclasts. The growth plate cartilage was disorganized and lacked normal chondrocyte columns. In the primary **spongiosa (fig. 3-2)**, there was hemorrhage and necrosis,

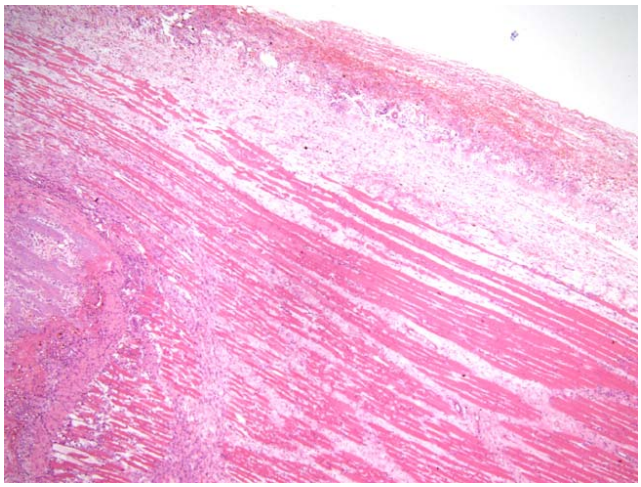
and predominance of spicules that were solely cartilage with no bony transformation. Active resorption of mineralized tissue was occurring and there were fragments of normal spongiosa present, surrounded by loose connective tissue, probably remnants of trabecular fractures. In the tibial metaphysis, there was a well developed scorbutic lattice.

The secondary spongiosa were normal.

Contributor's Morphologic Diagnoses: 1. Dysplasia of the proximal tibial growth plate and primary spongiosa with trabecular fractures, hemorrhage, necrosis, failure of ossification and development of a scorbutic lattice

2. Chronic active hemarthrosis of the stifle joint, with severe chronic active intra-periosteal, intramuscular and periarticular edema and hemorrhage

Etiological diagnosis: Chronic vitamin C deficiency (scurvy)



3-1. Bone (tibia and femur), guinea pig. Multifocally, the periosteum, adjacent muscle and fascial tissues are expanded and separated by hemorrhage, edema and fibrosis. (H&E 40X)

Contributor's Comment: Vitamin C deficiency, known as scurvy, is an ancient disease showing a modern recurrence. It is traditionally associated with sailors and long sea voyages, and was responsible for more deaths at sea in the 15th to 18th centuries than all other causes combined, accounting for up to 80% of the occupants of a ship on a long journey. It was not until the 20th century that a link was made between lack of fresh vegetables in the diet and the onset of "land scurvy".

Vitamin C (ascorbic acid, ascorbate) is water soluble and degraded by heat, ultraviolet radiation or free radical oxidation. Synthesis is widespread in nature, including micro-organisms and fungi. In those vertebrates which synthesize the vitamin, the site(s) of production is in the liver and/or kidneys. Fruit eating bats, red vented bulbul birds, guinea pigs, human and non human primates, most fish and insects lack gulonolactone oxidase to catalyze the last step of the synthesis, and require dietary intake as there is no storage within the body. Absorption occurs in the ileum via active transport.

The earliest signs of vitamin C deficiency are generally non specific, such as weakness, anorexia and weight loss. Guinea pigs fed on severely scorbutic diets will voluntarily decrease their intake and begin to lose weight after 2 weeks. After 3 weeks, serum $25OHD_3$, calcium and albumin levels are significantly reduced, bone mineral density and bone content are significantly lower than normal, and bone volume is reduced in long bones, with fewer and thinner trabeculae and a thinner growth plate. There is also bone loss with osteonecrosis, osteopenia and cortical thinning with periosteal proliferation.

The first histological signs in bone are flattening of osteoblasts and failure to lay down matrix. A lattice of vascularized, calcified cartilage is formed in the metaphysis and is not replaced by bone as it increases in thickness; vitamin C is required for the differentiation of osteoblasts from progenitors. Being relatively unresistant to mechanical forces, this "scorbutic lattice" develops numerous microfractures. Blood vessels of all bone regions dilate, with those of the metaphysis being particularly prominent. Active growth zones are severely hyperemic and microhemorrhages are common. Intercellular junctions between endothelial cells are wider in scorbutic vessels than those of normal animals.

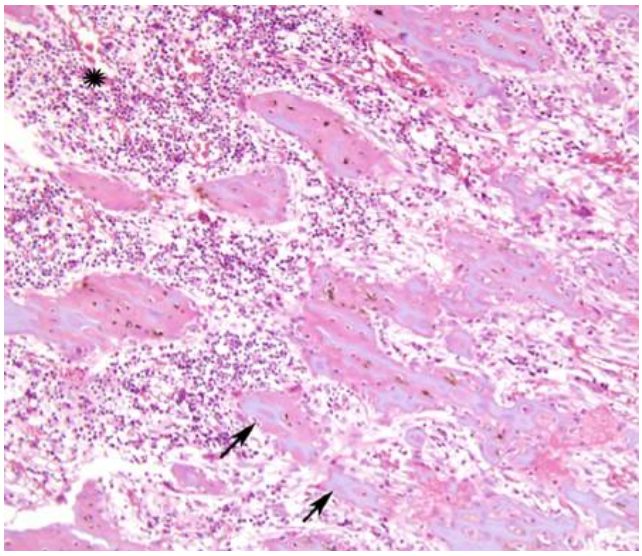
With time, hematopoietic tissue of the marrow is replaced by immature collagen-poor mesenchyme. In moderately advanced disease, vascularity of the bone is not a prominent feature but vascular fragility is increased. Chondrocyte columns of the growth plate become distorted and shortened as the disease progresses, the number of chondrocytes decreases and the growth plate becomes thin and uneven. Proliferation of spindle cells between the fibrous periosteum and the cortical bone surface thickens the periosteum of long bones, and metaphyseal infarction may occur.

Arthralgia and myalgia develop; eventually bleeding occurs into the joints due to damage to synovial vessels and microfracture of bone. Hemorrhage into stifle joints is among the most obvious signs of scurvy in guinea pigs.

There may also be non-hemorrhagic joint effusions. Hair loss and corkscrew hairs are reported; Vitamin C is important in the disulfide bonding of hair. Also reported are follicular hyperkeratosis, and pigmented ichthyosis, fibrosis of dental pulp, diarrhea and reproductive failure. Wound healing is delayed and incomplete. Conjunctival and intraocular bleeding are also common, and there is disruption of corneal epithelial and stromal organization with stromal vascularization in later stages.

Laboratory findings are nonspecific. Anemia is due less to bleeding than to the concomitant iron and folate deficiencies. Foods high in Vitamin C are also sources of folate and the deficiencies often coexist. Furthermore, Vitamin C increases the absorption of nonheme iron by reducing ferric iron in the stomach and enhances the amount of iron stored in ferritin. Guinea pigs with lowered levels of vitamin C but normal growth (phase 1 of scurvy) may have serum iron levels decreased to 50% of normal, and by the time clinical scurvy (phase 2) is evident, levels may be as low as 10 – 15% of normal. Intravascular hemolysis may also lower red cell counts. Leukopenia and hypoalbuminemia, a marker of malnutrition, are also common.

Response to treatment is rapid with most clinical signs reversed within a week of onset of adequate intake. In severely scorbutic guinea pigs, complete restoration of normal trabecular structure takes about 20 to 25 days.



3-2. Bone (tibia and femur), guinea pig. Multifocally within the primary spongiosa, there is retention of cartilaginous cores with a lack of ossification (arrows). Additionally, the marrow cavity is expanded by necrotic debris and hemorrhage (star). (H&E 100X)

Vitamin C is a major antioxidant, in co-operation with vitamin E and glutathione, and glutathione adminstration can delay the onset of clinical scurvy in guinea pigs. Persistant deficiency leads to hepatocyte apoptosis through endoplasmic reticulum stress as a result of its participation in oxidative protein folding. Oxidative injury in the absence of adequate vitamin has also been linked to motor neuron disease, demyelination of pyramidal tracts and consequent muscular atrophy.

Vitamin C is also an electron donor. It interacts with proline oxidase and lysine oxidase in the hydroxylation of procollagen, in two hydroxylation steps in the production of carnitine, which promotes transport of long chain fatty acids into mitochondria and assists the flux of substrates into the TCA cycle, and with β -monoxygenase in the conversion of dopamine to noradrenaline.

The interactions leading to scurvy are not entirely understood. Collagen related signs were thought to be due to failure of hydroxylation of pro-collagen proline and lysine with consequent failure of cross-linking, leading to fibril instability. However, starved animals with vitamin C supplementation show the same reduction in collagen production as scorbutic animals as a result of inhibition of insulin-like growth factor (IGF) by binding protein (IGFBP) induction, and deficiency of collagen type IV and elastin leads to defects in blood vessels with consequent hemorrhage in both scorbutic and vitamin supplemented starved animals. Similar effects of IGFBP on collagen are seen in bone, but the decreases in alkaline phosphatase activity are independent of the fasting effect. In cartilage, type II collagen production drops in the early stages of deficiency, but stabilizes to around 50% of normal.

The poor wound healing of scorbutic animals, however, is not due to inhibition of proline hydroxylation or induction of IGFBPs. Vitamin C is known to promote wound healing following irradiation through stimulation of collagen synthesis and deposition, and increased fibroblast density and tissue vascularity. Poor wound healing in scurvy may therefore be a consequence of failure of interstitial procollagen gene expression and blood vessel formation.

AFIP Diagnosis: Bone, tibia and femur: Osteochondrodysplasia, scorbutic, with lack of normal primary spongiosa, osteopenia, microfractures, and subperiosteal hemorrhage, guinea pig (*Cavia poricellus*), rodent.

Conference Comment: The contributor gives an excellent overview of Vitamin C/Ascorbic acid deficiency in

general and in the guinea pig in particular. Ascorbic acid is an important antioxidant and reducing agent. It is required for the hydroxylation of proline and lysine, a process that is essential in the formation of collagen.[5] Functional failure of these enzymes results in formation of collagen fibrils that are not cross-linked and lack tensile strength, leading to blood vessel fragility and poor wound healing.[5] Lesions associated with scurvy, such as subperiosteal, subcutaneous, intramuscular and gingival hemorrhages, reflect this defect in collagen synthesis.

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References:
1. Fain O: Musculoskeletal manifestations of scurvy. Joint Bone Spine 72:124-128, 2005
2. Mahmoodian F, Peterkofsky B: Vitamin C deficiency in Guinea pigs differentially affects the expression of type IV collagen, laminin, and elastin in blood vessels. J Nutr 129:83-91, 1999
3. Olmedo JM, Yiannias JA, Windgassen EB, Gornet MK: Scurvy: A disease almost forgotten. Int J Dermatol 45:909-913, 2006
4. Padayatty SJ, Levine M: New insights into the physiology and pharmacology of vitamin C. CMAJ 164:353-355, 2001
5. Thompson K: Bones and joints. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 136-145. Elsevier Limited, St. Louis, MO, 2007

CASE IV - H06-0308C (AFIP 3025649).

Signalment: 2-day-old, Landrace piglet, male, *Sus scrofa*, porcine

History: At a piggery with 500 sows a farm worker noted that there were one or two piglets in rare litters over the past few weeks that had bilateral thickened forelimbs. The piglets had a stilted forelimb gait. The sows were multiparous and had not had litters previously with this condition. The piglets did not survive more than a few days after birth.

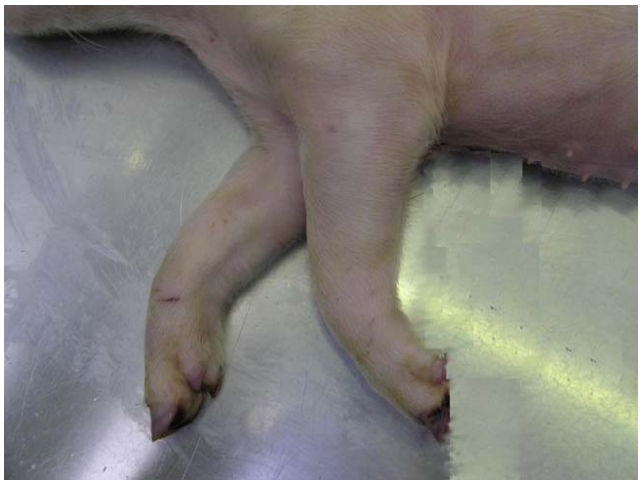
Gross Pathologic Findings: One piglet was submitted for post mortem examination. The forelimbs were bilaterally **thickened (fig. 4-1)**. On dissection the radius and ulna of both limbs were thickened uniformly through the diaphysis. There were no other significant post mortem findings.

Histopathologic Description: Ulna and Radius, diaphysis: There is a cross section of radius, ulna and attached fibrous tissue and skeletal muscle. The cortex of both bones is distended by interconnecting trabeculae of woven bone radiating from the periosteum at right angles to the existing cortical lamellar bone. Within the interstices of the new bone is myxodematous tissue without haemopoietic cells. The periosteum is irregularly expanded by polygonal to plump spindle cells set in eosinophilic fibrillar stroma extending from mature uniform fibrous stroma. The central cavity of the bone consists of multiple aggregates of haemopoietic cells set between trabeculae of bone. Within the connective tissue surrounding the bones there is separation of the adventitia of small arterioles from the attached collagen by proteinaceous material consistent with oedema.

Contributor's Morphologic Diagnosis: Ulna and radius: Hyperostosis

Contributor's Comment: Hyperostosis is a rare recessive autosomal disease seen in newborn piglets.[5] Most commonly these piglets die due to malnutrition, starvation or cardiac insufficiency.[5] The condition has been reported in Landrace and Duroc pigs.[1,3] Generally hy-

4-1. Landrace piglet. The forelimbs are diffusely thickened. Photograph courtesy of the Department of Veterinary Biology and Biomedical Sciences, School of Veterinary and Biomedical Sciences, Murdoch University, South St, Murdoch WA 6150, Australia



perostosis affects the forelimbs, most commonly the radius and ulna.[3]

The radioulnar region is thickened and may be twice normal diameter. The overlying skin is hyperaemic. At necropsy there is enlargement of the soft periosseous tissues mostly at the cranial surface of the limbs from proximal end of the radius down to and surrounding the metacarpal bone distally. Fibrous tissue may invade and partially fuse with muscles.[3]

Microscopically there are radiating osseous spicules associated with marked hyperplasia of periosteal osteoblasts. They are numerous, large and can appear as syncytium. Normal cortical bone is lamellar and forms concentric osseous plates consistent with rapid periosteal apposition. Osseous trabeculae of woven bone are orientated radially in relation to the medullary cavity.[3]

The pathogenesis of congenital hyperostosis is not known, however there are two suggested mechanisms reported in the piglet. These include 1) disruption of the growth of bone at the ossification groove of Ranvier or 2) local circulatory abnormality.

Dalton et al[3] proposed that radioulnar hyperostosis may be the result of an initial lesion situated at the anchor site of the periosteum to the epiphysis at the level of the perichondrial ossification groove of Ranvier. This true separation could be the cause of the fine supernumerary trabeculae of woven bone. The groove of Ranvier is an ossification groove (a component of the perichondrial ring supporting the zone of provisional calcification) that supplies chondrocytes to the physis for diametric growth of the foetal bone and also fibrous attach of the periosteum to the epiphysis.

A second study of piglets with hyperostosis, conducted by Roels et al[4] demonstrated distinct circular constrictions in the proximal antebrachial region of the median artery, in conjunction with the consistent finding of oedema within the connective tissue surrounding the thickened bone suggesting hypertension. In affected piglets the initial segment of the median artery (ie proximal antebrachial region) showed distinct circular constrictions (not seen in controls) suggesting acute hypertension. There was extensive smooth muscle fibre proliferation, intimal fibrosis and fibrinoid necrosis of tunica media and less narrowing of lumen in small arteries and arterioles of upper dermis.

AFIP Diagnosis: Bone, radius and ulna: Hyperostosis, periosteal, circumferential, severe, Landrace (*Sus scrofa*), porcine.

Conference Comment: Although not proven, hyperostosis is presumed to be an autosomal recessive inherited disease that has been described in Landrace swine.[1,3,5] The characteristic histology lesion associated with hyperostosis is proliferation of subperiosteal, radiating trabeculae of woven bone extending from the surface of apparently normal cortical bone, covered by a thickened periosteum.[5]

A similar hyperostotic condition has been reported in a single West Highland White Terrier dog, in which new bone formation involved the pelvis, scapulae, humeri, ulnae, femora, radii, and tibiae.[5]

The deeper, older portion of the examined cortex is formed of woven bone. This type of bone is formed in areas in which a support structure needs to be put in place quickly, such as in the developing fetus or at sites of fracture repair, inflammation, or neoplasia. It consists of collagen fibers within the bone matrix that are arranged in a haphazard interwoven fashion.[5] These haphazard arrangements are usually replaced with the more structurally sound lamellar bone during skeletal maturation.[5] In young rapidly growing animals, especially ruminants, a different type of lamellar bone is deposited along the surfaces of long bones. This type of bone is called laminar bone and consists of laminar arrays rather than the Haversian system seen more commonly in lamellar bone.[5]

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References:

1. Dalton JR: Hyperostosis in newborn pigs. Vet Rec 131:107-108, 1992
2. Dewey CE: Disease of the nervous and locomotor systems. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., p. 88, Blackwell Publishing, Ames, IA, 2006
3. Doize B, Martineau GP: Congenital hyperostosis in piglets: a consequence of a disorganization of the perichondrial ossification groove of Ranvier. Can J Comp Med 48:414-419, 1984
4. Roels S, Simoens P, Ducatelle R: Localised arteriosclerotic changes in congenital hyperostosis in pigs. Vet Rec 139:446-447, 1996
5. Thompson K: Bones and joints. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie

MG, 5th ed., vol. 1, pp. 6-7, 40-41, 106-110. Elsevier Limited, St. Louis, MO, 2007
6. Weisbrode SE: Bones and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1066-1067. Elsevier, St. Louis, MO, 2007

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 12

2 January 2008

Moderator:

Bridget Lewis, DVM, DACVP

CASE I – A7-005112 (AFIP 3069593).

Signalment: Ten-week-old, female, Border collie dog, *Canis familiaris*.

History: The 10-week-old puppy was euthanized and submitted for necropsy with a history of ascending progressive muscle weakness, neutrophilic leukocytosis, non-regenerative anemia, and elevated alkaline phosphatase levels.

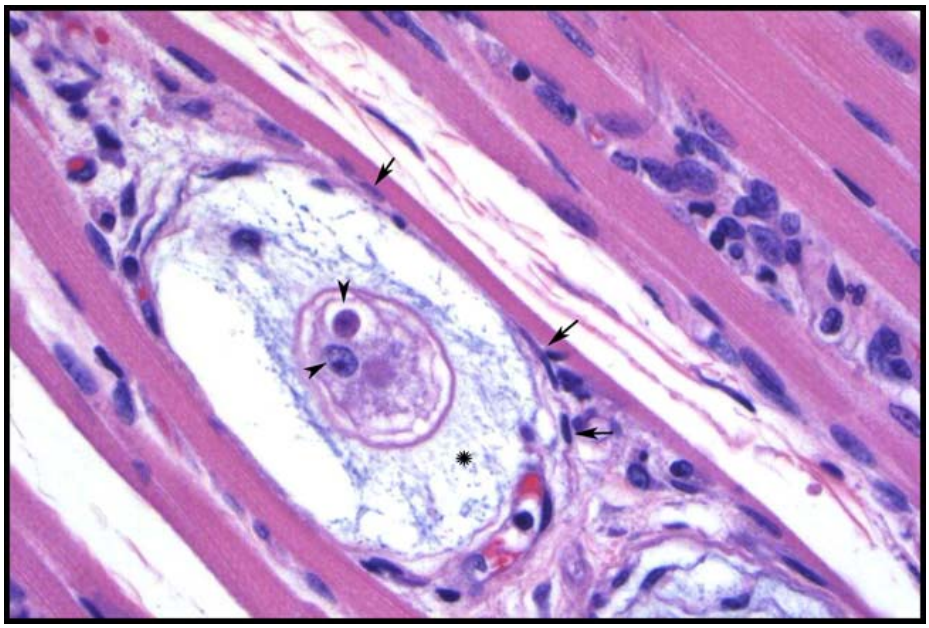
Gross Pathology: The carcass was in good physical condition and post mortem autolysis was mild. Mucopurulent ocular discharge and crusts were present bilaterally. Mucus membranes, subcutaneous tissues and viscera were uniformly pale. Small numbers of petechia were present in the peritoneum overlying the ventral abdominal muscles. A diffuse copper tint was present throughout the liver parenchyma. Intestinal contents were bright yellow and watery. No additional significant gross abnormalities were identified.

Laboratory Results: FA negative for canine distemper and infectious canine hepatitis viruses.

Histopathologic Description: Skeletal muscle: Endomysial areas are infiltrated by scattered small mixed populations of neutrophils and macrophages. Combinations of macrophages and neutrophils also commonly form multifocal, large, vascularized nodular aggregates containing myofiber fragments. Within these pyogranulomas, macrophages often possess an eccentric nucleus due to the presence of single, small, round, amphophilic, intracytoplasmic **parasite (fig. 1-1)**. Scattered throughout are numerous, round to oval, 50-100 µm structures containing a central, finely granular, pale eosinophilic core, with 1-2 ill-defined nuclei. Surrounding the core are multiple layers of lacy, pale basophilic material and a thin outer wall of flattened cells with elongated nuclei ("onion cysts"). These structures elicit little or no direct inflammatory response. Present in some sections are similarly sized structures (meronts) with a large pale eosinophilic core lined peripherally by either marginated nuclear material or elongated bodies (merozoites) with deep basophilic nuclei. Blood vessels contain increased numbers of neutrophils and macrophages.

Contributor's Morphologic Diagnosis: Skeletal muscle: Myositis, pyogranulomatous, widespread, chronic, severe, with multifocal intralesional *Hepatozoon americanum* zoites and meronts

Contributor's Comment: The history and histopathologic findings are consistent with American canine hepatozoonosis, a debilitating, tick-borne disease of dogs in the south-central and southeastern United States. The



1-1. Skeletal muscle, Boxer. Expanding and separating myocytes are 50 to 100 micrometer intracellular meronts, characterized by a central finely granular eosinophilic core with 1-2 nuclei (arrowheads). These are surrounded by layers of a myxomatous to lacy, pale basophilic material (star) and further bounded by a rim of compressed cells with flattened nuclei (arrows). Photomicrograph courtesy of the Department of Pathology, College of Veterinary Medicine, The University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602

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diagnosis was confirmed by PCR and sequence analysis. Discovered in 1978, *H. americanum* was advanced as a distinct species from its Old World counterpart, *H. canis*, in 1997.[1,8] At least 46 Hepatozoon species infect mammals and more than 120 infect snakes. Transmission of the apicomplexans to their vertebrate intermediate hosts occurs through hematophagous invertebrates. Like their *Plasmodium* and *Babesia* spp. relatives, many hepatozoons occur in erythrocytes. However, most of the mammalian parasites infect leukocytes and use acarines as definitive hosts or vectors. Interestingly, *H. americanum* is spread by the ingestion of infected ticks rather than through their feeding activities.[3]

It is believed that *H. americanum* crossed into canids from unknown vertebrates only recently, whereas *H. canis* has a long history with dogs. The vector for *H. americanum* is *Amblyomma maculatum*, gamonts are found in monocytes, and merogony occurs in host cells lodged between striated muscle fibers. In contrast, *Rhipicephalus sanguineus* is a primary vector for *H. canis*, the neutrophil is the favored host cell, and merogony takes place in a wide variety of tissues. Meronts of *H. americanum* develop most consistently in striated muscle within "onion skin" cysts created by layers of host secreted mucopolysaccharide. No similar lesion is associated with *H. canis*, which rarely occurs in muscle. Disease from *H. americanum* is more severe than that seen with *H. canis*. Developing organisms are shielded from the dog's immune system, but elicit intense local pyogranulomatous inflammation, systemic reaction, and overt illness when merozoites are released. Local lesions evolve into vascular granulomas. Diseased dogs are often febrile and lethargic, with stiff gaits, mucopurulent ocular discharge, and atrophy of the head muscles. Clinicopathological findings include mature neutrophilia, increased alkaline phosphatase, and hypoalbuminemia. Periosteal bone proliferation may be seen radiographically and at necropsy.[2,3,5]

AFIP Di agnosis: Skeletal muscle: Myositis, pyogranulomatous, multifocal, moderate, with fibrosis, and intracellular protozoal cysts and zoites etiology consistent with *Hepatozoon americanum*, Border collie (*Canis familiaris*), canine.

Conference Comment: The disease course of *Hepatozoon canis*, the causative agent of Old World hepatozoonosis, is generally mild with low levels of parasitemia, but severe illness occurs occasionally with nearly 100% of neutrophils containing parasites.[1,2] In the case of *Hepatozoon americanum* infection, the causative agent of American canine hepatozoonosis, parasitemia remains very low, and often less than 0.1% of leukocytes are infected, even in cases of severe illness.[1,2]

When *H. americanum* was first identified, *Rhipicephalus sanguineus* was thought to be the vector for transmission, as it is a known vector for *H. canis*. Even current literature has implied the role of *R. sanguineus* in transmission of *H. americanum*.[6] In fact, the primary vector for *H. americanum* appears to be *Amblyomma maculatum*, as *R. sanguineus*, *Dermacentor variabilis*, and *Amblyomma americanum* appear refractory to infection.[3,8]

In addition to the lesions within skeletal and cardiac mus-

cle, *H. americanum* is known to cause severe periosteal bone proliferation of proximal limbs.[3] Flat bones can be markedly, but less commonly, affected, and distal limbs are often spared.[3] The periosteal reaction shares common features with hypertrophic osteopathy in dogs.[3] The pathogenesis of the reaction is unknown, as there are no parasites identified with the bone lesions, and the inciting factors have not been identified.

Severe muscle wasting, especially of the temporal muscles, is also a feature of *H. americanum*.[3]

We thank Dr. C. H. Gardiner, PhD, veterinary parasitology consultant to the AFIP, for his review of this case.

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References:

1. Baneth G, Barta JR, Shkap V, Martin DS, MacIntire DK, Vincent-Johnson N: Genetic and antigenic evidence supports the separation of *Hepatozoon canis* and *Hepatozoon americanum* at the species level. J Clin Microbiol 38:1298-1301, 2000
2. Baneth G, Mathew JS, Shkap V, MacIntire DK, Barta JR, Ewing SA: Canine hepatozoonosis: Two disease syndromes caused by separate *Hepatozoon* spp. Trends Parasitol 19:27-31, 2003
3. Ewing SA, Panciera RJ: American canine hepatozoonosis. Clin Microbiol Rev 16:688-697, 2003
4. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., p. 4. Armed Forces Institute of Pathology, Washington, DC, 1998
5. Panciera RJ, Mathew JS, Cummings CA, Duffy JC, Ewing SA, Kocan AA: Comparison of tissue stages of *Hepatozoon americanum* in the dog using immunohistochemical and routine histologic methods. Vet Pathol 38:422-426, 2001
6. Valentine BA, McGavin MD: Skeletal muscle. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1035-1037. Elsevier, St. Louis, MO, 2007
7. Van Vleet JF, Valentine BA: Muscle and tendon. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 270-271. Elsevier Limited, St. Louis, MO, 2007
8. Vincent-Johnson NA, MacIntire DK, Lindsay DS, Lenz SD, Baneth G, Shkap V, Blagburn BL: A new hepatozoon species from dogs: Description of the causative agent of canine hepatozoonosis in North America. Am J Parasitol 83:1165-1172, 1997

CASE II – Pfizer-05/Case2 (AFIP 3024116).

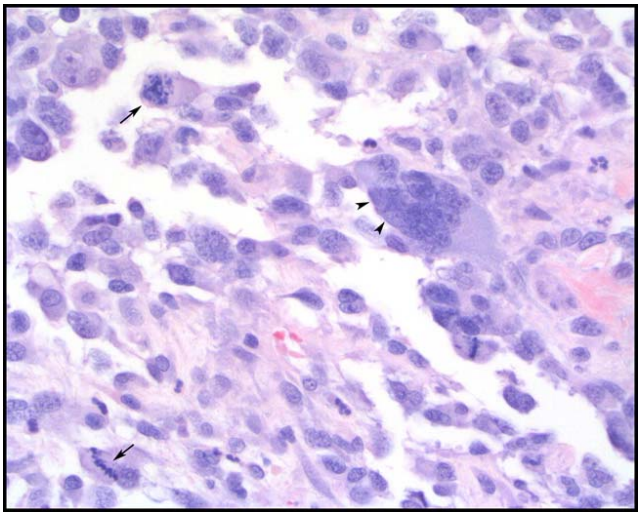
Signalment: Five-year-old, intact, beagle dog (*Canis familiaris*)

History: Dog had diarrhea for 2-3 days with unproductive vomiting starting on the third day. At the onset of vomiting a physical exam revealed a palpable abdominal mass and the dog was euthanized.

Gross Pathology: There were numerous multifocal 1-3 mm, white masses throughout the mesentery with 3-5, 3-10 mm white masses on right renal capsule.

Laboratory Results: Pan Cytokeratin (+), Vimentin (+), S-100 (-), SMA (-), Desmin (-)

Histopathologic Description: Overlying the serosa and throughout the mesentery are hypercellular papillary projections and disorganized mats of cells (forming layers and clumps) that overlay cores of fibrovascular stroma. The plump, pleomorphic, neoplastic mesothelial cells have indistinct cell margins, variable amounts of lightly eosinophilic cytoplasm, round nuclei with stippled chromatin and 1 nucleolus. There are 4-7 mitotic figures per high power field. Throughout the neoplastic tissues there



2-1. Mesentery, beagle. Neoplastic cells display marked anisocytosis and anisokaryosis with occasional multinucleate giant cells (arrowheads) and frequent bizarre mitoses (arrows). Photomicrograph courtesy of Pfizer Groton (PRGD), Groton, CT —>

is anisokaryosis, bizarre mitoses, **multinucleate g iant cells (fi g. 2-1)**, apoptotic cells and large cells with fragmented nuclei. Neoplastic cells are pancytokeratin and vimentin positive while negative for smooth muscle actin (SMA), desmin, and S-100.

Contributor's Morphologic Diagnosis: Biphasic mesothelioma

Contributor's Comment : Mesotheliomas are rare primary tumors of the squamous epithelium lining the pericardial, peritoneal, and pleural cavities. They rarely metastasize and are locally invasive.[8] Though they are most commonly reported in calves and dogs[8] they are also found in rodents[10,18], fish[6], horses, cattle, sheep, cats and pigs.[2] Peritoneal mesotheliomas are most frequent in a majority of species with the exception of pleural mesotheliomas in pigs.[2] Clinical signs vary with location. Pericardial signs include cardiac tamponade and congestive heart failure.[8] Pulmonary tumors may cause pleural effusion and dyspnea[1] while peritoneal tumors may cause ascites.[5]

Mesotheliomas in calves and other neonates are suspected to be due to spontaneous developmental disturbances.[9] Dog and human mesotheliomas linked to mineral fibers such as asbestos and erionite exposure may be due to physical irritation of mesothelium, disruption of the mitotic process leading to chromosome damage, increased reactive oxygen intermediate generation and/or persistent kinase-mediated signaling.[9,14] Mesotheliomas are also reported to be linked to exogenous hormones, metals and hydrocarbons as well as viruses (including SV40 virus).[6]

Mesotheliomas have three histological presentations with epitheloid, sarcomatous and biphasic forms. Epitheloid forms often have papillary structures lined by cuboidal basophilic mesothelial cells while sarcomatous forms have spindle cells and large anisocytotic cells with abundant eosinophlic cytoplasm and distinct cell margins.[5] Biphasic forms have characteristics of both.

Differential diagnosis include serous carcinomas and normal activated / reactive mesothelium. Differentiation of these three entities can be challenging. Reactive mesothelium usually is not invasive and has less cytologic atypia while carcinomas form acinar structures.[8] Immunohistochemistry is useful in differentiating mesotheliomas from carcinomas. Mesotheliomas often stain positive for vimentin, cytokeratin[2], LP 34 and also stain for tumor glycoprotein BER EP4, S-100 and HMB 45.[1] They stain negatively for carcinoembryonic antigen, CD15 (LEU M1).[1] Electron microscopy of mesotheliomas often reveals long, slender, branching and undulate microvilli on apical surfaces while serous carcinomas have fewer, variably lengthed straight microvilli.[11]

AFIP Diagn osis: Fibroadipose tissue, mesentery (per contributor): Mesothelioma, Beagle (*Canis familiaris*), canine.

Conference Com ment: Mesotheliomas are tumors of low grade malignancy that usually metastasize by exfoliation and implantation of neoplastic cells.[1] In humans mesotheliomas have been linked to exposure to asbestos fibers, and there is suggestive evidence in animals that a similar link occurs as well. Ferruginous bodies, asbestos fibers coated by ferritin and an amorphous protein[4], have been found in the lungs of dogs with mesotheliomas.[3,4] In addition to asbestos fiber exposure, mesotheliomas are reported to arise in response to non-fibrous and fibrous non-asbestiform agents.[8]

Cytologic diagnosis of mesotheliomas is difficult as neoplastic mesothelial cells are similar in appearance to reactive mesothelial cells in non-neoplastic effusions.[8] Immunohistochemistry can be useful in distinguishing mesotheliomas from other epithelial or non-epithelial neoplasms, although in some instances electron microscopy (EM) is necessary. Characteristic EM features of mesothelial cells include long slender, branching and undulating microvilli on all cell surfaces and prominent desmosomes.[1,11] The cytoplasm contains numerous bundles of tonofilaments that are arranged circumferentially around the nucleus.[8]

F344 rats are predisposed to mesotheliomas in the tunica vaginalis of the testes with occasional subsequent implantation on the serosal surfaces of the peritoneum.[1,10,12]

Other neoplasms that have positive immunoreactivity for both cytokeratin and vimentin include meningioma [8], chordoma[8], clear cell adnexal carcinoma[16], ciliary body adenoma/adenocarcinoma[7], anaplastic carcinoma[8], synovial cell sarcoma (biphasic)[8], and carcinosarcoma.[15]

Contributor: Pfizer Groton (PRGD), Groton, CT

References:

1. Armed Forces Institute of Pathology: P-N06 – mesothelioma – pleura – ox. Online Systemics. At: http://vetpath4.afip.org/systemic/show_page.php?id=577 2005
2. Barker IK: The peritoneum and retroperitoneum. In: Pathology of Domestic Animals. eds. Jubb KVF, Ken-

nedy PC, Palmer N, pp. 234. Academic Press Inc., San Diego, CA, 1993
3. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 294. Elsevier Limited, St. Louis, MO, 2007
4. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 578. Elsevier Limited, St. Louis, MO, 2007
5. Geninet C, Bernex F, Rakotovao F, Crespeau FL, Parodi AL, Fontaine JJ: Sclerosing peritoneal mesothelioma in a dog – a case report. J Vet Med A Physiol Pathol Clin Med 50: 402-405, 2003
6. Ilgren EB, Browne K: Background incidence of mesothelioma: animal and human evidence. Regul Toxicol Pharmacol 13:133-149,1991
7. Lieb WE, Shields JA, Eagle RC Jr, Kwa D, Shields CL: Cystic adenoma of the pigmented ciliary epithelium. Clinical, pathologic, and immunohistopathologic findings. Ophthalmol 97:1489-1493, 1990
8. Meuten DJ: Tumors in Domestic Animals, 4th ed., pp. 227-233, 477-478, 594, 721, 729. Blackwell Publishing, Ames, IA, 2002
9. Misdorp WP: Tumours in calves: comparative aspects. J Comp Pathol 127:96-105, 2002
10. Mitsumori K, Elwell MR: Proliferative lesions in the male reproductive system of F344 rats and B6C3F1 mice: incidence and classification. Environ Health Perspect 77:11-21, 1988
11. Ordonez NG: The diagnostic utility of immunohistochemistry and electron microscopy in distinguishing between peritoneal mesotheliomas and serous carcinomas: a comparative study. Mod Pathol 19:34-48, 2006
12. Percy DH, Barthold SW: Rat. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 174-176. Blackwell Publishing, Ames, IA, 2007
13. Robinson BW, Musk AW, Lake RA: Malignant mesothelioma. Lancet 366:397-408, 2005
14. Sánchez J, Buendía AJ, Vilafranca M, Velarde R, Altimara J, Martínez CM, Navarro JA: Canine carcinosarcomas in the head. Vet Pathol 42:828-833, 2005
15. Schulman FY, Lipscomb TP, Atkin TJ: Canine cutaneous clear cell adnexal carcinoma: histopathology, immunohistochemistry, and biologic behavior of 26 cases. J Vet Diagn Invest 17:403-411, 2005
16. Wilson DW, Dungworth DL: Tumors of the respiratory tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 398. Blackwell Publishing, Ames, IA, 2002
17. Wilson TM, Brigman G: Abdominal mesothelioma in an aged guinea pig. Lab Anim Sci 32:175-176, 1982

CASE III – 06-0115 (AFIP 3054023).

Signalment: 30-year-old, male, American flamingo (*Pheonicopterus ruber*)

History: Self-isolation from flock. On presentation, flamingo was weak and thin. Supportive care was given, but bird was found dead two days later.

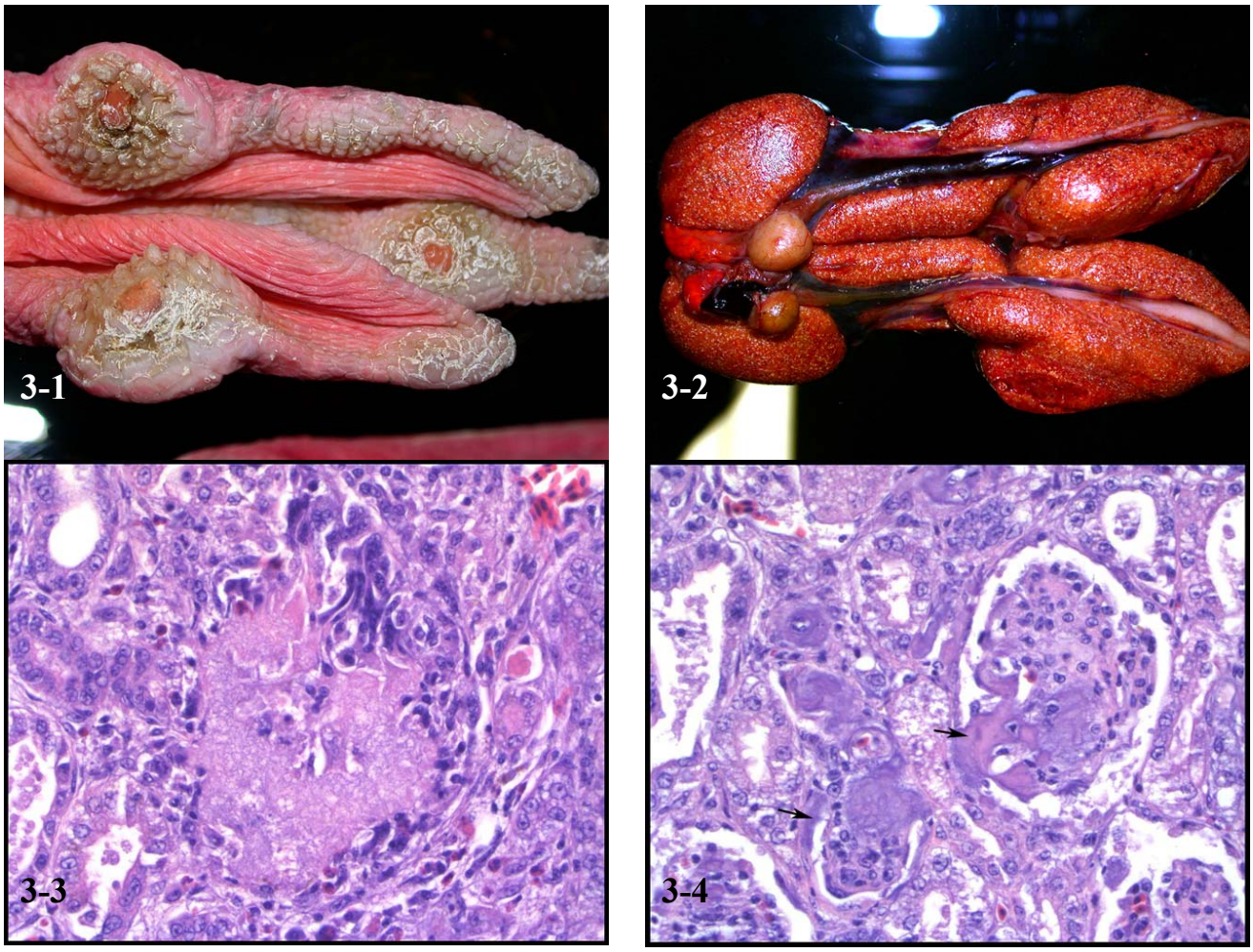
Gross Pathology: The plantar aspects of both **feet (fig. 3-1)** have thickened/calloused 1-1.5 cm diameter lesions with a small central crater over the proximal joints of digits one, two, and three. Associated joints contain cloudy, viscous fluid. The abdominal air sacs, primarily on the right side, have pinpoint gritty, white foci. The liver is firm and subtly mottled yellow brown to red brown, primarily on the edges and right lobe. On section, there are multifocal pinpoint, cream-to-yellow nodules in the hepatic parenchyma. The **kidneys (fig. 3-2)** are discolored yellow to light brown with pinpoint, gritty, palely yellow foci. The ureters are prominent.

Laboratory Results:
Joint fluid cytology at necropsy: Articular gout.
CBC revealed a leukocytosis (20.6 k/uL) with a heterophilia, lymphopenia and monocytosis. Hyperfibrinogenemia (700mg/dl), hypoglycemia (98mg/dl), hyperphosphatemia (11.2mg/dl), and hyponatremia (120mEq/L) were evident on CP. CP also showed elevations of uric acid (99.6mg/dl), CPK (3770 IU/L), and LDH (511 IU/L).

Histopathologic Description: Kidney: Multifocally, the renal architecture is disrupted by tubulocentric gouty **tophi (3-3)** that are composed of an inner, radiating array of eosinophilic, acellular spicules surrounded by macrophages and multinucleated giant cells with few heterophils. Rarely the centers of these tophi are mineralized. Within glomerular loops and occasionally expanding tubular basement membranes, there are lakes of homogenous, eosinophilic material **(amyloid-like) (fig. 3-4)**. There is a moderate increase in interstitial fibrosis and tubules are mildly ectatic and tortuous. Multifocally, lymphocytes and fewer macrophages are present in the interstitium.

Contributor's Morphologic Diagnoses: 1. Kidney: Gout, visceral, multifocal, marked, with tubular degeneration and necrosis, and chronic, interstitial nephritis, American flamingo (*Phoenicopterus ruber*)
2. Kidney: Amyloidosis, glomerular and interstitial, American flamingo (*Phoenicopterus ruber*)

Contributor's Comment: Of the many avian species,



3-1. Plantar surface of foot, American flamingo. Multifocally, the joints are markedly swollen with central craterform ulcers.
3-2. Kidneys, American flamingo. Multifocally, the kidneys have a military pattern of white to yellow pinpoint foci of mineralization.
3-3. Kidney, American flamingo. Multifocally, expanding renal interstitium, separating, surrounding and replacing uriniferous tubules and glomeruli are radiating acicular gouty tophi surrounded by granulomatous inflammation.
3-4. Kidney, American flamingo. Multifocally markedly expanding and replacing glomerular tufts, tubular and glomerular basement membranes and bowman's capsule is an amorphous, eosinophilic, acellular homogenous material (amyloid-like material) (arrows)

Gross photgraphs and photomicrograph courtesy of the Department of Pathology, National Zoological Park, 3001 Connecticut Ave NW, Washington DC, 20008

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waterfowl are most often affected by amyloidosis. Though mammals produce over 15 kinds of amyloid protein, birds are known only to deposit amyloid AA.[1] Accumulation of this protein in the extracellular spaces is a result of chronic antigenic stimulation and may be associated with such diseases as bumblefoot, chronic enteritis, or pneumonic aspergillosis. Deposition of amyloid AA occurs most frequently in the liver, spleen, and intestine, but may occur in any organ of the body.

Gout occurs in two forms: articular and visceral.[1] Articular gout is a chronic process with an uncertain etiology that results in deposition of urates and resultant granulomatous inflammation in the joint spaces, most

Extracted from Diseases of Poultry, Crespo, et al.[1]

	Visceral Gout	Articular Gout
Onset	Usually acute	Usually chronic
Frequency	Common	Rare
Kidney Lesions	Almost always involved, grossly abnormal, with white chalky deposits	May become involved with dehydrations
Joints	May or may not be involved	Always involved, especially the feet
Pathogenesis	Failure of urate excretion (renal failure)	Possibly due to metabolic defect in secretion of urates by kidney tubules
Causes	Dehydration Nephrotoxicity Infectious agents Vitamin A deficiency Urolithiasis Neoplasia Immune mediated glomerulonephritis Others	Genetics High protein diet Others

often of the feet. It is proposed that genetics or a high protein diet may contribute to this condition. Visceral gout occurs more acutely than articular gout and is characterized by small gouty tophi within the renal parenchyma and on the surface of the liver, heart and air sacs. In severe cases, gouty tophi may be seen within the liver and spleen parenchyma as well. These tophi incite little to no inflammatory response, as compared to deposits in articular gout, as their accumulation is more rapid. Visceral gout may occur due to dehydration or renal damage.

Renal function, in birds, is evaluated with the measurement of uric acid. This compound is the final result of nitrogen catabolism in avians and is produced in the liver.[1] If renal function is impaired, uric acid may build up in the bloodstream and precipitate as crystals in the tissues.[4] The validity of this test is not absolute as plasma uric acid may rise with normal feeding or ovulation, and may be normal in some cases of renal disease.[3]

We hypothesized that this aged flamingo developed articular gout over a period of time which led to severe amyloidosis in the kidney, liver and spleen. There was no other post mortem evidence of chronic disease, though this may have resolved by the time of death, leaving only amyloidosis as evidence of past pathology. The deposition of large amounts of protein in the interstitial spaces of the kidneys caused tubular degeneration and ischemia, leading to renal failure, decreased uric acid clearance, marked hyperuricemia and visceral gout.

Of the 62 American flamingos necropsied at the National Zoological Park since 1975, 21 (34%) have had amyloid deposition in at least one tissue. Amyloidosis was listed as the cause of death in 14 (23%) flamingos. This finding may indicate that this group of American flamingos, a closed flock since 1996, has a genetic predisposition to amyloid deposition.

AFIP Di agnosis: 1. Kidney, glomeruli, tubules and vessels: Amyloidosis, multifocal, marked, American flamingo (*Pheonicopterus rubber*), avian.
2. Kidney: Nephritis, tubulointerstitial, granulomatous and heterophilic, multifocal, moderate, with protein casts and urate tophi.

Conference Comment: Gout is the deposition of sodium urate crystals or urates in tissue; it occurs in species that lack the enzyme uricase such as humans, birds, and reptiles.[9] Uricase, or urate oxidase, is an enzyme that catalyzes the oxidation of uric acid to 5-hydroxyisourate. In those animals lacking this enzyme, uric acid is the final step in purine catabolism. Uric acid and urates are eliminated as semisolid urates in birds and reptiles.[4]

Visceral and articular gout are two separate syndromes with different etiologies, morphologies, and pathogenesis.

The diagnosis of visceral gout should not be considered a

disease entity itself, but rather a sign of severe renal dysfunction leading to hyperuricemia.[1]

True gout must be distinguished from pseudogout in which crystals other than sodium urate, such as calcium pyrophosphate dehydrate or hydroxyapatite, are deposited in joints. Grossly, pseudogout appears as cream-colored gritty material surrounding the joint capsule. This is in contrast to urates which are found inside the joint capsule and within the synovial fluid. Tophi are not present in pseudogout. Additionally, urates are radiolucent, whereas calcium deposits are radiopaque. True gout affects the kidneys, pericardium, liver, and other internal organs, whereas pseudogout only affects the joints and does not appear to occur in other locations. Pseudogout has been reported in humans, Rhesus macaques, dogs, and turtles.[5,6]

Contributing I nstitution: Department of Pathology, National Zoological Park, 3001 Connecticut Ave NW, Washington DC, 20008

References:

1. Crespo R, Shivaprasad HL: Non infectious diseases. In: Diseases of Poultry, ed. Saif YM, 11th ed., pp. 1058-1059, 1085-1086. Iowa State University Press, Ames, IA, 2003
2. Donoghue S: Nutrition. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., p. 281. Saunders Elsevier, St. Louis, Missouri, 2006
3. Gregory, CR: Urinary system. In: Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 253-255. Iowa State University Press, Ames, IA, 2003
4. Hochleithner M: Patient evaluation. In: Avian Medicine, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 228-241. Wingers Publishing Inc., Lake Worth, FL, 1994
5. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 60-61. Williams & Wilkins, Baltimore, Maryland, 1997
6. Mader DR: Gout. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 793-800. Saunders Elsevier, St. Louis, Missouri, 2006
7. Myers RK, McGavin MD: Cellular and tissue responses to injury. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 46-47. Mosby Elsevier, St. Louis, Missouri, 2007
8. Thompson K: Bones and joints. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 173-174. Elsevier Saunders, Philadelphia, Pennsylvania, 2007
9. Weisbrode SE: Bone and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1100. Mosby Elsevier, St. Louis, Missouri, 2007

CASE IV - W406/07 (AFIP 3074808).

Signalment: 12-month-old, female spayed, Bengal cat, *Felis catus*, feline.

History: Three week history of pyrexia, progressive inappetance, weight loss, unilateral uveitis, upper respiratory stertor with serous nasal discharge. Respiratory rate and effort was slightly increased. Thoracic **radiographs (fig. 4-1)** revealed a severe generalized mixed parenchymal pattern (fluffy increase in opacity throughout all lung lobes, coalescing into small nodules). The cat failed to respond to antimicrobial and supportive therapy and was euthanized.

Gross P athologic Fi ndings: The carcass of a young adult, spayed female, feline was in poor body condition and of adequate hydration. There was a tan-brown crust over the anterior nares. Transverse sectioning of the nasal cavity revealed almost complete **effacement (fig. 4-2)** of the turbinate architecture by soft pink moist tissue with smaller areas of green-brown discoloration. The trachea contained a small amount of cream-colored stable foam. At the level of the bifurcation there was a moderate amount of pink tinged clear viscid fluid. The pleural surface of the **lungs (fig. 4-3)** was extensively mottled dark red. On cut section the mottling extended throughout the parenchyma.

The liver had a pronounced acinar pattern. Multifocal irregularly shaped maroon discolorations, which were often depressed, were present on the capsular **surface (fig. 4-3)**.

All other organ systems were examined with no further gross lesions detected.

Laboratory Results: Routine hematology and biochemistry were within normal limits, except for elevated globulins (globulins 55 g/L, albumin 24 g/L, total protein 79 g/L).

Bronchoalveolar lavage contained large numbers of degenerate nucleated cells and an abundance of cellular debris. Markedly increased numbers of neutrophils (85%) and an abundance of mucous were also noted.

Serology

Toxoplasmosis IFAT (IgG)	negative
Toxoplasmosis IFAT (IgM)	negative
Feline Calicivirus IFA Titre	>= 1:80
Feline Herpes Virus IFA Titre	= 1:20
Feline Panleukopaenia Virus IFA Titre	= 1:20
Feline Coronavirus IFA Titre	>= 1:2560

PCR

Feline Immunodeficiency Virus PCR	negative

Using a monoclonal antibody against Feline Coronavirus (FCoV), immunohistochemistry was performed on several sections of paraffin embedded formalin fixed tissue. FCoV-infected macrophages were detected in large numbers throughout the nasal submucosa.

Histopathologic D escription: Nasal turbinates: The respiratory epithelium was generally intact with cilia present and sufficient numbers of goblet cells, however, there were small to locally extensive areas of epithelial erosion and surrounding attenuation and fibrinous exudation. There was moderate, predominately mononuclear inflammatory exocytosis. Turbinate structure was largely obliterated by a necrotizing inflammatory infiltrate. The submucosa contained a diffuse severe mixed, predominately lymphoplasmacytic inflammatory infiltrate. Numerous **veins (fi g. 4- 4)** were occluded by intense aggregations of monocytes and macrophages admixed with neutrophils. Many of these leucocytes were disintegrating. Vascular walls were often effaced by the inflammatory infiltrate. Bony trabeculae had irregular margins and were lined by abundant numbers of osteoclasts with surrounding proliferation of osteoprogenitor cells in some areas. The inflammatory reaction extended to cancellous marrow spaces and bone marrow of the maxilla and periodontal ligament and vessels of the dentine. Tooth dentine was necrotic.

Lung: There was a diffuse interstitial pneumonia with widely distributed foci of intense pyogranulomatous inflammation. Multifocal moderately sized areas of parenchyma were necrotic and surrounded by fibrinous effusion.

Liver: There was severe diffuse hydropic change of hepatocytes with mild bridging fibrosis and mild periportal lymphoplasmacytic inflammatory infiltrates. Multifocal moderately sized areas of telangiectasia were present and most noticeable towards the capsular surface. Leukocyte numbers were increased within the sinusoids.

Lymph nodes: Cortices contain numerous follicles with prominent germinal centers. The paracortices were expanded by numerous small mature lymphocytes. Numerous plasma cells were present within the medullary cords. There was a sinus histiocytosis. Multifocal granulomas of varying size were scattered throughout the nodes.

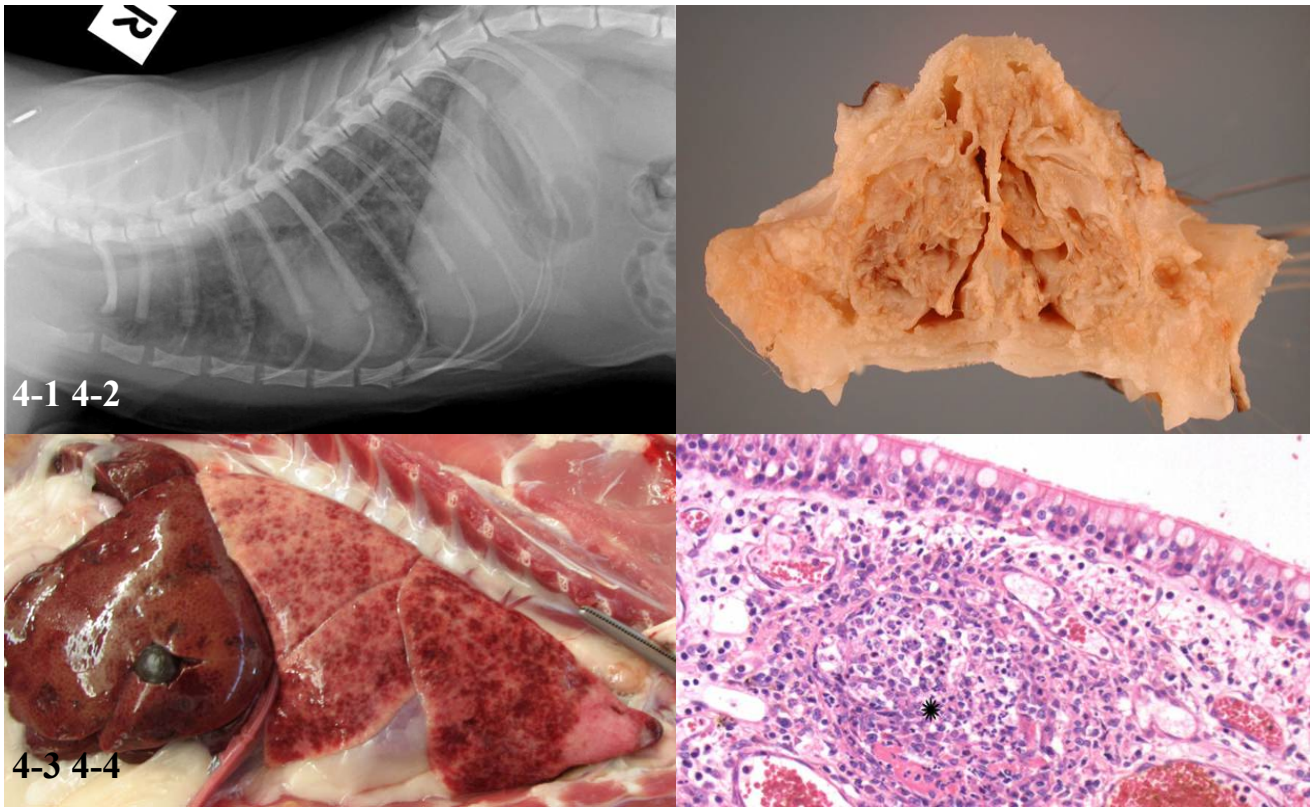
Bone marrow: There was a marked increase in myeloid to erythroid ratio

Contributor's Morphologic Diagnosis: 1. Nasal turbinates: Rhinitis and phlebitis, pyogranulomatous, chronic-active, diffuse, severe.
2. Lung: Bronchopneumonia, necrotising, granulomatous chronic, multifocal to coalescing, severe.
3. Lymph nodes: Lymphadenitis, granulomatous, chronic, multifocal, moderately severe.

Contributor's Com ment: Feline infectious peritonitis (FIP) is a worldwide fatal systemic disease of wild and domestic felids which is triggered by infection with FCoV. The disease syndrome was first described in 1963 with the etiological agent identified in 1978.[3] Granulomatous vasculitis and perivasculitis (especially of venules), fibrinous to granulomatous serositis, pyogranulomatous inflammation and granulomas in organs are characteristic histopathological lesions.[1,3,4] These result in the spectrum of "wet" (with large amounts of highly proteinaceous cavitory effusions) to "dry" (multifocal granulomas) clinical presentations.

FCoV is a member of the family Coronaviridae, genus *Coronavirus*. Coronaviruses are enveloped positive stranded RNA viruses averaging 100 nm in diameter and have characteristic petal shaped surface projections (peplomers) which are responsible for the crown like (corona) electron microscopic appearance.[3] Corona viruses have relatively low species specificity. Canine coronavirus is closely related to FCoV and can infect cats causing diarrhea. Exposed cats develop antibodies which cross-react with FCoV. Infection with FCoV results in a spectrum of outcomes, from asymptomatic enteric infection and healthy lifelong carrier status, through to systemic enteric infection to virulent systemic infection (FIP).

Coronavirus antibodies are present in up to 90% of cats in catteries and in up to 50% of cats in single cat households.[3] However, FIP morbidity is low, with only approximately 5% of FCoV infected cats developing FIP[3,4], and those cats that do develop the disease are usually under 2 years of age.[1] Infection usually takes place oral-nasally from exposure to FCoV-containing feces. Transmission in saliva can occur as the virus replicates in the tonsils in the early stages of infection.[3] Respiratory droplet and urine are also considered possible vehicles for



4-1. Thoracic lateral radiograph, Bengal cat. The radiograph shows a diffuse parenchymal nodular pattern of radiolucency within the lungs.
4-2. Transverse section, nasal turbinates, Bengal cat. Diffusely, the nasal turbinates are effaced by necrosis.
4-3. Thorax and abdomen, Bengal cat. Multifocally, both the lungs and liver have an irregular pattern of necrosis and hemorrhage characterized by a mottled discoloration.
4-4. Nasal turbinates, Bengal cat. Multifocally veins within the subepithelial connective tissue are expanded and occluded by fibrin thrombi characterized by a coagulum of fibrin, necrotic debris, viable and degenerate neutrophils (star).

Radiograph, photographs, and photomicrograph courtesy of the University of Melbourne, School of Veterinary Science, 250 Princess Highway, Werribee, Victoria, Australia 3030

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transmission.[3] Transplacental transmission has been shown to occur but is rare.[3] FCoV replicates in enterocytes either asymptomatically or causing transient and usually clinically mild diarrhea and/or vomiting.[3] A short episode of mild upper respiratory tract disease may also occur.[3]

FIP only ensues in individual affected animals when FCoV undergoes spontaneous mutation during replication. Deletions in open reading frames 3 and 7 which code for non structural proteins are responsible for surface changes which allow the virus phagocytozed by macrophages to bind to the ribosomes of the macrophages.[3,4] Mutated viruses have a 99.5% genetic homology with the parent virus.[3] The mutation allows the virus to replicate within macrophages. FCoV infected monocytes are considered responsible for viral dissemination within the host.[4] FCoV viremia is detectable in both virulent (mutated FIP infection) and non mutated FCoV infected cats.[3,4]

The pathogenesis of FIP is complex. The virus itself does not cause major cytopathic damage; rather lesions result from the host's own immune response.[3] Fibrinogen, C3 and viral antigen demonstrated within FIP lesions, and evidence of FCoV-specific immune complexes within the blood and vessels, together with high levels of gamma globulin in affected cats support an immune com-

plex-mediated, type III hypersensitivity pathogenesis.[3,4] However, an alternative pathogenesis has been proposed where lesion initiation appears to begin with endothelial adherence and extravasation of FCoV positive, activated monocytes and progresses to venous and perivenous, macrophage dominated, focal to circular infiltrates.[4] The presence of a peripheral rim of B lymphocytes in older FIP lesions, the paucity of neutrophils and T lymphocytes within the lesions and a lack of non-specific perivascular lymphocytic cuffing during one study are cited as significant differences between FIP and classical immune-complex mediated vasculitides.[4]

A definitive antemortem diagnosis of FIP is often challenging, complicated by the often insidious onset and non-specific signs displayed by infected animals. Laboratory tests including FCoV antibody titer (blood and CSF), RT-PCR to detect virus, ELISA to detect antibody-antigen complexes and measurement of acute phase proteins (mainly serum alpha-1 acid glycoprotein) are relatively insensitive and/or poorly specific.[3,4] This is mainly due to the inability to differentiate seroconversion or viremia caused by FCoV from that caused by mutated FCoV (FIP causing) strains. Serum alpha-1 acid glycoprotein has been shown to be elevated in other inflammatory conditions. Histopathology with immmunohistochemistry to detect FCoV infected macrophages remains the only conclusive means of diagnosing FIP.[1,4]

This case demonstrates classic characteristic FIP vasocentric granulomatous lesions. It is unusual in that the most severe lesions are within the nasal submucosa, this distribution has not previously been reported.

AFIP Diagnosis: Nasal turbinates, maxillary bone, and hard palate: Vasculitis, pyogranulomatous, multifocal, severe, with rhinitis, erosions, fibrin thrombi, and bone remodeling, Bengal cat (*Felis domesticus*), feline.

Conference Comment: The contributor provides an excellent overview of feline infectious peritonitis virus. Conference participants discussed the definition of vasculitis, the histomorphologic features needed for the diagnosis of vasculitis, and the differential diagnosis for vasculitis in various other species.

The diseases that cause vasculitis in animals are summarized in **table 4-1** below from Pathologic Basis of Veterinary Disease.[5]

Contributor: The University of Melbourne, School of

Table 4-1. Causes of Vasculitis in Animals

VIRAL
Equine viral arteritis (arterivirus), malignant catarrhal fever (gammaherpesvirus), hog cholera (porcine pestivirus), feline infectious peritonitis (coronavirus), bluetongue (orbivirus), African swine fever (asfarvirus), equine infectious anemia (lentivirus), bovine virus diarrhea (bovine pestivirus)

BACTERIAL
Salmonellosis, erysipelas (*Erysipelothrix rhusiopathiae*), *Hemophilus* spp. infections (*Hemophilus suis*, *Histophilus somni*, *Hemophilus parasuis*)

MYCOTIC
Phycomycosis, Aspergillosis

PARASITIC
Equine strongylosis (*Strongylus vulgaris*), dirofilariasis (*Dirofilaria immitis*), spirocercosis (*Spirocerca lupi*), onchocerciasis, elaeophoriasis (*Elaeophora schneideri*), filariasis in primates, aelurostrongylosis, angiostrongylosis

IMMUNE-MEDIATED
Canine systemic lupus erythematosus, rheumatoid arthritis, Aleutian mink disease (parvovirus), polyarteritis nodosa, lymphocytic choriomeningitis, drug-induced hypersensitivity

Veterinary Science, 250 Princess Highway, Werribee, Victoria, Australia 3030.
http://www.unimelb.edu.au/

References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 290-293. Elsevier Saunders, Philadelphia, Pennsylvania, 2007
2. Gelberb HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 380-381. Mosby Elsevier, St. Louis, Missouri, 2007
3. Hartmann K: Feline infectious peritonitis. Vet Clin Small Anim 35**:**39-79, 2005
4. Kipar A, May H, Menger S, Webber M, Leukert W, Reinacher M: Morphologic features and development of granulomatous vasculitis in feline infectious peritonitis. Vet Pathol 42**:**321-330, 2005
5. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 606. Mosby Elsevier, St. Louis, Missouri, 2007
6. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 883-884. Mosby Elsevier, St. Louis, Missouri, 2007

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 13

9 January 2008

Moderator:

Dr. Bruce Williams, DVM, DACVP

CASE I – 02-3323 (AFIP 3074949).

Signalment: 7-year-old, castrated male, *Mustela putorius furo*, ferret

History: In January of 2000, Eucchus presented with bilaterally symmetric hair loss over the dorsum, at that time both adrenals were at the upper range of normal size and nodular by ultrasound. Hormonal panels supported a clinical diagnosis of adrenal disease (Estradiol 174pmol/L (normal 30-180pmol/L); 17-OH-progesterone 0.85nmol/L (normal 0-0.8 nmol/L); androstenedione 30.7nmol/L (normal 0-15nmol/L). At that time the owner elected medical management with repeated injections of Depo-lupron. Depo-lupron is a GnRH analogue which inhibits production of LH and FSH. In September of 2000, the owner reported that Eucchus' belly would be soaked in urine after he urinated. Eucchus also repeatedly presented with alopecia and flaky skin. Urinary symptoms and alopecia resolved following increasing doses of Depo-lupron. In December of 2001 and again in June of 2002 Eucchus presented with difficulty urinating and was found to have an enlarged bladder. A urinary tract infection and enlarged prostate were diagnosed and Eucchus was started on antibiotics. At this time an insulinoma was also suspected clinically. Several weeks after discontinuing the antibiotics (August 2002), Eucchus again presented with straining to urinate and antibiotics were resumed. While still on antiobiotics, Eucchus presented in October of 2002, for straining to urinate. At this time the left adrenal gland was markedly enlarged (1cm) by ultrasound and surgery was elected. At surgery, the left adrenal gland was removed and 2 periprostatic cysts were identified which communicated with the urinary bladder. Additionally, 2 discrete nodules were noted in the pancreas and were removed. Following surgery, Eucchus became lethargic, dehydrated and anuric and was euthanized.

Biopsy results of the adrenal and pancreas were consistent with an adrenocortical adenocarcinoma and islet cell tumors (presumptive insulinomas), respectively.

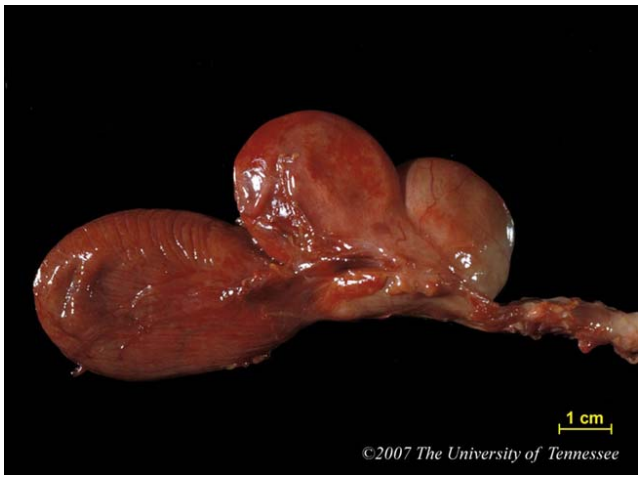
Gross Pathology: At necropsy, Eucchus was found to be obese and the abdomen contained 50ml of serosanguinous fluid. Two 3cm diameter cysts were found surrounding the prostate, just caudal to the **urinary bladder (fig. 1-1)**.

Histopathologic Description: The prostate is markedly expanded by a single large cyst and multiple smaller cysts lined by keratinizing stratified squamous epithelium, and containing variable amounts of keratin. In some sections, there is focal loss of the epithelial lining of one of the cysts with free keratin in the surrounding

stroma. This free keratin is surrounded by a variable infiltrate of neutrophils and macrophages. Throughout the prostate there are decreased amounts of glandular tissue. Remaining glandular structures are lined by a low cuboidal epithelium and rarely contain an eosinophilic secretory product. Scattered clusters of lymphocytes and rarely eosinophils are present in the surrounding fibrous connective tissue stroma.

Contributor's Morpho logic Diagn osis: Prostate: Severe glandular atrophy and squamous metaplasia with cyst formation.

Contributor's Com ment: Adrenal cortical lesions are the second most common neoplasm of ferrets, after pancreatic islet cell tumors. An increased incidence of proliferative adrenal lesions occurs in ferrets neutered at an early age (2-4months), and is likely due to chronic stimulation of the cells of the zona reticularis by luteinizing hormone.[4] Adrenal gland-associated endocrinopathy (AAE) is associated with the presence of hyperplastic or neoplastic adrenal lesions which produce high levels of estrogenic compounds (estradiol-17b, androstenedione, dehydroepiandrosterone sulfate, 17-hydroxyprogesterone, progesterone). Lesions associated with AAE include bone marrow toxicity[4] and Cushingoid features (thin skin, muscular atrophy, pot-bellied appearance)[1], bilateral symmetrical truncal alopecia, vulvar swelling in spayed females, reversion to sexual behavior in neutered animals, mammary gland hyperplasia in castrated males, and dysuria in males associated with squamous metaplasia of the prostate and prostatitis.



1-1. Prostate gland, ferret. Caudal to the urinary bladder and surrounding the prostate gland are two cysts measuring up to 3 cm in diameter. Photograph courtesy of the University of Tennessee, College of Veterinary Medicine, Department of Pathobiology; 2407 River Drive, Room A201; Knoxville, TN 37996-4542

The squamous metaplasia in the prostate of this ferret is likely due to increased levels of circulating estrogenic compounds. Six cases of prostatic squamous metaplasia with concurrent prostatitis have been reported in male ferrets with proliferative adrenocortical lesions. As in dogs, the prostatitis has been attributed to the presence of keratin.[2] The absence of significant prostatitis in this case may be unusual. The observed prostatic atrophy is likely a result of castration at a young age and failure of the prostate to develop normally.[3]

In dogs, squamous metaplasia of the prostatic glandular epithelium has been associated with estrogen-producing Sertoli cell tumors, or exogenous administration of estrogens. In such cases, squamous metaplasia affects the prostatic urethra, uterus masculinus and prostatic ducts. Similar changes have been reported in swine. In cats, exogenous estrogen results in prostatic enlargement due to epithelial hyperplasia and cystic dilation of the glands; squamous metaplasia and cornification, however, only occur in the urethral epithelium.[3] Enlargement of the prostate is most commonly associated with constipation, and less commonly stranguria.[3]

AFIP Diagn osis: 1. Prostate gland: Prostatic cysts, multiple, ferret (*Mustela putorius furo*), carnivore.
2. Prostate gland: Squamous metaplasia, multifocal, mild, with prostatitis and keratinizing cysts.

Conference Com ment: Squamous metaplasia of the prostate with keratinizing prostatic cysts is a common sequel in male ferrets diagnosed with adrenal-associated endocrinopathy.[1,8] Chronic elevation of circulating luteinizing hormone (LH), resulting from early neutering, is required for metaplastic transformation.[1] Elevated circulating LH acts on the zona reticularis[4], stimulating cellular proliferation as well as the production of high levels of circulating estrogenic compounds, including estradiol-17β, androstenedione, dehydroepiandrosterone, 17-hydroxyprogesterone, and progesterone.[2]

Luteinizing hormone receptors (LHRs) are usually present on ovarian thecal cells, granulosa cells, luteal cells, and testicular Leydig cells.[1] LHRs have also been identified in the adrenal gland of fetal (but not adult) mice, and low levels of LHR mRNA has been detected in the adrenal cortex of normal intact ferrets, indicating the presence of non-functional receptors.[1]

Tumors in the ferret adrenal gland include nodular hyperplasia, adrenocortical adenoma, and adrenocortical carci-

noma.[1,8] In the case of the latter, metastasis usually occurs late in the disease, and early complete removal of neoplastic adrenals carries a fair prognosis.[8] In contrast to other species, plasma concentrations of cortisol are only infrequently elevated in ferrets with AAE.[1,8]

Squamous metaplasia of glandular epithelium due to hyperestrogenism, has been documented in men, mice, dogs, and sheep.[1,2,3,6] Experimental induction of prostatic squamous metaplasia in the mouse model has revealed proliferation of basal cells with keratinization following injections with estrogen. In affected ferrets, squamous metaplasia of prostatic epithelium is followed by cyst formation and purulent inflammation as a result of keratin production[2] and may ultimately result in dysuria and post-renal azotemia.

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References:

1. Bielinska M, Kiiveri S, Parviainen H, Mannisto S, Heikinheimo M, Wilson DB: Gonadectomy-induced adrenocortical neoplasia in the domestic ferret (*Mustela putorius furo*) and laboratory mouse. Vet Pathol 43:97-117, 2006
2. Coleman GD, Chavez MA, Williams BH: Cystic prostatic disease associated with adrenocortical lesions in the ferret (*Mustela putorius furo*). Vet Pathol 35:547-549, 1998
3. Foster RA, Ladds PW: Male genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. ed. Maxie MG, 5th ed., vol. 3, pp. 605-611. Saunders Elsevier, Endinburgh, 2007
4. Peterson RA, Kiupel M, Capen CC: Adrenal cortical carcinomas with myxoid differentiation in the domestic ferret (*Mustela putorius furo*). Vet Pathol 40:136-142, 2003
5. Pollock CG: Urogenital diseases. In: Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery, eds. Quesenberry KE, Carpenter JW, 2nd ed., pp. 45-46. Saunders, St. Louis, Missouri, 2004
6. Risbridger GP, Wang H, Frydenberg M, Cunha G: The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. Endocrinology 142:2443-2450, 2001
7. Quesenberry KE, Rosenthal KL: Endocrine diseases. In: Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery, eds. Quesenberry KE, Carpenter JW, 2nd ed., pp. 83-87. Saunders, St. Louis, Missouri, 2004
8. Williams B: Pathology of the Domestic Ferret. At: http://www.afip.org/ferrets/ferret.path.html

CASE II – 07-A5 (AFIP 3064916).

Signalment: 130-140-day-old, laying hens

History: Thirty hens were submitted for post mortem examination with a history of respiratory distress and high mortality from a flock of 25,000. During a high wind storm the roofs of 2 houses were damaged; numerous birds were outside and intermixing of birds from both houses occurred.

Gross Pa thology: There was severe, catarrhal, hemorrhagic tracheitis. Caseous casts were present in the tracheas of several birds. Four had caseous plugs lodged in the larynx.

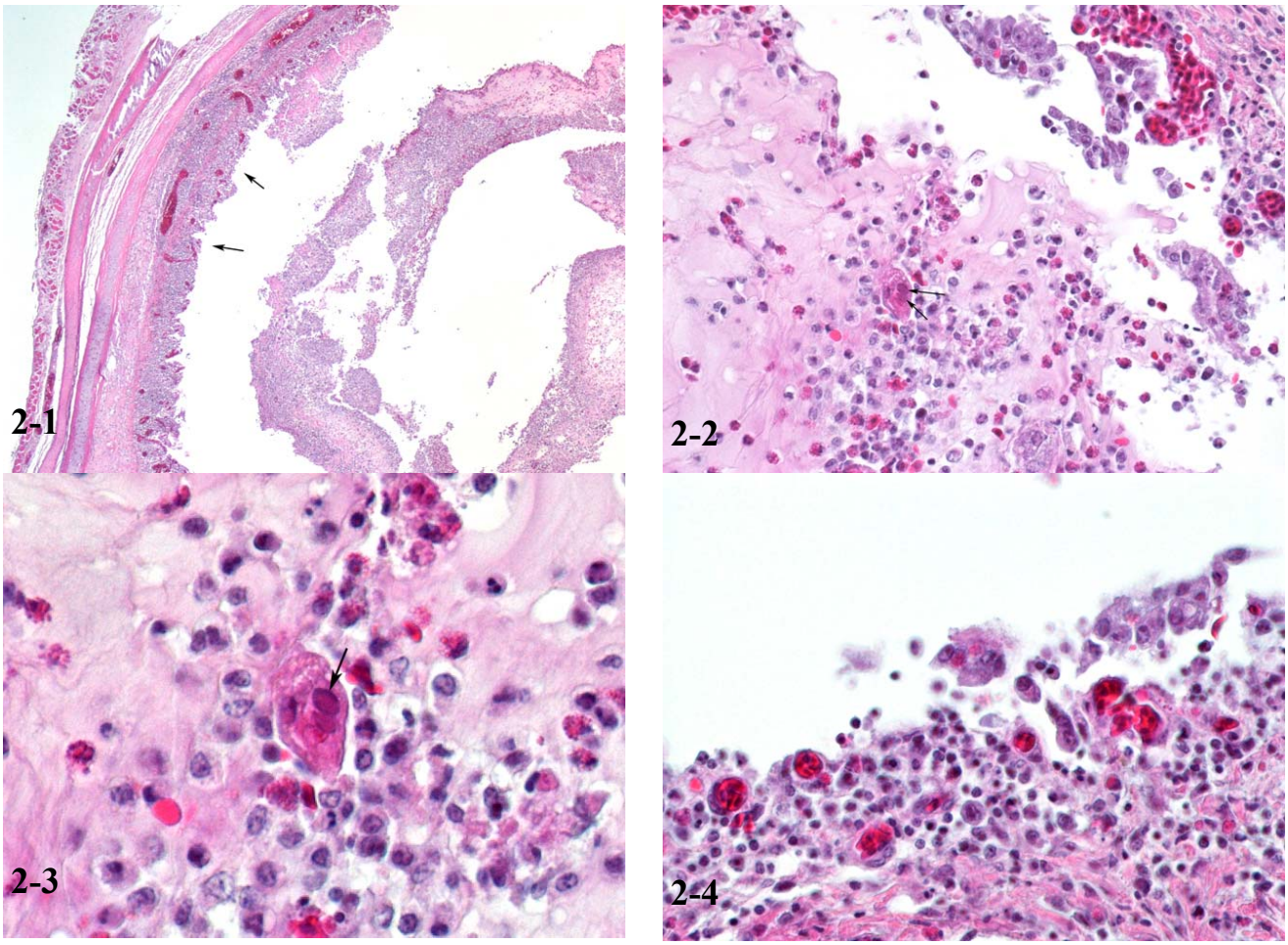
Laboratory Results: No significant bacterial pathogens were cultured.

PCR for Newcastle's Disease Virus (NDV) and Avian Influenza virus (AI) were negative. PCR for Infectious Laryngotracheitis virus (ILT) was positive.

Histopathologic Description: Trachea: The lumen contains large amounts of necrotic cellular **debris (fig. 2-1)**, fibrin and mats of bacterial colonies. The mucosa has diffuse erosion to ulceration of psuedostratified epithelium and formation of large, angular multinucleate **syncytia (fig. 2- 2)** within the mucosa and within sloughed luminal debris. Within syncytia, many nuclei contain large, eosinophilic nuclear **inclusion bodies (fig. 2-3)** that marginate chromatin. The submucosa is moderately expanded by congested blood vessels and dense lymphocytic **infiltrates (fig. 2-4)**.

Contributor's Morpho logic Diag nosis: Diffuse, severe, necrotizing and catarrhal tracheitis with syncytia formation and nuclear inclusions (Infectious Laryngotracheitis)

Contributor's Com ment: Infectious laryngotracheitis (ILT) is a disease primarily of chickens caused by *Gallid herpesvirus 1*, an alphaherpesvirus. The disease was first described in 1925 and was the first major avian viral disease for which an effective vaccine was developed. It has a worldwide distribution, causing the most characteristic signs in adult laying hens. Natural routes of infection are upper respiratory and ocular, although oral transmission can occur. Viral replication is limited to respiratory tissues. The trigeminal ganglion is the principle site of la-



2-1. Trachea, laying hen. The tracheal epithelium is effaced and replaced by abundant necrotic debris, fibrin, edema and hemorrhage (Arrows) admixed with colonies of bacterium. (H&E 40X).
2-2. Trachea, Laying hen. The tracheal epithelium is multifocally ulcerated and replaced by clumped epithelium (syncytia) (arrows).
2-3. Trachea, laying hen. Expanding the nuclei of sloughed epithelial cells and syncytial cells, marginating the chromatin are eosinophilic inclusion bodies (arrows).
2-4. Trachea, laying hen. Admixed within the necrosis and sloughed epithelium are moderate numbers of lymphocytes, plasma cells, heterophils and occasional macrophages.

Photomicrographs courtesy of the Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040

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tency. LTV can persist as latent infections in recovered birds, and virus can be re-excreted in birds under stress. The virus may also persist as endemic infections in backyard and fancier chicken flocks.

Clinical signs vary from severe epizootic forms (as in this case) to mild endemic forms. Coughing, gasping and expectoration of blood-stained mucus characterize severe forms; mild forms show unthriftiness, decreased egg production nasal discharge and hemorrhagic conjunctivitis. Severe forms have high morbidity (90 - 100%); mortality usually ranges from 10 - 20%. Endemic forms have low morbidity and mortality. Although antigenically homogeneous, different virus strains with differing virulence have recently been differentiated by PCR-RFLP techniques.[5] Differential diagnoses for respiratory disease in chickens include the diphtheritic form of avian pox, NDV, AI, infectious bronchitis, fowl adenovirus infec-

tions and aspergillosis.

The most consistent gross lesions of ILT are in the larynx and trachea. In mild cases, the only lesions may be conjunctivitis, sinusitis and mucoid tracheitis. In severe forms, diphtheritic changes can be striking, consisting of mucoid casts along the entire length of the trachea. Mucoid plugs in the larynx (as seen in this case) are also common. In some cases, hemorrhage predominates. Histologically, early lesions consist of loss of goblet cells and mononuclear inflammation. As the lesions progress, respiratory epithelial cells loose cilia, enlarge and form multinucleate syncytia. Nuclear inclusion bodies are present only in early stages (1 - 5 days). Confirmatory diagnostic procedures include viral isolation on embryonated chicken eggs, serology and PCR. Control of the disease in laying flocks is generally by vaccination, whereas tight biosecurity and a shorting growing cycle will often make vaccination of broiler flocks unnecessary. Vaccines are usually modified live virus, and mixing of flock with different immunity levels can cause disease outbreaks. In this case, birds from a non-vaccinated house were mixed with vaccinated birds, causing a disease outbreak. Newer deletion mutant vaccines are underdevelopment that will not only provide a safer ILT vaccine but show promise as vector vaccines for other avian infectious diseases, such as AI.[1]

AFIP Diagn osis: Trachea: Tracheitis, necrotizing, subacute, diffuse, moderate, with epithelial syncytia, intranuclear inclusion bodies, and intraluminal serocellular coagulum, chicken (*Gallus domesticus*), avian.

Conference Comment: The contributor gives an excellent overview of Infectious Laryngotracheitis (ILT). Chickens and pheasants are the only natural hosts, although isolation from peafowl and experimental infection of turkeys has been described.[1]

Ultrastructural features are those of a typical herpes virion and include a DNA-containing core within a 100nm icosahedral capsid surrounded by a variably sized proteinaceous tegument layer and an outer envelope with incorporated viral glycoproteins.[1] The viral glycoproteins appear as fine spikes projecting from the surface of the envelope.[2] Viral particle sizes vary between 200-350nm depending on the amount of incorporated tegument protein.[1]

Tegument proteins are common in enveloped viruses and are usually a combination of essential and non-essential proteins that are released shortly after viral entry into the cell. These proteins may aid in suppression of the immune response, suppression of host mRNA transcription, or transcribing/translating viral genes. Formation of tegument proteins is generally done late in the viral infectious cycle, following replication of viral genes.[3]

Viral replication of ILT is similar to that of other alpha-herpes virus.[1,2] Within the infected cell nucleus, viral capsids are formed and filled with viral DNA. These

Table 2-1. Alphaherpesviruses[4]

• Porcine herpesvirus-1: Pseudorabies, Aujeszky's disease
• Canine herpesvirus-1: Canine herpes
• Feline herpesvirus-1: Feline viral rhinotracheitis (FVR)

Bovine:
• BHV1: Infectious bovine rhinotracheitis
Infectious pustular vulvovaginitis
Infectious balanoposthitis
• BHV2: Bovine mammillitis virus/ Pseudo-lumpy skin disease
• BHV5: Bovine herpesvirus encephalitis (no inclusion bodies)

Equine:
• EHV1: Equine herpesviral abortion, rhinopneumonitis, neurologic disease
• EHV3: Equine coital exanthema
• EHV4: Equine rhinopneumonitis, abortion

Avian:
• Avian HV1: Infectious laryngotracheitis
• Avian HV2 (Gallid herpesvirus-2): Marek's disease
• Anatid HV1: Duck plague

Nonhuman primate:
• Herpesvirus simiae (Cercopithecine herpesvirus 1; B virus): Herpes B
• Herpesvirus tamarinus (Cebid herpesvirus 1; Herpes T): localized disease in
squirrel monkeys; generalized disease in marmosets, tamarins, owl monkeys
• Herpesvirus simplex, type 1: oral lesions in humans, apes, monkeys
• Herpesvirus simplex, type 2: genital lesions in humans, apes, monkeys
• Simian varicella: Simian varicella in macaques, African green monkeys,
Patas monkeys
• Herpesvirus papio 2: Oral and genital lesions in baboons

nucleocapsids are then enveloped by the inner nuclear membrane and deenveloped by the outer nuclear membrane when transported into the cytoplasm.[1] Within the cytoplasm the capsids associate with an electron dense tegument and are enveloped by a second budding event in the trans-Golgi region. The mature virus particles are then released by exocytosis.[1]

A list of common veterinary alpha-herpesviral infections is included in table 2-1.[4]

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References:

1. Fuchs W, Veits J, Helferich D, Granzow H, Teifke JP, Mettenleiter TC: Molecular biology of avian infectious laryngotracheitis virus. Vet Res 38:261-279, 2007
2. Guy JS, Bagust TJ: Laryngotracheitis. In: Diseases of Poultry, ed. Saif YM, 11th ed., pp. 121-131. Iowa State Press, Ames, IA, 1997
3. Yeung BJ, McHamstein HY, McHamstein M: Herpes simplex virus tegument Protein V1 elucidation and formation around the nucleocapsid. J Virol 28:1262-1274, 2007
4. Murphy FA, Gibbs EPJ, Horzinek M, Studdert MJ: Herpesviridae. In: Veterinary Virology, 3rd ed., pp. 301-325. Academic Press, San Diego, CA, 1999
5. Kirkpatrick NC, Mahmoudian A, Colson CA, Devlin JM, Noormohammadi AH: Relationship between mortality, clinical signs and tracheal pathology in infectious laryngotracheitis. Avian Pathol 35:449-453, 2006
6. Sellers HS, Garcia M, Glisson JR, Brown TP, Sander JS, Guy JS: Mild infectious laryngotracheitis in broilers in the southeast. Avian Dis 48:430-436, 2004

CASE III – W401/06 (AFIP 3034592).

Signalment: 10 to 12-month-old lambs (breed unknown), sex unknown, *Ovis aries*

History: Abattoir liver specimens derived from 260 lambs from New South Wales. Fifty percent of the livers were condemned at the abattoir. Lambs had no previous history of illness.

Gross P athology: Liver had retained their shape, but were small, yellow and the capsular surface was markedly granular in appearance. On cut section, nodular regeneration was apparent throughout.

Histopathologic D escription: The capsular surface of the liver was undulated. The normal acinar architecture of the liver was replaced by extensive nodular regeneration and segmental collapse and condensation. The portal triads showed increased mature biliary ductular profiles and there was a moderate mononuclear, primarily lymphocytic infiltration, which extended into the surrounding sinusoids. A moderate degree of fibrosis was present, radiating from the portal triads into the periphery of the regenerative nodules.

Periportal hepatocytes appeared large, and many appeared to be entrapped by collapsed stroma. The nuclei varied in sizes and shape, but were predominantly large and vesicular with dispersed chromatin. Other nuclear profiles included dark nuclei with smudged nuclear chromatin, fibrillary nuclear chromatin, and irregularly shaped nuclei.

There was a minimal degree of fatty change affecting hepatocytes within regenerative nodules. (Note: The degree of fatty change present may vary between submitted slides). The majority of nuclei within these nodules were unremarkable. Occasional apoptotic hepatocytes were scattered throughout the parenchyma.

Contributor's Morphologic Diagnoses: Chronic active hepatopathy with marked nodular regeneration, hepatic magalocytosis and karyomegaly, liver sheep.

Contributor's Comme nt: The histological changes present are indicative of a two-phase process. Previously there has been extensive loss of hepatocytes, resulting in condensation of parenchyma and liver shrinkage accompanied by nodular regeneration. Currently, residual periportal hepatocytes are undergoing degenerative changes with megalocytosis, karyomegaly and apoptosis.

Anecdotal history suggested that these lambs had been supplemented prior to slaughter with lupin grain. In addition, it is likely that these animals had been recently grazing lupin stubble.

Lupinosis is a sporadic disease reported primarily in Australia, New Zealand, South Africa and Europe.[4] It is a mycotoxic liver disease caused by infection of *Lupinus* spp with the fungus *Diaporthe toxica* (formerly *Phomopsis leptostromiformis*). In southern regions of Australia, *Lupinus* spp (primarily *L. cosentini*) are commonly used as fodder, either as stubble or as grain.[4] The fungus pro-

duces the toxic agents phomopsin A and B with A being two to three times more toxic than B.[3] These toxins bind to tubulin and interfere with the ability of hepatocytes to form microtubules and therefore undergo mitosis. The result is hepatic atrophy and fibrosis. Hepatocytes typically swell and have large vesicular nuclei.

Lupinosis is typically a sub-acute to chronic disease, and can affect other species including cattle, donkeys, goats, horses and pigs.[1] Clinically sheep show non-specific neurological signs and frequently die from misadventure or from copper poisoning. Other organ systems can exhibit cytotoxic effects, including adrenal glands, pancreas, kidneys, rumen and skeletal and cardiac musculature.[1]

Lupinus spp themselves also contain quinolizidine alkaloids that can cause teratogenic abnormalities such as crooked calf disease (due to angyrine) and neurotoxic signs.[5]

Although the history and pathological changes present in this case are suggestive of lupinosis, other causes of toxic hepatophy including pyrrolizidine alkaloids cannot be excluded.

AFIP Di agnosis: 1. Liver: Nodular regeneration, diffuse, with megalocytosis, biliary reduplication, and moderate portal bridging fibrosis, breed unspecified (*Ovis aries*), ovine.
2. Liver: Hepatitis, lymphocytic, subacute, multifocal, mild.

Conference Com ment: Conference participants suggested a differential diagnosis of lupin toxicosis, pyrrolizzidine alkaloid toxicosis and aflatoxicosis as potential causes of the changes noted in the distributed slides.

Phomopsis leptostromiformis, a fungus that grows on lupine (*Lupinus* sp.) plants, produces a toxic metabolite, phomopsin. Affected livers exhibit multifocal necrosis and remaining hepatocytes undergoing mitotic arrest in metaphase, resulting in a marked increase in mitotic figures.[2] Chronic affected livers are smaller than normal as a result of necrosis, inability to regenerate due to mitotic inhibition, and progressive fibrosis.[5] Nodular regeneration may occur with sporadic ingestion of the toxin.[5]

Following ingestion, pyrrolizidine alkaloids are converted to pyrrole esters by hepatic cytochrome p450 enzymes, which react with cytosolic and nuclear proteins and nucleic acids to inhibit DNA synthesis and mitosis in hepatocytes.[5] Megalocytosis, a characteristic finding in pyrollizidine alkaloid toxicosis, occurs when some hepatocytes are able to replicate their DNA yet are unable to divide.[5]

Aflatoxins are also metabolized by the hepatic mixed-function oxidase system to toxic and non-toxic metabolites.[5] The most potent of these is the 8,9-epoxide metabolite of aflatoxin B1, which binds to adenine in nucleic acids, resulting in very similar microscopic findings to animals metabolizing pyrollizzidine alkaloids.[5]

Chronic inconsistent ingestion of any of these toxic principles can result in end-stage liver disease over time. The characteristic micro- and macronodular regeneration seen in end-stage livers can have numerous causes other than toxicity:[2]

1. Chronic toxicity (therapeutic agents or naturally occurring toxins)
2. Chronic cholangitis and/or obstruction
3. Chronic congestion (right side heart failure)
4. Inherited disorders of metal metabolism (copper or iron)
5. Chronic hepatitis
6. Idiopathic

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References:

1. Allen JG: Proceedings No. 103 Veterinary Clinical Toxicology, pp. 113-131. The Post-Graduate Committee in Veterinary Science, The University of Sydney, Sydney, Australia, 1987
2. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 413, 444-446. Elsevier, St. Louis, MO, 2007
3. Culvenor, CCJ, Beck AB, Clarke M, Cockrum PA, Edgar JA, Frahn JL, Jago MV, Lanigan GW, Payne AL, Peterson JE, Petterson DS, Smith LW, White RR: Isolation of toxic metabolites of *Phomopsis leptostromiformis* responsible for lupinosis. Aust J Biol Sci 30:269-277, 1977
4. Everist SL: Poisonous Plants of Australia, pp. 465-469. Angus and Robertson Publishers, London, 1974
5. Stalker MJ, Hayes MA: The liver and biliary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 370-381. Elsevier Limited, St. Louis, MO, 2007

CASE IV - BB425/06 (AFIP 3032272).

Signalment: Adult (age unknown), female, Greyface sheep (*Ovis aries*)

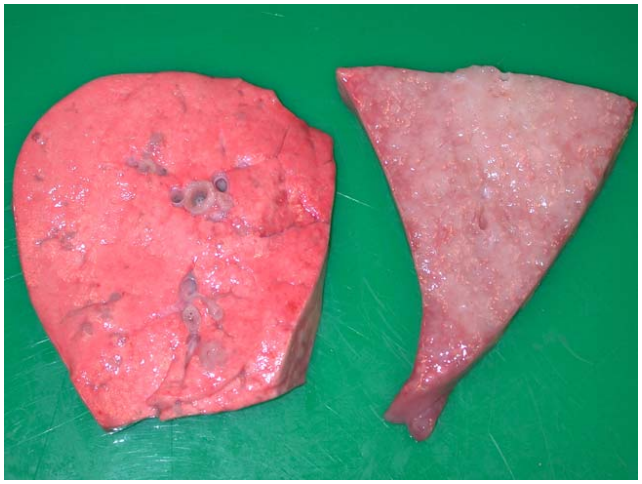
History: A mature Greyface ewe was culled due to prolonged respiratory distress. At necropsy, the only significant findings were in the lungs.

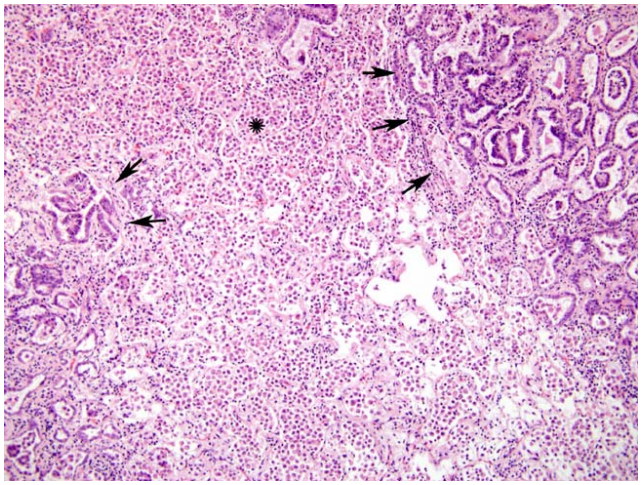
Gross Pathology: The lungs were almost diffusely much firmer and heavier than normal and failed to collapse. There were moderate to large amounts of frothy fluid in the tracheal and bronchial lumens. The cranioventral lung lobes and the caudal portions of the caudal lung lobes were expanded by fairly well demarcated, firm, ill-defined masses which were grey to pale purple. On cut section, the parenchyma in these areas was consolidated, firm and pale **grey (fig. 4-1)**. Both sections are from this sheep but the section on the left is relatively unaffected while the parenchyma in the section on the right is virtually replaced by slightly nodular, pale grey **homogeneous tissue (fig. 4-1)**. Affected areas also exuded frothy fluid, especially on cut section.

Histopathologic Description: Most of the normal lung parenchyma is replaced by multiple variably sized, nodular proliferations of well-differentiated cuboidal to low columnar **epithelial cells (fig. 4-2)**. The nodules are non-encapsulated, infiltrative and often coalesce with each other. The epithelial cells form tubuloacinar structures with occasional papillary projections, supported by a fibrovascular stroma. Some of the tubuloacinar structures contain pink, proteinaceous material. Individual cells have variably distinct cell borders with centrally to basally located hypochromatic nuclei, moderate amounts of faintly granular eosinophilic cytoplasm and indistinct nucleoli. Nuclear pleomorphism is mild and mitotic figures rare. Rare nodules are centrally necrotic or infiltrated by necrotic and viable neutrophils and cellular debris. In many areas, the surrounding alveolar spaces are flooded by very large numbers of alveolar and epithelioid macrophages, with fewer neutrophils, lymphocytes and plasma cells and occasional multinucleated giant cells. The macrophages are very plump with homogeneous, pink cytoplasm which sometimes causes peripheralization of the nucleus. Many bronchioles and bronchi are cuffed by large, discrete aggregates of large numbers of lymphocytes forming lymphoid follicles, many with distinct germinal centers. The bronchiolar and bronchial lining cells are variably hyperplastic or attenuated.

Contributor's Morphologic Diagnosis: 1. Ovine pulmonary adenocarcinoma (OPA)
2. Severe, diffuse, histiocytic interstitial pneumonia with marked BALT hyperplasia

Contributor's Comment: OPA has a number of syno-





4-1. Greyface sheep. On cut section, the right lung is variably consolidated, firm and pale in comparison to the lung on the left, also from the same animal. Photograph courtesy of the Veterinary Pathology Unit, Easter Bush Veterinary Centre, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, United Kingdom
4-2. Lung, Greyface sheep. Multifocally, markedly expanding and replacing the pulmonary architecture are nodular coalescing proliferations of epithelial cells which form variably sized tubules and acini (arrows). Adjacent less affected alveoli are expanded by high numbers of alveolar macrophages, lymphocytes, plasma cells and neutrophils (star). (H&E 100X)

nyms, including sheep pulmonary adenomatosis and jaagsiekte (Afrikaans for "driving sickness"). It is a naturally occurring, transmissible disease characterized by the development of pulmonary neoplasia and caused by an exogenous betaretrovirus called jaagsiekte sheep retrovirus (JSRV).[6] First recognized in South Africa, it now occurs worldwide, with the exception of the Antipodes. The incidence in the UK has been recorded to be as high as 30% and it can result in mortality rates of up to 50% in affected flocks.[4]

In this case, the gross changes were typical of the classical form of the disease. An atypical form occurs (although has apparently not been reported in Scotland) whereby the nodules are more discrete, harder and much drier. Lesions generally only occur in the lungs although metastasis to lymph nodes can arise and is one of the main features helping to classify the lung lesions as truly neoplastic, rather than simply proliferative. Extrathoracic metastases have also been reported.[4] Electron microscopy has confirmed that the alveolar proliferations are composed of type II pneumocytes, while those arising in bronchioles are composed of Clara cells. Both cell types are secretory, accounting for the copious amounts of frothy fluid produced, which tends to flood the respiratory passages in the classical form. This excessive fluid accumulation is absent from the atypical form.[6] Microscopically, the neoplasm is classified as a bronchioloalveolar carcinoma. The histological findings in this case were typical and tend to be identical between the two forms.[4]

The lung tropism of the JSRV and resultant neoplastic transformation of pulmonary epithelial cells is apparently unique in the retrovirus group. The exact mechanism of neoplastic transformation is the subject of much current research. Two genes appear to be important to this tropism and viral infectivity, the env gene and the long terminal repeat (LTR) gene. The env gene permits viral entry of cells because it encodes the viral glycoprotein which allows interaction with cell receptors. Thus, the virus can only infect cells which specifically express its receptor, although pulmonary epithelial cells are not the only cells to do so.[4] The LTR gene is integrated into the cellular genome after viral entry and induces viral expression by interacting with cellular transcription factors. It can activate proto-oncogenes via insertional mutagenesis, whereby provirus is inserted near a proto-oncogene and drives its overexpression.

Of these two genes, the env gene is gaining favor as the more likely oncogenic agent since it has been shown to function as an oncogene, at least experimentally in mammalian fibroblast and epithelial cell lines. The mechanism of cell transformation is unclear although it is believed to involve the cytoplasmic tail of the envelope transmembrane protein as well as two downstream cell signaling pathways, H/N-Ras-MEK-MAPK and Akt-mTOR.[2] The insertional mutagenesis theory seems less likely since the random nature and the infrequency of insertion in the desired place within the genome would not be efficient enough for proto-oncogene activation.[4]

The sheep genome also contains 15-20 copies of endogenous retrovirus which is very similar to the exogenous JSRV. The main difference lies in the LTR region of the genome such that the endogenous form of the virus does not have the same transcriptional efficiency in pulmonary epithelial cells as the exogenous, tumor-inducing form. The existence of the endogenous virus may explain the lack of an antibody response in OPA infected sheep, since the endogenous elements may promote immunotolerance during fetal development.[4]

There was marked BALT hyperplasia in this case, for which there could have been two main reasons. Firstly, it can occur in the atypical form of OPA. We felt this was less likely since the concomitant fibrosis and marked lymphoplasmacytic inflammation usually seen in the atypical form were not present.[6] Secondly, the possibility of concurrent maedi was considered since combined infections have been frequently recognized; no further testing was performed to confirm or refute this possibility.[5] There was also quite severe histiocytic inflammation. The widespread and marked infiltrate of plump macrophages is commonly found around neoplastic nodules; they are believed to be induced by the excessive surfactant protein production but their exact role in the pathogenesis is still unclear. Recent work suggests they reflect a cellular immune response to the presence of neoplastic cells. The apparent ineffectiveness of this response is believed to be due to putative immunosuppressive properties of the excess surfactant protein.[7]

AFIP Diagnosis: 1. Lung: Carcinoma, Greyface sheep (*Ovis aries*), ovine.
2. Lung: Lymphofollicular hyperplasia, diffuse, moderate.
3. Lung: Pneumonia, interstitial, multifocal, mild.

Conference Comment: The contributor gives an excellent overview of retroviral- induced ovine pulmonary adenocarcinoma (OPA). This section also exhibits the typical histologic finding of abundant macrophages located at the periphery of the neoplasm in OPAs which are presumably attracted by the abundant surfactant secreted by the neoplasm. We agree with the contributor that there is likely at least one other disease process occurring

in addition to OPA in the distributed section; ovine lentivirus pneumonia and a concomitant bacterial superinfection were also discussed in conference.

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References:

1. Caporale M, Cousens C, Centorame P, Pinoni C, De las Heras M, Palmarini M: Expression of the Jaagsiekte sheep retrovirus envelope glycoprotein is sufficient to induce lung tumors in sheep. J Virol 80:8030-8037, 2006
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 621-622. Elsevier Limited, St. Louis, MO, 2007
3. Maeda N, Fu W, Ortin A, de las Heras M, Fan H: Roles of the Ras-MEK-mitogen-activated protein kinase and phosphatidylinositol 3-kinase-Akt-mTOR pathways in Jaagsiekte sheep retrovirus-induced transformation of rodent fibroblast and epithelial cell lines. J Virol 79:4440-4450, 2005
4. Palmarini M, Fan H: Retrovirus-induced ovine pulmonary adenocarcinoma, an animal model for lung cancer. J Natl Cancer Inst 93:1603-1614, 2001
5. Rosadio RH, Sharp JM, Lairmore MD, Dahlberg JE, DeMartini JC: Lesions and retroviruses associated with naturally occurring ovine pulmonary carcinoma (sheep pulmonary adenomatosis). Vet Pathol 25:58-66, 1988
6. Salvatori D, De las Heras M, Sharp M: Ovine pulmonary adenocarcinoma: the story to date. In Practice 4:2004
7. Summers C, Norval M, De las Heras M, Gonzalez L, Sharp JM, Woods GM: An influx of macrophages is the predominant local immune response in ovine pulmonary adenocarcinoma. Vet Immunol Immunopathol 106:285-294, 2005

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 14

16 January 2008

Moderator:

Keith Steele, DVM, DACVP, PhD

CASE I – 03B 5415 (AFIP 3026837).

Signalment: 6-month-old, intact male, Dalmation, Canine

History: Chronic intermittent vomiting with a recent history of hematemesis and melena. Patchy alopecia on face, left elbow, and right foot.

Gross Pathology: Exploratory surgery revealed multiple acquired extrahepatic shunts. The liver had a greenish tint and accentuated lobular pattern.

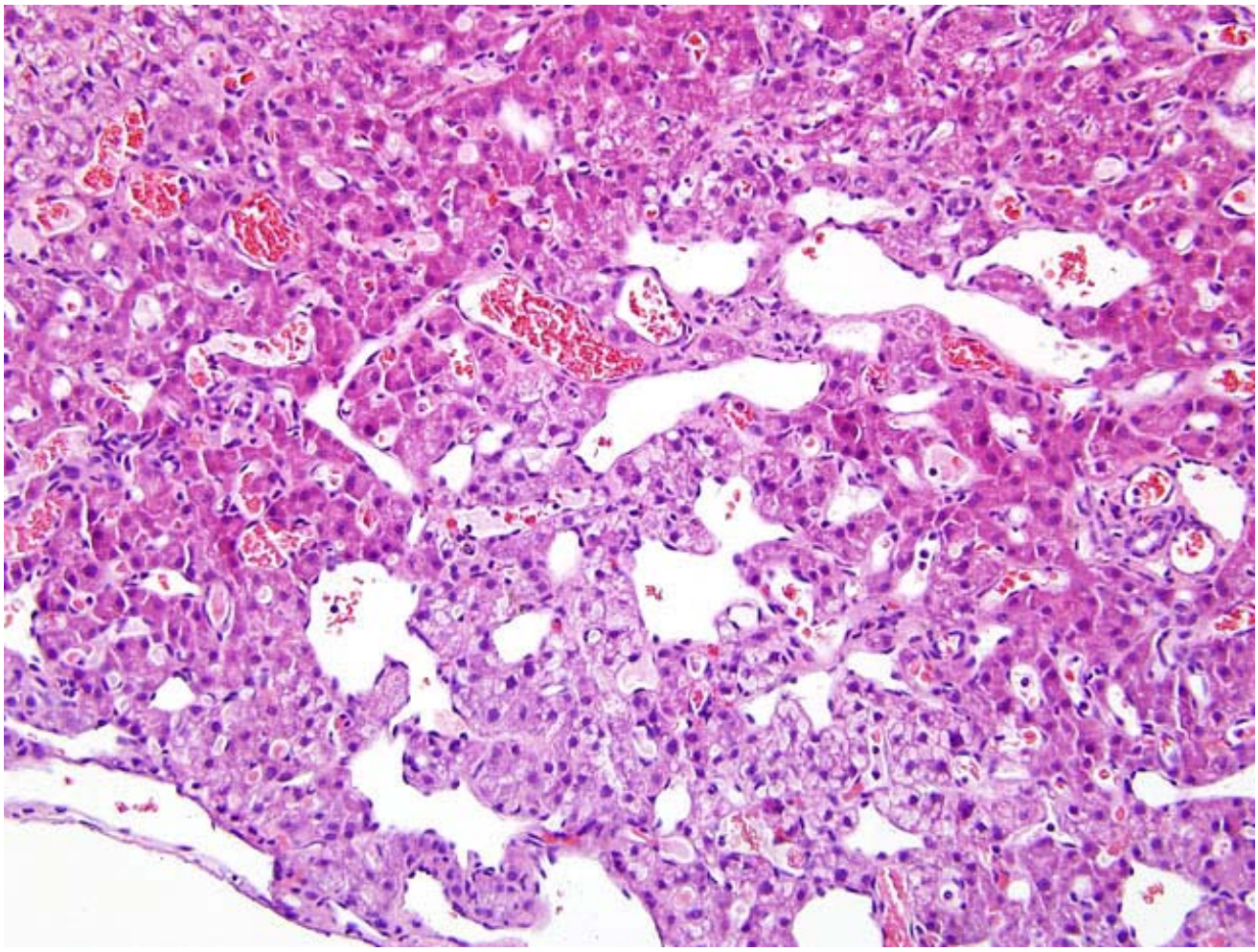
Laboratory Results:
Patient values are followed by reference interval. Anemia: Erythrocytes [3.15 (5.4-8.4)], Hemoglobin [6.9 (12-18)], Hematocrit [20.3 (35-54)], mild neutrophilia and monocytosis - increased ALP [620 (0-100)], ALT [172 (0-60)], AST [100 (0-50)], total bilirubin [0.7 (0.0-0.4)] and cholesterol [364(150-240)], prolonged PTT [24.4 (9.0-12.0)]. Hyperechoic enlarged liver and enlarged gall bladder on ultrasound.

Hepatic copper levels were 459 ppm.

Histopathologic Description: Widespread ectasia and reduplication of portal and central veins is prominent. The venous tunics are thickened by a combination of smooth muscle and fibrous connective tissue. Prominent vascularization and occasional arteriolization of hepatic sinusoids is apparent **(fig. 1-1).** Scattered mixed inflammatory cellular infiltrates are seen in portal and central areas. Centrilobular hepatocytes exhibit variable cell swelling and degeneration. Increased apoptotic bodies and hepatocellular pigmentation is also seen. There is extensive distension of subcapsular lymphatics and/or veins.

Contributor's Morphologic Diagnosis: Liver, severe microvascular dysplasia

Contributor's Comment: Hepatic microvascular dysplasia (HMD) is a syndrome of young to middle aged dogs, which present with signs of liver failure. The most common signs are CNS signs, vomiting, and/or diarrhea. The major differential diagnosis, both clinically and histopathologically, in the younger dogs is portosystemic shunts. In this case, the diagnosis of HMD was made primarily on the basis of the prominent vascularization of the hepatic sinusoids. Additionally, the dilation of the portal veins and minimal duplication of portal arterioles favors HMD over portosystemic shunt. The dilation of portal veins is presumed (though not proven in the literature reviewed by the submitter) to arise from portal hypertension, which could cause the secondary development of extrahepatic shunts as seen in this case.



1-1. Liver, Dalmatian. Sinusoidal ectasia with prominent vascularization (capillarization). (H&E 200X)

Yorkshire terriers and Cairn terriers have higher incidence of HMD, but it has been reported in numerous breeds. There are two hypotheses proposed for the cause of HMD. First is that the persistence of embryonic vitelline veins causes intrahepatic micro-shunts from the portal veins to the central veins. The other is that ultrastructural defects in the sinusoids cause reduced sinusoidal permeability and limited access of plasma components to the hepatocellular surfaces. The prognosis for uncomplicated HMD is better than that of portosystemic shunts. Many respond to dietary management alone and may survive for more than 5 years in good to excellent clinical condition.

AFIP Diagnosis: 1. Liver: Venous dilation, portal and central, diffuse, with lymphangiectasia, mild arteriolar and biliary reduplication, multifocal dissecting fibrosis, sinusoidal ectasia and capillarization, lobule atrophy, multifocal centrilobular hepatocellular degeneration and necrosis, and lipogranulomas, Dalmation (*Canis familiaris*), canine.
2. Liver: Hepatitis, neutrophilic, multifocal, mild.

Conference Comment: The case presented in conference is not typical of microvascular dysplasia or portal vein hypoplasia. Based on the degree of venous dilation and lymphangiectasia and the hepatocellular atrophy, abnormal circulation and portal hypertension are suspected. The process appears centered on the sinusoids with sinu-

soidal capillarization and possibly expanded basement membranes beneath them. Given the loss of lobular architecture, scattered mild fibrosis, mild inflammation, individual cell necrosis and pigment accumulation, this may be an example of lobular dissecting hepatitis in resolution with secondary portal hypertension. Lobular dissecting hepatitis, a form of cirrhosis of unknown etiology reported in young dogs, is characterized by dissection of the lobular architecture by fibroblasts and thin strands of extracellular matrix into small groups of hepatocytes, with accompanying mild to moderate inflammation and hepatocellular apoptosis or necrosis.[2]

Hepatic microvascular dysplasia is a poorly characterized condition with often confusing or contradictory descriptions in the literature on the disease etiology, description, and pathogenesis. The most current characterization, provided by the World Small Animal Veterinary Association (WSAVA) Working Group on Liver Disease and published in 2006, describes the condition as being no different from primary portal vein hypoplasia. The group prefers the latter term as more descriptive of the disease process.[2] Histologically, portal vein hypoplasia shares many features with congenital portosystemic shunts, intrahepatic arterioportal fistulas, and portal vein obstruction, including absent or diminished portal vein profiles and increased numbers of arteriolar profiles.[2] This standard was published after the submission of this case as a Wednesday Slide Conference submission, so this classification was not available for inclusion in the contributor's comments.

We thank Dr. John Cullen, Dr. Yvonne Schulman, and Dr. Thomas Lipscomb for their review and consultation of this case.

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References:

1. Christiansen JS, Hottinger HA, Allen L, Phillips L, Aronson LR: Hepatic microvascular dysplasia in dogs: a retrospective study of 24 cases (1987-1995). J Am Anim Hosp Assoc 36:385-389, 2000
2. Cullen JM, van den Ingh TSGAM, Bunch SE, Rothuizen J, Washabau RJ, Desmet VJ: Morphological classification of the circulatory disorders of the canine and feline liver. In: WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases, pp 51-52; 97-98. Elsevier Limited, St. Louis, MO, 2006
3. Phillips L, Tappe J, Lyman R, Dubois J, Jarboe J: Hepatic microvascular dysplasia in dogs. Prog Vet Neurol 7:88-96, 1996
4. Schermerhorn T, Center SA, Dykes NL, Rowland PH, Yeager AE, Erb HN, Oberhansley K, Bonda M: Characterization of hepatoportal microvascular dysplasia in a kindred of cairn terriers. J Vet Intern Med 10:219-230,1996

CASE II – CSU 067-69403 (AFIP 3065440).

Signalment: Eleven-month-old, female, intact, bobcat (*Lynx rufus*)

History: In summer, 2006, the kitten was found orphaned at approximately 2 months of age and taken to a wildlife rehabilitation facility. In September, the animal developed slowly progressive neurological disease including a head tremor, nystagmus, ataxia and loss of hind limb function. The animal was considered unsuitable for reintroduction, humanely euthanized and submitted to the Colorado State University veterinary diagnostic lab by the Colorado Division of Wildlife.

Gross Pathologic Findings: The animal was in moderate body condition with minimal autolysis. The brain had been removed immediately following euthanasia; both fresh and fixed brain was submitted with the carcass. No abnormalities were observed on gross post mortem examination.

Laboratory Results:
1. Rabies (FA, brain): Negative
2. Canine distemper virus (PCR, brain): Negative
3. *Toxoplasma gondii* (PCR, brain): Negative
4. Canine parvovirus/feline panleukopenia virus (PCR, brain): Positive
5. West Nile virus (IHC, brain and spinal cord): Positive
6. West Nile virus (PCR, brain): Negative

Histopathologic Description: Present on each slide are sections of spinal cord taken from the cervical and midthoracic region. Blood vessels in both grey and white matter are cuffed by lymphocytes, plasma cells and rare macrophages that often extend into adjacent neuropil. In rare sections perivascular hemorrhage is present. Glial nodules are present in both the grey and white matter. Most commonly in areas of gliosis, individual neuronal cell bodies are degenerate or necrotic characterized by hypereosinophilia, loss of Nissl substance, nuclear

pyknosis and variable neuronophagia. Occasionally perikaryonic vacuolization and rarefaction of the neuropil is present. Astrocytes numbers are moderately increased and often surround degenerate neurons (satellitosis). Within the white matter there is a variable amount of axonal degeneration, spongy change and occasional digestion chambers. The severity of lesion varies between submitted sections.

Contributor's Mor phologic Diagn osis: Spinal cord; lymphoplasmacytic myelitis, chronic, moderate with neuronal necrosis, astrocytosis, perivascular cuffing and glial nodules

Contributor's Com ment: West Nile virus emerged as a significant pathogen of birds, humans and horses in the northeastern United States in 1999. The arthropod-borne *Flavivirus* subsequently expanded north and westward resulting in widespread morbidity, and variable mortality, in susceptible species. Reports of WNV infection in non-avian wildlife are largely opportunistic and to our knowledge infection has not been previously reported in a bobcat.

Histologic lesions observed in this case are consistent with those previously reported in other mammals including horses,[1] fox squirrels,[2] white-tailed deer[4] and a dog[5]. The discordance between the WNV PCR and IHC results in this case may reflect the protracted nature of the disease and available tissue for testing. In the brain only very rare neurons stained weakly with IHC while neuronal cell bodies and occasional leukocytes in the spinal cord had abundant antigen; however only fresh brain, and no spinal cord, was available for PCR. The paucity of staining in the brain may represent remnant antigen while no RNA was present for amplification.

Classical gross and histological evidence of CPV or FPV infection in brain or gastrointestinal tract were absent in this case. The PCR product was sequenced and determined to be canine parvovirus 2b; the significance of this finding is unknown. Parvoviral infections have been reported in numerous wild carnivoirs[8] and it has been suggested that CPV 2a and 2b are more common in large, wild cats compared to domestic felids.[7] Recently, parvovirus infection has been reported in association with non-suppurative meningoencephalitis in dogs and cats and proposed as a new parvoviral disease pattern;[6] similar lesions were observed in the brain of this bobcat. Canine parvovirus is also widely distributed throughout the environment and the positive PCR result may be the result of contamination of the tissue sample during brain removal.

AFIP Diagnosis: Spinal cord, cervical and thoracic segments (per contributor): Myelitis, lymphoplasmacytic, multifocal, mild, with moderate axonal degeneration, bobcat (*Lynx rufus*), feline.

Conference Comment: Following its initial identification in the United States in 1999, West Nile Virus has subsequently spread throughout most of the United States and the southern parts of Canada. The virus is genetically divided into two lineages.[3] Lineage 1, occasionally highly virulent (clade 1a), is seen in North America and other areas of the world.[3] Lineage 2, usually non-pathogenic or only mildly virulent, is present primarily within enzootic areas of Africa.[3]

The virus is maintained in the environment within the wild bird population through a bird-mosquito-bird cycle. *Culex* spp. are the primary vectors of transmission, although the virus has been identified in ticks. Additionally, transmission has been documented through direct contact and via fomites.[3]

Histologic lesions often can be very mild even in severe disease and include nonsuppurative encephalomyelitis, gliosis, and glial nodule formation with occasional neuronal degeneration and necrosis.[3] The primary target cell is the neuron with additional damage to microglial cells.[9] Apoptotic cell death appears to be the mechanism of neuronal injury.[9] Conference participants' slides were quite variable in the presence and severity of perivascular cuffing and hemorrhage.

Primarily an infection of birds, WNV has also been documented in horses, humans, ruminants, cervids, canids, felids, squirrels, rodents, and swine.[2,3,4]

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References:

1. Cantile C, Del Piero F, Di Guardo G, Arispici M: Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. Vet Pathol 38:414-421, 2001
2. Kiupel M, Simmons HA, Fitzgerald SD, Wise A, Sikarskie JG, Cooley TM, Hollamby SR, Maes R: West Nile virus infection in Eastern fox squirrels (*Sciurus niger*). Vet Pathol 40:703-707, 2003
3. Maxie MG, Youssef S: Nervous system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.

ed. Maxie MG, 5th ed., vol. 1, pp. 421-422. Saunders Elsevier, Endinburgh, 2007
4. Miller DL, Radi ZA, Baldwin C, Ingram D: Fatal West Nile virus infection in a white-tailed deer (*Odocoileus virginianus*). J Wildl Dis 41: 246-249, 2005
5. Read RW, Rodriguez DB, Summers BA: West Nile virus encephalitis in a dog. Vet Pathol 42:219-222, 2005
6. Schwab S, Herden C, Seeliger F, Papaioannou N, Psalla D, Polizopulou Z, Baumgartner W: Non-suppurative meningoencephalitis of unknown origin in cats and dogs: an immunohistochemical study. J Comp Pathol 136:96-110, 2007
7. Steinel A, Munson L, van Vuuren M, Truyen U: Genetic characterization of feline parvovirus sequences from various carnivores. J Gen Virol 81:345-350, 2000
8. Steinel A, Parrish CR, Bloom ME, Truyen U: Parvovirus infections in wild carnivores. J Wildl Dis 37:594-607, 2001
9. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 882-883. Elsevier, St. Louis, MO, 2007

CASE III – TAMU-1 2005 (AFIP 2984049).

Signalment: Two-year-old, male, Weimaraner, *Canis familiaris*

History: The patient had a chronic vomiting problem of one-year duration. The dog was thin with atrophy of all muscles except those of the neck and tongue. Radiographs showed a hiatal hernia. The tongue was difficult to exteriorize for anesthesia due to a large sublingual "mass". Front limbs became spastic during anesthesia. Electromyogram demonstrated spontaneous activity, complex repetitive activity, and high frequency discharges, but motor nerve conduction velocities were normal. Myoglobinuria was noted. Due to the clinical diagnosis, this dog was euthanized.

Gross Pathology: The dog had left side abdominal cryptorchidism and right side renal agenesis. A left side esophageal hiatal hernia contained the stomach and duodenum. Most importantly, the dog had massive thickening of the muscles of the base of the tongue, and that musculature pulled the mandible caudally. The tongue was short and triangular with a base 10cm in diameter (the "mass" noted clinically). The neck muscles were thick, giving a "buffalo hump" appearance. The diaphragmatic muscle surrounding the central tendon was pale and 1.5cm thick; however, overall short. The body muscle mass was reduced and muscles were pale.

Laboratory Results: Serum Creatinine Kinase 32672 u/L (reference range – 68 – 400 u/L); Serum Alanine Aminotransferase 305 u/L (reference range 10 –130 u/L); WBC 21,500 cells/u/L (reference range – 6,000 – 17,000) with an absolute neutrophilia of 18,050 cells / u/L.

Histopathologic Description: The slide presented is of the diaphragm of the patient and a normal size and age-matched dog. On subgross, one notes the obvious and impressive difference in thickness of the longitudinal sections. The thickness is attributed to fibrosis, degenerating hypercontracted, hyalinized, broken and thick fibers with central fiber cysts and nuclei within fibers, as well as on-going regeneration and hypertrophy with proliferation of satellite muscle. The "resident" fat of the diaphragm remains. Mineralization is present.

Contributor's Morphologic Diagnoses: Diaphragm – Severe, diffuse, myodegeneration and necrosis with mineralization and fibrosis and on-going myoregeneration (muscular dystrophy).

Contributor's Comment: The lesions are typical of the muscular dystrophy described in Golden Retrievers.[6,7,8] Immunostaining for dystrophin showed absence of dystrophin (a membrane-associated protein) below the membranes of muscle fibers from the sublingual area, sternohyoideous, and infraspinatus. Thus, this case represents another breed with Duchenne-like muscular dystrophy. Similar X-linked muscular dystrophy has been demonstrated in Golden Retrievers, Labrador Retrievers, Irish Terriers, Samoyeds, Rottweilers and the Japanese Spitz.[1,5] Affected animals lack the subsarcolerminal protein, dystrophin. Clinically, they show progressive weakness and later cardiac abnormalities. This dog also had a dilated and hypertrophic myocardium with severe cardiomyopathy. The unusual presenting clinical complaint, chronic vomiting, is presumed due to the hiatal hernia. Interestingly, Duchenne-like muscular dystrophy researchers using Golden Retrievers found a left side hiatal hernia in their breeding colony.[9] Deficiency of the 427 KD dystrophin protein has been demonstrated in humans, cats, dogs and mice.[2,3,4,8]

The obvious difference in thickness of the longitudinal sections is attributed to hypercontracted, hyalinized, broken, swollen fibers, some having central cysts and central nuclei. These fibers are often separated by extensive fibrosis. Some fiber hypertrophy with sarcolemmal nuclei proliferation is ongoing.

3-1. Diaphragm, Weimaraner. Myofibril size variation with occasional large rounded myofibers. Skeletal muscle hypertrophy. (H&E 200X)
3-2. Diaphragm, Weimaraner. Skeletal muscle necrosis characterized by loss of cross striations, hypercontraction and fragmentation of cytoplasm. (H&E 200X)
3-3. Diaphragm, Weimaraner. Skeletal muscle regeneration characterized by myofibers with a small diameter, slightly basophilic cytoplasm and internal rows of large euchromatic nuclei. (H&E 200X)

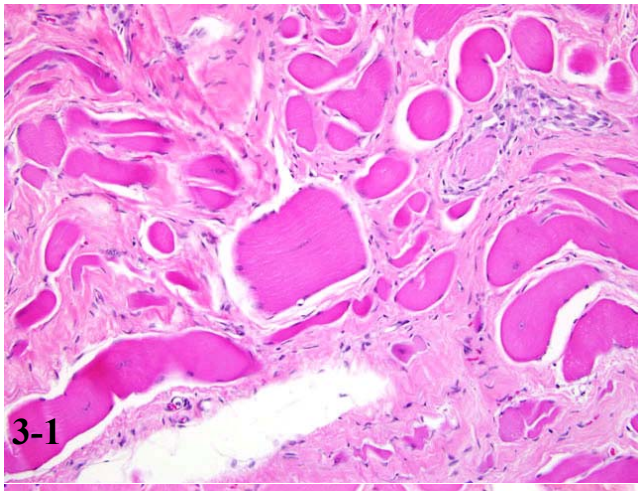
AFIP Dia gnosis: Skeletal muscle: Myocyte hypertrophy, degeneration, necrosis, regeneration, and mineralization, diffuse, severe, with fibrosis, Weimaraner, (*Canis familiaris*), canine **(fig. 3-1, 3-2, 3-3).**

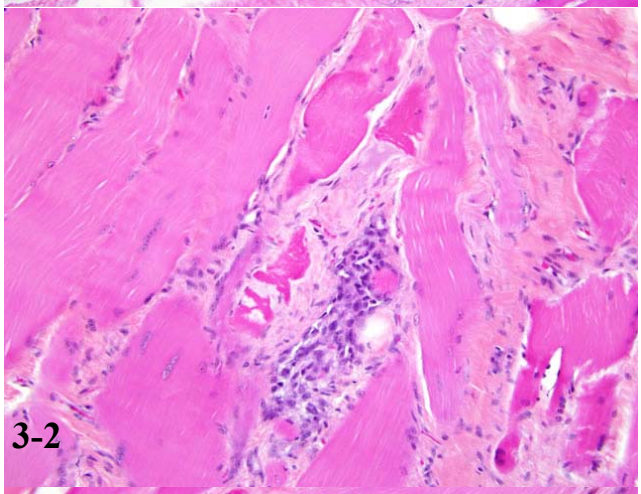
Conference Comment: X-linked muscular dystrophy, an X-linked recessive defect in the dystrophin gene, affects approximately 50% of males born to female carriers.[10] The dystrophin gene codes for a membrane-associated cytoskeletal protein that is present in skeletal and cardiac muscle. The lack of this gene increases the susceptibility of the muscle fibers to repeated bouts of necrosis, regeneration, and fibrosis.[10] Dystrophin deficiency generally results in progressive muscle atrophy of most breeds of dogs, but may cause marked muscle hypertrophy in cats, mice, and Rat Terrier dogs.[3,11]

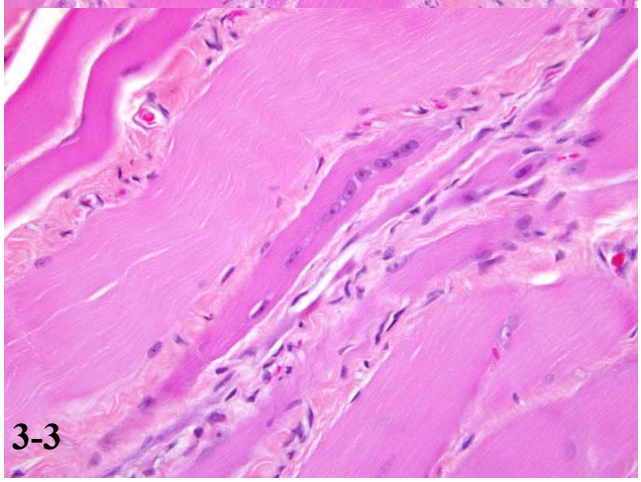
Characteristic gross pathological findings include severe degeneration of the diaphragm and strap muscles with pale white streaks within the affected muscles.[11]

Not all canine muscular dystrophies are X-linked. A defect in sarcoglycan, a component of the sarcolemmal dystrophin glycoprotein complex, occurs in both male and female dogs.[10]

Negative immunohistochemistry for the dystrophin protein is helpful in diagnosing muscular dystrophy, although a positive result will not rule out the entity. Partial expression of dystrophin may occur in Becker-type mutations or in revertant fibers, in which genetic mutation allows some dystrophin expression.[11]







Contributor: Dept. of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, 77843-4467

References:

1. Bergman RL, Inzana KD, Monroe WE, Shell LG, Liu LA, Engvall E, Shelton GD: Dystrophin-deficient muscular dystrophy in a Labrador retriever. J Am Anim Hosp Assoc 38:255-261, 2002
2. Collins CA, Morgan JE: Duchenne's muscular dystro-

phy: animal models used to investigate pathogenesis and develop therapeutic strategies. Int J Exp Pathol 84:165-172, 2003
3. Gaschen FP, Hoffman EP, Gorospe JRM, Uhl EW, Senior DF, Cardinet III GH, Pearce LK: Dystrophin deficiency causes lethal muscle hypertrophy in cats. J Neurol Sci 110:149-159, 1992
4. Gaschen L, Lang J, Lin S, Ade-Damilano M, Busato A, Lombard CW, Gaschen FP: Cardiomyopathy in dystrophin-deficient hypertrophic feline muscular dystrophy. J Vet Intern Med 13:346-356, 1999
5. Jones BR, Brennan S, Mooney CT, Callanan JJ, McAllister H, Guo LT, Martin PT, Engvall E, Shelton GD: Muscular dystrophy with truncated dystrophin in a family of Japanese spitz dogs. J Neurol Sci 217:143-149, 2004
6. Valentine BA, Cooper BJ, Cummings JF, deLahunta A: Progressive muscular dystrophy in a Golden Retriever dog: light microscope and ultrastructural features at 4 and 8 months. Acta Neuropathol (Berl) 71:301-310, 1986
7. Valentine BA, Cooper BJ, Cummings JF, deLahunta A: Canine X-linked muscular dystrophy: morphologic lesions. J Neurol Sci 97:1-23,1990
8. Valentine BA, Cooper BJ, Dietze AE, Noden DM: Canine congenital diaphragmatic hernia. J Vet Intern Med 2:109-112, 1988
9. Valentine BA, Winand J, Pradhan D, Moise NS, deLahunta A, Korneygay JN, Cooper BJ: Canine X-linked muscular dystrophy as an animal model of Duchenne muscular dystrophy: a review. Am J Med Genet 42:352-356, 1992
10. Valentine BA, McGavin: Skeletal muscle. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1026-1029, 1035-1037. Elsevier, St. Louis, MO, 2007
11. Van Vleet JF, Valentine BA: Muscle and tendon. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. ed. Maxie MG, 5th ed., vol. 1, pp. 210-216. Saunders Elsevier, Endinburgh, 2007

CASE IV - 2006 AFIP #1 (AFIP 3031544).

Signalment: 7-month-old, female, neutered, German Shepherd Dog (*Canis familiaris*).

History: This dog was singly kenneled in a training center which had 60 other dogs. This dog was found weak and ataxic, with vomiting and diarrhea and was euthanized. It was reported to be eating and drinking and to have had normal feces the day before. Ovariohysterectomy had been performed on this animal 10 days prior to euthanasia. A transitory diarrhea that resolved with oral metronidazole was present for a few days after surgery but no other complications were reported. All other dogs at the facility remained clinically normal.

Gross Pathologic Findings: Approximately 150 ml of sero-sanguineous fluid and large fibrinous clots were present in the thoracic cavity. There were numerous fibrinous adhesions between the visceral and parietal pleura and petechial hemorrhages were present in the intercostal muscles. The ventral 50-80% of all lung lobes was firm, black and depressed. Randomly scattered within these dark areas, were numerous white to pale pink, 1-4mm diameter irregular foci. The pericardial sac was thickened and edematous, with fibrinous adhesions to the visceral pleura.

Laboratory Results: A pure culture of hemolytic *Escherichia coli* was isolated from the lung. Serotyping classified the isolate as O4:H5 or O4:H56 and it tested positive for cytotoxic necrotizing factor 1 (CNF1). Samples of lung were negative for canine influenza by reverse-transcription PCR (RT-PCR).

Histopathologic Description: Most alveoli and bronchioles contain extravasated erythrocytes, eosinophilic proteinaceous fluid, fibrin and an inflammatory exudate of viable and necrotic neutrophils, with fewer macrophages and lymphocytes. There is extensive coagulative septal necrosis, with foci of complete parenchymal dissolution and replacement by necrotic cellular debris. In some sections focally extensive hemorrhage disrupts the normal architecture of the lung. Blood vessel necrosis and fibrin thrombosis are prominent in these areas. Colonies of short rod-shaped bacteria are present in many bronchioles and scattered throughout the necrotic parenchyma **(fig. 4-1).** There are scattered clumps of amorphous, basophilic material (consistent with mineral) in alveolar spaces. There are extensive subpleural hemorrhages.

In the tracheobronchial lymph nodes there was lymphocellular necrosis, hemorrhage and medullary hemosiderosis. Widespread, acute centrilobular hepatic necrosis was the only other significant finding.

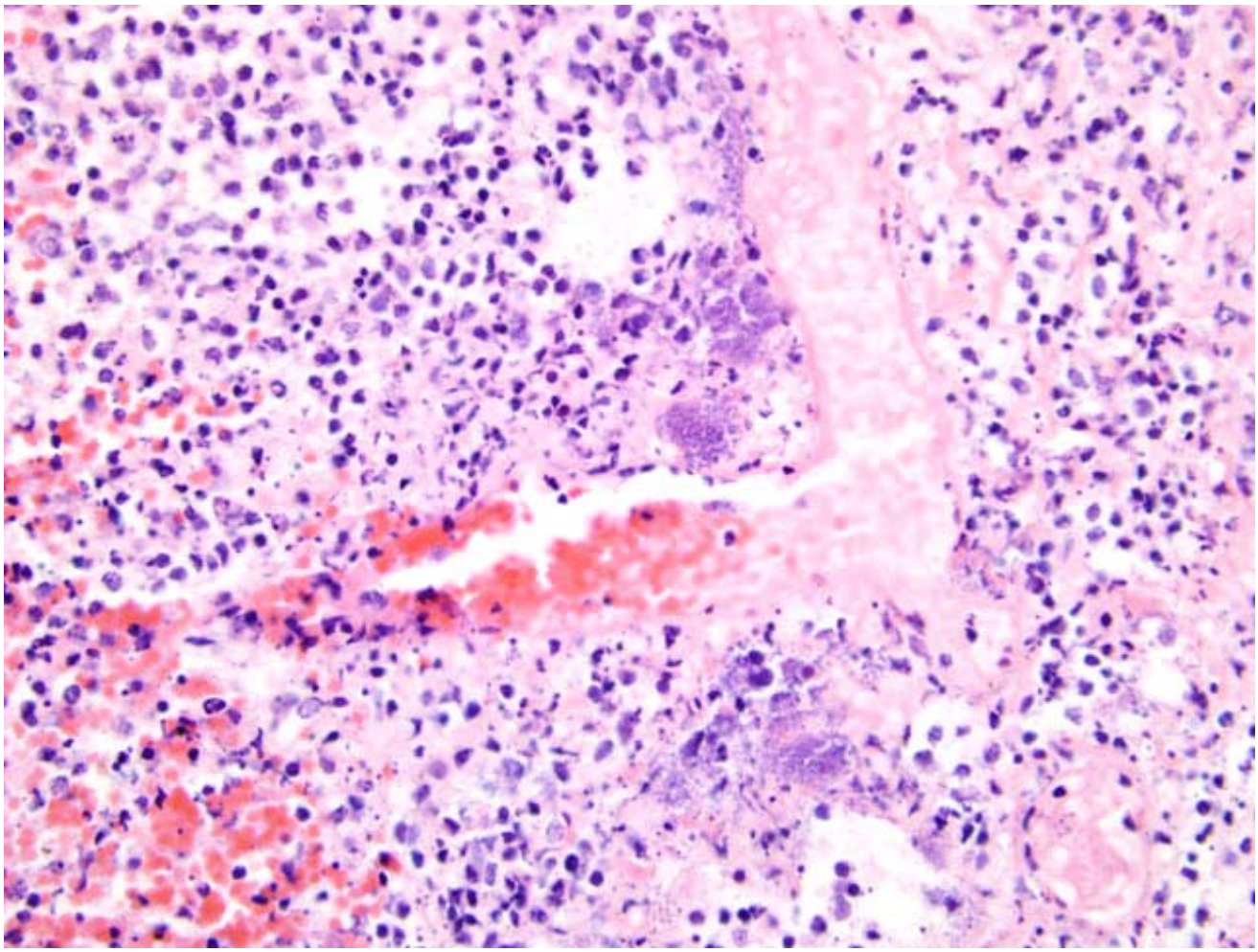
Contributor's Morphologic Diagnosis: Lung: Pneumonia, necrotizing and fibrinohemorrhagic, acute, diffuse, severe with thrombosis and rod-shaped bacteria, etiology consistent with necrotoxigenic *Escherichia coli*.

Contributor's Comment: *Escherichia coli* is the predominant facultatively anaerobic enteric bacterium of

most vertebrates and is frequently isolated from diagnostic specimens. Most strains are either commensals or opportunistic pathogens of immunocompromised individuals, but other strains are well recognized pathogens. The pathogenic strains are historically classified into enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, necrotoxigenic and enteroaggregative strains according to the O (lipopolysaccharide) and H (flagellum) serotype, and are further characterized by their production of virulence factors.[5] Cytotoxic necrotizing factor 1 (CNF1) is consistently produced by necrotoxigenic *E. coli* and some isolates also produce CNF2 and alpha hemolysin. Necrotoxigenic strains are an important cause of extraintestinal disease such as urinary tract infections, pyometra, meningitis, septicemia and pneumonia in humans and mammals.[5]

Hemorrhagic and necrotizing pneumonia caused by necrotoxigenic *E. coli* has recently been described in dogs.[1,4] In common with the present case, affected dogs have been young (<1year), the clinical illness is usually less than 24 hours and immunodeficiency or concurrent illness were not identified. In our case, parainfluenza virus and adenovirus testing were not performed and while no inclusion bodies were identified, concomitant infection with these agents cannot be excluded. *E. coli* of both O4 and O6 serotypes have been reported to cause these lesions, which irrespective of serotype were positive for CNF1.

The main differential diagnoses for hemorrhagic pneumonia in dogs are canine influenza and bacterial septicemias including streptococcal septicemia.[2,3] Microscopically, canine influenza is characterized by a pneumonia that is more broncho-interstitial and suppurative with less necrosis[2], but RT-PCR or virus isolation is best per-



4-1. Lung, German Shepherd. Colonies of rod-shaped (bacilli) admixed with inflammatory cell infiltrates and cellular debris in a necrotic focus. (H&E 200X)

formed for definitive exclusion. Samples of lung from this case were negative for canine influenza by RT-PCR.

Little is known about the source and route of infection, means of transmission and pathogenesis of this disease in dogs.

AFIP Diagnosis: Lung: Pneumonia, necrohemorrhagic, neutrophilic and histiocytic, diffuse, severe, with fibrin, edema, and numerous bacilli, German Shepherd Dog (*Canis familiaris*), canine.

Conference Comment: Strains of *E. coli* are identified by the various antigens they express, primarily using the O and H antigens.

- O antigens (somatic): Determines the serogroup, lipopolysaccharide molecule
- H antigens (flagellar): Determines the serotype
- K antigens (capsular): Made up of polysaccharides and proteins; may also be used for classification purposes
- Fimbrial or pili antigens: Important in adhesion and colonization of epithelium

Extraintestinal pathogenic *E. coli* have been associated with pyometra, mastitis, otitis, prostatitis, bacteremia, skin diseases, cholecystitis, and pneumonia. Strains producing the cytotoxic necrotizing factor (CNF) are referred to as necrotoxic *E. coli*.[4] These strains produce either CNF1, identified in humans and domestic animals, or CNF2, identified only in ruminants.[4,5] The genes that code for CNF-1 and alpha hemolysin are genetically linked and have a tendency to occur with O4 and O6 groups.[1,4]

The primary fimbrial antigen in extraintestinal pathogenic *E. coli* is the P fimbriae and is encoded by the *pap* (pilus-associated pyelonephritis) gene.[1,4] The *papG* (fimbrial tip adhesion) and *papA* (major fimbrial subunit) alleles have also been associated with necrotoxic *E. coli*.[1,4]

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References:

1. Breitschwerdt EB, DebRoy C, Mexas AM, Brown TT, Remick AK: Isolation of necrotoxigenic *Escherichia coli* from a dog with hemorrhagic pneumonia. J Am Vet Med Assoc 226:2016-2019, 2005
2. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AI, Katz JM, Donis RO: Transmission of equine influenza virus to dogs. Science 310:482-485, 2005
3. Garnett NL, Eydelloth RS, Swindle MM, Vonderfecht SL, Strandberg JD, Luzarraga MB: Hemorrhagic streptococcal pneumonia in newly procured research dogs. J Am Vet Med Assoc 181:1371-1374, 1982
4. Handt LK, Stoffregen DA, Prescott JS, Pouch WJ, Ngai DT, Anderson CA, Gatto NT, DebRoy C, Fairbrother JM, Motzel SL, Klein HJ: Clinical and microbiologic characterization of hemorrhagic pneumonia due to extraintestinal pathogenic *Escherichia coli* in four young dogs. Comp Med 53:663-670, 2003
5. Songer JG, Post KW: The genera *Escherichia* and *Shigella*. In: Veterinary Microbiology - Bacterial and Fungal Agents of Animal Disease, eds. Songer JG, Post KW, pp. 113-119. Elsevier Saunders, St. Louis, MO, 2005

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 15

30 January 2008

Moderator:

Dr. Elizabeth Mauldin, DVM, DACVP, DACVD

CASE I – N06-274 (AFIP 3074696).

Signalment: Mature, female spayed, Miniature Schnauzer

History: The animal presented with a two day history of generalized skin erythema. This rapidly progressed to skin thickening, which began around the ears, head and neck, and soon involved the whole body. Severe depression and dehydration ensued (8% dehydrated), with vomiting and foul smelling diarrhea. Extreme pain was noted all over the body. There was prominent pitting edema which was most pronounced over the abdomen, base of the ears, sternum and vulva. Gentle manipulation of the skin revealed detachment from the underlying tissues (Nikolsky's sign). Blood biochemistry and hematology results are detailed below. Attempts at supportive therapy were unsuccessful and the owners elected for euthanasia on humane grounds.

Gross Pathology: Gross necropsy revealed generalized, moderate cutaneous erythema; the epidermis was easily detached from the underlying dermis on gentle manipulation (Nikolsky's sign). There were no other significant findings in other organs.

Laboratory Results: Provided in **table 1-1**.

Histopathologic Description: Haired skin: Diffusely, keratinocytes within all layers of the epidermis are hypereosinophilic with pyknotic to karyorrhectic nuclei (cell death). Frequently, this extends into the follicular infundibulum. Randomly scattered throughout the dermis, there are small to moderate numbers of lymphocytes, admixed with plasma cells, neutrophils, macrophages and numerous melanin-laden macrophages (pigmentary incontinence). Lymphocytes are occasionally present within the epidermis, however this is variable. In places, there is clefting between the epidermis and dermis, both beneath the basement membrane within the suprabasilar epithelium (this differs between sections). Superficial dermal vessels are prominent and lymphatics occasionally contain moderate numbers of neutrophils.

Contributor's Morphologic Diagnosis: Haired skin: Severe, diffuse, sub-acute epidermal necrosis with mild lymphoplasmacytic dermatitis and pigmentary incontinence (consistent with Toxic Epidermal Necrolysis)

Contributor's Comment: Toxic epidermal necrolysis (TEN) is a rare, and often life-threatening skin disease.[1,3-5,11] The distinction between this, erythema multiforme (EM) and Stevens-Johnson syndrome (SJS) remains a source of controversy. This partly stems from the criteria

Routine Blood Chemistry
Sodium: 148 mEq/L (142-151)
Potassium: 3.1 mEq/L (3.9-5.3)
Chloride: 115 mEq/L (107-117)
Bicarb: 19 mEq/L (15-25)
Anion gap: 17 mEq/L (13-25)
Na:K: 48
Urea: 40mg/dL (8-30)
Creat: 1.7mg/dL (0.5-1.3)
Calcium: 9.1 mg/dL (9.3-11.6)
Phosphate: 6.4 mg/dL (2.8-5.3)
Magnes: 2.8 mEq/L (1.4-2)
Tot Prot: 4.3 g/dL (5.6-7.1)
Alb: 2.1 g/dL (3.1-4.1)
Glob: 2.2 g/dL (1.9-3.6)
A/G: 0.95
Glucose: 183 mg/dL (60-120)
ALT/P5P: 85 U/L (25-106)
AST/P5P: 120 U/L (16-50)
Alk Phos: 348 U/L (12-122)
GGT: <3 U/L (0-10)
TotBili: 5 mg/dL (0-0.3)
Dir Bili: 4 mg/dL (0-0.1)
Ind Bili: 1 mg/dL (0-0.3)
Amylase: 934 U/L (286-1124)
Cholesterol: 269 mg/dL (124-335)
CK: 3633 U/L (58-241)
Iron: 93 ug/dL (98-220)
TIBC: 213 ug/dL (249-496)
%SAT: 44% (28-62)

Hematology
HCT: 34% (42-57)
HB: 11.5 g/dL (14.6-19.7)
RBC: 4.6 mill/uL(6.1-8.5)
MCV: 73 fL (63-74)
MCH: 25 pg (21-26)
MCHC: 34 g/dL (32-37)
RDW: 12.8% (11.3-14)
Retic: 0.4% (0.2-1.1)
Retic-abs: 18.4 thou/ul (10.1-75.9)
Nucl RBC: 1/100WBC (0-1/100WBC)
WBC: 24.8 thou/ul (6.2-14.4)
Seg Neuts: 19.1 thou/ul (3.4-9.7)
Band Neuts: 2.5 thou/ul (0-0.1)
Lymph: 0.5 thou/ul (1.2-4.7)
Mono: 0.7 thou/ul (0.1-1)
Eosin: 2 thou/ul (0.1-2)
Baso: 0 thou/ul (0-0.1)
Plat smear: Low
Plat: 67 thou/ul (179-483)
MPV: 12.3 fL (8.4-13.2)
TP: 6.6 g/dL (5.9-7.8)
RBC Morphology: No significant abnormalities
WBC Exam: Toxic changes in neutrophils (moderate)
Plasma appearance: Icterus (moderate)

Coagulation Panel
Activated Partial Thromboplastin Time: 21.5s (10-17)
Antithrombin 3: 48% (75-120)
D-dimer: 250-500 ng/ml (<250)
Fibrinogen: 1414 mg/dL (150-480)
Protein C: 70%
Prothrombin Time: 17s (14-18)
Thrombin Clotting Time: 5s (5-9)

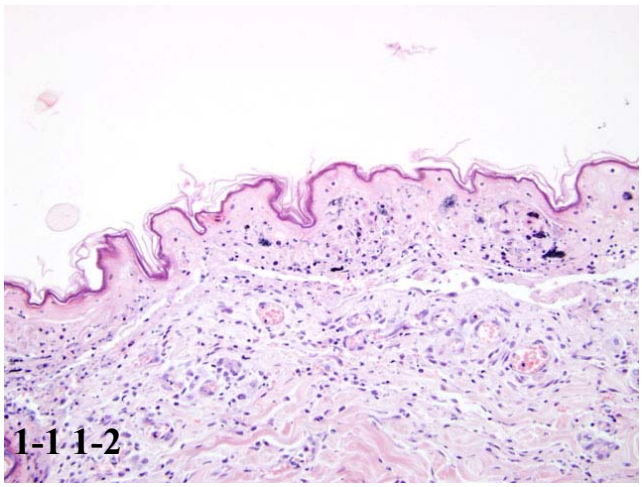
Ancillary Tests
Antinuclear antibody test: Negative

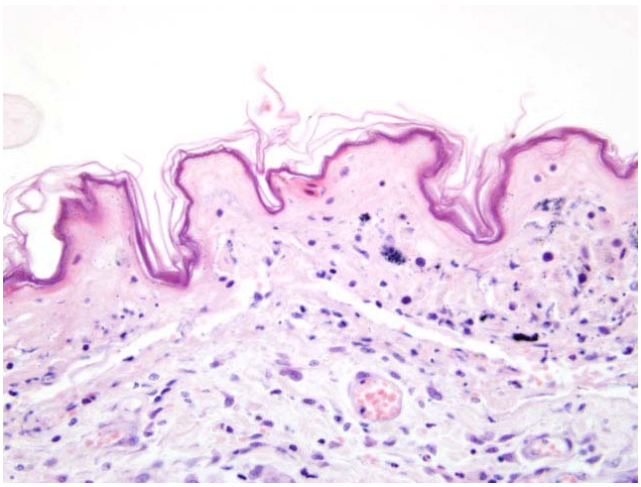
Table 1-1. Laboratory Values, Case 1.

applied to the different conditions, which for some time, was poorly defined. In dogs, Hinn *et al* divided the clinical lesions into five categories: EM-minor, EM-major, SJS, SJS-TEN 'overlap', and TEN.[5]

Clinically, TEN represents the most severe of the three syndromes.[3,4,11] This distinctive clinical presentation has led many to believe this is a pathologically distinct entity from EM.[3,4,11] Microscopically, TEN is characterized by extensive, full thickness, **epidermal cell de ath (fig. 1-1, 1-2).** In the classical sense, this is associated with little to no inflammation. However more advanced cases can develop a degree of inflammation, particularly following dermal-epidermal detachment.[3] SJS bears similarity to TEN, though has less extensive epidermal detachment.[5]

Many authors now consider EM to be clinically and histologically distinct from SJS and TEN.[3,4] Classically,





1-1. Haired skin, Miniature Schnauzer. Diffuse epidermal necrosis at all levels of the epidermis. H&E X400
1-2. Haired skin, Miniature Schnauzer. Higher magnification of keratinocyte necrosis. H&E X100

EM is characterized by multiple 'target' lesions (present in 15.9% of cases)[11], and these affect less than 50% of the body. The severity of disease can be variable, but removal of the inciting causes usually produces a clinical resolution within three weeks.[11] Histologically, EM is characterized by multi-level, single cell-death surrounded by lymphocytes or macrophages (satellitosis). Severe cases of EM (EM-major) can develop to transepidermal necrosis, whereby lesions may resemble those of TEN.

A diagnosis of EM, with confluent areas of epidermal necrosis, was considered as a differential in this case. However, we feel that the clinical and histologic findings differ from EM on a number of levels. Importantly, the clinical disease in this animal was severe and rapidly progressive: dermatologic lesions affected large areas of the body (significantly greater than 50%) and there were large areas of epidermal detachment. This was accompanied by profound depression and lethargy, with vomiting and diarrhea. Blood biochemistry and hematology also revealed electrolyte abnormalities; a coagulation panel revealed late stage changes consistent with Disseminated Intravascular Coagulation. Although animals with EM can also be systemically unwell, the extent of antemortem lesions would favor a diagnosis of TEN. From a histologic perspective, the lymphocytes could represent a more chronic change, and possibly a sequel to the extensive epidermal changes.

All of the above syndromes have been associated with an immune reaction against a hematogenous antigen. Drug administration appears to be an important predisposing factor, particularly in cases of TEN where there was a temporal association in over 80% of human cases.[4,11] A similar association seems to exist in the dog.[5] Medication has also been associated with EM and SJS: however the study by Hinn *et al*. found that unlike TEN, the majority of EM cases were not associated with previous drug administration. Other antigens such as infections, vaccination, food, and neoplasms are therefore thought to account for disease in animals.[11] The remaining cases are thought to be idiopathic.

Given the infrequency of the disease in veterinary species, recent studies regarding pathogenesis are largely derived from the human literature. It is generally agreed that the disease represents massive, immune mediated apoptosis of epidermal keratinocytes; however the mechanism by which this occurs is controversial. A number of different theories have been proposed. Some papers have suggested a primary role for both T-cells and NK cells in the development of disease.[2,8,10] Part of the basis for this is the presence of CD8+ lymphocytes within blister fluid and the epidermis in the early stages of disease. Blister fluid is also found to contain high levels of soluble IL-2 receptor, indicative of activated T-cells.[10] Other cytokines and chemokines may also play a role in human cases of SJS and TEN.[2,8,10] In particular, over expression of TNF-α has been shown in many cases of SJS/TEN.[10] IL-5, IL-13 and IFN-γ, may also contribute to the inflammation.[2]

A primary role of apoptotic mechanisms has also been proposed.[1,7,12,13] The activation of Fas (CD95), through binding of FasL, may be fundamental to the development of disease. Viard *et al*. showed that blocking Fas/FasL

interactions, through the antibodies in pooled human sera, could prevent keratinocyte death *in vitro*.[13] In one report, a single dog with SJS was successfully treated with human immunoglobulin, and the authors attributed this to the same mechanism.[9] Initial studies suggested that FasL was translocated to the surface of affected keratinocytes. However subsequent publication have failed to consistently demonstrate surface expression of FasL in affected patients.[1] One study found increased levels of soluble Fas ligand (sFasL) in the peripheral blood of patients with SJS-TEN, and this was derived form peripheral blood mononuclear cells(PBMCs).[1] In this same study, keratinocyte apoptosis could be induced *in vitro*, following treatment with serum from SJS and TEN patients. Single nucleotide polymorphisms in Fas and FasL have been suggested to confer susceptibility to these diseases in humans.[7]

Following the appropriate stimulation, apoptosis may occur via activation of death receptors, of which Fas is the most widely studied.[6] In the Fas pathway, binding with FasL (from either autocrine, paracrine or endocrine route) induces receptor triimerization. This facilitates binding of a Fas-associated death domain (FADD), leading to conversion of procaspase 8 and subsequent caspase activation. Other potential mechanisms of apoptosis include activation of TNF-related apoptosis-inducing ligand (TRAIL), which may be important given the high concentrations of TNF-α in lesions.[10] Activated cytotoxic T-cells may also induce apoptosis by perforin/ granzyme B.[10] The contribution of these various pathways is unclear, however there is evidence to suggest that multiple pathways may be involved.

AFIP Diagnosis: Haired skin: Epidermal necrosis, diffuse with subepidermal clefting, mild subacute dermatitis, dermal edema, and congestion, Miniature Schnauzer (*Canis familiaris*), canine.

Conference Comment: The contributor gives an excellent review of toxic epidermal necrolysis (TEN), the controversy over its association with erythema multiforme (EM) and Stevens-Johnson syndrome (SJS), and the proposed mechanism of epidermal cell death.

In general the separation of these disease entities depends on the clinical as well as the histological criteria. **Table 1-2** lists general characteristics of EM, SJS, and TEN.[4]

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References:

1. Abe R, Shimizu T, Shibaki A, Nakamura H, Watanabe H, Shimizu H: Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. Am J Pathol 162:1515-1520, 2003
2. Caproni M, Torchia D, Schincaglia E, Volpi W, Frezzolini A, Schena D, Marzano A, Quaglino P, De Simone C, Parodi A, Barletta E, Fabbri P: Expression of cytokines and chemokine receptors in the cutaneous lesions of erythema multiforme and Stevens-Johnson syndrome/ toxic epidermal necrolysis. Br J Dermatol 155:722-728, 2006

Table 1 –2.[4]

Condition	Location	Characteristics
EM, Minor	No signs of systemic illness At least 1 mucosal surface affected < 10% of body surface	Lymphohistiocytic, perivascular and interface Lymphocytic sattelitosis
EM, Major	Signs of systemic illness >1 mucosal surface affected 10-50% of body surface <10% epithelial detachment	High degree of epidermal inflammation, vesiculobullous lesions
Steven-Johnson Syndrome	50% of body surface 10-30% epithelial detachment	Severe epithelial necrosis
TEN	Generalized disease More than 30% epithelial detachment	None, or minimal except when ulcerated

3. Ginn PE, Mansell JEKL, Rakich PM: Skin and appendages. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 656-658. Elsevier Saunders, Philadelphia, PA, 2007
4. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Necrotizing diseases of the epidermis. In: Skin Diseases of the Dog and Cat, 2nd ed., pp. 65-68, 75-78, 80-84. Blackwell, Ames, IA, 2005
5. Hinn AC, Olivry T, Luther PB, Cannon AG, Yager JA: Erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis in the dog: clinical classification, drug exposure and histopathological correlations. J Vet Allergy Clin Immunol 6:13-20, 1998
6. Kumar V, Abbas AK, Fausto N: Cellular adaptations, cell injury, and cell death. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 26-32. Elsevier Saunders, Philadelphia, PA, 2005
7. Murata J, Abe R: Soluble Fas ligand: is it a critical mediator of toxic epidermal necrolysis and Stevens-Johnson syndrome? J Invest Dermatol 127:744-745, 2007
8. Nassif A, Moslehi H, Le Gouvello S, Bagot M, Lyonnet L, Michel L, Boumsell L, Bensussan A, Roujeau JC: Evaluation fo the potential role of cytokines in toxic epidermal necrolysis. J Invest Dermatol 123:850-855, 2004
9. Nuttall TJ, Malham T: Successful intravenous human immunoglobulin treatment of drug-induced Stevens-Johnson syndrome in a dog. J Small Anim Pract 45:357-361, 2004
10. Pereira FA, Mudgil AV, Rosmarin DM: Toxic epidermal necrolysis. J Am Acad Dermatol 56:181-200, 2007
11. Scott DW, Miller WH, Griffin CE: In: Muller and Kirk's Small Animal Dermatology, 6th ed., pp. 729-742. Saunders, Philadelphia, PN, 2001
12. Stur K, Karlhofer FM, Stingl G: Soluble FAS ligand: a discriminating feature between drug-induced skin eruptions and viral exanthemas. J Invest Dermatol 127:802-807, 2007
13. Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, Hunziker T, Saurat JH, Tschopp J, French LE: Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. Science 282:490-493, 1998

CASE II – 22923-05 (AFIP 3066306).

Signalment: Adult, female, spayed, domestic shorthaired, cat, *Felis catus*

History: The cat had initially presented with multiple cutaneous and subcutaneous nodular swellings on the paws. Over a 2 year period, these nodules slowly increased in size, coalesced and the overlying skin often ulcerated and developed crusts. Similar, large nodular, firm, coalescing, often ulcerated lesions also developed on the head, deforming the face and along the trunk. There was no response to several courses of antibiotic treatment. The cat was eventually euthanized.

Gross Pathologic Findings: The cat was in good body condition having moderate visceral fat stores. Large, 4-5 cm in greatest diameter areas of the dorsal skin surface of both metatarsals were ulcerated and the skin was markedly thickened measuring up to 2.5-3 cm thick due to the presence of coalescing tan-colored nodules. A similar, thickened tan colored, fleshy, ulcerated nodule measuring 3.5 cm in greatest diameter was present on the dorsal surface of the left front metacarpals (**Fig. 2-1**). The skin extending medially and proximally to this area was alopecia and also mildly thickened. Multifocal to coalescing 5-10 mm in greatest diameter firm, nodules expanded the skin below the eyes. The bridge of the nose was ulcerated and the skin was markedly thickened, multinodular and firm (**Fig. 2-2**). A large, 9 cm in greatest diameter and 2 cm thick, flattened, oval, ulcerated, nodule expanded the dermis and subcutis on the left flank (**Fig. 2-3**). These lesions, with the exception of those on the bridge of the nose were freely moveable and on cut surface, were all pale, tan and had a homogenous texture. On the nose, this homogenous tissue extended into and partially effaced the underlying cartilage of the rostral, bridge of the nose and the nasal planum. In the area of the left popliteal lymph node, there was a pale, tan, 4-5 cm in greatest diameter tan nodule. The overlying skin was alopecic. There were several, freely movable, 3-4 cm in greatest diameter, subcutaneous, soft nodules in the mid to caudal ventral abdomen. The visceral organs were grossly unremarkable.

Laboratory Results: At postmortem, samples of skin and enlarged regional lymph nodes were sampled. Aerobic and anaerobic culture did not reveal significant pathogens.

Histopathologic Description: Sections of skin from lesions from the face, flank, left front and hind feet were examined. Sections of enlarged subcutaneous lymph node were included in some sections. Lesions were all similar in appearance and consisted of poorly-defined, infiltrative, densely cellular, monomorphic populations of large, polygonal to plump, slightly spindloid cells (resembling fibrohistiocytic populations) which had large, oval to sometimes slightly bean-shaped, nuclei

2-1. Tan-colored, fleshy mass measuring 3.5 cm in greatest diameter was present on the dorsal surface of the left metacarpals.
2-2. Multifocal to coalescing firm nodules expanded the skin below the eyes. The bridge of the nose was ulcerated and the skin was markedly thickened, multinodular and firm.
2-3. A large, 9 cm in greatest diameter and 2 cm thick, flattened, oval, ulcerated, nodule expanded the dermis and subcutis on the left flank.

Photographs courtesy of Department of Pathology/Microbiology, Atlantic Veterinary College, University of Prince Edward Island, www.upei.ca

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with coarse chromatin, often a single, prominent nucleoli and moderate amounts of variably well-defined, eosinophilic cytoplasm. Anisokaryosis within these cell populations was mild and mitotic figures are occasionally seen (1-2 per 6 HPF). These cellular populations were interspersed with small numbers of lymphocytes, plasma cells, mast cells, occasional small lymphoid follicles and were supported by small amounts of fine, collagenous stroma. These infiltrates extended from the dermoepidermal junction to the panniculus markedly expanding and often effacing the tissue. The overlying epidermis was mildly to moderately acanthotic and often extensively ulcerated. The left popliteal lymph node was markedly enlarged and adherent to the overlying skin. The normal nodal architecture was largely replaced by similar, dense infiltrates of mononuclear cells in which these fibrohistiocytic cellular proliferations predominated. Small residual cortical lymphoid nodules remained, as did the outline of the capsule in areas. There were multifocal large areas of pale lytic necrosis within the node. Subcutaneous nodules from the caudal abdominal or inguinal area represented similarly affected and enlarged lymph nodes. Modified acid-fast and PAS staining of sections of skin and lymph node do not reveal infectious agents. Toluidine blue staining did not reveal cytoplasmic, metachromatic granules. Immunohistochemistry was performed. The majority of mononuclear cell infiltrates were CD18 positive, MHC II positive and negative for T and B cell markers. These findings would be typical of a histiocytic cell population. Numerous CD3 positive T lymphocytes and rare single and clusters of CD45 B cells were also scattered within these infiltrates.

Contributor's Morphologic Diagnosis: Severe, multifocal to locally extensive, cutaneous, atypical histiocytic proliferation with ulceration and regional lymph node infiltration, skin of distal limbs, face, and flank

Contributor's Comment: The large ulcerated, nodular skin lesions that were so grossly prominent were composed of densely cellular infiltrates of histiocytes or macrophages-like cells interspersed with fewer lymphocytes. Special stains did not reveal infectious agents (such as mycobacterium or fungi) and these infiltrates did not form classic granulomas but instead were arranged in dense sheets. Regional lymph nodes were also multifocally enlarged due to prominent infiltrates of these same histiocytic populations which largely respected lymph node architecture. The postmortem findings, immunohistochemical results and the clinical history in this case are highly suggestive of a rare condition recently reported in cats called Feline Progressive Dendritic Cell Histiocytosis (FPDCH)[1,2]. Very few cases have been reported but the clinical features of those reported cats are very similar to those reported in this cat. Affected cats initially present with a solitary skin nodule, typically located on the head, neck or distal limbs. These lesions progress to multiple, non-painful nodules which may occur anywhere and which commonly become ulcerated. Nodules may wax and wane but complete spontaneous regression has not been reported. In general, nodules progress in size and may coalesce to form large plaques. Regional lymph node involvement is common in chronic cases. The cause of this rare condition has not been determined.

Immune dysregulation and proliferation of cutaneous dendritic cells, such as in Canine reactive histiocytosis, is one possible pathogenesis. However, unlike the canine cases, rare reported attempts to treat affected cats with immunomodulatory drugs has been unrewarding. FPDCH may represent a low grade neoplastic proliferation of dendritic cells which slowly progresses over time.

AFIP Diagnosis: 1. Haired skin and panniculus: Atypical histiocytic proliferation, diffuse, severe, with low to moderate numbers of lymphocytes, plasma cells and mast cells, domestic shorthair (*Felis catus*), feline.
2. Lymph node: Atypical histiocytic proliferation, severe (not included in all sections).

Conference Comment: Feline progressive dendritic cell histiocytosis (FPDCH) resembles Langerhans cell histiocytosis in humans and is divided into two subgroups, epitheliotropic and nonepitheliotropic.[1] In epitheliotropic form, the cellular infiltrate extends from the dermis up to the basement membrane, with single or clusters of cells located within the epidermis. In nonepitheliotropic form, the infiltrate extends within the dermis up to but not beyond the basement membrane.[1] The exact lineage of proliferating dendritic cells is not known.[1,2]

Histiocytic proliferative diseases may be reactive or neoplastic. Chronically, FPDCH clinically and morphologically resembles histiocytic sarcoma and may affect one or more internal organs.[1,2] This suggests that FPDCH should be considered an indolent, slowly progressive, cutaneous neoplasm that may disseminate to various organs.[2]

The etiology of FPDCH is currently unknown but it is thought to be related to chronic antigen stimulation, although animals do not respond to immunomodulatory therapy.[2]

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References:
1. Feline progressive Dendritic Cell Histiocytosis. In: Skin Diseases of the Dog and Cat, Clinical and Histopathologic Diagnosis. ed. Gross TL, Ihrke PJ, Walder EJ, Affolter VK, 2nd ed., pp. 845-847. Blackwell Publishing Limited, 2005
2. Affloter, VK, Moore PF: Feline Progressive Histiocytosis. Vet Pathol 43:646-655, 2006

CASE III – CPC07-043; H07-388A (AFIP 3063259).

Signalment: A 6-year-old, female, Labrador cross dog (*Canis familiaris*).

History: This dog has a ~3 year history of atopy which has been managed using allergen specific immunotherapy and symptomatic anti-pruritic therapy. At the time of biopsy the dog was receiving cyclosporin 30mg q 24hrs, ketoconazole 250mg q 24 hrs, prednisolone 6.25mg q 48hrs, and Episoothe® shampoo baths once a week.

Gross P athology: On clinical examination, there were multiple erythemic dermal papular to nodular lesions located on the head, trunk and limbs ranging from 0.5cm to 4cm in diameter.

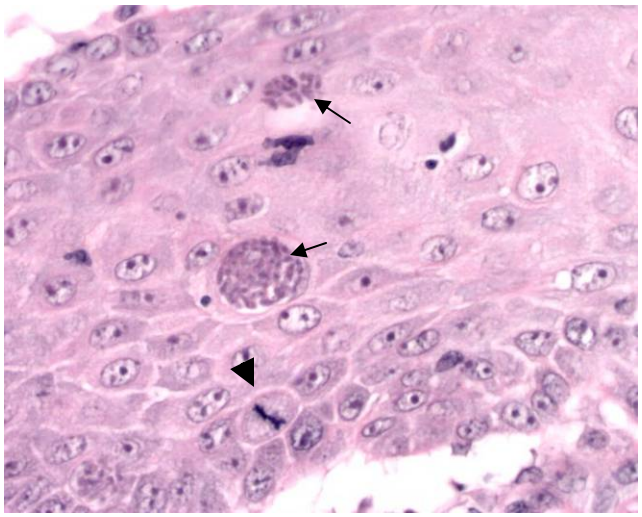
Laboratory Results:
Anti-*Toxoplasma* IgG Indirect Immunofluorescence (IFAT) $\geq$ 1:512
Anti-*Toxoplasma* IgM Indirect Immunofluorescence (IFAT) < 1:32
Anti-*Neospora* Indirect Immunofluorescence (IFAT) $\geq$ 1:25600

Indirect Immunohistochemistry:
Anti-*Neospora* Antibody (monoclonal): Positive
Anti-*Toxoplasma* Antibody (polyclonal).

Microbiology: Negative for fungal and bacterial growth

The two samples from the skin of the dog were strongly positive for *Neospora caninum* with a *Neospora* qPCR and a nested *Neospora* PCR.

Histopathologic D escription: Ten tissue sections were examined from the biopsies submitted, only one was submitted as our Wednesday Slide Conference submission. In all sections there was moderate to marked hyperplasia of the epidermis and outer root sheath of the follicular infundibulum. Within basal and spinous layer keratinocytes of predominately the infundibulum, but also the epidermis were frequent, scattered protozoal pseudocysts containing numerous (~5 to 100) rectangular to banana-shaped **zoites measuring ~2 x 5 microns (fig. 3- 1)**. There were occasional nodular aggregates of neutrophils and eosinophils within the follicular epithelium and epi-



3-1. Haired skin, Labrador cross. Protozoal pseudocysts containing numerous zoites (arrows) within hyperplastic epidermis. Note mitotic figure in stratum basale (arrowhead) H&E X400.

dermis associated with the protozoal pseudocysts. Follicular infundibulae were distended with keratin, aggregates of degenerate neutrophils and sheets of free protozoal zoites. There was also occasional follicular rupture. Within the dermis was a diffuse interstitial infiltrate of neutrophils, macrophages, lymphocytes and plasma cells. Associated with the inflammatory infiltrate were modest numbers of free and phagocytosed protozoal zoites, which in some areas may have been within vascular endothelium.

Contributor's Morphologic Diagnoses: Haired Skin:
1. Moderate to severe, chronic, follicular and epidermal hyperplasia and hyperkeratosis with intra-keratinocyte protozoal pseudocysts DDx: *Neospora*
2. Moderate to marked, diffuse, interstitial pyogranulomatous dermatitis with intrahistiocytic, intraendothelial and free protozoal zoites: DDx: *Neospora*
3. Moderate to marked, multifocal, neutrophilic folliculitis and pyogranulomatous furunculosis with numerous intrakeratinocyte and free protozoal zoites: DDx: *Neospora*

Contributor's Com ment: Although indirect immunohistochemistry was positive using a monoclonal primary antibody against *Neospora* and a polyclonal primary antibody against *Toxoplasma*, the very high anti-*Neospora* titre supports a diagnosis of cutaneous Neosporosis in this case. Further work using PCR to further discriminate between *Neospora* and *Toxoplasma* is underway.

Cutaneous Neosporosis has been reported in dogs before, although it is more commonly recognized as a cause of neuromuscular disease in young dogs and abortion in cattle.[2] The predominately cutaneous manifestation of Neosporosis demonstrated in this case may be associated with the concurrent immunotherapy this dog was receiving. CD4+ T-cells have been shown to play an important role in mice in protecting them against *N. caninum* infection.[8] Although cyclosporine is recognized to be an effective treatment for canine atopy with minimal side effects[9], the combination of prednisolone and cyclosporine treatment in this dog would have had a suppressive effect upon cell mediated immunity which is important in protecting dogs against *N. caninum* infection.[8] Neosporosis has been previously reported in an adult dog receiving prednisone and azathioprine.[6]

AFIP Di agnosis: Haired skin: Dermatitis and furunculosis, pyogranulomatous, multifocal, moderate, with neutrophilic folliculitis, and intraepithelial intrahistiocytic and free protozoa, Labrador cross (*Canis familiaris*), canine.

Conference Com ment: *Neospora caninum* is an apicomplexan that up until 1988 was often misdiagnosed as *Toxoplasma gondii*.[3] There are three infective stages:

Table 3-1. Ultrastructural features of Neospora and Toxoplasma.

Neospora caninum	Over 11 rhoptries Tachyzoites often not within a parasitophorous vacuole Tissue cysts are relatively uncommon
Toxoplasma gondii	Few rhoptries Always found in membrane-bound vacuole in cytoplasm

oocysts, tachyzoites, and tissue cysts. Canids, in addition to acting as an intermediate host, are considered the primary definitive host.[5] Oocysts are only found in and shed by the definitive host.[5] Tissue cysts, 110µm diameter with a 1-4µm thick cyst wall, are usually found in the brain, spinal cord, and rarely muscle, and contain numerous 2 X 8 µm bradyzoites.[1] Tachyzoites are 4-7µm X 1.5-5 µm and may be located within macrophages, keratinocytes, neutrophils, endothelial cells, or fibroblasts.[5]

Neosporosis affects a variety of species including sheep, goats, and deer, but most importantly, dogs and cattle.[4] It is reported to be one of the most important causes of bovine abortion, and transplacental transmission can occur.[4] In dogs, neurological disease in puppies is common, and cutaneous manifestations have been reported in immunosuppressed animals.[4] Histologically, cutaneous lesions are composed of pyogranulomatous and eosinophilic to necrotizing and hemorrhagic dermatitis.[7] Diagnosis primarily relies on serologic testing.[7]

N. caninum can be distinguished from *T. gondii* based on ultrastructural features **(table 3-1).**[1]

We thank Dr. C. H. Gardiner, PhD, veterinary parasitology consultant to the AFIP, for his review of this case.

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References:

1. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 272-273. Elsevier Saunders, Philadelphia, PA, 2007
2. Dubey JP: Review of *Neospora caninum* and neosporosis in animals. Korean J Parasitol 41:1-16, 2003
3. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 59-60. Armed Forces Institute of Pathology, Washington, DC, 1998
4. Ginn PE, Mansell JEKL, Rakich PM: Skin and appendages. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, p. 711. Elsevier Saunders, Philadelphia, PA, 2007
5. Maie MG, Youssef S: Nervous system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 436-437. Elsevier Saunders, Philadelphia, PA, 2007
6. Ordeix L, Lloret A, Fondevila D, Dubey JP, Ferrer L, Fondati A: Cutaneous neosporosis during treatment of pemphigus foliaceus in a dog. J Am Anim Hosp Assoc 38:415-419, 2002
7. Scott DW, Miller WH, Griffin CE: Viral, rickettsial, and protozoal skin diseases. In: Muller and Kirk's Small Animal Dermatology, 6th ed., pp. 532-533. Saunders, Philadelphia, PN, 2001
8. Steffan J, Favrot C, Mueller R: A systematic review and meta-analysis of the efficacy and safety of cyclosporin for the treatment of atopic dermatitis in dogs. Vet Dermatol 17:3-16, 2006
9. Tanaka T, Hamada T, Inoue N, Nagasawa H, Fujisaki K, Suzuki N, Mikami T: The role of $CD4^+$ or $CD8^+$ T cells in the protective immune response of BALB/c mice to *Neospora caninum* infection. Vet Parasitol 90:183-191, 2000

CASE IV - Case #1 (AFIP 3065823).

Signalment: 6-year-old, male, neutered, mixed breed dog (*Canis familiaris*).

History: The animal had severe non-responsive skin lesions on the left hind leg.

Gross Pathologic Findings: The specimen was a formalin-fixed, excisional biopsy of a haired skin mass. The mass was firm, raised and measured 1.7 x 1.0 x 0.3 cm. On cut surface the mass was grey-white.

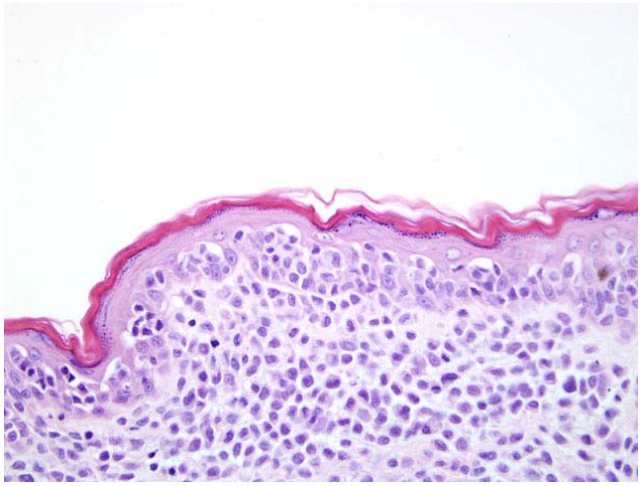
Histopathologic D escription: Expanding the dermis, elevating the overlying epidermis, which is ulcerated in some sections, and infiltrating the subcutis is an unencapsulated, highly cellular mass composed of round cells that infiltrate the superficial dermis and surround hair follicles and adnexa. The cells infiltrate the follicular and glandular epithelium in some foci. Individual cells have distinct cytoplasmic borders, scant to moderate eosinophilic cytoplasm, and irregularly round to indented nuclei with fine lacy chromatin and one or two nucleoli. Mitotic figures range from 1 to 4 per high power field. In some sections, the deeper dermis is infiltrated by neutrophils together with fibrin and edema. In one of the sections provided, there is ulceration of the epidermis and segments of exposed dermis are covered by lytic collagen, protein, necrotic cells (neutrophils) and cellular debris. Within the epidermis are small clusters of lymphocytes and individual round cells surrounded by clear halos. Small numbers of plasma cells and macrophages are dispersed throughout the round cell population.

Contributor's Morp hologic Di agnosis: Haired skin**:** Epitheliotropic lymphoma.

Contributor's Comment: Cutaneous epitheliotropic lymphoma (mycosis fungoides) is an uncommon, slowly progressive disease characterized by neoplastic infiltration of the epidermis and adnexal structures. It represents only 3-8 % of all canine lymphoma cases.[3] This disease has a T-cell origin and includes a wide spectrum of diseases such as mycosis fungoides, sezary syndrome, and pagetoid reticulosis.[4]

Two representative samples of the biopsy of the haired skin were submitted. In one section, there is marked epidermal ulceration with superficial bacterial colonization. The histopathologic lesions in each sample of the biopsy were characteristic of cutaneous **epitheliotrophic lymphoma (abnormal l ymphocytes loc alized in epidermis (fig. 4 -1)**.[4,5] This condition is thought to be analogous to mycosis fungoides (MF) in humans[1], except that in dogs, lymphocytic infiltrates consistently express CD3 and CD8, whereas in human cases, expression of CD 4 predominates.[9] This condition is commonly observed in older dogs; however, its etiology is unknown.[1,3]

In this case, immunohistochemical stains were performed on paraffin-embedded sections with antibodies to the T-cell marker CD3 and the B-cell and plasma cell marker CD 79a. The lymphocytes that compose the population within the dermis and infiltrating the epidermis were strongly positive for CD3 antigen but were negative for CD 79a, indicating that this tumor had a T-lymphoid lineage consistent with immunohistochemical staining patterns previously reported in dogs.[4]



4-1. Haired skin, mixed breed, canine. Intraepidermal clusters of neoplastic ymphocytes (Pautrier's microabscesses). H&E X400

The disease progresses from erythematous patches and plaques to nodules and tumors, which are initially localized to the skin and mucous membranes but eventually spread to lymph nodes and metastasize.[9] Due to the uncommon nature of the disease, diagnosis is often difficult. Differential diagnosis for skin lesions in canines include diseases like pemphigus vulgaris and discoid lupus or systemic lupus erythematosus lupus (immune-mediated diseases), histiocytoma, cutaneous histiocytosis and mastocytoma.[5]

This disease has a poor prognosis in the dog. Palliative treatment is often resorted to in order to prolong survival and maintain some reasonable quality of life for the animal.[3,8] Anti-neoplastic drugs may induce temporary remission but are accompanied by side effects like contact dermatitis or cutaneous neoplasia.[2,3,6] Radiotherapy is another option for treatment due to the radiosensitive nature of lymphoma cells.[6]

AFIP Dia gnosis: Haired skin: Lymphoma, epitheliotropic, mixed breed dog (*Canis familiaris*), canine.

Conference Comment : Cutaneous lymphoma is traditionally divided into epitheliotropic (mycosis fungoides) and nonepitheliotropic forms. The key histological feature of epitheliotropic lymphoma is the tropism of the neoplastic cells for the epidermal and adnexal structures, particularly that of the follicular epithelium and the sweat glands.[7] The neoplastic lymphocytes may be distributed within the epithelium diffusely or as aggregates termed 'Pautrier's microabscesses.'[7]

The epitheliotropic form is further subclassified in the human literature into 4 forms, and although these forms do not generally correlate well clinically, they have been adapted by veterinary literature for histopathological classification (**table 4-1**).[7]

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References:

1. Ackerman L: Cutaneous T-cell like lymphoma in the dog. Compend Contin Educ Pract Vet 6:37-42, 1984
2. Baker JL, Scott DW: Mycosis fungoides in two cats. J Am Anim Hosp Asso 25:97-101, 1989
3. Beale KM, Bolon B: Canine cutaneous lymphosarcoma: epitheliotropic and non- epitheliotropic, a retrospective study. In: Advances in Veterinary Dermatology,

Table 4-1.

Pagetoid reticulosis Woringer-Kolopp (localized) Ketron-Goodman (generalized)	Localized or generalized exfoliative erythroderma with scaling, alopecia, erosions or ulcerations without palpable masses Generalized form usually predominates in dogs and cats
Classical mycosis fungoides	Most common form seen in dogs Three stages (patch, plaque, and tumor) Patch and plaque stage usually seen simultaneously Progresses to tumor stage with spread to lymph nodes and other organs
d'emblée form	Tumor formation without a previous patch or plaque stage
Sézary syndrome	Circulating tumor cells in peripheral blood with epitheliotropic lymphoma and peripheral lymphadenopathy

eds. Ihrke PJ, Mason IS, White SD, vol. 2, pp. 273-283. Oxford: Pergamon Pr, 1993
4. Bhang DH, Choi US, Kim MK, Choi EH, Kang MS, Hwang CY, Kim DY, Youn HY, Lee CW: Epitheliotropic cutaneous lymphoma (mycosis fungoides) in a dog. J Vet Sci 7:97-99, 2006
5. Bouchard H: Epitheliotropic lymphoma in a dog. Can Vet J 41:628-630, 2000
6. DeBoer DJ, Turrel JM, Moore PF: Mycosis fungoides in a dog: demonstration of T-cell specificity and response to radiotherapy. J Am Anim Hosp Assoc 26:566-572, 1990
7. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Lymphocytic tumors. In: Skin Disease of the Dog and the Cat, 2nd ed., pp. 866-890, Blackwell Science, Ames, IA, 2005
8. Holloway KB, Flowers FP, Ramos-Caro FA: Therapeutic alternatives in cutaneous T-cell lymphoma. J Am Acad Dermatol 27:367-378, 1992
9. Moore PF, Olivry T, Naydan D: Canine cutaneous epitheliotropic lymphoma (mycosis fungoides) is a proliferative disorder of CD8+ T cells. Am J Pathol 144:421-429, 1994

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 16

6 February 2008

Moderator:

Dr. Fabio Del Piero, DVM, DACVP

CASE I – 05-7666 (AFIP 3026263).

Signalment: 3.5-year-old cow

History: Animal with a suppurative osteomyelitis of the right mandible

Gross Pathology: In the right mandible, there was a hard mass 12 cm in diameter with ulceration of the adjacent gum. The mass was composed of many confluent fibrous nodules and several suppurative tracts. In the fibrous nodules, there were several cavities 1 mm to 1 cm in diameter containing variable amounts of a yellowish pus with many sulfur granules.

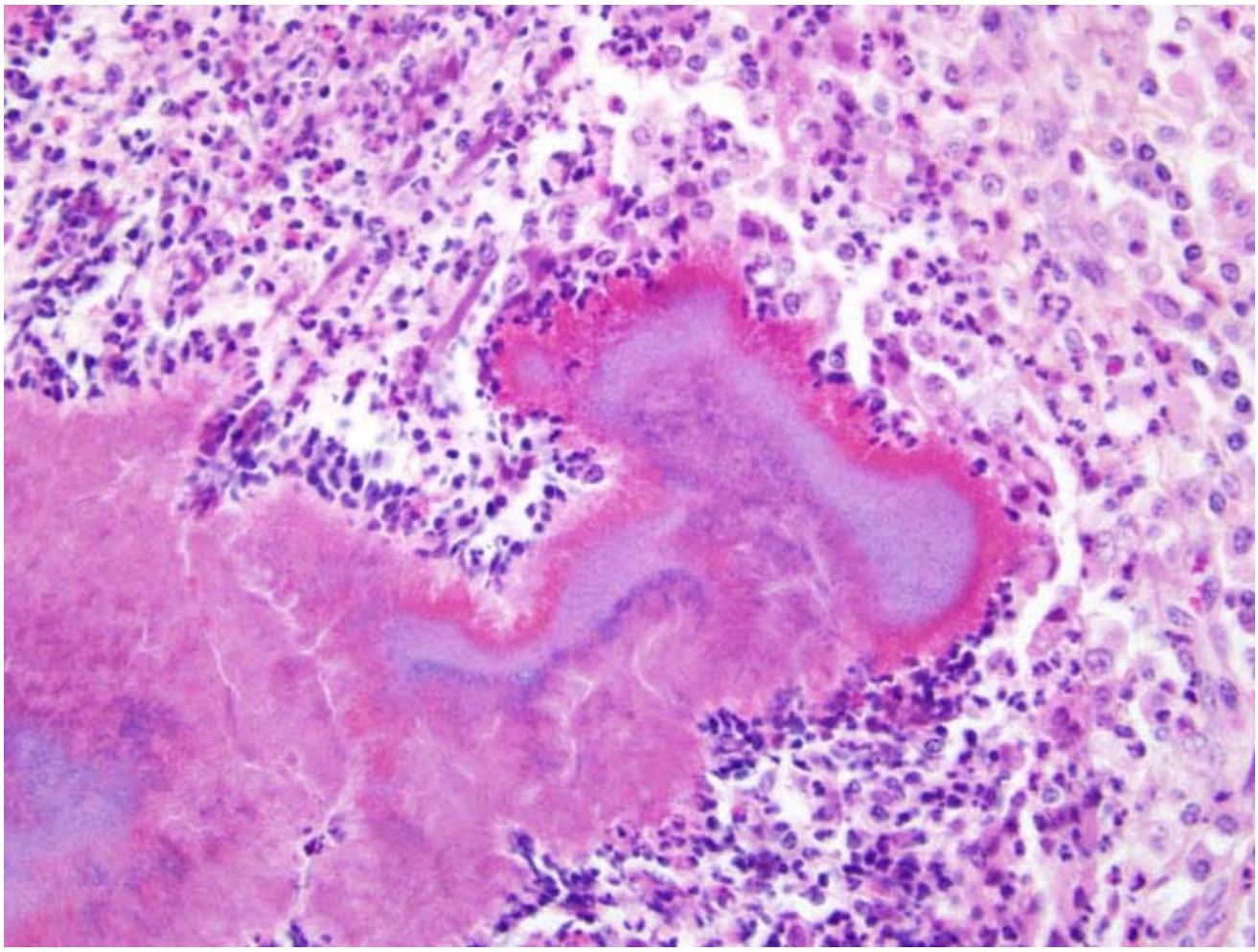
Histopathologic Description: Most of the mandibular bone is replaced by a granulation tissue infiltrated by macrophages and plasma cells. The granulation tissue is surrounding many small abscesses with granules composed of large bacterial colonies (Gram positive rod or coccoid-shaped and branching filamentous organisms) surrounded by radiating eosinophilic clubs (Splendore-Hoeppli material) **(Fig. 1-1).** There is a zone of neutrophils around the granules, surrounded by many large macrophages and plasma cells.

Contributor's Morphologic Diagnosis: Chronic pyogranulomatous mandibular osteomyelitis, with large colonies of Gram-positive filamentous organisms.

Contributor's Comment: This pyogranulomatous mandibular osteomyelitis with the presence of colonies of Gram-positive branching filamentous organisms forming sulfur granules is a good example of mandibular actinomycosis in cattle caused by *Actinomyces Bovis*.[1,2] The osteomyelitis would result from an extension of the infection of the gums or peridontium by the bacteria, following injury by foreign bodies or as a complication of periodontitis.[1] The excessive periosteal proliferation and the granulation tissue induced by the chronic inflammatory process, can cause a marked enlargement of the affected mandible (lumpy jaw).

AFIP Diagnosis: Bone; skeletal muscle; fibrous connective tissue, right mandible (per contributor): Pyogranulomas, multifocal to coalescing, with Splendore-Hoeppli material and colonies of Gram-positive filamentous bacteria, cow (*Bos taurus*), bovine.

Conference Comment: Actinomycetes are Gram-positive, non-acid-fast, branching filamentous rods. They are facultative anaerobes and normal inhabitants of the oral mucous membranes, tooth surfaces, and gastrointestinal tract.[1,2] Actinomycosis, or lumpy jaw, is primarily a disease of cattle, although it has been reported in horses, pigs, deer, sheep and dogs.[2] Infections usually



1-1. Mandible, bovine. Large bacterial colonies admixed with brightly eosinophilic Splendore-Hoeppli material. (H&E 400X).

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are restricted to the bone of the mandible resulting in a chronic suppurative and fibrosing osteomyelitis, although infections have been reported to involve the maxilla, regional lymph nodes or tongue.[2,3]

Infection usually occurs secondary to trauma with subsequent extension into the periosteum.[2] The normal architecture of the mandible is progressively destroyed inciting an extensive proliferative periosteal reaction.[2] The purulent exudate may contain necrotic trabecular bone (bone sand), or soft yellow granules containing mats of tangled, filamentous bacteria and Splendore-Hoeppli material (sulfur granules).[2]

Residents at AFIP utilize the mnemonic "YACS" to develop a differential diagnosis when large colonies of bacteria are present in hematoxylin and eosin stained sections.

YACS stands for:
Y *Yersinia sp.*
A *Actinomyces sp., Actinobacillus sp. Arcanobacter sp.*
C *Corynebacterium sp.*
S *Staphylococcus sp., Streptococcus sp.*

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References:
1. Jones TC, Hunt RD, King NW: Diseases caused by bacteria. In: Veterinary Pathology, eds. Jones TC, Hunt RD, and King NW, 6th ed., pp. 482-484. Williams and Wilkins, Baltimore, MA, 1997
2. Thompson K: Bones and joints. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 98-99. Elsevier Saunders, Philadelphia, PA, 2007
3. Valentine BA, McGavin MD: Skeletal muscle. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1020. Elsevier, St. Louis, MO, 2007

CASE II – 06L-0264 (AFIP 3028612).

Signalment: 2-3 years, female, pony, equine, *Equus caballus*

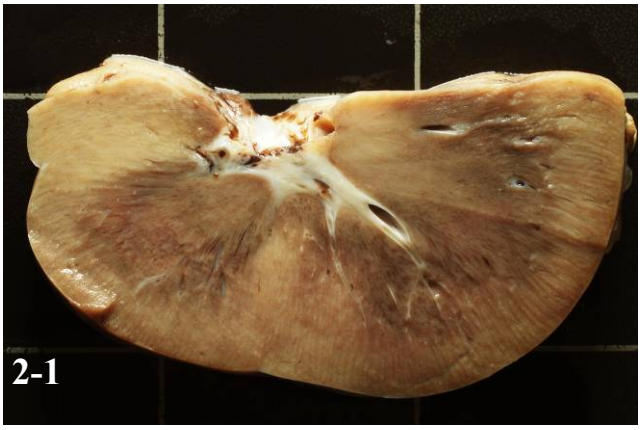
History: The case was part of an investigation in an animal cruelty case. The pony was cachectic and deteriorated. Due to poor body condition, severe elevated levels of urea and creatinine (azotaemia), the horse was euthanased. Multiple formalin-fixed fragments of right and left kidney were submitted for histopathology.

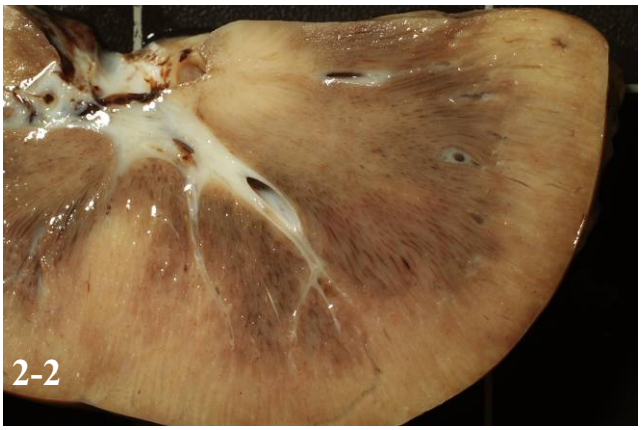
Gross Pathology: Kidney moderately firm, with prominent, pale beige glomerula, mild diffuse hyperaemia, moderate diffuse cortical and medullary interstitial fibrosis and focally indistinct cortico-medullary transition zone **(Fig. 2-1, 2-2).**

Histopathologic Description: The renal architecture is preserved. Within the epithelium of proximal and distal tubular epithelia of the cortical and medullary zone, numerous intracytoplasmic apicomplexan coccidian trophozoites and sporoblasts are seen. Few tubular epithelia are desquamated. Multifocal, mild to moderate tubular epithelial necrosis, intratubular, Von Kossa stain-positive mineralisation (calcification), and Von Kossa stain-negative, birefringent crystals (oxalate) are seen. Multifocally, distal tubules contain moderate to large amounts of cellular debris (desquamated, necrotic epithelia ?) and show mild dilation. There is focal mild glomerular fibrosis with glomerular synechia formation and multifocal mild to focally moderate interstitial fibrosis. A focal mild interstitial infiltration by lymphocytes, plasma cells and small numbers of eosinophils is seen.

Contributor's Morphologic Diagnosis: Glomerular and interstitial fibrosis, tubulonecrosis with (dystrophic) mineralisation, oxalate crystal formation, mixed cellular interstitial nephritis; diffuse, moderate (end-stage renal disease); with intracellular sporogenic and gametogenic stages of apicomplexan coccidian parasites, consistent with *Klossiella equi*, (sporozoa, apicomplexa, coccidia) infection, kidney, horse, *E. caballus*.

Contributor's Comment : The parasitic genus *Klossiella* belongs to the subphylum sporozoa which is characterised by intracellular life-cycle and an apical complex at some point during its development. The trophozoites have no cilia or flagella. The reproduction

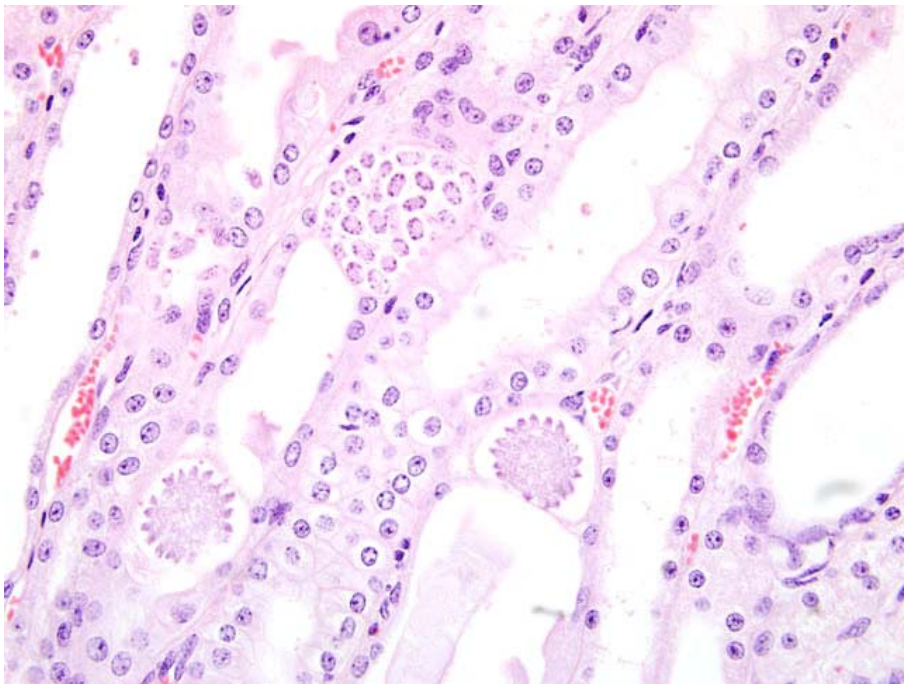




2-1. Kidney, equine. Moderate diffuse cortical and medullary interstitial fibrosis and focally indistinct cortico-medullary transition zone.
2-2. Kidney, equine. Higher magnification.

Gross photographs courtesy of Department of Veterinary Pathology, Faculty of Veterinary Science, University of Liverpool, Crown Street, Liverpool L69 7ZJ, United Kingdom
http://pcwww.liv.ac.uk/vets

involves both asexual (schizogony) and sexual (gametogony) phases. Following gametogony a zygote is formed which divides to produce spores (sporogony). Klossiellidae can be found in the kidneys of equids, mice, guinea pigs, bats, opossums, and snakes.[2,7] Renal infections with *K. equi* are often clinically inapparent. Mild infections, usually self-limiting, are followed by a predetermined cycle of development, with the production of infective stages that are shed in the host urine, and are thought to be ingested by another host. There are few reports on *K. equi*-related nephritis in severely infected equids.[1,3] Both sporogenic and gametogenic stages of *Klossiella equi* were identified in the kidneys. All stages developed in individual tubular epithelia cells. Schizonts were seen mostly in the proximal convoluted tubules, but also free within the lumen of a tubule. Macrogametocytes and microgametocytes were present in syzygy in the loop of Henle and collecting ducts. Sporont and budding sporont stages were also seen in the loop of Henle. All stages of development of sporoblasts were observed protruding into the tubular lumen. Sporocysts were identified rupturing out of the sporoblast membrane into the lumen of tubules. Renal tubules were greatly dilated and contained cellular debris. The tubulonecrosis and desquamation of tubular epithelia in this case most likely can be ascribed to the infection by *K. equi*. Whether the additionally described chronic renal alterations are due to the parasitic infection or are a separate underlying pathomechanism, cannot be stated.



2-3. Kidney, equine. Klossiella equi sporonts with radiating sporoblasts and mature sporocyst containing sporoblasts. (H&E 400X).

AFIP Diagnosis: 1. Kidney, tubules: Degeneration and necrosis, multifocal, moderate, with cellular casts and protozoa **(Fig. 2- 3),** etiology consistent with *Klossiella equi*, pony (*Equus caballus*), equine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, multifocal, moderate, with intratubular crystals.

Conference Comment: The contributor gives an excellent description of *Klossiella equi*. *Klossiella equi* is the only known coccidian parasite of the equine urinary tract, with various stages of development located in the kidney.[3] The life cycle is not currently known, although infection is presumed to occur via ingestion of infective sporocysts that were shed in the urine.[6] It is also believed that one schizont generation develops in the glomerular endothelium and another in the proximal tubular epithelium, with sporogony occurring in the epithelium of the thick limb of Henle's loop.[4] Infection is thought to be an incidental finding although it has been associated with nephrosis and nephritis in immune-compromised individuals.[1,6] We agree with the contributor that it cannot be determined if the interstitial nephritis and crystals are the result of the *K. equi* infection.

Ultrastructurally, developing sporoblasts are encased by a bilaminated cell membrane composed of an overlying thin granular layer, and an underlying dense inner layer.[1]

We thank Dr. C. H. Gardiner, PhD, veterinary parasitology consultant to the AFIP, for his review of this case.

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References:

1. Anderson WI, Picut CA, eorgi ME: *Klossiella equi* induced tubular nephrosis and interstitial nephritis in a pony. J Comp Pathol 98:363-366, 1988

2. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 61-62. Armed Forces Institute of Pathology, Washington, DC, 1998
3. Karanja DNR, Ngatia TA, Wandera JG: Donkey Klossiellosis in Kenya. Vet Parasitiol 53:1-5, 1994
4. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 498. Elsevier Saunders, Philadelphia, PA, 2007
5. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 657-658. Elsevier, St. Louis, MO, 2007
6. Suedmeyer WK, Restis E, Beerntsen BT: *Klossiella equi* infection in a Hartmann's Mountain zebra (*Equus zebra hartmannae*). J Zoo Wildl Med 37:420-423, 2006
7. Taylor JL, Wagner JE, Kusewitt DF, Mann PE: Klossiella parasites of animals: a literature review. Vet Parasitol 5:137-144, 1979

CASE III – R06-120 (AFIP 3027084).

Signalment: Two-month-old, Duroc-Hampshire crossbred, barrow, hog (*Sus scrofa domestica*)

History: The animal presented and culled for necropsy was from a farrow to finish swine herd in central Taiwan. Piglets were born healthy and began developing skin lesions and weight loss 6-7 days prior to necropsy. Four piglets from a batch of 60 piglets in the nursery pen were affected. Two piglets were found dead. Recent management changes included new farrowing and nursery houses and pens for batch production.

Gross Pathology: The piglet was in fair body condition. The integument of the piglet had multiple 1 to 3cm diameter, slightly raised, crusted skin lesions affecting all parts of the body. Lesions had dark-brown friable surfaces. There were multiple, black, raised, smooth, shiny nodules disseminated throughout the thoracic viscera, affecting the lungs and myocardium. Some dark, melanin like lesions were noted on the liver. All other organs were normal.

Laboratory Results: Specimens of skin, lung and liver were submitted for aerobic bacterial culture. No bacterial organism was cultured from these organs.

Histopathologic Description: Microscopically, the skin mass is well demarcated and nonencapsulated. The intact epidermis of the skin section exhibits mild epidermal hyperkeratosis while a large coalescing zone of superficial epidermal necrosis with inflammatory infiltration of neutrophils and histiocytes is observed in the affected skin. The skin mass is focally expanding the subcutaneous fat, compressing the underlying subcutaneous tissue and elevating the overlying dermis. The mass is composed of closely packeted large, polygonal to spindle-shaped cells arranged in sheets and short bundles contained within a scant intervening fibrous stroma. Most tumor cells (melanocytes) contain variable amount of brown to black intracytoplasmic pigment. The pigmentation varies from fine dusting to large quantities of granular to coarse material. Some of the spindle cells are less pigmented. Nuclei vary considerably in size; many nuclei are large, round to oval. Most nuclei contain one or rarely two large and round nucleoli. Cells along the superficial margins of the dermis abut on and occasionally surround the bulbs of hair follicles. The morphology of the tumor cells (melanocytes) is applicable to the metastatic focus in myocardium. Some myocardial tissue is destroyed and replaced by the growing metastases.

Contributor's Morphologic Diagnoses:
Skin: Melanoma and moderate subacute necrotizing epidermatitis, Duroc-Hampshire cross, swine.
Myocardium: Melanoma, metastatic.

Contributor's Comment: Melanomas have been reported in a variety of domestic and wild animals.[4,7,8] It is a devastating disease frequently encountered with both veterinary and human medicine. The Sinclair miniature and Duroc breeds have a genetic predisposition for melanomas; in addition, the Sinclair miniature pig has served as a model for spontaneous cutaneous melanoma in humans. Melanomas occur as congenital lesions and sporadically in all ages of Duroc-Jersey, Hormel, Sinclair and their crossbreeds, whereas these tumors are rare in other swine breeds. Regression of such tumors are common and in some breeds may occur *in utero* and at various times after birth.[1] Other tumors arising from the skin may look clinically very similar to melanoma. These include melanocytoma, dermal hemangioma and hemangiosarcoma as well as pigmented lesions of the epidermis and adnexa.[2,4]

Specific immunohistochemistry (IHC) to identify melanocytic tumors of swine is needed. In a recent study, normal and neoplastic porcine melanocytes were vimentin positive, cytokeratin negative, S-100 positive and alpha-1-antitrypsin (AIAT) negative, similar to the immunophenocyte reported for human normal and neo-

plastic melanocytes.[6]

AFIP Dia gnosis: 1. Haired skin and subcutis: Melanoma, Duroc-Hampshire crossbreed (*Sus scrofa domestica*), porcine.
2. Heart: Melanoma, metastatic.

Conference Comment: The contributor provides a complete, concise description of melanomas in pigs. In dogs, 56% of melanomas develop in the oral cavity.[3,5] It is also the second most common subungual neoplasm.[3,7] Known as the "great imitator", melanoma may appear with or without melanin granules; in an interwoven, whorled, or nested pattern; with round, polygonal, and/or spindled cells; or any combination of these types.[3,7] Malignancy of canine cutaneous melanocytic neoplasms is often determined by number of mitoses (>3/10 HPF).[3,7] Melanocytic neoplasms involving the oral cavity, subungual region, and mucocutaneous junctions are almost always malignant.[7] In feline cutaneous melanocytic neoplasms, extensive nuclear atypia, high mitotic activity, and an epithelioid cell type are suggestive of malignancy.[7] When numbers and size of melanin granules obscure the mitotic rate, an H&E stained slide pre-treated with bleach can aid in evaluation. In this Wednesday Slide Conference case, the neoplasm has a mitotic rate of 1 per HPF, with some fields containing up to 3 mitotic figures.

Melanocytes are dendritic cells that are derived form neuroectodermal melanoblasts, and are normally found within the basal layer of the epidermis.[7] Neoplastic transformations have been linked to various molecular changes such as mutation in the *INK4a* and *INK4b* and *Waf-1* genes resulting in malfunction of two tumor suppressor proteins (retinoblastoma protein and p53), proto-oncogene mutation to oncogene, altered expression of epithelial cadherin and CD44 adhesion molecules, and upregulation of angiogenic and other growth factors.[7] A recent study by van Kempen *et al.* has linked the increased expression of Type I collagen to the angiogenic switch that facilitates the progression of microinvasive to deeply invasive tumors in a porcine cutaneous melanoma model.[9]

Malignant melanomas in canines and humans may show chondroid or osseous metaplasia.[3,5] Oyamada *et al.* shows that the cartilaginous matrix transitions from the myxoid matrix produced by dedifferentiated neoplastic melanocytes.[5] Since the osseous matrix is not associated with either the cartilagenous matrix or the myxomatous matrix, it is theorized that the osteoid matrixes are formed form dense collagenous connective tissue that is also produced by the dedifferentiated neoplastic melanocytes.[5]

Melanomas are common in gray or white horses.[7] More than 90% of these tumors are benign at initial presentation, but approximately two-thirds are thought to progress to malignancy.[7] German Shepherd Dogs and Boxers are more prone to develop oral melanoma.[7] Sinclair miniature and Duroc breeds of swine have a genetic predisposition to developing melanomas.[7] Melanomas have also been reported in cats, cattle, sheep, and alpaca.[7]

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References:

1. Das Gupta TK, Ronan SG, Beattie CW, Shilkaitis A, Amos MS Jr: Comparative hiotopathology of porcine and human cutaneous melanoma. Pediatr Dermatol 6:289-299, 1989
2. Fisher LF, Olander HJ: Spontaneous neoplasm of pigs-a study of 31 cases. J Comp Path 88:505-517, 1978
3. Goldschmidt MH, Dunstan RW, Stannard AA, von Tscharner C, Walder EJ, Yager JA: Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals, Second series, vol. III, pp. 39-40. Armed Forces Institute of Pathology, Washington D.C., 1998
4. Goldschmidt MH, Hendrick MJ: Tumors of the skin and soft tissues. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 78-84. Blackwell Publishing, Ames, IA, 2002
5. Oyamada T, Tanaka H, Park C-H, Ueki H, Komiya T, Arai S: Pathology of canine oral malignant melanoma with cartilage and/or osteoid formation. J Vet Med Sci 69:1155-1161, 2007
6. Pérez J, Garcia PM, Bautista MJ, Millán Y, Ordas J, de las Mulas M: Immunohistochemical characterization of tumor cells and inflammatory infiltrate associated with cutaneous melanocytic tumors of Duroc and Iberian swine. Vet Pathol 39:445-451, 2002
7. Smith SH, Goldschmidt MH, McManus PM: A comparative review of melanocytic neoplasms. Vet Pathol 39:651-678, 2002
8. Thirloway L, Rudolph R, Leipold HW: Malignant melanomas in a Duroc boar. J Am Vet Med Assoc 170:345-347,1997
9. van Kempen LC, Rijntjes J, Mamor-Cornelissen I, Vincent-Naulleau S, Gerritsen MJ, Ruiter DJ, van Dijk MC, Geffrotin C, van Muijen GN: Type I collagen expression contributes to angiogenesis and the development of deeply invasive cutaneous melanoma. Int J Cancer 122:1019-1029, 2008

CASE IV - 04-0843 (AFIP 2985667).

Signalment: Adult, 55 lb. male pygmy goat, *Capra hircus*

History: The pygmy goat presented with ulcerated, oozing, pustular lesions on the face and muzzle prior to euthanasia.

Gross Pa thologic Findings: Numerous confluent ulcerative, scabby, verrucous and proliferative oozing lesions are present on the muzzle, commissures of the lips, surrounding the eyes, left lateral tongue and the dental pad. A circular ulcer is also present on the left cheek below the left eye. Creamy white exudate drains from some of the larger lesions. The thorax contains creamy tan pus and the pleural surfaces are lined with thick exudate forming adhesions to the thoracic wall and diaphragm. The lungs are consolidated ventrally with multifocal variably sized abscesses containing thick tan pus.

Laboratory Results:
Aerobic bacterial cultures of the muzzle yielded heavy growths of *Arcanobacterium pyogenes* and moderate growths of *Pseudomonas aeruginosa* and *Staphylococcus intermedius*.

Aerobic bacterial cultures of the lungs yielded heavy growths of *Pasteurella trehalosi* and *Arcanobacterium pyogenes*.

Electron microscopy: Tissues from muzzle and lips yielded Parapoxvirus (179 X 300nm)

Histopathologic D escription: The lesions consist of locally extensive papillary projections of acanthotic, hyperkeratotic epidermis and extensive dermal (or submucosal) inflammatory infiltrate of neutrophils, histiocytes, and lymphocytes with occasional epidermal or dermal pustules and microabscesses. Numerous small capillaries course throughout the dermis. Occasional ballooning vacuolation of keratinocytes with rare eosinophilic intracytoplasmic inclusions are seen. The epidermis is covered with thick serocellular crusts containing degenerating cells and small clusters of bacteria. Deep anastomosing rete pegs extend into the dermis (or submucosa).

Contributor's Morphologic Diagnosis:
1. Muzzle: Lymphocytic, neutrophilic, histiocytic, pustular and proliferative dermatitis and stomatitis with papillomatous epidermal hyperplasia, acanthosis, hyperkeratosis and occasional eosinophilic intracytoplasmic inclusions in keratinocytes
2. Lungs: Severe fibrinosuppurative bacterial bronchopneumonia (not included)

Etiologic Diagnosis: Parapoxvirus

Contributor's Comment: Contagious pustular dermatitis (contagious ecthyma, sore mouth, orf) is an infectious dermatitis of sheep and goats with worldwide distribution, caused by *Parapoxvirus*. The genus *Parapoxvirus* is a member of the *Poxviridae* family, and includes orf virus, bovine papular stomatitis virus, and pseudocowpox virus. It is an ovoid, enveloped, double stranded, DNA virus. Transmission is into skin abrasions through aerosols, direct contact, or through mechanical transmission via arthropods.[6] Lesions typically develop on commissures of lips and buccal cavity, but also develop on feet, teats (from nursing an affected kid), and genitals. Lambs and kids are at greatest risk because they are immunologically naïve, and the colostrum from a previously infected animal does not provide protection. Due to its tropism for epithelial cells, *Parapoxvirus* will cause epidermal hyperplasia, producing papular lesions usually within 7 days. Papular lesions progress to vesicles, pustules, and then crusty scabs. In a 2002 study of 16 persistently infected goat kids, lymph node enlargement, premature thymic involution, and a number of secondary bacterial infections were present. It is suggested in this study that individual susceptibility factors of the host, such as breed, genetic susceptibility and immune defects, are contributing factors in orf virus persistence and progression.[1] Infections typically last 3-4 weeks, depending on the severity of systemic disease. Cell mediated immunity is of high importance in recovery from infection. Antibiotics are recommended to prevent secondary complications such as cellulitis, mastitis, aspiration pneumonia, and necrotizing stomatitis. Animals that do recover have transient to solid immunity. Mortality rates in lambs is reported to be 15%.[2] Transmission between sheep and goats can occur, but is uncommon. *Parapoxvirus* may also be transmitted to humans causing similar pustular lesions, commonly on the forearm, hands and face.[3]

Diagnosis of *Parapoxvirus* is based on the recognition of characteristic lesions and lesion distribution. Microscopically, eosinophilic intracytoplasmic inclusion bodies are visible, along with vacuolation and swelling of keratinocytes. The virus particles can also be photographed with an electron microscope. The virus can survive in the environment for months in the scab material shed

from affected animals. Virulent, live virus vaccines do exist but are only recommended for use in persistently infected herds.[4] Contagious pustular dermatitis is of economic significance because lambs and kids become reluctant to eat or suckle, causing weight loss and reduced growth rates. Differential diagnosis for contagious pustular dermatitis should include Foot and Mouth disease, Rinderpest, and Bluetongue.

AFIP Dia gnosis: 1. Mucocutaneous junction, lip: Cheilitis, proliferative and necrotizing, focally extensive, severe, with intracytoplasmic eosinophilic inclusion bodies, pygmy goat (*Capra hircus*), caprine.
2. Haired skin, lip: Abscess, focal, with foreign material and fungal hyphae.

Conference Comment : Members of the parapoxvirus genus include orf virus, papular stomatitis virus, peseudocowpoxvirus, parapoxvirus of red deer in New Zealand, and squirrel parapoxvirus.[5] Other species that have been tentatively included include auzduk disease virus, chamois contagious ecthyma virus and seal parapoxvirus.[5] Seal parapoxvirus is the preferred term used rather than 'sealpox virus' to distinguish it from the orthopoxviruses that cause similar clinical diseases.[7]

Characteristic ultrastructural features of parapoxvirus include 250nm X150nm particles, with an oval- to dumbbell-shaped core surrounded by a membrane, lateral bodies, and a surface membrane.[5]

Histopathologic lesions of contagious ecthyma are typical of other poxviral lesions except they usually have a very brief vesicle stage, a prominent ulcer and crust stage, and inclusion bodies present for only a brief period of time during the vesicular stage.[2]

There is variation in sections. Some sections have a focal ulcer with bacterial colonies and neutrophilic mural folliculitis with fungal arthrospores, both likely secondary to the ulcerative lesions induced by the orf virus.

Contributor: San Diego County Animal Disease Diagnostic Laboratory 5555 Overland Avenue Suite 4103, San Diego, CA 92123

References:

1. de la Concha-Bermejillo A, Guo J, Zhang Z, Waldron D: Severe persistent orf in young goats. J Vet Diagn Invest 15:423-431, 2003
2. Hargis AM, Ginn PE. The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1174-1175. Elsevier, St. Louis, MO, 2007
3. Jones T, Hunt R: Diseases caused by viruses. In: Veterinary Pathology, 5th ed., p. 303. Lea & Febige, Philadelphia, Pa, 1983
4. Michelsen PG: Diseases of the alimentary tract, contagious ecthyma. In: Large Animal Internal Medicine, ed. Smith BP, 2nd ed., p. 805. Mosby Inc., St. Louis, Missouri, 1996
5. Müller G, Gröters S, Siebert U, Rosenberger T, Driver J, König M, Becher P, Hetzel U, Baumgärtner W: Parapoxvirus infection in Harbor seals (*Phoca vitulina*) from the German North Sea, Vet Pathol 40:445-454, 2003
6. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC: Poxviridae. In: Veterinary Microbiology and Microbial Disease, p. 335. Blackwell Science Ltd, Oxford, UK, 2002
7. Tryland M, Klein J, Nordøy ES, Blix AS: Isolation and partial characterization of a parapoxvirus isolated form a skin lesion of a Weddell seal, Virus Res 108:83-87, 2005

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 17

13 February 2008

Moderator:

Dr. Tabitha Viner, DVM, DACVP

CASE I – ND1 (AFIP 3065879).

Signalment: 4-year-old, female, River Otter (*Lutra canadensis*)

History: Found dead on display at the local zoo. The body was found under water.

Gross Pathology: The referring veterinarian performed the necropsy and reported pulmonary congestion, multifocal areas of pallor on the surface of the liver, and thickened myocardium.

Histopathologic Description: Liver sections show unencapsulated, moderately well-defined inflammatory foci characterized by infiltrates of eosinophils, lymphocytes, macrophages and multinucleate giant cells which affect approximately 10% of the tissue **(Fig. 1-1).** Within these inflammatory foci are occasional curvilinear nematode parasites with discernable nuclear columns consistent with microfilarial forms **(Fig. 1-2).** Smaller, primarily eosinophilic foci are randomly present within the parenchyma as well. Periportal zones contain increased numbers of mononuclear cells.

Contributor's Morphologic Diagnosis: Hepatitis, eosinophilic and granulomatous, multifocal, marked with intralesional microfilaria.

Contributor's Comment: *Dirofilaria immitis* infection has been previously reported in the River otter (*Lutra canadensis*), however it is not known if the species serves as a definitive host for the parasite.[8] This is in contrast to the Eurasian otter (*Lutra lutra*) which appears to be able to support the filarial infection.[3] The gross necropsy in this case was performed by the referring veterinarian who did not observe adult worms. However, numerous microfilaria were observed histologically within pulmonary and myocardial blood vessels. Eosinophilic granulomatous hepatitis has been reported in River otters previously, therefore the lesion may be a manifestation of the infection in this species.[6] The parasite was not speciated, however is consistent with the genus *Dirofilaria*.

Specific syndromes associated with heartworm disease include asymptomatic infection, glomerulonephritis, allergic pneumonitis, eosinophilic granulomatosis, pulmonary embolization, congestive heart failure, caval syndrome, and aberrant migration.[1] Changes in this case to correlate best with eosinophilic granulomatosis, however the organ most severely affected was the liver, not the lung. Microfilaria lodged in the sinusoids causing a striking inflammatory response. While microfilaria were clearly present in the circulation, similar foci of inflammation were not observed in lung, kidney or heart, suggesting that the liver may be predilection site for this infection in otters.

AFIP Diagn osis: 1. Liver: Hepatitis, granulomatous, eosinophilic, multifocal to coalescing, moderate, with hepatocellular degeneration and microfilaria, River otter (*Lutra canadensis*), carnivore.
2. Liver, hepatocytes: Vacuolar change, glycogen-type, diffuse, mild.

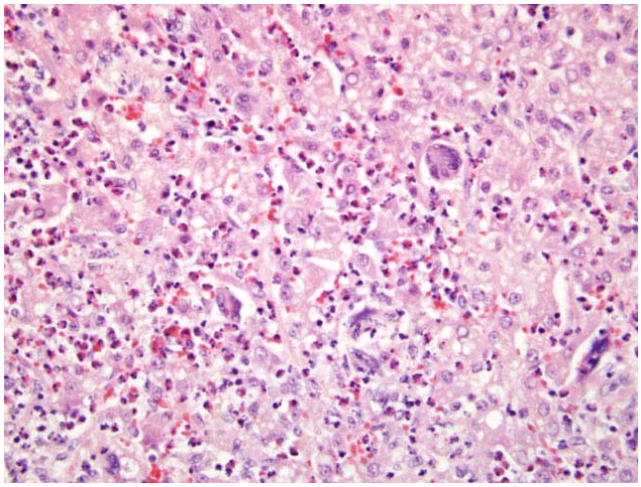
Conference Comment : It is not possible to determine the genus and species of an organism through examination of microfilaria only in tissue cross sections. Body length, the shape of the head and tail, presence or absence of a sheath, and curvature of the body are all used to aid in identification, and even then that may not be sufficient to classify even to the genus level.[9] Obtaining an entire microfilarial organism from a blood or tissue specimen, examination through antigen or antibody tests on serum samples from infected animals or PCR on microfilaria may all aid in identification. Even with the presence of adult worms, their specific speciation may be difficult to impossible if the parasite has not been well described and characterized.[7]

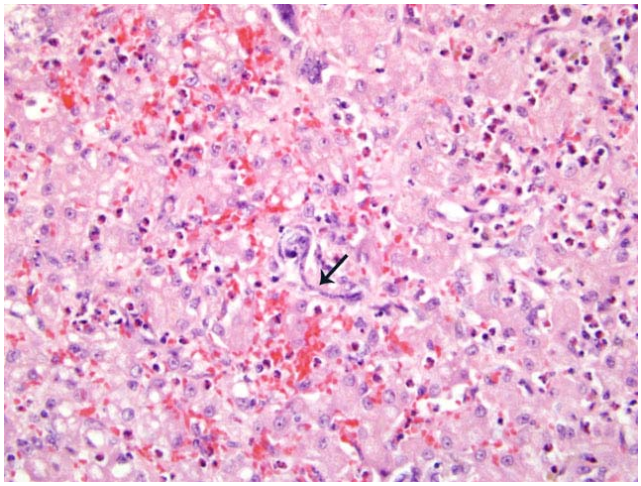
There are numerous species of Dirofilaria that primarily infect particular host species. The most well known cause of microfilariasis of animals in North America is *Dirofuilaria immitis*, which is primarily found in the dog.[4] With the exception of *D. immitis*, most species of Dirofilaria adults are found within the subcutaneous tissues.[7] Morbidity and mortality associated with *D. immitis* infections have been reported in mustelids, canids, felids, otariids, and other domestic and nondomestic carnivores[6], including the river otter.[8] *D. lutrae* infections have only been reported in North American river otters, with adults occurring in subcutaneous spaces and very rarely in the cardiopulmonary vasculature.[6,7] Adult forms of *D. repens* are found within the subcutaneous tissues of canines, other carnivores and occasionally humans.[4] *D. tenuis* is primarily found in raccoons of the southern USA, and *D. striata* within bobcats.

Characteristic histopathologic lesions in the lungs of dogs with dirofilariasis include villous endarteritis with luminal occlusion caused by villous intimal proliferation and medial hypertrophy.[2] Nitric oxide (NO) production has been implicated in the inflammatory response during filarial infections.[5] NO production can be induced by a recombinant *Wolbachia* surface protein.[5] *Wolbachia* is an intracellular endosymbiont bacteria that resides within some filarial organisms, including *Dirofilaria immitis*.[4]

In a recent Journal of Wildlife Diseases article, an unidentified filarial organism was isolated from wild populations of the black-footed ferret.[9] Although this unidentified microfilaria elicited a positive ELISA reaction for *D. immitis*, it only shared approximately 76% molecular identity with *D. immitis*, while sharing approximately 97% identity with *Acanthocheilonema viteae*.[9]

Contributor: North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND, 58105
http://www.vdl.ndsu.edu/





1-1. Liver, river otter. Inflammatory infiltrate characterized by eosinophils, lymphocytes, macrophages and multinucleate giant cells. (H&E 400X).
1-2. Liver, river otter. Microfilarid nematode within focus of inflammation (arrow). (H&E 400X).

Table 1-1. Morphologic characteristics of some microfilariae of onchocercids adapted from Wisely et. al.[9]

Filarial species	Length µm	Sheath	Host
Dirofilaria immitis	233-322	no	Dog
Dirofilaria striata	230-371	no	Bobcat
Dipetalonema reconditum	270-290	no	Dog
Loaina uniformis	285	yes	Eastern cottontail
Dirofilariaeformia pulmoni	213-288	no	Eastern gray squirrel
Brugia beaveri	300	yes	Raccoon
Brugia lepori	210-330	yes	Swamp rabbit
Mansonella llewellyni	290 ± 5	no	Raccoon
Monsonella interstitium	250 ± 5	no	Eastern gray squirrel
Molinema arbuta	280-297	no	North American porcupine
Acanthocheilonema reconditum	269-283	no	Dog
Acanthocheilonema mephitis	186-218	no	Striped skunk

References:
1. Atkins C: Canine heartworm disease. In: Textbook of Veterinary Internal Medicine, eds. Ettinger SJ, Feldman EC, 6th ed., pp. 1118-1137. Elsevier Saunders, St. Louis, Missouri, 2005
2. Kawabata A, Nakagaki K, Yoshida M, Shirota K: Histopathological comparison of pulmonary artery lesions between Raccoon Dogs (*Nyctereutes procyonoides*) and domestic dogs experimentally infected with *Dirofilaria immitis*. J Vet Med Sci 70:301-303, 2008
3. Matsuda K, Baek B, Lim C: Eurasian Otter (*Lutra lutra*), a definitive host for *Dirofilaria immitis*. J of Zoo and Wildl Med 34:200-201, 2003
4. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 87-89. Elsevier Limited, St. Louis, MO, 2007
5. Morchón R, Bazzocchi C, López-Belmonte J, Martín-Pancho JR, Kramer LH, Grandi G, Simón F: iNOs expression is stimulated by the major surface protein (rWSP) from *Wolbachia* bacterial endosymbiont of *Dirofilaria immitis* following subcutaneous injection in mice. Parasitol Int 56:71-75, 2007
6. Neiffer DL, Klein EC, Calle PP, Linn M, Terrell SP, Walker RL, Todd D, Vice CC, Marks SK: Mortality associated with melarsomine dihydrochloride administration in two North American River Otters (*Lutra canadensis*) and a Red panda (*Ailurus fulgens fulgens*). J of Zoo and Wildl Med 33:242-248, 2002
7. Orihel TC, Eberhard ML: Zoonotic filariasis. Clin Microbiol Rev 11:366-381, 1998
8. Snyder DE, Hamir AN, Nettles VF and Rupprecht CE: *Dirofilaria immitis* in a River Otter (*Lutra canadensis*) from Louisiana. J of Wildl Dis 25:629, 1989
9. Wisely SM, Howard J, Williams SA, Bain O, Santymire RM, Bardsley KD, Williams ES: An unidentified filarial species and its impact on fitness in wild populations of the black-footed ferret (*Mustela nigripes*). J Wildl Dis 44:53-64, 2008

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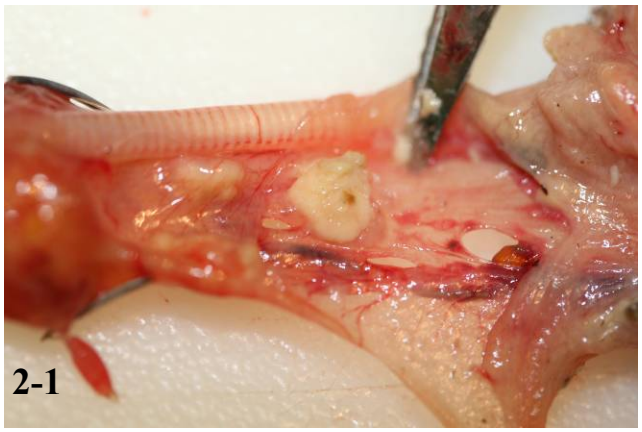
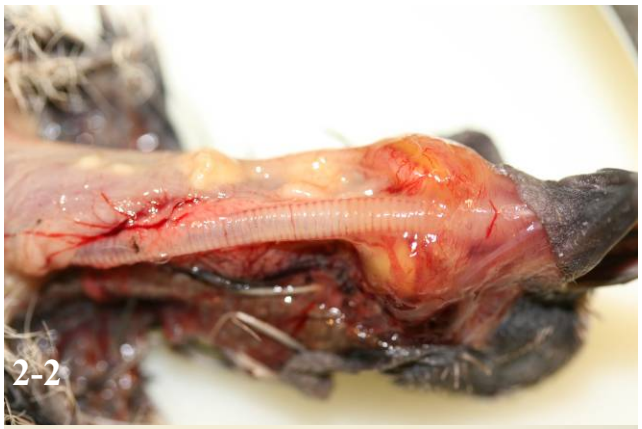
CASE II – X8593 (AFIP 3075563).

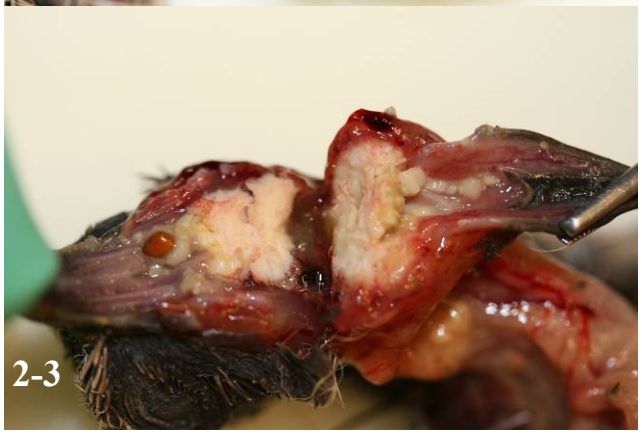
Signalment: Juvenile, mourning dove (*Zenaida macroura*), Columbiforme

History: Found dead on zoo grounds

Gross Pa thology: A white, caseous, 5mm diameter mass is apparent within the oral cavity extending into the pharynx. Ventral to the mandible there are two bilateral,







5mm diameter caseous nodules on either side of the trachea. Within the esophagus on the mucosal surface there are multifocal, 3mm diameter plaques of white caseous material **(Fig. 2-1, 2-2, 2-3).**

Histopathologic D escription: 1. Esophagus: In a focally extensive area, the esophageal epithelium is ulcerated and there is a transmural infiltration of inflammatory cells. Replacing the epithelium is a band of macrophages interspersed with 5 – 7 µm diameter, ovoid to round protozoa, and a mat of bacteria at the luminal surface. Subjacent to this layer are many heterophils with few macrophages, necrotic debris and scattered protozoa. This inflammatory combination extends through the tunica muscularis to the serosa, which is expanded by heterophils and moderate amounts of mucinous material. The adjacent epithelium is moderately acanthotic with prominent intracellular edema. The serosa below the adjacent epithelium is expanded by a proliferation of capillaries with plump endothelial cells and diffusely the serosa is expanded by heterophils and mucinous material. Small clusters of macrophages, lymphocytes, and few plasma cells are present in the mucosal connective tissue of the remaining, intact esophageal mucosa.
2. Trachea: Essentially normal tissue for a juvenile bird with unmineralized tracheal rings.

Contributor's Morpho logic Diagn osis: Esophagus: Esophagitis, histiocytic, heterophilic and necrotizing, acute, focally extensive and transmural, severe, with intralesional protozoa, mourning dove (*Zenaitha macroura*), columbiform, avian

Contributor's Comme nt: *Trichomonas gallinae* is carried subclinically by rock doves (*Columba livia*)[5] but may become pathogenic in immunocompromised individuals and mourning doves. The characteristic "canker", or caseous plaque of the dove oral cavity, esophagus, and crop, may also be seen in birds that prey upon or scavenge dead columbiformes, such as raptors and crows.[2] Falconers that keep these birds call the con-

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2-1 Mourning dove. Caseous nodules on either side of the trachea.
2-2 Mourning dove. Caseous nodules in area near the trachea.
2-3. Oropharnygeal region, Mourning dove. A mass is apparent within the oral cavity extending into the pharynx.

Gross photographs courtesy of Department of Pathology, National Zoological Park, 3001 Connecticut Ave. NW, Washington, D.C., 20008
http://nationalzoo.si.edu/default.cfm?ref=index.htm

dition "frounce". Additionally, the protozoan may be passed to a chick from the parental crop milk.[5]

At necropsy, the flagellates may be seen on a wet mount from carcasses that are up to 24 hours old.[6] A cytologic flagellum stain or silver stains on histologic preps may also elucidate the organism.

AFIP Diagnosis: Esophagus: Esophagitis, necrotizing, histiocytic and heterophilic, subacute, transmural, multifocal, severe with protozoa, mourning dove (*Zenaitha macroura*), avian.

Conference Comment: *Trichomonas gallinae* is a flagellated protozoan that contains four free anterior flagellae and one attached flagellum that trails along an undulating membrane, but does not extend beyond it.[3,8] In Columbiformes, transmission occurs directly through crop milk from infected adults to nestlings, through drinking water and food, as well as between adult birds during courtship behavior.[1,5] Transmission to raptors primarily occurs through ingestion of infected pigeons and doves.[2] *Trichomonas gallinae* has also been reported in Passeriformes (particularly canaries and zebra finches) and Psittaciformes (particularly budgerigars and cockatiels).[4]

Ultrastructural features of Trichomonads include piriform to spherical shapes, four anterior flagella of unequal size, well-developed undulating membrane, an axostyle, and a pelta surrounding the periflagellar canal.[8] The anterior flagellae emerge from the periflagellar canal which is reinforced by the pelta.[8]

Gross differentials for *Trichomonas gallinae* within the esophagus and crop include avian poxvirus, oral capillariasis, and candidiasis. Fibrinonecrotic lesions of wetpox, caused by avian poxvirus, are characterized by small white nodules to coalescing raised plaques with a diphtheritic membrane within the mouth, esophagus, trachea, pharynx, and larynx. Oral capillariasis produces almost identical gross lesions to that of *T. gallinae* and requires further testing to differentiate.[2] *Candida albicans* causes gray-white pseudomembranous patches in the mouth, pharynx, esophagus, and, most frequently, the crop.[7]

Contributor: Department of Pathology, National Zoological Park, 3001 Connecticut Ave. NW, Washington, D.C., 20008
http://nationalzoo.si.edu/default.cfm?ref=index.htm

References:

1. Bunbury N, Jones CG, Greenwood AG, Bell DJ: *Trichomonas gallinae* in Mauritian columbids: implications for an endangered endemic. J Wildl Dis 43:399-407, 2007
2. Heidenreich, M: Birds of Prey, Medicine and Management, pp. 131-132. Blackwell, Wissenschafts-Verlag GmbH, Berlin, 1995
3. Kocan RM, Herman CM: Trichomoniasis. In: Infectious and Parasitic Diseases of Wild Birds, eds. Davis JW, Anderson RC, Karstad L, Trainer DO, pp. 282-287. Iowa State University Press, Ames, IA, 1971
4. Lumeij JT: Gastroenterology, the esophagus and crop. In: Avian Medicine: Principles and Applications, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 491-497. Wingers Publishing Inc., Lake Worth, FL, 1994
5. Ostrand, WD, JA Bissonette, MR Conover: Trichomoniasis as a factor in mourning dove population decline in Fillmore, Utah. J Wildl Dis 31:87-89, 1995
6. Schulz, JH, AJ Bermudez, JJ Millspaugh: Monitoring presence and annual variation of trichomoniasis in mourning doves. Av Dis 49:387-389, 2005
7. Saif YM: Diseases of Poultry, 11th ed., pp. 896-898, 1006-1008, 1029-1030. Iowa State Press, Ames, Iowa, 2003
8. Tasca T, de Carli GA: Scanning electron microscopy study of *Trichomonas gallinae*. Vet Parasitol 118:37-42, 2003

CASE III – 7-1907 (AFIP 3065931).

Signalment: Adult beaver, *Castor canadensis*

History: Adult, male beaver, found dead near a pond, March 2007.

Gross Pathology: The animal presented in poor body condition. Throughout the plantar aspect of the hindlimbs and lateral margins of the tail, there are multiple cutaneous ulcers. On incision of the abdominal wall, there is approximately 5 ml of clear serosanguinous fluid. There was moderate enlargement of the spleen and liver and throughout the parenchyma, there are focally disseminated pale yellow white nodules.

Laboratory Results: PCR for *Francisella tularemia* was negative. Aerobic culture yielded heavy growth of *Yersinia pseudotuberculosis* from the spleen and liver.

Histopathologic Description: Immediately below the capsule and randomly throughout the parenchyma, there is multifocal to coalescing hepatocellular necrosis with

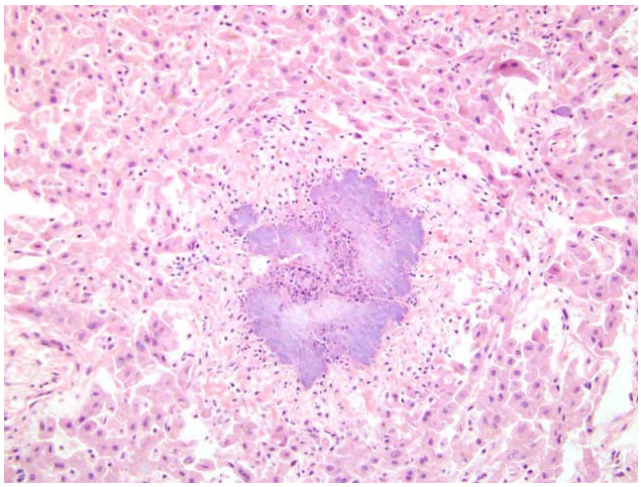
lobulated colonies of coccobacilli frequently bound by variable neutrophilic infiltrates **(Fig. 3-1).**

Contributor's Morphologic Diagnoses:
Liver: Hepatitis, marked, necrotizing, multifocal to coalescing, acute, with florid lobulated colonies of extracellular coccobacilli

Contributor's Comment: The cause of death of this animal is attributed to the cumulative effects of the necrotizing hepatitis and splenitis and generalized emaciation. The heavy growth of *Yersinia pseudotuberculosis* from the liver and spleen was considered significant. Although *Y. pseudotuberculosis* is endemic worldwide, infections are more commonly recognized in temperate zones, particularly in Europe, and have been reported in a wide variety of domestic and wild mammals, including rodents, rabbits, deer, cattle, goats and sheep and birds, such as turkeys, ducks, geese, pigeons, pheasants and canaries. *Y. pseudotuberculosis* had previously been recovered in wildlife species between 1962 and 1973 (1 crow, 2 purple martens, 1 snowshoe hare, and 7 beavers) in Ontario, Canada and rat feces have been implicated as a source of human infection in Japan.[4,5] Exposure is predominantly by consumption of fecal contaminated food or water, or alternatively, ingestion of infected prey.[4] The epidemiology of infection is complex and not yet fully resolved; however, isolation of this bacterium from a beaver has important public health implications and the regional health authority has been notified and appropriate actions implemented. In humans, infection may result in mesenteric lymphadenitis, ulcerative ileitis, septicemia and erythema nodosum and most presenting patients are 4-15 year old males. From a wildlife population perspective, yersiniosis typically occurs in only select individuals in an area and, thus presumably has few implications for free ranging and marine mammals. In more densely populated communities, infection may be exacerbated by stress and reduced transmission distance.

AFIP Dia gnosis: Liver: Hepatitis, necrotizing, acute, random, multifocal to coalescing, severe, with large colonies of coccobacilli, beaver (*Castor canadensis*), rodent.

Conference Com ment: There are three species of *Yersinia* that are pathogenic for rodents and humans: *Y. pestis* (etiologic agent of bubonic and pneumonic plague), *Y. pseudotuberculosis*, and *Y. enterocolitica*.[6] All pathogenic *Yersinia* spp. produce *Yersinia* Outer Proteins (Yop), which enable extracellular survival and proliferation **(Table 3-1).** A combination of various translocator, recognition, and effector Yops combine with a type III secretion system called Ysc to disarm macrophages and contribute to delaying the development of a cell-mediated immune response.[6]



3-1. Liver, beaver. Large colonies of coccobacilli within areas of hepatocellular necrosis. (H&E 200X).

Type III secretion systems are found within a wide variety of Gram-negative bacteria, and are used to deliver bacterial proteins into host cells.[7] It consists of a base structure that spans the inner and outer bacterial membranes and a needle-like structure that provides a conduit for the transfer of bacterial proteins into the host cell.[7]

There are 6 effector Yops, 5 of which are found in all three pathogenic *Yersinia* species: YopH, YopM, YopE, YpkA/YopO and YopJ/YopP. YopT is exclusively found in *Y. enterocolitica*.[3,7] YopB, YopD, and LcrV are required to translocate effector proteins into the host cell.[3,6] YopN and LcrG are part of a control and recognition system.[6]

Contributor: Animal Health Center, BC Ministry of Agriculture and Lands, Abbotsford, British Columbia

References:
1. Adkins I, Köberle M, Gröbner S, Bohn, E, Autenrieth IB, Borgmann: *Yersinia* outer proteins E, H, P, and T differentially target the cytoskeleton and inhibit phagocytic capacity of dendritic cells. Int J Med Microbiol 297:235-244, 2007
2. Day JB, Ferracci F, Plano GV: Translocation of YopE and YopN into eukaryotic cells by *Yersinia pestis* YopN, tyeA, sycN, YscB and lcrG deletion mutants measured using a phosphorylatable peptide tag and phosphospecific antibodies. Mol Microbiol 47:807-823
3. Fisher ML, Castillo C, Mecsas J: Intranasal inoculation of mice with *Yersinia pseudotuberculosis* causes a

***Table 3-1. Yersinia* Virulence Factors[2,3,6,7]**

Type III secretion system	Composed of transmembrane base structure, and needle like conduit
PhoP	Plays a crucial role in ability to replicate within macrophages[3]
Translocation Yops	Required for translocation of Yops into the host cell, but is not required for excretion of Yops into extracellular space[3]
YopB	
YopD	
LcrV	
Control and Recognition Yops	Prevents Yop secretion prior to host cell attachement[2]
YopN	
LcrG	
Effector Yops	
YopH	Disrupts actin structures including focal adhesions and prevents phagocytosis by macrophages and neutrophils[3,7] , affects oxidative burst of macrophages and inhibits T- and B-cell signaling, and T-cell proliferation[6]
YopM	Scaffolding protein, only *Yersinia* effector that lacks catalytic activity[7]
YopE	Disrupts actin structures[6], prevent phagocytosis by macrophages and neutrophils[3]
YpkA/YopO	Disrupts actin skeleton, prevent phagocytosis by macrophages and neutrophils[3,7]
YopJ/YopP	Induces programmed cell death in macrophages, and functions as an acetyltransferase[7] resulting in inhibition of mitogen-activate protein kinase pathway[3]
YopT (*Y. enterocolitica*)	Inactivates RhoA[6], prevent phagocytosis by macrophages and neutrophils[3]

lethal lung infection that is dependent on *Yersinia* outer proteins and PhoP. Infect Immun 75:429-442, 2007

4. Fukushima H, Gomyoda M, Shiozawa K, Kaneko S, Tsubokura M: *Yersinia pseudotuberculosis* infection contracted through water contaminated by a wild animal. J Clin Microbiol 26:584-585, 1988

5. Hacking MA, Sileo L: *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from wildlife in Ontario. J Wild Dis 10:452-453, 1974

6. Monnazzi LGS, Carlos IZ, de Medeiros BMM: Influence of *Yersinia pseudotuberculosis* outer proteins (Yops) on interleukin-12, tumor necrosis factor alpha and nitric oxide production by peritoneal macrophages. Immunol Lett 94:91-98, 2004

7. Trosky JE, Liverman ADB, Orth K: Microreview: *Yersinia* outer proteins: Yops. Cell Microbiol 10: 557-565, 2008

CASE IV - 95-702 (AFIP 3080450).

Signalment: 2-year-old, female, Giant marine toad (*Bufo marinus*)

History: Found dead; no observable problems prior to death. Two cagemates have died of mycobacteriosis in the past.

Gross Pathologic Findings: Expanding the caudal portion of the base of the tongue and laryngeal/pharyngeal area are multiple, up to 5 mm cross sectional diameter, white to tan, caseous nodules that extend to the cranial portion of the left lung. There are multiple, up to 4 mm in diameter, white to tan, caseous nodules throughout the tissue of both lungs. The spleen is enlarged and measures 1.0 cm x 1.0 cm x 0.7 cm; there are multiple, up to 2 mm in diameter, caseous, white to tan nodules throughout the splenic parenchyma. The kidneys are uniformly dark red to purple and contain a few, small, up to 1 mm in diameter, white to gray foci, predominantly on the surface of the cranial portion of both kidneys.

Laboratory Results:
Lung cytology revealed acid fast bacilli.
Culture of the lung grew *Mycobacterium gordonae.*

Histopathologic D escription: Lung: Multifocally expanding the intersitium are many granulomas that are up to 2 mm in diameter and are composed of a core of macrophages and few lymphocytes surrounded by a variably thick capsule of loose, fibrous connective tissue. Epithelium overlying these granulomas is often thinned and has lost the ciliated border. Occasionally, the epithelium is ulcerated. Between the faveolar walls are frequent infiltrates of macrophages, few lymphocytes and neutrophils and rare multinucleated giant cells. Rarely macrophages have peripheralized nuclei and peripheral cytoplasmic pallor.

Acid fast: There are scattered, positive bacilli throughout granulomas **(Fig. 4-1).**

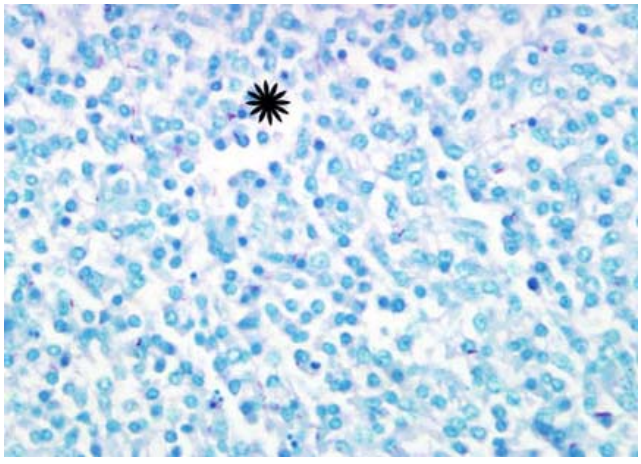
Contributor's Morphologic Diagnosis:
Lung: Pneumonia, granulomatous, chronic, multifocal, moderate, with intralesional acid fast bacilli consistent with *Mycobacterium* sp., giant marine toad (*Bufo marinus*)

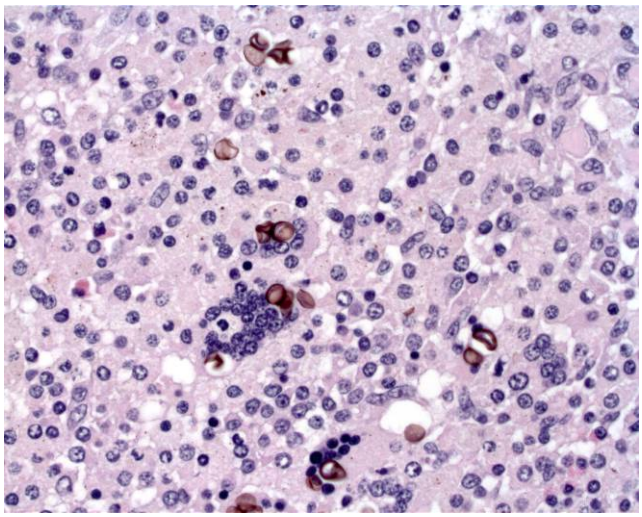
Contributor's Comment: Mycobacteriosis is common in animals and may be associated with a variety of pathogenic or environmental species. Historically, *Mycobacterium tuberculosis* and *M. leprae* most commonly affect humans, but *M. tuberculosis* has also been isolated from a number of non-human primates and elephants, and *M. leprae* may be enzootic in 9-banded armadillos.[5] *Mycobacterium gordonae* is commonly found in water and is not considered pathogenic.[5] However, compromise of the immune system can leave an individual open to opportunistic invasion by this organism. The toad in this case had systemic chromoblastomycosis which may have been associated with immunocompromise.

In amphibians, *Mycobacterium* induces granulomas similar to those precipitated by *M. tuberculosis* in humans. Key differences for amphibians include few multinucleated giant cells, a lack of mineralization, and a propensity for development in the skin.[4] Additionally, cytology and histology of amphibian lesions reveal numerous acid fast organisms, in contrast to the paucibacillary lesions of human TB. Cutaneous mycobacteriosis in the amphibian is most often associated with *M. marinum,* another waterborne mycobacterial species. The initial inflammatory response is disorganized and histiocytic and can be seen histologically by 2 weeks post-infection.[1] Mature granulomas composed of sheets of interdigitating epithelioid macrophages surrounded by a fine, fibrous capsule are seen by 8 weeks post-infection. Caseation of the core may occur in some granulomas[3] effectively walling off the bacteria from the host immune system. In this way, latent infection may be maintained.

AFIP Dia gnosis: Lung: Pneumonia, granulomatous, multifocal, moderate, with acid fast bacilli consistent with *Mycobacterium* sp., giant marine toad (*Bufo marinus*), amphibian.

4-1. Lung, Giant Marine Toad. Scant positive bacilli scattered within granulomas (asterisk). (Acid fast 400X).





4-2. Lung, Giant Marine Toad. Multifocally, rarely within granulomas, there are few, 8-12 um, brown, thick-walled, dematiaceous (pigmented) fungal organisms (sclerotic bodies). They were not present in all sections. (H&E 400X)

Conference Com ment: Mycobacteria are non-motile, nonspore-forming, pleomorphic, acid-fast, weakly Gram-positive coccobacilli.[3] Their cell walls contain a large hydrophobic layer of mycolic acids which allows environmental and antimicrobial resistance. Increased amounts of trehalose dimycolate (cord factor) within the cell wall is also associated with increased virulence.[3] The *Mycobacterium* genus contains numerous strict pathogens (i.e. *M. tuberculosis* and *M. leprae*), as well as opportunistic pathogens (i.e. *M. avium* and *M. marinum*).[5] Tuberculosis is a term reserved for diseases caused by *M. tuberculosis* or *M. bovis* with all other conditions referred to as mycobacteriosis.[3]

The initiation of the cell-mediated immune response consists of activated macrophages secreting interleukin-12, which in turn skews the immune system to secrete interferon-g and interleukin-2 by CD4+ T-helper-1 lymphocytes.[3] Tumor necrosis factor-α and interferon-g act together to promote the formation of the tuberculoid granuloma.[3]

Granulomas in the amphibians induced by *Mycobacterium marinum* share many features with human tuburculosis granulomas.[1] They contain mature macrophages, epithelioid cells, and extracellular matrix components. Yet caseation does not appear to be a feature in frog granulomas even though it is seen in *M. marinum* infections of humans, goldfish and toads.[1]

Fungi consistent with a diagnosis of chromoblastomycosis **(Fig. 4-2)** were not present in all lung sections, so the entity was not discussed by the contributor. Chromoblastomycosis (chromomycosis) can be caused by a wide variety of brown pigmented, dematiaceous fungi, and infections have been seen in mammals and amphibians.[2] In amphibians the infection is often systemic with lesions in the skin, liver, lungs, and kidneys of stressed animals.[2]

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http://nationalzoo.si.edu/default.cfm?ref=index.htm

References:

1. Bouly, DM, N Ghori, KL Mercer, S Falkow, L Ramakrishnan: Dynamic nature of host-pathogen interactions in *Mycobacterium marinum* granulomas. Infect Immun 69:7820-7831, 2001
2. Bube A, Burkhardt E, Weiss R: Spontaneous chromomycosis in the marine toad (*Bufo marinus*). J Comp Path 106:73-77, 1992
3. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 606-610. Elsevier Limited, St. Louis, MO, 2007
4. Green, DE: Infectious etiologies – Mycobacteria – Mycobacteriosis. In: Amphibian Medicine and Captive Husbandry, eds. Wright KM, Whitaker BR, pp. 427-429. Kreiger Publishing Co., Malabar, FL, 2001
5. Rastogi N, Legrand E, Sola C: The mycobacteria: an introduction to nomenclature and pathogenesis. Rev Sci Tech 20:21-54, 2001
6. Shively JN, Songer JG, Prchal S, Keasey MS 3rd, Thoen CO: *Mycobacterium marinum* infection in *Bufonidae*. J Wildl Dis 17:3-8, 1981
7. Taylor SK, Williams ES, Thorne ET, Mills KW, Withers DI, Pier AC: Causes of mortality of the Wyoming toad. J Wildl Dis 35:49-57, 1999

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 18

27 February 2008

Moderator:

Dr. James Raymond, DVM, MS, DACVP

CASE I – 47678 (AFIP 3066297).

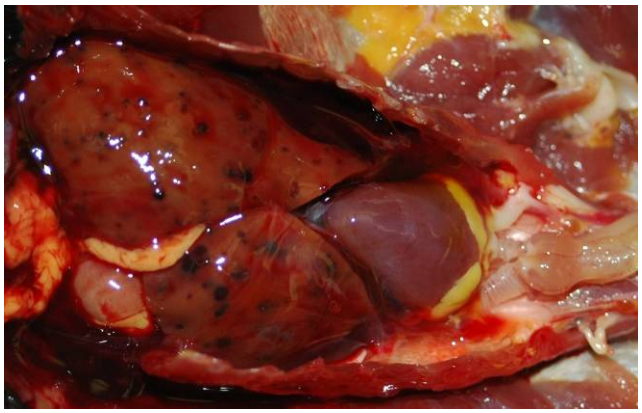
Signalment: 10-year-old, male, Magnificent Bird of Paradise (*Diphyllodes magnificus hunsteini)*

History: This Magnificent Bird of Paradise was housed in a bird breeding facility, where it was introduced to a female once daily. The bird had no history of medical problems, and was found on the ground, "puffed up, disoriented, and unable to fly", with blood on the beak. The bird was subsequently transported to the hospital where it died soon thereafter (less than 6 hours later).

Gross Pathology: The caudal coelom contained a moderate amount of unclotted blood. The liver was diffusely mottled green to brown with approximately two to three dozen, 1 to 3 mm diameter black foci scattered randomly throughout the parenchyma (hemorrhage) **(Fig. 1-1).** The intestines were diffusely pale. Body condition was assessed as fair.

Laboratory Results: Polymerase chain reaction (PCR) of fresh frozen liver was performed, using consensus primers for an avian malarial mitochondrial cytochrome B gene segment, and was positive in 9 of 10 cases. Sequencing of the gene product revealed that the protozoa were consistent with *Haemoproteus* spp., and not consistent with *Leukocytozoon* or *Plasmodium. In situ* hybridization was done in two animals with a mitochondrial cytochrome B probe and was positive only in megaloschizonts. Phylogenetic analyses showed that the protozoan was related to *Haemoproteus* spp. reported from asymptomatic native North American passerine birds.

Histopathologic Description: The liver parenchyma is multifocally disrupted by variably sized poorly demarcated regions of hemorrhage and necrosis ranging from 0.5 to 2 mm in diameter, typically with intralesional protozoal organisms (megaloschizonts) **(Figs. 1- 2).** Protozoal megaloschizonts range from 200 to 500 µm in diameter and consist of a thin, 1-2 µm wide basophilic wall bordering a 30-60 µm thick peripheral basophilic rim. This rim delineates the perimeter of the schizont, and is comprised of numerous round to oval often poorly preserved, coalescing basophilic 1-5 µm diameter zoites and larger cytomeres. Cytomeres range from 6 to 15 µm in diameter, within which are often clear acicular clefts. Central cystic regions of the megaloschizonts contain amorphous wispy amphophilic to eosinophilic material. Rare megaloschizonts do not have a central cystic region, and are entirely comprised of well demarcated cytomeres **(Fig. 1- 3).** In few locations, amorphous basophilic to amphophilic material resembling the protozoal zoites conforms to and expands sinusoids. Surrounding the megaloschizonts and intermixed within the regions of hemorrhage, there are small to moderate numbers of in-



1-1. Liver, Bird of Paradise. Scattered foci of hemorrhage. Photograph courtesy of Zoological Society of San Diego, San Diego, CA, USA

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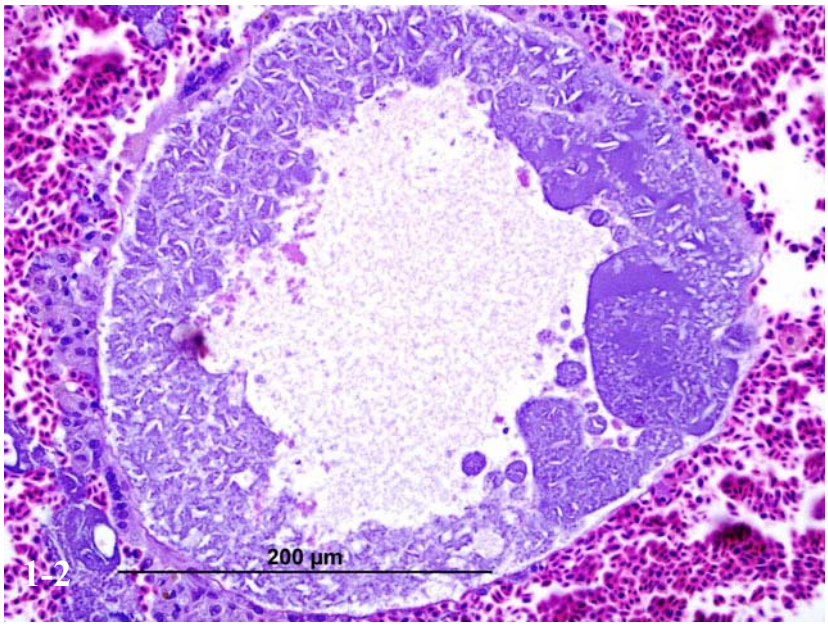
flammatory cells, including lymphocytes, plasma cells, macrophages and multinucleate giant cells. Multifocally, hepatocytes surrounding the parenchymal hemorrhage exhibit degenerative and necrotic features characterized by hypereosinophilia, anisocytosis, anisokaryosis, pyknosis, karyolysis and karyorrhexis. Golden brown granular anisotropic pigment is extracellular and intracellular, found within hepatocytes, erythrocytes and Kupffer cells, typically at foci of hemorrhage (acid hematin or malaria pigment, Fig. 4). Inflammatory cell populations including lymphocytes, plasma cells, macrophages and fewer heterophils are randomly disseminated or present at portal regions. Capsular mesothelial cells are multifocally hypertrophied. Diffusely, hepatocytes contain abundant golden brown granular pigment (hemosiderin).

Contributor's Morphologic Diagnosis: 1. Liver: Severe, acute multifocal hemorrhage and necrosis with intralesional protozoal megaloschizonts (*Haemoproteus*-like spp.) and moderate multifocal lymphoplasmacytic histiocytic hepatitis
2. Liver: Moderate diffuse hemosiderosis

Contributor's Comment: Slides vary with respect to number and morphology of protozoal megaloschizonts. Ten cases of protozoal infection associated with multifocal hepatic hemorrhage, hemocoelom and intralesional protozoal schizonts have been observed in passerine birds at this zoological park. The gross lesions were particularly striking, as multifocal hemorrhages were disseminated throughout the hepatic parenchyma, and the coelom contained unclotted blood. Severe blood loss into the abdominal cavity was the proposed mechanism of sudden death. Other lesions consistently present included non-suppurative endocarditis, epicarditis, myocarditis, and occasional endarteritis. Also common were coelomitis and airsacculitis, possibly secondary to widespread migration of the hemoparasites, or a reaction to the coelomic hemorrhage. Splenomegaly was a common finding, with reticuloendothelial hyperplasia or lymphoid hyperplasia diagnosed in the majority of cases. Although Isosporoid coccidia (formerly called *Atoxoplasma*)[14] were found in lung impression smears in the bird presented in this case and two others, no other hemoprotozoal parasites were found in lung impression smears of any bird. In this case and all but one of the ten cases, hepatic protozoa were identified as *Haemoproteus* via polymerase chain reaction (PCR) and sequencing of the gene product. *In situ* hybridization was done in three birds with a mitochondrial cytochrome B probe and was positive only in megaloschizonts.

Hemoparasitism in birds typically consists of 3 genera of Apicomplexan parasites in the family Plasmodiidae: *Leukocytozoon*, *Haemoproteus* and *Plasmodium.*[4] These protozoa can cause severe clinical disease, or hosts may be asymptomatic. *Haemoproteus* and *Plasmodium* are distributed almost worldwide,[16] with high species diversity. Over 120 species of *Haemoproteus* have been reported in birds.[4] *Haemoproteus* spp. are characterized by schizogony (merogony) within visceral endothelial cells (typically the lung, liver or spleen), with gametocyte development in circulating erythrocytes.[4,5] Biting flies, characteristically louse flies (Hippoboscidae) and biting midges (Ceratopogonidae) transmit the protozoa. In our cases, Ceratopogonidae are considered to be the more likely vector. Sexual development of *Haemoproteus* occurs in the intermediate host (insects), with asexual development in the bird.[5] Clinical disease is typically associated with anemia due to erythrocytic parasitism, frequently in a compromised or immunocompromised host. Few species of *Haemoproteus* are reported to induce clinical disease, including *H. meleagridis* in turkeys, *H. mettionis* in ducks and geese, and *H. columbae* in pigeons and doves.[13]

In these cases, disease manifestation is presumed to occur with the pre-erythrocytic stages, rather than circulating intra-erythrocytic gametocytes, as no gametocytes were identified with lung impression smears (a representation of peripheral blood). Pre-erythrocytic schizont stages of *Haemoproteus* spp. have been reported to occur in many organs including the lung, liver, spleen, heart, kidney and cecum.[2] Schizont development within capillary endothelial cells and myofibroblasts suggests that the parasite can use a variety of host cells.[2] The reason for the apparent tropism to the liver in our cases is unknown. Because of the location of the schizont formation and sequelae to



1-2. Liver, Bird of Paradise. Megaloschizont with central lumen containing proteinaceous fluid and a peripheral basophilic rim of protoplasm containing variable numbers of merozoites.
1-3. Liver, Bird of Paradise. Megaloschizont containing numerous merozoites.

Photomicrographs courtesy of Zoological Society of San Diego, San Diego, CA, USA. (H&E 400X).

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hepatic parenchymal disruption (hemorrhage and hemocoelom), it is plausible that there is not enough time for erythrocytic forms to be identified. Experimental *Haemoproteus* infection in turkey poults, a naturally infected wild turkey, and naturally infected bleeding heart doves caused severe myositis with intralesional megaloschizonts, muscle necrosis and lameness.[2] Significant myositis was not found in any of our ten cases.

Descriptions of lesions virtually identical to those found in our cases have been reported in psittacine and nonpsittacine birds,[12] with the designation of "Haemosporozoa of undetermined taxonomic status". As described by Gardiner, this group of protozoal organisms is characterized by protozoal cysts within viscera and skeletal muscle without identification of gamonts or gametes in peripheral blood cytology.[8] A recent report describing similar hepatic lesions in passerine and nonpasserine birds used PCR to confirm *Haemoproteus* as the etiologic agent.[7] Interestingly, DNA sequence analysis of a conserved area of the cytochrome B gene reveals that the *Haemoproteus* sp. in the aforementioned cases is identical to the *Haemoproteus* in our cases. The presence of the same species of *Haemoproteus* in different orders of birds is a surprising finding, which challenges the previous designation of high host specificity of this protozoal disease.

Host sharing involving both *Haemoproteus* and *Plasmodium* was reported to occur between populations of migrating European birds and resident African birds,[16] suggesting that these hemoparasites may be less host specific than previously believed. Host switching of protozoal hemoparasites into naïve populations has been linked with changes in virulence and increased morbidity.[1,3,14] These findings imply that host switching is a conceivable mechanism for *Haemoproteus* infection of birds from different localities assimilated into a novel environment, as is typical in a zoological setting. Although avian species from similar regions of the world are typically grouped together in a zoo, insect vectors can move between groups, and may play a role in transmission of infectious organisms. Interestingly, the majority of the birds affected (9 of 10) at our institution are from South America, excluding the bird from this case, the magnificent bird of paradise. In general, hemoprotozoa of neotropical birds are rare.[15] Therefore, birds from this

region of the world may have an increased susceptibility to hemoprotozoal infections. Sequence analysis of PCR products from our cases revealed that the sequences were identical and most consistent with *Haemoproteus* spp. from North American passerine birds. Thus, it is possible that infection with North American *Haemoproteus* in South American birds (aberrant hosts) may result in an aberrant parasitic form or a more prominent or virulent pre-erythrocytic form of the parasite. A similar example may be the introduction of new *Plasmodium* species to Hawaii, which resulted in high mortality rates and limited range habitation of native passerine species[1], illustrating the impact engendered by introduction of a novel parasite to a naive population.

Iron stains confirmed the presence of hemosiderin within hepatocytes. Hemosiderosis is a common finding in many avian species, particularly in frugivorous or insectivorous birds. Birds of paradise are listed as a family within the order Passeriformes (Paradisaeidae) in which hepatic or multisystemic hemosiderosis is often found.[10] Other commonly affected families include Ramphastidae and Sturnidae. Iron storage disease in these families may be primary or due to vulnerability to dietary overload, as opposed to secondary causes, including infection or inflammation.[10] One must be careful to differentiate between hemosiderosis, meaning that there is excessive stainable iron in parenchymal or phagocytic cells, and hemochromatosis, meaning that the excess iron damages the cell, tissue or organ. In this case, although hemosiderin is widespread throughout the liver, no cellular damage or reactive changes attributable to the hemosiderin are apparent, which warrants a diagnosis of hemosiderosis.

AFIP Diagn osis: 1. Liver: Hemorrhage and necrosis, multifocal with megaloschizonts, Magnificent Bird of Paradise (*Diphyllodes magnificus hunsteini*), avian.
2. Liver: Hepatitis, portal, lymphoplasmacytic, multifocal, moderate.
3. Liver, hepatocytes: Hemosiderosis, diffuse, moderate.

Conference Comment: The contributor gives an excellent overview of *Haemoproteus* infections. The typical life cycle of *Haemoproteus* consists of gametocytes within the host erythrocyte cytoplasm that are taken up by blood-sucking vectors (hippoboscid flies or midges of the genus *Culicoides*).[6] The parasite undergoes several stages of development within the insect host to become sporozoites within the insect's salivary gland. These sporozoites are injected into a new susceptible host when the insect feeds.[6] The sporozoites enter the bird's endothelial cells and tissues (lung, liver, bone marrow, and spleen), undergo schizogony, and form large round cysts containing numerous multinucleated bodies (cytomeres) that in turn produce numerous merozoites.[6] The merozoites escape into the bloodstream and enter erythrocytes to become gametocytes (macrogametes and microgametes).[9] Occasionally extraerythrocytic macrogametes and microgametes are found within the peripheral blood.[6]

Haemoproteus, *Plasmodium*, and *Leucocytozoon* gametocytes can all be found within the peripheral blood, although several differences exist among the three groups. In *Leucocytozoon* sp., gametocytes may also be found in leukocytes and will severely distort host cells.[9] Mature *Haemoproteus* gametocytes within the erythrocytes of birds are located within the cytoplasm and partially encircle the nucleus without causing nuclear displacement (halter shape).[6] Megaloschizonts are frequently present in tissues in cases of leucocytozoonosis and with infection of some species of *Haemoproteus*, but are not characteristic of *Plasmodium* sp.

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http://cres.sandiegozoo.org/

References:

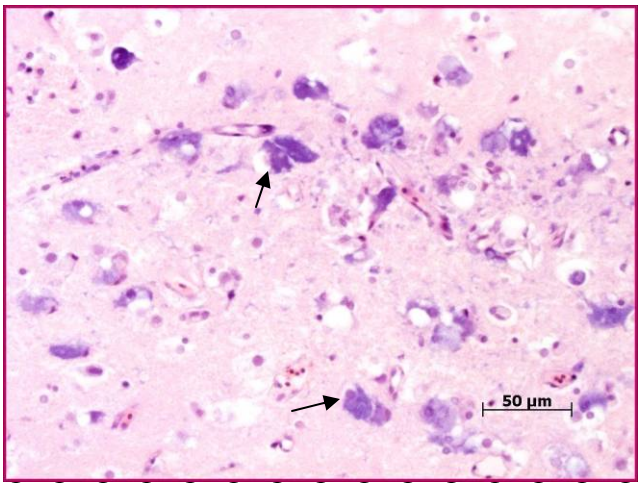
1. Atkinson CT, Dusek RJ, Woods KL, Iko WM: Pathogenicity of avian malaria in experimentally infected Hawaii amakihi. J Wildl Dis 36:197-204, 2000
2. Atkinson CT, Greiner EC, Forrester DJ: Pre-erythrocytic development and associated host responses to *Haemoproteus meleagridis* (haemosporina: Haemoproteidae) in experimentally infected domestic turkeys. J Protozool 33:375-381, 1986
3. Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, Pinheiro RT: Host specificity in avian blood parasites: A study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proc Biol Sci 267:1583-1589, 2000
4. Burmudez, AJ: Miscellaneous and sporadic protozoal infections. In: Diseases of Poultry, eds. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, 11th ed., pp 1014-15. Iowa State Press, Ames, IA,2003
5. Campbell TW: Common avian blood parasites. In: Avian Hematology and Cytology, 2nd ed., pp. 30-31. Iowa State Press, Ames, IA, 1995
6. Campbell TW: Section III: Hematology of common non-domestic animals. In: Vetenirary Hematology and Clinical Biochemistry, ed. Thrall MA, pp. 245-248. Lippincott Williams & Wilkins, Philadelphia, PA, 2004
7. Ferrel ST, Snowden K, Marlar AB, Garner M, Lung NP: Fatal hemoprotozoal infections in multiple avian

species in a zoological park. J Zoo Wildl Med 38:309-316, 2007
8. Gardiner CH, Fayer R, Dubey JP: Apicomplexa: *Haemoproteus* and Haemosporozoa of undetermined taxonomic status. In: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 74-75. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, 1998
9. Greiner EC, Ritchie BW: Parasites. In: Avian Medicine: Principle and Applications, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 1019-1021. Wingers Publishing Inc., Lake Worth, FL, 1994
10. Lowenstine LJ and Munson, L: Iron Overload in the Animal Kingdom. In: Zoo and Wildlife Medicine, Current Therapy, eds. Fowler ME, Miller RE, 4th ed., pp. 260-265. WB Saunders Company, Philadelphia, PA, 1999
11. Macwhirter P: Passeriformes. In: Avian Medicine: Principle and Applications, eds. Ritchie BW, Harrison GJ, Harrison LR, p. 1197. Wingers Publishing Inc., Lake Worth, FL, 1994
12. Panigrahy B, Harmon BG, Grumbles LC: Hemorrhagic Disease in Canaries (*Serinus canarius*). Av Dis 28:536-541, 1984
13. Peirce, MA: Hematozoa. In: Avian Medicine, ed. Samour J, pp. 247-248. Mosby, Elsevier Science Limited, Philadelphia, PA, 2003
14. Schrenzel M.D, Maalouf GA, Keener LL, Gaffney PM: Molecular characterization of malarial parasites in captive passerine birds. J Parasitol 89:1025-33, 2003
15. Valkiunas G, Salaman P, Iezhova TA: Paucity of hematozoa in colombian birds. J Wildl Dis 39:445-448, 2003
16. Waldenstrom J, Bensch S, Kiboi S, Hasselquist D, Ottosson U: Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Mol Ecol 11:1545-54, 2002

CASE II – U24048-04A-A1, C; U24883 A1-B2 (AFIP 2988631).

Signalment: Atlantic cod (*Gadus morhua*)

History: The samples are from approximately 12-month-old fingerlings, 5 cm-in-length, taken from a population of fish in grow out tanks held indoors at 15 degrees Celsius. Over a 2 week period, there was an increase in mortalities. Grossly the skin was darkened.



2-1. Brain, Atlantic cod. Neuronal vacuolation (arrows). (H&E 400X).
Photomicrograph courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada. www.upei.ca

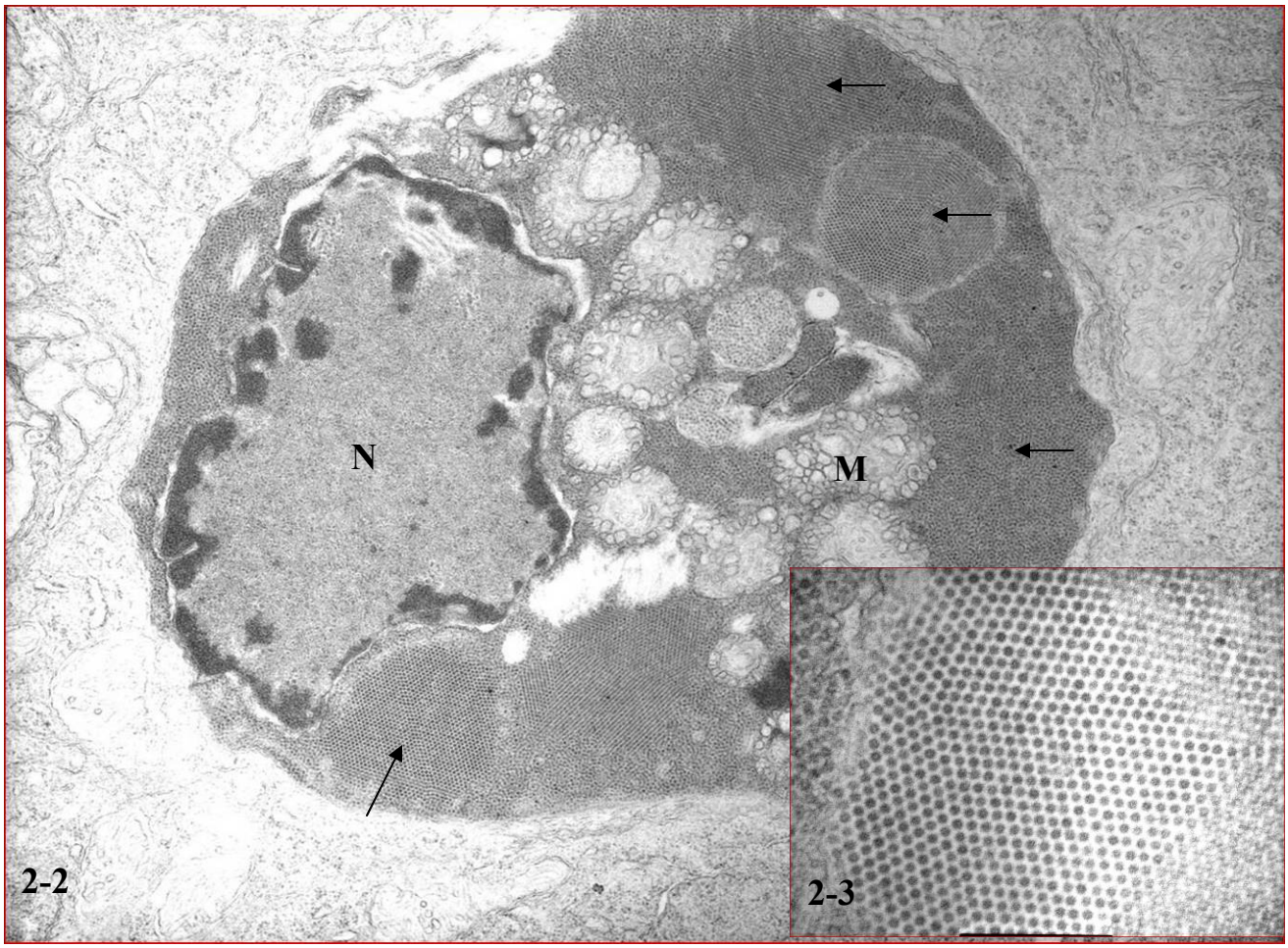
Laboratory Results:
Viral isolation – Nodavirus recovered
Immunohistochemistry – Positive for nodavirus

Histopathologic D escription: Each slide contains a tangential section through the cranium of 4 fish. All slides have between 1 and 4 visible sections of brain and associated optic structures.

Telecephalon: The most prominent changes are present in the olfactory lobe of the telencephalon. There are neurons present with clear cytoplasmic vacuoles, often with scalloped edges **(Fig. 2 -1).** The affected neurons are typically markedly basophilic, and in more advanced cases there is more prominent neuronal degeneration with mild gliosis. Some cases have similar changes in more caudal aspects of the brain, often extending into the cranial spinal cord.

Eye: The posterior chamber commonly contains a lightly eosinophilic fluid admixed with loose histiocytic cells. There are commonly adherent to the surface of the lens, and occasionally to the retina. Within the retina, sparsely dispersed vacuolated neurons are present predominantly in the inner nuclear layer and nerve fiber layer.

Contributor's Morpho logic Diagn osis: 1. Multifocal neuronal vacuolation and degeneration, with focal gliosis – telencephalon.
2. Multifocal neuronal vacuolation and degeneration,



2-2. Retina, Atlantic cod. Large numbers of intracytoplasmic viral particles (arrows) and several degenerating mitochondria (M) within retinal cell; nucleus (N). Transmission electron micrograph.
2-3 (inset). Higher magnification of virions.
Electron micrographs courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada.
www.upei.ca

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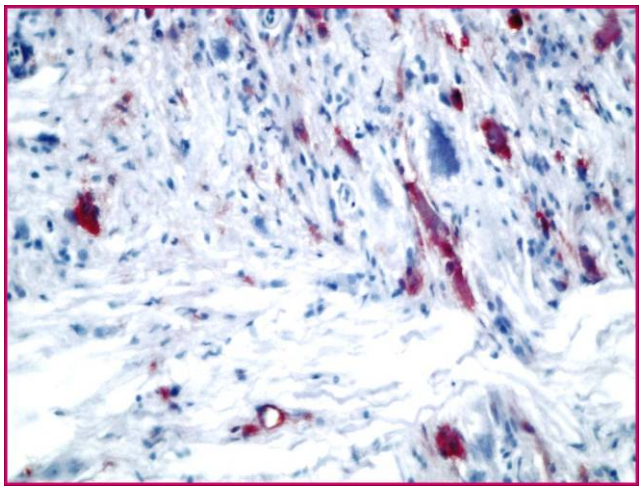
retina (variable)
3. Diffuse histiocytic uveitis, mild to moderate, (variable)

Contributor's Comment : The changes in the telencephalon and retina are considered highly compatible with a disease condition referred to as viral encephalopathy and retinopathy, caused by an aquatic Nodavirus.[1]

Nodaviruses are species specific non-enveloped, icosahedral agents, 25-30nm in diameter.[1,5] A large number of marine species are affected by this family of viruses which cause significant mortality in juveniles. The viruses have been a major impediment to the commercialization of numerous fish species. Consistently, the virus affects the central nervous system (with some exceptions noted – spinal ganglia of Japanese parrotfish).[5] Characteristically the lesions are in the anterior section of the brain (telencephalon – in most fish, the olfactory lobe comprises the largest portion) and consist of the presence of vacuoles in the grey matter which appear to be cytoplasmic. Other lesions included pyknosis and basophilia of affected cells. Similar lesions are noted in the neural component of the retina.[1,4,5] The relative lack of gliosis is likely due to the acute nature of the infection.



2-4. Forebrain, Atlantic cod. Neurons exhibit positivity in immunohistochemical staining for nodavirus. Photomicrograph courtesy of Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada. www.upei.ca

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Participants are referred to the electron microscopic images **(Figs. 2-2 and 2-3 [inset])** which display large numbers of intracytoplasmic viral particles. Note the degenerating mitochondria (M) in figure 2-2. An immunohistochemistry image is also submitted demonstrating strongly positive neurons in the telencephalon **(Fig. 2-4).**

Nodavirus encephalitides are common in the Mediterranean and Indo-Pacific regions, however, recently (2002) a nodavirus of Atlantic cod was demonstrated along the Atlantic seaboard of Canada.[4]

From a comparative view point, the inflammatory response in teleosts differs somewhat from the higher vertebrates. Whilst teleosts possess oligodendrocytes and astrocytes, it has not been reported that they possess microglia. Additionally, in teleosts even mature ependymal cells retain the capacity for differentiation, implying that neuronal regeneration is possible.[3]

AFIP Di agnosis: Brain, telencephalon: Encephalitis, histiocytic, multifocal, moderate with necrosis, neuronal vacuolation, and spongiform change, Atlantic cod (*Gadus morhua*), piscine.

Conference Com ment: Nodaviruses were originally isolated from insects (termed alpha-nodavirus), then from fish (termed beta-nodavirus).[7] Betanodavirus are the agents causing viral encephalopathy and retinopathy (VER), also referred to as viral nervous necrosis (VNN).[8] The brain, spinal cord and retina are the primary target organisms for infection, causing vacuolation and neuronal degeneration.[7] The virus has been described in over 40 species of fish, affecting primarily larval and juvenile fishes[2], and has been a major limiting factor of marine aquaculture development world wide.[7] Transmission is not fully understood, although it is believed to occur vertically from eggs or sperm, or horizontally from water or feed.[7]

Characteristic histologic features of vacuolation and degeneration occur most frequently in the anterior brain. Additional lesions include focal pyknosis and karyorrhexis of neural cells, granularity of neuropil, and mononuclear cell infiltrates.[6] There are conflicting reports in the literature on the extent of optic involvement among different species of fish.[6] Optical lesions, when described, include vacuolation of the rod and cone layer, as well as ophthalmitis of both the anterior and posterior chambers.[6] The cells that most often contain the virus, as identified through electron microscopy, are the neurons, astrocytes, oligodendrocytes, and microglia.[6]

There is multifocal, moderate histiocytic inflammation of the vitreous body or humor (hyalitis) which was not evident in all sections. Retinal and uveal lesions were not evident in the sections evaluated at AFIP.

Contributor: Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada.
www.upei.ca

References:

1. Anonymous. Chapter 2.1.7. Viral encephalopathy and retinaopathy. In: OIE World Organization for Animal Health. Manual of Diagnostic Tests for Aquatic Animals, 2003, Available at:
www.oie.int/eng/normes/fmanual/A_00024.htm
2. Barker DE, MacKinnon AM, Boston L, Burt MD, Cone DK, Speare DJ, Griffiths S, Cook M, Ritchie R, Oliver G: First report of piscine nodavirus infecting wild winter flounder *Pleuronectes americanus* in Passamaquoddy Bay, New Brunswick, Canada. Dis Aquat Org 49:99-105, 2002
3. Ferguson HW: Systemic Pathology of Fish, pp. 158-167. Iowa State University Press, Ames, IA, 1989
4. Johnson SC, Sperker SA, Leggiadro CT, Groman DB, Griffiths SG, Ritchie RJ, Cook MD, Cusack RR: Identification and characterization of a piscine neuropathy and nodavirus from juvenile Atlantic cod from the Atlantic coast of North America. J Aquat Anim Health 14:124-

133, 2002
5. Munday BL, Nakai T: Special topic review: nodaviruses as pathogens in larval and juvenile marine fish. World J Microbiol Biotechnol 13:375-381, 1997
6. Munday BL, Kwang J, Moody N: Betanodavirus infections of teleost fish: a review. J Fish Dis 25: 127-142, 2002
7. Samuelsen OB, Nerland AH, Jørgensen T, Schrøder MB, Svåsand T, Bergh Ø: Viral and bacterial diseases of Atlantic cod *Gadus morhua*, their prophylaxis and treatment: a review. Dis Aquat Org 71:239-254, 2006
8. Starkey WG, Ireland JH, Muir KF, Shinn AP, Richards RH, Ferguson HW: Isolation of nodavirus from Scottish farmed halibut, *Hippoglossus hippoglossus* (L). J Fish Dis 23:419-422, 2000

CASE III – 05-3426 (AFIP 3027074).

Signalment: 10-year-old, female Alpaca (*Lama pacos)*

History: The alpaca had been healthy until last month, had a successful pregnancy the past year. Started with anorexia, and lethargy with rapid weight loss over the last 2 weeks.

Gross Pathology: Mass within small intestine (jejunum) and small circular white nodules in liver

Histopathologic Description: Received 2 fragments of tissue, one a section of liver 5 x 5 cm and the other a fragment of small intestinal tract 16 cm long with attached mesentery. The section of liver has a multifocal infiltrate of neoplastic cells throughout the hepatic parenchyma (not included on the slides). The intestinal sections reveal the origin of the tumor within the mucosa on the majority of the slides. The cells exhibit marked anisokaryosis and have large oval open vesicular nuclei with a variable mitotic rate. This rate is highest in the sections of the mucosa that have the neoplastic process. Necrosis is common to the center of the masses present throughout the supporting mesentery and within the muscle layers. There is a connective tissue reaction within the supporting mesentery and cells in this area vary from nodular masses to individual cells within the connective tissue stroma. A mucinous matrix is apparent mainly with in the supporting mesentery. The cells extend full thickness through the submucosa, muscle layers, serosal surfaces and within the mesentery**.**

Contributor's Morphologic Diagnoses: Intestinal adenocarcinoma poorly differentiated; jejunum.

Contributor's Comment: Tumors in llamas and alpacas are reported to be relatively rare.[5] There are few reported cases in the literature and although this may reflect the population of alpacas in North America, it may also reflect inherent differences within the immune system of New World camelids as compared to other species.

Small intestinal primary epithelial tumors are rare in most species, and in man are more likely to be benign rather than malignant. Extensive research has been done on colonic neoplasia or colorectal neoplasia in man including identification of genetic alterations, familial tendencies and chromosomal abnormalities. The low number of small intestinal adenocarcinomas has precluded this type of evaluation. In man the majority of small intestinal adenocarcinomas can be surgically resected with substantial benefits in terms of 5-year survival.[1]

In domestic animals, small intestinal adenocarcinomas are also considered to be rare, but cats do tend to have mid-jejunal and ileocecal origins for this tumor at a higher rate than the other domestic species. Tumors are classified as adenocarcinoma, mucinous, undifferentiated or solid, and signet ring carcinomas.

In the cat, surgical excision of the tumors yields a reasonable prognosis.[2]

AFIP Diagnosis: 1. Small intestine; mesentery: Carcinoma, anaplastic, alpaca (*Lama pacos*), camelid.
2. Small intestine: Enteritis, necrotizing, acute, diffuse, severe, with fibrin, hemorrhage, edema, vasculitis, and fibrin thrombi.

Conference Comment: According to the World Health Organization International Histological Classification of Tumors of the Alimentary System of Domestic Animals[3], there are six categories of malignant intestinal epithelial neoplasia in domestic animals: Acinar adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, undifferentiated carcinoma, and adenosquamous carcinoma (table 3-1).

A recent review of neoplasia in llamas and alpaca conducted by Valentine et. al.[6] indicated that although the overall prevalence of neoplasia was higher in llamas, the mean age of affected alpacas was significantly lower. The most common malignant neoplasm in camelids was cutaneous and mucocutaneous squamous cell carcinoma with lymphoma being the second most common.[6]

In intestinal adenocarcinoma cells of sheep, there is altered expression of b-catenin, E-cadherin, cycloxygenase-2, and p53 protein.[4] The rates of these altered expressions were lower than that of corresponding rates in human colonic neoplasms, but these findings suggest the use of sheep as potential animal models.[4] b-catenin is a component of the WNT signaling pathway, and increased concentrations of this protein promote genes that regulate the cell cycle.[4] Neoplasm dedifferentiation, invasion and metastasis are promoted by the loss of E-cadherin.[4] COX-2 is often found in increased levels in colonic neoplasm, although the influence it has on tumor behavior is currently under investigation.[4] p53 protein is one of the key regulators of cell cycle regulation and apoptosis.[4]

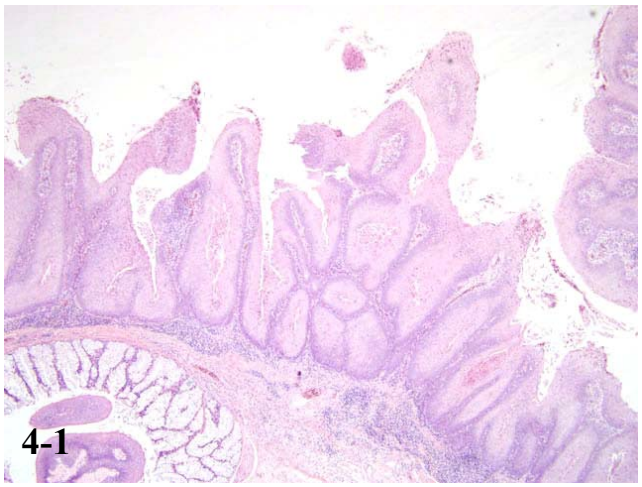
Contributor: Central Laboratory for Veterinarians Ltd, 5645 199th street, Langley B.C. V3A 1H9

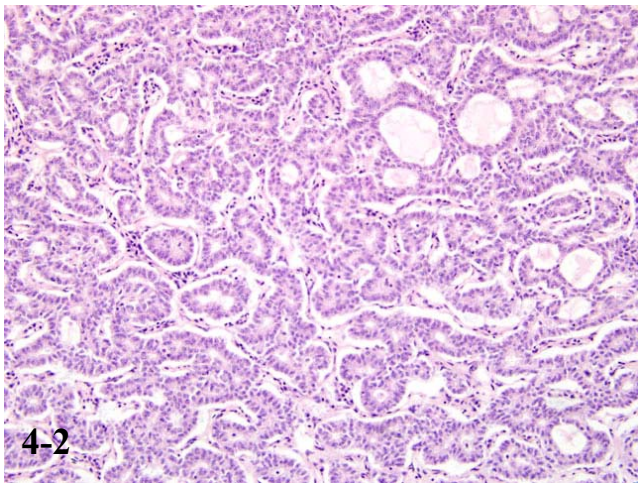
References:

1. Crawford James M. The gastrointestinal tract: Tumors of the small and large intestine. *In*: Robbins Pathologic basis of disease. 6th Edition Cotran RS; Kumar V; Collins T. pp 826-835. WB Saunders Co. 1999.
2. Head KW, Else RW, Dubielzig RR: Tumors of the intestine. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 461-467. Blackwell Publishing, Ames, IA, 2002
3. Head KW, Cullen JM, Dubielzig RR, Else RW, Misdorp W, Patnaik AK, Tateyama S, van der Gaag I: Histological Classification of Tumors of the Alimentary System of Domestic Animals, 2nd series, vol. X, pp. 89-94. Armed Forces Institute of Pathology, Washington, DC, 2003
4. Munday JS, Brennan MM, Kiupel M: Altered expression of b-catenin, E-cadherin, cycloxygenase-2, and p53 protein by ovine intestinal adenocarcinoma cells. Vet Pathol 43:613-621, 2006
5. Sartin EA, Crowe DR, Whitley EM, Treat RE Jr, Purdy SR, Belknap EB: Malignant neoplasia in four alpacas. J Vet Diagn Invest 16:226-229, 2004
6. Valentine BA, Martin JM: Prevalence of neoplasia in llamas and alpacas (Oregon State University, 2001-2006). J Vet Diagn Invest 19:202-204, 2007

Table 3-1. Malignant intestinal epithelial neoplasms. Adapted from the WHO classification[3]

Type of Neoplasm	Characteristic Histologic Features
Acinar adenocarcinoma	Variably sized **acinar structures** replacing intestinal mucosa, arise from hypercellular crypts, infiltrate submucosa and musclular layers, tumor cells may contain occasional goblet cells. In colon, infiltrates Peyer's patches at the primary site
Papillary adenocarcinoma	**Papillary projections** lined by multiple layers of anaplastic columnar cells with little stroma, may have cribriform pattern, mostly intraluminal
Mucinous adenocarcinoma	Acinar or irregular crypts **filled or distended with mucin** replacing the mucosa, mostly goblet cells are seen, infiltrates intestinal wall and mesentery, mostly annular lesions
Signet ring cell carcinoma	**Signet-ring cells** characterized by mucin-filled cytoplasm and peripheralized crescent-shaped nuclei, replace mucosa, infiltrate bowel wall, severe desmoplasia, rare multinucleated cells, must differentiate from adenocarcinoid (which contains neuroendocrine and signet-ring cells)
Undifferentiated carcinoma	Solid sheets of large **anaplastic or pleomorphic cells** with little stroma, desmoplasia, may be rare mucin or signet-ring cells
Adenosquamous carcinoma	Glandular forming adenocarcinoma with **areas replaced by squamous cells** with varying degrees of keratinization





4-1. Cloaca, Scarlet Macaw. Multifocally extending from the mucosa are multiple epithelial lined fibrovascular papillary frondlike projections. Papilloma. (H&E 200X).

4-2. Liver, Scarlet Macaw. Infiltrating and replacing normal hepatic architecture are numerous irregular, branching and anastomosing tubules of neoplastic biliary epithelium. Bile duct carcinoma. (H&E 200X).

CASE IV - H06-976B; 06-998 (AFIP 3063514).

Signalment: Adult, female, *Ara macao*, scarlet macaw

History: The caged scarlet macaw was presented to the consulting veterinarian with a history of weight loss and also straining to pass faeces and urates.

Gross Pathologic Findings: The bird was in poor body condition and there was evidence of wasting of the pectoral muscles. There were multiple small 2-4mm broad based papillomatous masses extending over the mucosa of the cloaca. A low number of papillomatous masses were seen in the choana. Within the left lobe of the liver was a firm white demarcated nodular mass (approximately 4-5mm across) extending to the capsular surface.

Laboratory Results: PCR analysis of emulsified cloacal tissue was positive for psittacid herpesvirus.

Histopathologic Description: 1. Cloaca: The normal epithelium is replaced by abundant papillary structures. The papillary structures are supported by a fibrovascular stroma and lined by markedly hyperplastic stratified squamous epithelium **(Fig. 4-1).** There is marked parakeratotic hyperkeratosis. There are an increased number of mitotitic figures among the basal epithelial cells (36 mitotic figures/10hpf). Between the folds of the papillary structures and extending along the surface is necrotic cellular debris, and aggregates of cocci bacteria. Within the fibrous stroma there is a mild diffuse mixed inflammatory infiltrate consisting of macrophages, plasma cells, heterophils and lymphocytes.

The second section of cloaca: There is mild erosion of the overlying mucosal epithelium.

2. Liver: There is a focally extensive mass with an invasive pattern of growth at the margins replacing the normal hepatic architecture. The mass consists of pleomorphic cells forming tubular structures supported by a fine fibrovascular stroma **(Fig. 4-2).** The cells vary from columnar to cuboidal, have a moderate to scant amount of eosinophilic cytoplasm, oval to round nucleus, reticular chromatin and prominent nucleolus. There is moderate anisokaryosis and increased nuclear to cytoplasmic ratio. Mitotic figures are rare. There are multiple small aggregates of lymphocytes within the mass.

Contributor's Morphologic Diagnosis:
1. Cloaca: Marked diffuse epidermal hyperplasia, cloacal papillomatosis
2. Liver: Bile duct carcinoma

Contributor's Comment: Internal papillomatosis of parrots is believed to be an infectious disease which results in papillomatous tumours of the cloaca and oral cavity.[5] Some macaws also show severe lesions within the oesophagus, ventriculus and proventriculus. Studies

of internal papillomatosis of parrots have shown a correlation between this disease and intercurrent carcinomas of the bile duct and pancreas.[5]

Recent studies have demonstrated an alphaherpesvirus, within cloacal, oral and cutaneous papillomatous lesions and normal cloacal tissue.[4,6] An alphaherpesvirus was identified by PCR analysis of cloacal tissue in the scarlet macaw presented in this case. Styles D and co-workers demonstrated the virus isolated from cutaneous & cloacal papillomas and the normal cloacal mucosa of African grey parrots (*Psittacus erithacus erithacus*) was most closely related, phylogenetically, to the psittacid herpesvirus (which causes Pacheco's disease, psittacid herpesvirus 1), but demonstrated sufficient nucleotide and amino acid diversity to be considered a new alphaherpesvirus, psittacid herpesvirus 2.[6]

Pacheco's disease is a devastating disease with acute onset. Histopathological findings include marked hepatic necrosis, with intranuclear inclusion bodies. Splenic necrosis, enteritis, pancreatitis, tracheitis and air sacculitis are other lesions which are seen variably.[1]

AFIP Diagnosis: 1. Liver: Bile duct carcinoma, scarlet macaw (*Ara macao*), avian.
2. Cloaca: Papilloma.

Conference Comment : Internal papillomatosis of parrots (IPP) is characterized by the progressive development of tumors in the oral and clocal mucosa.[4] Cloacal lesions are most commonly found in the Amazon parrot. Oral papillomas are most common in the oral cavity with occasional extension into the esophagus, proventriculus and ventriculus.[4] Herpesvirus inclusion bodies, virions or PCR products identified as psittacid herpesvirus-2 have been recognized within cutaneous and mucosal papillomas or from healthy cloacal mucosa in African grey parrots, macaws, and a conure.[3,4,8] It is well documented in the literature that there is an association between the presence of papillomatous lesions in Amazon parrots with an increased incidence of pancreatic or bile duct carcinomas, although the exact relationship between these two has not been determined.[2,4]

Psittacid herpesvirus-2 DNA sequence differs from psittacid herpesvirus-1, the cause of Pacheco's disease, by more than 20%.[8] All four psittacid herpesvirus-1 geneotypes have been shown to cause Pacheco's disease in Amazon parrots, but only genotypes 2, 3 and 4 result in disease in African grey parrots.[8]

Other causes of cloacal papillomas or papilloma-like lesions include: papillomavirus; chronic irritation with cell hypertrophy or hyperplasia; and malnutrition with vitamin A deficiency.[2] Papillomavirus infections in birds have been demonstrated in an African grey parrot, finch, and Cuban Amazon parrot.[3,6]

Contributor: Division of Health, Murdoch University, South St, Murdoch, Western Australia, 6150, Australia
http://wwwvet.murdoch.edu.au/

References:

1. Chartwright M, Spraker TR, McCluggage D: Psittacine inclusion body hepatitis in an aviary. J Am Vet Med Assoc 187:1045-1046, 1985
2. Gerlach H: Viruses. In: Avian Medicine: Principle and Applications, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 886-888. Wingers Publishing Inc., Lake Worth, FL, 1994
3. Gibbons PM, Busch MD, Tell LA, Graham JE, Lowenstine LJ: Internal papillomatosis with intrahepatic cholangiocarcinoma and gastrointestinal adenocarcinoma in a peach-fronted conure (*Aratinga aurea*). Avian Dis 46:1062-1069, 2002
4. Johne R, Konrath A, Krautwald-Junghanns ME, Kaleta ER, Gerlach H, Muller H: Herpesviral, but no papovaviral sequences, are detected in cloacal papillomas of parrots. Arch Virol 147:1869-1880, 2002
5. Latimer KS: Oncology. In: Avian Medicine: Principle and Applications, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 657-658. Wingers Publishing Inc., Lake Worth, FL, 1994
6. Styles DK, Tomaszewski EK, Phalen DN: A novel psittacid herpesvirus found in African grey parrots (*Psittacus erithacus erithacus*) Avian Pathol 34:150-154, 2005
7. Tomaszewski EK, Kaleta EF, Phalen DN: Molecular phylogeny of the psittacid herpesviruses causing Pacheco's disease: Correlation of genotype with phenotypic expression. J Virol 77:11260-11267, 2003
8. Tomaszewski EK, Wigle W, Phalen DN: Tissue distribution of psittacid herpesviruses in latently infected parrots, repeated sampling of latently infected parrots and prevalence of latency in parrots submitted for necropsy. J Vet Diagn Invest 18:536-544, 2006

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 19

5 March 2008

Moderator:

Dana Scott, DVM, DACVP

CASE I – 05-9705 (AFIP 2987057).

Signalment: 13-year-old, gelding, Quarter Horse, *Equus caballus*

History: 2 weeks prior to euthanasia, the horse exhibited signs of colic, was dehydrated and had a temperature of 102.8°F; CBC and serum chemistries were normal. Horse was treated with Banamine and mineral oil. His temperature and temperament returned to normal. 10 days later, the horse became anorexic, had difficulty breathing, was very weak and developed dependent ventral edema. Severe pleural effusion found on physical exam. The horse treated with lasix. There was no improvement. On 05/23/05, one fourth of a gallon of straw colored fluid was drained from the chest. The horse was euthanized and submitted to the diagnostic laboratory. Necropsy was performed on the same day.

Gross Pathology:
1. Subacute, severe fibrinous serosanguinous thoracic and peritoneal effusion
2. Subacute, severe, proliferative, fibrinohemorrhagic pericarditis
3. Subacute, severe, bilateral pulmonary congestion and edema
4. Subacute, severe, focally extensive, ventral subcutaneous edema

Laboratory Results: *Actinobacillus* spp. was isolated in pure culture from a swab of the pericardial sac contents.

Histopathologic D escription: The epicardium is diffusely congested, hyperplastic and inflamed. The epicardial surface is covered by edematous, well vascularized fibroblastic tissue which contains a marked infiltrate of degenerative neutrophils and scattered macrophages. This proliferative tissue is covered by laminations of fibrin which contains degenerative neutrophils. Within this material scattered clusters of gram negative coccobacilli are detected in replicate tissue sections stained with Brown and Brenn.

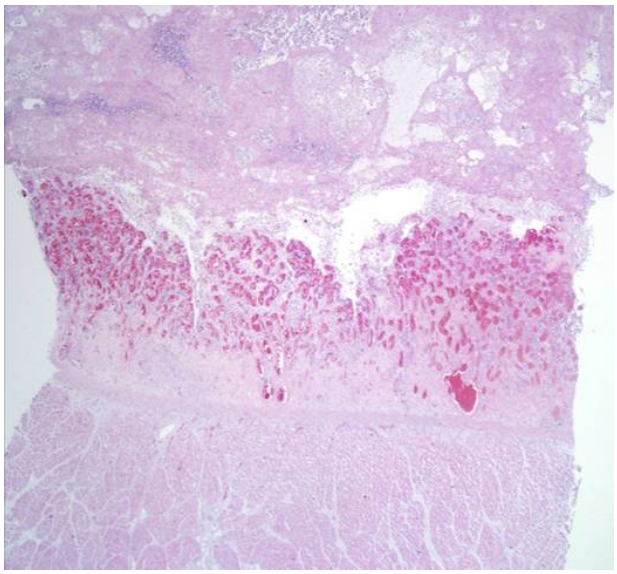
Contributor's Morp hologic Di agnosis: Subacute, severe, proliferative fibrinosuppurative epicarditis with intralesional bacteria.

Contributor's Comment: The severe pleural effusion noted at necropsy is attributed to heart failure secondary to severe, restrictive, fibrinous pericarditis. Fibrinous pericarditis is a fairly uncommon condition in horses.[3] This condition can result from hematogenous bacteria, extension of inflammation from the surrounding tissues via lymphatic spread or by direct inoculation of the pericardial sac by a puncture wound.[3,4] Historically, streptococci have been incriminated in equine cases.[4] However,

a retrospective study of cases of terminal equine pericarditis associated with the mare reproductive loss syndrome during the spring and summer of 2001 confirmed that *Actinobacillus* species played a significant role in this disease.[1]

AFIP Diagnosis: Heart, epicardium: Epicarditis, fibrinous, chronic-active, diffuse, severe **(Fig. 1 -1),** with abundant granulation tissue, Quarter Horse (*Equus caballus*), equine.

Conference Comment: There are three forms of pericarditis: effusive, fibrinous, and constrictive.[6,7] Fibrinous pericarditis usually occurs via hematogenous spread of infectious agents.[4,7] The fibrin exudate covers the epicardium and pericardium and forms gray-white shabby projections when the two are pulled apart (bread and butter pericarditis).[4] Suppurative or purulent pericarditis occurs in the presence of pyogenic bacteria.[4] It is seen mainly in cattle with hardware disease, and occasionally in cats and horses with pyothorax. In dogs it can be associated with migrating grass awns.[4] Constrictive pericarditis occurs following extensive fibrous proliferation and adhesions forming between the pericardium and epicardium.[7] Blunt dissection is usually not sufficient to break down the adhesions formed. The lesions obliterate the pericardial space and impair diastolic filling often leading to right sided heart failure.[4,7]



1-1. Heart, equine. Diffusely the epicardium is markedly expanded by variably sized blood small caliber blood vessels admixed with eosinophilic homogenous material which blends into an overlying thick fibrinous mat. (HE 20X)

Recent articles concerning an epidemic of fibrinous pericarditis, primarily caused by *Actinobacillus* spp., indicate a strong relationship with mare reproductive loss syndrome (MRLS).[1,2,6] MRLS is a syndrome of abortion in horses that occurred in Kentucky in 2001 and 2002. Features of the syndrome include little to no signs of premonitory illness in the mare, hemorrhages in the chorion, amnion, and amniotic segment of the umbilical cord, pleura, and heart.[5] Non-b-hemolytic *Streptococcus* spp. and/or *Actinobacillus* spp. were isolated in 50% and 20% of the cultured specimens.[5] MRLS has been associated with the Eastern tent caterpillar (*Malacosoma americanum*), specifically the worm exoskeleton and attached setae.[2,5] The exact relationship between exposure to the

Conditions potentially associated with fibrinous pericarditis Table extracted from Maxie et al.[4] and Van Vleet et al.[7]

Cattle: Pasteurellosis (*Mannheimia haemolytica* and *Pasteurella multocida*), blackleg (*Clostridium chauvoei*), sporadic bovine encephalomyelitis (*Chlamydophila pecorum*), contagious bovine pleuropneumonia (*Mycoplasma mycoides mycoides* small colony type), clostridial hemoglobinuria (*Clostridium haemolyticum*), neonatal coliform infections (via umbilicus)
Swine: Glasser's disease (*Haemophilus suis*), pasteurellosis (*Pasteurella multocida and Mannheimia haemolytica*), porcine enzootic pneumonia (*Mycoplasma hyopneumoniae* and other agents), salmonellosis, streptococcal infection of piglets
Sheep: Pasteurellosis (*Mannheimia haemolytica* and *Pasteurella trehalosi*)
Lambs: Pasteurellosis, streptococci
Horses: *Mycoplasma felis*, streptococcal polyarthritis with pericarditis, mare reproductive loss syndrome

Eastern tent caterpillar, MRLS, and fibrinous pericarditis is not known.[1,2,5,6]

Contributor: Tennessee Department of Agriculture, Regulatory Services, State Veterinarian Office – C.E. Kord Laboratory
http://www.state.tn.us/agriculture/regulate/labs/kordlab.html

References:

1. Bolin D, Donahue J, Vickers M, Harrison L, Sells S, Giles R, Hong C, Poonacha K, Roberts J, Sebastian M, Swerczek T, Tramontin R, Williams N: Microbiologic and pathologic findings in an epidemic of equine pericarditis. J Vet Diagn Invest 17:38-44, 2005
2. Donahue JM, Sells SF, Bolin DC: Classification of *Actinobacillus* spp. isolates from horses involved in mare reproductive loss syndrome. Am J Vet Res 67:1426-1432, 2006
3. Knottenbelt DC, Pascoe RR: Disorders of the cardiovascular system. In: Color Atlas of Diseases and Disorders of the Horse, pp. 170-171. Mosby, New York, New York, 2003
4. Maxie MG, Robinson WF: Cardiovascular system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 22-30. Elsevier Limited, St. Louis, MO, 2007
5. Schlafer DH, Miller RB: Female genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 506-507. Elsevier Limited, St. Louis, MO, 2007
6. Seahorn JL, Slovis NM, Reimer JM, Carey VJ, Donahue JG, Cohen ND: Case-control study of factors associated with fibrinous pericarditis among horses in central Kentucky during spring 2001. J Am Vet Med Assoc 223:832-838, 2003
7. Van Vleet, JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 575-578. Elsevier, St. Louis, MO, 2007

CASE II – 07-12027 (AFIP 3075555).

Signalment: Adult, male, domestic longhair, *Felis domesticus*

History: The cat was hospitalized for signs attributable to acute renal failure. The cat had elevated BUN and creatinine levels. The cat was euthanized for acute renal failure and submitted to the diagnostic laboratory for necropsy.

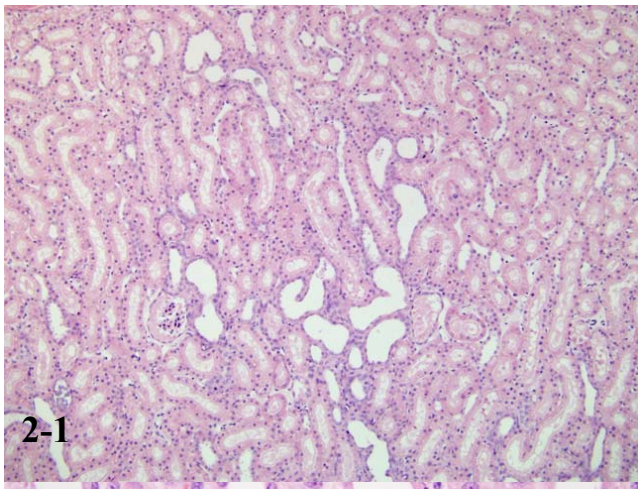
Gross Pathology: The cat was in good body condition with normal amounts of body fat and only mild postmortem autolysis. The lungs were mildly congested and edematous. The urinary bladder was empty. Cut sections of both kidneys had a diffuse lightly pale appearance in the cortices. No other significant gross changes were observed in the carcass.

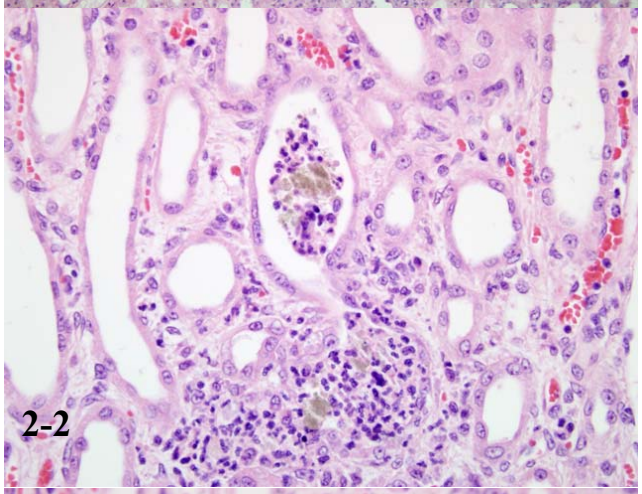
Histopathologic Description: H&E sections of kidney were submitted. There is some mild variability within the slides submitted. Numerous cortical and medullary tubules are moderately dilated **(Fig. 2-1).** Tubular lining epithelium of these tubules is flattened and attenuated. Occasional tubules contain clusters of necrotic epithelial cells and rare neutrophils. Rare granular casts are present. Variable, but usually low numbers of intratubular irregular shaped greenish brown birefringent crystals **(Fig. 2-2)** that fluoresce under polarized light are present within cortical and medullary tubules. Occasional crystals also contain variable amounts of basophilic staining material interpreted as partial mineralization **(Fig. 2-3).** In some sections, the cortical interstitium has multifocal mild infiltrates of lymphocytes and macrophages with mild foci of interstitial fibrosis.

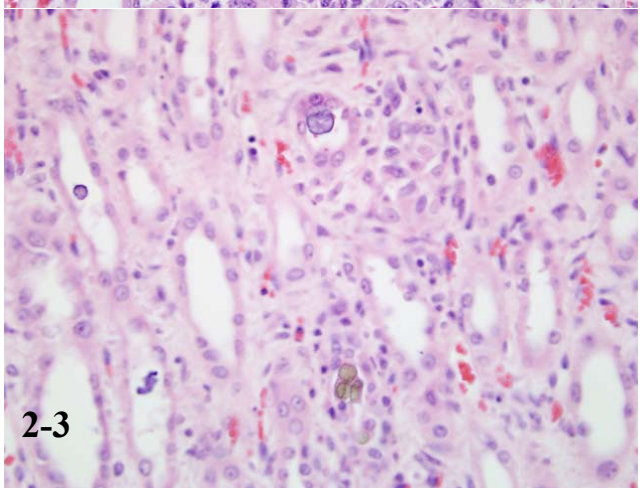
Contributor's Morphologic Diagnosis: Kidney, acute tubular necrosis (nephrosis), multifocal, moderate with nephritis, interstitial, lymphohistocytic, multifocal, mild.

Contributor's Comment: The findings in the kidney are consistent with acute toxic tubular necrosis (nephrosis). The history of elevated BUN and creatinine also supports the histological findings. The crystals present in scattered tubules have a greenish brown color, fluoresce under polarized light and are generally less in number compared to most cases of ethylene glycol (antifreeze) toxicity. The crystals do not have the typical palisading appearance as usually seen with oxalate crystals associated with ethylene glycol toxicity.

The cat had been eating one of the pet food brands (Menu foods) which had been recently recalled from the market due to suspicion of toxic compounds in the food.[6] Current thoughts regarding etiology of this pet food toxicity are focused on melamine. Melamine has been identified as a component in the wheat gluten imported from China which was a component of the recalled pet foods. Current speculation is that the melamine was intentionally added to the wheat gluten to increase the apparent protein concentration of the product. At the time of this report (April, 2007), there is no standardized toxicologic test for







melamine in tissue specimens.

Acute toxicity studies of melamine in mice and rats suggest that this compound is of lower toxicity and has been rated as slightly toxic in acute toxicity ratings when administered by oral route.[7] The LD50 of this compound in mice and rats are 3.3g/kg and 3.2 g/kg respectively.[3,7]

The pathogenesis of crystalluria and the formation of bladder stones in rodents in this syndrome is not fully understood.[2,3] Melamine is excreted in the dog or rat partly as crystalline dimelaminemonophosphate. This can be isolated from warm urine by precipitation with oxalic acid as crystalline monomelaminemonooxalate.[2] In experimental studies, 60-86.5 per cent of the melamine fed to dogs was recovered in the urine in 24 hours.[2]

AFIP Diagnosis: Kidney, corticomedullary junction and medulla: Nephritis, tubulointerstitial, acute, multifocal, mild, with tubular necrosis and degeneration, and numerous intratubular crystals, domestic longhair, (*Felis domesticus*), feline.

Conference Com ment: On March 16, 2007, Menu Foods Inc. issued a recall on more than 60 million containers of pet food that was manufactured between December 3, 2006 and March 6, 2007.[6] This recall occurred due to numerous instances of animal deaths attributed to food related nephrotoxicosis.[5] Over the course of several months, this recall expanded to include several major commercial pet food companies and affected large numbers of dogs and cats in the United States.[1] The toxic compounds contaminating wheat flour were isolated as melamine and cyanuric acid.[1,4,5] Both of these compounds are considered relatively nontoxic when administered separately, but when combined, they form insoluable crystals nearly identical to the ones found in the cases of melamine associated renal failure (MARF).[4,5]

Up to three different crystals have been identified in the kidneys of animals affected by MARF: calcium oxalate monohydrate, calcium phosphate, and melamine-containing. On H&E, melamine-containing crystals within the lumen of renal tubules are up to 80µm in diameter, birefringent, pale yellow to brown, and vary from fan-shaped to starburst radial spokes arranged in concentric circles.[4,5] Calcium oxalate crystals are also birefrin-

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2-1. Kidney, cat. Multifocally, within the cortex and medulla there are ectatic tubules. (HE100X).
2-2. Kidney, cat. Fragmented dense green melamine/cyanuric acid crystals often admixed with necrotic tubular epithelium and cellular debris within tubules. (HE 400X).
2-3. Kidney, cat. Variably-sized light green to slightly basophilic, round, globular crystals with radiating striations. (HE 400X).

Table 2-1. Comparison of staining characteristics of melamine-containing, calcium oxalate, and calcium phosphate crystals.[5]

Stain	Melamine-Containing	Calcium Oxalate	Calcium Phosphate
Oil Red O (72 hour)	Positive	Negative	Negative
Von Kossa	Negative	Positive	Positive
Alizarin Red S (pH 4.1-4.3)	Negative	Negative	Positive
Hematoxylin and Eosin	Pale yellow-brown, radiating spokes, birefringent	Colorless, prismatic effect	Basophilic

gent on H&E, but have a smoother surface and a slight blue tinge due to a prismatic effect.[5] Calcium phosphate crystals on H&E appear as non-birefringent, basophilic particles within the lumen of renal tubules as well as within the walls of blood vessels (not apparent in the present WSC case).[5] Staining characteristics of the crystals are listed in table 2-1.

Prolonged formalin fixation results in dissolution of the melamine-containing crystals within 6 weeks.[1] Therefore, it is recommend that fixation in formalin be kept to a minimum or preserved in 100% (absolute) ethanol. Although more commonly associated with cases of ethylene glycol toxicity, the calcium oxalate crystals in cases of MARF are likely the result of a secondary oxalosis.[1,5]

It was brought to our attention by Dr. Wayne Corapi at Texas A&M University College of Veterinary Medicine that the intratubular crystals in WSC 2004-2005, Conference 12, Case 3, are histomorphologically similar to the melamine-containing crystals recently identified in the kidneys of cats and dogs that were fed pet food on the Menu Foods recall list manufactured between December 3, 2006 and March 6, 2007. Upon reviewing the case and performing special stains, we concur with Dr. Corapi and believe it is a case of pet food-associated nephrotoxicosis with melamine-containing crystals. This association with the outbreak of renal toxicity in Asia was also reported by Puschner et al.[4] and Brown et al.[1] Conference participants are encouraged to review the WSC 2004-2005 case and compare it with the crystals presented in the current case.

Contributor: Tifton Veterinary Diagnostic and Investigational Laboratory, The University of Georgia, Tifton, Georgia.

http://www.vet.uga.edu/vpp/index.php

References:

1. Brown CA, Jeong KS, Poppenga RH, Puschner B, Miller DM, Ellis AE, Kang KI, Sum S, Cistola AM, Brown SA: Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J Vet Diagn Invest 19:525-531, 2007

2. Lipschitz W, Stokey E: The mode of action of three new diuretics: Melamine, adenine and formoguanine. J Pharmacol Exp Ther 83:235-249, 1945

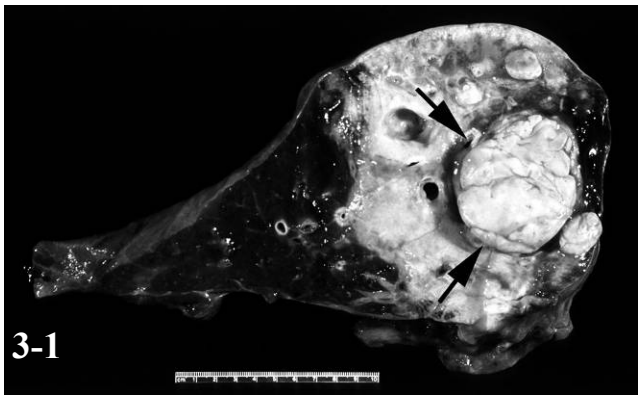
3. Melnick RL, Boorman GA, Haseman JK, Montali RJ, Huff J: Urolithiasis and bladder carcinogenicity of melamine in rodents. Toxicol Appl Pharmacol 72:292-303, 1984

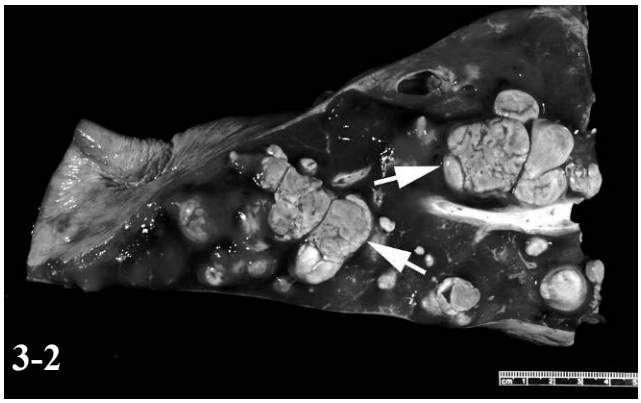
4. Puschner B, Poppenga RH, Lowenstine LJ, Filigenzi MS, Pesavento PA: Assessment of melamine and cyanuric acid toxicity in cats. J Vet Diagn Invest 19:616-624, 2007

5. Thompson ME, Lewin-Smith MR, Kalasinsky VF, Pizzolato KM, Fleetwood ML, McElhaney MR, Johnson TO: Characterization of melamine-containing and calcium oxalate crystals in three dogs with suspected pet food induced nephrotoxicosis. Vet Path 45, 2008 (in press)

6. Pet Food Recall (Melamine)/Tainted Animal Feed. http://www.fda.gov/oc/opacom/hottopics/petfood.html originally accessed on April 23, 2007

7. NTP Acute Toxicity Studies for Melamine http://www.pesticideinfo.org/List_NTPStudies.jsp?Rec_Id=PC35459 originally accessed on April 15, 2007





3-1. Lung, warmblood mare. A yellow-tan mass expands and distorts the caudal lobar bronchus.
3-2 Lung, warmblood mare. Variably-sized masses within the lung parenchyma.

Gross photographs courtesy of Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN 46140

CASE III – 2 (AFIP 2985231).

Signalment: 7-year-old, warmblood mare (*Equus caballus*).

History: The mare presented with a 6-month history of coughing. The veterinarian suspected respiratory infection and allergic pneumonitis; however, the horse did not improve with treatment. Thoracic radiography and pulmonary endoscopic examination were performed (see below).

Gross Pathology: The right lung was surgically excised. The resected lung had a large intraluminal pale yellow-to-tan firm mass expanding and distorting the right caudal lobar bronchus **(Fig. 3- 1)** and had many variably-sized masses protruded into the airways and within the pulmonary parenchyma **(Fig. 3-2).**

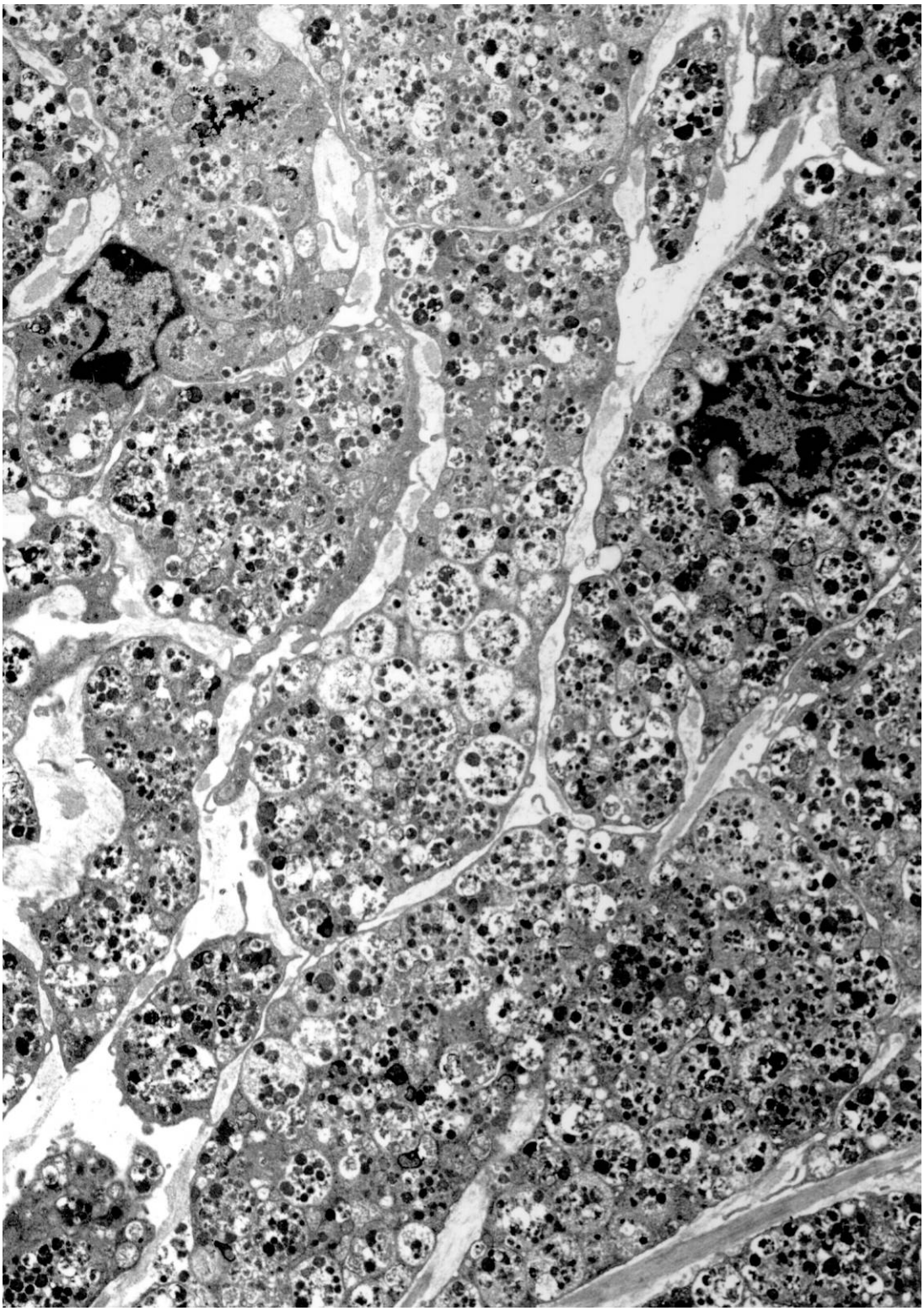
Histopathologic D escription: [Submitted tissue: Tissue from one of the lung masses fixed in glutaraldehyde]. Transmission electron micrograph. The tumor **(Fig. 3-3)** consisted of a homogenous population of neoplastic mononuclear cells. The neoplastic cells were elongate to polygonal with moderate to abundant amounts of cytoplasm, thin elongate cytoplasmic extensions, irregularly shaped nuclei and small nucleoli. The cytoplasm was filled with single membrane-bound secondary lysosomes filled with slightly electron dense amorphous granular material, membranous debris, and spherical moderately electron dense material. Some of the lysosomal contents were consistent with degenerate organelles. Cell-cell junctions, basement membranes, and angulate bodies were not identified. Cytoplasmic organelles were difficult to evaluate because of the abundant number of secondary lysosomes. The neoplastic cells were separated by electron lucent spaces and extracellular bundles of fine fibrils.

Contributor's Mor phologic Diag noses: Lung mass: Granular cell tumor.

Contributor's Comment: Granular cell tumor (GCT) has been reported to occur in multiple species, including horses.[2,6,8] In the horse GCT occurs primarily in the lungs.[8] Similar to the horse of the current case, affected horses may be dyspneic and may be misdiagnosed with allergic airway disease. GCT in horses typically presents as single to multiple masses that may obstruct airways. Masses protruding into the airways can be visualized and sampled via endoscopic examination of the bronchial tree. The clinical course is typically benign, and in the current case surgical excision of the affected lung was curative.

The exact cell of origin of GCTs is not known.[1,3,6,8] Morphologic and immunohistochemical analyses of a variety of GCTs in multiple species suggest that at least some GCTs may be of neural origin (Schwann cells, neuroectoderm).[6,7] The cell of origin may depend on where in the body the tumor originated. Immunohistochemistry was not performed in the current case.

The ultrastructural features of GCT are similar across species.[1-3,6-8] The main morphologic finding is that of neoplastic cells with abundant amounts of secondary ly-



3-3 Lung, warmblood mare. Granular cell tumor. Transmission electron micrograph courtesy of Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN 46140

sosomes containing granular to membranous to amorphous material. The contents likely are remnants of degraded organelles and cell membranes (autophagosomes). In the current case a few lysosomes were found to contain degraded mitochondria. Cell-cell junctions, basal lamina, extracellular collagen, and angulate bodies are variably present in GCTs of animals and humans; however, in the current case cell-cell junctions, basal lamina, and angulate bodies were not identified. The amounts of collagen was minimal (not present in the submitted micrograph). The identity of the extracellular finely fibrillar material present in the current case is not known but may be a product of the neoplastic cells.

AFIP Diagnosis: Lung (per contributor): Granular cell tumor, Warmblood (*Equus caballus*), equine.

Conference Comment: In the horse, granular cell tumors (GCTs) are found primarily within the lower trachea and bronchi as airway associated peri-and endobronchial tumors.[4,8] They are often slow growing, benign neoplasms that over time may result in airway obstruction.[4] GCTs can arise in any tissue, and some are thought to be of neuroectodermal origin. They are characterized by neoplastic cells containing abundant cytoplasm with numerous small, eosinophilic, PAS positive, diastase resistant, non-argyrophilic granules identified as secondary lysosomes or phagosomes (myelin figures) on transmission electron microscopy.[4,8] Small secondary lysosome granules have been associated with active Golgi apparatus, while larger granules are characteristic of multivesicular autophagocytic vacuoles.[8]

Although granular cell tumors have been reported to occur in many locations, in dogs they generally occur in the oral cavity, particularly the tongue, while in rats they occur within the meninges and brain.[6] They have also been reported in the reproductive tract of rodents and a rabbit.[6,8]

Contributor: Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN 46140

References:

1. Barnhart KF, Edwards JF, Storts RW: Symptomatic granular cell tumor involving the pituitary gland in a dog: a case report and review of the literature. Vet Pathol 38:332-336, 2001
2. Facemire PR, Chilcoat CD, Sojka JE, Adams SB, Irizarry AR, Weirich WE, Morrisset SS, Dutweiler VA: Treatment of granular cell tumor via complete right lung resection in a horse. J Am Vet Med Assoc 217:1522-1525, 2000
3. Higgins RJ, LeCouteur RA, Vernau KM, Sturges BK, Obradovich JE, Bollen AW: Granular cell tumor of the canine central nervous system: two cases. Vet Pathol 38:620-627, 2001
4. Irizarry-Rovira AR, Lennox AM, Ramos-Vara JA: Granular cell tumor in the testis of a rabbit: cytologic, histologic, immunohistochemical, and electron microscopic characterization. Vet Pathol 45:73-77, 2008
5. Kagawa Y, Hirayama K, Tagami M, Tsunoda N, Yoshino T, Matsui T, Furuoka H, Taniyama H: Immunohistochemical analysis of equine pulmonary granular cell tumours. J Comp Pathol 124:122-127, 2001
6. Markovits JE, Sahota PS: Granular cell lesions in the female reproductive tract of aged Sprague-Dewley rats. Vet Pathol 37:439-448, 2000
7. Pusterla N, Norris AJ, Stacy BA, Smith P, Fielding CL, Moore PF, Watson JL: Granular cell tumors in the lungs of three horses. Vet Rec 153:530-532, 2003
8. Wilson DW, Dungworth DL: Tumors of the respiratory tract. In: Tumors in Domestic Animals, ed. Meuten DJ, pp. 365-399. Iowa State Press, Ames, IA, 2002

CASE IV - EM for AFIP (AFIP 3050834).

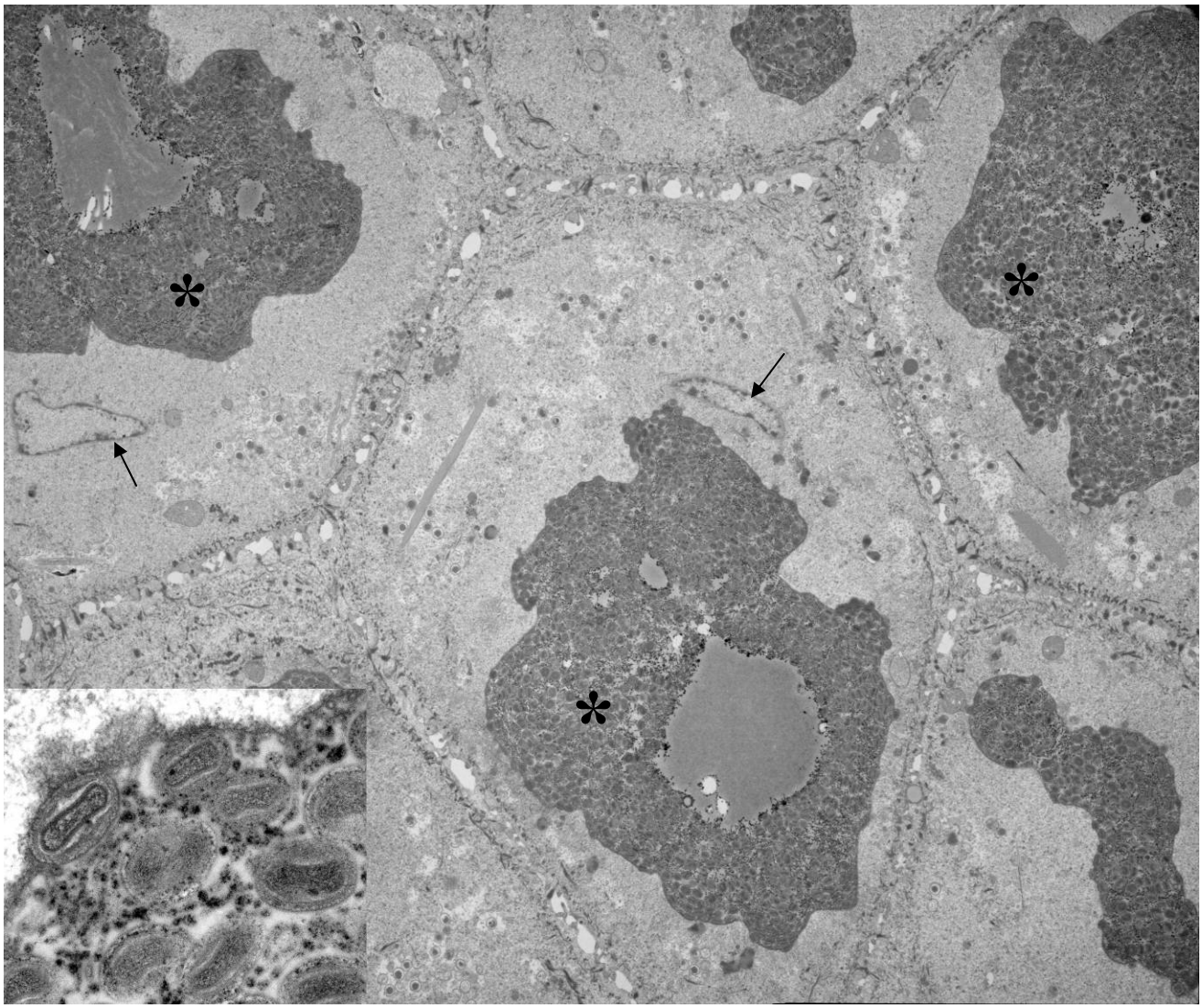
Signalment: Adult, male, rosy finch (*Leucosticte* spp.)

History: A wild-caught research flock of outdoor-housed rosy finches (*Leucosticte* spp.) captured in California and white-crowned sparrows (*Zonotrichia leucophrys*) captured in eastern Washington State presented during the winter of 2001 with history of periocular ulceration, pododermatitis and death.

Gross Pathologic Findings: Numerous birds had similar lesions that included proliferative and necrotizing palpebral dermatitis with conjunctivitis and/or fibrinonecrotic and proliferative stomatitis and glossitis. One rosy finch had a large (5-7mm) mass on the right wing. Numerous mites were noted within the feathers.

Laboratory Results:
The mites were not further characterized.
Candida albicans and *Staphylococcus aureus* were cultured from the tongue.

Histopathologic Description: EM Skin of Wing (5,000X) **(Fig. 4-1):** Numerous polygonal cells with desmosomes, few discernable organelles consistent with squamous epithelial cells, have displaced and flattened



4-1. Skin, wing, rosy finch. Polygonal cells with desmosomes, few discernable organelles, and displaced and flattened nuclei (arrows). In each squamous cell, there is a large electron dense cytoplasmic inclusion body (*) that contains abundant virions.
Inset (60,000X): Enveloped virions approximately 300nm long with brick-shaped nucleocaspid and biconcave core consistent with pox virions. Transmission electron micrographs courtesy of Department of Comparative Medicine, School of Medicine, University of Washington, Seattle, WA 98195-7190

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nuclei, loss of organelle detail, granular homogenous cytoplasm and widened intercellular spaces. In each squamous cell, there is a large electron dense cytoplasmic inclusion body that contains abundant virions. **Fig. 4- 1 Inset** (60,000X): Enveloped virions approximately 300nm long with brick-shaped nucleocaspid and biconcave core consistent with pox virions.

Contributor's Morpho logic Di agnosis: Squamous epithelial degeneration, intra- and intercellular edema with single large intracytoplasmic inclusion bodies composed of virions consistent with pox.

Contributor's Comm ent: The gross, histological lesions are consistent with avipox infection that was confirmed by electron microscopy. Several strains of poxvirus infect a variety of avian hosts including passerines. Transmission is via direct contact, ingestion or mechani-

cal vectors such as mosquitoes or other insects. Pox infection results in cutaneous necroproliferative lesions (cutaneous form or dry pox) and/or fibrinonecrotic and proliferative lesions affecting the mucous membranes of the upper gastrointestinal and respiratory systems (diphtheritic form or wet pox). Both forms can cause significant morbidity by interfering with bodily functions. Secondary bacterial or fungal infections also increase morbidity and mortality. Songbirds are more commonly affected in the winter months as seen in this epornitic. Diagnosis is via gross and histological findings, electron microscopy, viral isolation serology and PCR. Distinctive microscopic findings include epithelial hyperplasia and enlargement of epithelial cells with the characteristic eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies). Electron microscopy can confirm diagnosis by identifying 250 x 354 nm virions with a distinctive brick or dumbbell shape. The presumptive introduction of poxvirus into this research aviary was through the recently caught white-crowned sparrows. The stress of the capture may have exacerbated the infection and the mechanical vectors such as the mites, feather dander, and mosquitoes in the environment likely resulted in the spread if the infection to the finches. Vaccination of the remainder of the flock with a canary pox vaccine has reduced incidence of new disease.

AFIP Dia gnosis: Skin, epithelium (per contributor): Intracytoplasmic inclusions, with mature virions, etiology consistent with poxvirus, rosy finch (*Leucosticte spp*.), avian.

Conference Comment: The Avipoxvirus genus contains many members that are mostly species-specific, although there are some that may cross over species, genus or family barriers.[1] Avipoxvirus strains vary in virulence and protective immunity appears to be strain specific.[4] Characteristic histopathologic lesions of avipoxvirus infection include intracytoplasmic, eosinophilic inclusion bodies (Bollinger bodies) of the epithelial cells in the integument, respiratory tract, and oral cavity.[1] Transmission occurs primarily via innoculation, primarily through mosquitoes, although stable flies and blowflies have also been implicated.[3]

Three forms of the disease have been described: cutaneous form (dry pox), diphtheroid form (wet pox), and septicemic form.[1,4] The cutaneous form is most commonly characterized by cutaneous proliferative lesions around the eyes, beak, nares, vent, and distal to the tarsometatarsus. It is the most common form of the disease in raptors and Passeriformes, but not the Psittaciformes.[1,4] The diphtheroid form consists of multifocal to coalescing fibrinous and caseous lesions on the mucosa of the tongue, pharynx and larynx. Grossly, these lesions are similar to lesions caused by vitamin A deficiency, infectious laryngotracheitis, *Trichomonas gallinae*, *Capillaria* sp., and *Candida albicans*.[1] The septicemic form occurs most commonly in canaries and canary finch crosses, and is characterized by small pneumonic foci and hemorrhages, with cutaneous lesions occuring only rarely.[1] All three forms may occur simultaneously in the same individual.[1]

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References:

1. Gerlach H: Viruses. In: Avian Medicine: Principles and Application, eds. Ritchie BW, Harrison GJ, Harrison LR, pp. 865-874. Wingers Publishing Inc., Lake Worth, FL, 1994

2. Hukkanen RR, Richardson M, Wingfield JC, Treuting PM, Brabb T: Avipox in a grey-crowned rosy finch (*Leucosticte tephrocotis*) Colony. Comp Medicine 53:548-52, 2003.

3. Pledger A: Avian pox virus infection in a mourning dove. Can Vet J 46:1143-1145, 2005

4. Thiel T, Whiteman NK, Tirapé A, Baquero MI, Cedeño V, Walsh T, Uzcátegui GJ, Parker PG: Characterization of canarypox-like viruses infecting endemic birds in the Galápagos Islands. J Wildl Dis 41:342-353, 2005

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 20

12 March 2008

Moderator:

Dr. Don Gardner, DVM, DACVP

CASE I – H06/344/110 #2 (AFIP 3041763).

Signalment: 13-year-old, female, Freiberger horse (*Equus caballus*)

History: The horse had colic for 6 days. The horse showed obstipation at the flexura pelvis. Its colon was displaced to the right at the flexura pelvis. The horse showed slight icterus. With ultrasound examination the liver was inconspicuous. The clinicians suspected a primary or secondary hepatopathy.

Gross P athology: The liver showed severe atrophy of the Lobus hepatis dexter; the whole liver was mildly more friable and was marbled dark and pale brown; on cut section, pus was coming out of the bile ducts; several small (up to 0.5 cm in diameter) white, hard superficial nodules were present (calcifications). In the stomach few *Gasterophilus intestinalis* were present on the pars non-glandularis. The wall of the ileum was mildly thickened (up to 1 cm thick). The left colon ascendens ventralis contained large amounts of dry, dark green, fibrous content (impaction); the mucosa was mildly edematous.

Laboratory Results: The blood values showed elevated liver enzymes.
In the liver, a high content of *Pasteurella* sp. and of a mixed flora were isolated (Institut für Veterinär-Bakteriologie of the University of Bern).

Histopathologic Description:
Liver: Multifocally in the lumen of the bile ducts, in the portal triads and in the surrounding liver parenchyma, there are many degenerate neutrophils and cell debris. The bile ducts are surrounded by moderate to large amounts of connective tissue and many lymphocytes, macrophages, plasma cells and degenerate neutrophils. Multifocally the bile ducts are increased in size and in number (bile duct hyperplasia). They are lined by columnar to cuboidal epithelial cells. Epithelial cells show rarely pyknotic nuclei or karyorrhexis and karyolysis. Many epithelial cells are plump and contain large amount of amphophilic cytoplasm and plump, vesiculated, pale basophilic nuclei. Multifocally there are a lot of bile plugs. There is mild lymphangiectasia and periportal edema. Multifocally there is a mild infiltration of the liver capsule with few lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis:
1. Liver: Cholangiohepatitis, suppurative, multifocal to coalescing, severe, chronic
2. Atrophy of the Lobus hepatis dexter
3. Colon ascendens ventralis, left: Impaction

Contributor's Comme nt: Cholangiohepatitis is a sporadic, but common disease of adult horses. Generally, the disease process is initiated by an ascending biliary tract infection due to a gram negative rod. In the chronic state choleliths can develop, causing obstruction of the biliary tree and leading to icterus and colic. Cholangiohepatitis may also occur secondary to cholelithiasis.[4]

The exact etiology and pathogenesis of cholangiohepatitis in large animals is unknown. The early stages of the disease are often associated with periportal inflammation as well as inflammation in the bile ducts. In suppurative cholangiohepatitis, the bacterial infection may be distributed through portal circulation or extend through the bile ducts. In non-suppurative cholangiohepatitis, disease progression is more likely due to an immune-mediated processes.[7]

Because the hepatocytes are being destroyed more rapidly than they can be replaced, fibrosis begins to bridge the affected areas of the liver. As the fibrosis becomes more extensive, cholestasis and failure of hepatic function may occur. The bile ducts and bile duct epithelium undergo proliferation, which may impair bile excretion.

On necropsy, the liver will appear firm, pale brown to green, with prominent irregular markings on the cut surface.

Histopathologically, two forms are described. A suppurative form in which there is extensive neutrophilia in the periportal area. The neutrophils often contain bacteria. Biliary hyperplasia, loss of hepatocytes, and fibrosis are also evident in the periportal areas. A non-suppurative form occurs in which the primary cellular infiltrate is composed of mononuclear cells, primarily lymphocytes, and plasma cells.[2,6]

Early in the disease, clinical signs are referable to the inflammatory processes occurring in the liver. These inflammatory signs include fever and hepatomegaly, and may lead to colic and biliary obstruction. Anorexia follows in cases which present for colic. Biliary obstruction may then lead to icterus and hepatic photosensitization. Hepatic encephalopathy and related signs are rare except in cases of chronic hepatic fibrosis. Clinical pathologic changes include significantly elevated GGT (600-2500 U/L), slight elevations in AST and SDH relative to the GGT levels, elevated bile acids, leukocytosis with neutrophilia, hyperfibrinogenemia and hyperproteinemia; coagulation parameters should be within normal range.[1,5]

AFIP Diagn osis: Liver: Cholangiohepatitis, chronic-active, diffuse, severe, with bile duct hyperplasia, diffuse bridging fibrosis, and cholestasis, Freiberger horse (*Equus caballus*), equine.

Conference Comment: The contributor gives an excellent overview of cholangiohepatitis in horses. Cholangiohepatitis in horses has been reported to occur as a primary disease, or secondarily due to cholelithiasis, duodenal inflammation, intestinal obstruction, neoplasia, parasitism, and certain toxins such as pyrrolizidine alkaloid and those of *Trifolium hybridum* (alsike clover).[3,7,8]

Suppurative cholangiohepatitis in horses is most commonly associated with cholelithiasis, which is thought to result from ascending infections from the small intestine.[2] Cholangiohepatitis and/or pancreatitis secondary to reflux of duodenal contents may occur acutely as in cases of duodenal obstruction or more chronically, due to either intermittent outflow obstruction or spasm of the major duodenal papilla secondary to inflammation.[1] It has also been found that bacteria, bacterial products, and endotoxins may enter the liver through the portal circulation resulting in periportal inflammation.[2] The bacteria most commonly associated with cholelithiasis and cholangiohepatitis in horses are *Escherichia coli*, *Salmonella* sp., *Aeromonas* sp., and *Citrobacter* sp.[2]

Whether choleliths occur prior to or following the development of cholangiohepatitis has not been determined.[4] The pathogenesis of cholelith formation is not clear, although most choleliths are reported to contain a mixed amount of bilirubin, bile pigments, cholesterol esters, esters of cholic and carboxylic acid, calcium phosphate, and sodium taurodeoxycholate.[3]

In cattle and sheep, cholangiohepatitis has been reported to occur due to sporidesmin, a fungal toxin produced by *Pithomyces chartarum*[3], and liver flukes such as *Fasciola hepatica*.

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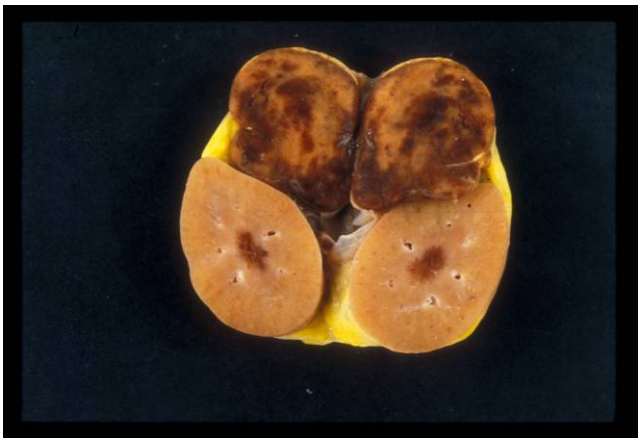
http://www.vetmed.unibe.ch/content/tierpathologie/index_ger.html

References:

1. Buote M: Cholangiohepatitis and pancreatitis secondary to severe gastroduodenal ulceration in a foal. Can Vet J 44:746-748, 2003
2. Davis JL, Jones SL: Suppurative cholangiohepatitis

and enteritis in adult horses. J Vet Intern Med 17:583-587, 2003
3. Gerros TC: Gallbladder and biliary tract disease. In: Large Animal Internal Medicine, ed. Smith BP, 2nd ed., pp. 946-948. Mosby-Year Book Inc., New York, NY, 1996
4. Peek SF, Divers TJ: Medical treatment of cholangiohepatitis and cholelithiasis in mature horses: 9 cases (1991-1998). Equine Vet J 32:301-306, 2000
5. Ryu SH, Bak UB, Lee CW, Lee YL: Cholelithiasis associated with recurrent colic in a Thoroughbred mare. J Vet Sci 5:79-82, 2004
6. Schulz KS, Simmons TR, Johnson R: Primary cholangiohepatitis in a horse. Cornell Vet 80:35-40, 1990
7. Smith MR, Stevens KB, Durham AE, Marr CM: Equine hepatic disease: the effect of patient- and case-specific variables on risk and prognosis. Equine Vet J 35:549-552, 2003
8. Stalker MJ, Hayes MA: Liver and biliary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 345-348. Elsevier Limited, St. Louis, MO, 2007



2-1. Adrenal gland, cotton-top tamarin. An 1.5 cm diameter tan and red mass in the typical location of the right adrenal gland compresses the adjacent kidney. Pheochromocytoma. Gross photograph courtesy of New England Primate Research Center, Harvard Medical School, Southborough, MA, USA http://www.hms.harvard.edu/nerprc/main.html

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CASE II – A03-255 (AFIP 2890562).

Signalment: 17-year-old, male, Cotton-top tamarin (*Saguinus oedipus*), nonhuman primate

History: This elderly tamarin was weak, lethargic and ataxic. He was dyspneic under ketamine anesthesia. On auscultation there was a cardiac arrhythmia and a systolic ejection murmur that was loudest on the left and an EKG demonstrated occasional skipped beats and abnormal QRS complexes. An ultrasound revealed cystic areas in the liver and increased mineralization in the right renal calyxes. A mass was palpated in the right cranial abdomen. The animal was euthanized due to a poor prognosis.

Gross P athology: In the region of the right adrenal gland is a large tan and red mass **(Fig. 2- 1)** approximately 1.5 cm in diameter. The mass has a slightly irregular surface and is compressing the subjacent renal parenchyma. In the left adrenal gland is a focal, tan, round approximately 4 mm in diameter mass. There are numerous cysts in the liver, especially in regions near the diaphragm, filled with clear liquid. The cystic regions are coalescing in areas replacing large portions of the hepatic parenchyma. There are a few tan masses in the liver measuring 2-4 mm in diameter. Bilaterally the cortex of the kidneys is light tan and contains many small cysts. The lungs are edematous and there is pink foam in the trachea. The left AV valve of the heart has mild, irregular thickening of the valve leaflet (endocardiosis).

Laboratory Results:
Immunohistochemistry (adrenal mass):

- Tumor cells were positive for chromogranin A, neuron specific enolase (NSE) and synaptophysin.
- Tumor cells were negative for GFAP, VIP, somatostatin, S-100, cytokeratin, NFP and substance P.

Contributor's Morphologic Diagnosis:
Adrenal gland:
1) Pheochromocytoma
2) Myelolipoma (not in all sections)

Contributor's Comment: Pheochromocytomas are neuroendocrine neoplasms derived from chromaffin cells of the adrenal medulla and are most common in dogs, cattle and rats.[2] The adrenal medulla derives from the neural crest and consists of three types of cells: Chromaffin, neuronal (ganglion-like), and sustentacular cells.[8] Chromaffin and ganglion-like cells are descendent from a common sympathoadrenal neuroblastic precursor, express neuronal cytoskeletal proteins and exhibit catecholaminergic properties.[8] Sustentacular cells are stromal or supportive cells and possess morphologic, functional and antigenic properties similar to those of

Schwann and satellite cells.[8]

A diagnosis of a malignant pheochromocytoma in domestic animals is based on invasion of the capsule and adjacent structures (e.g. vena cava) and/or metastasis. In humans, both capsular and vascular invasion may be encountered in benign lesions. Therefore, a diagnosis of malignancy is based exclusively on the presence of metastases.[7] The metastases may involve regional lymph nodes, as well as more distant sites including liver, lung, spleen and bone.[3,7] There are only a few reports of malignant pheochromocytomas with multiple metastases in domestic animals.[10]

Functional pheochromocytomas have been reported infrequently in animals.[2] These tumors may occasionally be associated with clinical signs as a result of the continuous or episodic secretion of one or more of the catecholamines: epinephrine, norepinephrine or dopamine.[2,7] Elevations of blood pressure induced by the sudden release of catecholamines can precipitate acute congestive heart failure, pulmonary edema, myocardial infarction, ventricular fibrillation and cerebral hemorrhage.[2,7] In this case, clinical cardiac abnormalities and histologic findings of myocardial fibrosis (slide not submitted) might suggest catecholamine production by the tumor. Human pheochromocytomas are known to produce sustained hypertension in one-third of cases and experimental catecholamine administration induces myofibrillar degeneration and interstitial fibrosis in animals.[1] However, plasma catecholamine levels and urinary excretion of catecholamines and their metabolites were not measured in this case.

In nonhuman primates adrenal gland tumors are rare. Recognized tumors include: myelolipomas, pheochromocytomas, cortical adenomas, cortical adenocarcinomas, paragangliomas, medullary fibromas and hemangioma/angiomas.[1] In New World nonhuman primates with spontaneous endocrine neoplasia the adrenal gland is most frequently affected with pheochromocytomas reported most often.[5] Other adrenal gland tumors in New World monkeys include myelolipomas.[1] Among prosimian and Old World primates pheochromocytomas have been reported in the ring-tailed lemur (*Lemur catta*), rhesus monkey (*Macaca mulatta*) and cynomolygus monkey (*Macaca fascicularis*).[5] In humans, pheochromocytoma is an uncommon neoplasm and is usually a benign tumor affecting one or both adrenals.[5]

At the New England Primate Research Center, myelolipomas and pheochromocytomas are the two most commonly recognized neoplasms in the adrenal glands of aged Cotton-top tamarins. In humans, most pheochromocytomas occur sporadically in adults with a slight female preponderance.[7] About 10% occur in several, mostly autosomal dominant, familial syndromes including the multiple endocrine neoplasia (MEN) syndromes, type I neurofibromatosis, von Hippel-Lindau disease and Sturge-Weber syndrome.[7] In the familial syndromes, many arise in childhood with a strong male preponderance. Most of the tumors in the syndromes are bilateral (70%), but in the nonfamilial setting only 10-15% are bilateral.[7]

In some of the sections of adrenal from this animal a myelolipoma was also recognized. Myelolipomas are a benign lesion commonly encountered in the adrenal glands of cattle and nonhuman primates and infrequently in other animals.[2] They are composed of accumulations of well-differentiated adipose cells and hematopoietic tissue, including both myeloid and lymphoid elements. Focal areas of mineralization or bone formation may occur. Although the origin of these lesions is uncertain, they appear to develop by metaplastic transformation of cells in the adrenal cortex or cells lining adrenal sinusoids.[2]

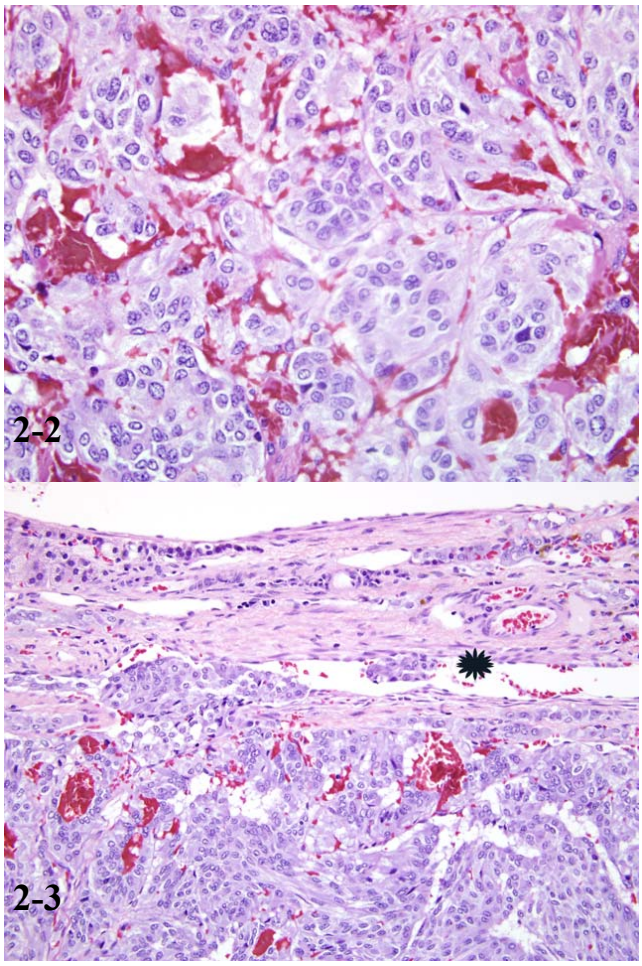
AFIP Di agnosis: Adrenal gland: Pheochromocytoma, Cotton-top tamarin (*Saguinus oedipus*), primate **(Fig. 2-2 and 2-3).**

Conference Comment: The contributor gives an excellent overview of pheochromocytomas and their origin. They occur most often in cattle, dogs and some laboratory rats.[2] In dogs, Boxers appear to be overrepresented, and F344 rats with more severe chronic progressive glomerulopathy have been found to have an increased incidence.[2,3] In bulls and humans, pheochromocytomas have been associated with calcitonin secreting C-cell (ultimobranchial) tumors of the thyroid gland.[2]

Tumor development within the adrenal medulla has been associated with multiple factors, including genetics, dietary factors, chronic high levels of growth hormone or prolactin associated with pituitary tumors, and autonomic nervous system stimulation.[3]

In dogs, caval thrombi occur more frequently with pheochromocytomas than adrenal cortical tumors. The caval thrombus primarily develops as an intraluminal extension from the phrenicoabdominal veins rather than by direct invasion of the vena cava.[6]

Rats have three types of chromaffin cells: epinephrine cells, norepinephrine cells, and small granule-containing cells, unlike human chromaffin cells, which contain both epinephrine and norepinephrine granules within a single



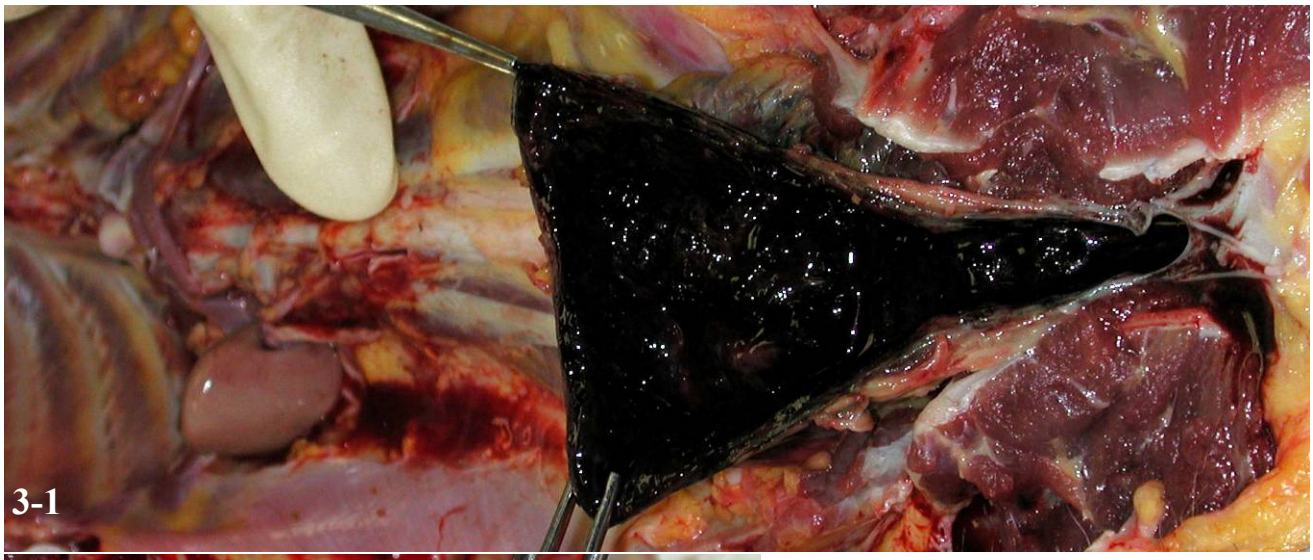
2-2. Adrenal gland, cotton-top tamarin. Neoplastic cells, arranged in nests and packets and supported by a fine fibrovascular stroma, are polygonal with indistinct cell borders, moderate amount of finely granular eosinophilic cytoplasm. There is mild anisokaryosis. (HE 400X).
2-3. Adrenal gland, cotton-top tamarin. There are small variably-sized nests of neoplastic cells within the subcapsular vasculature (star). (HE 400X).

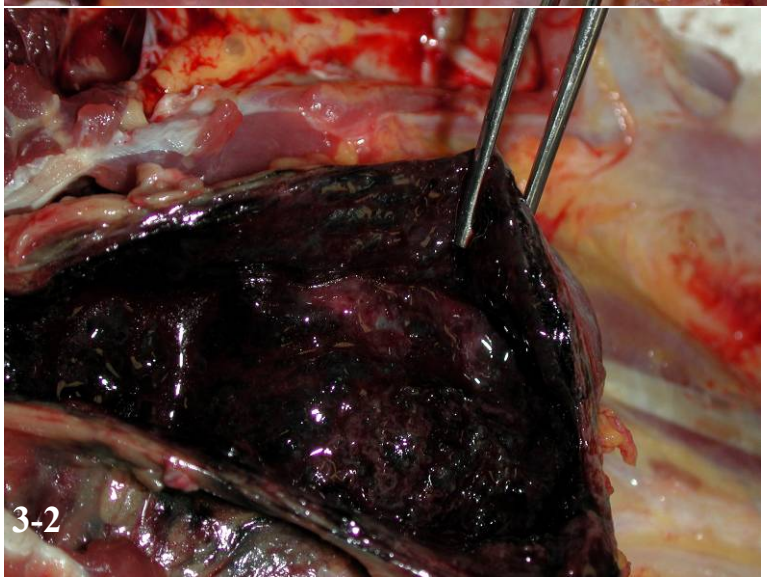
cell.[8] Either epinephrine or norepinephrine secreting cells, or both may be found within a pheochromocytoma. Ultrastructurally, norepinephrine granules have an eccentrically placed, small, electron dense core that is surrounded by a wide submembranous space.[2] Epinephrine granules have a coarse granular core that is less dense than that of norepinephrine granules, and has a narrower submembranous space.[2] In dogs, norepinephrine appears to be the principle catecholamine secreted by pheochromocytomas.[2]

Contributor: New England Primate Research Center, Harvard Medical School, Southborough, MA, USA http://www.hms.harvard.edu/nerprc/main.html

References:

1. Brack M: Adrenal gland tumors in two cotton-top tamarins (*Saguinus oedipus oedipus*). Lab Anim 34:106-110, 2000
2. Capen CC: Tumors of the endocrine gland. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 629-638. Iowa State Press, Ames, Iowa, 2002
3. Capen CC: Endocrine glands. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 419-422. Elsevier Limited, St. Louis, MO, 2007
4. De Cock HE, MacLachlan NJ: Simultaneous occurrence of multiple neoplasms and hyperplasias in the adrenal and thyroid gland of the horse resembling multiple endocrine neoplasia syndrome: case report and retrospective identification of additional cases. Vet Pathol 36:633-636, 1999
5. Dias JLC, Montali RJ, Strandberg JD, Johnson LK, Wolff MJ: Endocrine neoplasia in new world primates. J Med Primatol, 25:34-41, 1996
6. Kyles AE, Feldman EC, De Cock HE, Kass PH, Mathews KG, Hardie EM, Nelson RW, Ilkiw JE, Gregory CR: Surgical management of adrenal gland tumors with and without associated tumor thrombi in dogs: 40 cases (1994-2001). J Am Vet Med Assoc 223:654-662, 2003
7. Maitra A, Abbas AK: The endocrine system. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 1219-1223. Elsevier Saunders, Philadelphia, PA, 2005
8. Pace V, Perentes E, Germann P: Pheochromocytomas and ganglioneuromas in the aging rats: Morphological and immunohisochemical characterization. Toxicol Pathol, 30:492-500, 2002
9. Raue F, Frank-Raue K: Multiple endocrine neoplasia type 2: 2007 update. Horm Res, 68:101-104, 2007
10. Sako T, Kitamura N, Kagawa Y, Hirayama K, Morita M, Kurosawa T, Yoshino T, Taniyama H: Immunohistochemical evaluation of a malignant pheochromocytoma in a wolfdog. Vet Pathol, 38:447-450, 2001
11. Vogel P, Fritz D: Cardiomyopathy associated with angiomatous pheochromocytoma in a Rhesus macaque (*Macaca mulatta*). Vet Pathol 40-468-473, 2003





3-1. Urinary bladder, cynomolgus macaque. The urinary bladder contains a mixture of clotted blood and bloody urine.
3-2. Urinary bladder, cynomolgus macaque. The mucosa and muscular wall are necrotic and friable.

Gross photographs courtesy of U.S. Army Medical Research Institute of Infectious Diseases Fort Detrick, MD 21702-5011

CASE III – 040739 (AFIP 3073369).

Signalment: 4-year-old, female, cynomolgus macaque (*Macaca fascicularis*).

History: Animal caretakers observed a 4-year-old, female, cynomolgus macaque (*Macaca fascicularis*), colony animal, as being in estrous and not eating well. During physical examination by the laboratory animal veterinarian, the animal was lethargic, dehydrated, and there was a moderate amount of perineal bloody discharge. Initial treatment included oral non-steroidal anti-inflammatory (aspirin) and oral electrolyte replacement. There was no clinical improvement and the regimen was changed to intramuscular flunixin meglumine, intramuscular enrofloxacin, and 300 ml of subcutaneous fluids. The animal's physical condition continued to deteriorate and she became unresponsive and hypothermic. Additionally, increased capillary refill time, bilateral nystagmus, mucoid diarrhea, persistent bleeding from the perineum and significant abdominal pain were observed. The macaque was treated for shock and suspected sepsis using intravenous fluids with 2.75% dextrose and continued intramuscular enrofloxacin and flunixin meglumine. Despite these aggressive therapies, the animal succumbed and a complete necropsy was performed.

Gross P athology: Gross necropsy findings included moderate bloody discharge from the vulva. The mucus membranes of the lips, mouth, conjunctiva, and sclera were diffusely pale white. The urinary bladder was diffusely green to black, distended, and contained a mixture of dark red to greenish black fluid that was irregularly

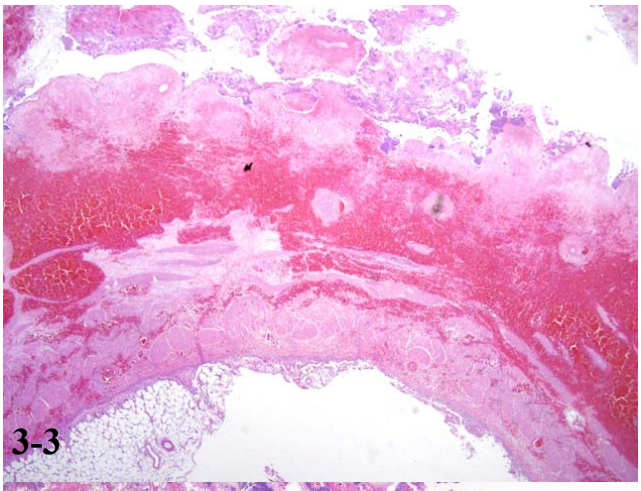
granular to clumped (clotted blood and bloody urine) **(Fig. 3-1).** The mucosa and muscular wall of the urinary bladder were friable and easily torn (necrotic) **(Fig. 3-2).** The hemorrhagic contents from the urinary bladder and the heart blood were sampled at necropsy for microbial culture; the heart blood was extremely thin and watery during collection.

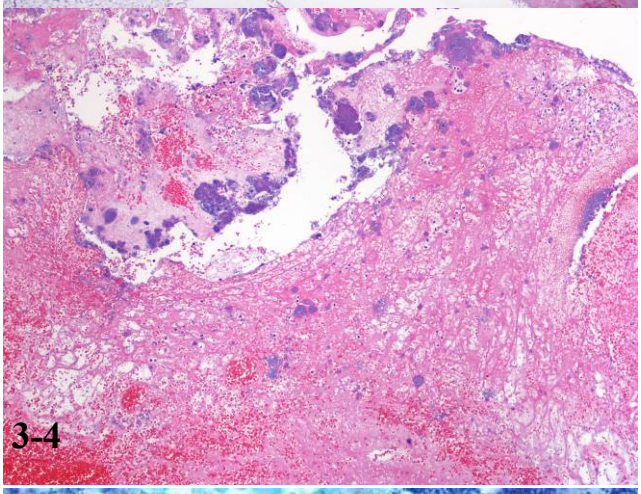
Laboratory Results: Sections were stained by the Gram-Twort staining method. Deparaffinized sections were immersed in crystal violet for 1 minute and stained with Lugol's iodine and neutral red/fast green. Gram-positive organisms stain blue to black and Gram-negative organisms stain pink to red.

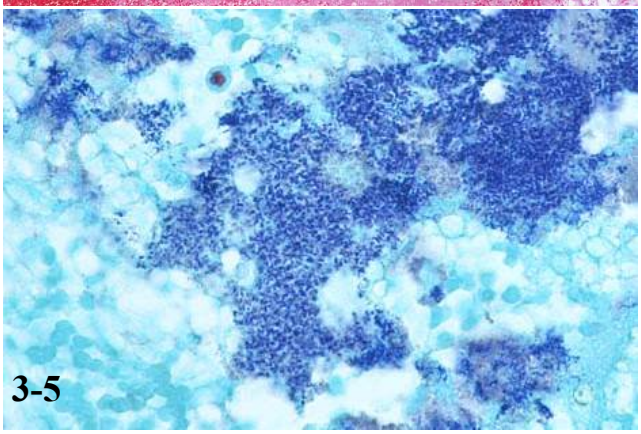
Heart blood culture: No growth after 72 hours
Urine culture: *Corynebacterium* sp.

Histopathologic D escription: Within the section of urinary bladder there is transmural necrohemorrhagic to fibrinosuppurative cystitis that is diffuse and severe **(Fig. 3-3).** The urinary bladder mucosa is replaced by a fibrinonecrotic (diphtheritic) membrane or pseudomembrane, composed of abundant fibrin, hemorrhage, necrotic transitional epithelial cells, degenerate neutrophils, and many coryneform bacteria **(Fig. 3-4).** The wall of the urinary bladder (submucosa, muscular tunic, and serosa) is expanded three to four time's normal thickness by abundant fibrin, edema, hemorrhage, and viable and degenerate neutrophils. Blood vessels are congested and there is multifocal necrotizing vasculitis with disruption of architecture in some/all vessel tunics (intima, media, and adventitia) and multifocally blood vessels contain fibrin thrombi.

Additional significant histologic findings in the cynomolgus macaque included acute to subacute fibrinous polyserositis that included the urinary bladder, stomach, pancreas and mesentery. The inflammatory lesions on the serosal surfaces of the viscera were attributed to leakage of the contents from the necrotic urinary bladder into the abdomen (peritonitis).







3-3. Urinary bladder, cynomolgus macaque. Diffusely, there is transmural hemorrhage and loss of urothelium. (HE 20X).
3-4. Urinary bladder, cynomolgus macaque. Multifocally within areas of hemorrhage and necrosis there are large colonies of bacteria. (HE 100X).
3-5. Urinary bladder, cynomolgus macaque. Pleomorphic short rods are Gram-positive. (Gram-Twort method, 400X).

Photomicrographs 3-4 and 3-5 courtesy of U.S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, MD 21702-5011

Contributor's Morphologic Diagnoses:
1. Urinary bladder: Cystitis, necrohemorrhagic, transmural, subacute, diffuse, severe, with fibrinonecrotic membrane, hemorrhage, edema, necrotizing vasculitis, thrombi, structural loss (perforation), and many Gram positive coryneform bacteria **(Fig. 3-5).**
2. Urinary bladder, serosa and mesentery: Serositis and peritonitis, necrotizing, subacute, diffuse, severe, with neutrophilic and histiocytic inflammation, marked mesothelial cell hypertrophy and hyperplasia, hemorrhage, fibrin and edema.

Contributor's Comme nt: Corynebacterial infections are important causes of disease, morbidity, and death in humans and various wild and domestic animal species. *Corynebacterium diphtheriae* causes diphtheria in humans, a highly contagious upper respiratory tract disease characterized by a pseudomembrane within the tonsil, pharynx, and nose. However, many non-diphtheritic coryneform bacteria are a component of the bacterial flora in the skin and mucous membranes, ubiquitous environmental organisms, and potential opportunistic pathogens.[6,7,8] With a few exceptions, the opportunistic infections caused by non-diphtheritic coryneform bacteria are frequently characterized by necrotizing tissue lesions with suppurative inflammation in the affected host.[7,9]

Of the non-diphtheritic coryneform bacteria, several members of the *Corynebacteria renale* group, including *C. renale* (I), *C. pilosum* (II), and *C. cystitidis* (III), are opportunistic urinary tract pathogens in domestic animals and natural causes of cystitis, ureteritis, and ascending pyelonephritis in cattle. This condition in the bovid is commonly known as bacillary pyelonephritis.[6,8,9] Animals may become predisposed to bacillary pyelonephritis through physical or chemical damage to the lower genitourinary tract caused by dystocia, urinary bladder paralysis, and urinary catheterization. Urinary tract infections are more common in females. These predisposing factors disrupt the host's natural defenses, such as the mucosal barrier, and may allow initial colonization of tissue with coryneform bacteria. Hemorrhagic urethritis, cystitis, and pyelonephritis develop as a result of ascending urinary tract infection.[6,8,10,14]

This case displayed similarities to corynebacterial urinary tract infections in other animal species, including natural and experimental infections in cattle, goats, mice and rats.[2,5,6,7,12] Infection with *C. renale* group bacteria in cattle in particular may affect all or part of the urinary tract. In the urinary bladder, typical lesions include mural thickening from infiltrating leukocytes and hemorrhage, vasculitis and fibrin thrombi, mucosal necrosis, ulceration and perforation, hemorrhage; and replacement of the mucosa by a fibrinonecrotic (diphtheritic) membrane.[6,8]

Several bacterial virulence factors may predispose animals to infection and progression of necrohemorrhagic

Table of common pathogenic Corynebacteriae adapted from Jones et al.[6] and Quinn et al.[9]

Organism	Principle Species	Disease
Corynebacterium diphtheriae	Human	Diptheria
C. renale (Type I)	Bovine	"Bacillary" pyelonephritis, ureteritis, cystitis, may be recovered from healthy individuals
C. cystitidis (Type II)	Bovine	Hemorrhagic cystitis and pyelonephritis
C. pilosum (Type III)	Bovine	Cystitis and pyelonephritis
C. pseudotuberculosis	Ovine and caprine	Caseous lymphadenitis, produces phospholipase D exotoxin
	Equine	Ulcerative lymphangitis and pectoral abscesses
C. bovis	Bovine	Mastitis (rare), found in teat canal of 20% of apparently healthy dairy cows
C. kutscheri	Rodents	Pseudotuberculosis
C. ulcerans	Non-human primates, and many other species	Bite wounds and abscesses

urinary system lesions due to *C. renale* group organisms. *Corynebacteria renale* group organisms possess pili, allowing attachment to the urogenital mucosa and facilitating ascension of microbes into the bladder and kidneys. The bacteria produce urease and hydrolyze urea, which may be contributory to extensive ulceration of the mucosa and necrosis in the affected tissues.[5,8,9,14] Thus, high protein diets and subsequently elevated urinary urea levels may predispose animals to disease because of the ability to hydrolyze urea.[9]

Host factors may also predispose animals to infection. In cattle, females are more predisposed to infection than males due to anatomic structure, hormonal influences, and risks associated with pregnancy or iatrogenic procedures; infection in bulls is rare.[8] Females have short urethras, which decreases the anatomic barrier bacteria must overcome to reach the urinary bladder and kidneys, and urethral trauma associated with dystocia or catheterization may serve as initiating events for infection.[8,10,14] Hormone-induced changes in female animals may serve as predisposing factors; high estrogen levels may affect the functional integrity of the epithelium in the urethra and urinary bladder; cattle with high estrogen levels that graze pastures are reportedly prone to infection with *C. renale*.[8,9] In some animal species, such as the sow, estrogen causes an elevation in the urine pH, which may produce an alkaline environment optimal for expression of bacterial pili and enhanced microbial survival and proliferation.[8,14] Spontaneous and experimentally induced *C. renale* infections in animals are frequently associated with alkaline urine, although this may be reflective of post infection bacterial hydrolysis of urea and production of ammonia rather than preexisting alkaluria.[5,8,14]

In this case, the histopathologic findings of necrotizing and hemorrhagic cystitis, and the microbial culture results from the contents of the urinary bladder support the underlying cause of death as complications from infection by non-diphtheritic coryneform bacteria. The signalment, clinical history, pathological findings and microbial culture results in this case of necrohemorrhagic cystitis show similarities to spontaneous and experimentally induced corynebacterial urinary tract disease observed in several animal species. The perineal bleeding was presumed to be normal estrous bleeding, therefore, diagnosis and treatment were delayed in this case. Severe urinary tract infection due to corynebacteria should be included in the clinical differential diagnosis for protracted perineal bleeding in macaques.

AFIP Dia gnosis: Urinary bladder (per contributor): Cystitis, necrohemorrhagic, transmural, diffuse, severe, with fibrin, edema, and large colonies of bacilli, cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: The contributor gives an extensive overview of *Corynebacterium* infections, their pathogenesis and virulence factors. Although members of the *Corynebacterium renale* group are commonly associated with cystitis and pyelonephritis in cows, they are not generally associated with cases in non-human primates.[13]

In sheep, ulcerative posthitis of wethers (also known as sheath rot or pizzle rot)[9], is a disease that occurs due to the presence of a transmissible urea-hydrolyzing bacterium and the excretion of urine rich in urea.[3] The lesions begin as an ulceration of the prepuce that may progress to destruction of the urethral process and ulceration of the glans penis.[3] *C. renale*, *Rhodococcus equi*, and *C. hofmanni* have all been isolated from infections.[3]

In dogs and cats *Corynebacterium urealyticum*, a urease-producing bacteria, is associated with alkaline urine of pH > 8 and struvite and calcium phosphate precipitations that form encrustations along the bladder wall.[1,11] *Staphylococcus* sp. and some strains of *Proteus mirabilis* are more commonly associated with alkaline urine and struvite production in dogs, but do not produce the mucosal encrustations seen with *C. urealyticum*.[1]

Contributor: U.S. Army Medical Research Institute of Infectious Diseases
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Research at USAMRIID is conducted in compliance with the Animal Welfare Act and other principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. USAMRIID is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

References:

1. Bailiff NL, Westropp JL, Jang SS, Ling GV: *Corynebacterium urealyticum* urinary tract infection in dogs and cats: 7 cases (1996-2003). J Am Vet Med Assoc 226:1676-1680, 2005
2. Elias S, Abbas B, El San-Ousi SM: The goat as a model for *Corynebacterium renale* pyelonephritis. Br Vet J 149:485-493, 1993
3. Foster RA, Ladds PW: Male genital system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 3, pp. 614-615. Elsevier Limited, St. Louis, MO, 2007
4. Hayashi A, Yanagawa R, Kida H: Adhesion of *Corynebacterium renale* and *Corynebacterium pilosum* to the

epithelial cells of various parts of the bovine urinary tract from the renal pelvis to vulva. Vet Microbiol 10:287-292, 1985
5. Jerusik RJ, Solomon K, Chapman WL, Wooley RE: Experimental rat model for *Corynebacterium renale*-induced pyelonephritis. Infect Immun 18:828-832, 1977
6. Jones TC, Hunt RD, King NW: Diseases caused by bacteria. In: Veterinary Pathology, eds. Thomas CJ, Hunt RD, King NW, pp. 479-481. Lippincott Williams & Wilkins, Boston, MA, 1997
7. Lipsky BA, Goldberger AC, Tompkins LS, Plorde JJ: Infections caused by nondiphtheria corynebacteria. Rev Infect Dis 4:1220-1235, 1982
8. Maxie MG, Newman SJ: Urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 490-494. Elsevier Limited, St. Louis, MO, 2007
9. Quinn PJ, Markey BK, Cater ME, Donelly WJC, Leonard FC: *Corynebacterium* species. In: Veterinary Microbiology and Microbial Disease, pp. 55-59. Blackwell Science Ltd., Oxford, 2002
10. Rebhun WC, Dill SG, Perdrizet JA, Hatfield CE: Pyelonephritis in cows: 15 cases (1982-1986). J Am Vet Med Assoc 194:953-955, 1989
11. Saulez MN, Cebra CK, Heidel JR, Walker RD, Singh R, Bird KE: Encrusted cystitis secondary to *Corynebacterium matruchotii* infection in a horse. J Am Vet Med Assoc 226:246-248, 2005
12. Shimono E, Yanagawa R: Experimental model of *Corynebacterium renale* pyelonephritis produced in mice. Infect Immun 16:263-267, 1977
13. Stevens EL, Twenhafel NA, MacLarty AM, Kreiselmeier: Corynebacterial necrohemorrhagic cystitis in two female macaques. J Am Assoc Lab Anim Sci 46:65-69, 2007
14. Yeruham I, Elad D, Avidar Y, Goshen T: A herd level analysis of urinary tract infection in dairy cattle. Vet J 171:172-176, 2006

CASE IV – G7344 (AFIP 3034502).

Signalment: 5-year-old, intact male, rhesus macaque (*Macaca mulatta*), non-human primate.

History: This monkey was inoculated with simian immunodeficiency virus ($SIV_{mac}239$) on 25/08/05 via the tonsillar route. There was a high virus load after two weeks post infection. Twenty-five weeks after the tonsillar challenge the animal showed a pustular skin rash, deteriorating general condition and reduced appetite. The monkey was euthanized on 15/02/06 due to a poor prognosis.

Gross Pathology: At necropsy the rhesus macaque was in a good nutritional condition. The skin was covered with multiple single to coalescing umbilicated pustules. Pustules were preferentially found within the inguinal region, the lips, the hands and feet but they also affected the tongue, the gingiva and the oropharyngeal mucosa. The skin pustules were umbilicated, covered with a central invaginated crust and surrounded by peripheral hyperemia.

Further findings included a severe, necrotising pneumonia and splenitis, a generalized hyperplasia of the lymph nodes and severe follicular hyperplasia of the spleen.

Laboratory Results: Immunohistochemistry: *Herpes simplex* Type 1 and 2: negative

Histopathologic Description: At microscopic examination the skin and the mucous membranes revealed focal areas with epidermal vesiculation, epidermal acanthosis, acantholysis and ballooning degeneration as well as full thickness epidermal necrosis and ulceration. A mixed inflammatory infiltrate composed of neutrophilic and eosinophilic granulocytes, few histiocytes and lymphocytes accompanied the process. In some locations hair follicles and sebaceous glands were involved in the dermal process. Intact affected cells of the vesicle base or margin contained single round to oval intracytoplasmatic inclusion bodies identical with Guarnieri bodies. The Guarnieri bodies were eosinophilic and lay close to the nuclei of infected cells. They were randomly distributed within the altered epithelium. Rare syncytia formations were found close to the basal epithelial layer. At the skin of soles and palms the lesion was arrested in the vesicular stage, covered with a thick intact epidermal cell layer (not included in all sections).

Transmission electron microscopy of skin samples revealed single orthopox like particles in the cytoplasma of keratinocytes.

Monkey pox virus was diagnosed by PCR and cell culture.

Contributor's Morphologic Diagnosis: 1. Tongue: Dermatitis, erosive-ulcerative, subacute, multifocal, severe, with single intracytoplasmatic eosinophilic inclusion bodies, rhesus macaque (*Macaca mulatta*), non-human primate.
2. Skin: Dermatitis, proliferative, pustular, subacute,

multifocal, severe, with single eosinophilic intracytoplasmatic basophilic inclusion bodies and rare syncytia, rhesus macaque (*Macaca mulatta*), non-human primate.

Contributor's Comme nt: Monkeypox is a rare viral disease that is found mostly in the rainforest countries of Central and West Africa. The disease is called "monkeypox" because it was first discovered in laboratory monkeys in 1958.

Monkeypox virus belongs to the orthopox virus group of pox-viruses. Other orthopox viruses that can cause infection in humans and non-human primates include variola (smallpox), vaccinia (used in smallpox vaccine), and cowpox viruses. Pox-viruses are large, complex double stranded DNA viruses.

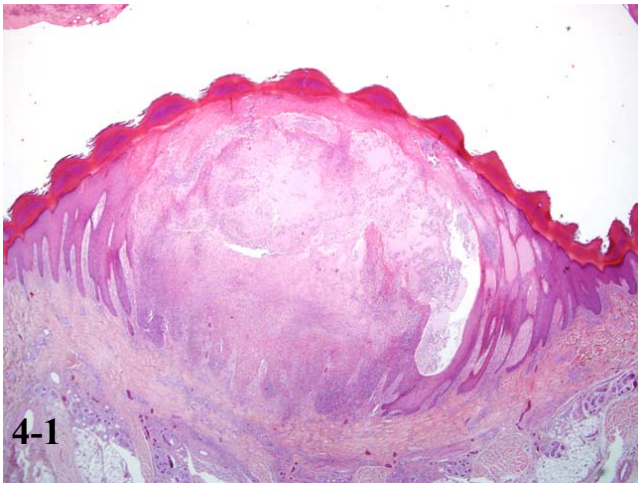
Naturally monkeypox virus only occurs in the tropical rain forest of Western and Central Africa, where it causes subclinical endemic infections in several non-human primate (NHP) species. In the past outbreaks have been reported in captive NHP's, primarily rhesus and cynomolgus, involving institutes importing large numbers of macaques. But the disease has also been reported in marmosets, squirrel monkeys, langurs, baboons, orangutans, gorillas, gibbons and chimpanzees.[6,7,10] The first human case of monkeypox was reported in 1970. Till then several human cases of monkeypox appeared in the tropical rain forest areas of West and Central Africa as isolated cases or as small epidemics. In these regions the infection causes a serious, sometimes fatal smallpox-like disease among young people. Transmission occurs probably aerogenously, by biting or other contacts. People can get monkeypox from an infected animal through a bite or direct contact with the infected animal's blood, body fluids, or lesions (bush meat problem). In 2003 monkeypox was reported among several residents in the United States who became ill after having contact with sick imported prairie dogs. The disease can be spread from person to person too, but it is much less infectious than smallpox.

In non-human primates the disease in usually exhibit a high morbidity and low mortality. Clinical signs may be inapparent or animals may exhibit fever, lymphadenopathy and cutaneous eruptions. Death is uncommon except in infant monkeys. Typical pocks appear as papules of 1 to 4 mm in diameter, which then develop into pustules containing cell debris. The pustules become umbilicated and covered by crusts. The most common sites of pock formation in the monkeys are the face, hands and feet, the mucous membranes of the oral cavity and the genital tract, but also pharynx, larynx trachea, lung, spleen and lymph nodes are commonly involved.

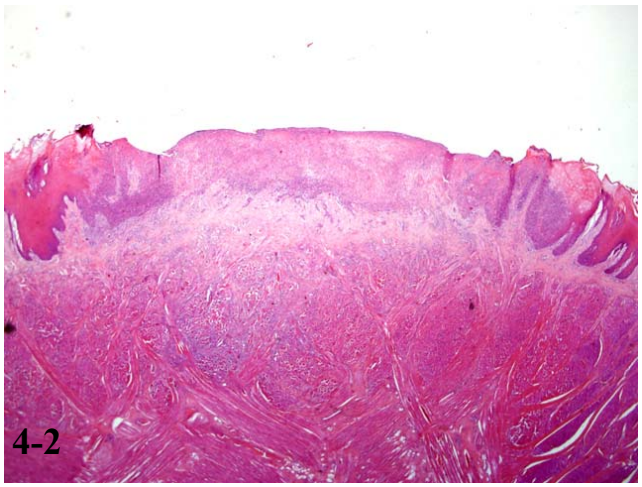
Today we gained experience with the outcome of the disease in immunocompromised monkeys. This accidental case of monkeypox in an immunocompromised animal described here showed that the disease outcome was characterized by severe vesicular exanthema. The skin rash was accompanied by severe respiratory tract involvement and progression of the disease was fatal. Till now it is not clear how transmission occurred in this case. Diagnosis was complicated due to the minimal content of inclusion bodies indicative for poxvirus infection. By electron microscopy typical orthopox like viral particles were demonstrable. An *Eczema herpeticatum* was considered as differential diagnosis, but immunohistochemistry for *Herpes simplex* type 1 and 2 was negative.

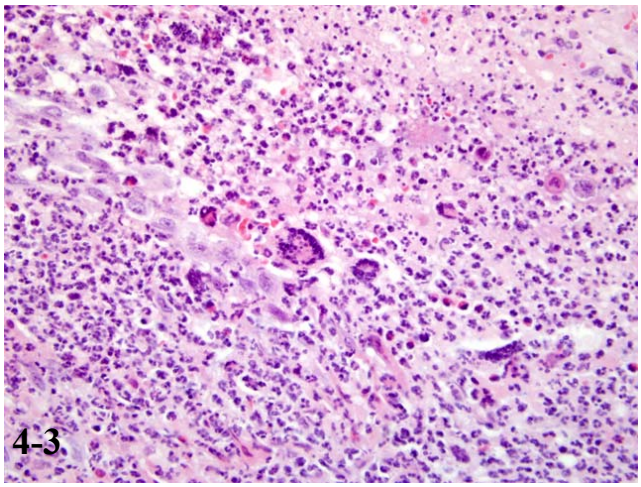
AFIP Diagnosis: 1. Glabrous skin: Dermatitis, vesiculopustular, focally extensive, marked, with acanthosis and ballooning degeneration, rhesus macaque (*Macaca mulatta*), primate **(Fig. 4-1).**
2. Haired skin: Dermatitis, necroulcerative, neutrophilic and eosinophilic, focally extensive, severe with ballooning degeneration.
3. Tongue: Glossitis, necroulcerative, neutrophilic and eosinophilic, multifocal, marked, with ballooning degeneration and intralesional cocci **(Fig. 4-2 and 4-3)**.

Conference Comment : In 2003, several people in the Midwestern United States were diagnosed with monkeypox virus infection. All affected individuals were associated with exposure to captive prairie dogs that had been housed with Gambian giant pouched rats (*Cricetomys* sp.), rope squirrels (*Funiscuirus* spp.), and/or dormice



4-1. Foot, rhesus macaque (Macaca mulatta). Within the epidermis there is a focally extensive vesiculopustule. (HE 20X).





4-2. Tongue, rhesus macaque (Macaca mulatta). Within the mucosal epithelium there is a focally extensive ulcer. (HE 20X).
4-3. Tongue, rhesus macaque (Macaca mulatta). Within necrotic areas there are high numbers of nondegenerate neutrophils admixed with fewer histiocytes, lymphocyte, eosinophils and rare multinucleated syncytial cells. (HE 400X).

(*Graphiurus* sp.) that originated from Ghana.[2,4] As of 30 July 2003, 72 human cases had been reported of human monkeypox virus infection.[2] Affected individuals included veterinarians, pet store personnel, an animal distributor, and children and parents that bought the infected rodents.[2]

Ultrastructurally, orthopox viruses are 375 X 200 nm particles, located free in the cytoplasm, composed of an outer membrane enclosing a characteristic dumbell-shaped inner electron lucent core that is bounded by two lateral bodies.[4]

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http://dpz.gwdg.de

References:

1. Arita T, Jezek Z, Khodakevich L, Ruti K: Human monkey pox: a newly emerged orthopoxvirus zoonosis in the tropical rain forests of Africa. Am J Trop Med Hyg 34:781-789, 1985
2. Bernard SM, Anderson SA: Qualitative assessment of risk for monkeypox associated with domestic trade in certain animal species, United States. Emerg Infect Dis 12:1827-1833, 2006
3. Ginn PE, Mansell JE, Rakich PM: Skin and appendages. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 664-674. Elsevier Limited, St. Louis, MO, 2007
4. Langohr IM, Stevenson GW, Thacker HL, Regnery RL: Extensive lesions of monkeypox in a prairie dog (*Cynomys* sp.). Vet Pathol 41-702-707, 2004
5. Mätz-Rensing K, Ellerbrok H, Ehlers B, Pauli G, Floto A, Alex M, Czerny CP, Kaup FJ: Fatal poxvirus outbreak in a colony of new world monkeys. Vet Pathol 43:212-218, 2006
6. McConnell SJ, Hickman RL, Wooding WL, Huxsoll DL: Monkey pox: experimental infection in chimpanzees and immunization with vaccinia virus. Am J Vet Res 29:1675-1680, 1968
7. Marennikova SS, Shelukhina EM, Maltseva VI, Ladnnyj ID: Poxviruses isolated from clinically ill and asymptomatically infected monkeys and a chimpanzee. Bull WHO 46:613-620, 1976
8. Percy DH, Barthold SW: Mouse. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 25-28. Blackwell Publishing, Ames, IA, 2007
9. Peters JC: An epizootic of monkey pox at Rotterdam Zoo. Int Zool Yearb 6:274-275, 1966
10. Von Magnus P, Andersen EK, Petersen KB, Birch-Anderson A: A pox like disease in cynomolgus monkeys. Acta Pathol Microbiol Scand 46:156-176, 1959

Orthopoxvirus diseases of animals. Table extracted from Ginn et al.[3]

Orthopoxvirus	**Key points**
Camelpox virus	Dromedary camels; clinically identical to camel contagious ecthyma (parapox)
Cowpox virus	Cutaneous and occasionally respiratory lesions in domestic cats; on face and forepaws; affects wild and domestic Felidae, cattle, dogs, rodents, humans; not endemic in cattle, and infections in cattle are uncommon; wild rodents are the reservoir; severe fatal pneumonia in elephants
Ectromelia virus (mousepox virus)[8]	Limb amputation in surviving mice; systemic infection
Monkeypox virus	Rodents, New Wold monkeys, and great apes; systemic disease
Buffalopox virus	Affects waterbuffalo in India; Zebu cattle apparently refractory to infection; closely related to Vaccinia virus
Uasin Gishu disease virus (unassigned)	Horsepox became naturally extinct in 19th century; recent uncharacterized orthopox viruses isolated from horses with equine papular dermatitis, and in equines with Uasin Gishu disease in Kenya; are found closely related to Vaccinia virus and cowpox virus
Vaccinia virus	Does not cause natural infection in domestic animals
Variola virus (human smallpox)	Affects humans and non-human primates; irradicated?

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 21

2 April 2008

Moderator:

Dr. F. Yvonne Schulman, DVM, Diplomate ACVP

CASE I – 05-317 (AFIP 3026796).

Signalment: Five-month-old, female, Yorkshire terrier, canine

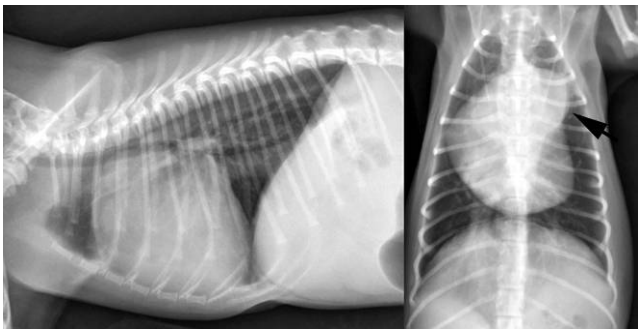
History: Presented to referring veterinarian at 3 months of age with V/VI continuous murmur.
Referred to University of Tennessee Veterinary Teaching Hospital 2 months later:

- Dyspnea - RR 40 bpm - Increased bronchovesicular sounds
- HR 132 bpm, no murmur at this time
- Vulvar mucosa gray-pink
- Lethargy, Weakness, Anorexia

Radiographs: Right-sided cardiomegaly with enlarged right pulmonary artery segment **(Fig. 1-1)**
Echocardiogram and Doppler measurements used to estimate pulmonary artery pressures:

- Patient = 74 mmHg Systolic, 42 mmHg Diastolic (Pulmonary hypertension)
- Normal = 20 to 25 mmHg, 8 to 10 mmHg
- Severely dilated RA and RV
- Severely dilated proximal pulmonary artery
- Decreased LV and LA filling

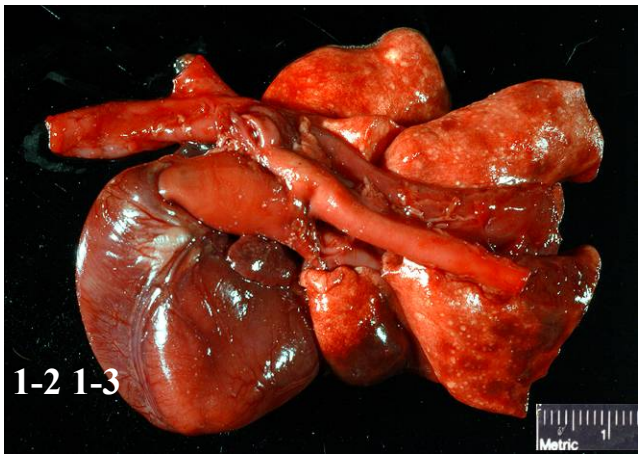
First Pass Cardiac Nuclear Scintigraphy: Cephalic vein bolus injection traced first to right atrium and then to liver and other abdominal organs - very little went to the lungs - interpreted to reflect high magnitude right to left shunt.

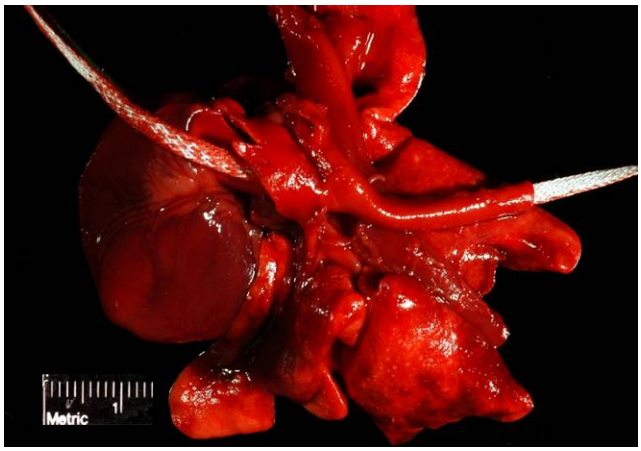


1-1 Thoracic radiograph, Yorkshire terrier. Right-sided cardiomegaly with enlarged right pulmonary artery segment.

Radiograph courtesy of University of Tennessee College of Veterinary Medicine, Department of Pathobiology, Knoxville, TN 37996-4542, http://www.vet.utk.edu/departments/path/

Patient was euthanized after developing apnea and bradycardia. Necropsy was delayed due to scintigraphy study, resulting in some autolysis. Owners requested cosmetic exam restricted to thorax.





1-2 Heart, great vessels and lungs, Yorkshire terrier. The right atrium and ventricle and main pulmonary artery segment are markedly dilated. Patent ductus arteriosus (PDA) is present approximately 2 cm from the heart base.
1-3 Heart, great vessels and lungs, Yorkshire terrier. Patent ductus arteriosus.

Gross photographs courtesy of University of Tennessee College of Veterinary Medicine, Department of Pathobiology, Knoxville, TN 37996-4542, http://www.vet.utk.edu/departments/path/

Gross Pa thology: Two ml of a clear red-tinged fluid was present in the pericardial sac. The right atrium and ventricle were markedly dilated, the right auricle approximately 2-3 times the size of the left. The main pulmonary arterial segment (PAS) was greatly dilated with a diameter of approximately 1 cm extending 2 cm from the heart base to the area of the patent ductus arteriosus (PDA) where the PAS and proximal descending aorta appeared to be fused from the exterior aspect **(Fig. 1-2).** The connection between the two vessels was 0.33 cm externally and the internal diameter was approximately 3 mm **(Fig. 1-3)**. Distal to the PDA the pulmonary artery narrowed abruptly entering the lung.

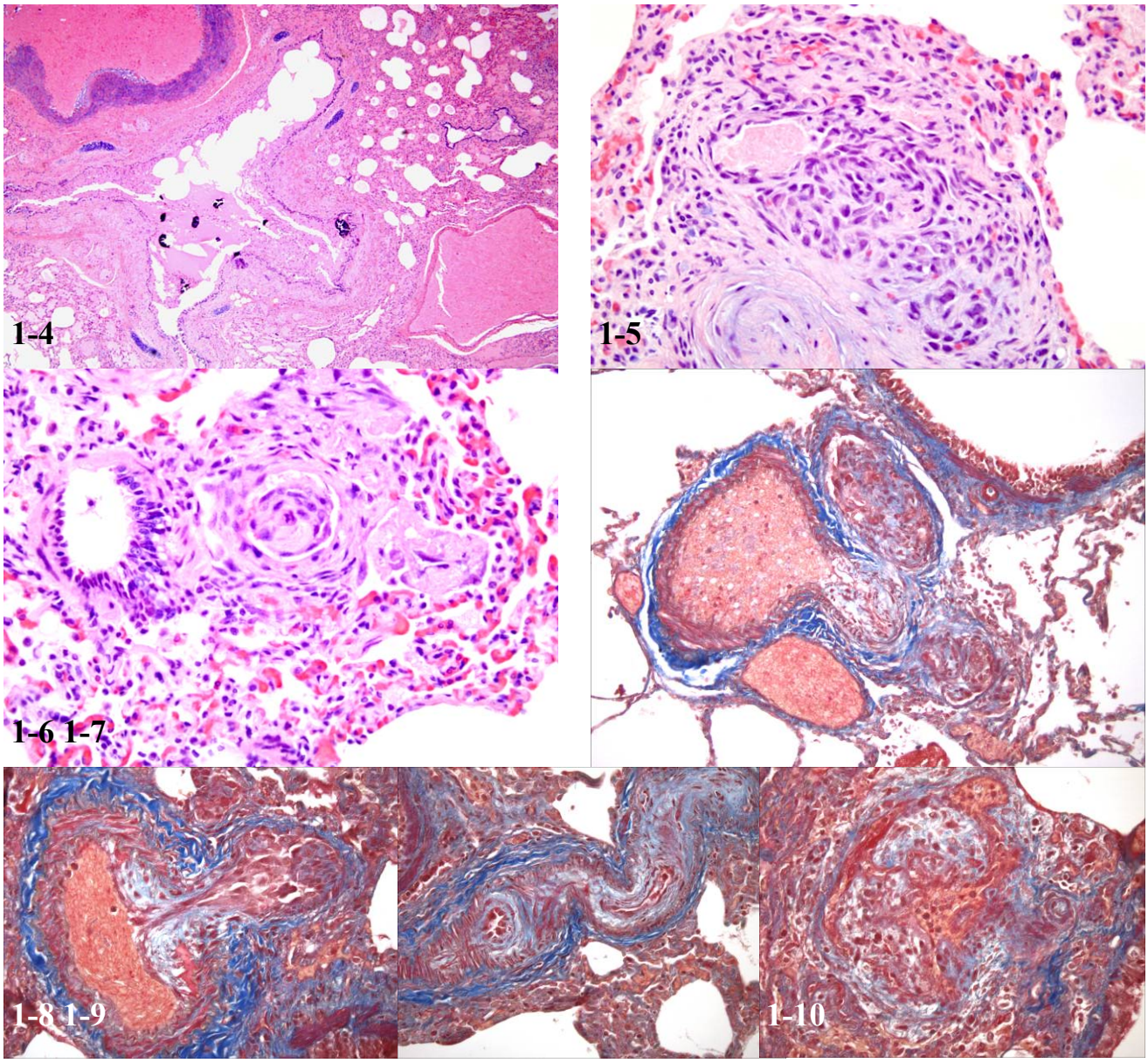
Laboratory Results:
CBC, blood glucose, electrolytes and coagulation panel unremarkable except for low platelet count
Urinalysis: SG 1.019, pH 5.5, 2+ proteinuria, 0-1 WBC/hpf, 1-3 RBC/hpf, many granular casts, trace bacteria

Histopathologic Description:
Lung: There are scattered areas of soft tissue mineralization (alveolar walls, bronchial basement membranes) with some emphysema, edema, proliferation of type II pneumocytes and accumulation of alveolar macrophages **(Fig. 1-4)**. Some large to medium sized pulmonary arteries contain asymmetric areas of intimal thickening and basophilia (reactive myxomatous matrix) or dense proteinic eosinophilia **(Fig. 1-5, 1-6)** . Scattered smaller arteries have segmental areas of intimal to medial basophilia or marked smooth muscle thickening of the wall - in some cases vessels are largely obscured by knots of proliferating plump spindle cells, sometimes forming capillaries (fibroblasts and/or endothelium) and sometimes forming bulging "masses" apparently at branch points. Trichrome stains reveal alveolar wall collagen deposition in scattered areas, sometimes associated with emphysema, increased collagen around some affected small arteries and the fibrotic nature of intimal proliferative lesions **(Fig. 1-7, 1-8, 1-9, 1-10)**.

Contributor's Morphologic Diagnosis:
1. Marked multifocal pulmonary arterial intimal sclerosis and medial hypertrophy with fibro-endothelial proliferation
2. Mild multifocal alveolar fibrosis and emphysema
3. Moderate multifocal bronchial and alveolar mineralization

Contributor's Comment: The clinical and necropsy findings, including pulmonary arterial lesions, are essentially identical to those described by J.W. Buchanan in dogs with hereditary PDA and a right to left pressure gradient.[2] The clinical work up clearly indicated a right to left shunting of blood flow in this case. There are two possible explanations for this right to left pressure gradient in the context of a PDA:

1. The pulmonary vasculature does not respond (dilate) as it should at birth, with inflation of the lung, and the prenatal pressure gradient from right heart to left is maintained after birth.



1-4. Lung, vessel, Yorkshire terrier. There is multifocal mineralization within the large caliber vessel walls and within bronchial subepithelial connective tissue. Occasional mineralized concretions are within the bronchial lumen. (HE 40 X).
1-5. Lung, vessel, Yorkshire terrier. Focally mature plexiform lesions characterized by endothelial proliferation admixed with tightly packed vasoformative cell proliferation and extracellular matrix proliferation and occludes the lumen. (HE 400X)
1-6. Lung, vessel, Yorkshire terrier. Multifocally within intra-acinar arterioles there is concentric nonlaminar medial hypertrophy and intimal thickening which occludes the lumen. (HE 400X).
1-7, 1-8, 1-9, 1-10. Lung, Yorkshire terrier. Increased collagen around some affected small arteries and the fibrotic nature of intimal proliferative lesions is demonstrated using trichrome stain. (Trichrome stain).

Fig. 1-7 to 1-10 courtesy of University of Tennessee College of Veterinary Medicine, Department of Pathobiology, Knoxville, TN 37996-4542, http://www.vet.utk.edu/departments/path/

2. After birth the normal shift in pressure gradient occurs across the ductus and shunting of blood across the PDA from the left to right outflow tracts results in increased circulation through the pulmonary vasculature. Eventually this over-circulation induces arterial sclerosis, hypertension and reversal of the gradient across the ductus from right to left.

While either scenario is possible with this clinical presentation, the histologic arterial changes and pulmonary arterial pressures, as per analysis of echocardiograms, are more consistent with the latter. The vascular lesions in the lung are most consistent with a reactive change and would also exacerbate the progression of hypertension over time. The distinct murmur detected at 3 months of age and absent 2 months later, just before euthanasia, likely reflects this dynamic process as hypertension developed and resulted in changes in blood flow through the ductus.

The etiology of pulmonary mineralization is most likely related to azotemia. Urinalysis suggested significant tubular damage but BUN and creatinine values were not available and kidneys were not examined because necropsy was restricted to the thorax.

AFIP Diagnosis: 1. Lung, artery: Pulmonary arteriopathy characterized by subintimal and medial hypertrophy, intimal fibrosis and cellular thickening, plexiform lesions, and arteritis, Yorkshire terrier (*Canis familiaris*), canine.
2. Lung: Mineralization, interstitial, vascular, multifocal.
3. Lung: Edema, multifocal, moderate.

Conference Comment: Pulmonary hypertension occurs when the mean pulmonary arterial pressure is greater than 25 mmHg at rest or more than 30 mmHg during exercise.[9,13] Secondary pulmonary hypertension may occur following conditions that lead to increased left atrial pressure or increased pulmonary vascular resistance (heartworm disease, chronic respiratory disease, thromboembolism, and vascular remodeling).[5,6,8] Primary pulmonary hypertension on the other hand is defined as pulmonary hypertension of unknown cause.[3,6]

Pulmonary arteriopathy (plexogenic pulmonary arteriopathy) is a condition characterized by constrictive and complex, obstructive, and proliferative vascular lesions in the pre- and intra-acinar pulmonary arteries that results in pulmonary arterial hypertension and eventually right-sided heart failure.[20] Pulmonary arterial hypertension with pulmonary arteriopathy can be subdivided into idiopathic, familial and associated with risk factors or conditions. Most cases in dogs have been idiopathic or associated with congenital heart disease, particularly patent ductus arteriosus.[9,16] Histologic lesions include plexiform lesions of the small arterioles with concentric intimal cellular proliferation and fibrosis, non-specific medial hypertrophy, muscularization of arterioles, fibrinoid degeneration and arteritis.[1,2,7,9,15,16] The association between pulmonary hypertension and the development of pulmonary arteriopathy is not fully understood as each may contribute to the formation of the other.

The pathogenesis of the changes within the small and medium sized pulmonary arteries seen in cases of pulmonary hypertension is not clear. Potential factors associated with this condition may be due to a genetically based hyperreactivity of pulmonary arteries[18], sheer stresses on the pulmonary arteries, injury to the pulmonary endothelium, or changes induced by toxins, drugs and infections.[6,11,14,19] Chronic changes within the pulmonary arteries due to increased flow have been associated with altered nitric oxide and endothelin respones.[4] Endothelin-1, a potent vasoconstrictor, has been associated with increased pulmonary flow in left-to-right shunts independent of pulmonary artery pressure.[4,17] Pulmonary arteries exposed to high flow and pressure also have been reported to have increased levels of VEGF, which suggests the ongoing process of tissue remodeling.[10,12]

Contributor: University of Tennessee College of Veterinary Medicine, Department of Pathobiology, Knoxville, TN 37996-4542
http://www.vet.utk.edu/departments/path/

References:

1. Berger RMF, Geiger R, Hess J, Bogers AJ, Mooi WJ: Altered arterial expression patterns of inducible and endothelial nitric oxide synthase in pulmonary plexogenic arteriopathy caused by congenital heart disease. Am J Respir Crit Care Med 163:1493-1499, 2001
2. Buchanan, JW: Patent ductus arteriosis morphology, pathogenesis, types and treatment. J Vet Cardiol 3:7-16, 2001
3. Fishman AP: Pulmonary hypertension. In: Hurst's The Heart, eds. Alexander RW, Schlant RC, Fuster V, 9th ed., pp. 1699-1717. McGraw-Hill, New York, NY, 1998
4. Gavaghan BJ, Lapointe JM, Thomas WP: Acute onset of pulmonary necrotising arteritis in a dog with a left-to-right patent ductus arteriosus. Aust Vet J 76:786-791, 1998
5. Glaus TM, Hauser K, Hassig M, Lipp B, Reusch CE: Non-invasive measurement of the cardiovascular effects of chronic hypoxaemia on dogs living at moderately high altitude. Vet Rec 152:800-803, 2003

6. Glaus TM, Soldati G, Maurer R, Ehrensperger F: Clinical and pathological characterisation of primary pulmonary hypertension in a dog. Vet Rec 154:786-789, 2004
7. Heath D, Edwards J: The pathology of hypertensive pulmonary vascular disease: a description of six grades of structural changes in the pulmonary arteries with special reference to congenital cardiac septal defects. Circulation 18:533-547, 1958
8. Johnson L, Boon J, Orton EC: Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992-1996. J Vet Intern Med 13:440-447, 1999
9. Kolm US, Amberger CN, Boujon CE, Lombard CW: Plexogenic pulmonary arteriopathy in a Pembroke Welsh corgi. J Sm Anim Pract 45:461-466, 2004
10. Lam CF, Peterson TE, Croatt AJ, Nath KA, Katusie ZS: Functional adaptation and remodeling of pulmonary artery in flow-induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 289:H2334-H2341, 2005
11. Lee S, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM: Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. J Clin Invest 101:927-934, 1998
12. Mata-Greenwood E, Meyrick B, Soifer SJ, Fineman JR, Black SM: Expression of VEGF and its receptors Flt-1 and Flk-1/KDR is altered in lambs with increased pulmonary blood flow and pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 285:L222-L231, 2003
13. Perry LA, Dillon AR, Bowers TL: Pulmonary hypertension. Comp Cont Educ Pract 13:226-232, 1991
14, Rubin LJ: Primary pulmonary hypertension. N Engl J Med 336:111-117, 1997
15. Tuder RM, Groves B, BAdesch DB, Voelkel NF: Exuberant endothelial cell growth and elements of inflammation are present in plexiform lesions of pulmonary hypertension. Am J Pathol 144:275-285, 1994
16. Turk JR, Miller JB, Sande RD: Plexogenic pulmonary arteriopathy in a dog with ventricular septal defect and pulmonary hypertension. J Am Anim Hosp Assoc 18:608-612, 1982
17. Vincent JA, Ross RD, Kassab J, Hsu JM, Pinsky WW: Relation of elevated plasma endothelin in congenital heart disease to increased pulmonary blood flow. Am J Cardiol 71:1204-1207, 1993
18. Wagenvoort CA: Open lung biopsies in congenital heart diseases for evaluation of pulmonary vascular disease: predictive value with regard to corrective operability. Histopathol 9:417-436, 1985
19. Yeager ME, Halley GR, Golpon HA, Voelkel NF Tuder RM: Microsatellite instability of endothelial cell growth and apoptosis genes within plexiform lesions in primary pulmonary hypertension. Circ Res 88:e2-e11, 2001
20. Zabka TS, Campbell FE, Wilson DW: Pulmonary arteriopathy and idiopathic pulmonary arterial hypertension in six dogs. Vet Pathol 43:510-522, 2006

CASE II – 07-0613 (AFIP 3066389).

Signalment: An adult, female, German shepherd dog (*Canis familiaris*)

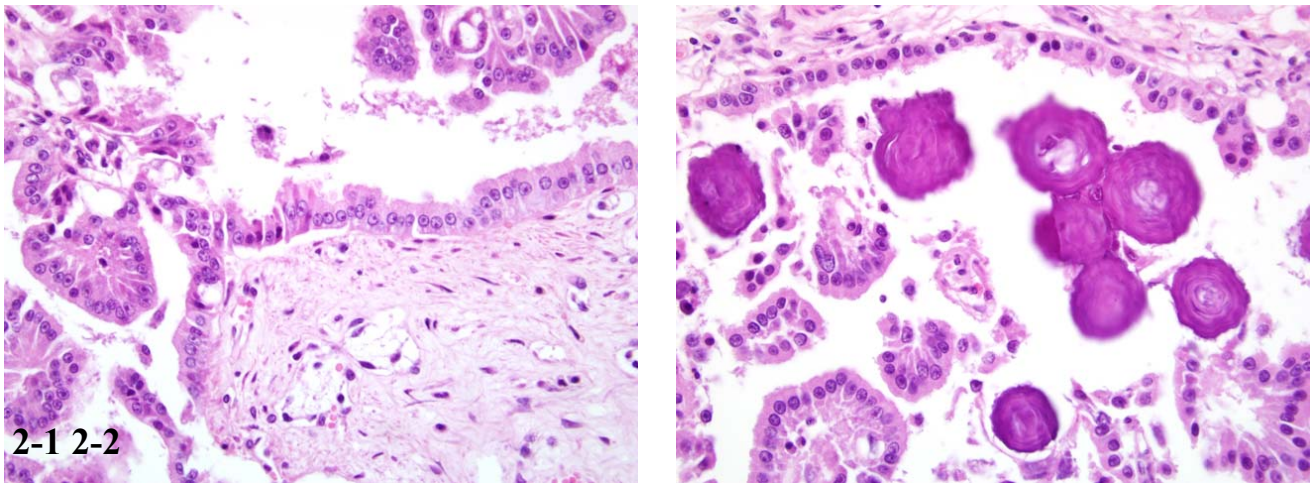
History: A 6-year-old female German shepherd dog was presented for a posterior ataxia and a bilateral quadriceps amyotrophy. At neurological examination, the posterior patellar reflex was bilaterally increased and the posterior proprioceptive reflexes were decreased. The dog was euthanized for humane reasons and a complete necropsy examination was undertaken.

Gross P athology: At necropsy, a nonencapsulated, grayish granular mass was observed within the 4th ventricle. Nodular and multifocal intradural dull gray lesion with a granular cut surface was also observed on the ventral face of the brain stem scattering along the spinal cord. The nodules were firm, and had expansive growth without evidence of infiltration of the brain and spinal cord parenchyma. They compressed and displaced the spinal cord.

Laboratory Results: No significant bacterial pathogens were culture.

White blood cells count: 67 x 10^3 cells/mL (leucocytosis)
Red blood cells count: 2,3 x 10^6 cells/mL (anemia)
Hematocrit : 18%
MCH: 63 g/L
Reticulocyte count : 50 %

Histopathologic D escription: Spinal cord cross and sagittal sections: At histopathological examination, the ventral subarachnoidal space of the spinal cord and the nerve roots were severely infiltrated by a multinodular, poorly-demarcated, partially encapsulated mass with a moderate cellularity. The tumor was composed of papillary structures supported by a delicate fibrovascular stroma **(Fig. 2- 1)**. The neoplastic cells were cuboidal or columnar, measuring 15 to 20 µm with well-defined cytoplasmic borders. The cytoplasm was abundant and pale eosinophilic. The nucleus was round, basally located, euchromatic with coarse chromatin. The mitotic index



2-1. Meninges, German Shepherd Dog. Neoplastic cell form fibrovascular papillary projections which are lined by a single row of cuboidal to low columnar epithelium. (HE 400X).
2-2. Meninges, German Shepherd Dog. Within neoplasm there are moderate numbers of deeply basophilic, round, often lamellated concretions (psammoma bodies). (HE 400X).

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was 3 to 4 mitoses per high power field and was dependent on examined area. Atypia cellular was moderate to strong: anisokaryosis and anisocytosis, nuclear gigantism, prominent nucleoli and multinucleated cells. No embolus was found. Numerous psammoma bodies and foci of mineralization were observed and focal perivascular accumulation of lymphoid cells was noted **(Fig. 2-2)**.

Contributor's Mor phologic Diagn osis: Spinal cord: Meningeal metastasis of choroid plexus carcinoma (meningeal carcinomatosis)

Contributor's Com ment: Choroid plexus tumors are usually rare and benign tumors. They have been described in man, cattle, horses, goats, cats, mice and dogs. The average age of affected dogs is 6 years and male dogs are up to three times more commonly affected than females, but there is no breed predisposition. In human pathology, choroid plexus carcinoma occurs mainly in children under 3 years of age.

The fourth ventricle is the most common site for these tumors in man and dog. In our case, a primary mass was detected microscopically in the choroid plexus of the fourth ventricle. No significant lesion was detected at post mortem examination so the possibility of metastasis of another tumoral process was ruled out. The spinal cord tumor was multiple without embolus so the hypothesis of a meningeal carcinomatosis due to diffusion of the neoplastic cells through cerebrospinal pathways is highly probable. Concerning immunohistochemistry, it is reported that Pankeratin and CK AE1 positivity is observed in choroid plexus carcinomas with marked cell anaplasia, whereas CK AE3 is expressed by well-differentiated neoplastic cells.[1] Vimentin positivity is observed in a large number of neoplastic cells. EMA and S-100 give negative results in all cases of choroid plexus carcinoma.

AFIP Diagn osis: Meninges, spinal nerve root: Metastatic choroid plexus carcinoma, German shepherd dog (*Canis familiaris*), canine.

Conference Comment: While some have proposed including a category of choroid plexus papilloma with atypia,[4] two major forms of choroid plexus tumors are generally recognized: choroid plexus carcinoma and papilloma. Any form of anaplasia and/or metastasis, including metastasis of well-differentiated tumors within the ventricular system and along the neuraxis, is sufficient for a diagnosis of choroid plexus carcinoma.[3] Anaplastic features include nuclear atypia, loss of papillary architecture with transition to patternless cellular sheets, an increased mitotic index, and necrosis.[2,3]

Papillary ependymomas can be included in the differential diagnosis for choroid plexus tumors. On H&E, ependymomas contain pseudorosettes, occasionally true rosettes with cilia, and have a glial rather than a fibrovascular core.[2,3] Immunohistochemistry may be necessary to differentiate the two.

By immunohistochemistry performed at the AFIP, neo-

Table adapted from Ribas et al.[4] and Koestner et al.[2]

Immunohistochemical stain	Ependymoma (Papillary)	Choroid plexus tumor
Cytokeratin	Usually negative	Positive
Vimentin	Positive	Positive
GFAP (glial fibrillary acidic protein)	Positive	Usually negative, but rarely positive

---

plastic epithelial cells in this tumor had positive cytoplasmic immunoreactivity to cytokeratin and GFAP. While most reported cases of canine choroid plexus tumor are negative for GFAP, at least one case in the literature was positive for GFAP.[1] GFAP positivity of some canine choroid plexus tumors is not surprising based on their histogenesis and the findings in human choroid plexus tumors. Human choroid plexus tumors are often positive for both epithelial markers and glial markers reflecting their hybrid nature. Ependymomas should be widely positive for GFAP and are generally negative for cytokeratin.[5]

Contributor: Department of Veterinary Pathology, Nantes Veterinary School, Atlanpôle-la Chantrerie, Nantes, France.

References:

1. Cantile C, Campani D, Menicagli M, Arispici M: Pathological and immunohistochemical studies of choroid plexus carcinoma of the dog. J Comp Pathol 126:183-93, 2002

2. Koestner A, Higgins RJ: Tumors of the nervous system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 709-712. Blackwell Publishing, Ames, IA, 2002

3. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Tumors of neuroepithelial tissue. In: WHO International Histological Classification of Tumors of the Nervous System of Domestic Animals. 2nd series, vol. V, pp. 17-24, Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC, 1999

4. Ribas JL, Mena H, Braund KG, Sesterhenn IA, Toivio-Kinnucan M: A histologic and immunocytochemical study of choroid plexus tumors of the dog. Vet Pathol 26:55-64, 1989

5. Summers BA, Cummings JF, de Lahunta A: Tumors of the central nervous system. In: Veterinary Neuropathology. pp. 351-401, Mosby, St. Louis, Missouri, 1995

CASE III – 06-11733 (AFIP 3067218).

Signalment: 10-month-old, castrated male, Newfoundland, *Canis familiaris*, canine.

History: The patient was presented to the referring veterinarian with a history of decreased appetite, cough, gagging and regurgitation not associated with eating. The mother and two littermates had died recently after showing similar clinical signs.

There was an episodic fever most apparent during morning and evening associated with an increase in his coughing and regurgitation episodes. The referring veterinarian sent out ANA, ACH receptor, and T4 titers. The ANA was positive at 1:50 and the other results are not available. Thoracic radiographs were taken and an initial diagnosis of megaesophagus was made. An endoscopy was performed and some degrees of esophageal and gastric mucosal scarring were detected. The dog was started by the referring veterinarian on antibiotics, antacids and gastric protectants and then transferred to the Foster Hospital for Small Animals at the Tufts University Cummings School of Veterinary Medicine.

On initial physical exam at Tufts the animal was depressed and gagging on palpation of his trachea. There were harsh lung sounds dorsally and bilaterally. Tho-

racic radiographs showed patchy interstitial to alveolar lung pattern in the right lung lobes and left cranial lung lobe, consistent with aspiration pneumonia. The initial therapy at Tufts included IV fluids, antibiotics and Vitamin E and Selenium. A presumptive diagnosis of polymyositis was made and due to the overall poor prognosis and rising expenses the owner elected euthanasia and an autopsy was performed. Muscle biopsies were submitted to the neuromuscular laboratory at UC Davis.

Gross Pathology: The retropharyngeal and mediastinal lymph nodes were enlarged. The esophageal mucosa and serosal were normal and there was no evidence of megaesophagus. The lung lobes were mostly (70%) dark red to brown heavy and wet.

Laboratory Results:
CBC: WNL.
Taurine:

- Plasma 19 nmol/l (normal values: 60-120)
- Whole blood: 212 nmol/l (normal values: 300-600)

UA: Specific gravity (1.017)
Toxoplasma and neospora titers – cancelled

Histopathologic Description: Esophagus (Submitted slides contain a full-thickness section of distal esophagus, and many include a portion of the gastric cardia): There is mild, multifocal hyperplasia of the stratified squamous epithelium. The muscularis externa is markedly thinned and distorted. Lymphocytes, epithelioid macrophages and occasional neutrophils infiltrate the outer longitudinal muscular layer. Fibroblasts dissect and efface the myofibers and the interstitium is expanded by immature, pale basophilic connective tissue stroma (endomysial fibrosis). Skeletal muscle fibers are markedly distorted and irregular with hyalinization, loss of striation, cytoplasmic pallor and occasional multiple large internalized nuclei. Few hypereosinophilic myocytes with pyknotic nuclei are also present.

Contributor's Morphologic Diagnoses: Esophagus: Severe, diffuse, chronic, lympho-histiocytic myositis with myocyte degeneration, necrosis and regeneration.

Contributor's Comment: The histological findings in this 11-month-old, Newfoundland dog are consistent with a polymyositis and the localization of the lesion (tongue, esophagus, larynx and pharynx) explain the clinical signs exhibited by this animal. Skeletal muscles from the limbs and trunk were unaffected. No bacterial, fungal or protozoal organisms were detected. In addition the histological diagnosis of polymyositis was confirmed by Dr. Diane Shelton at U.C. Davis. [Immunohistochemical staining with staphylococcal protein A-HRP revealed sarcolemmal positive staining consistent with autoantibodies (staphylococcal protein A binds to the FC region of immunoglobulin heavy chains)]

Inflammatory myopathies are a group of disorders characterized by non suppurative inflammation of skeletal muscles. Polymyositis is an immune-mediated, generalized skeletal muscle disorder characterized by muscular weakness, muscular atrophy, elevated serum concentration of creatine kinase, abnormal electromyography, negative serologic tests for infectious disease and lymphocytic infiltrate.[4] Some forms of generalized myositis are associated with protozoal, rickettsial or bacterial infections.[1] In human medicine and in few veterinary medicine cases, inflammatory myopathies have been associated with malignant neoplasia.[2,6]

Some inflammatory myopathies can be localized to particular groups of muscles such as the masticatory muscles[15] and extraocular muscles.[3] Such particular distributions are likely related to molecular characteristics of particular muscle groups. Masticatory muscles myositis for example affects all muscles innervated by the mandibular branch of the trigeminal nerve (masseter, temporalis, pterygoids, tensor tympani and tensor veli palatine muscles) and is characterized by clinical signs such as jaw pain, inability to open the jaw and masticatory muscles atrophy. Masticatory muscles contain a distinctive muscle fiber, type 2M, which is biochemically and histocemically different from the muscle fibers contained within other skeletal muscles.[15] Serum antibody titer for type 2M fibers is negative in extraocular myositis. The targeting of specific muscles in the current case suggests that these muscles may be distinct in some way from other skeletal muscle in the body.

Inflammatory polymyositis has been most thoroughly reported in veterinary medicine in Collies, Shetland sheepdogs and Newfoundlands, and it seems to be related to a generalized immune-mediated disorder.[9] The association of inflammatory myositis with malignant neoplasia in dog has not been completely demonstrated, while it in human medicine it is commonly reported.[2]

AFIP Diagnosis: Esophagus: Myositis, lymphoplasmacytic, histiocytic, subacute to chronic, diffuse, moderate, with muscle degeneration, necrosis and regeneration, Newfoundland dog (*Canis familiaris*), canine **(Fig. 3-1 3-2, 3-3)**.

Conference Comment: Inflammatory myopathies can be divided into two broad categories based on whether an underlying cause can be identified. The idiopathic or presumably immune-mediated neuromuscular diseases

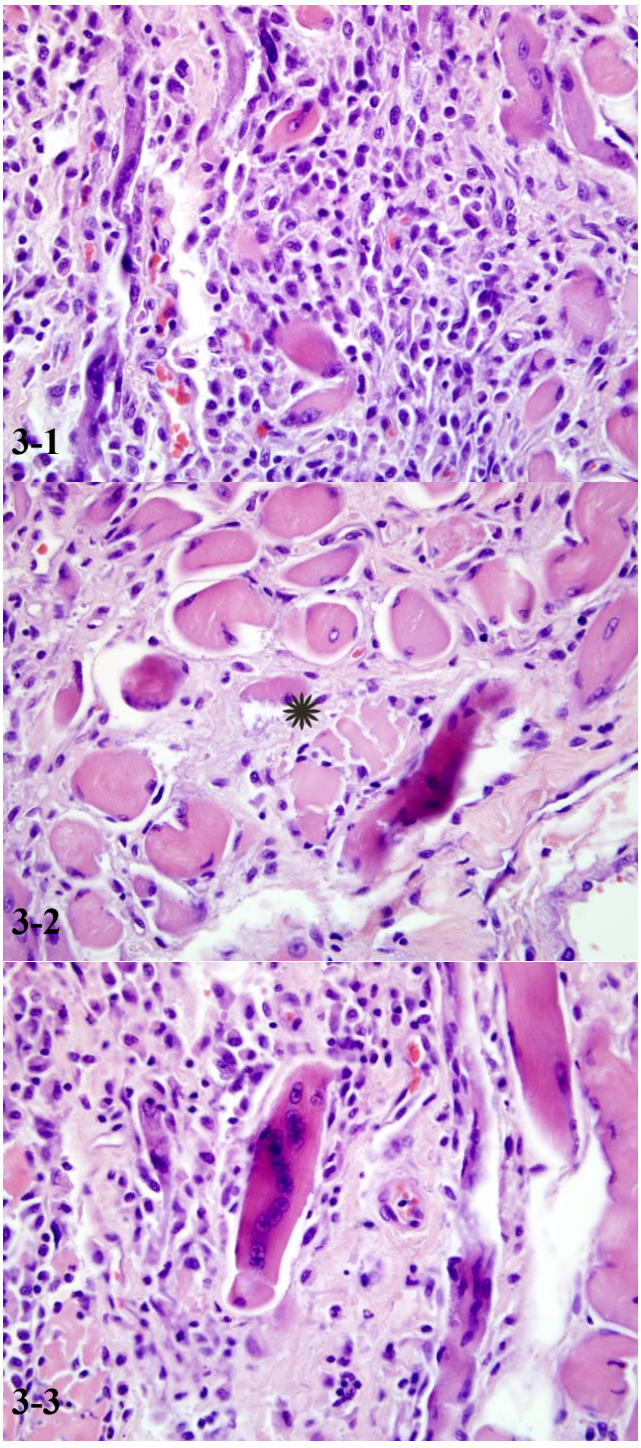
include polymyositis (PM), masticatory muscle myositis (MMM), extraocular myositis, and dermatomyositis.[13,15] Secondary inflammatory myopathies may include those secondary to infectious agents (*Neospora caninum*, *Toxoplasma gondii*, *Hepatozoon americanum*, *Clostridium chauvoei*, *Ehrlichia canis*), paraneoplastic diseases (thymoma), drug-induced myopathies (D-penicillamine, Cimetidine, Trimethoprim-sulfadiazine), or connective tissue diseases (systemic lupus erythematosus).[4,13]

Generally, the muscles affected in MMM are specific to the muscles of mastication and spare the extraocular, esophageal, and limb muscles. In addition to autoantibodies against type 2M fibers, autoantibodies against myositigen, a masticatory muscle variant of the myosin binding protein C family, has been identified in cases of MMM.[18] The cellular infiltrate in cases of MMM has some distinct differences between those seen in other types of inflammatory myopathies. In MMM, B-cells, dendritic cells, and macrophages are seen in greater numbers than T-cells, and the CD4+ T cells are seen in greater numbers than the CD8+ T cells.[14,16]

In polymyositis (PM), B cells are not a prominent feature, while CD8+ T cells are present in great numbers than CD4+ T cell. Both MHC class I and class II antigens are upregulated in cases of PM as well as MMM.[12,14,16] In the Boxer and Newfoundland breeds, a sarcolemma-specific autoantibody has been identified in some dogs with PM. In general, dogs with PM will not have autoantibodies against type 2M fibers, although there have rare reports of on overlap syndrome in which dogs will have features of both PM and MMM.[4,15]

Extraocular myositis is an inflammatory condition restricted to the extraocular muscles; Golden retrievers may be more susceptible. Bilateral exophthalmos due to swelling of the extraocular muscles may be the only clinical sign, and may resemble the acute form of MMM.[3, 16]

Dermatomyositis is a breed-related (Collies, Shetland sheepdogs), autoimmune disorder of skeletal muscle,



3-1. Esophagus, Newfoundland. Diffusely separating, surrounding and replacing myocytes are high numbers of lymphocytes, plasma cell and histiocytes. Within this area individual myocytes are often atrophied. (HE 400X).
3-2. Esophagus, Newfoundland. There is multifocal myocytes necrosis characterized by loss of cross striations and fragmented sarcoplasm (star). (HE 400X).
3-3. Esophagus, Newfoundland. There is multifocal myocyte regeneration characterized by myofibers with lightly basophilic sarcoplasm, multiple, centrally located, linearly arranged euchromatic nuclei with prominent nucleoli. (HE 400X).

skin, and the vasculature.[5,10,11,16,17] A perifascicular pattern of muscle fiber atrophy is considered a characteristic component of this disease.[8,16] Cutaneous lesions are characterized by mild, perifollicular, mixed inflammation with follicular atrophy, follicular basal cell degeneration to the level of the isthmus, ulceration, crusting, smudging of the dermal collagen, and occasional vesiculations.[7,13]

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References:

1. Barber JS, Trees AJ: Clinical aspects of 27 cases of neosporosis in dogs. Vet Rec 139:439-443, 1996
2. Buchbinder R, Hill CL: Malignancy in patients with inflammatory myopathy. Curr Rheumatol Rep 4:415-426, 2002
3. Carpenter J, Schmidt G, Moore F, Albert DM, Abrams KL, Elner VM: Canine bilateral extraocular polymyositis. Vet Pathol 26:510-512, 1989
4. Evans J, Levesque D, Shelton DG: Canine inflammatory myopathies: a clinicopathologic review of 200 cases. J Vet Intern Med 18:679-691, 2004
5. Ferguson EA, Cerundolo R, Lloyd DH, Rest J, Cappello R: Dermatomyositis in five Shetland sheepdogs in the United Kengdom. Vet Rec 146:214-217, 2000
6. Griffiths IR, Duncan ID, McQueen A: Neuromuscular disease in dogs: some aspects of its investigation and diagnosis. J Small Anim Practice 14:533-554, 1973
7. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Interface diseases of the dermal-epidermal junction. In: Skin Diseases of the Dog and Cat, 2nd ed., p49-52. Blackwell Science Ltd., Ames, IA, 2005
8. Hargis AM, Haupt KH, Hegreberg GA, Prieur DJ, Moore MP: Familial canine dermatomyositis: initial characterization of the cutaneous and muscular lesions. Am J Pathol 116:234-244, 1984
9. Hargis AM, Haupt K, Prieur DJ, Moore MP: A skin disorder in three Shetland sheepdogs: comparison with familial canine dermatomyositis of collies. Compend Cont Ed 7:306-315, 1985
10. Hargis AM, Prieur DJ, Haupt KH, Collier JJ, Evermann JF, Ladiges WG: Postmortem findings in four litters of dogs with familial canine dermatomyositis. Am J Pathol 123:480-496, 1986
11. Haupt KH, Prieur DJ, Moore MP, Hargis AM, Hegreberg GA, Gavin PR, Johnson RS: Familial canine dermatomyositis: clinical, electrodiagnostic, and genetic studies. Am J Vet Res 46:1861-1869, 1985
12. Morita T, Shimada A, Yashiro S, Takeuchi T, Hikasa Y, Okamoto Y, Mabuchi Y: Myofiber expression of class I major histocompatibility complex accompanied by CD8+ T-cell-associated myofiber injury in a case of canine polymyositis. Vet Pathol 39:512-515, 2002
13. Podell M: Inflammatory myopathies. Vet Clin North Am Small Anim Pract 32:147-167, 2002
14. Pumarola M, Moore PF, Shelton GD: Canine inflammatory myopathy: analysis of cellular infiltrates. Muscle Nerve 29:782-789, 2004
15. Shelton GD, Cardinet GH III, Bandmam E, Cuddon P: Fiber type-specific antibodies in a dog with eosinophilic myositis. Muscle Nerve 8:783-790, 1985
16. Shelton GD: From dog to man: the broad spectrum of inflammatory myopathies. Neuromuscul Disord 17:663-670, 2007
17. White SD, Shelton GD, Sisson A, McPherron M, Rosychuk RA, Olson PJ: Dermatomyositis in an adult Pembroke Welsh corgi. J Am Anim Hosp Assoc 28:398-401, 1992
18. Wu X, Li Z, Brooks R, Komives EA, Torpey JW, Engvall E, Gonias SL, Shelton GD: Autoantibodies in canine masticatory muscle myositis recognize a novel myosin binding protein-C family member. J Immunol 179:4939-4944, 2007

CASE IV - 05-9637 (AFIP 2986812).

Signalment: A mixed breed (miniature schnauzer) dog, 12 years, spayed female

History: The dog was presented in 2003 with a growth in the ventral neck region (approximately 3cm diameter). The mass was excised at that time and in 2005 the mass had re-grown. Again the tumor was excised and a diagnosis similar to the 2003 evaluation was made.

Gross P athology: A round mass, 2-2.5cm in diameter was attached to the left thyroid gland.

Laboratory Results: CBC and chemistries were all unremarkable except for slight elevation of ALP and ALT, both in the 300's. The animal had slightly elevated ionized calcium at 1.5. Total calcium was normal. Urinalysis was unremarkable. Thoracic and abdominal radiographs and abdominal ultrasound were unremarkable. No evidence of metastatic disease was found. Sections of the neoplasm were immunostained to confirm the biologic activity of the tumor.

Histopathologic D escription: A soft tissue mass from

the ventral neck consists of a very cellular mass, a neuroendocrine neoplasm **(Fig. 4-1)**. The cells are in packets separated by a thin vascularized stroma. Peripheral to the mass are numerous satellite nodules with tumor invasion of the lymphatics. The lesion was immunohistochemically positive for thryocalcitonin and negative for thyroglobulin and parathormone.

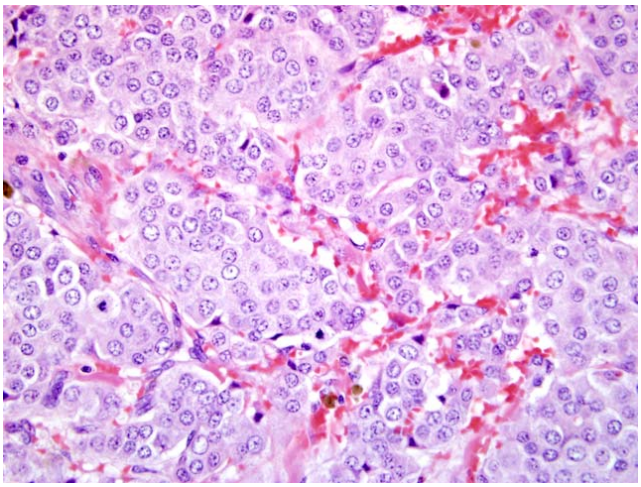
Contributor's Morphologic Diagnosis: Mass: Thyroid carcinoma parafollicular

Contributor's Comment: Incidence: The C-cell thyroid parafollicular cells are seen most frequently in adult cattle[2,11], aged horses[9] and infrequently in other domestic species.[12,22] As bulls age there is an increased incidence of neoplastic C-cells, especially where bulls are fed high calcium diets.[13] Multiple endocrine tumors have been associated with pheochromocytoma in bulls with C-cell tumors.[19] In the dog, a pheochromocytoma has been also associated with parathyroid chief cell hyperplasia.[15] In a histochemical study of 33 thyroid carcinomas in the dog, 36% were of C-cell original and 64% were from thyroid follicle cells.[4] The affect on the elevated calcium is uncertain. In adult bulls, various skeletal lesions have been associated with C-cell neoplasia.[20] In man, prominent bone lesions have not been reported as the result of excessive calcitonin.[14]

AFIP Diagnosis: Fibrovascular tissue, ventral neck (per contributor): C-cell (parafollicular) carcinoma, mixed breed dog (*Canis familiaris*), canine.

Conference Comment: In domestic animals, most thyroid neoplasms are thyroid follicular cell tumors or C-cell (also called parafollicular or medullary) tumors. These tumors can have similar (endocrine/neuroendocrine) histologic features, ie. packets and trabeculae of epithelioid cells supported by a fine fibrovascular stroma. While C-cell carcinomas may have pallisading of columnar cells along the periphery of the lobules, dense bands of connective tissue, and/or amyloid deposits, and thyroid follicular cell tumors usually have some follicular differentiation, immunohistochemistry may be needed to differentiate between thyroid follicular cell tumors and C-cell tumors. One study[4] suggested that C-cell tumors have been underdiagnosed when the diagnosis was based on histologic evaluation of H&E stained sections alone.

C-cell neoplasms exhibit positive cytoplasmic immunoreactivity for calcitonin and are negative for thyroglobulin. The sensitivity for thyroglobulin for thyroid carcinomas is 90.5% alone, but if it is combined with TTF-1, that sensitivity increases to 95.2%.[16] TTF-1, thryoid transcription factor, is expressed in the thyroid, brain, and lung during early embryogenesis, and the thyroid cells and bronchioloalveolar epithelial cells following birth. In the lung, it activates surfactant proteins and Clara cell secretory protein gene promoters.[1] In the thyroid gland, it activates many factors including thyroglobulin, thyroperoxidase, thyrotropin receptor, and thyroid peroxidase.[5,6,8,10,18] When positive, TTF-1 is diffusely located within the nucleus and never in the cytoplasm. In one study, approximately 50% of the C-cell neoplasms also stained positive for TTF-1, therefore it is not suitable to use as a single marker.[16]



4-1. Thyroid gland, mixed breed dog. Neoplastic cells are polygonal with indistinct cell boarder moderate amounts of finely granular cytoplasm with round nucleoli, finely stippled chromatin, variably distinct nucleoli and arranged in nests and packets supported by a fine fibrovascular stroma. (HE 400X).

C-cell tumors in bulls, often occur concurrently with bilateral pheochromocytomas and pituitary adenomas.[4] Multiple endocrine tumors are thought to arise due to a simultaneous neoplastic mutation of multiple endocrine cell populations of neural crest origin in the same individual.[3,23] In humans, multiple endocrinen neoplasia type 2 (MEN-2) occurs in an autosomal dominant pattern, and is classified into three clinical manifestations.[17] MEN-2A is characterized by the presence of a medullary thyroid carcinoma in addition to a pheochromocytoma and multiple tumors of the parathyroid gland.[7] MEN-2B consists of a medullary thyroid carcinoma, a pheochromocytoma, ganglioneuromatosis, and marfanoid habitus.[21] FMTC syndrome, is the third form of MEN-2 and is defined as the development of a medullary thyroid carcinoma and a low incidence of other clinical manifestations of either MEN-2A or MEN-2B.[7]

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http://www.state.tn.us/agriculture/regulate/labs/kordlab.html

References:

1. Bohinski RJ, Di Lauro R, Whitsett JA: The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. Mol Cell Biol 14:5671-5681, 1994
2. Capen CC and Block H E; Calcitonin secreting ultimobranchial neoplasms of the thyroid gland in bulls: an animal model for medullary thyroid carcinoma in man. Am J Pathol. 1974; 74:371-380
3. Capen CC: Tumors of the endocrine glands. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 657-664. Blackwell Publishing, Ames, IA, 2002
4. Carver JR, Kapatkin A, Patnaik AK: A comparison of medullary thyroid carcinoma and thyroid adenocarcinoma in dogs: a retrospective study of 38 cases. Vet Surg 24:315-319, 1995
5. Civitareale D, Castelli MP, Falasca P, Saiardi A: Thyroid transcription factor 1 activates the promoter of the thyrotropin receptor gene. Mol Endocrinol 7:1589-1595, 1993
6. Civitareale D, Lonigro R, Sinclair AJ, Di Lauro R: A thyroid-specific nuclear protein essential for tissue-specific expression of the thyroglobulin promoter. EMBO J 8:2537-2542, 1989
7. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA: Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 2:851-856, 1993
8. Endo T, Kaneshige M, Nakazato M, Ohmori M, Harii N, Onaya T: Thyroid transcription factor-1 activates the promoter activity of rat thyroid Na^+/I^- symporter. Mol Endocrinol 11:1747-1755, 1997
9. Hillage CJ, Sanecki RK, Theodorakis MC: Thyroid carcinoma in a horse. J Amer Vet Med Assoc 181:711-714, 1982
10. Kikkawa F, González FJ, Kimura S: Characterization of a thyroid-specific enhancer located 5.5 kilobase pairs up-stream of the human thyroid peroxidase gene. Mol Cell Biol 10:6216-6224, 1990
11. Krook L., Lutwak L, McEntee K: Dietary calcium and osteopetrosis in the bull: syndrome of calcitonin excess? Am J Clin Nutr 22:115-118, 1969
12. Leav I, Schiller AL, Rijiiberk A, Kegg MB, der Kinderen PJ: Adenomas and carcinomas of the canine and feline thyroid. Am J Pathol 83:61-64, 1976
13. McEntee K, Hall CE, Dunn HO: The relationship of calcium intake to the development of vertebral osteophytosis and ultimobranchial tumors in bulls. In: Proceedings of the Eight Technical Conference on Artifical Insemination Reproduction, pp. 45-47, 1980
14. Melvin KW, Miller HH, Tashjean AH Jr: Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. N Engl J Med 285:1115-1120, 1971
15. Peterson ME, Randolph JF, Zaki FA, Heath H: Multiple endocrine neoplasia in a dog. J Amer Vet Med Assoc 180:1476-1478, 1982
16. Ramos-Vara JA, Miller MA, Johnson GC, Pace LW: Immunohistochemical detection of thyroid transcription factor-1, thyroglobulin, and calcitonin in canine normal, hyperplastic, and neoplastic thyroid gland. Vet Pathol 39:480-487, 2002
17. Raue F, Frank-Raue K: Multiple endocrine neoplasia type 2: 2007 update. Horm Res 68:101-104, 2007
18. Saji M, Shong M, Napolitano G, Palmer LA, Taniguchi S-I, Ohmori M, Ohta M, Suzuki K, Kirshner SL, Giuliani C, Singer DS, Kohn LD: Regulation of major histocompatibility complex class I gene expression in thyroid cells. J Biol Chem 272:20096-20107, 1997
19. Spoonenberg DP, VcEntee K: Phoechromocytoma and ultimobranchial (C Cell) neoplasms in the bull: evidence of autosomal dominant inheritance in the Guernsey breed. Vet Pathol 20:369-400, 1983
20. Thomas RG: Vertebral body osteophytes in bulls. Pathol Vet 6:1-46, 1969
21. Vasen HF, van der Feltz M, Raue F, Nieuwenhuyzen Kruseman A, Koppeschaar HPF, Pieters G, Seif FJ, Blum WF, Lips CJM: The natural course of multiple endocrine neoplasia type 2b. Arch Intern Med 152:1250-1252, 1992
22. Wadsworth PF, Lewis DJ, Jones DM: Medullary carcinoma of the thyroid in a Mouflon (Ov*is musimon*). J Comp Pathol 91:313-316, 1981
23. Weichert RF III: The neural ectodermal origin of the peptide-secreting endocrine gland: a unifying concept for the etiology of multiple endocrine adenomatosis and the inappropriate secretion of peptide hormones by nonendocrine tumors. Amer J Med 49:232-241, 1970

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 22

16 April 2008

Moderator:

Dr. Thomas Van Winkle, DVM, Diplomate ACVP

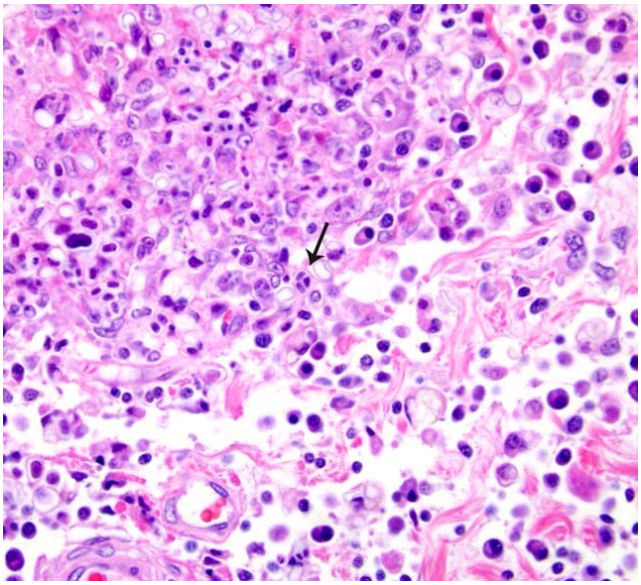
CASE I – 3148216 (AFIP 3063505).

Signalment: This was a 2 years of age, male, Collie dog (*Canis familiaris*) that weighed 55 pounds.

History: This dog was reported to have inappetence and neurologic signs for 3 weeks duration. The neurologic signs included inability to stand, nystagmus, torticollis, and fine tremors when moving the head.

Gross P athology (Per submitting veterinarian): The lungs had a diffusely gritty consistency. The other reported gross findings were considered to likely be insignificant: the kidneys were slightly softer than normal, the brainstem was slightly softer than normal, and the valve leaflets of the heart were slightly roughened.

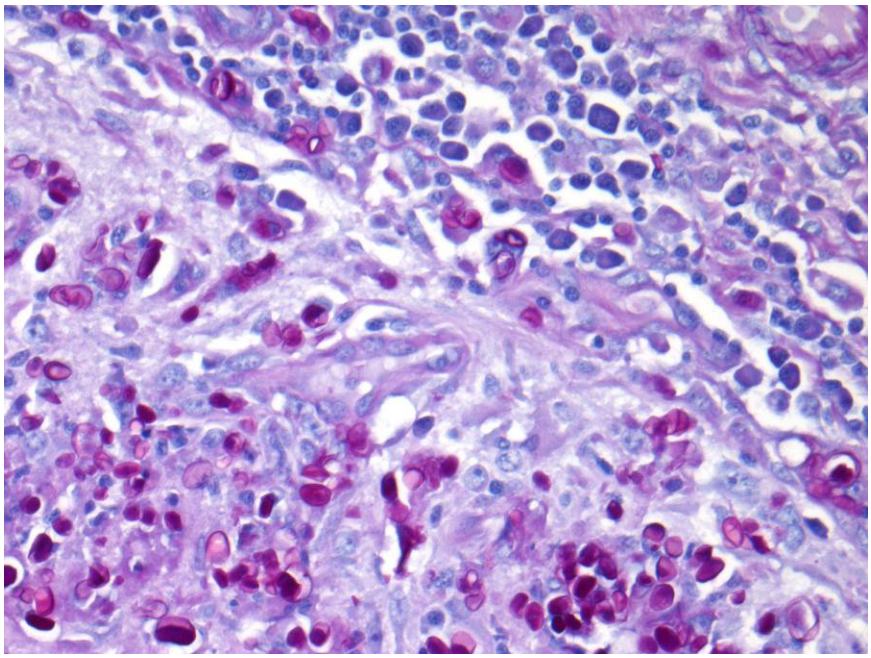
Histopathologic D escription: In sections of brain and brainstem, the meninges were thickened by multiple locally extensive infiltrates of mononuclear cells—which were predominately plasma cells, occasional foci of dense fibrin, scattered cellular debris, and numerous round to oval and angular, colorless and refractile organisms measuring 5-15 micrometers. The organisms had a thin, distinct, poorly staining cell wall and contained a nucleus. There were morulae with 2-6 internal round daughter cells measuring 3-8 micrometers and containing 1 nucleus **(Fig. 1-1)**. The organisms were free, as well as phagocytized by macrophages. These organisms were



1-1. Cerebellum, meninges, Collie. Admixed within the inflammatory cell infiltrate and necrotic debris there are numerous algal forms in different stages of development. Occasional organism is in the intermediate form of development characterized by nuclear and cytoplasmic cleavage (arrow). (HE 400X). →

PAS positive **(Fig. 1- 2)** and consistent with Prototheca sp. Variably sized multifocal aggregates of these organisms and identical nonsuppurative inflammatory reactions and necrosis were occasionally within the neuropil and extending into the neuropil from the meninges.

Sections of pancreas (not submitted) contained multifocal to coalescing areas of necrosis with predominate histiocytes and occasional plasma cells, lymphocytes and neutrophils. Round to oval and angular, 5-15 microns, colorless, refractile organisms were phagocytized in macrophages and free within these necrogranulomatous aggregates.



1-2. Cerebellum, meninges, Collie. Numerous PAS-positive algal organisms. (Periodic acid-Schiff stain). Photomicrograph courtesy of Diagnostic Center for Population and Animal Health, Michigan State University.

Contributor's Mor phologic D iagnosis: 1. Brain: Severe, locally extensive and multifocal, granulomatous meningoencephalitis with intralesional organisms consistent with *Prototheca* sp.
(Following tissues not included in submitted histologic sections)
2. Pancreas: Moderate to severe, multifocal to coalescing, necrogranulomatous pancreatitis with intralesional organisms consistent with *Prototheca* sp.
3. Lung: Moderate, multifocal, pyogranulomatous interstitial pneumonia

Contributor's Com ment: *Prototheca* is a saprophytic achlorophyllic algae related to the green algae *Chlorella*. *Prototheca* thrives in moist environments, and is commonly contracted from lakes and environments with organic debris. The two species associated with disease are *Prototheca wickerhamii* and *Prototheca zopfii*. In animals, protothecosis most commonly develops as a systemic infection with or without cutaneous involvement. The organism often has a predilection for the brain and the eyes with resulting neurologic signs or blindness being a common clinical presentation.[2] Infection usually develops in immunocompromised patients, and Collie dogs are over-represented in reported cases of natural infections. It is believed that with disseminated protothecosis, the organism typically infects the gastrointestinal tract with rapid spread to other organs.[3]

Prototheca sp. are round, oval, or angular cells that are 8-20 micrometers in diameter, have a refractile wall and contain granular cytoplasm. The organism reproduces by endosporulation and can be seen histologically as morula of 2-20 daughter cells within a single organism. The morula ruptures and releases the individual daughter cells. The cell wall of *Prototheca* stains poorly with hematoxylin-and-eosin, but stains strongly positive to stains

Table extracted from Stenner et al.[4]

	Shape	**Sporangia**	**Sporangiospores**
Prototheca zopfii	oval or cylindrical	14-25 µm	up to 20
Prototheca wickerhamii	round	7-13 µm	up to 50

for carbohydrate, such as PAS, Gridley, Bauer, and GMS.

Prototheca sp. resembles *Chlorella* sp., but can be distinguished by PAS-positive starch granules in the cytoplasm of *Chlorella*. In fresh smears of lesions that contain *Chlorella*, the organisms are green due to the presence of chlorophyll.[2]

AFIP Diagn osis: Brain, cerebrum and cerebellum: Meningoencephalitis, granulomatous, multifocal, moderate, with algae, etiology consistent with *Prototheca* sp., Collie dog (*Canis familiaris*), canine.

Conference Comment : *Prototheca* sp. are saprophytic organisms that are ubiquitous in the environment, and have been identified on 4 continents.[3] They grow in moist environments including sewage, animal feces, slime flux of trees, soil, standing and flowing water, and food.[3,4]

Infection is thought to occur through traumatic inoculation, ingestion, or wound contamination. The immune status of an individual appears to play a role in acquiring the infection as well as lesion extent and distribution, although infections have been found in both immunocompetent and immunosuppressed patients.[4]

Clinical manifestation of protothecosis in mammals is to a certain extent species specific. In cattle, mastitis due to *P. zopfii* is the most common presentation of protothecosis in cattle[1,4] with occasional spread into adjacent lymph nodes, while protothecosis in the dog, also primarily due to *P. zopfii*[4], is principally a systemic disease with multiorgan involvement and a predilection for the eyes and brain.[2,3] Protothecosis in cats, due to *P. wickerhamii*, localizes in the skin, and may be successfully treated by wide surgical excision.[3,4]

In dogs the most consistent presenting clinical sign of systemic protothecosis is hemorrhagic colitis.[4] In fact, the colon and the rectum appear to be primary sites of replication even without clinical evidence of colitis.[4] A proposed pathogenesis includes initial colonization of the colonic mucosa following ingestion of large numbers of infective organisms, followed by penetration of the gut wall and systemic spread via blood vessels and lymphatics.[4]

Contributor: Diagnostic Center for Population and Animal Health, Michigan State University

References:

1. da Costa EO, Ribeiro MG, Ribeiro AR, Rocha NS, de Nardi Junior G: Diagnosis of clinical bovine mastitis by fine needle aspiration followed by staining and scanning electron microscopy in a *Prototheca zopfii* outbreak. Mycopathologia 158:81-85, 2004

2. Jones T, Hunt R, King N: Diseases caused by fungi. In: Veterinary Pathology, 6th ed., pp. 534-535. Williams & Wilkins, Baltimore, MD, 1997

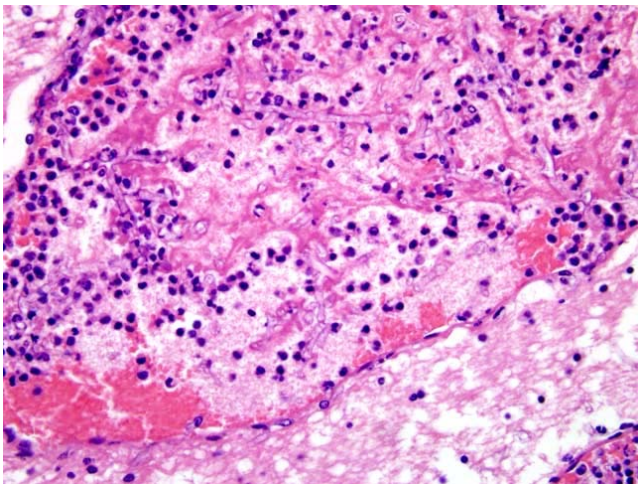
3. Schultze AE, Ring RD, Morgan RV, Patton CS: Clinical, cytologic and histopathologic manifestations of protothecosis in two dogs. Vet Ophthalmol 1:239-243, 1998

4. Stenner VJ, Mackay B, King T, Barrs VR, Irwin P, Abraham L, Swift N, Langer N, Bernays M, Hampson E, Martin P, Krockenberger MB, Bosward K, Latter M, Malik R: Protothecosis in 17 Australian dogs and a review of the canine literature. Med Mycol 45:249-266, 2007

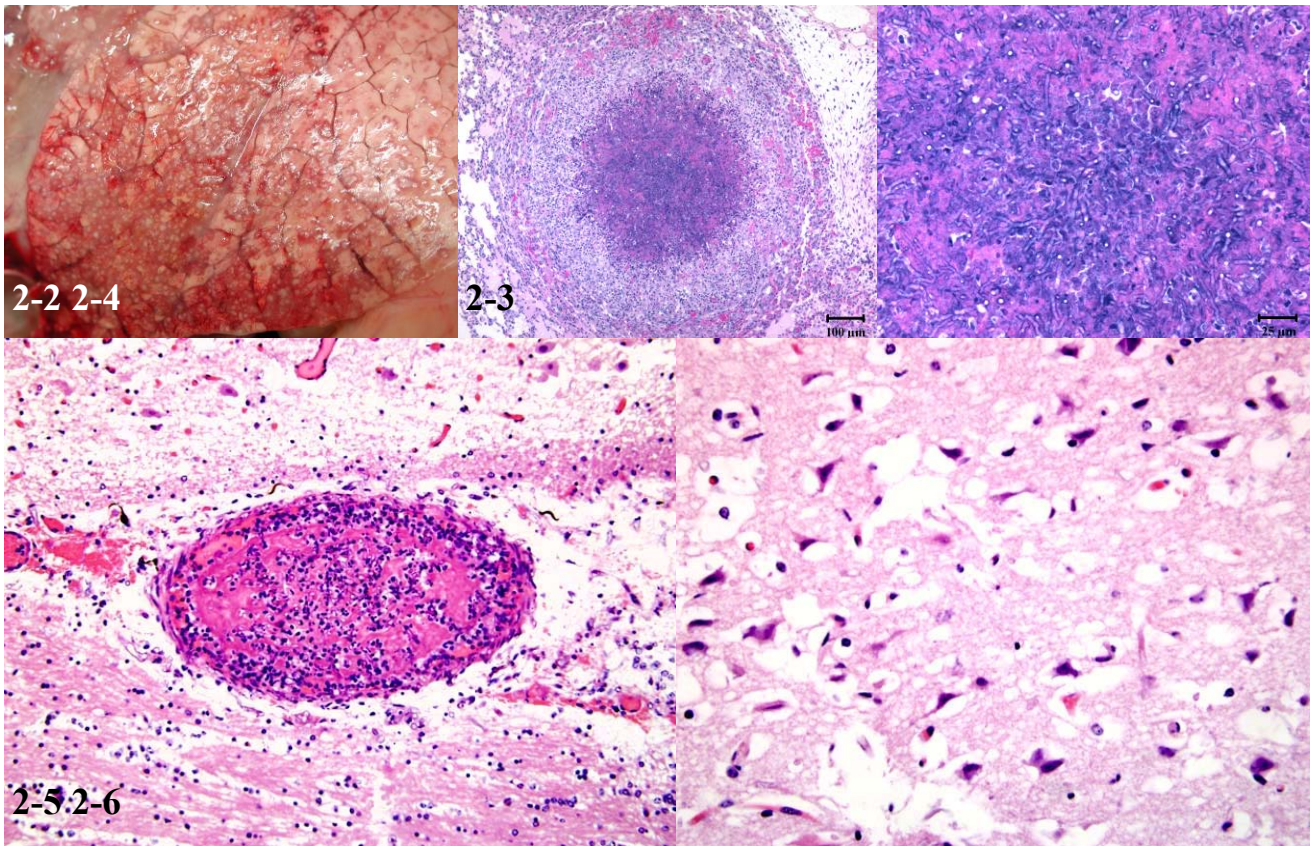
CASE II – NADC MVP-1 2007 (AFIP 3065886).

Signalment: White-tailed deer (*Odocoileus virginianus*), female, 10-days-old

History: This fawn had been removed from the dam 24-48 hrs after birth and moved inside a barn for bottle-feeding. The fawn was kept in a pen, bedded with oat straw. The fawn appeared normal until 9 days of age when there was an acute onset of respiratory signs including elevated respiratory rate, clear nasal discharge,



2-1. Cerebrum, deer. Thrombi contain numerous parallel-walled, dichotomous branching hyphae. (HE 400X)



2-2. Lung, deer. Multifocal to coalescing pyogranulomatous pneumonia.
2-3. Lung, deer. Pyogranuloma containing numerous hyphae consistent with Aspergillus sp.
2-4. Lung, deer. Higher magnification of Fig. 2-3 showing myriad hyphae admixed with necrotic debri.
2-5. Cerebrum, deer. Multifocally within many vessels there are thrombi occluding the vessels and there is a vasculitis characterized by disruption and fragmentation of the vessels wall with replacement by numerous inflammatory cells and eosinophilic fibrillar material. (HE 200X).
2-6. Cerebrum, deer. Within the neural parenchyma there are areas of infarction with numerous shrunken, eosinophilic neurons with pyknotic nuclei (neuronal necrosis). (HE 400X).

Photomicrographs (Fig. 2-2, 2-3, 2-4) courtesy of National Animal Disease Center, ARS, USDA, 2300 Dayton Avenue, Ames, IA 50010, www.nadc.ars.usda.gov

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anorexia, and fever (103°F). The fawn was treated with antibiotics and antipyretics. There was no response to treatment and within 24 hrs of onset the animal died.

Gross Pathology: The lung had multifocal areas of consolidation and failed to deflate. There were multiple fibrinous adhesions to the internal thorax and numerous pin-point (2-4 mm) white to translucent nodules visible on the pleural surface. Nodules were surrounded by a narrow hyperemic border. The renal cortex contained several variable sized wedge-shaped areas of pallor (infarcts). Within the brain there was a gray to white, soft area (3 cm in size) in the cortex of the right frontal lobe that was especially pronounced after formalin fixation.

Laboratory Results: Lung submitted for bacteriological culture: No bacteria isolated, heavy pure growth of *Aspergillus flavus*.

Histopathologic D escription: Within the section of brain there is accentuation of the vasculature and meninges due to edema, accumulation of fibrin and infiltrates of moderate to large numbers of inflammatory cells including neutrophils, macrophages and lymphocytes. Numerous vessels are characterized by infiltrates of neutro-

phils, lymphocytes and macrophages within the vessel wall, fibrinoid degeneration and partially occluding fibrinocellular thrombi. Associated with the inflammatory infiltrate and especially prominent within vessels are numerous intralesional fungal hyphae **(Fig. 2-1)** . Hyphae have parallel sides, are 3-6 microns in width and are characterized by frequent septation and dichotomous, progressive branching. Inflammatory cells and fungal hyphae are also found within the neuropil.

Within the lung **(Fig. 2-2)** (not submitted) are multifocal to coalescing nodular infiltrates **(Fig. 2-3)** of macrophages and lymphocytes surrounding cores of numerous neutrophils, necrotic debris and aggregates of fungal hyphae with morphology similar to that seen in the brain **(Fig. 2-4)**. Similar lesions were present in the kidney (not submitted).

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis, pyogranulomatous, focally extensive, subacute, moderate, with thrombosis and intralesional, angioinvasive fungal hyphae consistent with *Aspergillus* sp.

Contributor's Comment: Organisms of the genus *Aspergillus* are ubiquitous in the environment and opportunistic pathogens. Although *A. fumigatus* is most commonly associated with infection in mammals, infections with *A. flavus*, *A. terreus*, *A. nidulans* and *A. niger* have also been described. Fungal spores may be inhaled from moldy bedding or feed and implant on the mucous membranes of the upper or lower respiratory tract. Although most often a respiratory disease, dissemination of infection can occur, with the meninges and kidneys being most commonly involved.

Disseminated aspergillosis, due to *A. fumigatus*, with involvement of the lungs, brain and kidneys has been previously described in an adult white-tailed deer.[10] Pulmonary aspergillosis has been reported in fallow deer (*Dama dama*) due to *A. fumigatus* and *A. corymbifera*.[4] Disseminated aspergillosis is often associated with debilitation, immunologic suppression or prolonged antibiotic or corticosteroid administration. In the present case, debilitation, or prolonged use of antibiotics or corticosteroids were not factors; however, an unknown immunologic deficiency cannot be ruled out.

The two main portals of entry for fungal spores that cause systemic aspergillosis in cattle are the respiratory and gastrointestinal tracts. Mycotic placentitis in cattle can lead to abortion. Systemic aspergillosis in 4-day-old calves where lesions included well developed hepatic granulomas with intralesional hyphae also suggests that a local or transitory mycotic placentitis could lead to calves that are born alive and survive.[2] In mature cows it is suggested that the gastrointestinal tract is almost exclusively the portal of entry for *A. fumigatus* and that placentitis and pneumonia are secondary to hematogenous dissemination from the gastrointestinal lesions.[8]

The precise virulence factors of *Aspergillus* spp. are not well characterized. However, the common features of necrosis, angioinvasion and hematogenous dissemination may serve as clues to key factors in pathogenesis.

AFIP Diagnosis: Brain, cerebrum: Vasculitis **(Fig. 2-5)** and meningoencephalitis, necrotizing, subacute, multifocal, marked, with hemorrhage, edema, fibrin thrombi, focally extensive cortical coagulative necrosis (infarct) **(Fig. 2-6)**, and numerous hyphae, etiology consistent with *Aspergillus* sp., white-tailed deer (*Odocoileus virginianus*), cervid.

Conference Comment : Histologically, *Aspergillus* sp. infections are characterized by vasculitis, often with numerous hyphae, and resultant thrombosis and infarction. More chronic lesions are granulomas with central cores of necrotic debris. Although hyphae are often present within the lesions and may be seen as negative images with hematoxylin and eosin stains, special stains, such as periodic acid-Schiff (PAS) or Gomori methenamine-silver (GMS), may be required to visualize their characteristic morphology.[5]

Aspergillus sp. can produce several virulence factors including adhesins, antioxidants, enzymes, and toxins. The role of these virulence factors has not been fully defined. Restrictocin and mitogillin are two ribotoxins produced by *Aspergillus* that degrade host mRNA, thereby inhibiting host-cell protein synthesis. In addition, melanin pigment, mannitol, catalases, and superoxide dismutases are all antioxidant defenses produced by *Aspergillus*.[7]

Aspergillosis, primarily caused by *A. fumigatus*, is commonly encountered in birds.[6,9] Captive penguins, turkeys, raptors and waterfowl appear to be particularly susceptible to infection. Certain physical and immunological characteristics of avians may make them more susceptible to infection. Birds lack an epiglottis to prevent particulate matter from being inhaled. Also, they are not able to produce a strong cough reflex due to their lack of a diaphragm. Avian heterophils use cationic proteins, hydrolases and lysosymes to kill fungal hyphae, which may be less effective than mammalian myeloperoxidase and oxidative destruction mechanisms. A unique feature of avian aspergillosis is the presence of reproductive phases of the fungus in tissue. This unique finding

maybe due to the presence of cavernous air sacs, a warm core body temperature, or birds' sensitivity to gliotoxin, which results in tissue necrosis and thus produces a nutrient rich environment for fungus growth.[9]

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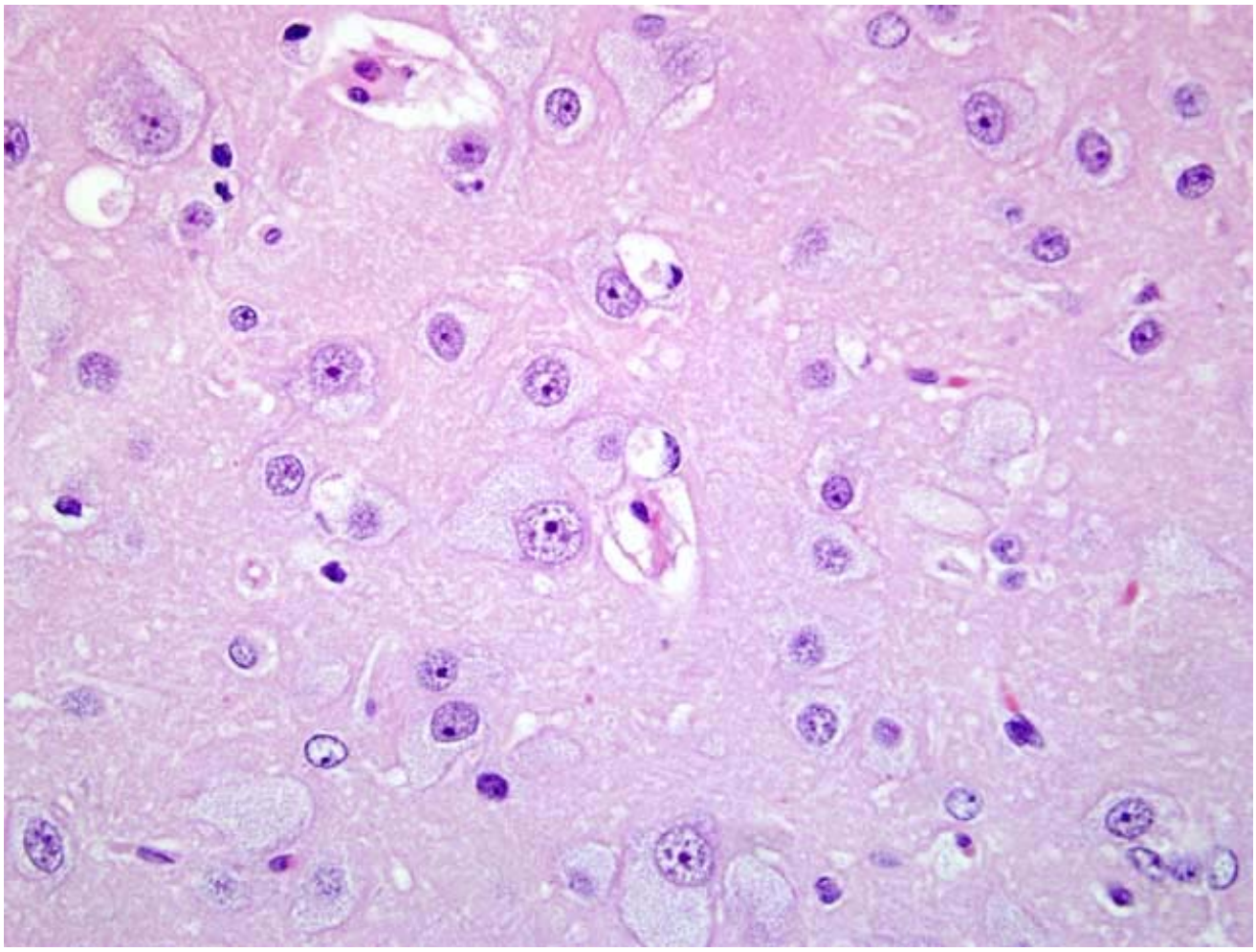
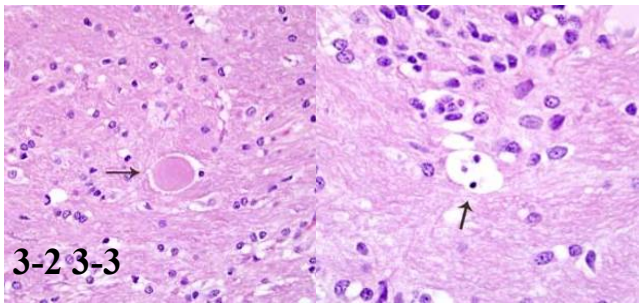
www.nadc.ars.usda.gov

References:

1. Al-Doory Y: Laboratory Medical Mycology, Lea & Febiger, Philadelphia, PN, 1980
2. Cordes DO, Royal WA, Shortridge EH: Systemic mycosis in neonatal calves. NZ Vet J 15:143-149, 1967
3. Emmons CW, Binford CH, Utz JP, Kwon-Chung KJ: Medical Mycology, 3rd ed, pp. 285-304. Lea & Febiger, London, Great Britain, 1977
4. Jensen HE, Jorgensen JB, Schonheyder H: Pulmonary mycosis in farmed deer: allergic zygomycosis and invasive aspergillosis. J Med Vet Mycol 27:329-334, 1989
6. Martin MP, Bouck KP, Helm J, Dykstra MJ, Wages DP, Barnes HJ: Disseminated *Aspergillus flavus* infection in broiler breeder pullets. Avian Dis 51:626-631, 2007
7. McAdam AJ, Sharpe AH: Infectious diseases. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas, AK, Fausto N, 7th ed., pp. 399-400. Elsevier Saunders, Philadelphia, PA, 2005
8. Safarti J, Jensen HE, Latge JP: Route of infections in bovine aspergillosis. J Med Vet Mycol 34:379-383, 1996
9. Tell LA: Aspergillosis in mammals and birds: impact on veterinary medicine. Med Mycol Suppl 1:S71-S73, 2005
10. Wyand DS, Langheinrich K, Helmboldt CF: Aspergillosis and renal oxalosis in a white-tailed deer. J Wild Dis 7:52-56, 1971



3-1. Cerebrum, cat. Diffusely, neurons are moderately swollen with finely granular to microvacuolated cytoplasm. Multifocally, glial cells often contain large, discrete, clear vacuoles. (HE 400X).



3-2. Cerebrum, cat. Rarely within the white matter there are spheroids characterized by swollen, hypereosinophilic axons (arrow). (HE 400X).
3-3. Cerebrum, cat. Within the white matter there are rare digestions chambers characterized by swollen axon sheaths containing gitter cells and cellular debris (arrow). (HE 400X).

CASE III – 05-26548 (AFIP 3027410).

Signalment: An approximately 14-week-old, male, European Burmese kitten (*Felis catus*) presented for euthanasia.

History: Nervous signs including ataxia, dysmetria, head and limb tremors and constant loud purring

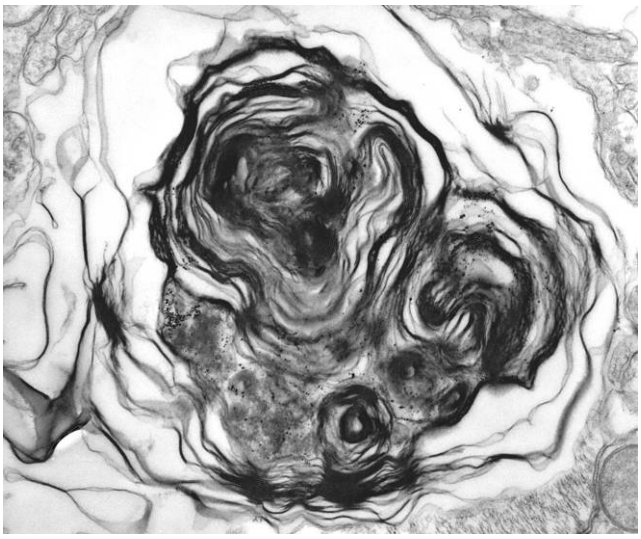
Gross Pathology: Necropsy was unremarkable with no gross lesions observed.

Laboratory Results: Enzyme assays for total hexosaminidase, α-hexosaminidase and control lysosomal enzymes, β-galactosidase and mannosidase and molecular analysis was conducted under Dr. Henry J. Baker at Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University. The tests revealed a deficiency in total hexosaminidase activity, identifying the disease as a variant of GM2 gangliosidosis. Further molecular analysis identified a deletion in the β subunit of the hexosaminidase gene as the cause of disease. Though virology was not conducted on samples from this kitten, no virus could be isolated from a previously submitted kitten from the same litter with similar clinical signs and lesions.

Histopathologic Description: Histologically neurons throughout the brain are diffusely swollen with abundant finely granular foamy cytoplasm which often displaces the nucleus to the periphery **(Fig. 3-1)**. Small numbers of individual neurons are shrunken, with deeply basophilic clumped chromatin material and with variable degree of cytoplasmic hypereosinophilia. Many glial cells contain a single, large, clear discrete vacuole which displaces the nucleus to the periphery. Additional lesions include many hypereosinophilic and swollen axons with dilated myelin sheaths **(Fig. 3-2)**. The fragmented myelin sheath of a few axons form ellipsoids arranged in a row which contain small amounts of eosinophilic granular necrotic debris and macrophages (digestion chambers) **(Fig. 3-3)**. Electron microscopy revealed the presence of characteristic electron-dense concentric lamellar membranous whorls, separated by clear spaces (membranous cytoplasmic bodies) within the neuronal somata **(Fig. 3-4)**.

Contributor's Morphologic Diagnosis: Cerebrum: Neuronal vacuolation, cytoplasmic, diffuse, severe, with neuronal degeneration and necrosis and axonal degeneration, European Burmese, feline

Contributor's Comment: Lysosomal storage disorders are a diverse group of mainly autosomal recessive inherited diseases that can result from the lack of any protein that is necessary for the normal functioning of lysosomal degradation pathways.[5] Lysosomal acid hydrolases are important for the catabolism of a variety of macromolecules. Faulty breakdown of these macromolecules results in their accumulation within the lysosomes, which progressively enlarge and lead to interference with normal cell functions. Although initially described as disorders that are caused by the deficiency of lysosomal enzymes, lysosomal storage disorders can also occur through de-



3-4. TEM, cerebrum, cat. Intraneuronal concentric lamellar membranous whorls. Transmission electron micrograph courtesy of Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, http://www.cvm.uiuc.edu/path/

fective posttranslational modification of lysosomal enzymes, lack of enzyme activator, lack of substrate activator, or the lack of transport proteins required for the removal of digested material from the lysosomes.[5] The organs affected by particular lysosomal storage diseases are determined by the tissue in which the substrate to be degraded is found and where it is degraded.[5]

Biochemically, the inherited lysosomal storage diseases can be broadly divided into sphingolipidoses, cholesterol ester storage disease, glycoproteinoses, glycogenoses, mucopolysaccharidoses, mucolipidoses and ceroid-lipofuscinoses.[3] Sphingolipidoses comprise diseases in which there is a failure to properly catabolize various complex lipids derived from ceramide.[3] These include GM1 and GM2 gangliosidosis, galactocerebrosidosis, glucocerebrosidosis, sphingomyelin lipidosis and galactosialidosis.[3] GM2 ganglioside is a cell membrane glycolipid which is catabolized by the action of N-acetylyhexosaminidase and GM2 activator (GM2A) protein.[6,7] N-acetylyhexosaminidase exists as a dimer in two forms, αβ (hexosaminidase A) and ββ (hexosaminidase B). Improper functioning of either one of these subunits (α or β) or GM2A results in accumulation of GM2 ganglioside in the lysosomes of neurons leading to a progressive deterioration of the central nervous system (GM2 gangliosidosis).[6,7] Tay-Sachs disease of humans is a GM2 gangliosidosis caused by a mutation in the α subunit resulting in the deficiency of hexosaminidase A, whereas Sandhoff disease is caused by mutation of β subunit which affects both hexosaminidase A and B.[3] GM2 gangliosidosis has been previously described in German shorthaired pointer and Japanese Spaniel dogs, domestic short haired and Korat cats, and Muntjak deer.[1,3]

GM1 gangliosidosis (generalized gangliosidosis) is caused by the deficiency of β galactosidase which cleaves the terminal galactose from GM1 ganglioside. GM1 gangliosidosis has been described in dogs, cats, cattle and sheep.[3] Galactocerebrosidosis (globoid cell leukodystrophy, Krabbe disease of humans) is primarily a leukodystrophy and has been described in dogs, cats and sheep. Galactocerebrosides are found in myelin sheaths and the deficiency of galactocerebrosidase causes accumulation of the lipids in the macrophage-like 'globoid cells' and in oligodendrogial cells.[3] Glucocerebrosidosis (Gaucher disease of humans) is caused by a deficiency in the lysosomal enzyme glucocerebrosidase, a β-glucosidase, which results in the accumulation of the lipid glucocerebroside in neurons and in macrophages. The disease has been described in Sydney Silky dogs.[3] Sphingomyelin lipidosis (Niemann-Pick disease of humans) is caused by a deficiency of sphingomyelin phosphodiesterase (sphingomyelinase) which results in the accumulation of sphingomyelin in neurons and macrophages. The disease has been described in cats and a single case in miniature Poodle dog.[3]

AFIP Dia gnosis: Brain, cerebrum, neurons and glia: Cytoplasmic vacuoles, diffuse, moderate, European Burmese (*Felis catus*), feline.

Conference Comment: The contributor gives an excellent overview of the sphingolipidoses. GM2 gangliosidosis results from a defect in only one of three gene products, the α-subunit, the b-subunit or the GM2 activator. Three variant forms of GM2 gangliosidosis have been described and are based on the specific subunits that retain functionality: Variant B (α-subunit deficiency), variant 0 (b-subunit deficiency), and variant AB (GM2 activator deficiency).[7]

Neurons of the central and autonomic nervous system and retina are primarily affected.[5] Typical histopathologic findings in GM2 gangliosidosis include swollen neurons with cytoplasmic vacuoles consisting of lysosomes distended with accumulated gangliosides. Oil red O and Sudan black B may stain storage material in astrocytes and macrophages.[3,5] With electron microscopy (EM), the cytoplasmic inclusions are visualized and consist of membrane layers forming "onion-skin" whorls within the lysosomes.[5]

Lysosomal storage bodies visualized on EM are not considered characteristic of a particular storage disorder. In general, GM1 and GM2 may form similar concentric lamellations, whereas in mucopolysaccharide storage diseases, membranous stacks (zebra bodies) are more common.[3] Abnormal twisted tubular structures are often seen in glucocerebrosidosis and galactocerebrosidosis, while glycogen particles may be seen in the glycogen storage diseases.[3]

Contributing Institu tion: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign
http://www.cvm.uiuc.edu/path/

References:

1. Fox J, Li YT, Dawson G, Alleman A, Johnsrude J, Schumacher J, Homer B: Naturally occurring GM2 gangliosidosis in two Muntjak deer with pathological and biochemical features of human classical Tay-Sachs disease (type B GM2 gangliosidosis). Acta Neuropathol 97: 57-62, 1999
2. Hasegawa D, Yamato O, Kobayashi M, Fujita M, Nakamura S, Takahashi K, Satoh H, Shoda T, Hayashi D,

Yamasaki M, Maede Y, Arai T, Orima H: Clinical and molecular analysis of GM2 gangliosidosis in two apparent littermate kittens of the Japanese domestic cat. J Feline Med Surg 9:232-237, 2007
3. Jolly RD, Walkley SU. Lysosomal storage diseases of animals: An Essay in Comparative Pathology. Vet Pathol 34:527-548, 1997
4. Kroll RA, Pagel MA, Roman-Goldstein S, Barkovich J, D'Agostino AN, Neuwelt EA: White matter changes associated with feline GM2 gangliosidosis (Sandhoff disease): Correlation of MR findings with pathologic and ultrastructural abnormalities. Am J Neuroradiol 16:1219-1226, 1995
5. Kumar V, Abbas, AK, Fausto N: Genetic disorders. In: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 158-168. Elsevier Saunders, Philadelphia, PA, 2005
6. Martin DR, Krum BK, Varadarajan GS, Hathcock TL, Smith BF, Baker HJ. An inversion of 25 base pairs causes feline GM2 gangliosidosis variant. Exp Neurol. 187:30-37, 2004
7. Martin DR, Cox NR, Morrison NE, Kennamer DM, Peck SL, Dodson AN, Gentry AS, Griffin B, Rolsma MD, Baker HJ. Mutation of the GM2 activator protein in a feline model of GM2 gangliosidosis. Acta Neuropathol 110:443-50 2005
8. Yamato O, Satoh H, Matsuki N, Ono K, Yamasaki M, Maede Y: Laboratory diagnosis of canine GM2-gangliosidosis using blood and cerebrospinal fluid. J Vet Diagn Invest 16:39-44, 2004
9. Yamato O, Matsunaga S, Takata K, Uetsuka K, Satoh H, Shoda T, Baba Y, Yasoshima A, Kato K, Takahashi K, Yamasaki M, Nakayama H, Doi K, Maede Y, Ogawa H: GM2-gangliosidosis variant O (Sandhoff-like disease) in a family of Japanese domestic cats. Vet Rec 155:739-744, 2004

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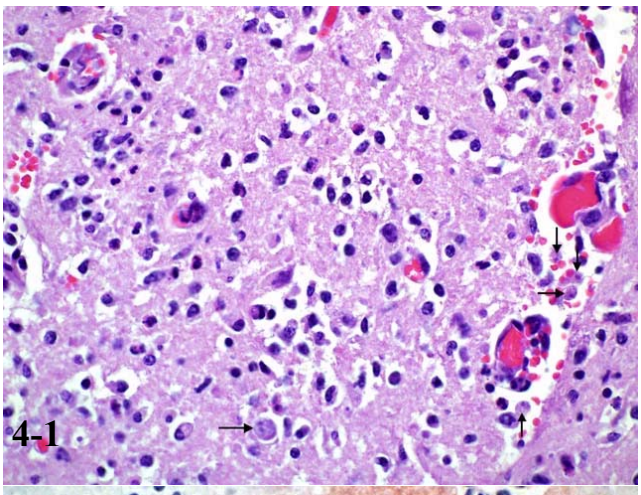
CASE IV - S0610573 (AFIP 3071896).

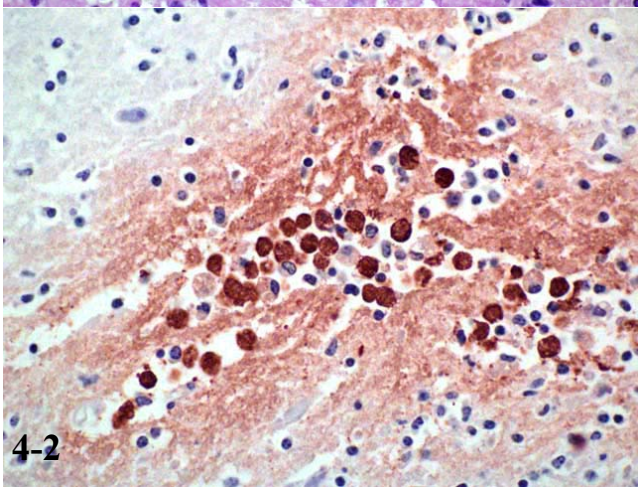
Signalment: Holstein heifer, unknown age, bovine.

History: Between July 30 and September 28, 1998 and 1999, specimens from nine 10-20-month-old Holstein heifers with a history of acute CNS disease were submitted to our laboratory. All submissions were from one large heifer-raising operation located in southern California in an arid but highly productive agricultural area irrigated by Colorado River water supplied through a system of canals and ditches. The ranch relied on untreated canal water for livestock drinking water. These heifers had been diagnosed with fatal meningoencephalitis caused by *Naegleria fowleri*. Sporadic specimens from animals with similar clinical signs have been submitted in the successive years, including the formalin-fixed brain we are now examining.

Gross Pathology: No gross lesions.

Laboratory Results: Immunohistochemistry: Positive





4-1. Cerebrum, bovine. Multifocally, often perivascularly, there are low number of amoebic trophozoites with visible nuclei and karyosomes (arrows).
4-2. Cerebrum, bovine. Positive immunostaining for Naegleria fowleri within focus of necrosis and inflammation..

Photomicrographs courtesy of the California Animal health and Food Safety laboratory, U.C.Davis

for *Naegleria fowleri* **(Fig. 4-2).**

Histopathologic Description: Multifocal necrosuppurative hemorrhagic encephalitis, predominating within neuropil bordering the cerebral aqueduct, includes foci of malacia or necrosis, populated by degenerative neutrophils, hemorrhage, interspersed with neuropil populated by scattered lymphocytes and macrophages, and rare multinucleated giant cells. Numerous blood vessels are mildly (1-2 cell thickness) to markedly (9-10 cells thick) cuffed by lymphohistiocytic cells. Clustered and individual amoebic trophozoites **(Fig. 4 -1)** are observed within and surrounding areas of necrosis/malacia and perivascularly. The amoebae are round to oval, approximately 5-10 µm in diameter, and have a pale eosinophilic, finely granular cytoplasm. The nuclei are small (approximately 2-3 µm in diameter), poorly delineated, mostly eccentric and weakly basophilic with a prominent basophilic karyosome.

Contributor's Morphologic Diagnosis: Encephalitis, hemorrhagic, multifocal, necrosuppurative, severe, with presence of multifocal amoebic trophozoites.

Contributor's Comment: Primary amoebic meningoencephalitis is a fulminant infection of the human central nervous system caused by *Naegleria fowleri*, a free-living amoeba that thrives in artificially or naturally heated water. The infection usually is acquired while bathing or swimming in such waters. There are 4 genera of amoebae that cause CNS disease in mammals, namely Acanthamoeba (several species), *Naegleria fowleri*, *Balamuthia mandrillaris*, and the recently described *Sappinia diploidea*.[9] Acanthamoeba and Naegleria are ubiquitous in soil and fresh water, including lakes, streams, and hot springs. These amoebae have also been isolated from various artificial water sources, such as swimming pools, tap water, heating and ventilation units, air conditioners, cooling water, sewage, contaminated cell cultures, and contact lens-storing fluid.[1,9] Their cysts have even been demonstrated in dust during dust storms.[1]

Primary amoebic meningoencephalitis (PAM) is the term for the human disease caused by *N. fowleri*. It is an acute, usually fatal, necrotizing, and hemorrhagic meningoencephalitis.[9] Although CNS infections due to Acanthamoeba and Balamuthia have been recorded in animals, such as dogs, sheep, cattle, primates, and horses, there has been only 1 report of naturally acquired PAM in animals, namely in a South American tapir at a zoo in Arizona.[1-3,7,8,10] Primary amoebic meningoencephalitis has been experimentally induced in mice, sheep, and monkeys.[1,10] *Naegleria fowleri* is thermophilic and tolerates temperatures of up to 45° C; hence, the frequent association of PAM with a history of contact with naturally warm or artificially heated waters.[1] The portal of entry is the olfactory mucosa. The parasite migrates to the brain via olfactory nerves. Incubation period is short, with onset of clinical signs several days following exposure. The disease rapidly progresses and usually culminates in death within 5-7 days.[5]

The life cycle of *N. fowleri* includes a trophozoite stage (amoebic form), a temporary flagellate stage, and, in unfavorable environments, a cyst stage. The cyst stage is susceptible to desiccation.[1] Only the trophozoite stage of *N. fowleri* has been detected in CNS lesions. This is in contrast with other free-living opportunistic amoebae, where identification of cyst stages aids in their differentiation from *Naegleria.*[8]

Gross lesions vary and may or may not be present. Findings may include multifocal meningeal hemorrhages and tan-gray thickening of meninges, tan-gray deposits obscuring cerebellar cortical surface, and light brown and malacic brain parenchyma. Lesions may be bilateral and symmetrical.

Histopathology shows mainly a multifocal, necrosuppurative, and hemorrhagic meningoencephalitis. Most severely affected areas were the anterior cerebra, olfactory bulbs, and cerebella. Amoebae are not easily detected microscopically because they resemble degenerate macrophages and may be few in number. The tendency of the amoebae to accumulate in aggregates or in perivascular spaces within areas of necrosis is most helpful, as is the small, weakly basophilic karyosome within a poorly delineated, small nucleus. There are very few or no multinucleated giant cells or eosinophils to direct the pathologist toward parasitic etiology. Some amoebae form cysts, visualized by periodic acid-Schiff stain, but *N. fowleri* does not, possibly because death occurs during the acute stage of the disease. Routine special stains to identify bacteria, such as Giemsa, Brown-Brenn-modified Gram, and Steiner silver stain were not helpful in identifying amoeba. Immunohistochemistry of the brain is an important tool in the confirmation of the diagnosis and also the isolation of the organism from animal tissues and suspected sources can be attempted. Finally, it is advantageous to know that the disease is seasonal, occurs in areas with high ambient summer temperatures, and may be associated with the use of consumption of untreated surface water.[1]

AFIP Diagnosis: Brainstem: Encephalitis, necrotizing, subacute, multifocal, moderate, with hemorrhage and amoebic trophozoites, Holstein (*Bos taurus*), bovine.

Conference Comment: The contributor gives an excellent overview of *Naegleria fowleri* infection. The full pathogenesis and virulence factors of the organism are not completely understood. These infections are believed to be transmitted when water containing the organisms comes in contact with the host nasal mucosa. The amoebic trophozoites then migrate along the olfactory nerves and pass through the cribiform plate to enter the brain.[4,9] *N. fowleri* contains a surface protein, similar to the human integrin-like receptor, that facilitates adhesion to fibronectin, an extracellular matrix glycoprotein found at the basal lamina and surrounding cells.[4] Two pore-forming proteins termed naegleriapores A and B, as well as proteases and phospholipases have all been implicated in host cell destruction.[4,9]

Most animals infected with *N. fowleri* die before mounting a detectable adaptive immune response. The innate immune response, consisting of complement, neutrophils, and macrophages, appears to be a frequently inadequate defense mechanism. In vitro studies have shown that *N. fowleri* is able to evade complement damage by either expressing complement-regulatory proteins (CD59-like protein), shedding the MAC complex (C5b-C9) on surface membrane blebs, internalizing and degrading the MAC complex, or preventing the insertion of the MAC into the amoebic membrane.[4] Although neutrophils play a major role in antiamoebic activity, their exact function is not clearly defined. TNF-α does not have a direct effect on *N. fowleri*, but it appears that organism destruction by neutrophils cannot occur without TNF-α being present.[4] Production of high levels of proinflammatory cytokines such as IL-1α, IL-1b, IL-6, and TNF-α do not appear to provide protection against infection.[4] The roles of humoral and cell mediated immunity are also uncertain.

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References:

1. Daft MD, Visvesvara GS, Read DH, Kinde H, Uzal FA, Manzer MD: Seasonal meningoencephalitis in Holstein cattle caused by *Naegleria fowleri*. J Vet Diag Invest 17:605-609, 2005
2. Fuentealba IC, Wikse SE, Read WK, Edwards JF, Visvesvara GS: Amebic meningoencephalitis in a sheep. J Am Vet Med Assoc 200:363-365, 1992
3. Kinde H, Visvesvara GS, Barr BC, Nordhausen RW, Chiu PHW: Amebic meningoencephalitis caused by *Balamuthia mandrillaris* (leptomyxid ameba) in a horse. J Vet Diag Invest 10:378-381, 1998
4. Marciano-Cabral F, Cabral GA: The immune response to *Naegleria fowleri* amebae and pathogenesis of infection. FEMS Immunol Med Microbiol 51:243-259, 2007
5. Martinez JA, Visvesvara GS: Free-living, amphizoic and opportunistic amebas. Brain Pathol 7:583-598, 1997
6. Morales JA, Chaves AJ, Visvesvara GS, Dubey JP: *Naegleria fowleri*-associated encephalitis in a cow from Costa Rica. Vet Parasitol 139:221-223, 2006
7. Pearce JR, Powell HS, Chandler FW, Visvesvara GS: Amebic meningoencephalitis caused by *Acanthamoeba castellani* in a dog. J Am Vet Med Assoc 187:951-952, 1985
8. Rideout BA, Gardiner CH, Stalis IH, Zuba JR, Hadfield T, Visvesvara GS: Fatal infections with *Balamuthia mandrillaris* (a free-living amoeba) in Gorillas and other old world primates. Vet Path 34:15-22, 1997
9. Visvesvara GS, Moura H, Schuster FL: Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol 50:1-26, 2007
10. Wong MM, Karr SL, Balamuth WB: Experimental infections with pathogenic free-living amebae in laboratory primate hosts: I. (B) A study on susceptibility to *Acanthamoeba culbertsoni*. J Parasitol 61:682-690, 1995

Notes:

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 23

23 April 2008

Moderator:

Dr. Don Nichols, DVM, Diplomate ACVP

CASE I – 07-253 (AFIP 3065807).

Signalment: 1-year-old, male, *Petaurus breviceps,* sugar glider

History: A 1-year-old, male, sugar glider was presented to the Emergency Service at MJR-VHUP for weakness and lethargy. The owner reported the acquisition of a new sugar glider during the previous week. The animal was emaciated and dehydrated. He vomited during the physical exam and died while the doctor was placing an IV catheter.

Gross Pathology: On gross examination, the animal was emaciated with a poor hair coat. The liver was firm, diffusely red tan with disseminated light tan foci ranging from 1 to 4 mm in diameter.

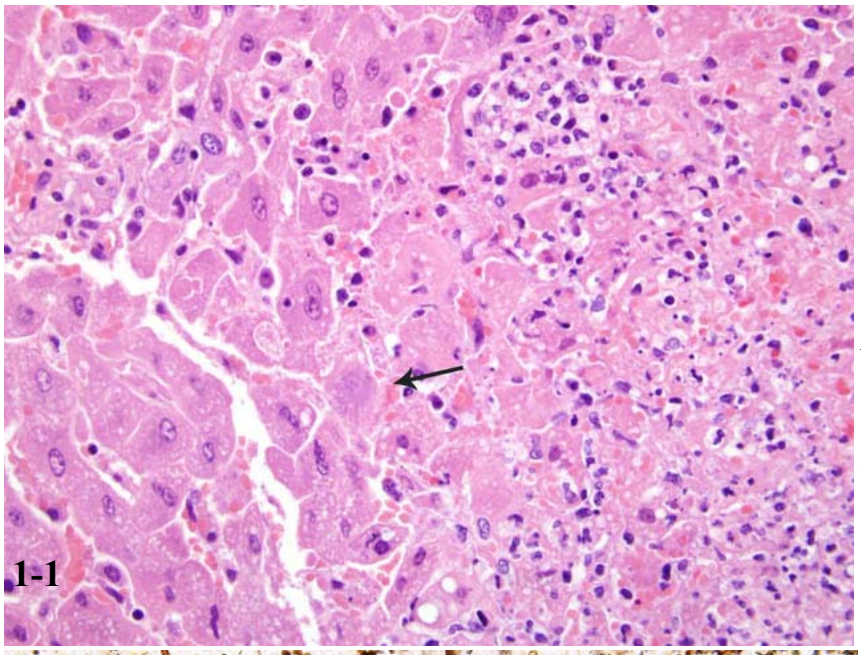
Laboratory Results: A small amount of blood obtained immediately postmortem revealed low blood glucose.

Histopathologic Description: Histology revealed multifocal random hepatocellular coagulative necrosis with associated neutrophils, fibrin and hemorrhage. Hepatocytes occasionally contained 5 to 10 micron long filamentous basophilic bacteria, which were haphazardly arranged within the cytoplasm **(Fig. 1- 1)**. No lesions were observed in the heart, and the gastrointestinal tract was severely autolyzed.

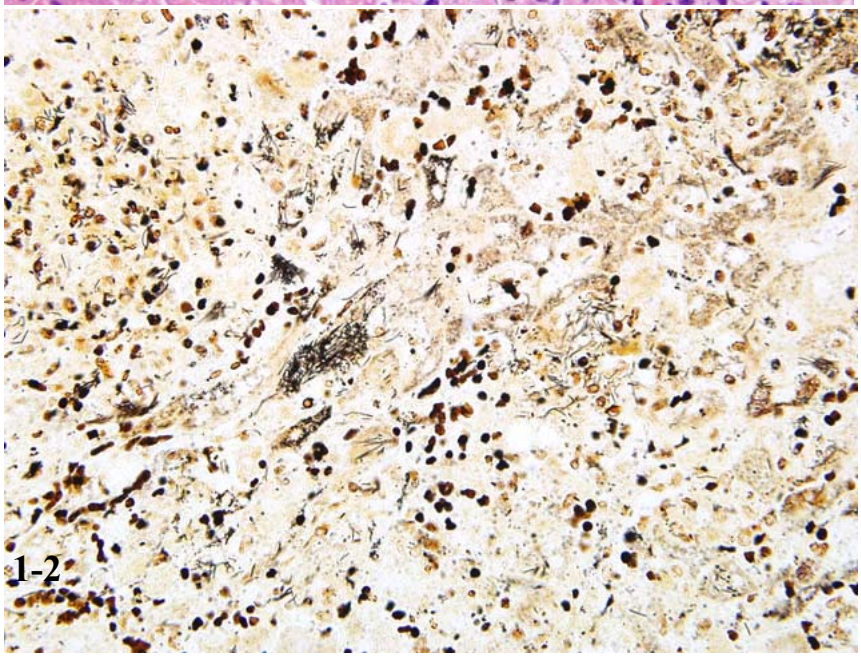
Contributor's Morphologic Diagnosis: Severe multifocal random acute necrotizing hepatitis with hemorrhage and hepatocellular cytoplasmic filamentous bacteria consistent with *Clostridium piliforme*.

Contributor's Comment: Tyzzer's disease is caused by *Clostridium piliforme*, a gram negative, filamentous, obligate intracellular bacterium. Clinical infection results in 1) hepatic necrosis, 2) myocardial necrosis, and 3) enterocolitis (specifically the distal ileum, cecum and proximal colon)[5] resulting in the "triad within a triad" lesion distribution. Although infection has been reported in many species (dogs[5], cats[5], horses[4], bovids[4], white tailed deer[1], cotton-top tamarins[10], Australian possums[3], snow leopards[12], muskrats[19], grey foxes[15], a red panda[6], a rainbow lorikeet[9], a serval[8], a cockatiel[11], a koala[3], a wombat[3], a dasyurid[3], a raccoon[20], and two coyotes[7]), it has not, to our knowledge, been reported in a sugar glider. Clinical disease often occurs in young (neonatal) animals or in immunocompromised individuals. The first reported occurrence of *C. piliforme* infection in a human was a subcutaneous infection in an immunocompromised HIV patient.[13]

Tyzzer's disease is common in laboratory animals including mice, rats, guinea pigs, and rabbits. *C. piliforme* is



1-1. Liver, Sugar Glider. Immediately adjacent to areas of necrosis, there are few to moderate numbers of hepatocytes that contain numerous, intracytoplasmic, 1-2 um diameter, long, filamentous bacilli. (HE 400X).



1-2. Liver, Sugar Glider. Numerous argyrophilic, 1 x 7 um, filamentous bacilli that occur singly or arranged in parallel or perpendicular sheaves, haystacks or bundles. (Warthin-Starry 400X).

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considered a commensal organism in the rodent intestinal tract which may spread to the liver via the portal circulation.[5] In laboratory mice systemic infection is often subclinical with fulminate disease occurring sporadically. Susceptibility varies among mouse strains. Likewise, virulence varies among *C. piliforme* isolates. Factors involved in the immunity and virulence have been investigated in mice.[16-18] Infection of both susceptible and resistant strains of mice with either toxigenic (virulent) or non-toxigenic *C. piliforme* isolates resulted in strain-independent and isolate-independent elevations in serum IFN γ and IL-6 from day 1 to 14 and in serum TNF α from day 1 to 28.[16,17] All mice had serologic evidence of inflammation; however, only mice infected with the toxigenic bacterial isolate had histologic lesions in the liver (day 7 to 14). A similar experiment revealed that IL-12 levels were significantly higher in resistant mice than susceptible mice.[18] The importance of IL-6 and IL-12 in mediating the immune response to *C. piliforme* was also demonstrated in these experiments; histopathologic lesions in the liver were more severe if polyclonal antibodies against these cytokines were injected immediately prior to infection with the bacteria.[17,18]

The possibility of latent infections has been debated. Presence of bacterial DNA in hepatocytes from animals with elevated serum cytokines and normal liver histology suggests that the infection may persist in a latent state for long periods of time (at least 28 days).[16]

Special stains to identify the organisms are occasionally required. Warthin-Starry, Giemsa, Gomori Methamine Silver (GMS), and gram stains are commonly used for these purposes.[5] In our laboratory, Warthin-Starry demonstrated the organisms **(Fig. 1- 2)** as well as occasional short rods. This second infectious organism may represent a secondary bacterial infection, possibly one that ascended from the gastrointestinal tract.

AFIP Di agnosis: Liver: Hepatitis, necrotizing, acute, random, severe, with fibrin, hemorrhage, and hepatocel-

lular intracytoplasmic bacilli, etiology consistent with *Clostridium piliforme*, sugar glider (*Petaurus breviceps*), marsupial.

Conference Comment: *Clostridium piliforme*, first described in 1917 in Japanese waltzing mice[10] was originally classified as *Bacillus piliformis*, but based on 16S rRNA analysis, has since been reclassified as a *Clostridium* sp.[18] Unlike other Clostridial species, *C. piliforme* is an obligate intracellular pathogen and will consistently stain gram negative.[18]

Transmission of Tyzzer's disease is presumed to occur through ingestion of contaminated feces, with colonization of the intestine followed by hematogenous spread to the liver via the portal circulation.[4] The mechanism of attachment and entry into the host cell is not currently known.[4,5] *C. piliforme* is considered difficult to grow on artificial media and requires eggs or cell culture to proliferate.[5,14] Diagnosis of infection is dependent on identification of the organism within the cytoplasm of degenerate and apparently healthy cells at the periphery of areas of necrosis. The bacteria have often been described as being arranged in characteristic sheaves, haystacks, or bundles. Immunohistochemistry, immunofluorescence, and PCR have all been used to aid in identification.[14]

Susceptibility to infection and disease progression has been linked to genetics, immune status, age, and bacterial virulence factors. T lymphocyte, natural killer cell, neutrophil cell function and cytokine responsiveness have all been linked to disease progression.[10,18] The pathogenesis of *C. piliforme* infection does not appear to be clearly dependent on toxin production. Some strains do produce cytotoxic proteins and these isolates are generally more virulent than the non-toxic isolates.[2,16]

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References:

1. Brooks JW, Whary MT, Hattel AL, Shaw DP, Ge Z, Foz JG, Poppenga RH: *Clostridium piliforme* infection in two farm-raised white-tailed deer fawns (*Odocoileus virginianus*) and association with copper toxicosis. Vet Pathol 43:765-768, 2006
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 214. Elsevier Limited, St. Louis, MO, 2007
3. Canfield PJ, Hartley WJ: Tyzzer's disease (*Bacillus piliformis*) in Australian marsupials. J Comp Pathol 105:167-173, 1991
4. Cullen JM: Liver, biliary system and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds McGavin MD, Zachary JF, 4th ed., pp 433-434. Mosby Elsevier, St. Louis, Missouri, 2007
5. Jones BR, Greene CE: Tyzzer's disease. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed., pp. 362-363. Saunders Elseiver, St. Louis, Missouri, 2006
6. Langan J, Bemis D, Harbo S, Pollock C, Schumacher J: Tyzzer's disease in a red panda (*Ailurus fulgens fulgens*). J Zoo Wildl Med 31:558-562, 2000
7. Marler RJ, Cook JE: Tyzzer's disease in two coyotes. J Am Vet Med Assoc. 169:940-941, 1976
8. Poonacha KB: Naturally occurring Tyzzer's disease in a serval (*Felis capensis*). J Vet Diagn Invest 9:82-84, 1997
9. Raymond JT, Topham K, Shirota K, Ikeda T, Garner MM: Tyzzer's disease in a neonatal rainbow lorikeet (*Trichoglossus haematodus*). Vet Pathol 38:326-327, 2001
10. Sasseville VG, Simon MA, Chalifous LV, Lin KC, Mansfield KG: Naturally occurring Tyzzer's disease in cotton-top tamarins (*Sanguinus oedipus*). Comp Med 57:125-127, 2007
11. Saunders GK, Sponenberg DP, Marx KL: Tyzzer's disease in a neonatal cockatiel. Avian Dis 37:891-894, 1993
12. Schmidt RE, Eisenbrandt DL, Hubbard GB: Tyzzer's disease in snow leopards. J Comp Pathol 94:165-167, 1984
13. Smith KJ, Skelton HG, Hilyard EJ, Hadfield T, Moeller RS, Tuur S, Decker C, Wagner KF, Angritt P: *Bacillus piliformis* infection (Tyzzer's disease) in a patient infected with HIV-1: confirmation with 16S ribosomal RNA sequence analysis. J Am Acad Dermatol 34:343-348, 1996
14. Stalker MJ, Hayes MA: Liver and biliary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 356. Elsevier Limited, St. Louis, MO, 2007
15. Stanley SM, Flatt RE, Daniels GN: Naturally occurring Tyzzer's disease in the gray fox. J Am Vet Med Assoc 173:1173-1174, 1978
16. Van Andel RA, Franklin CL, Besch-Williford, CL, Hook RR, Riley LK: Prolonged perturbations of tumor necrosis factor-α and interferon-γ in mice inoculated with *Clostridium piliforme*. J Med Microbiol 49:557-563, 2000
17. Van Andel RA, Franklin CL, Besch-Williford, CL, Hook RR, Riley LK: Role of interleukin-6 in determining the course of murine Tyzzer's disease. J Med Microbiol 49:171-176, 2000

18. Van Andel RA, Hook RR, Franklin CL, Besch-Williford, CL, Riley LK: Interleukin-12 has a role in mediating resistance of murine strains to Tyzzer's disease. Infection and Immunity 66:4943-4946, 1998
19. Wobeser G, Barnes HJ, Pierce K: Tyzzer's disease in muskrats: re-examination of specimens of hemorrhagic disease collected by Paul Errington. J Wildl Dis 15:525-527, 1979
20. Wojcinski ZW, Barker IK: Tyzzer's disease as a complication of canine distemper in a raccoon. J Wildl Dis 22:55-59, 1986

CASE II – 03-399 (AFIP 2941570).

Signalment: 12-year-old, female, Pigtail macaque, *Macaca nemestrina*

History: The animal was part of a small research colony housed in outdoor/indoor gang cages. Following an outbreak of diarrhea, she had tested positive for fecal culture of *Yersinia pseudotuberculosis* and treated successfully with Enrofloxacin 8 years previously. During another diarrhea episode 2 years ago, she tested positive for *Balantidium coli* and was treated successfully with metronidazole. She recently developed diarrhea in which *Balantidium coli* was again identified. She was treated with metronidazole, but continued to have diarrhea that began to contain some fresh blood. Gentamycin and fluid therapy were quickly added to treatments, but she developed retching of food and continued to have diarrhea and dehydration until it was decided she should be euthanized approximately 10 day after onset of the initial signs.

Gross P athology: At necropsy there was loose stool adhered to the perineal region and signs of dehydration based upon skin turgor and dryness of subcutaneous tissues. The stomach contained liquid contents, the small and large intestines contained pale greenish mucoid stool. There were petechial hemorrhages in the mucosa of the small intestine and paintbrush type hemorrhages on the mucosa and also serosa of the large intestines.

Laboratory Results: *Shigella flexneri* type IV was isolated from large intestines.

Contributor's Mor phologic Diagn osis: Acute hemorrhagic purulent colitis - Shigellosis

Contributor's Comment : Shigellosis represents a "classic entity" of nonhuman primates, an infection long associated with exposure to humans.[2] For this reason, it is still a disease threat to captive primates and a good example of the need for employee health monitoring of the animal caretakers. This outbreak was associated with renovation of the gang cages by outside contractors. Pub MED currently lists over 12,500 citations for *Shigella* and 255 citations for *Shigella* and primates.

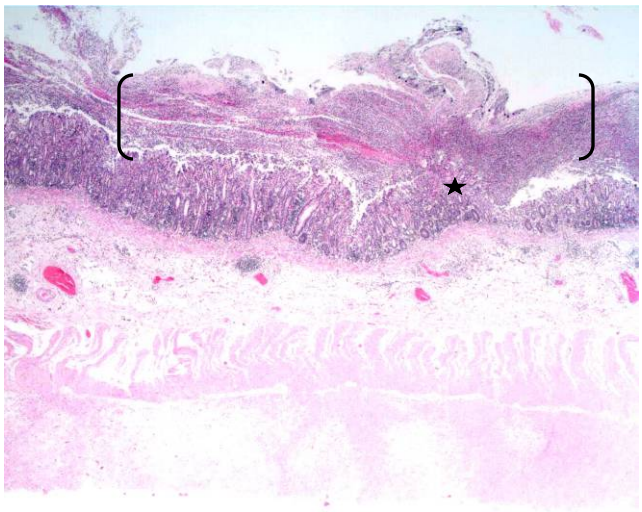
Shigella flexneri, *Yersinia enterocolitica*, S*trongyloides fulleborni*, adenovirus, *Campylobacter coli* and *C. jejuni* were recently reported to be associated with chronic diarrhea in Rhesus macaques.[7] Enteric agents not associated with chronic diarrhea within the same population included *Balantidium coli*, *Giardia lamblia*, *Trichuris trichiura*, *Enterocytozoon bieneusi* and enteropathogenic *E. coli* carrying the eaeA intimin or Stx2c Shiga toxin virulent genes. Chronic diarrhea was associated with increased CD69+ cells and CD4+ T lymphocytes as well as upregulation of IL-1-a, IL3, and TNF-a cytokine genes. Chronic diarrhea is the leading cause of morbidity requiring veterinary care in captive primates and is not currently a criterion of specific pathogen-free colonies. The enteric infections and associated modulation of the immune system is a complicating variable in the study of AIDS and SIV in the primate models.

AFIP Di agnosis: Colon: Colitis, fibrinonecrotic, subacute, diffuse, severe with edema and pseudomembrane **(Fig. 2- 1)**, Pigtail macaque (*Macaca nemestrina*), primate.

Conference Comment: Shigellosis is a common disease in non-human primates, caused by a gram negative, nonmotile, nonspore forming, bacilli.[2] The most common isolate is *Shigella flexneri*, although *S. sonnei*, *S. dysenteriae* and *S. boydii* have also been less frequently identified.[2,3,5] Clinical signs of shigellosis may vary depending on the species of Old World monkey infected. Rhesus macaques generally present with either an acute foul-smelling, watery diarrhea with frank blood, or a more chronic intermittent semi-soft diarrhea with occasional episodes of frank blood. Tamarins and marmosets, on the other hand, will primarily demonstrate lethargy, depression, dehydration, and dried blood around the anus, rather than diarrhea.[3]

Infection and disease does not provide immunity. Animals with shigellosis may be chronically reinfected, and colonies endemically infected my not demonstrate overt clinical disease unless challenged by a stressful event or compromised immune system.[3]

Upon ingestion of the bacteria, it is proposed that inva-



2-1. Colon, Pig-tailed macaque. Overlying and focally contiguous (star) with the mucosa, there is a thick fibrinonecrotic and suppurative exudate, or pseudomembrane (brackets). The submucosa and tunica muscularis are expanded by edema. (HE 20X).

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sion of the M cells overlaying lymphoid follicles within the colon, allows the bacteria to reach the basolateral pole of the epithelial cells, which is where they induce their entry.[5]

Shigellosis in humans is caused by both *Shigella* spp. and enteroinvasive *Escherichia coli*.[5] These bacteria contain a virulence plasmid that encodes most proteins directly involved in host cell entry, bacterial dissemination, and induction of apoptosis in infected macrophages.[4] This region encodes a type III secretion apparatus (Mxi-Spa TTS apparatus), translocators (IpaB and Ipa C), effectors (IpaD, IpgB1, IpgD and IcsB), their dedicated chaperones (IpgA, IpgC, IpgE and Spa15), and two transcriptional activators (VirB and MxiE). The current theory suggests that upon contact of the bacterium with the host cell, translocators are inserted into the host cell membrane which forms a pore, effectors are then transmitted through this pore and enter into the host cell cytoplasm.

Genes encoding a type III secretory apparatus have been identified in a number of mammalian and plant pathogens including enterohemorrhagic and enteropathogenic *Escherichia coli*, *Shigella*, *Salmonella*, *Yersinia*, *Chlamydia*, *Bordetella*, *Pseudomonas*, *Xanthomonas*, *Ralstonia*, and *Erwinia* spp.[6]

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References:

1. Adams MM, Allison GE, Verma NK: Type IV O antigen modification genes in the genome of *Shigella flexneri* NCTC 8296. Microbiol 147:851-860, 2001
2. Benirschke K, Garner FM, Jones TC: Pathology of Laboratory Animals, vol. II, pp 1449, Springer-Verlag, New York, NY, 1978
3. Bernacky BJ, Gibson SV, Keeling ME, Abee CR: Nonhuman primates. In: Laboratory Animal Medicine, eds. Fox JG, Anderson LC, Loew FM, Quimby FW, 2nd ed., pp. 730-734. Elsevier Science, San Diego, CA, 2002
4. Gall TL, Mavris M, Martino MC, Bernardini ML, Denamur E, Parsot: Analysis of virulence plasmid gene expression defines three classes of effectors in the type III secretion system of *Shigella flexneri*. Microbiol 151:951-962, 2005
5. Parsot C: *Shigella* spp. and enteroinvasive Escherichia *coli* pathogenicity factors. FEMS Microbiol Let 252:11-18, 2005
6. Schuch R, Maurelli AT: The Mxi-Spa Type III secretory pathway of *Shigella flexneri* requires an outer membrane lipoprotein, MxiM, for invasion translocation. Infect Immun 67:1982-1991, 1999
7. Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, Didier ES, Didier PJ, Plauche G, Bohm RP, Aye PP, Alexa P, Ward RL, Lackner AA: Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun 71:4079-4086, 2003

CASE III – 05-14824 (AFIP 2984543).

Signalment: Female, 11-year-old, Taita falcon (*Falco fasciinucha*).

History: This animal was one of seven captive Taita falcons (*Falco fasciinucha*) that died within 3 weeks during the breeding season of 2005. The falconer had 7 breeding pairs. Four of his females and three of his males died after being lethargic and anorexic for few hours to one day. Three of the four female animals died about one day after laying an egg.

Gross Pathology: The animal was in a good to fair post-

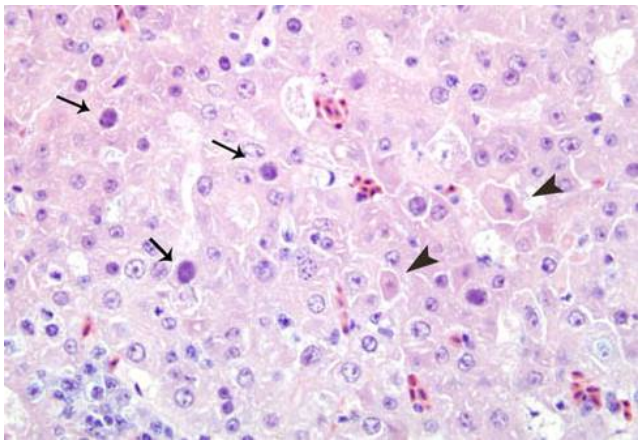
mortem preservation state. It was anemic and had marked hemorrhage into the reproductive tract. The liver was diffusely beige. The spleen was mildly to moderately enlarged and had multiple beige well demarcated foci, up to 2 mm in diameter.

Laboratory Results: The animal was negative for adenovirus-specific antibodies in 2002. Nucleic acid of falconid adenovirus was detected by PCR in the liver. The liver was negative for dependovirus (parvovirus) nucleic acid, herpesvirus nucleic acid, polyomavirus nucleic acid and chlamydial nucleic acid. Aviadenoviral nucleic acid was also detected in the liver by *in situ* hybridization. Adenoviral particles were present in the intestinal content. Few non-hemolytic *E. coli* were isolated from the liver by aerobic culture.

Histopathologic D escription: The nuclei of numerous hepatocytes contained one large basophilic inclusion body **(Fig. 3-1)**. Occasionally, a halo was present around the intranuclear inclusion and the nuclei membrane was hyperchromatic. A low to moderate number of necrotic individual hepatocytes were scattered within the parenchyma. Multiple portal fields were infiltrated by a moderate number of lymphocytes, plasma cells and macrophages.

Contributor's Morpho logic Diag noses: 1. Hepatitis, necrotizing, multifocal, moderate with abundant hepatocellular intranuclear inclusion bodies.
2. Hepatitis, portal, lymphoplasmacytic and histiocytic, mild to moderate.

Contributor's Comment: Primary histological differential diagnosis in this case included herpesvirus hepatitis and adenovirus hepatitis. The clinical presentation and finally the results of electron microscopy, *in situ* hybridization and PCR supported and confirmed a diagnosis of adenovirus hepatitis. Recently, a falconid adenovirus has been identified.[3] This virus may be fairly widespread in indigenous species, such as peregrine falcons in which it does not seem to cause clinical disease.[2] However, if naive tropical falcon species become infected with the virus, it may cause fatal peracute disease.[2,3,5]



3-1. Liver, falcon. Several hepatocytes contain large, deeply basophilic, intranuclear inclusions that often peripheralize the chromatin (arrows). There is scattered individual hepatocyte necrosis characterized by shrunken, deeply eosinophilic cytoplasm with pyknotic nuclei (arrowheads). (HE 400X).

AFIP Dia gnosis: 1. Liver, hepatocytes: Degeneration and necrosis, single cell, random, moderate, with basophilic intranuclear inclusion bodies, Taita falcon (*Falco fasciinucha*), avian.
2. Liver: Hepatitis, portal, lymphoplasmacytic, multifocal, moderate.

Conference Comment: The recently identified falcon adenovirus, most closely related to fowl adenovirus type

Major groups of the Aviadenoviruses, adapted from Schrentzel et al.[3] and Tomaszewski et al.[4]

Name		
Fowl adenovirus 1 (FAV-1)	Chickens	Includes chicken embryo lethal orphan strain (CELO). Tropic for hepatocytes, pancreatic acinar cells, and gizzard epithelium
Hemorrhagic enteritis virus (HEV)	Turkeys	Non-pathogenic in pheasants; Produces severe enteritis in turkeys and guinea fowl
Egg drop syndrome (EDS)	Chickens	Little or no disease in waterfowl; Reproductive abnormalities in chickens
Falcon adenovirus	Falcons	Necrotizing hepatitis and splenitis

1 and 4, has been associated with disease in a variety of falcons, with a particularly severe course of infection within falcons originating from more isolated island/ tropical regions or within the smaller falcon species such as kestrels and merlins.[2,3] The disease appears to be widespread within the Peregrine falcons (*Falco peregrinus*) population which are considered to be a potential reservoir species for the falcon adenovirus.[1,2,4,5]

There are four genera within the family Adenoviridae (mastadenovirus, Aviadenovirus, Atadenovirus, and Siadenovirus).[3] Aviadenovirus is further subdivided into subgenera and groups based on shared neutralizing epitopes.

Characteristic histopathological findings include a multifocal, necrotizing hepatitis and splenitis, with hepatocytes, biliary epithelium, and mononuclear cells resembling lymphocytes containing intranuclear basophilic inclusion bodies that frequently marginate the chromatin.[1,3,4] Electron microscopic evaluation will demonstrate a 70-90nm nonenveloped icosahedral viral particle occasionally arranged in paracrystalline arrays.[3] In one report of infection in gyrfalcon and peregrine falcon hybrids, intranuclear inclusions were identified within renal tubular epithelial cells and not hepatocytes, suggesting that disease presentation may be species dependent.[5] Other lesions reported in different species include, vasculitis, heterophilic nephritis, myocardial necrosis, and hemorrhagic enteritis.[1-3,5]

Conference participants are encouraged to examine a similar case in a Northern aplomado falcon (*Falco femoralis septentrionalis*) that was presented in the 1996-1997 Wednesday Slide Conference number 29, Case 4, and subsequently published by Schrenzel et al.[3]

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References:

1. Dean J, Latimer KS, Oaks JL, Schrenzel M, Redig PT, Wünschmann A: Falcon adenovirus infection in breeding Taita falcons (*Falco fasciinucha*). J Vet Diagn Invest 18:282-286, 2006
2. Oaks JL, Schrenzel M, Rideout B, Sandfort C: Isolation and epidemiology of falcon adenovirus. J Clin Microbiol 43:3414-3420, 2005
3. Schrenzel M, Oaks JL, Rotstein D, Maalouf G, Snook E, Sandfort C, Rideout B: Characterization of a new species of adenovirus in falcons. J Clin Microbiol 43:3402-3413, 2005
4. Tomaszewski EK, Phalen D: Falcon adenovirus in a n American kestrel (*Falco sparverius*). J Avian Med Surg 21:135-139, 2007
5. Van Wettere AJ, Wünschmann A, Latimer KS, Redig PT: Adenovirus infection in Taita falcons (*Falco fasciinucha*) and hybrid falcons (*Falco rusticolus x Falco peregrinus*). J Avian Med Surg 19:280-285, 2005

CASE IV - 061127/061129/061130 (AFIP 3073388).

Signalment: Adult, female and male, thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*)

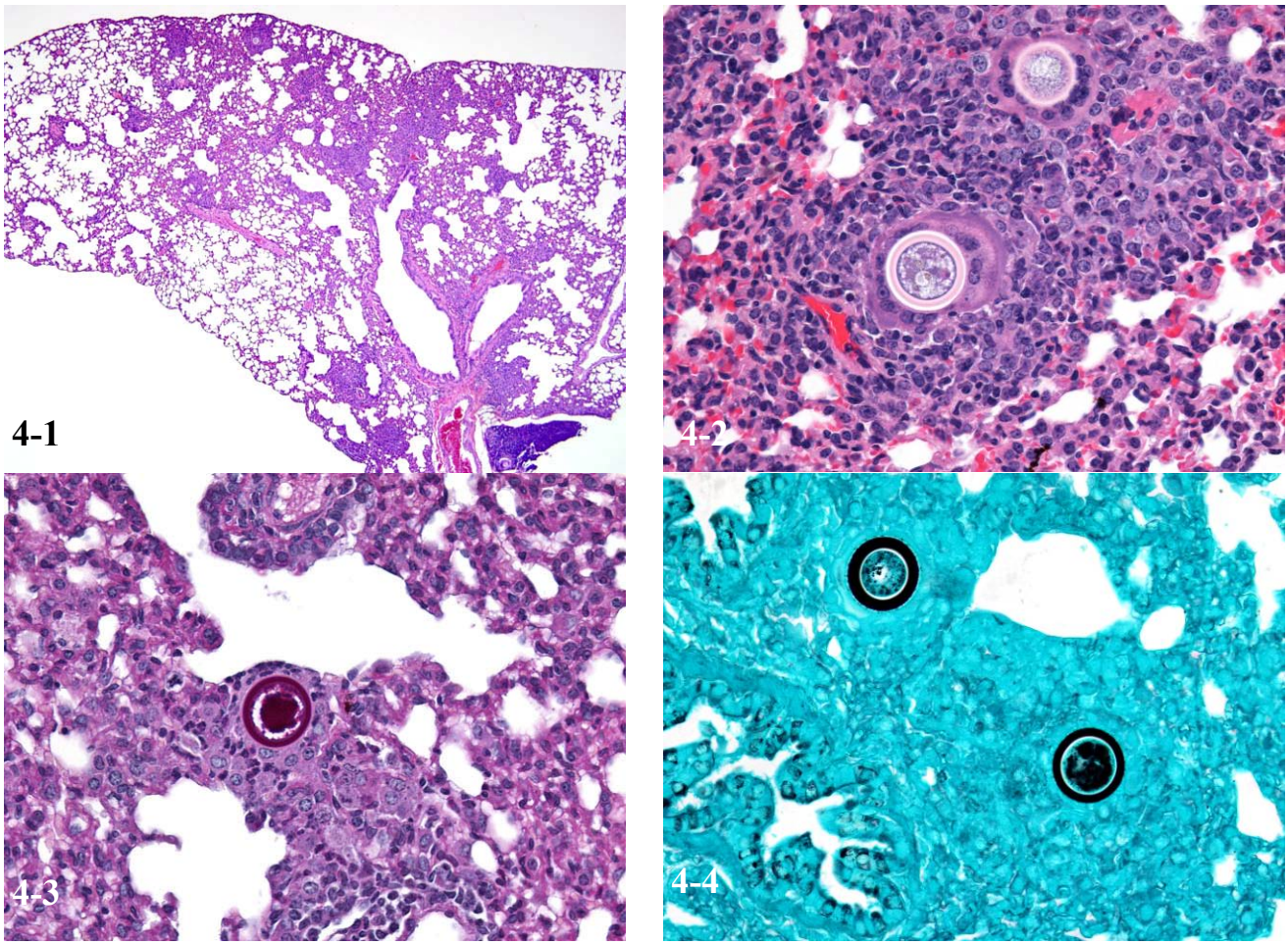
History: Selected tissue samples were collected from approximately fifty wild rodents as part of a plague surveillance project in Colorado during a plague outbreak in prairie dogs in July 2006. The animals were humanely trapped, euthanized, and selected fresh tissue samples were collected for PCR, ECL, microbial culture, histopathology, and immunohistochemistry.

Gross Pa thology: No gross lesions were observed during necropsy.

Laboratory Results: Immunonegative for *Yersinia pestis*

Histopathologic D escription: Scattered throughout all lung lobes are coalescing pyogranulomas that disrupt and efface up to 20% of alveolar septae and bronchioles. The pygranulomas **(Fig 4- 1)** are up to 1mm in diameter and each is centered upon an adiaspore which is surrounded by degenerate and nondegenerate neutrophils, epithelioid macrophages, and foreign body and Langhans multinucleate giant cells in varying proportion. Lymphocytes and plasma cells are rare. The inflammatory cells extend into and expand adjacent alveolar septae. The adiaspores are round, up to 250 µm in diameter with a 20-30 µm thick refractile non-staining cell wall **(Fig. 4- 2).** The center contains amophophilic globular material. The cell wall stains dark purple with the Periodic Acid-Schiff (PAS) stain **(fig. 4-3)** and black with Grocott's Methenamine Silver (GMS) **(Fig. 4-4)** .

Contributor's Morpho logic Diagn osis: Lung: Bronchopneumonia, pyogranulomatous, multifocal, mild, with fungal conidia (adiaspores), etiology consistent with *Chrysosporium parvum* (adiaspiromycosis).



4-1. Lung, ground squirrel. Multifocal to coalescing granulomatous inflammation. (HE 20X).
4-2. Lung, ground squirrel. Multifocally, often within multinucleated giant cells and bounded by high numbers of macrophages and fewer lymphocytes and neutrophils, there are approximately 100-150 micron diameter adiaspores. (HE 400X)
4-3. Lung, ground squirrel. Adiaspores stain purple with periodic acid-Schiff staining method . (PAS 400X)
4-4. Lung, ground squirrel. Grocott's methenamine silver stain method further demonstrates the thick capsule of Chrysosporium parvum. (GMS 400X)

Photomicrographs (Fig. 4-2, 4-3, 4-4) courtesy of U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011 .

Contributor's Comment: Adiaspiromycosis is primarily a pulmonary fungal infection of small animals.[1] Infection occurs when the conidia (spores) are inhaled.[1] The name, adiaspore, refers to a spore that grows in size without replicating in tissues.[3] Therefore, the degree of infection is determined by the number of spores inhaled. The inhaled conidia simply enlarge in the lung tissue and are eventually removed by the immune system; thus, the disease is not contagious. [1,4] The most susceptible animals are those that live in close contact with soil that contains the saprophytic stage of the fungus, such as burrowing rodents.[1]

Two varieties of the fungal genus *Chrysosporium*, formerly known as *Emmonsia*, cause adiaspiromyosis: *C. parvum* var. *parvum* and *C. parvum* var. *crescens*. The latter has the widest host range and distribution.[1] *C. parvum* var. *crescens* is a dimorphic fungus with spores that are 2-4µm in the environment. They enlarge to 200-400µm when inhaled and incubated at body temperature.

At lower temperature (20-30°C) the spores will develop into a mycelial form.[4]

The typical pathologic feature of the infection is the granuloma or pyogranuloma which appear grossly as gray white nodules in the lungs.[1] The lung is the only organ known to be infected.[6] Histologically, these nodules contain a central adiaspore surrounded by varying degrees granulomatous to pyogranulomatous inflammation. Ruptured spores incite the most severe reactions.[1] Adiaspores range from 200-400µm in diameter for *C. parvum* var. *crescens* and 20-40µm *C. parvum* var. *parvum*.[2] *C. parvum* var. *crescens* has a characteristic thick cell wall that is up to 70µm thick.[4] The center of the adiaspore contains a mass of amphophilic to basophilic small globules.[3] There is a single nucleus in spores of *C. parvum* var. *parvum*, however *C. parvum* var. *crescens* develops multiple nuclei as it enlarges.[3] There is no budding or endosporulation.[1]

Differential diagnosis includes other fungi of similar size and morphology, such as *Coccidioides immitis* and *Rhinosporidium seeberi*. Morphologically, *C. parvum* (20-70µm) has a thick capsule while *C. immitis* (1–2µm) and *R. seeberi* (3–5µm) have relatively thin capsules.[4] The presence of endospores occurs with *C.immitis* and *R. seeberi*, but not *C. parvum*.[4] *C. parvum* infection does not produce hyphae, unlike *C. immitis*.[4] Histochemically, the capsules of all three stain with PAS and GMS.[4] The capsule of *R. seeberi* also stains with mucicarmine, unlike the other two.[4]

The infection has been reported in mice, moles, rats, rabbits, ground squirrels, weasels, martens, minks, armadillos, wallabies, skunks, opossums, dogs, cats, raccoons, and humans.[6]

The disease in immunocompetent animals (including humans) is typically benign, self-limiting, and confined to the lungs. However, clinical signs can occur with heavy infections.[1] Most infections are considered incidental findings during the course of histopathologic evaluation of the lungs – as in these ground squirrels.

Of the 20 ground squirrels examined during this study, adiaspiromycosis was detected in 13 of them (60.5%). Interestingly, only 1 of 18 (5.1%) prairie dogs in this study was infected. The reasons that two species of burrowing rodents from the same geographical area had such different prevalences of infection were not determined.

AFIP Diagnosis: 1. Lung: Pneumonia, pyogranulomatous, multifocal, moderate with fungal conidia, thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), rodent.
2. Kidney: Essentially normal tissue.

Conference Comment: Adiaspiromycosis caused by the dimorphic fungi *Emmonsia parva* (=*Chrysosporium parvum* var. *parvum*) or *Emmonsia crescens* (=*C. parvum* var. *crescens*) is a rare mycotic condition in small mammals and humans worldwide. In the literature, the names *Emmonsia* and *Chrysosporium* are considered synonyms with significant controversy occurring concerning their appropriate use. In 1962, Charmichael reclassified *Emmonsia* as a *Chrysosporium* and reduced the two species to a variety of *E. parva*.[9,10] This was later refuted by von Arx who retained *Emmonsia* as a single species with two varieties based on the reasoning that *Emmonsia* produces blastic conidia and adiaspores, and *Chrysosporium* produces thallic conidia and no adiaspore at elevated temperature.[9,10] To confuse taxonomic matters further, it has recently been found that *E. parva* is phylogenetically closer to *Blastomyces dermatitidis* (the anamorph of *Ajellomyces dermatitidis*) than it is to *Emmonsia crescens*.[7] At this time, both genera names continue to be used in the literature.

Inhaled dust-borne aleurioconidia (2-4 um) do not germinate in the host, but instead dramatically enlarge into thick-walled adiaspores. Clinically, infection may range from asymptomatic to severe necrogranulomatous pneumonia depending on adiaspore load and immunocompetence of the host.[5] Infection is not considered transmissible between individuals.[7,8]

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References:

1. Burek K: Bacterial and mycotic diseases. In: Infectious Diseases of Wild Animals. 3rd ed., pp 522-523, London, Manson Publishing, 2001
2. Caswell JL, Williams KJ: Respiratory system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 645. Elsevier Limited, St. Louis, MO, 2007
3. Chandler FW, Kalpan W, Ajello L: Adiaspiromycosis. In: Color Atlas and Text of the Histopathology of Mycotic Diseases, pp. 30–33. Year Book Medical Publishers, Chicago, IL, 1980
4. Chandler FW, Watts JC: Adiaspiromycosis. In: Pathologic Diagnosis of Fungal Infections, pp. 35–41. ASCP Press, Chicago, IL, 1987
5. Chantrey JC, Borman AM, Johnson EM, Kipar A:

Emmonsia crescens infection in a British water vole (*Arvicola terrestris*). Med Mycol 44:375-378, 2006
6. Hamir AN: Pulmonary adiaspiromycosis in raccoons (*Procyon lotor*) from Oregon. J Vet Diagn Invest 11:565–567, 1999
7. Hubálek Z, Burda H, Scharff A, Heth G, Nevo E, Šumbera R, Peško J, Zima J: Emmonsiosis of subterranean rodents (*Bathyergidae*, *Spalacidae*) in Africa and Israel. Med Mycol 43:691-697, 2005
8. Hubálek Z: Emmonsiosis of wild rodents and insectivores in Czechland. J Wild Dis 35:243-249, 1999
9. Kwon-Chung KJ, Bennett JE: Infections due to miscellaneous molds. In: Medical Mycology, pp. 733-739. Lea & Febiger, Philadelphia, PA, 1992
10. Sigler L: *Ajellomyces crescens* sp. nov., taxonomy of *Emmonsia* spp., and relatedness with *Blastomyces dermatitidis* (telomorph *Ajellomyces dermatitidis*). J Med Vet Mycol 34:303-314, 1996
11. Sun Y, Bhuiya T, Wasil T, Macias A, Wasserman PG: Fine needle aspiration of pulmonary adiaspiromycosis. Acta Cytol 51:217-221, 2007

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 24

7 May 2008

Moderator:

Dr. Thomas P. Lipscomb, DVM, Diplomate ACVP

CASE I – C-33738-06 (AFIP 3083594).

Signalment: Adult, spayed, female Chihuahua

History: This dog developed vomiting, diarrhea and anorexia one day following routine vaccinations and was treated symptomatically with fluids and antibiotics. However, the patient continued to deteriorate with evidence of a progressively worsening liver disorder and was euthanized eight days following the vaccinations.

Gross P athology: The submitting veterinarian did a necropsy on the patient but did not report abnormal findings. Fixed specimens of spleen, kidney and liver were received from the referring veterinarian for histopathology.

Histopathologic D escription: The section of liver is characterized by marked centrilobular and midzonal hepatic necrosis **(Fig. 1 -1)** with sparing of hepatocytes located adjacent to portal triads. Canalicular plugging with bile is frequently observed between surviving hepatocytes **(Fig. 1- 2)**. In the necrotic tissue, ghost-like remnants of necrotic hepatocytes and the accompanying sinusoids can generally be visualized (coagulative necrosis). Inflammatory cell activity is minimal in all areas.

Contributor's Morpho logic Diagn osis: Marked acute hepatic necrosis with periportal sparing and periportal intrahepatic cholestasis, Chihuahua, canine.

Contributor's Comment: Upon further investigation, the referring veterinarian discovered that the patient had inadvertently been vaccinated by injection with an intranasal trivalent *Bordetella bronchiseptica*–canine parainfluenza-canine adenovirus-2 vaccine product due to an error in vaccine preparation by a newly hired technician. The product package insert warns that subcutaneous or intramuscular administration of the intranasal product may result in icterus or death from liver failure, but we were unable to find any information in the scientific literature to explain the mechanism of hepatic injury or which component in the vaccine might be responsible for the injury. There is one published report of acute hepatic necrosis associated with subcutaneous administration of an intransasal canine Bordetella-canine parainfluenza vaccine, but the authors did not speculate as to pathogenesis. The patient survived and hepatocellular disease was still present two months later based on hepatic biopsy and serum bile acid concentrations.[5] Equine serum hepatitis, sometimes known as Theiler's disease, occurs subsequent to vaccination with biologics that contain equine serum and has a similar pattern of marked hepatic necrosis with periportal sparing. However, after nearly one hundred years since equine serum hepatitis was first reported, the pathogenesis of the disorder remains elu-

sive.[2] Ordinarily, massive hepatic necrosis in dogs suggests a toxic etiology. Although many drugs, toxins and chemicals have been shown to cause hepatic injury in dogs[2], it is difficult to find a comprehensive list of substances in which the toxicosis in dogs is predominately manifested by acute, severe hepatic necrosis. In our laboratory, ingestion of xylitol, cycad palm, poisonous mushrooms (particularly *Amanita* sp.), or water containing blue-green algae are our first considerations as causes of marked hepatic necrosis when there has been no known exposure to drugs or chemicals.

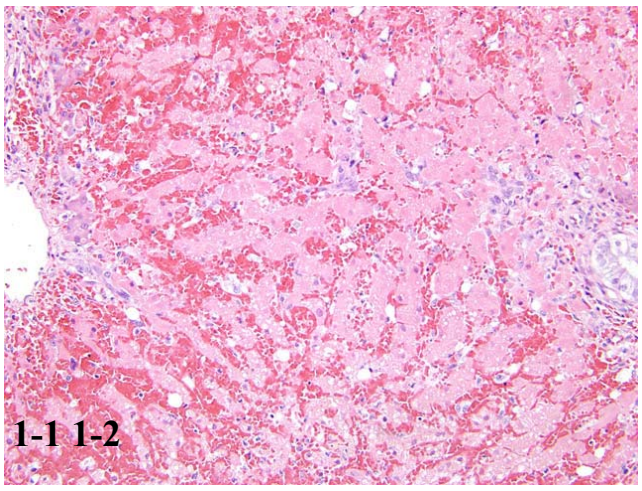
AFIP Diagnosis: Liver: Hepatocellular necrosis, acute, submassive to massive, diffuse, with hemorrhage and canalicular cholestasis, Chihuahua (*Canis familiaris*), canine.

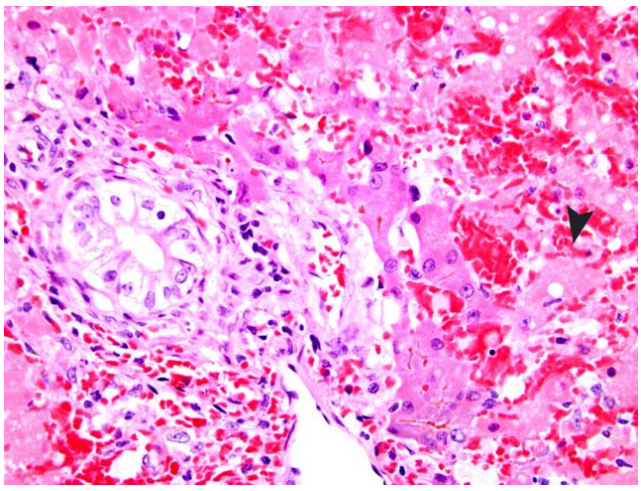
Conference Comment: Massive hepatic necrosis is defined as necrosis of entire acini. In the sections examined at the conference, there are acini that are entirely necrotic as well as acini that are largely necrotic with a rim of surviving hepatocytes around the portal areas. Massive necrosis leads to collapse of the remaining stroma, impaired regeneration and postnecrotic scarring. It is usually, but not always, caused by toxins. Hepatosis dietetica is a nutritionally induced form of massive hepatic necrosis. [4]

Hepatotoxic agents can be divided into two broad categories based on their predicted activity. Predictable hepatotoxins are those that produce a generally consistent activity in the majority of the animals that are exposed. The extent of injury produced in an individual animal by a predictable hepatotoxin may differ depending on various factors including age, sex, diet, and endocrine function. Idiosyncratic drug reactions are caused by those agents that produce an effect in a small minority of the animals exposed, such as carprofen occasionally causing acute hepatic necrosis in Labrador retrievers and diazepam causing acute fatal hepatic injury in some, but not all, cats.[2]

Hepatotoxic agents can be classified into six different categories based on their cellular target.[2]

1. Production of toxic metabolites by the cytochrome p450 system is the most common form of hepatocellular injury. The enzymes of this system are located in the smooth endoplasmic reticulum and are found in the highest concentration of centrilobular hepatocytes. They function to metabolize lipid-soluble chemicals into water-soluble compounds for excretion.
2. Drugs and cellular enzymes may combine together to form neoantigens. When transported to the cell surface and presented as antigens, these neoantigens may stimulate both cellular and humoral immune responses resulting in either direct cellular cytotoxicity or antibody-dependent cellular cytotoxicity. (halothane)
3. Some toxins may directly initiate apoptosis by stimulating proapoptotic pathways within hepatocytes. (hydrophobic bile acids)
4. Certain toxins may directly damage cellular membranes disabling calcium homeostasis and resulting in cell death. (carbon tetrachloride)
5. There are chemicals that will bind and disrupt the ca-





1-1. Liver, Chihuahua. Diffuse coagulative necrosis of the hepatic cords. (HE 40X).
1-2. Liver, Chihuahua. Multifocally, hepatocytes of the limiting plate are often degenerate characterized by swollen, pale, vacuolated cytoplasm (arrowhead) and/or contain green-brown intracanilicular bile plugs (cholestasis). (HE 400X).

Selected hepatotoxins extracted from Cullen[2]

Category	Members	Mechanism of action	Remarks
Blue-green algae	Anabaena, Aphanizomenon, Microcystis	Microcystin LR (cyclic heptapeptide)	More closely related to bacteria
Pyrrolizidine alkaloids	Senecio, Cynoglossum, Crotalaria, Heliotropium	Ingested alkaloids converted to pyrrolic esters by cytochrome p450 enzymes	Esters are alkylating agents that act on cytosolic and nuclear proteins. Megalocytes due to antimitotic effect.
Aflatoxin	*Aspergillus flavus*	Aflatoxin B_1 (toxic intermediates produced by cytochrome p450 enzymes)	Toxin and carcinogen. Sheep more resistant.
Sporidesmin	*Pithomyces chartarum* (fungus growing on dead rye grass)	Necrosis of the epithelium of large intrahepatic and extrahepatic biliary ducts	Results in cholestasis with failure to excrete phylloerythrin leading to photosensitization
Mushroom	*Amanita* sp.	Toxic cyclopeptides Palloidin (toxic heptapeptide)	Inhibition of RNA polymerase II function disrupting DNA and RNA transcription Disruption of intracellular actin filaments

nalicular pumps that normally secrete bile into the canaliculi. This disruption results in cholestasis. (estrogen, erythromycin)
6. Direct damage to mitochondria decreases production of adenosine triphosphate as well as resulting in the release of cytochrome-c leading to apoptosis or necrosis. (antiviral nucleosides, intravenous tetracycline)

Certain toxic compounds may affect cells other than hepatocytes.[2] Damage to biliary epithelium may be caused by trimethoprim-sulfa or sporidesmin, while damage to Kupffer cells can be caused by endotoxin. Arsenicals damage endothelial cells of the liver, and vitamin A excess causes activation of hepatic stellate cells.

Equine serum hepatitis is an idiopathic condition most closely associated with administration of equine-origin biologics.[1,3] It is generally reported 41-60 days following administration of a biologic product, and is characterized by acute hepatic centrilobular necrosis.[1]

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References:

1. Aleman M, Nieto JE, Carr EA, Carlson GP: Serum hepatitis associated with commercial plasma transfusion in horses. J Vet Intern Med 19:120-122, 2005
2. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 440-447. Elsevier, St. Louis, MO, 2007
3. Guglick MA, MacAllister CG, Ely RW, Edwards W: Hepatic disease associated with administration of tetanus antitoxin in eight horses. J Am Vet Med Assoc 11:1737-1740, 1995
4. Stalker MJ, Hayes MA: Liver and biliary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 322-333. Elsevier Limited, St. Louis, MO, 2007
5. Toshach K, Jackson MW, Dubielzig RR: Hepatocellular necrosis associated with the subcutaneous injection of

an intranasal *Bordetella bronchiseptica*-canine parainfluenza vaccine. J Am Anim Hosp Assoc 33:126-128, 1997

CASE II – S61/07 (AFIP 3063785).

Signalment: Two and a half-year-old, male beagle, canine.

History: The dog died with multiple bite wounds inflicted by other dogs kept in the same kennel.

Gross P athology: At necropsy the animal displayed multifocal, severe epidermal ulcers and excoriations of the skin of the neck, thorax and both hind limbs associated with multifocal subcutaneous and intramuscular hemorrhages. The mandibular and retropharyngeal lymph nodes were enlarged and severely hyperemic. The endocardium had multiple petechial hemorrhages and the atrioventricular valves had mild nodular endocardiosis. The liver and lung were moderately congested. In addition, the lung had mild alveolar edema and emphysema.

Histopathologic D escription: Within the kidney there was multifocal vacuolation, degeneration and necrosis with sloughing and loss of tubular epithelial cells. Epithelial degeneration and necrosis were frequently associated with small cytoplasmic granular deposition of a brown-greenish pigment. Tubuli were multifocally moderately dilated and contained hyalin or coarsely granular eosinophilic to brown-greenish casts **(Fig 2 -1)**. The Bowman´s capsule spaces contained abundant eosinophilic, proteinaceous material. Interstitial and glomerular blood vessels were moderately congested with multifocal prominent dilation of cortical veins.

A Turnbull blue stain identified iron in the tubular casts, the brush border and in cytoplasmic granular deposits of the tubular epithelium, consistent with chromoproteinuria.

Contributor's Morphologic Diagnosis: Kidney: Tubular degeneration and necrosis **(Fig. 2-2)**, acute, moderate, multifocal with cytoplasmic pigment deposition and intratubular chromoprotein casts.

Contributor's Comme nt: The lesions are consistent with acute tubular necrosis following traumatic rhabdomyolysis and chromoproteinuria.
Myoglobinuria as a consequence of elevated myoglobin serum concentration can be seen in metabolic dysfunction (e.g. equine exertional rhabdomyolysis, tying up), stress (e.g. capture myopathy) or severe direct trauma to muscles. In cases of traumatic injury, animals commonly also have renal hypoperfusion due to hypovolemic shock. The proposed mechanisms involved in myoglobinuria-induced renal injury include renal vasoconstriction, intraluminal cast formation and direct intra- and/or extracellular toxicity of myoglobin.

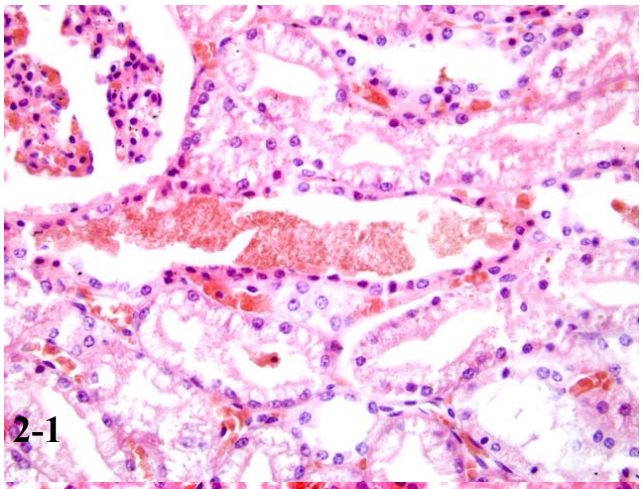
It has been argued that renal vasoconstriction is due to extravasation of fluid in areas of damaged muscle tissue leading to intravascular volume depletion. Furthermore, activation of cytokine cascades and scavenging of nitric oxide as an important endogenous vasodilatator by heme protein contribute to renal hypoperfusion.

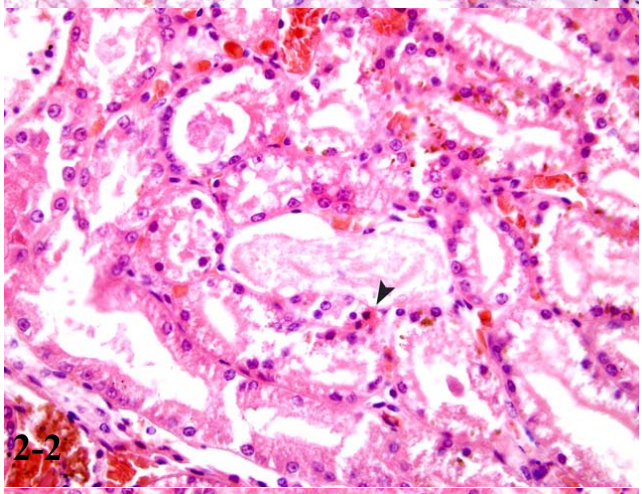
In contrast to earlier views, it has been shown that the intraluminal cast formation during myoglobinuria enhances the dose dependent toxicity of heme by its accumulation and uptake and not by intratubular obstruction. Additionally, hypovolemia, renal vasoconstriction and loss of myoglobin solubility in acidic urine facilitate the formation of casts.

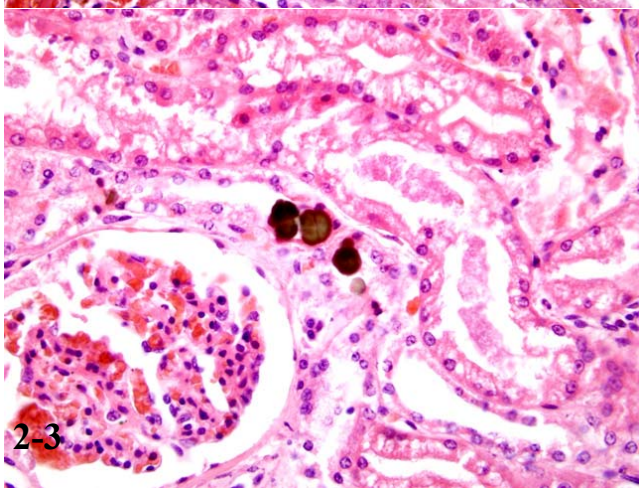
The exact mechanisms of direct myoglobin toxicity are still under investigation. It has been hypothesized that by heme protein endocytosis, tubular plasma membranes become more vulnerable to the effects of phospholipase A_2. Furthermore, iron dependent mechanisms of cellular damage including formation of free radicals, subsequent oxidative stress and lipid peroxidation have been proposed.

Acute tubular necrosis is the most common reason for acute renal failure. Early degenerative lesions commonly seen with acute renal failure include loss of brush borders, flattening of the epithelium, detachment of cells, disruption of tubular basement membranes, formation of intratubular casts, and dilation of the lumens. These changes are observed predominantly in proximal tubules, but injury can also be demonstrated in the distal nephron and may progress to signs of necrosis like hypereosinophilia and loss of cellular detail. The distal nephrons seem to be secondarily damaged by obstruction with desquamated cells, cellular debris, hemoglobin, myoglobin, and other plasma proteins. Tubular regeneration, represented by flattened to elongated epithelial cells with hyperchromatic nuclei and mitosis can be seen after about three days. Within 2-3 weeks after toxin exposure, recovery of normal renal structure may be completed.

Other conditions damaging renal tubular epithelium may result in morphologic changes similar to the lesions de-







scribed here. However, the pigment deposition seen in this case is regarded as specific for hemoglobinuria, myoglobinuria or bilirubinuria. Other common nephrotoxins producing specific acute tubular necrosis in domestic animals include heavy metals (e.g. mercury, lead, arsenic), antibiotics, antifungal agents, anti-inflammatory drugs, and fungal, bacterial and plant toxins.

AFIP Diagnosis: 1. Kidney: Degeneration and necrosis, tubular, acute, multifocal, moderate, with orange-red-brown casts, Beagle (*Canis familiaris*), canine.
2. Kidney: Anisotropic green-brown crystals, intratubular, multifocal **(Fig. 2-3).**

Conference Comment: The contributor gives an excellent overview of myoglobinuric nephrosis. Hemoglobin and myoglobin are chromoproteins that have been associated with hemoglobinuric nephrosis or myoglobinuric nephrosis respectively. Hemoglobin is normally bound to the carrier protein haptoglobin, which is too large to be filtered by the glomerulus. Therefore, hemoglobin is not excreted in the urine unless supplies of the carrier molecule are depleted. Hemoglobin and myoglobin have little nephrotoxicity by themselves [4,6], but when associated with renal ischemia, acidic urine, and decreased glomerular filtration rate, they contribute to acute renal failure.[6]

It is generally accepted that vasoconstriction, lipid peroxidation, and acidification of the urine all play roles in acute tubular necrosis. Cast formation is thought to result from decreased urine flow associated with a decreased GFR.[1,3] In vitro studies of myoglobin toxicity in Fischer 344 rats suggest primary mechanisms of damage result from diminished pyruvate-stimulated gluconeogenesis, decreased total glutathione levels and induction of lipid peroxidation.[5] The exact mechanisms for these actions and their effect in vivo are not fully known.

Hematuria, hemaglobinuria, and myoglobinuria will all generate a positive occult blood test. They can be differentiated by various diagnostic tests.[2] Centrifugation will cause sedementation of erythrocytes leaving a clear supernatant with hematuria. Red-brown urine that does not clear upon centrifugation may be either hemoglobinuria or myoglobinuria. These may be differentiated by adding saturated ammonium sulfate solution, which will precipi-

2-1. Kidney,Beagle. Numerous ectatic tubules and ducts contain moderate amounts of red-orange granular casts. Often these tubules are lined by attenuated epithelium. (HE 400X).
2-2. Kidney, Beagle. There is multifocal tubular epithelial necrosis characterized by hypereosinophilic, shrunken epithelial cells with pyknotic nuclei (arrowhead). Multifocally within the interstitium there is mild hemorrhage. (HE 400X).
2-3. Kidney, Beagle. Few tubules contain variably- sized, green-brown, irregularly round crystals. (HE 400X).

Pigmentary changes in the kidney, extracted from Maxie et al.[4] and Newman et al.[6]

Condition	Pigment	Gross lesion	Histologic lesion
Hemoglobinuric nephrosis (acute hemolytic crisis)	Hemoglobin	Dark red-brown to blue-black with radial streaks	Fine red granular speckling within epithelial cells or granular casts
Myoglobinuric nephrosis (acute rhabdomyolysis)	Myoglobin	Dark red-brown to blue-black with radial streaks	Fine red granular speckling within epithelial cells or granular casts
Hemosiderosis (chronic hemolytic anemia)	Hemosiderin	Brown discoloration of cortex	Pigment within the epithelial cells of proximal tubules
Cloisonné kidney (non-clinical condition)	Ferritin and hemosiderin	Brown to black renal cortices	Brown pigmentation of basement membrane, convoluted portions of proximal tubules
Lipofuscinosis	Brown iron-free pigments	Radial dark lines on the cut surface of cortex, sparing the medulla	Fine brown granules in epithelial cells of convoluted tubules

tate hemoglobin. A clear supernatant following ammonium sulfate addition is indicative of hemoglobinuria, while a red-brown color indicates myoglobinuria.

The green-brown intratubular crystals were identified by scanning electron microscopy with energy dispersive x-ray analysis (SEM-EDXA) and infrared spectroscopy (IR) as consistent with calcium oxalate monohydrate. The calcium oxalate crystals in this case are unusual in appearance because of the green-brown color in H&E. The crystals stained positive for Von Kossa and negative for Alizarin red. It is possible that protein and iron deposition within the crystals could account for their abnormal appearance. We would like to thank the AFIP Department of Environmental and Toxicologic Pathology for their assistance in evaluating this case.

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References:

1. Efstratiadis G, Voulgaridou A, Nikiforou D, Kyventidis A, Kourkouni E, Vergoulas G: Rhabdomyolysis updated. Hippokratia 11:129-137, 2007
2. Gregory CR: Urinary system. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 240-241. Iowa State Press, Ames, Iowa, 2003
3. Holt S, Moore K: Pathogenesis of renal failure in rhabdomyolysis: the role of myoglobin. Exp Nephrol 8:72-76, 2000
4. Maxie MG, Newman SJ: The urinary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 475476. Elsevier Limited, St. Louis, MO, 2007
5. Minigh JL, Valentovic MA: Characterization of myoglobin toxicity in renal cortical slices form Fischer 344 rats. Toxicol 187:77-87, 2003
6. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Disease, McGavin MD and Zachery JF, 4th ed., pp. 644-658. Mosby Elsevier, St. Louis, Missouri, 2007
7. Zager RA: Rhabdomyolysis and myohemoglobinuric acute renal failure. Kidney Int 49:314-326, 1996

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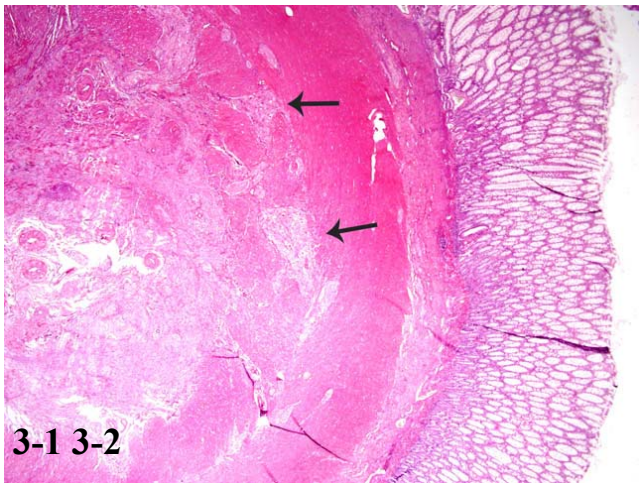
CASE III – CAS 2 (AFIP 2991412).

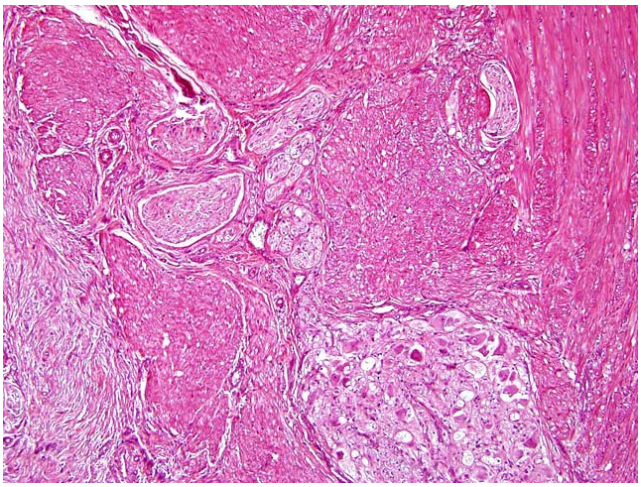
Signalment: 1-year-old, male, Beagle dog.

History: This dog was part of a 10-day oral toxicological study and was euthanized at the end of the study. There were no relevant clinical signs.

Gross Pathology: An abnormal shape of the cecum was the only relevant macroscopic finding.

Histopathologic Description: There is invagination of





3-1. Cecum, Beagle. Tthere are increased numbers of large, irregularly shaped ganglia (arrows) within the tunica muscularis and serosa of the intussusceptum. (HE 100X).
3-2. Cecum, Beagle. Higher magnification demonstrating increased numbers of nerve bundles and ganglia. (HE 200X).

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the tip of the cecum within its lumen. All parts of the cecum wall are diffusely, moderately thickened (about twice normal thickness). The muscularis mucosa, the submucosa, some parts of the muscular layers (particularly the longitudinal layer), and the serosa are replaced by a poorly demarcated tissue, primarily in the same location as the myenteric (Auerbach's) and the submucous (Meissner's) plexuses **(Fig. 3-1)**. This tissue is composed of irregularly-arranged wavy fascicles of nerve fibers with round and spindle cells, and some clusters of enlarged ganglion cells **(Fig. 3-2)**. The mucosa is moderately hyperplastic, with multifocal to coalescing hemorrhages in the lamina propria, and multifocal minimal degeneration of some glands. Scattered in the submucosa and the proliferative neural tissue are some cells containing large pigmented brown granules (hemosiderin).

Contributor's Morpho logic Di agnoses: Cecum: Transmural ganglioneuromatosis, locally extensive, with intussusception.

Contributor's Comment: Intestinal ganglioneuromatosis refers to a hyperplastic proliferation of ganglion cells, nerve fibers, and supporting cells of the enteric nervous system. In humans, intestinal ganglioneuromatosis is most often part of multiple tumor syndromes, particularly the multiple endocrine neoplasia (MEN) 2B syndrome.[12] MEN-2B is inherited in an autosomal dominant fashion and is caused by a single mutation in the RET proto-oncogene. This heritable endocrine disorder is characterized by medullary thyroid carcinoma, pheochromocytoma, multiple mucosal neuromas, gastrointestinal ganglioneuromatosis, corneal nerve thickening and skeletal abnormalities.[8] Gastrointestinal symptoms are common in patients with MEN-2B, and are secondary to the pseudo-obstruction caused by the ganglioneuromatosis.[6] The pathogenesis of ganglioneuromatosis is not well understood, but some studies in humans indicate that it may be related to the overproduction of some nerve growth factors.

Immunohistochemically, some cases of ganglioneuromatosis were shown to be a complex hyperplasia of several peptidergic, cholinergic, and probably adrenergic nerve fibers instead of a selective overgrowth of one type of nerve fibers.[4]

Some rare cases of intestinal ganglioneuromatosis or ganglioneuromas have been reported, most often in young animals: in a horse[1], a steer[3], a cat[9], and 3 dogs.[5,11,13] In all cases, there were clinical signs (e.g. colic, impaction, anorexia, vomiting, diarrhea, rectal prolapse) that led to surgical resection of the masses. Masses were located in the small intestine (3 cases), colon (1 case), colorectum (2 cases) or Vater's papilla (1 case). This is the first reported case of asymptomatic ganglioneuromatosis in a dog.

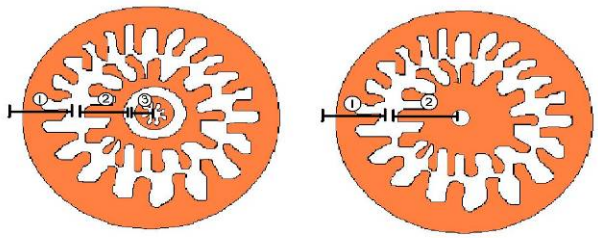
AFIP Diagnosis: Cecum (per contributor): Ganglioneuromatosis, with intussusception, Beagle (*Canis familiaris*), canine.

Conference Comment: Ganglioneuromas are composed of mature autonomic ganglion cells, satellite cells, un-

myelinated and occasionally myelinated axons, Schwann cells and a fibrous stroma. They are generally considered benign neoplasms. Intestinal ganglioneuromatosis is considered a hyperplasia of similar elements; it is typically transmural. As noted by the contributor, both lesions are rare in animals and have not been found to be associated with MEN-like syndromes.[7] Some have suggested that ganglioneuromas may actually represent hamartomas (benign, nonneoplastic, tumor-like nodules consisting of an overgrowth of mature cells that normally occur in the affected organ) rather than benign neoplasms.[10]

Intussusceptions are described as having three layers: (1) outer wall of the receiving segment, (2) middle returning segment of invaginated bowel, and (3) inner entering segment. The intussusception seen in this lesion is unusual in that it contains only two of the three layers, a feature that will occur only through the invagination of a blind pouch (in this case, the tip of the cecum). Cecal inversion is another term for such a lesion **(Fig. 3 -3)**. Various causes of intussusception may include linear foreign bodies, heavy parasitism, previous intestinal surgery, enteritis, and intramural lesions. It may also develop as a terminal, agonal or postmortem event.[2]

We appreciate the assistance from the Departments of Gastrointestinal Pathology, Neuropathology, and Soft Tissue Pathology at the Armed Forces Institute of Pathology in consultation on this case.



A B

Fig 3-3. A. Intussusception of tubular section of bowel consisting of three layers: (1) outer wall of the receiving segment, (2) middle returning, segment of invaginated bowel, and (3) inner entering segment.
B. Intussusception of a blind pouch consisting of two layers: (1) outer wall of the receiving segment, and (2) the middle returning, segment of invaginated bowel.

Contributing Institution: Pfizer PGRD, Department of Pathology, Z.I. Pocé-sur-Cisse, B.P. 159, 37401 Amboise Cedex, France

References:

1. Allen D, Swayne D, Belknap JK: Ganglioneuroma as a cause of small intestinal obstruction in the horse: a case report. Cornell Vet 79:133-141, 1989
2. Brown CC, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 96-97. Elsevier Limited, St. Louis, MO, 2007
3. Cole DE, Migaki G, Leipold HW: Colonic ganglioneuromatosis in a steer. Vet Pathol 27:461-462, 1990
4. D'Amore ES, Manivel JC, Pettinato G, Niehans GA, Snover DC: Intestinal ganglioneuromatosis: mucosal and transmural types. A clinicopahologic and immunohistochemical study of six cases. Hum Pathol 22:276-286, 1991
5. Fairley RA, McEntee MF: Colorectal ganglioneuromatosis in a young female dog (Lhasa Apso). Vet Pathol 27:206-207, 1990
6. Goyal RK, Hirano I: The enteric nervous system. N Engl J Med 334:1106-1115, 1996
7. Head KW, Else RW, Dubielzig: Tumors of the alimentary tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., p. 470. Blackwell Publishing, Ames, IA, 2002
8. Komminoth P: Multiple endocrine neoplasia type 1 and 2: from morphology to molecular pathology. Vehr Dtsch Ges Path 81:125-138, 1997
9. Patnaik AK, Lieberman PH, Johnson GF: Intestinal ganglioneuroma in a kitten – a case report and review of literature. J Small Anim Pract 19:735-742, 1978
10. Porter BF, Storts RW, Payne HR, Edwards JF: Colonic ganglioneuromatosis in a horse. Vet Pathol 44:207-210, 2007
11. Ribas JL, Kwapien RP, Pope ET: Immunohistochemistry and ultrastructure of intestinal ganglioneuroma in a dog. Vet Pathol 27:376-379, 1990
12. Shekitka KM, Sobin LH: Ganglioneuromas of the gastrointestinal tract. Relation to Von Recklinghausen disease and other multiple tumor syndromes. Am J Surg Pathol 18:250-257, 1994
13. Van Den Ingh TSGAM, Rothuizen J: Ganglioneuroma of Vater's papilla and extrahepatic cholestasis in a dog. Vet Pathol 21:254-256, 1984

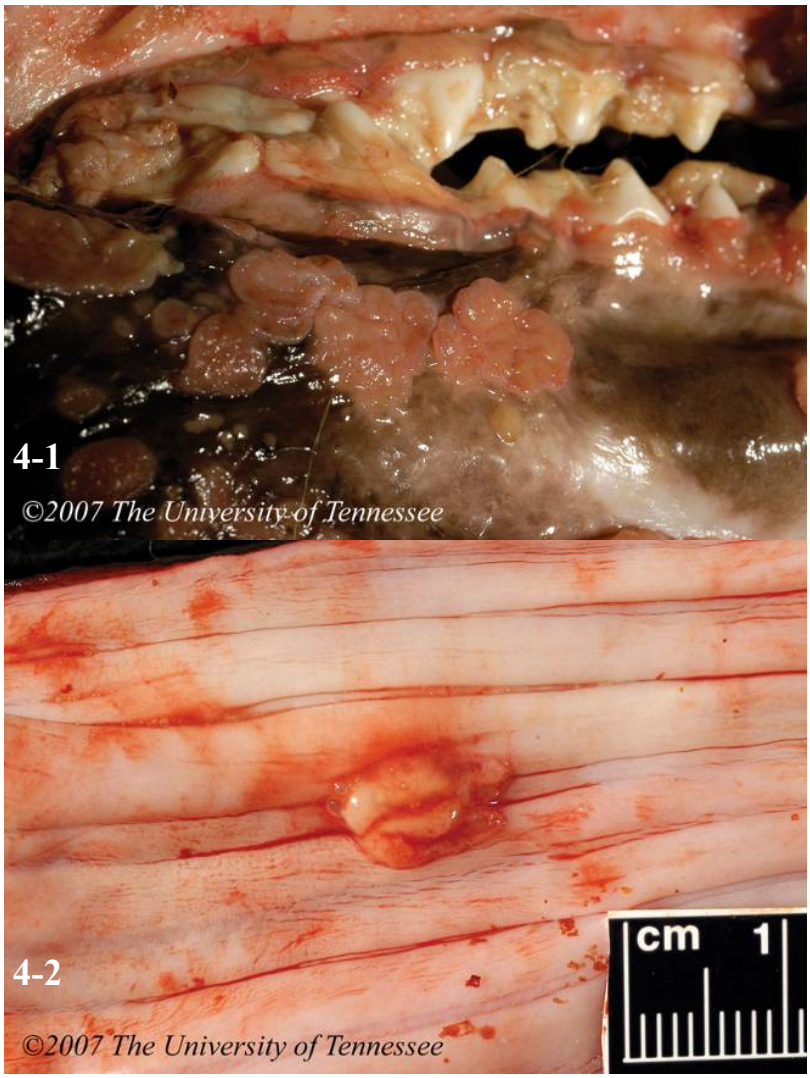
CASE IV - 07-45 (AFIP 3074806).

Signalment: Seven-month-old, female, Golden Retriever mixed breed, *Canis familiaris*, dog

History: The dog was presented with chronic conjunctivitis, gingival lesions, and respiratory disease. The clinical signs had begun at 4.5 weeks of age and had progressed. Previous diagnostics including conjunctival biopsies, cytology and bacterial culture of conjunctival swabs, canine distemper serology, virus isolation, and routine bloodwork failed to establish a diagnosis. On presentation there were circular, raised, pink, fleshy, mucosal lesions of the conjunctiva, throughout the oral cavity and naso-pharynx and an ulcer on the soft palate. Thoracic auscultation revealed harsh referred upper airway sounds. A repeat biopsy of the ocular conjunctiva identified a profuse accumulation of fibrin in areas of ulceration and under-running the epithelium. A diagnosis of ligneous conjunctivitis was made. Based on this diagnosis and involvement of other mucosal surfaces, a presumptive diagnosis of plasminogen deficiency was made. This was confirmed by a low plasminogen functional activity assay of 35% (compared to a normal age-matched control of 111% and pooled samples from normal dogs of 118%). The conjunctival lesions recurred after the excisional biopsy. A 2-week round of topical and intravenous treatment with fresh frozen plasma diminished the conjunctival lesions; however, four weeks later the dog had a lower plasminogen activity assay (10%), weight loss, inappetance and lethargy. The owners requested euthanasia.

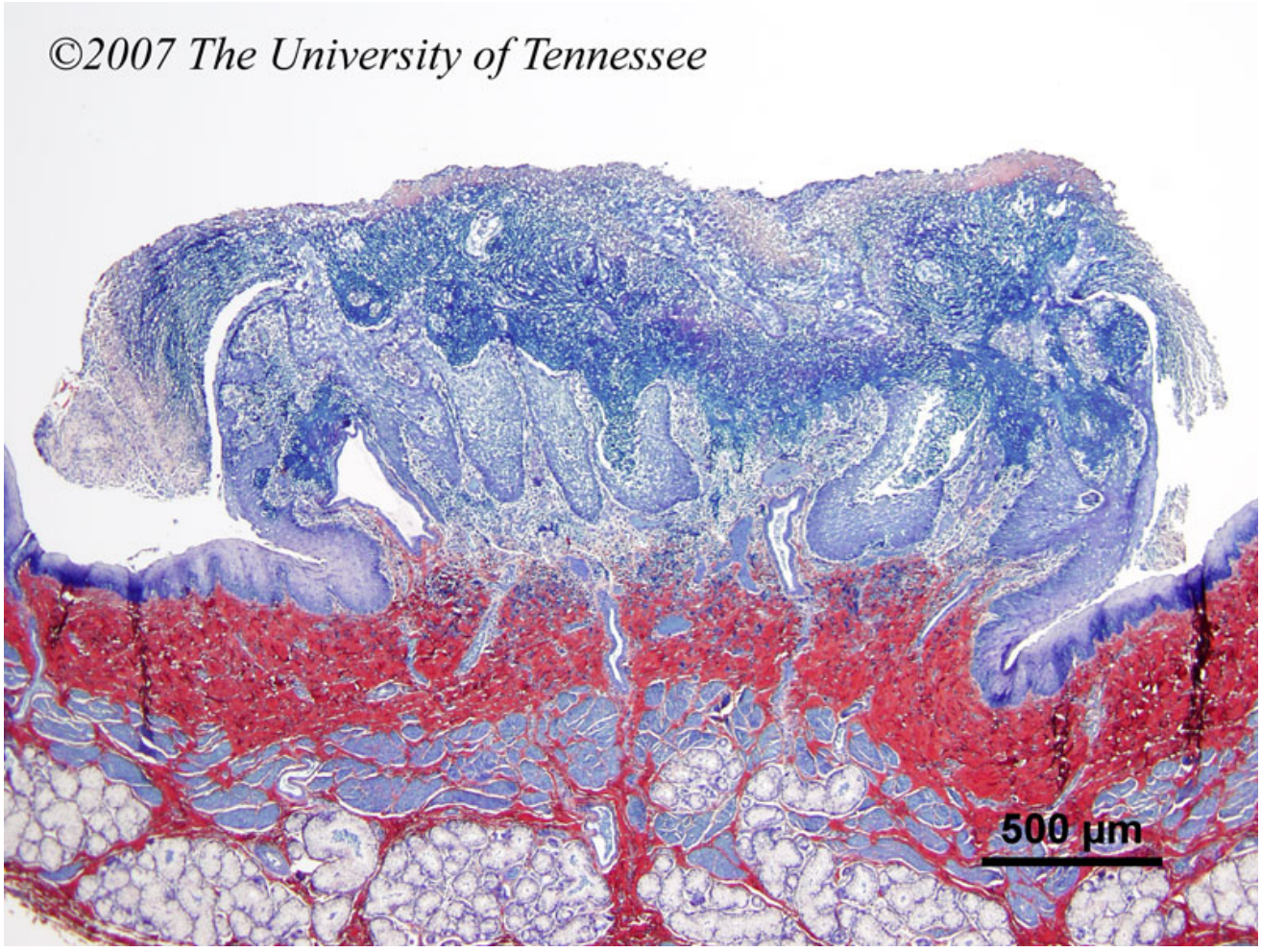
Gross Pathology: Multifocal to coalescing, 0.2cm to 2.0 cm in diameter, raised, white to gray, granular, plaques decorated the glossal, buccal and gingival surfaces **(Fig 4 -1)**. In the soft palate, there was a 2 x 2cm ulcerated area, covered by a thick layer of a granular yellow material. There were multifocal, 0.5cm to 1cm diameter, gray plaques on the esophageal mucosa **(Fig. 4-2)**. Gray to yellow, granular, fibrinous plaques were disseminated over the length of the tracheal mucosa. Multifocally slightly elevated plaques covered the epicardium of the right and left ventricles. Additionally there was mild hydrocephalus, rare intestinal mucosal hemorrhages, and a mild fibrinous perihepatitis

Histopathologic D escription: The sections of esophagus submitted have focal erosion to ulceration of locally hyperplastic epithelium covered by an exophytic coagulum of fibrin and cellular debris **(Fig 4 -3)**. The exophytic coagulum is supported by a pedunculated to broad base of fibrin irregularly infiltrated by granulation tissue



4-1. Oral cavity, Golden retriever mix, canine. Raised, white to gray, granular, plaques decorate the glossal, buccal and gingival surfaces.
4-2. Esophagus, Golden retriever mix, canine. 0.5cm to 1cm diameter gray plaques on the esophageal mucosa. →

Gross photographs courtesy of The University of Tennessee, Collage of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996



4-3. Esophagus, Golden retriever mix, canine. Exophytic plaque composed of markedly thickened lamina propria which elevates the overlying moderately hyperplastic and ulcerated epithelium. Superficially, these plaques are covered by a fibrinocellular mat. (PTAH).

Photomicrograph courtesy of The University of Tennessee, Collage of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996

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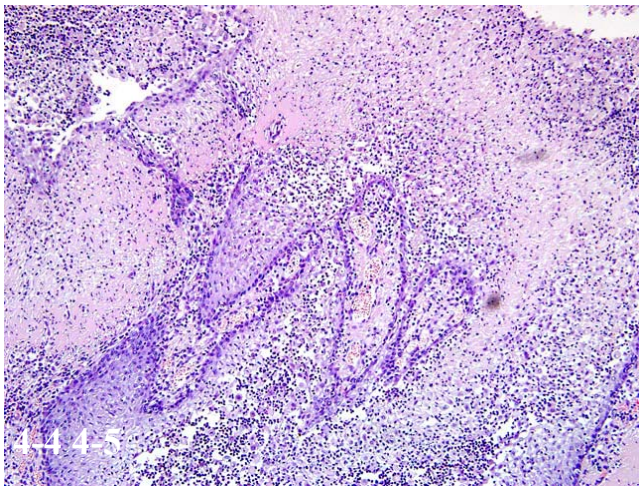
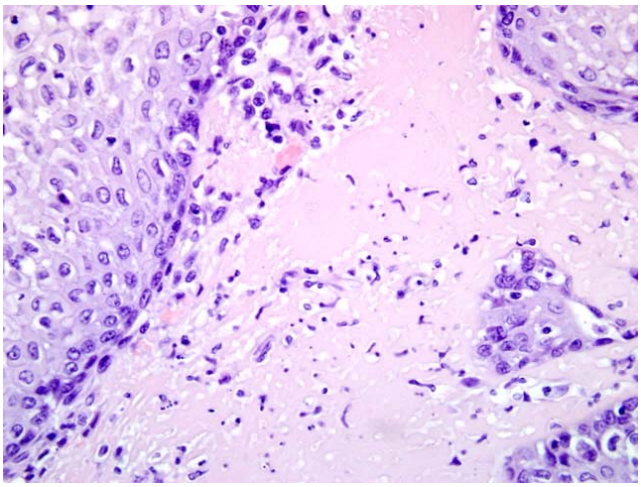
with macrophages and neutrophils and a few lymphocytes, plasma cells and eosinophils admixed. In some areas, this thick layer of fibrin and inflammatory cells forms finger-like projections partially overlain by squamous epithelium **(Fig. 4-4)**. In immediately adjacent esophagus there is limited focal to multifocal subepithelial fibrin deposition **(Fig. 4-5)**.

Contributor's Mor phologic Di agnosis: Severe, chronic, ulcerative and proliferative, fibrinomembranous esophagitis

Contributor's Comme nt: This patient was initially presented to the Ophthamology service because of bilateral conjunctival lesions. The clinical diagnosis of ligneous conjunctivitis was made based on the histologic appearance of the conjunctival biopsy and functional plasminogen activity assay. This form of conjunctivitis is so named because of the wood-like consistency of the membranes. Reports of this condition in canines are rare, predominantly in the Doberman Pinscher breed.[9] It is more commonly reported in females in both the veterinary and human literature.[5,9] The condition is linked to a type I plasminogen deficiency and an autosomal-recessive genetic mutation has been identified as a common cause of this functional deficiency.[10] The pathogenesis of the lesions in the conjunctiva and other mucosal sites involves the coagulation of fibrin following minor mechanical





4-4. Esophagus, Golden retriever mix, canine. Diffusely, overlying the ulcerated plaques, is a thick fibrinous mat admixed with numerous inflammatory cells. (HE 200X).
4-5. Esophagus, Golden retriever mix, canine. Subepithelial fibrin deposition admixed with neutrophils, lymphocytes, and macrophages. (HE 400X).

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injury to tissues. This fibrin rich matrix provides hemostasis and is subsequently replaced by granulation tissue in normal individuals.

Impaired proteolysis due to deficiency in plasminogen results in an inability to remove the fibrin rich matrix and remodel granulation tissue, thus arresting wound healing at the granulation tissue stage and resulting in the accumulation of fibrin rich membranes.

The condition is typically diagnosed in neonates but may develop at any age. The palpebral conjunctiva is affected most frequently but the bulbar conjunctiva and cornea may be affected as well. Other mucosal sites such as the gingiva, ear, respiratory tract, gastrointestinal tract and female reproductive tract may also be affected with or without the presence of conjunctival lesions.[3,4,9,12] As was the case in this dog, hydrocephalus has been reported in infants with plasminogen deficiency.[4,12] In this case there were multiple venous thrombi associated with lesions in the oral cavity and present in the pulmonary field. While the primary respiratory signs in this dog were likely due to tracheal obstruction, the pulmonary thrombi may have played some role in this animal's respiratory condition. Although not reported in the human literature in association with ligneous conjunctivitis, severe decreases in plasminogen activity, when coupled with other insults or precipitating events such as operations, trauma or infection have been reported to increase the risk for thromboembolic events.[8]

AFIP Diagnosis: Esophagus: Esophagitis, proliferative, fibrinous, neutrophilic and lymphoplasmacytic, multifocal, marked, with ulceration, acantholysis, granulation tissue and multifocal subepithelial fibrin, Golden retriever mix (*Canis familiaris*), canine.

Conference Comment: The contributor gives an excellent overview of plasminogen deficiency associated with ligneous conjunctivitis. Conference participants are encouraged to review the article on this case published by Johnstone McLean et al.[7] Plasminogen plays a vital role in intravascular and extravascular fibrinolysis, wound healing, cell migration, tissue remodeling, angiogenesis, and embryogenesis.[2] Plasminogen may be converted to plasmin by cleavage with either tissue-type plasminogen activator (tPA) leading to lysis of fibrin clots in the blood stream or urokinase-type plasminogen activator (uPA) associated with wound healing and tissue remodeling.[1]

It is interesting to note that in humans and animals diagnosed with type I plasminogen defficiency, there is little to no increase in the risk of developing intravascular thrombosis, which implies the existence of an alternative pathway for intravascular fibrinolysis.[7,11,13]

The pseudomembranous deposits on mucous membranes occurs primarily in areas of previous damage. The hyaline material may contain scattered neutrophils, eosinophils, T-lymphocytes, plasma cells, mast cells and/or foreign material. Immunohistochemistry may be positive for fibrin, albumin and immunoglobulins (IgG, IgA).[3,6]

Contributor: The University of Tennessee, College of Veterinary Medicine, 2407 River Dr., Knoxville, TN, 37996

References:

1. Bugge TH, Flick MJ, Danton MJ, Daugherty CC, Romer J, Dano K, Carmeliet P, Collen D, Degen JL: Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. Proc Natl Acad Sci USA 93:5899-5904, 1996

2. Castellino FJ, Ploplis VA: Structure and function of the plasminogen/plasmin system. Thromb Haemost 93:647-654, 2005

3. Chai F, Coates H: Otolaryngeal manifestations of ligneous conjunctivitis. Int J Pediatr Otorhinolaryngol 22:760-762, 2003

4. Cifti E, Ince E, Akar N, Dogru U, Tefs K, Schuster V: Ligneous conjunctivitis, hydrocephalus, hydrocele and pulmonary involvement in a child with homozygous type I plasminogen deficiency. Eur J Pediatr 162:462-465, 2003

5. Cohen SR: Ligneous conjunctivitis: an opthalmic disease with potentially fatal tracheobronchial obstruction. Am Otol Rhinol Laryngol 99:509-512, 1990

6. Wilcock BP: Eye. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, p. 481. Elsevier Limited, St. Louis, MO, 2007

7. Johnstone McLean NS, Ward DA, Hendrix DV, Donnell RL, Ilha MR: Ligneous conjunctivitis secondary to a congenital plasminogen deficiency in a dog. J Am Vet Med Assoc 232:715-721, 2008

8. Lottenberg R, Dolly JR, Kitchens CS: Recurrent thromboembolic disease and pulmonary hypertension associated with severe hypoplasminogenia. Am J Hematol 168:181-193, 1985

9. Ramsey DT, Ketring KL, Glaze MB, Knight B, Render JA: Ligneous conjunctivitis in four doberman pinschers. J Am Anim Hosp Assoc 32:439-447, 1996

10. Schuster V, Zeitler P, Seregard S, Ozcelik U, Anadol D, Lutchman-Jones L, Meire F, Mingers AM, chambeck C, Kreth HW: Homozygous and compound–heterozygous type I plasminogen deficiency is a common cause of ligneous conjunctivitis. Thromb Haemost 85:1004-1010, 2001

11. Schuster V, Hugle B, Tefs K: Plasminogen deficiency. J Thromb Haemost 5:2315-2322, 2007

12. Scully C, Allen C, Ervesen G, Hodgson T, Speight P: Oral lesions indicative of plasminogen deficiency (hypoplasminogenemia). Oral Surg Oral Med Oral Pathol Radiol Endod 91:334-337, 2001

13. Shigekiyo T, Uno Y, Tomonari A, Satoh K, Hondo H, Udea S, Saito S: Type I congenital plasminogen deficiency is not a risk factor for thrombosis. Thromb Haemost 67:189-192, 1992

The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 25

14 May 2008

Moderator:

JoLynne Raymond, DVM, Diplomate ACVP

CASE I – 04L-1129 (AFIP 2937349).

Signalment: Discus fish (*Symphysodon aequifasciata*) adult female, in moderate body condition.

History: Zoo owned fish held in a community fish tank with other discus and tropical fish. This individual had been identified with an increased respiratory rate for approximately 18 months but problems with isolation of this fish meant that it was left on display. Other individuals in tank had a much lower respiration rate and were clinically normal. This female fish was submitted live for necropsy.

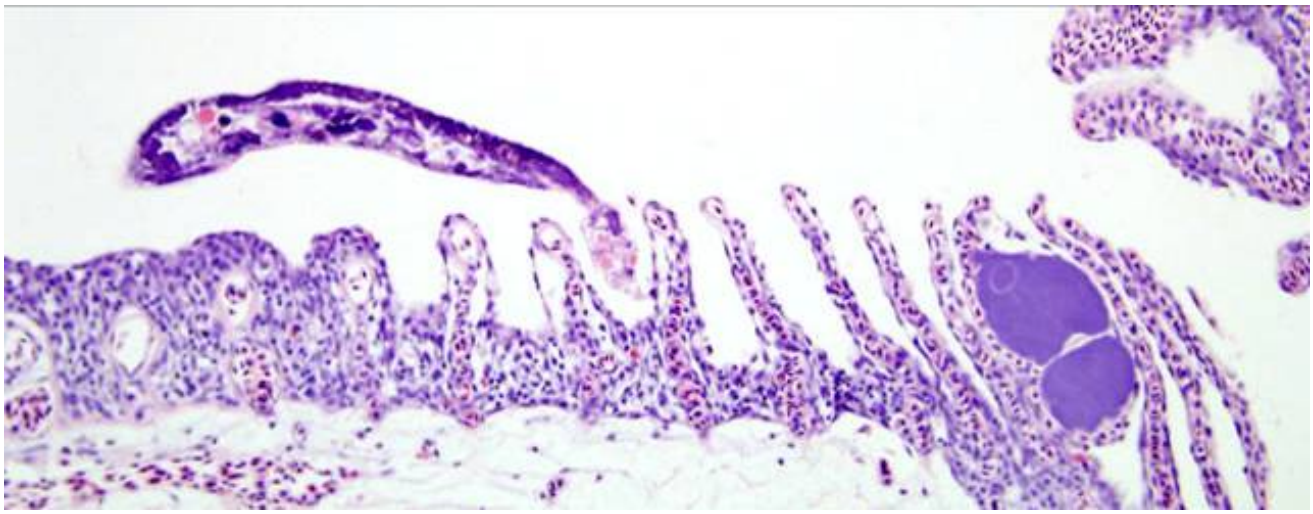
Gross P athology: No gross abnormalities identified at necropsy.

Laboratory Results: Wet preparation of gills: Numerous motile parasites attached to the gill epithelium. Appearance was consistent with *Dactylogyrus* species, monogenetic gill trematodes, due to its shape being flattened and leaf-like, with four anterior eyespots; a cephalic end which was scalloped and had an attachment organ (haptor).

Histopathologic D escription: Multifocally, the gills are moderately thickened by oedema and show mild hyperplasia with stunted, fused or complete lack of secondary lamellae. Gill interstitial tissue is multifocally infiltrated by moderate to severe numbers of inflammatory cells, predominantly lymphocytes with lesser numbers of eosinophilic granulocytes. There is multifocal moderate external haemorrhage and in between gill filaments are numerous (50 x 300um) multicellular parasites showing a thin (~2um) eosinophilic tegument, poorly discernable basophilic parenchyma and occasionally an oral sucker by which they are attached to the gill epithelium (trematodes). Multifocally, at the base of gill filaments, arterial vascular walls are moderately to severely thickened and show hyalinisation (fibrinoid necrosis) and infiltration by mild to moderate numbers of degenerate and viable leucocytes with much cell debris. Occasional clusters of basophilic, finely granular material are present in the secondary lamellae (bacterial colonies).

Contributor's Morphologic Diagnosis:
Gill – multifocal moderate to severe gill inflammation with oedema, mild hyperplasia, a leukocytoclastic fibrinonecrotic vasculitis and epithelial attached adult trematodes, Discus fish, aetiology consistent with *Dactylogyrus* sp.

Contributor's Comment: Although monogenean gill flukes are commonly found on wild fish, they are rarely a direct cause of disease or death in free-ranging populations. In captivity, epidemics can occur with significant



1-1. Gill, Discus fish. Within secondary lamellae, there are few, up to 300 um diameter, microsporidian cysts (right) characterized by a thin, 1 um thick eosinophilic wall and filled with numerous lightly basophilic oval spores. Adjacent to the secondary lamellae there is a trematode (left) characterized by a thin tegument, spongy parenchyma, digestive tracts and reproductive organs. (HE 400X)

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morbidity and mortality especially in cultured fish with excessive parasite loads under conditions of overcrowding, inadequate sanitation and poor water quality.[1,2] Under these circumstances, the parasites rapidly multiply. Gill fluke infection affects many fresh and saltwater species across a variety of temperatures, but is especially common in carp, goldfish and discus fish.[4] There is a diverse range of fluke species, most are host- and site-specific, requiring only one host to complete an entire life cycle.[5]

Freshwater fish infected with heavy gill infestations result in respiratory disease. Clinical signs can include opaque mucus covering the gills, protrusion of the gill filaments from under the gill covers and gills may be swollen and pale. Infected fish are less tolerant of low oxygen conditions and have an increased respiratory rate with gulping of air at the water surface. Fish become anoxic with flaring of the gill opercula.[1] At a very advanced stage, the fish will isolate itself and spend long periods lying on the bottom with its fins clamped to its body. Acute infections are characterized by a short period of dyspnoea followed by sudden death.[1]

Most monogenean flukes are browsers, moving about the body surface and feeding on dermal mucus and gill debris. Monogeneans have a series of hooks that enable them to attach while feeding.[2] Flukes anchoring to the gills induce a variety of lesions, depending on the density and species of parasite. Lesions can range from excessive mucous secretions, hyperplasia of gill epithelium with fusion of secondary lamellae to the presence of haemorrhage, necrosis / ulceration and inflammation.[3,6] Secondary infection by bacteria and fungi are commonly established at damaged epithelial sites.[4]

In this case, there is a widespread arterial vasculitis, which we propose may be mediated by immune complexes. Other discus fish in the collection are almost certainly affected by flukes but the involvement of a vasculitis, in other cases, remains to be determined.

The Dactylogyrus gill fluke life cycle is direct, not requiring intermediate hosts. The adults are oviparous and produce eggs with long filaments. The eggs are usually attached to the gills and develop into a free-swimming onchomiricidium, which then locates and attaches to the fish within a few hours.[5]

AFIP Diagnosis: 1. Gill: Branchitis, lymphocytic and granulocytic, multifocal, moderate with blunting, fusion, and loss of lamellae, mild epithelial hyperplasia and adult trematodes **(Fig. 1- 1)**, Discus fish (*Symphysodon aequifasciata*), Pisces.
2. Gill: Vasculitis, necrotizing, multifocal, moderate with edema and hemorrhage **(Fig. 1-2)**.

Conference Comment: The contributor gives an excellent overview of *Dactylogyrus* sp. gill infections. Gills are composed of two sets of four holobranchs that are located on either side of the pharynx. Each holobranch is composed of two hemibranchs that project from the pos-

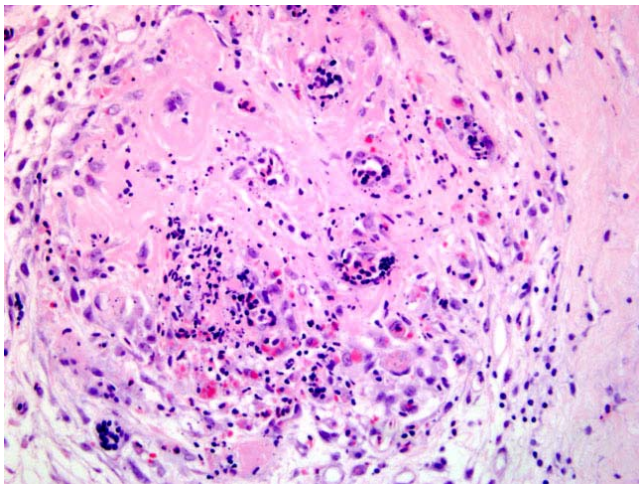
terior edge of the branchial arch. These hemibranchs contain numerous primary lamella and their secondary lamella.[4,8] Cells on the primary and secondary lamellae include melanocytes, lymphocytes, macrophages, endothelial cells, mucous cells, rodlet cells, and chloride cells.

Conference participants noted a microsporidian-like organism within some of the sections examined. Coinfections are not uncommon within compromised gill epithelium.[7]

Other diseases of importance that may affect the gills include the following list adapted from Moeller[4] and Wootten[8]

Bacterial
Flexibacter columnaris, *Flexibacter psychrophilus*, *Cytophaga psychrophilia*, and *Flavobacterium* - prominent hyperplasia, clubbing adn fusion of lamella, necrosis of gill lamella

Fungal
Branchiomyces sanguinis and *B. demigrans* (gill rot) - fungal disease of carp, trout and eels, prominent gill necrosis, with hyphae
Saprolegnia, *Achyla*, *Aphanomyces* (Saprolegniasis) - white to brown cotton-like growths on skin, fins, and gills



1-2. Gill, Discus fish. The tunica intima and media of small and medium-sized vessels are disrupted and replaced by eosinophilic fibrillar material admixed with numerous inflammatory cells and necrotic cellular debris (vasculitis) which occludes most of the lumen. Multifocally, within the occlusion there are small caliber, endothelial lined vascular spaces (recanalization). (HE 400X)

Protozoal
Ichthyophthirius multifiliis (Ich) - ciliated protozoan with horseshoe nucleus, hyperplasia with encysted trophozoites on skin and gills
Aurantiactinomyxo sp (myxosporidean) - hamburger gill disease, granulomatous inflammation and swelling of gills, with epithelial hyperplasia and gill necrosis surrounding cysts
Microsporidians (*Glugea*, *Pleistophora*, *Loma*) - cysts contain 1-2 um spores

Trematode
Diplostomum spathaceum - (eye fluke) - digenetic trematode, gulls/pelicans definitive host, metacercaria in the anterior chamber, vitreous body, and lens, snails 1st intermediate host, salmonids 2nd intermediate host
Gyrodactylus sp. - a monogenetic trematode that attaches to skin, fins, and gills
Uvulifer ambloplitis (black spot disease) - digenetic fluke, numerous black to brown spots over skin, gills, and eyes, snails 1st intermediate host, fish 2nd intermediate host

Other
Argulus sp. - (fish louse) parasite of the skin and buccal cavity resulting in cutaneous ulcers, contains a retractile preoral stylet used to pierce the skin
Lernea sp. (at base of fins) & *Ergasilus* sp. (on the gills) - Copepod, invades the skin, forms ulcers that are slow to heal
Cryptosporidiosis - intracellular extracytoplasmic protozoan,

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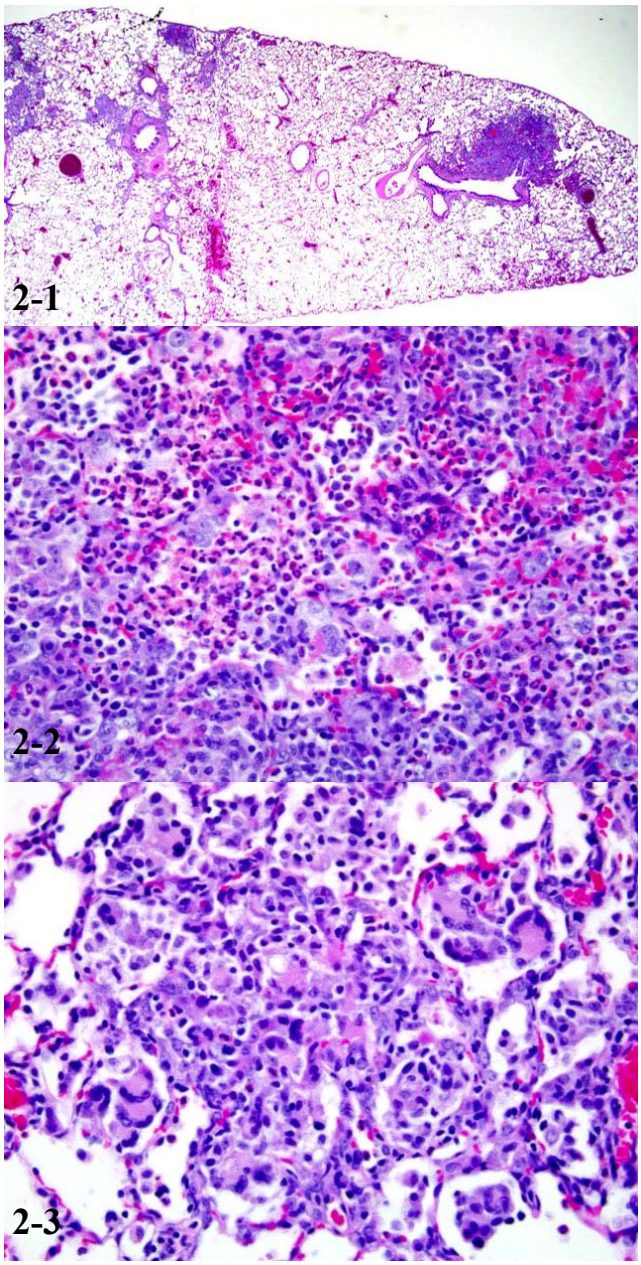
www.liv.ac.uk/vets/general/vpath/vpath.htm

References:

1. http://article.dphnet.com/catagory-02.shtml
2. Ferguson HW: Gills and pseudobranchs. In: Systematic Pathology of Fish, eds. Herman RL, Meade JW, pp. 11-40. Iowa State University Press, Ames, IA, 1989
3. Herbert BW, Shaharom FM, Anderson IG: Histopathology of cultured sea bass (*Lates calcarifer*) (Centropomidae) infected with *Cruoricola lates* (Trematoda: Sanguinicolidae) from Pulau Ketam, Malaysia. Int J Parasitol 25:3-13, 1995
4. Moeller RB: Diseases of Fish. In: 49th Pathology of Laboratory Animals Conference, pp. 568– 611, American Registry of Pathology, Washington DC, 2005
5. Roberts RJ: The pathophysiology and systemic pa-

thology of teleosts. In: Fish Pathology, ed. Roberts RJ, pp. 55-132. WB Saunders, London, 2001
6. Roubal FR: Microhabitats, attachment of eggs and histopathology by the monogenean *Allomurraytrema robustum* on *Acanthopagrus australis* (Pisces: Sparidae). Int J Parasitol 25:293-298, 1995
7. Smith SA, Noga EJ: General parasitology. In: Fish Medicine, ed. Stoskopf MK, pp. 132-148. W.B. Saunders Company, Philadelphia, PA, 1993
8. Wooten R: The parasitology of teleosts. In: Fish Pathology, ed. Roberts RJ, pp. 242-288. WB Saunders, London, 2001



CASE II – 06-0768 (AFIP 3031295).

Signalment: One-year-old, male, Brown Norway rat

History: This animal came from a room where rats had a history of respiratory lesions. It was euthanized and submitted for pathologic examination.

Histopathologic D escription: Multifocally there are numerous, variably sized, occasionally coalescing nodules **(Fig. 2-1)** composed of numerous epitheliod macrophages and fewer multinucleated giant cells admixed with numerous eosinophils and fewer neutrophils and lymphocytes **(Fig. 2-2, 2-3)**. Often, granulomas contain areas with many degenerate inflammatory cells, fibrin, and necrotic debris. Multifocally there is a perivascular infiltrate of low numbers of eosinophils and neutrophils.

Contributor's Morphologic Diagnosis: Lung: Inflammation, granulomatous and eosinophilic, multifocal, moderate to marked.

2-1. Lung, Brown Norway rat. Multifocal, often peribronchiolar and perivascular, variably-sized aggregates of cellular infiltrate. (HE 20X)
2-2. Lung, Brown Norway rat. The cellular infiltrate is characterized by numerous eosinophils admixed with moderate numbers of histiocytes, lymphocytes, fewer neutrophils and plasma cells. (HE 400X)
2-3. Lung, Brown Norway rat. Multifocally, within the alveoli and often admixed with other inflammatory cells, there are multinucleated giant cells. (HE 400X)

Contributor's Comme nt: The pulmonary immune response of Brown Norway (BN) rats is unique and often differs from other strains. Following ovalbumin challenge, BN rats develop airway hyperresponsiveness where as Sprague-Dawley rats do not. In addition, Sprague-Dawley rats develop only a neutrophilic inflammation, while BN rats develop neutrophilic and eosinophilic inflammation.[1] In a separate experiment, ovalbumin sensitization produced marked eosinophil infiltration into the lungs of BN rats while no pulmonary inflammation was observed in Lewis or Fisher rats.[10] When fed hexachlorobenzene for at least 21 days, BN rats develop extensive eosinophilic and granulomatous lung inflammation that correlated with airway hyperresponsiveness.[5]

Because of this unique immunologic response, the Brown Norway (BN) rat has served as a model for asthma in humans. BN rats also tend to develop a spontaneous eosinophilic and granulomatous pneumonia that is most frequently observed in young females. The lesion consists of epitheliod cells and numerous multinucleated giant cells admixed with an eosinophilic inflammatory infiltrate. To date the pathogenesis had not been discovered and no infectious agent has been identified.[9]

AFIP Diagnosis: Lung: Pneumonia, granulomatous and eosinophilic, multifocal, moderate, Brown Norway rat (*Rattus norvegicus*), rodent.

Conference Comment: The Brown Norway rat strain is widely used as an animal model for allergic asthma because they share many immunological and physical responses seen in human asthma, such as high production of IgE antibody, contraction of airway smooth muscle, airway hyperresponsiveness, involvement of leukotrienes in lung reactions, and infiltration of eosinophils and lymphocytes into the airway.[8] Untreated Brown Norway rats have also been reported to have a high incidence of eosinophilic granulomatous pneumonia.[6] These lesions are usually seen after 7 weeks of age and do not appear to be related to foreign material, fungi, or bacteria.[4,7]

In the Brown Norway strain, submucosal glands are larger and more numerous, particularly in the middle and lower trachea, as compared to F344, but the histochemical nature of the mucin produced by these cells is the same. The relationship between the quantitative difference in the number of submucosal glands and the Brown Norway rat's predisposition for hyperresponsiveness to allergens is still under investigation.[6] It has been proposed that mucosal IgA and interleukin-8 stimulate the activity of eosinophils.[2,11] Following exposure to 1% ovalbumin (OVA) solution, Brown Norway rats exhibited increased levels of Th1- (IFN-γ and IL-2) and Th2-related cytokines (IL-4 and IL-5) and chemokines (eotaxin and MCP-1) as compared to exposed F344 rats.[8] Alveolar macrophages have been shown to produce significantly more nitric oxide, IL-10 and TNF when exposed to ovalbumin than similarly challenged Sprague-Dawley rats, indicating that alveolar macrophages have a role in expanding the Th2 response in allergic inflammation.[1]

Other strain-specific responses of the Brown Norway rat include a high susceptibility to Th2-mediated autoimmune disease such as mercury ($HgCl_2$)-induced autoimmune glomerulonephritis. They have a vigorous Th2 immune response, producing cytokines IL-4, IL-6, and IL-10, along with antibody isotypes IgG1 and IgE on antigenic stimulation.[3]

Contributor: Walter Reed Institute of Research/Naval Medical Research Center
http://wrair-www.army.mil/
http://www.nmrc.navy.mil/

References:

1. Careau E, Sirois J, Bissonnette EY: Characterization of lung hyperresponsiveness, inflammation, and alveolar macrophage mediator production in allergy resistant and susceptible rats. Am J Respir Cell Mol Biol 26:579-586, 2002
2. Erger RA, Casale TB: Interleukin-8 is a potent mediator of eosinophil chemotaxis through endothelium and epithelium. Am J Physiol 268:L117-L122, 1995
3. Jennings VM, Dillehay DL: Immunology. In: The Laboratory Rat, eds. Suckow MA, Weisbroth SH, Franklin CL, 2nd ed., p. 854. Elsevier Inc., New York, NY, 2006
4. Kohn DF, Clifford CB: Biology and diseases of rats. In: Laboratory Animal Medicine, eds. Fox JG, Anderson LC, Loew FM, Quimby FW, 2nd ed., p. 156. Elsevier Science, San Diego, CA, 2002
5. Michielsen CP, Leusink-Muis A, Vos JG, Bloksma N: Hexachlorobenzene-induced eosinophilic and granulomatous lung inflammation is associated with in vivo airways hyperresponsiveness in the Brown Norway rat. Toxicol Appl Pharmacol 172:11-20, 2001
6. Ohtsuka R, Doi K, Itagaki S: Histological characteristics of respiratory system in Brown Norway rat. Exp Anim 46:127-133, 1997
7. Ohtsuka R, Doi K: Environmental effect on eosinophilic granulomatous pneumonia (EGP) in Brown Norway rats. J Toxicol Pathol 16:129-131, 2003
8. Ohtsuka R, Shutoh Y, Fujie H, Yamaguchi S, Takeda M, Harada T, Doi K: Changes in histology and expression of cytokines and chemokines in the rat lung follow-

ing exposure to ovalbumin. Exper Toxicol Pathol 56:361-368, 2005
9. Percy DH, Barthold SW: Rat. In: Pathology of Laboratory Rodents and Rabbits, 3rd ed., pp. 125-177121. Blackwell Publishing, Ames, IA, 2007
10. Schneider T, van Velszen D, Moqbel R, Issekutz AC: Kinetics and quantitation of eosinophil and neutrophil recruitment to allergic lung inflammation in a Brown Norway rat model. Am J Respir Cell Mol Biol 17:702-712, 1997
11. Shute JK, Lindley I, Peichl P, Holgate ST, Church MK, Djukanović R: Mucosal IgA is an important moderator of eosinophil responses to tissue-derived chemoattractants. Int Arch Allergy Immunol 107:340-341, 1995

CASE III – 03-27 (AFIP 2936161).

Signalment: A wild-caught, male, owl monkey (*Aotus vociferans*)

History: This owl monkey was found dead in its cage. The animal was involved in a malarial study. A splenectomy had been performed and the monkey had received two inoculations of *Plasmodium falciparum* two weeks apart approximately one month before its death.

Gross Pathology: The abdominal cavity contained 2mL of clear yellow fluid. The left and right kidneys revealed a pale tan surface. The left kidney was small (2.42g) and had a bumpy cortical surface compared to the right kidney (5.87g). *The average weight of a normal owl monkey kidney is 4-5g.*[1] All other gross findings were within normal limits.

Laboratory Results: Abdominal cavity fluid specific gravity: 0g/dl

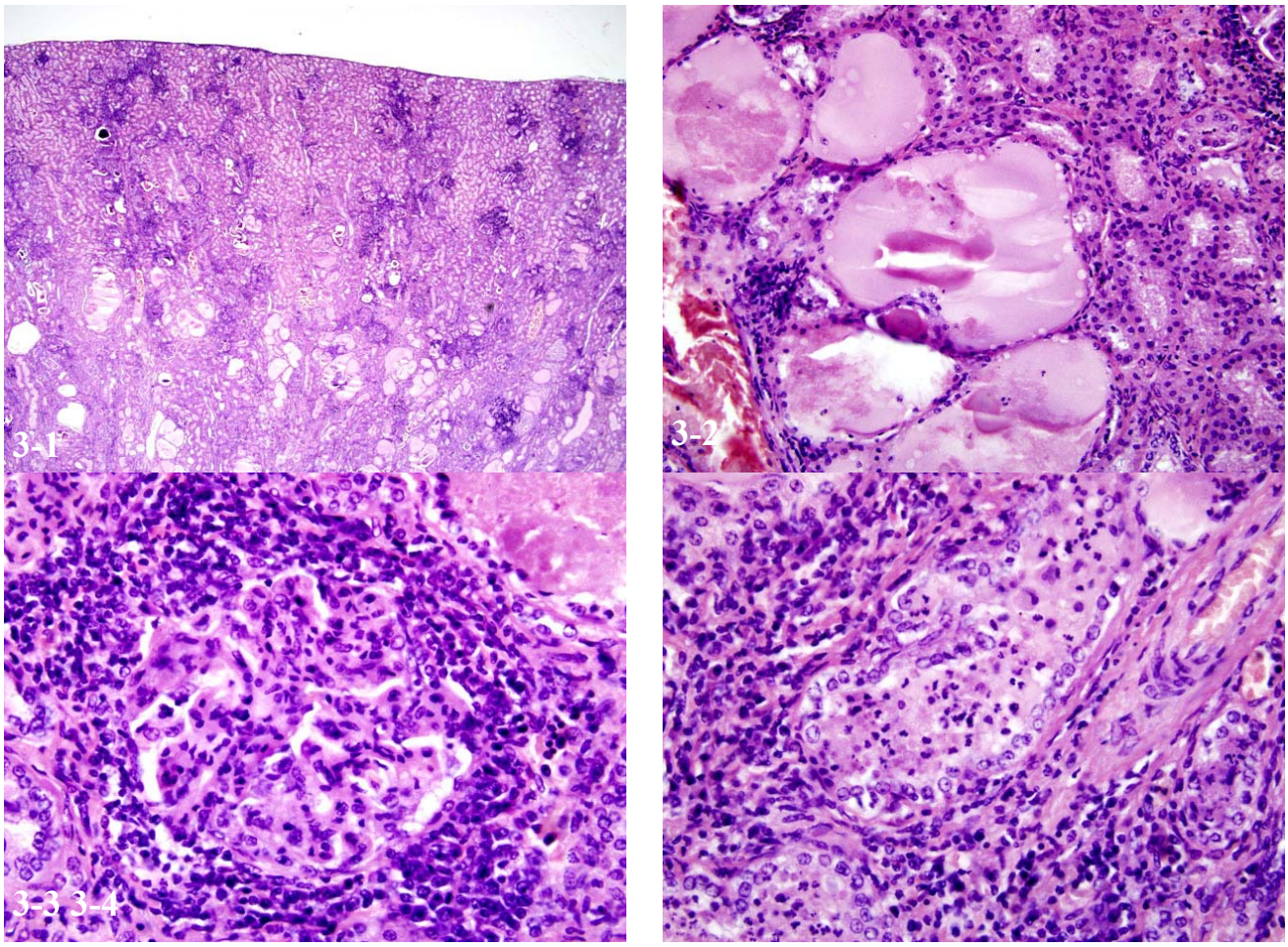
Contributor's Morphologic Diagnoses:
1) Kidney, nephritis, interstitial **(Fig. 3-1)**, lymphocytic, multifocal, severe, owl monkey (*Aotus vociferans*), non-human primate.
2) Kidney, tubular ectasia **(Fig. 3-2)**, diffuse, severe with glomerulosclerosis and membranous glomerulopathy with mesangial proliferation **(Fig. 3-3)**, multifocal, moderate.

Contributor's Comment: Malarial infection continues to be a leading cause of morbidity and mortality in the world today. The importance of this disease has led to a great amount of research within the field. A murine model using a rodent malarial protozoa, *Plasmodium berghei*, was once used as an animal model for human malarial studies, but there was lack of evidence for similarity to the human disease.[1] The owl monkey is now the gold standard animal model for malarial studies. There are four species of human malarial parasites: *Plasmodium falciparum, P. vivax, P. malariae, and P. ovale*.[2] The splenectomized *Aotus* monkey is susceptible to all but the latter and is used to test new antimalarial drugs and vaccines and to study mechanisms of immunity and immunopathology of the disease.[1]

The relationship between malaria and renal disease has been long understood and was first described in a malarial patient with edema in 1905.[1] There are two major renal syndromes associated with malarial infection. The first is a chronic, progressive glomerulopathy most common in children in Africa with *P. malariae* infection and the other an acute renal failure associated with falciparum malaria in Southeast Asia, India, and sub-Saharan Africa.[2] Falciparum malaria can also be associated with a severe disease in humans characterized by intravascular hemolysis, hemoglobinurea, acute tubular necrosis, and renal failure and is termed blackwater fever.

Although there are differences between *P. malariae* and *P. falciparum* infection in humans, the histological changes within the kidney of experimentally infected owl monkeys can be similar. Histological features include glomerular hypercellularity, infiltration of polymorphonuclear leukocytes, and thickening of the glomerular basement membrane and mesangium.[1,5,6] Immunofluorescent microscopy reveals IgG, IgA, IgM, and C3 within the mesangium and along the basement membrane and *Plasmodium sp.* antigens and corresponding antibodies.[1,5,6] Interstitial inflammation is a common finding in inoculated monkeys.

A condition exists that complicates studying malarial associated renal disease and nephrotic syndrome in owl monkeys. Owl monkeys commonly have spontaneous renal disease.[1,3,8] Renal disease such as glomerulonephropathies and interstitial nephritis are some of the most common causes of death in owl monkeys. The cause is unknown, but it has been speculated that it may be due to immune responses to infectious agents in their natural habitat of Central and South America. Histologically, lesions are characterized by increased mesangium, glomerulosclerosis, and interstitial nephritis with lymphocytic, eosinophilic, and plasma cell infiltration.[1,3]



3-1. Kidney, Owl monkey. Multifocal interstitial nephritis and tubular ectasia. (HE 20X)
3-2. Kidney, Owl monkey. Tubules are ectatic, lined by attenuated epithelium and contain eosinophilic homogenous material (proteinosis). (HE 400X)
3-3. Kidney, Owl monkey. Diffusely, glomeruli are often surrounded by high numbers of lymphocytes, few histiocytes and neutrophils; glomerular tufts are hypercellular with increased mesangium. (HE 400X)
3-4. Kidney, Owl monkey. Multifocally, tubular epithelium is often disrupted and necrotic characterized by shrunken, eosinophilic cytoplasm with pyknotic nuclei. (HE 400X)

AFIP Dia gnosis: Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, severe, with multifocal tubular degeneration and necrosis **(Fig. 3-4)**, suppurative tubulitis, tubular ectasia and proteinosis, and lymphoplasmacytic interstitial nephritis, owl monkey (*Aotus vociferans*), primate.

Conference Comment: The contributor gives an excellent review of *Plasmodium* infection in owl monkeys and the confounding lesions seen in that particular animal model. *Plasmodium* are intracellular protozoan parasites in the family of Plasmodiidae that can affect a wide rage of mammals, birds, and reptiles.[7,10] *Hemoproteus*, *Leucocytozoon*, and *Hepatocystis* are other members of the family Plasmodiidae.

Diagnosis may be made on a blood smear stained with Giemsa or Wright-Giemsa stains. Hemazoin, a brownish malarial pigment formed from the incomplete catabolism of hemoglobin by the parasite[7], can be seen in Kupffer cells of the liver, within macrophages in the bone marrow, and in the red pulp of the spleen.[10]

The life cycle[4, 9] consists of an infected mosquito feeding on a susceptible host injecting sporozoites into the host circulation. These sporozoites invade hepatocytes by binding hepatocyte receptors for the serum proteins thrombospondin and properdin.[9] They develop in hepatocytes to form schizonts that release up to 30,000 merozoites when the hepatocytes rupture. Merozoites enter erythrocytes by binding a parasite lectinlike molecule to sialic residues on glycophorin molecules on the surface of red blood cells.[9] There, they reside within a parasitophorous vacuole, until it enlarges into a trophozoite stage, divides into schizonts containing 24 or more merozoites, and lyses the erythrocyte to then infect other RBCs. A few merozoites will develop into gametocytes (both micro- and macrogametocytes) which are taken up by a mosquito during a blood meal. Within the mosquito, the gametocytes develop into gametes, forming a motile zygote. Through repeated nuclear divisions, the oocyst ruptures releasing sporozoites into the hemolymph, where they then migrate to the salivary gland for injection into a susceptible vertebrate host.

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References:

1. Aikawa M, Broderson JR, Igarshi I, Jacobs G, Pappaioanou M, Collins W, Campbell C: An Atlas of Renal Disease in *Aotus* Monkeys with Experimental Plasmodial Infection, pp 1-22. American Institute of Biological Sciences, Arlington, VA, 1988
2. Barsoum RS: Malarial acute renal failure. J Am Soc Nephrol 11:2147-2154, 2000
3. Chalifoux LV, Bronson RT, Sehgal P, Blake BJ, King NW: Nephritis and hemolytic anemia in owl monkeys (*Aotus trivirgatus*). Vet Pathol 18(S6):23-37, 1981
4. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, pp 65-66. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC 1998
5. Hutt MSR, Davies DR, Voller A: Malarial infections in *Aotus trivirgatus* with special reference to renal pathology. Br J Exp Path 56:429-438, 1975
6. Iseki M, Broderson JR, Pirl KG, Igarashi I, Collins W, Aikawa M: Renal pathology in owl monkeys in *Plamodium falciparum* vaccine trials. Am J Trop Med Hyg 43:130-138,1990
7. Jones TC, Hunt RD, and Norval WK: Veterinary Pathology, pp. 590-593. Williams and Wilkins, Baltimore, MD 1997
8. Roberts JA, Ford EW, Southers JL: Urogential system. In: Nonhuman Primates in Biomedical Research, Diseases, eds. Bennett BT, Abee, CR, Henrickson R, pp. 311-333. Academic Press, San Diego, CA, 1998
9. Samuelson J: Infectious diseases. In: Robbins Pathologic Basis of Disease, eds. Cotran RS, Kumar V, Collins T, 6th ed., pp. 389-391. WB Saunders Company, Philadelphia, PA 1999
10. Toft JD, Eberhard ML: Parasitic diseases. In: Nonhuman Primates in Biomedical Research: Diseases, ed. Bennett BT, Abee CR, Henrickson R, pp.124-126, 249. Academic Press, San Diego, CA, 1998

CASE IV - 2005-126 (AFIP 3050843).

Signalment: A 2.8-year-old, female, pig-tailed macaque (*Macaca nemestrina*)

History: Tissue from a 2.8 year old female *M. nemestrina* which underwent experimental total body irradiation (TBI) and bone marrow reconstitution 11 months prior. The animal presented with mild dermal hyperemia in the axillary region. Over the subsequent 24 hours, lesions progressed to cover the trunk, face, and extremities. At physical examination, the animal was hyperthermic; oral ulcerations were noted. A skin scraping demonstrated suppurative inflammation. Serology was negative for Mumps, Measles, Monkey pox, Cercopithecine Herpes virus-1, and Simian Varicella Virus. Despite aggressive medical management, the clinical condition remained unchanged for the subsequent 48 hours. Ninety-six hours after initial presentation, the animal became lethargic and refused oral medications and food; euthanasia was elected.

Gross Pathology:
1. Diffuse, vesiculoulcerative and pustular dermatitis
2. Diffuse necroulcerative and hemorrhagic gastroenterocolitis
3. Multifocal splenic, hepatic, and renal necrosis and hemorrhage

Laboratory Results: Serology obtained at necropsy demonstrated antibodies to Simian Varicella Virus (SVV). Skin and pustule fluid were positive for SVV by PCR.

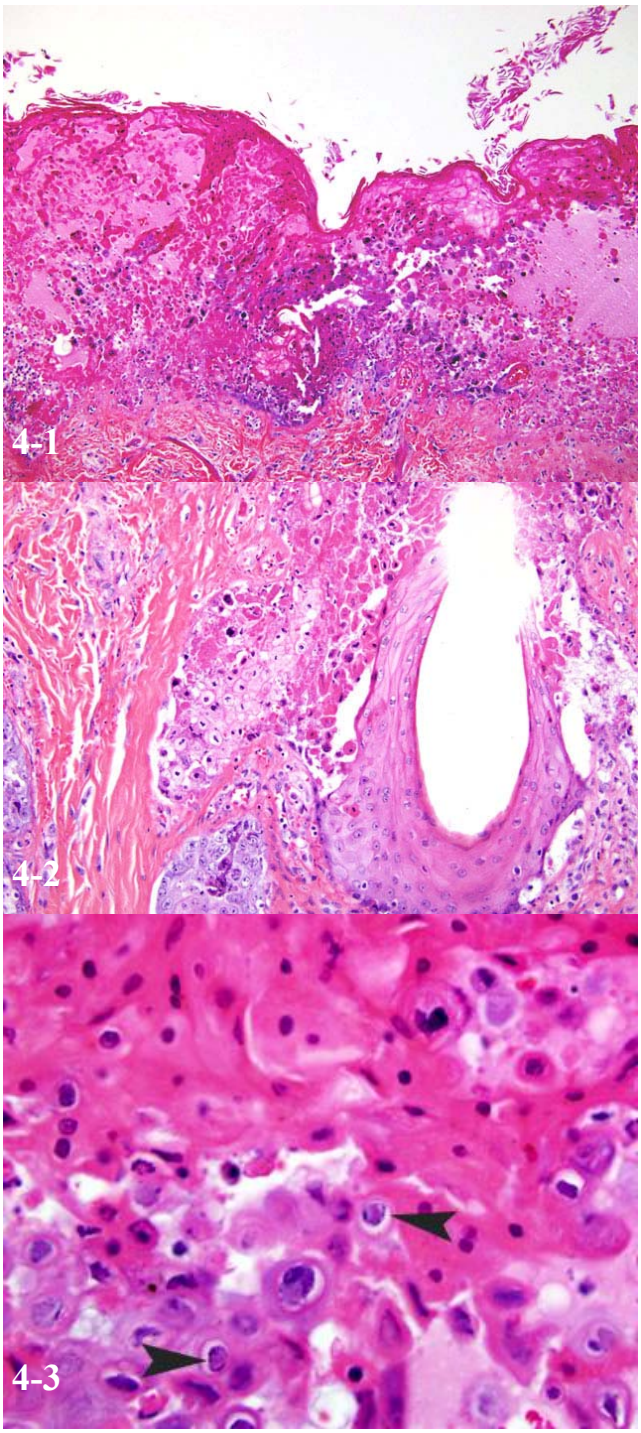
Histopathologic D escription: Buccal skin and abdominal skin were submitted. The integrity of the epidermis is

disrupted by multiple vesicles, pustules, and ulcerations. Multifocally, pustules and vesicles contain viable and degenerate neutrophils, lymphocytes, plasma cells, and variable amounts of homogenous eosinophilic material, cellular and karyorrhectic debris. Occasional vesicles and pustules are intact; most vesicles have lost the surface epithelium (dermal ulcerations) and are covered by serosanguinous debris. Multifocally adjacent epithelial cells are swollen and contain abundant pale eosinophilic, homogenous, sometimes vacuolated, cytoplasm (intracellular edema), occasional "glassy" basophilic intranuclear inclusions, and are separated by clear space that accentuates intercellular bridges (intercellular edema; spongiosis).

Contributor's Mor phologic Diagn osis: Multifocal to diffuse, severe, necroulcerative and vesicular suppurative dermatitis **(Fig. 4- 1, 4-2)** with intraepithelial herpetic inclusion bodies **(Fig. 4-3)**

Contributor's Comment: Submitted tissue is from one of two cases of SVV occurring in *M. nemestrina* at the WaNPRC in 2005. Both animals had similar signalments (TBI 11-15 months prior) and clinical presentations. Both cases were housed in same room for greater than 6 months prior to presentation. Four weeks prior to clinical presentation, animals from domestic quarantine were co-housed with the two clinical cases.

Simian Varicella Virus (SVV) is a naturally occurring herpes virus of Old World Primates characterized by fever, vesicular skin lesions, hemorrhagic ulceration throughout the gastrointestinal tract, and multifocal hemorrhagic necrosis of the liver, spleen, lymph nodes, and endocrine organs.[4,6] SVV is responsible for sporadic epizootics in biomedical research facilities.[1-3,5-8,10] Simian Varicella is antigenically related to Varicella Zoster Virus (VZV) in man; the two viruses must be distinguished from one another through PCR (serology from this animal was negative for VZV by PCR and nested PCR). Both SVV and VZV demonstrate a high incidence of asymptomatic or mild disease with seroconversion in immunocompetent animals. A colony survey performed at the WaNPRC demonstrated a 20% incidence of SVV antibodies within *M. nemestrina*.



4-1. Lip, macaque. Within the epidermis there are areas of epithelial necrosis and degeneration. (HE 400X)
4-2. Lip, macaque. Multifocally, there is necrosis of the follicular epithelium and adjacent sebaceous glands. (HE 400 X)
4-3. Lip, macaque. Within areas of epithelial necrosis, there are oval, 4-6 micron, eosinophilic intranuclear inclusion bodies which often peripheralize the chromatin (arrowheads). (HE 400X)

The reason for overt clinical disease in these two animals remains unclear. Both VZV and SVV may induce symptomatic disease with immunosuppression; VZV is an important pathogen in man post TBI and chemotherapy. It is hypothesized that the stress of new animal introductions into an established housing room may have precipitated viral shedding in seropositive animal(s). Despite normal blood profiles, the TBI animals may not have been fully immunocompetent, and thus more susceptible to systemic disease.

AFIP Diagnosis: Haired skin and oral mucosa: Dermatitis and stomatitis, necroulcerative, neutrophilic and lymphoplasmacytic, multifocal, marked, with vesiculopustules, epithelial dyscohesion, syncytia, and intranuclear inclusion bodies, pig-tailed macaque (*Macaca nemestrina*), primate.

Conference Comment: Simian varicella virus (SVV) is a naturally occurring disease in non-human primates that closely resembles human varicella (Varicella-zoster virus) infections. SVV is an alphaherpesvirus, classified as a single species (Cercopithecine herpesvirus 9) within the Varicellovirus genus.[9]

Natural transmission is primarily through inhalation and/or direct contact with infected skin lesions. The virus is disseminated throughout the body during a transient viremia that clears by day 11 post-infection. SVV DNA has been detected within B and T cells, but not monocytes.[4] The virus becomes latent in the neural ganglia, although infectious virus has not yet been recovered from these sites. This could indicate an unknown intermediate stage between acute infection and latency. SVV DNA has been identified in cervical, trigeminal, thoracic and lumbar ganglia, but not in the brain, liver or lung tissues of naturally infected monkeys.[4]

Clinical signs, which occur 10-15 days following inoculation, usually consist of a skin rash starting in the inguinal area and becoming generalized over the course of 48 hours, developing into macules, papules, and vesicles. Areas of the skin affected include the face, thorax, and abdomen, but not the palms or soles.[4] Symptoms begin to subside by day 14 post-infection. Lesions can range from mild and easily overlooked to severe infection resulting in pneumonia, hepatitis, and death. Reactivation following latency may occur spontaneously or in response to immune suppression or stress months to years after the initial infection.[4]

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References:

1. Blakely GA, Lourie B, Morton WG, Evans HH, Kaufmann AF: A varicella-like disease in macaque monkeys. J Infect Dis 127:617-625, 1973
2. Dueland AN, Martin JR, Devlin ME, Wellish M, Mahalingam R, Cohrs R, Soike KF, Gilden DH: Acute simian varicella infection. Clinical, laboratory, pathologic, and virologic features. Lab Invest 66:762-773, 1992
3. Gard EA, London WT: Clinical history and viral characterization of Delta herpesvirus infection in a Patas monkey colony. In: Viral and Immunological Diseases in Nonhuman Primates, p. 211-212. Alan R. Liss, Inc., New York, NY, 1983
4. Gray WL: Simian varicella: a model for human varicella-zoster virus infections. Rev Med Virol 14:363-81, 2004
5. Mahalingam R, Traina-Dorge V, Wellish M, Smith J, Gilden DH: Naturally acquired simian varicella virus infection in African green monkeys. J Virol 76:8548-8550, 2002
6. Roberts ED, Baskin GB, Soike K, Gibson SV: Pathologic changes of experimental simian varicella (Delta herpesvirus) infection in African green monkeys (*Cercopithecus aethiops*). Am J Vet Res 45:523-530, 1984.
7. Schmidt NJ, Arvin AM, Martin DP, Gard EA: Serological investigation of an outbreak of simian varicella in *Erythrocebus patas* monkeys. J Clin Microbiol 18:901-904, 1983
8. Treuting PM, Johnson-Delaney C, Birkebak TA: Diagnostic exercise: vesicular epidermal rash, mucosal ulcerations, and hepatic necrosis in a cynomolgus monkey (*Macaca fascicularis*). Lab Anim Sci 48:384-386, 1998
9. van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB: Virus taxonomy: the classification and nomenclature of viruses. In: The Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, CA, 2000
10. Wenner HA, Abel D, Barrick S, Seshumurty P: Clinical and pathogenetic studies of Medical Lake macaque virus infections in cynomolgus monkeys (simian varicella). J Infect Dis 135:611-622, 1977

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