The Armed Forces Institute of Pathology
Department of Veterinary Pathology
Wednesday Slide Conference
2010-2011
Conference 25
23 March 2011

Conference Moderator:
JoLynne Raymond, DVM, Diplomate ACVP

CASE I: 03N2865 (AFIP 2936141).

Signalment: 10-month-old, female, Siamese, feline (*Felis catus*).

History: Previous history includes persistently thin body condition despite a voracious appetite. This young cat presented to the VMTH Emergency Service after being found obtunded and seizuring by the owners. On physical examination the cat exhibited generalized seizures and was soaked in urine. The cat was hypothermic (84.5°F), dehydrated (10%), and had weak femoral pulses. Initial diagnostic procedures revealed metabolic and respiratory acidosis with elevated blood lactate. Urine specific gravity was 1.010. Seizures were controlled with IV phenobarbital; however, the cat went into respiratory and cardiovascular arrest three hours after presentation and resuscitation was unsuccessful.

Gross Pathology: On post-mortem examination, there was severe, segmental, nodular thickening of medium-sized blood vessels throughout the mesentery. Intercostal vessels and the coronary arteries along the coronary and interventricular groove were similarly affected. Multiple triangular depressions were noted bilaterally in the renal cortices that extended to a point at the corticomedullary junction. On cut section of the kidneys, the arcuate blood vessels were prominent and the vessel walls were markedly thickened. Lymphoid hyperplasia was noted in the mesenteric lymph nodes and duodenal mucosal lymphoid patches.

Laboratory Results:
Body Temp: 96.0°F.
PH: 7.015.
CO_2 total: 18.1.
Glucose: 60 mg/dL.
Aerobic and anaerobic culture of the lung yielded no significant growth.

Histopathologic Description: Kidney: In the examined sections of kidney, there is a severe proliferative and inflammatory process affecting medium and small arteries, primarily at the corticomedullary junction and hilus. In affected vessels the tunica media and intima are markedly expanded by increased numbers of plump spindloid cells (smooth muscle cells and fibroblasts), greatly reducing or obstructing the vessel lumen. The connective tissue and smooth muscle in the vessel walls are hyalinized and separated by wispy basophilic material, hemorrhage, brightly eosinophilic fibrillar material (fibrin/fibrinoid necrosis), and occasional globular, amorphous eosinophilic deposits (amyloid via Congo red staining). The internal elastic lamina is often irregular or split, endothelial cells are plump and reactive, and there are profiles of small arterioles in the tunica media of severely affected vessels. There is a variable inflammatory infiltrate within and surrounding affected vessels characterized by moderate numbers of plasma cells and lymphocytes, with fewer neutrophils, eosinophils and mast cells. Clusters of hemosiderin-laden macrophages are within and surrounding many affected vessels. There are several wedge-shaped regions of intense interstitial inflammation and fibrosis extending from the corticomedullary junction. In these areas the inflammation is comprised of lymphocytes, plasma cells, and fewer eosinophils and dense bands of fibrous connective tissue surround remaining tubules and variably sclerotic glomeruli. Prominent lymphoid follicles with central necrosis surround blood vessels throughout the sections.

Contributor's Morphologic Diagnosis: 1. Kidney, small and medium arteries: Severe, segmental, chronic-ongoing, necrotizing and proliferative arteritis
2. Kidneys: Multifocal subacute infarction and multifocal severe lymphoplasmacytic interstitial nephritis.

3. Kidneys Marked perivascular lymphoid hyperplasia with lympholysis.

Contributor's Comment: The number of tissues affected by the vasculopathy in this cat was extensive and included the arcuate arteries in the kidney, the coronary arteries, the mesenteric and serosal arteries, the cervical arteries adjacent to the thyroid gland, and the intercostals arteries. In all affected tissues the arteriopathy was characterized by extensive intimal and medial smooth muscle hyperplasia and fibrosis, segmental fibrinoid necrosis, variable non-suppurative arteritis and segmental amyloidosis. As small arteries in the brain were similarly affected, the seizures and eventual death in this cat were attributed to vascular compromise and hypoxia in the brain and heart. Amyloid deposition was noted in many tissues, including the spleen, thyroid gland, liver, stomach and intestinal mucosa and affected vasculature. The amyloid was confirmed to be type AA, or reactive amyloid, via potassium permanganate staining and was attributed to long-standing vascular inflammation. Marked lymphoid hyperplasia, with lymphoid necrosis and lympholysis, was also a prominent finding in many lymphoid and parenchymal organs, including the kidneys. These changes are reminiscent of lymphoid tissue response in viral infections, such as Type D retroviral infection in non-human primates[7] and feline immunodeficiency virus (FlV) in cats.[11]

The severe vascular lesions in this cat resemble those found in polyarteritis nodosa in humans and other animal species.[5,13] Classic polyarteritis nodosa is characterized by segmental, transmural, necrotizing inflammation of medium and small muscular arteries. The most commonly affected blood vessels are in the kidneys, heart, liver, and gastrointestinal tract, sparing the pulmonary circulation, similar to the distribution of lesions in this cat. Affected vessels are in varying stages of necrosis, and in chronic stages there is marked fibrous thickening of the vessel walls creating a nodular appearance to affected vasculature. In humans, segmental erosion and weakening of arterial walls can lead to aneurysmal dilatation and rupture. It is hypothesized that an inflammatory stimulus (autoimmune disease, viral, bacterial, fungal or protozoal infection) cites the vascular lesions; however, no one etiology has been implicated as a definitive cause of the disease in either humans or animal species. In humans, polyarteritis nodosa is associated with hepatitis B virus in approximately 30% of cases, and is rarely associated with anti-neutrophil cytoplasmic antibodies (ANCA).[13] There are several older reports of proliferative vascular lesions in cats; however, in all of these reports an underlying etiology for the vasculopathy was not determined.[1,4] A proliferative arteriopathy has also been described in cows affected by malignant catarrhal fever[10] and in non-human primates with simian immunodeficiency virus (SlV) infection.[3] Polyarteritis nodosa has also been reported in beagle dogs[14] and laboratory rats;[12] however, the etiology remains unknown.

Numerous histochemical stains (Giemsa, PAS, acid fast, Brown and Benn, and GMS) were completed on affected tissues in this cat in an attempt to identify possible infectious agents; however, all stains were negative. Immunohistochemical stains were also completed on affected tissue using antibodies against *Sarcocysis neurona*, *Neospora* spp., *Toxoplasma gondii*, feline enteric coronavirus (FlP), feline leukemia virus (FeLV), and bacillus of Calmette-Guerin (BCG). Antibodies against IgG (1:2000 dilution) were not immunoreactive in these sections. Other antiboidies against other immunoglobulins were not available. In sections of the thymus, spleen, and bone marrow a few lymphoid cells expressed FeLV antigen. As the number of positive staining cells was minimal and the FeLV status is unknown for this cat, it is not possible to definitively determine whether the positive staining is indicative of viral infection. All other stains were negative. A section of kidney submitted for PCR analysis for FeLV, feline immunodeficiency virus (FlV), and feline herpesvirus-1 was negative. Serum was not available for diagnostic procedures.

AFIP Diagnosis: 1. Kidney, medium and small arteries: Arteritis, proliferative and necrotizing, multifocal, marked with periarterial lymphoid hyperplasia
2. Kidney: Nephritis, cortical, interstitial, lymphoplasmacytic, multifocal to coalescing, moderate with synechiae, senescent glomeruli, and periglomerular fibrosis.

Conference Comment: Participants engaged in discussion of the pathogenesis of immune-mediated arteritis. The authors of *Robbins and Cotran Pathologic Basis of Disease* describe three phases in type III hypersensitivity.[6] The first phase involves generation of antigen-antibody complexes around one week after antigen exposure. Secreted antibody binds to antigen present in the systemic circulation. Antigen-antibody complexes are deposited in a variety of tissues during the second phase. The final phase involves immune-mediated damage to the affected tissues via immune complex binding and complement fixation; these complexes are recognized by neutrophil Fc and complement C3b receptors. Release of neutrophil contents results in local tissue damage, necrosis and subsequent inflammation. As the vascular inflammatory process progresses, neutrophils are replaced by mononuclear leukocytes. In many cases, antigen complexes are rapidly cleared from the lesion, and therefore are not detected

during testing for the presence of IgG and/or IgM; thus, failure to detect immunoglobulin does not exclude an immune-mediated process.[6]

In response to the immune-mediated inflammation and damage, the vasculature must begin the reparative process. The two cell types involved in vascular repair are the endothelial cells and vascular smooth muscle cells of which the smooth muscle cells are most prominent. Regulation of smooth muscle proliferation is accomplished through a balance of promoters and inhibitors of proliferation. Platelet-derived growth factor, thrombin, fibroblast growth factor, IFN-γ, and IL-1 promote proliferation, while heparin sulfate, nitric oxide, and TGF-β are inhibitory.[9] Vascular injury with endothelial damage or loss stimulates smooth muscle cell proliferation. The increase in smooth muscle cells results from recruitment from the tunica media; recruitment and differentiation of smooth muscle precursor cells; and proliferation of local smooth muscle cells through mitosis.[9] In addition to proliferation, smooth muscle cells also produce extracellular matrix, much the same as occurs in wound healing, resulting in a "scar" of fibrous connective tissue and smooth muscle cells.[8]

Conference participants reviewed some of the diseases resulting in proliferative arteritis in veterinary species. [8]

- Cow: Malignant catarrhal fever (ovine herpesvirus 2)
- Rat: Polyarteritis nodosa
- Non-human primates: Associated with Simian immunodeficiency virus in macaques
- Dog: Beagle pain syndrome
- Mink: Aleutian disease of mink

Contributor: Veterinary Medical Teaching Hospital (VMTH), Anatomic Pathology, University of California, Davis

References:

1. Altera KP, Bonasch H. Periarteritis nodosa in a cat. *J Am Vet Med Assoc*. 1966;149:1307-1311.
2. Campbell LH, Fox JG, Drake DF. Ocular and other manifestations of periarteritis nodosa in a cat. . *J Am Vet Med Assoc*. 1972;161:1122-1126.
3. Chalifoux LV, Simon MA, Pauley DR, MacKey JJ, Wyand MS, Ringler DJ. Arteriopathy in macaques infected with simian immunodeficiency virus. *Lab Invest.* 1992;67:338-349.
4. Curtis R, Laing PW. Polyarteritis in a cat. *Vet Rec*. 1979;105:354.
5. Jubb KVF, Kennedy PC, Palmer N. *Pathology of Domestic Animals*. 4th ed. Vol. 3. San Diego, CA: Academic Press Inc; 1993:67-68.
6. Kumar V, Abbas AK, Fausto N, Aster JC. Diseases of the immune system. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:204-205
7. Lowenstin LJ. Type D retroviral infection, macaques. In: Jones TC, Mohr U, Hunt RD, eds. *Nonhuman primates I*, Berlin, Germany: Springer-Verlag; 1993:20-32.
8. Maxie MG, Robinson WF. Cardiovascular system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 1, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:69-71.
9. Mitchell RN, Schoen FJ. Blood vessels. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:490-492.
10. O'Toole D, Li H, Roberts S, et al. Chronic generalized obliterative arteriopathy in cattle: A sequel to sheep-associated malignant catarrhal fever. *J Vet Diagn Invest.* 1995;7:108-121.
11. Parodi AL, Femenia F, Morillon A, Crespeau F and Fontaine JJ. Histopathological changes in lymph nodes of cats experimentally infected with the feline immunodeficiency virus. *J Comp Pathol.* 1994;111(2):165-174.
12. Percy DH, Barthold SW. *Pathology of Laboratory Rodents and Rabbits*. 2nd ed. Ames, IA: Iowa State University Press; 2001:153-154.
13. Schoen FJ, Cotran RS. Blood vessels. In: Cotran R S, Kumar V, Collins T, eds. *Robbins Pathologic Basis of Disease.* 6th ed. Philadelphia, PA: W.B. Saunders; 1999:515-521.
14. Synder PW, Kazacos EA, Scott-Moncrieff JC, et al. Pathologic features of naturally occurring juvenile polyarteritis in beagle dogs. *Vet Pathol.* 1995;32:337-345.

CASE II: 09-093 (AFIP 3162471).

Signalment: 5-month-old, male, New Zealand white, rabbit (*Oryctolagus cuniculi*).

History: This animal had not been used for any experimental purposes. On a Saturday, the animal was noted to have bilateral mucopurulent nasal discharge, lethargy, dehydration, and an unkempt coat with fecal and urine staining. A culture of the nasal exudate was taken, and the animal was empirically started on parenteral enrofloxacin. On Monday, the animal was anesthetized with intramuscular ketamine and xylazine and euthanatized with intravenous pentobarbital.

Gross Pathology: The forepaws and muzzle area were stained with a moderate amount of dried yellow purulent exudate apparently originating from the nares. Nares were crusted over with mucopurulent discharge. Bilaterally, the nasal cavities contained approximately 0.5 mL viscous creamy suppurative exudate each, with mild atrophy of the nasal turbinates. Neither tympanic bulla contained any exudate or evidence of inflammation.

In the thoracic cavity the cranioventral lung lobes, particularly the left cranial and middle lobes, were affected by multifocal to coalescing areas of necrosuppurative inflammation with extension to the pleura. No exudate was noted within the trachea. A culture was taken from the lung parenchyma. The caudodorsal lung lobes were noted to be slightly enlarged and firm with rib impressions. There was no evidence of infection or inflammation in the brain or meninges.

Laboratory Results:

1. Nasal culture: 4+ *Bordetella bronchiseptica*; 3+ *Pasteurella* spp. (not *P. multocida*); 3+ coagulase negative *Staphylococcus*; 2+ non-hemolytic *Streptococcus*; 2+ microaerophilic *Streptococcus*; 1+ *Actinobacter* species.
2. Lung culture: No growth.
3. Serology was negative for *Pasteurella multocida*.

Histopathologic Description: Lung: There are two discrete pathologic processes present in the lung, with variable proportions on different slides. Filling the lumina of bronchi, bronchioles, and alveoli, and occasionally effacing normal alveolar architecture, are large numbers of predominantly degenerate heterophils admixed with necrotic cellular debris and variable amounts of fibrillar eosinophilic proteinaceous material (fibrin). In some sections, there is focally extensive coagulative necrosis of the lung parenchyma with preservation of tissue architecture and hypereosinophilic ghost cells (infarction) as well as overt abscessation. The Brown and Hopps tissue Gram stain shows low to moderate numbers of intra-lesional Gram negative rods.

Multifocally to diffusely alveolar spaces are filled with flocculent eosinophilic proteinaceous material with low to moderate numbers of intra-alveolar macrophages, often with abundant foamy cytoplasm. Alveoli are lined by plump cuboidal epithelial cells, creating an adenomatous appearance (type II pneumocyte hyperplasia). Scattered sloughed necrotic pneumocytes with hypereosinophilic cytoplasm and pyknotic, karyorrhectic or karyolytic nuclei are present in alveoli. Within the clear spaces of the intra-alveolar proteinaceous material there are 1-2 um faintly basophilic coccoid organisms. These organisms are visualized with Wright-Giemsa, toluidine blue, and Gomori methenamine silver stains.

Low numbers of heterophils and eosinophils can be seen in the adventitia and intima of scattered small and medium pulmonary arteries. This is a commonly observed change in rabbits, and has been associated with the use of ketamine/xylazine anesthetic[4].

Contributor's Morphologic Diagnosis: 1. Lung, bronchopneumonia, necrosuppurative, subacute, multifocal, severe.
2. Lung, alveolitis, histiocytic, chronic, multifocal to coalescing, severe, with type II pneumocyte hyperplasia, eosinophilic intra-alveolar flocculent exudate and intra-lesional fungal trophic forms.

Contributor's Comment: Clinically, grossly, and histologically, this animal presented with the classic lesions of pasteurellosis (snuffles).[6] However, *Pasteurella multocida* was not isolated from the nasal culture, nor have routine deep nasal cultures of diagnostic rabbit necropsies at this institution yielded any animals positive for *P. multocida*. Additionally, this animal had negative titers for *P. multocida* in serum obtained before necropsy. *Bordetella bronchiseptica* was isolated in a mixed culture from the nasal exudate. This agent typically causes a less severe and more indolent disease in rabbits, characterized by a more purulent and less necrotizing pneumonic process.[6] It is suspected that the same factors allowing the overgrowth of *Pneumocystis oryctolagi* resulted in the more aggressive clinical course of *B. bronchiseptica* in this animal.

Pneumocystosis was an unexpected finding in this animal, as there were no experimental manipulations or known reasons for immunosuppression. *Pneumocystis oryctolagi* (formerly *P. carinii* f.sp. *oryctolagi*) specifically colonizes the type I alveolar epithelial cells (pneumocytes) of Old World rabbits, and Dei-Cas *et al.* provide an excellent review of this[4] and other *Pneumocystis* species.[1] *Pneumocystis* species are highly host adapted, and display genetic co-phylogeny/co-speciation with their hosts.[1] No significant cross-species colonizations have been reported to occur, even in SCID mice and nude rats. The trophic forms of *Pneumocystis* organisms have filopodia that penetrate host epithelial cell cytoplasm without disruption of the cell membrane or apparent alteration of host cell structure or function.[4] The ultrastructural characteristics of these filopodia are species-specific.
Rabbits have a characteristic and consistent age-dependent pattern of colonization with *P. oryctolagi*.[4,8] Although initial experiments in rabbits dating back to the 1950s used corticosteroids to visualize pneumocystosis, it was later observed that spontaneous subclinical pneumocystosis was histologically evident at the time of weaning (approximately 4-6 weeks of age). Transplacental infections may occur.[7] Colonization results in diffuse lung histology changes as well as serum biochemical abnormalities, but these changes spontaneously resolve within 3-4 weeks, such that very few organisms are detectable in 60 to 90 day old rabbits. This pattern of colonization is hypothesized to be due to the decline of maternal antibodies (passive immunity) prior to the acquisition of active immunity.[8] Spontaneous pneumocystosis in weanling rabbits typically presents a unique and characteristic histologic pattern wherein individual widely spaced organisms line the alveolar epithelium, in contrast to the typical appearance of pneumocystosis in mice, rats, non-human primates and man (as well as this case), where crowded organisms fill the alveolar lumen[3]. Additionally, the flocculent eosinophilic (honeycomb) proteinaceous exudate typical of pneumocystosis in other species is rarely seen in weanling rabbits. It should be noted that the age of this rabbit (5 months) is well beyond that of weaning.

Pneumocystis organisms have two distinct forms in tissue sections: the trophic form (formerly trophozoite); and the cystic form. Trophic forms are irregular in size and shape, 2-8 µm, with a well stained nucleus. Cystic forms are 4-7 µm with a thick cell wall. This cell wall remains unstained with Wright-Giemsa and H&E stains, creating a halo effect around the organism. The number of nuclei present varies from 1-8, depending upon maturity (8 in mature cysts, fewer in sporocyte stages).

Other described species of *Pneumocystis* include *P. murina* in mice, *P. carinii* and *P. wakefieldiae* in rats, and *P. jirovecii* in man. *Pneumocystis* has also been identified in non-human primates, ferrets, horses, pigs, shrews, and dogs[1,2].

AFIP Diagnosis: 1. Lung: Pneumonia, necrosuppurative, multifocal, moderate.
2. Lung: Pneumonia, interstitial, histiocytic, multifocal to coalescing, marked with type II pneumocyte hyperplasia and myriad intralesional fungal trophic forms, etiology consistent with *Pneumocystis* species.

Conference Comment: All conference participants readily diagnosed and agreed with necrosuppurative pneumonia; participants commented on section variability, with some slides having evidence of bronchopneumonia. Few participants recognized the additional histiocytic interstitial pneumonia associated with *Pneumocystis oryctolagi*. The moderator commented that the findings of eosinophilic flocculent material within alveoli and type II pneumocyte hyperplasia are highly suggestive of pneumocystosis, regardless of the host species. The moderator commented that rabbits are unique as an animal model of pneumocystosis because they can develop spontaneous disease without the administration of glucocorticoids, which is required in models utilizing mice, rats and ferrets. The contributor provides an excellent review of pneumocystosis in the rabbit, as well as a brief review of the disease in other veterinary species.

Bordetella bronchiseptica has several virulence factors which contribute to the development of disease. Upon colonization of the host, a group of genes regulated by the *Bordetella* virulence group (*bvg*) operon is expressed, particularly adhesion proteins such as filamentous hemagglutinin (FHA), pertactin, and fimbriae. Expression of virulence factors regulated by the *bvg* operon depends on environmental conditions; activation of virulence factors occurs at 37°C, whereas a temperature of 25°C or the presence of sulfate or nicotinic acid is inhibitory. The expression of adhesion proteins allows bacterial attachment to the ciliated epithelium of the respiratory tract, which is followed by another round of expression of *bvg*-regulated genes. These proteins are involved in motility, iron scavenging, and the activity of various enzymes like urease and phosphatases.[2]

Several exotoxins also are involved in the pathogenesis of primary *B. bronchiseptica* infection. One of the most important and destructive toxins is a hemolysin, adenylate cyclase, which is an RTX toxin with similar activity to the RTX toxin produced in bovine mannheimiosis and the Apx toxin of porcine pleuropneumonia. The toxin forms

pores in host leukocytes which allows the functional part of the adenylate cyclase to enter the host cell. This results in increased production of cyclic adenosine monophasphate and suppresses phagocytosis and the oxidative burst necessary for clearing the infection. There is also production of peptidoglycan-derived tracheal toxin which stimulates host cell nitric oxide production; nitric oxide is ciliostatic and induces apoptosis of respiratory epithelium. Bacterial FHA also binds to histiocyte complement receptors, resulting in uptake of the bacteria which subsequently survive by inhibiting the oxidative burst. *Bordetella bronchiseptica* bacteria also have the ability to secrete adenylate cyclase while in histiocytes, resulting in apoptosis.[2]

Participants reviewed causes of necrosuppurative pneumonia in other veterinary species.

- Pig: *Actinobacillus pleuropneumoniae*
- Ox, sheep: *Mannheimia haemolytica*
- Guinea pigs: *Bordetella bronchiseptica*
- Rat: CAR bacillus; *Corynebacterium kutscheri*
- Non-human primate: *Streptococcus pneumonia*; *Klebsiella pneumoniae*

Contributor: Department of Comparative Medicine, H054, Penn State Milton S. Hershey Medical Center, Penn State College of Medicine, 500 University Dr, Hershey, PA, 17033-0850
http://www.hmc.psu.edu/comparativemedicine/

References:

1. Aliouat-Denis CM, Chabe M, Demanche C, et al. *Pneumocystis* species, co-evolution and pathogenic power. *Infect Genet Evol.* 2008;8:708-726.
2. Caswell JL, Thompson KJ. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 2, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:638-639.
3. Dei-Cas E, Brun-Pascaud M, Bille-Hansen V, Allaert A, Aliouat EM. Animal models of pneumocystosis. *FEMS Immunol Med Microbiol.* 1998;22:163-168.
4. Dei-Cas E, Chabe M, Moukhlis R, et al. *Pneumocystis oryctolagi* sp. nov., an uncultured fungus causing pneumonia in rabbits at weaning: Review of current knowledge, and description of a new taxon on genotypic, phylogenetic and phenotypic bases. *FEMS Microbiol Rev.* 2006;30:853-871.
5. Marini RP, Li X, Harpster NK, Dangler C. Cardiovascular pathology possibly associated with ketamine/xylazine anesthesia in Dutch belted rabbits. *Lab Anim Sci.* 1999;49:153-160.
6. Percy D, Barthold, SW. *Pathology of Laboratory Rodents and Rabbits*. 3rd ed. Ames, IA: Blackwell Publishing; 2007:65-67,143-146.
7. Sanchez CA, Chabe M, Aliouat el M, et al. Exploring transplacental transmission of *Pneumocystis oryctolagi* in first-time pregnant and multiparous rabbit does. *Med Mycol.* 2007;45:701-707.
8. Tamburrini E, Ortona E, Visconti E, et al. *Pneumocystis carinii* infection in young non-immunosuppressed rabbits. Kinetics of infection and of the primary specific immune response. *Med Microbiol Immunol*. 1999;188:1-7.

CASE III: RB040-2959 (AFIP 3164422).

Signalment: 3.5-year-old, male, New Zealand white (NZW), rabbit (*Oryctolagus cuniculus*).

History: A 3.5-year-old, intact male, NZW, apo-AI and LCAT (apolipoprotein and lecithin:cholesterol acyltransferase) double transgenic rabbit initially presented with a discrete raised 0.5 x 1.0 x 1.0 cm mass on the face approximately one centimeter rostral to the medial canthus of the left eye. The facial mass was surgically removed as an excisonal biopsy. Within three months the mass had recurred and was raised and ulcerated, measuring 4.0 x 2.0 x 1.5 cm. A second surgery was performed to resect the tumor with wider margins. Over the next three weeks the rabbit's condition declined and the rabbit developed respiratory distress and died acutely.

Gross Pathology: At necropsy, there was a healed incision at the site of the excisional biopsy. One facial mass was 1.5 x 2.5 x 3.5 cm white, resilient, somewhat lobulated and a similar, 1.0 x 1.0 x 1.5 cm mass present caudal to the first mass. Two subcutaneous masses were present between the rami of the mandible. Within all lung lobes there were multifocal to coalescing, firm, grayish-white variably sized masses which measured up to 7 mm in diameter. The masses extended into the parietal pleura and multifocally within the diaphragm. Lesions were not observed in any other major organs.

Histopathologic Description: Haired skin: Histological examination of the original excised skin biopsy revealed a raised mass that invaded into the dermis which was partially ulcerated, non-encapsulated and well-delineated composed of tightly packed polygonal to spindloid cells with a fine fibrovascular stroma. The cells were moderately anisocytotic and anisokaryotic with moderate, lightly eosinophilic finely granular cytoplasm with indistinct cell borders. Nuclei were round to oval, with finely stippled chromatin and 1-4 nucleoli; mitoses were frequent at 5 to 7/ hpf. Vascular invasion was evident, with tumor cells present within multiple venules and lymphatic vessels in multiple tissues. Within the skin, neoplastic cells were seen fingering into the adjacent dermis and extended to the cut border of the facial mass. Similar neoplastic foci with a packeting of cells were present in the submandibular lymph nodes, lungs, parietal pleura, and liver. Tumor cell morphology in these metastatic sites resembled the morphology of the tumor from the excisional biopsies.

The masses from the biopsies and necropsy were stained for Fontana-Masson method (melanin stain); results were negative. Immunohistochemistry (IHC) demonstrated positive cytoplasmic staining for vimentin in both polygonal and spindloid cells. There was positive cytoplasmic staining for Mart-1 and S-100 protein in the polygonal cells, but negative staining in the spindloid cells. Staining with actin, HHF35, AE1/AE3, HMB-45, SMA and tyrosinase were negative.

Transmission electron microscopy (TEM) of the neoplastic cells revealed small numbers of clustered stage II melanosomes with a size range of 100-900 nm with myelin-like membranes in sheets.

Contributor's Morphologic Diagnosis: Skin, Melanoma, amelanotic.

Contributor's Comment: This case of an amelanotic melanoma in an albino rabbit is rare. Albinism is an autosomal recessive disorder in which there are an adequate number of normally distributed melanocytes, but the melanin is not synthesized in a great enough quantity due to a point mutation in the gene for tyrosinase. Amelanotic melanomas have been reported in human albinos and experimentally induced in albino guinea pigs.[18,22] Human amelanotic melanomas are rare, only accounting for 5% of all melanoma cases.[22] While melanomas are most commonly found in skin, any tissue that has melanin can be a site of tumor development.[11]

Melanomas are uncommon in rabbits.[2,3,4,9,10,21] Amelanotic melanomas are extremely rare, with only one report in the literature.[10] The first case of melanoma in a rabbit was reported over 80 years ago.[20] Melanomas have been reported in pet and laboratory rabbits, with the NZW strain being over-represented.[10] In a retrospective study at the School of Veterinary Medicine at the University of Pennsylvania of 179 pet rabbits with a total of 190 tumors, eight were diagnosed as malignant melanoma by H&E and IHC.[3] These melanomas were located on the skin of the pinna, eyelid, head, limb and scrotum. These melanomas had the typical appearance of aggressively growing tumors with high cellular pleomorphism and high numbers of mitotic figures. All tumors contained abundant melanin.[3]

Melanomas have been reported in other species, including the cat,[15] opossum,[15] monkey,[19] rat,[14] dog,[11] ferret[24] and horse.[16] Melanomas account for 7% of all malignant tumors in dogs, and are most often found in the oral cavity with metastases commonly occurring in the lung and lymph nodes. Metastases also can be found in the brain, heart and spleen and, in rare cases, the bone marrow.[13] Melanomas are very rare in cats, with only 4 diagnosed out of 3145 cats or 0.1% necropsied at the Animal Medical Center in New York. The most common site was intraocular with metastasis in 63% of the cases. Tumors thicker than two mm in human cases often metastasize to lymph nodes, skin, subcutaneous tissue, lung, liver, small intestine, pancreas, heart, brain, and spleen.[11]

The ability for melanomas to express a wide range of antigens complicates the diagnosis. Antibodies key to differentiating melanomas include S-100, HMB-45, Mart-1 and MiTF.[23] Many times IHC is necessary for a definitive diagnosis of melanomas due to their ability to be the "great pretender". In the present case, the neoplasm was undifferentiated and contained both spindloid and polygonal cells. The initial biopsy closely mimicked a basal cell carcinoma; however, the pan-cytokeratin stain was negative. To rule out melanoma, a Fontana-Masson stain was performed and was negative for melanin. Next, a panel of antibodies was used, revealing the mass to be diffusely vimentin positive; yet, only Mart-1 and S-100 were positive in the polygonal cells. The inconclusiveness of negative spindloid staining warranted TEM.

Transmission electron microscopy can be utilized to show melanin granules that may be few in number and small in size.[5] The melanosomes generally are granular, myelin-like with shapes including oval, round, spindle-shaped, rod-like, or irregular, and are found singular not in groups. The present case demonstrated features of type II melanosomes.

AFIP Diagnosis: Haired skin and subcutis: Malignant melanoma, amelanotic.

Conference Comment: Participants reviewed common spontaneous cutaneous neoplasms in the rabbit, including those induced by viruses. The recent publication cited by the contributor provides a comprehensive review of the more common cutaneous neoplasms in rabbits. Briefly, trichoblastomas are most common, followed by Shope fibroma, lipoma, myxosarcoma, malignant peripheral nerve sheath tumor, and fibrosarcoma. Of those tumors, Shope fibroma is induced by viral infection with rabbit fibromavirus. Shope papillomas, another viral-induced skin tumor, is caused by a papovavirus. The following chart summarizes key points of these two important viral-induced neoplasms in rabbit species:[20]

Disease name	**Rabbit (Shope) papillomatosis**	**Rabbit (Shope) fibromatosis**
Species affected	Cottontail rabbits (*Sylvilagus*); domestic rabbits (*Oryctolagus*)	Cottontail rabbits; European domestic rabbits
Virus*	Cottontail rabbit papillomavirus	Rabbit fibroma virus (leporipoxvirus)
Gross findings	Pedunculated, cornified mass(es) on the eyelids and ears	Firm, flat, circumscribed tumors of the feet and legs ± muzzle, periorbital, perineal
Histologic findings	Consistent with squamous papilloma	Fibroblast proliferation with mixed inflammatory infiltrate, may be myxoid, large intracytoplasmic eosinophilic inclusion bodies in proliferative cells
Progression/Regression	May progress to SCC[#]	Benign, self-limiting infection

***** Virus nomenclature is based on the most recent International Committee on Taxonomy of Viruses taxonomy list.[12]
[#] Squamous cell carcinoma

In addition, participants reviewed the features of melanocytic neoplasia in several domestic and laboratory animal species.[1,6,7,8]

- Dog: Cutaneous, digit, oral cavity, and eye; melanocytic neoplasms of the conjunctiva tend to be malignant, while those involving the anterior uvea are typically benign.
- Cat: Rare, other than the eye; in the eye, found particularly frequent in the iris.
- Cow: Uncommon; can occur as benign congenital tumors, as well as at any age.
- Horse: Old gray horses; perineum, genitalia, tail head, distal limbs.
- Pig: Most common in Durocs and Hormel crosses; congenital tumors in the Sinclair miniature pig.
- Angora goat: dorsal ear, face, perineum.
- Fish: Most common in platyfish and swordfish.

Contributor: National Institutes of Health, Division of Veterinary Resources, Office of Research Services, 9000 Rockville Pike, Building 28A, Room 106, Bethesda, Maryland, 20892

References:

1. Baumann PC, Okihiro MS. Cancer. In: Ostrander GK, ed. *The Laboratory Fish*. San Diego, CA: Academic Press; 2000:604.
2. Beniashvili DS. Spontaneous rabbit melanoblastoma. *Voprosy Onkologii.* 1972;18:84-85.
3. Bomhard W, Goldschmidt MH, Shofer FS, Perl L, Rosenthal KL, Mauldin EA. Cutaneous neoplasms in pet rabbits: A retrospective study. *Vet Pathol*. 2007;44:579-588.
4. Brown WH, Pearce L. Melanoma (sarcoma) of the eye in a syphilitic rabbit. *J. Exp Med.* 1926;43:807-813.
5. 4 B, Moss J, Shore I, Banerjee SS. Metastic small cell malignant melanoma: A case requiring immunoelectronmicroscopy for the demonstration of lattice-deficient melanosomes. *Ultrastruct Pathol.* 2005;29:71-78.
6. Ginn PE, Mansell JEKL, Rakich PM. Skin and appendages. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 1, 5th ed. Philadelphia, PA: Elsevier Ltd; 2007:759-760.
7. Goldschmidt MH, Hendrick MJ. Tumors of the skin and soft tissues. In: Meuten DJ, ed. *Tumors In Domestic Animals*. 4th ed. Ames, IA: Blackwell Publishing; 2002:78-83.
8. Hargis AM, Ginn PE. The integument. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 4th ed. St. Louis, MO: Elsevier; 2007:1255.
9. Holz K, Heutgens W. Multiple Melanombildungen bein einem Kaninchen. *Stsch Tierarztl Wochenschr.* 1955;62:146-148.

10.Hotchkiss CE, Norden H, Collins BR, Ginn PE. Malignant melanoma in two rabbits. *Lab Anim Sci.* 1994;44:377-79.
11.Hussein MR. Extracutaneous malignant melanomas. *Cancer Investigation*. 2008;26:516-534.
12.International Committee on Taxonomy of Viruses. http://www.ictvonline.org/index.asp?bhcp=1. Accessed 13 April 2011.
13.Kim DY, Royal AB, Villamil JA. Disseminated melanoma in a dog with involvement of leptomeninges and bone marrow. *Vet Pathol*. 2009;46:80-83.
14.Kurotaki T, Tomonari Y, Kanno T, Wako Y, Tsuchitani M. Malignant amelanotic melanoma behind the left eye in a female Crj:CD(SD)IGS rat: A case report. *Vet Pathol*. 2008;45:681-84.
15.Kusewitt DF, Applegate LA, Bucana CD Ley RD. Naturally occurring malignant melanoma in the South American opossum (*Monodelphis domestican*). *Vet Pathol*. 1990;27:66-68.
16.Murphy J, Young S. Intraocular melanoma in a horse. *Vet Pathol*. 1979;16:539-42.
17. Patnaik AK, Mooney S. Feline melanoma: A comparative study of ocular, oral, and dermal neoplasms. *Vet Pathol*. 1988;25:105-112.
18.Pawlowski A, Heaberman HF, Menon A. Skin melanoma induced by 7, 12-dimethylbenzanthracene in albino guinea pigs and its similarities to skin melanoma of humans. *Cancer Res.* 1980;40:3652-3660.
19.Pellegrini, G, Bienvenu JG, Meehan JT, et al. Cutaneous melanoma with metastasis in a cynomolgus monkey (*Macaca fascicularis*). *J Med Primatol.* 2009;38:444-47.
20.Percy DH, Barthold. *Pathology of Laboratory Rodents and Rabbits*. 3rd ed. Ames, IA: Blackwell Publishing; 2007:255,258.
21.Sustmann. Multiple melanombildungen bein kaminchen. *Dtsch Tierarztl Wochenschr.* 1922;30:402.
22.Terenziani M, Spreafico F, Serra A, Podda M, Cereda S. Amelanotic melanoma in a child with oculocutaneous albinism. *Med Petiatr Oncol*. 2003;41:179-80.
23.Tong LCB, Kamil ZS, Habeeb A Al, Ghazarian D. Non-melanocytic mimics of melanoma, part II: Intradermal and intraepidermal mimics. *J Clin Pathol*. 2008;62:290-307.
24.Tunev SS, Wells MG. Cutaneous melanoma in a ferret (*Mustela putorius furo*). *Vet Pathol.* 2002;39:141-43.

CASE IV: B10-10951 (AFIP 3167236).

Signalment: 3-month-old, male, beagle dog (*Canis familiaris*).

History: The dog had two episodes of vomiting and diarrhea and presented with weight loss, depression, and dehydration. A fecal antigen test for parvovirus was reported to be negative and CBC and chemistry results were unremarkable. The dog was treated with subcutaneous fluid, vitamin B, clavamox, and albon. It is unknown whether the dog was euthanized or succumbed to a natural death. Samples of jejunum, duodenum, and mesenteric lymph node were submitted for histological evaluation.

Gross Pathology: An approximately 2 inch portion of the jejunum was dark red and the mesenteric lymph nodes were enlarged.

Histopathologic Description: Small intestine: There is multifocal loss of crypts with collapse of the overlying mucosa. Villi are multifocally blunted, fused, eroded or ulcerated and lining epithelial cells are cuboidal, attenuated, or dysplastic. The lamina propria is edematous, congested, and infiltrated by neutrophils, lymphocytes, and macrophages. Crypts are occasionally ectatic, lined by attenuated epithelial cells, and multifocally contain mucin admixed with necrotic cellular debris. There is extensive crypt regeneration characterized by cytoplasmic basophilia, an increased nuclear to cytoplasmic ratio, a prominent nucleolus, and frequent mitoses. Scattered crypt epithelial cells contain 4 x 6 µm polygonal eosinophilic to amphophilic intranuclear inclusions that peripheralize the chromatin. Within Peyer's patches, there is marked germinal center lymphoid depletion and necrosis with infiltration by neutrophils and macrophages. In several slides, clusters of basophilic short bacilli (gram negative) are adhered to the apical surface of superficial villus epithelial cells. The mesenteric lymph node (not submitted) is edematous and the subcapsular and medullary sinuses are expanded by draining neutrophils, macrophages, and red blood cells.

Contributor's Morphologic Diagnosis: Small intestine: severe acute to subacute necrotizing enteritis with crypt loss and regeneration, villus collapse, Peyer's patch lymphoid depletion and necrosis, epithelial intranuclear inclusions (canine parvovirus 2) and superficial bacteria.

Contributor's Comment: Parvoviruses are small, nonenveloped, single-stranded DNA viruses that are extremely resistant to environmental inactivation.[1,6] Capsid proteins, such as viral protein 1 (VP-1) and VP-2, play a critical role in determining host range, tissue tropism, and antigenicity of specific parvoviruses.[2,6] These viruses require the host cell machinery to replicate, but cannot induce mitosis. Therefore, they often infect rapidly dividing cells, such as intestinal crypt cells; hematopoietic and lymphoid tissues; and developing tissues in the fetus.[1,2] The canine parvovirus type 2 (CPV-2) originated in 1978 either from feline parvovirus (FPV) or a wild carnivore strain, and has quickly evolved to develop new strains (2a, 2b, 2c). The feline panleukopenia virus (FPV) and canine parvovirus 1 (canine minute virus) have remained fairly stable; however, mutations in CPV-2 occur relatively frequently and have enabled the virus to spread to other species, including the domestic cat.[2,3,6] The CPV-2c variant, containing a Glu-426 mutation in the capsid protein, is currently the most prevalent form of CPV-2.[6]

Canine parvovirus 2 (CPV-2) exposure occurs oronasally. The virus initially infects the epithelium over tonsils and Peyer's patches, then replicates in local lymphoid tissues before disseminating throughout the body via infected lymphoblasts. Approximately 3-4 days post infection, lymphocytolysis leads to release of the virus and cell free viremia. Neutralizing antibodies appear by 5-7 days post infection and correlates with termination of viremia.[1] Parvovirus infection most commonly occurs in 4-12-week-old puppies, when maternal antibodies decline, but can affect older dogs.[6] A myocardial form of the disease characterized by necrosis and intranuclear inclusions in cardiomyocytes occurs in neonates, most commonly born to naïve bitches.[1]

Infection of crypt epithelial cells occurs secondary to virus dissemination and viremia and is most common in areas over or close to Peyer's patches. Maximum infection of intestinal crypt cells occurs during days 5-9 post-infection. The most important factors dictating the severity of the intestinal disease include the availability of the virus, determined by the rate of lymphocyte proliferation, as well as the rate of proliferation of progenitors in the crypts.[1] Bone marrow cells are also often infected, leading to depletion of myeloid and erythroid lineages. Transient neutropenia due to increased consumption along with damaged bone marrow precursors occurs commonly in cats with FPV, but is less frequent in dogs. Megakaryocytes are less sensitive, but can also be decreased. Lymphopenia due to viral lymphocytolysis is common.[1]

Peyer's patches are often grossly evident from the serosal and/or mucosal surfaces as dark red oval depressions with CPV-2 infection. Other gross findings in the intestine may include mucoid to fluid contents that can contain blood; patchy fibrinous exudate; segmental to widespread mucosal congestion and mural hemorrhage; and fibrin deposition on the serosa. Similar changes can less commonly be observed in the colon. There is often thymic atrophy and enlargement of mesenteric lymph nodes.[1] Intestinal histologic features of CPV-2 infection include necrosis and depletion of Peyer's patches; crypt necrosis, loss, and regeneration; collapse and blunting of mucosal villi; mild to moderate proprial inflammation; epithelial intranuclear inclusions; and possibly secondary infectious agents. Similar lymphoid involution and crypt necrosis can be observed with canine distemper virus infection.[1]

The predominant lesions and presence of inclusions can vary significantly, depending on the stage and severity of disease and the presence of concurrent or secondary infections. Furthermore, a negative rapid fecal parvovirus test result, as was reported in this case, cannot reliably rule out parvovirus infection in cases with typical clinical signs.[9] A culture was not performed in this case; however, the gram negative bacteria adhered to the apical surface of superficial enterocytes resemble attaching and effacing *Escherichia coli*.[4]

AFIP Diagnosis: Small intestine: Enteritis, necrohemorrhagic, diffuse, severe with villar blunting and fusion, crypt necrosis and regeneration, Peyer's patch necrosis, and epithelial intranuclear amphophilic viral inclusions.

Conference Comment: As noted by the contributor, parvoviruses require actively dividing cells for viral replication. The virus hijacks the host cell DNA polymerases during the S-phase of the cell cycle.[7] Participants discussed the phases and checkpoints in the cell cycle. Cells not in a senescent state (G_0) are in one of four phases of the cell cycle:[5]

- G_1: Pre-synthetic phase
- S: Synthesis of DNA phase
- G_2: Pre-mitotic phase
- M: Mitotic phase

In order to detect and respond to DNA damage, there are checkpoints between the G_1/S transition to check for DNA integrity and a G_2/M transition to check replicated DNA for errors.[5] The G_1/S transition is the rate-limiting step in

the cell cycle and is the "point of no return" for the cell, as once the cell enters the S-phase it is committed to completing replication. The G_1/S transition is tightly controlled by cyclins and cyclin-dependent kinases (CDK) which in turn act on retinoblastoma susceptibility protein (RB) and transcription factor E2F.[5] In the normal, non-cycling state, RB is tightly bound to E2F rendering E2F inactive. When RB is phosphorylated by activated CDK, it releases E2F, which then stimulates transcription of genes necessary for cell cycling.[5] The cyclin-CDK complexes responsible for regulating the cell cycle are listed in the chart below along with their respective CDK inhibitors:[10]

Cyclin-CDK Complex	**Cell Cycle Phase Regulated**	**CDK Inhibitors**
Cyclin D-CDK4	G_1/S transition	p15, p16, p18, p19; p21, p27, p57
Cyclin D-CDK6	G_1/S transition	p15, p16, p18, p19; p21, p27, p57
Cyclin E-CDK2	G_1/S transition	p21, p27, p57
Cyclin A-CDK2	S-phase	p21, p27, p57
Cyclin A-CDK1	S-phase	p21, p27, p57
Cyclin B-CDK1	G_2/M transition	p21, p27, p57

Conference participants briefly discussed parvoviruses relevant to other veterinary species.[7,8]

- Feline panleukopenia virus: Generalized disease in kittens manifested as panleukopenia and enteritis; cerebellar hypoplasia
- Porcine parvovirus: Stillborn; mummification; embryonic death; infertility (SMEDI); abortion
- Mink enteritis virus: Leukopenia; enteritis
- Aleutian mink disease virus: Chronic immune complex disease; encephalopathy; neonatal interstitial pneumonia
- Goose parvovirus: Hepatitis; myocarditis; myositis
- Duck parvovirus: Hepatitis; myocarditis; myositis
- Mice minute virus and mouse parvovirus 1: Subclinical infection; congenital malformations
- Kilham's rat virus (RV), H-1 virus, and rat parvovirus (RPV): Scrotal and testicular hemorrhage (RV); congenital malformations; hemorrhagic syndrome
- Hamster parvovirus: Enamel hypoplasia of incisor teeth; cerebral mineralization; testicular atrophy; and high mortality

Contributor: Department of Pathobiology, University of Pennsylvania, School of Veterinary Medicine, Suite 4005 MJR-VHUP, 3800 Spruce Street, Philadelphia, PA 19104
http://www.vet.upenn.edu/FacultyandDepartments/Pathobiology/PathologyandToxicology/tabid/412/Default.aspx

References:

1. Brown CC, Baker DC, Barker IK. Alimentary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol. 2. Philadelphia, PA: Saunders Elsevier: 2007:177-182.
2. Hoelzer K, Parrish CR. The emergence of parvoviruses of carnivores. *Vet Res*. 2010;41:39.
3. Ikeda Y, Nakamura K, Miyazawa T, Tohya Y, Takahashi E, Masami. Feline host range of canine parvovirus: Recent emergence of new antigenic types in cats. *Emerg Infect Dis*. 2002;8:341-846.
4. Janke B, Francis DH, Collins JE, Libal MC, Zeman DH, Johnson DD. Attaching and effacing *Escherichia coli* infections in calves, pigs, lambs, and dogs. *J Vet Diagn Invest.* 1989;1:6-11.
5. Kumar V, Abbas AK, Fausto N, Aster JC. Tissue renewal, repair, and regeneration. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:86-87.
6. Lamm CG, Reebok GB. Parvovirus infection in domestic companion animals. *Vet Clin North Am Small Anim Pract*. 2008;38:837-850.
7. Parrish CR. Parvoviridae. In: In: MacLachlan NJ, Dubovi, eds. *Fenner's Veterinary Virology*. 4th ed. San Diego, CA: Elsevier; 2011:225-228.
8. Percy DH, Barthold. *Pathology of Laboratory Rodents and Rabbits*. 3rd ed. Ames, IA: Blackwell Publishing; 2007:24-25,127-128, 181.
9. Schmitz S, Coenen C, Konig M, Thiel H, Neiger, R. Comparison of three rapid commercial canine parvovirus antigen detection tests with electron microscopy and polymerase chain reaction. *J Vet Diagn Invest*. 2009;21:344-345.

10.Stricker TP. Kumar V. Neoplasia. In: Kumar V, Abbas AK, Fausto N, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. 8th ed. Philadelphia, PA: Elsevier Saunders; 2009:284-286.