The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2006-2007

SLIDE 1
CONFERENCE 1 / CASE I – 0600288 (AFIP 3024111)

Signalment: 9.5-year-old, black, neutered male domestic short-haired cat

History: Shadow was euthanized after a 2-3 week history of breathing difficulty. The respiratory distress was not responsive to antibiotics and there was suspicion of widely disseminated metastatic neoplasia. There were multiple masses on the upper and lower left eyelids.

Gross Pathology: A mildly autolyzed adult gray neutered male cat has body weight 4.65 and is in good body condition. Mild autolysis is present. Multiple, 1-2 mm diameter circular masses are visible on the upper and lower right eyelids. When incised, these nodules have yellow-tan, soft centers. A larger 5 mm diameter, well defined, dark red, abraded mass is present in the upper eyelid of the left eye. Mucoid, reddish yellow serous fluid is present in the trachea. The lung lobes fail to collapse, are generally dark red, and firm. They contain 2-5 mm diameter disseminated yellow tan nodules, similar to those seen in the eyelids. The nodules continue throughout the underlying parenchyma. Lung sections sink in formalin. The heart weighs 0.27% body weight, within normal reference range. Both kidneys contain numerous disseminated tan to white raised nodules throughout the cortex. No other gross lesions were detected.

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

Contributor's Morphologic Diagnosis: Multifocal granulomatous chorioretinitis, and anterior uveitis, with intralesional yeasts and retinal detachment.

Contributor's Comment: Not all ocular elements are present on all slides, and the focal nature of the lesions may result in a more limited distribution of inflammation. The animal also has granulomatous inflammation in the lung, kidney and eyelid caused by *Blastomyces*. Pulmonary disease was thought related to centrilobular degeneration in the liver due to hypoxia. *Blastomyces* infection is much less common in cats than in dogs but can produce lethal multi-organ granulomas.

Blastomycosis is caused by the dimorphic fungus *Blastomyces dermatitidis*, found in the Ohio, Missouri, and Mississippi river valleys, Virginia, the Carolinas, and Georgia.[1] Severe pyogranulomatous uveitis is the most common histopathologic finding in feline

blastomycosis. Blindness can result from ocular or central nervous system involvement. The posterior segment of the eye is more commonly affected than the anterior segment. A distribution of lesions in the ciliary body, choroid and tapetum has been previously noted.[2] As in other systemic mycoses, intraocular inflammation occurs in the presence of organisms in the eye, not as a secondary inflammatory phenomenon. The general character of the inflammation is similar to that seen in dogs, including necrosis, but organisms are quite numerous compared to many canine cases.[3,4]

Blastomyces can infect and cause ocular disease in a variety of species. Ocular disease is most often seen in a setting of multi-systemic disease, and this patient was presented for its respiratory distress rather than blindness.[5,6] Ocular involvement occurred in 2 of 5 cats in that series, while others have reported 18%.[7] The overall prevalence of this infection is much less in the feline than canine population. Forty one percent of canine cases have ocular involvement.[8]

AFIP Diagnosis: Eye: Uveitis, pyogranulomatous, multifocal, moderate, with retinitis, retinal detachment, and numerous yeasts, etiology consistent with *Blastomyces dermatitidis*, domestic shorthair (*Felis domesticus*), feline.

Conference Comment: The contributor provides an excellent overview of Blastomycosis. Deep mycotic infections are rare in cats and are not always associated with immunosuppressive conditions.[7] The most common feline disseminated fungal infection is cryptococcosis.[3]

Conference attendees briefly reviewed the anatomy of the eye and discussed how to differentiate the systemic mycoses. Below is a chart to help identify the common systemic mycoses.

Common Systemic (Deep) Mycoses

Yeast	Size	Wall or Capsule	Reproduction
Blastomyces dermatitidis	5-25 µm in diameter	double-contoured, refractile	Broad-base budding
Cryptococcus neoformans	5-20 µm in diameter	2-8 µm thick mucopolysaccharide carminophilic capsule	Narrow-base budding
Histoplasma capsulatum	*var. capsulatum* 2-5 µm in diameter *var. duboisii* 8-15 µm in diameter	thin cell wall, no capsule	Narrow-base budding
Coccidioides immitis	Spherules 20-200 µm in diameter	double-contoured, refractile	Endosporulation

Discussion was directed at the eye as an immunologically privileged site with no resident lymphocytes antigen presenting cells, or draining lymphatics and the presence of the blood-eye barrier.[10] The two major components of the blood-eye barrier are the blood-retinal barrier and the blood-aqueous barrier. These barriers prevent inward and outward movement of proteins and low molecular-weight solutes.[9] The blood-retinal barrier is composed of the endothelium of retinal capillaries and the retinal pigment

epithelium. This barrier separates the choroidal and retinal tissue fluids. The blood-aqueous barrier is primarily maintained by the tight junctions between the nonpigmented ciliary body epithelium.[9] Discussion about this case was concluded with a review of the four types of hypersensitivity reactions. A table created directly from Robbins and Cotran has been included below to help residents remember the immune mechanisms and pathologic lesions associated with each type of hypersensitivity reaction.

Mechanisms of Immunologically Mediated Diseases

Type	Prototype Disorder	Immune Mechanisms	Pathologic Lesions
Immediate (type I)	Anaphylaxis; allergies; bronchial asthma (atopic forms)	Production of IgE antibody → immediate release of vasoactive amines and other mediators from mast cells; recruitment of inflammatory cells (late-phase reaction)	Vascular dilation, edema, smooth muscle contraction, mucus production, inflammation
Antibody-mediated (type II)	Autoimmune hemolytic anemia; Goodpasture syndrome	Production of IgG, IgM → binds to antigen on target cell or tissue → phagocytosis or lysis of target cell by activated complement or Fc receptors; recruitment of leukocytes	Cell lysis; inflammation
Immune complex-mediated (type III)	Systemic lupus erythematosus; some forms of glomerulonephritis; serum sickness; Arthus reaction	Deposition of antigen-antibody complexes → complement activation → recruitment of leukocytes by complement products and Fc receptors → release of enzymes or other toxic molecules	Necrotizing vasculitis (fibrinoid necrosis); inflammation
Cell-mediated (type IV)	Contact dermatitis; multiple sclerosis; type I diabetes; transplant rejection; tuberculosis	Activated T lymphocytes → i) release of cytokines and macrophage activation; ii) T-cell mediated cytotoxicity	Perivascular cellular infiltrates; edema; cell destruction; granuloma formation

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References:

1. A.M. Legendre: Blastomycosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd ed., pp. 371-377. WB Saunders, Philadelphia, Pennsylvania,1998
2. Alden CL, Mohan R: Ocular blastomycosis in a cat. J Amer Vet Med Assoc 164:527-528, 1974
3. Gionfreddo JR: Feline systemic fungal infections. Vet Clin North Am Small Anim Pract 30:1029-1050, 2000
4. Krohne SG: Canine systemic fungal infections. Vet Clin North Am Small Anim Pract 30:1063-1090, 2000
5. Nasisse MP, Van Ee RT, Wright B: Ocular changes in a cat with disseminated blastomycosis. J Amer Vet Med Assoc 187:628-631, 1985
6. Breider MA, Walker TL, Legrende AM, VanEe RT: Blastomycosis in cats: five cases (1979-1986). J Am Vet Med Assoc 193:570-572, 1988
7. Davies C, Troy GC: Deep mycotic infections of cats. J Am Anim Hosp Assoc 32:381-391, 1996
8. Legendre AM, Walker M, Buyukmihci N, Stevens R: Canine blastomycosis: a review of 47 clinical cases. J Am Vet Med Assoc 178:1163–1168, 1981
9. Gum GG, Gelatt KN, Ofri R: Physiology of the eye. In: Veterinary Ophthalmology, ed. Gelatt KN, 3rd ed., pp. 161-162, Lippincott Williams and Wilkins. Baltimore, Maryland,

1999
10. Wilcock BP: The eye and ear. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 477. Academic Press, Inc., San Diego, California, 1993
11. Abbas AK: Diseases of immunity. In: Robbins and Cotran Pathologic Basis of Disease, eds. Abbas AK, Fausto N, Kumar V, 7th ed., p. 206. Elsevier Saunders, Philadelphia, Pennsylvania, 2005

SLIDE 2
CONFERENCE 1 / CASE II – 04-5029 (AFIP 2944785)

Signalment: 9-month-old, female, Rag knockout mouse (*Mus musculus*)

History: 20 out of 26 mice in a breeding colony died over the course of 2 days.

Gross Pathology: At necropsy, the mouse examined had a firm, red-tan liver with a diffusely pitted surface.

Laboratory Results: PCR performed on internal organs and feces was positive for mouse hepatitis virus (MHV).

Contributor's Morphologic Diagnosis: Massive hepatocellular necrosis with syncytia – liver, murine.

Contributor's Comment: MHV is the most important virus that infects laboratory mouse colonies. A coronavirus, MHV is ubiquitous and highly contagious. It is also highly mutable, with many strains, and variable organotropism. Infection with MHV is dependent upon the virulence of the specific strain, as well as many host factors – age, genotype, and immune status.[1] Though most infections are subclinical, very young mice (less than 2 weeks of age), genetically susceptible mice, and immunocompromised mice are vulnerable to MHV, with high mortality.[1] Virulent strains can be problematic even for adult mice with normal immune system function.

Strains of MHV have tropism for either enteric or respiratory mucosa. Viral tropism is believed to be mediated by the spike (S) protein, a gylcoprotein of the viral envelope.[2] Enteric strains, which are currently the most commonly isolated, have a predilection for the intestinal mucosal epithelium, causing villus attenuation, mucosal necrosis, and syncytia.[3] Severity of infection is very much age-dependent, with young mice being most susceptible.[4] Polytropic strains, which were more prevalent in the 1950s and 1960s, replicate in nasal mucosa, and then disseminate and replicate in the endothelium and parenchyma of other organs, including liver, brain, and lymphoid organs.[3] The virus causes necrosis with syncytia formation in these sites.

In the case presented, the most severe lesions are present in the liver, but necrosis with syncytia is also present in the spleen, and there are some syncytia present in the small intestinal mucosa. Some strains of MHV affect both liver and intestine.[3] In some of the sections of liver provided, there are foci of extramedullary hematopoiesis.

AFIP Diagnosis: Liver: Necrosis, multifocal to coalescing, with syncytia, etiology consistent with murine coronavirus, Rag knockout mouse (*Mus musculus*), rodent.

Conference Comment: The contributor provides a thorough review of MHV. Variation in morphologic diagnoses prompted a discussion about the definition of massive hepatic necrosis. Robbins and Cotran defines necrosis of entire lobules as submassive and of most of the liver as massive; whereas, Thomson's defines massive necrosis as that of an entire hepatic lobule or contiguous lobules without reference to a submassive pattern.[5,6]

Further discussion was directed at the variability in the susceptibility and clinical outcome associated with the polytropic (respiratory) and enteroptropic strains, respectively, depending on the age and immune status of affected mice. Infection with polytropic MHV in neonatal, genetically susceptible, or immunocompromised mice results in viremia and dissemination of the virus with viral replication in the endothelium and parenchyma of multiple tissues throughout the body to include the brain, liver, lymphoid organs, bone marrow, and other sites. The virus is cleared without persistence or a carrier state by 3-4 weeks post-infection. Infection of post-weaning age mice is usually subclinical. Nude or SCID mice cannot clear the virus and develop severe multisystemic disease. In contrast, enterotropic strains tend to infect only the intestinal mucosa with minimal or no dissemination to other organs, even in immunocompromised mice. Mice of all ages are susceptible to disease; however, the development of disease is age related. Neonatal mice infected with enterotropic MHV develop a severe necrotizing enterocolitis with high mortality. With advancing age, lesion severity and mortality progressively decrease. Infected adult mice develop minimal lesions, including adult SCID and nude mice. This prompted discussion about what determines the severity of infection in entertropic strains. The moderator emphasized that severity of intestinal disease is associated with the age-related rapid turn over of intestinal epithelial mucosal cells rather than immune-related susceptibility accounting for the severe infection in neonatal mice.[1]

The differential diagnosis for random hepatic necrosis in the mouse includes Tyzzer's disease (*Clostridium piliforme*), salmonellosis, and mousepox (ectromelia virus). Tyzzer's disease and salmonellosis do not have syncytial cells as a characteristic microscopic finding. If present in sections, salmonella organisms may be demonstrated by tissue Gram stains as short Gram-negative bacilli. Necrosis of Peyer's patches frequently occurs with enteric salmonellosis. Silver stains, such as the Warthin-Starry, best demonstrate the filamentous bacilli within hepatocytes at the periphery of necrotic

areas, typical of Tyzzer's disease. In the liver lesions of mousepox, intracytoplasmic inclusions are evident in hepatocytes at the periphery of necrotic foci, and syncytial cells are not present. Cutaneous lesions, splenic necrosis, and necrosis of lymph nodes and Peyer's patches are often present in cases of mousepox.

The gallbladder is present in some sections.

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References:

1. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, 2nd ed., pp. 28-31. Iowa State University Press, Ames, Iowa, 2001
2. Homberger FR: Enterotropic mouse hepatitis virus. Lab Animals 31:97-115, 1997
3. Homberger FR, Zhang L, Barthold SW: Prevalence of enterotropic and polytropic mouse hepatitis virus in enzootically infected mouse colonies. Lab Animal Science 48(1):50-54, 1998
4. Barthold SW, Beck DS, Smith AL: Enterotropic coronavirus (mouse hepatitis virus) in mice: influence of host age and strain on infection and disease. Lab Animal Science 43(4):276-284, 1993
5. Crawford JM: Liver and biliary tract. In: Robbins and Cotran Pathologic Basis of Disease, eds. Abbas AK, Fausto N, Kumar V, 7th ed., pp. 877-936. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
6. Cullen MJ, MacLachlan JN: Liver and biliary system, and exocrine pancreas. In: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 81-124. Mosby, St. Louis, Missouri, 2001

SLIDE 3
CONFERENCE 1 / CASE III – 06-17282 (AFIP 3026714)

Signalment: 1.5-year-old, female, Yorkshire (*Sus scrofa*)

History: Two of ten non-bred sows recently moved to a pasture developed diarrhea, lost body weight, and died.

Gross Pathology: The sow was thin with a body condition score of 2. The stomach contained forage and a small amount of ground feed. The mucosa of the pyloric region was congested with mild hemorrhage. Intestinal contents in the duodenum and jejunum were grayish brown and had normal consistency. Segmental congestion was noted on the serosa of the ileum. The ileum was not rigid or thickened. The mucosa of the ileum was necrotic and hemorrhagic with a ragged appearance. No lesions were observed in the cecum or colon.

Laboratory Results: No pathogens were isolated by aerobic culture of the small and large intestine, lung, liver, and kidney.

Histopathologic Description: The villi were denuded and covered by a thick layer of exudate composed of cellular debris, thick-walled oocysts, mucus, and myriads of bacteria. The villi were necrotic and expanded by proliferation of numerous coccidia organisms to include schizonts, gamonts, and oocysts. The oocysts were ellipsoidal with a rough, golden wall. They were approximately 50 by 35 microns. Many degenerate gamonts and oocysts were surrounded by exudate and bacteria. Blood vessels in the lamina propria often contained poorly-formed fibrin clots. Necrotic mucosal tissue with degenerate gamonts and oocysts was herniated into a few partially depleted foci of gut associated lymphoid tissue (Peyer's patches). Some of the degenerate oocysts were surrounded by multinucleated giant cells.

Contributor's Morphologic Diagnosis: Subacute severe necrotizing ileitis with numerous intralesional protozoa (*Eimeria* spp.) and bacteria.

Contributor's Comment: Although many species of *Eimeria* have been identified in swine, most are considered nonpathogenic.[5] Reports of disease in swine are rare. *Eimeria* was considered the primary pathogen in this case and a previously diagnosed and reported case from our laboratory.[1] Both cases involved animals maintained on pasture or dirt lots with sandy soils. Attempts to experimentally reproduce the disease in confinement produces minimal or no significant lesions.[3] In naturally-occurring cases, the animals are likely exposed to heavily contaminated soil with the opportunity for repeated exposure to the organism.

The size of the oocyst is one parameter used to determine the species of *Eimeria*. A mixed population of *Eimeria* was isolated in the other case diagnosed at our laboratory. Another report involved lesions associated with *E. spinosum.*[4] This species has smaller oocysts (20.4 by 14.2) with spiny walls. The size of the oocyst in this case is larger than the size reported for *E. scabra* (31.9 by 22.5 microns).[6] *E. scabra* has the largest oocysts of the coccidia associated with swine.

Silver stained sections of intestine in this case and the other case diagnosed at our laboratory revealed no intracellular organisms with morphologic features consistent with *Lawsonia intracellularis*. Numerous mixed bacteria were observed in this case. Some of the bacteria were slender long bacilli arranged in sheaves suggestive of *Fusobacterium* spp. Damage to the mucosal epithelium by coccidia provides a portal of entry for the numerous bacteria present in the lower GI tract.[2] The bacteria may have been synergistic or secondary opportunists. Bacterial toxins may have been a factor in lesion promotion and death.

AFIP Diagnosis: Ileum: Ileitis, subacute, diffuse, moderate, with myriad coccidia, etiology consistent with *Eimeria* spp., Yorkshire pig (*Sus scrofa*), porcine.

Conference Comment: The Phylum Apicomplexa includes intracellular parasites characterized by a sporozoite stage with a typical apical complex of organelles. Genera include: *Eimeria*, *Isospora*, *Caryospora*, *Hammondia*, *Toxoplasma*, *Besnoitia*, *Sarcocystis*, *Cystoisospora*, *Frenkelia*, *Cryptosporidium*, *Neospora*, *Klossiella*, *Haemogregarina*, *Hepatozoon*, *Calyptospora*, *Haemoproteus*, *Leucocytozoon*, *Hepatocystis*, *Plasmodium*, *Babesia*, *Theileria*, and *Cytauxzoon*.[7]
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 below for quick reference.

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

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.[8]

Conference attendees also reviewed the ultrastructural features of Apicomplexans, specifically *Toxoplasma* to include the following: 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. Dr. Gardiner adds, “There are myriads of macro- and microgametocytes throughout with associated oocysts. And, it (this case) shows the segmental nature of the infection, i.e., that the trophozoites would have been more proximal in the intestines. *Eimeria* is the only genus of coccidians I know to have the very large gametocytes and oocytes.”

The contributor provides an overview of the *Eimeria* species infecting swine. We are grateful to Dr. Gardiner for his comments on this interesting case.

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References:

1. Hill, JE, Lomax LG, Lindsay DS, Lynn BS: Coccidiosis caused by *Eimeria scabra* in a finishing hog. J Am Vet Med Assoc 186:981-983, 1985
2. Koudela B, Vitovec J, Stebra J: Concurrent infection of enterocytes with *Eimeria scabra* and other enteropathogens in swine. Vet Parasitol 35:71-77, 1990
3. Lindsay DS, Blagburn BL, Boosinger TS: Experimental *Eimeria dubliecki* infections in nursing and weaned pigs. Vet Parasitol 25:39-45, 1987
4. Lindsay DS, Neiger R, Hildreth M: Porcine enteritis associated with *Eimeria spinosa* Henry, 1931 infection. J Parasitol 88:1262-1263, 2002
5. Linsay DS, Dubey JP: Coccidia and other protozoa. In: Diseases of Swine, eds. Straw BS, Zimmermann JJ, D’Allaire S, Taylor DJ, 9th ed., p. 865. Blackwell publishing, Ames, Iowa, 2006
6. Veterling JM: Coccidia (Protozoa: Eimeridae) of swine. J Parasitol 75:172-173, 1965

7. Jones TC, Hunt RD, King NW: Diseases due to protozoa. In: Veterinary Pathology, 6th ed., pp. 549-567. Williams & Wilkins, Baltimore, Maryland, 1997
8. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 20, Armed Forces Institute of Pathology, Washington, D.C., 1998

SLIDE 4
CONFERENCE 1 / CASE IV – E969/03 (AFIP 2940309)

Signalment: Four-year-old, female, canine (*Canis familiaris*), mixed-breed

History: Two weeks after whelping, a severe persistent bloody vaginal discharge occurred suddenly. After clinical examination an ovariohysterectomy was performed.

Gross Pathology: The placental sites were enlarged and irregularly thick. The luminal surface was rough, grey to brown, and foci of hemorrhages were detectable. The interplacental endometrium was inconspicuous.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Subinvolution of placental sites (SIPS).

Contributor's Comment: The luminal epithelium of the placental sites was partly detached. The residual epithelial cells were mostly cuboidal but in some regions columnar with a vacuolated cytoplasm. Intraepithelial remnants of secretion and intraluminal projections were detectable. Large amounts of eosinophilic collagen masses in a lobular arrangement were found in the subepithelial layer. Numerous intact large polygonal cells with multiple nuclei and a foamy cytoplasm (so-called "trophoblast-like cells") lay within the collagen masses as well as inside the layers underneath. Multifocal hemorrhages accompanied by hemosiderophages and an accentuated periglandular mononuclear inflammatory reaction were recognized. The secretorically active endometrial glands showed a moderate to severe dilation with retention of mucus and cell detritus. Furthermore, a marked periglandular fibrosis was visible. The endometrium of the interplacental zones, the myometrium and perimetrium showed no obvious changes.

Although in the literature subinvolution of placental sites (SIPS) is described at later stages of the puerperium, in the present case the alterations observed in the endometrium differ in their degree from normal postpartum involution and correspond to those described in other reports on subinvolution.[1,2,3,4,5,7] Therefore, an involution abnormality is assumed in the present case too. Subinvolution of placental sites occurs predominantly in younger bitches.[1,4,7] The etiology and pathogenesis of subinvolution are unknown as well as the fetal or maternal origin of the prominent "trophoblast-like cells".[1,6] The relevance of these cells for the pathogenesis of SIPS is discussed.[1,8]

AFIP Diagnosis: Endometrium: Fibrosis, hemorrhage, and subacute inflammation, focally extensive, moderate, with trophoblast-like cells, consistent with placental site involution, mixed breed (*Canis familiaris*), canine.

Conference Comment: Thomson's defines subinvolution of placental sites (SIPS) as longer than normal persistence and deeper than normal penetration of trophoblast-like cells in the uterus after parturition.[9] In some cases, the trophoblast- like cells can perforate the uterine wall.[9-11]

Typical gross findings include multiple ellipsoidal enlargements of the endometrium that are visible from the serosal surface. The enlargements correspond with areas of previous placental attachment. The endometrial surface is characterized by hemorrhagic, irregularly thickened, rough, gray to brown plaques up to twice the size of a normal placental site from the same breed at the same stage after parturition. The endometrium between the enlarged sites is normal.[1,9-11]

The key histologic finding is the presence of syncytial masses of trophoblast-like cells with abundant vacuolated, eosinophilic cytoplasm in the endometrium, often surrounding blood vessels. These cells may invade the myometrium and perforate the serosa. Other characteristic light microscopic findings include a plaque (placental site) that protrudes into the uterine lumen composed of amorphous eosinophilic necrotic debris, fibrin, hemorrhage, and regenerating endometrium. Deeper within the plaque, there is collagen deposition and dilated endometrial glands.[1,9-11]

The contributor points out that SIPS is usually diagnosed at a later stage of the post-parturient period than the two weeks stated in the clinical history. Conference participants discussed the process of involution and the criteria required to make the diagnosis of SIPS. Given a 12-week involution interval, participants had difficulty accepting this case as an example of SIPS. Conferees agreed that the histological features were consistent with involution, but that two weeks postpartum was too early to make a definitive diagnosis.

This case was reviewed in consultation with Dr. Donald Schlafer, Cornell University College of Veterinary Medicine, who adds, "I couldn't tell a normal involution at two weeks from subinvolution of placental sites unless there was massive or marked deep invasion of trophoblast cells. I know of no accepted criteria for making a distinction between the two." We are grateful to Dr. Schlafer for his comments on this thought provoking case.

Readers are encouraged to review reference number two below.

Contributor: Institute of Veterinary Pathology, Website: www.patho.vetmed.uni-muenchen.de

References:
1. Al-Bassam MA, Thomson RG, O'Donnell L: Involution abnormalities in the postpartum uterus of the bitch. Vet Pathol 18:208-218, 1981
2. Al-Bassam MA, Thomson RG, O'Donnell L: Normal postpartum involution of the uterus in the dog. Can J Comp Med 45:217-232, 1981
3. Beck AM, McEntee K: Subinvolution of placental sites in a postpartum bitch. A case report. Cornell Vet 56 (2):269-277, 1966
4. Burke TJ: Post-parturient problems in the bitch. Vet Clin North Am 7:693-698, 1977
5. Dickie MB, Arbeiter K: Diagnosis and therapy of the subinvolution of placental sites in the bitch. J Reprod Fert Suppl 47:471-475, 1993
6. Fernández PE, Portiansky EL, Barbeito CG, Gimeno EJ: Characterisation of cytotrophoblastic-like cells present in subinvolutioned placental sites of the bitch. Histol Histopathol 13:995-1000, 1998
7. Schall WD, Finco DR: Spontaneous recovery after subinvolution of placental sites in a bitch. J Am Vet Med Assoc 159 (12):1780-1782, 1971
8. Wheeler SL: Subinvolution of placental sites in the bitch. In: Current Therapy in Theriogenology II, pp. 513–515. Morrow and Saunders, Philadelphia, Pennsylvania 1986
9. Acland HM: Reproductive system: Female. In: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., p. 607. Mosby, St. Louis, Missouri, 2001
10. Kennedy PC, Miller RB: The female genital system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 444-445. Academic Press, Inc., San Diego, California, 1993
11. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielsen S: Histological Classification of the Tumors of the Genital System of Domestic Animals, Second Series, vol. IV, pp. 33-34. Armed Forces Institute of Pathology, Washington, D.C., 1998

SLIDE 5
CONFERENCE 2 / CASE I – 8688 (AFIP 2985463)

Signalment: 2-year-old, female, crossbreed goat (*Capra hircus*)

History: The animal underwent surgery as part of a research study to evaluate components of a synthetic cervical disc implant. She had been anesthetized with diazepam and ketamine IV and maintained on isoflurane delivered via positive pressure ventilation. The surgical procedure went smoothly, and the animal was stable throughout surgery; total anesthetic time was approximately 3.5 hours. Shortly after discontinuation of gas anesthesia, the goat quickly awoke and was extubated. She soon became apneic, and within minutes entered cardiac arrest.

Gross Pathology: The animal was submitted fresh and was in good body condition. The mouth contained a small amount of rumen contents, which extended to the mid-trachea. The larynx was slightly edematous. Atelectasis was present in both lungs; the left side was 80% atelectatic, and the right side was 30% affected. The bronchi and large airways contained a large amount of blood, seen post-fixation. The mediastinum contained an enlarged, firm lymph node filled with caseous material.

Laboratory Results: Culture from the mediastinal lymph node grew *Corynebacterium pseudotuberculosis*.

Histopathologic Description: The enlarged mediastinal lymph node is centrally effaced by necrotic cellular debris with foci of mineralization. The necrotic debris is surrounded by laminated layers of epithelioid macrophages (some multinucleate), neutrophils, fibrosis, and a peripheral rim of lymphocytes and plasma cells.

There is multifocal to diffuse atelectasis and congestion in the lungs. The bronchial-associated lymphatic tissue is centrally abscessed as was seen grossly in the mediastinal lymph node. In addition, there is multifocal hyperplasia of the terminal bronchial epithelial cells. The bronchioles and bronchi are multifocally to diffusely filled with blood and necrotic cellular debris.

Contributor's Morphologic Diagnoses:

1. Mediastinal lymph node: Granulomatous lymphadenitis with abscessation, severe, chronic, focally extensive.
2. Lung: Bronchial and bronchiolar hemorrhage, severe, acute, diffuse.
3. Lung: Bronchial-associated lymphoid tissue hyperplasia, with multifocal abscessation, moderate, chronic, multifocal.

Contributor's Comment: *Corynebacterium pseudotuberculosis* is a non-motile Gram-positive aerobic (and facultatively anaerobic) coccobacillus bacteria.[6] It has been documented to cause disease in several species, including horses, cattle, swine, mice, deer, camels, zebras, alpacas, hedgehogs and humans.[1,4,6] In sheep and goats, caseous lymphadenitis (CLA) is the most common disease syndrome caused by *C. pseudotuberculosis*.

The organism most commonly gains entry to the body via wounds in the skin or mucous membranes, though inhalation and ingestion can also be forms of exposure.[6] After tissue infiltration, the organism then disseminates via the lymphatic system to local lymph nodes and internal organs, where local colonization and abscessation can occur.[6] Due to presence of an external lipid coat and secretion of the potent exotoxin phospholipase D, the bacteria are able to survive phagocytosis and spread through the body.[6]

Internal and external forms of caseous lymphadenitis can occur in small ruminants, sometimes concurrently. The external form of CLA is characterized by abscessation of superficial lymph nodes and/or subcutaneous tissues. Animals with this form generally

present with visible swellings that may rupture and drain. The internal form of CLA causes abscessation of internal organs and internal lymph nodes. Most commonly affected organs include the liver, kidneys, lungs, spleen and uterus. Additionally, the mediastinal, bronchial and/or lumbar lymph nodes are often involved. Clinical findings can include: unthriftiness ("thin-ewe" syndrome), poor fertility, decreased milk production, poor growth, decreased weaning weights of lambs, and a decrease in wool quality and production.[5,6] In rare cases, the hematogenous spread to atypical organs can cause osteomyelitis, mastitis, or CNS disease.[2,3,5]

Though *C. pseudotuberculosis* causes similar disease in both sheep and goats, there are a few differences in general disease patterns seen between the two species. For example, the external form of disease is more common in goats, while sheep are more likely to develop internal CLA. The distribution pattern of external abscesses also varies; lymph nodes of the head and neck are more commonly affected in goats, while sheep tend to present with external abscessation of the subiliac and superficial cervical lymph nodes.[6] The character of the abscesses also differs. In sheep, the abscesses often contain a pale green material that may be centrally mineralized. On cross-section, the abscess has a layered morphology that is often referred to as an "onion-ring" appearance.[5,6] The center of the mass is composed of proliferating bacteria and dying phagocytes. Surrounding the central abscess are layers of fibrosis and inspissated caseous material.[5] In goats, this laminated appearance generally does not occur. Rather, goat abscesses are typically uniform in character, with a cream to pale-green pasty exudate.

Clinical signs are highly suggestive of the external form of caseous lymphadenitis, but bacteriologic culture should be obtained to make a definitive diagnosis. *C. pseudotuberculosis* is a catalase-positive, urease-negative, phospholipase-D-positive, pyrazinamide-negative organism.[6] Diagnosis of the internal form of CLA, however, is not as straightforward. Serologic tests are available, such as the synergistic hemolysin inhibition test (SHI) performed at the California Animal Health and Food Safety Laboratory System at the University of California—Davis. The value of serological screening is a controversial topic, as false positives and false negatives occur. If serological screening is performed, comparison of paired titers taken 2-4 weeks apart is recommended. Several animals from the flock associated with this goat tested positive with the SHI test, two of which have presented with external CLA.

This case is unusual in that the goat was affected by the internal form of caseous lymphadenitis, and rupture of the abscess resulted in acute death. After an uneventful surgical and anesthetic event, it is likely that the abrupt anesthetic awakening caused a sudden increase in thoracic pressure, which ruptured pulmonary abscesses. There was secondary severe pulmonary hemorrhage, causing rapid death. Though small ruminants are commonly used models in biomedical research, there is a general lack of available specific-pathogen free (SPF) herds. Researchers and veterinary staff need to be cognizant of this fact when designing experiments and providing care for these species, particularly when the animals will be exposed to stressful events, such as surgery and anesthesia.

AFIP Diagnosis: Lung: Pneumonia, granulomatous, multifocal to coalescing, moderate, with hemorrhage and lymphoplasmacytic peribronchiolar inflammation, goat (*Capra hirucus*), caprine.

Conference Comment: The contributor provides an excellent overview of *Corynebacterium pseudotuberculosis* to include the notable differences in the general disease pattern seen between sheep and goats. Additionally, *C. pseudotuberculosis* causes ulcerative lymphangitis and pectoral abscesses in horses.[7]

C. pseudotuberculosis typically forms large colonies in hematoxylin and eosin (H & E) stained sections. Residents at AFIP utilize the mnemonic "YACS" to develop a differential diagnosis when large colonies of bacteria are present in H & E stained sections.
YACS stands for:

Y *Yersinia* sp.
A *Actinomyces* sp., *Actinobacillus* sp.
C *Corynebacterium* sp.
S *Staphylococcus* sp., *Streptococcus* sp.

Some residents include *Arcanobacter* sp. and *Clostridium* sp. on this list since these bacteria are large in size; however, they do not typically form large colonies.

Conference attendees discussed potential etiologies that incite granuloma formation to include fungi, foreign bodies, and higher bacteria. The higher bacteria belong to the order Actinomycetales and include the following genera: *Corynebacterium*, *Actinomyces*, *Nocardia*, *Rhodococcus*, *Dermatophilus*, *Streptomyces*, and *Mycobacterium*.[7]
Surprisingly, special stains run at the AFIP did not reveal any Gram-positive or Gram-negative bacteria. Although this does not definitively rule in or rule out *C. pseudotuberculosis* as the cause of the multifocal pulmonary granulomas, other potential etiologies were discussed since only the mediastinal lymph node, and not the lung itself, was cultured. Attendees discussed mycobacteria as a potential etiology; however, Ziehl-Neelsen staining did not reveal any acid-fast bacteria.
Additionally, in some sections, the granulomatous response was centered on brightly eosinophilic material leading to a discussion of a potential infestation by *Muellerius capillaris*.

M. capillaris, also known as the nodular lungworm, is the most common lung parasite of sheep in Europe and Northern Africa and also occurs in goats. Sheep develop multifocal, subpleural nodules that tend to be most numerous in the dorsal caudal lung lobes. Microscopically, adults, eggs, and larvae reside in the subpleural alveoli and are surrounded by a focal, eosinophilic, and granulomatous reaction. In contrast, goats develop diffuse interstitial rather than focal lesions.

Microscopically, the reaction to the lungworms varies from almost no lesions to a severe interstitial pneumonia with heavy mononuclear cell infiltrates in alveolar walls resembling caprine arthritis-encephalitis virus (CAE) or mycoplasmal infections.[8]

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References:
1. Anderson DE, Rings DM, Kowalski J: Infection with *Corynebacterium pseudotuberculosis* in five alpacas. J Am Vet Med Assoc 225**:**1743-1747, 1702, 2004
2. Hancock RB, Rashmir-Raven AM, Thomas MW, Marti JM, Fleming SA: What is your diagnosis? A large, oval, radiolucent defect in the proximolateral portion of the humerus. J Am Vet Med Assoc 217**:**823-824, 2000
3. Hogan PM, Crabill MR, Walker M: What is your diagnosis? Focal osteolysis involving the third and fourth metacarpal bones. J Am Vet Med Assoc 212**:**1543-1544, 1998
4. Perkins SL, Magdesian KG, Thomas WP, Spier SJ: Pericarditis and pleuritis caused by *Corynebacterium pseudotuberculosis* in a horse. J Am Vet Med Assoc 224**:**1133-1138, 1112, 2004
5. Pugh DG: Sheep & Goat Medicine, 1st ed., pp. xii, 468. Saunders, Philadelphia, Pennslyvania, 2002
6. Williamson LH: Caseous lymphadenitis in small ruminants. Vet Clin North Am Food Anim Pract 17**:**359-371, vii, 2001
7. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 479-482, Williams & Wilkins, Baltimore, Maryland, 1997
8. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 534. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 6
CONFERENCE 2 / CASE II – K (AFIP 2816006)

Signalment: Adult female *Lepus timidus,* hare (varying hare)

History: The hare was found dead.

Gross Pathology: Normal body condition; liver light brown; spleen slightly enlarged.

Laboratory Results: No specific bacteria were isolated from liver, spleen, lung or intestine. Liver was positive for European brown hare syndrome (EBHS) virus with RT-PCR.

Contributor's Morphologic Diagnosis: Liver: Severe periportal necrotizing hepatitis. Etiology: calicivirus (EBHS-virus).

Contributor's Comment: European brown hare syndrome is a disease caused by a calicivirus that is closely related to the rabbit hemorrhagic disease (RHD) virus. The disease affects many hare species e.g. *Lepus europaeus* (the European brown hare) and *Lepus timidus* (varying hare). Gross lesions can be minimal, but typically pulmonary edema, a slightly enlarged spleen and a yellowish or mottled friable liver are present. Hemorrhages sometimes occur on the serosal surfaces of the lungs and intestine.

Histologically the disease is characterized by necrotizing hepatitis. The degree of hepatic necrosis varies from periportal coagulation necrosis to massive acute liver necrosis with hemorrhage. In less acute cases, there is usually mild to moderate fatty degeneration of hepatocytes and mild mononuclear inflammatory cell infiltrate in the portal areas. There is usually some mineralization of the necrotic periportal hepatocytes.

Affected hares are usually adults. Both sexes are affected. The disease appears to be highly infectious and fatal; however, it does not seem to affect significantly the size of the hare population.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, periportal, diffuse, marked, with hemorrhage, varying hare (*Lepus timidus*), lagomorph.

Conference Comment: The contributor mentions that European brown hare syndrome is closely related to the rabbit hemorrhagic disease (RHD) virus. Both are caliciviruses and cause periportal to massive hepatocellular necrosis.[2] Only lagomorphs of the species *Oryctolagus cuniculus* are affected by rabbit hemorrhagic disease virus. North American lagomorphs, such as the cottontail rabbit (*Sylvilagus floridanus*), the snowshoe hare (*Lepus americanus*), and the black-tail jackrabbit (*L californicus*) are not susceptible to disease.[5] Similarly, only hares are affected by the European brown hare syndrome virus. Attempts to infect rabbits and hares with the heterologous virus have failed to produce clinical disease.[4] Terminal DIC is seen less frequently in hares with EBHS than in rabbits with RHD. Hares with chronic disease often develop jaundice and chronic hepatitis. Hares that recover may have liver fibrosis and remain in poor body condition. The characteristic histological liver lesion is single-cell necrosis in periportal areas. Acidophilic bodies are often present, formed as a result of single cell acidophilic degeneration and coagulative necrosis. Hepatocytes expel fragments of condensed cytoplasm into the sinusoids where they are phagocytized by Kupffer cells. Acidophilic bodies are characteristically present in a number of viral hepatic infections such as yellow fever (Flavivirus), where they are referred to as Councilman bodies.[2] Mitochondrial mineralization is frequently observed in hepatocytes undergoing necrosis.[6] The basophilic granules can be viewed by vonKossa staining. The heptatic calcium content of hares with EBHS is increased up to 20 times the normal value.[2]

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References:

1. Bascunana CR, Nowotny N, Belak S: Detection and differentiation of rabbit hemorrhagic disease and European brown hare syndrome viruses by amplification of VP60 genomic sequences from fresh and fixed tissue specimens. J Clin Microbiol 35:2492-2495, 1997
2. Gavier-Widen D: Morphologic and immunohistochemical characterization of the hepatic lesions associated with European brown hare syndrome. Vet Pathol 31: 327-334, 1994
3. Salmela P: The occurrence of European brown hare syndrome in Finland. Acta Vet Scand 34:215-217, 1993
4. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., pp. 255-256, Iowa State University Press, Ames, Iowa, 2001
5. Campagnolo ER, Ernst MJ, Berninger ML, Gregg DA, Shumaker TJ, Boghossian AM: Outbreak of rabbit hemorrhagic disease in domestic lagomorphs. JAVMA 223(8):1151-1154, 2003
6. Lenghaus C, Studdert MJ, Gavier-Widén D: Calicivirus infections. In: Infectious Disease of Wild Mammals, eds. Williams ES, Barker IK, 3rd ed., pp. 280-285. Iowa State University Press, Ames, Iowa, 2001

SLIDE 7
CONFERENCE 2 / CASE III – 056-39214 (AFIP 3028040)

Signalment: 1-year-old female intact, mixed-breed Caprine (*Capra hircus*)

History: Animal presented for a history of coughing, diarrhea and foot and skin lesions. Lesions had been present for five months. There is a farm history of both contagious ecthyma and caseous lymphadenitis.

Gross Pathology: The coronary bands of all four limbs, the base of the ears bilaterally and commisure of the lip contain, multiple, raised, proliferative crusty lesions measuring up to 3 cm in diameter, that are associated with superficial areas of hemorrhage on cut surface. The hard palate and tongue contain similar lesions measuring up to 1 cm.

There is regionally extensive fibrinous peritonitis of the cranial abdominal cavity. The rumen and omasal mucosa contain multiple raised, plaque-like lesions measuring up to 2 cm in diameter with occasional central areas of hemorrhage on cut surface.

There is generalized lymphadenopathy.

The lungs have pronounced cranioventral bronchopneumonia with diffuse edema and congestion. There is effusive fibrinous pleuritis.

Remaining organ systems appear grossly normal.

Histopathologic Description: Rumen: The rumen mucosa contains multifocal mildly proliferative lesions with severe ballooning degeneration and epithelial necrosis. Scattered through the mucosal epithelium are degenerative neutrophils and cellular debris. There are occasional superficial mucosal colonies of mixed, predominately rod-shaped, bacteria. At the periphery of the lesion epithelial cells contain eosinophlic 5-10 um intracytoplasmic inclusion bodies. There is submucosal fibrovascular proliferation and infiltrate of lymphocytes and plasma cells. Underlying muscle is mildly edematous.

Some sections contain underlying omentum with lymph node. There is minimal infiltration of the omentum with lymphocytes and plasma cells. The lymph node is reactive with sinus histiocytosis.

Contributor's Morphologic Diagnosis: Rumen: Rumenitis, proliferative with ballooning degeneration, moderate, and intracytoplasmic inclusion bodies.

Contributor's Comment: Gross and histological lesions are consistent with the parapoxviral disease, contagious ecthyma caused by the Orf virus. Orf virus is a double-stranded DNA parapox virus causing an acute skin disease of sheep and goats, which may be debilitating.[1] Of importance is the zoonotic character of the Orf virus and other viruses in the genus *Parapoxvirus*.[2] The disease generally has high morbidity with low mortality. Mortality has decreased substantially since the eradication of screwworm flies from the USA.[3] Severe persistent Orf in young Boer goats has been previously reported suggesting that individuals in this breed may have, as yet, unidentified susceptibility factors to the disease.[3] This particular case had similar pneumonia and lymphoid changes similar to that reported in the previous study. Lesions usually spontaneously heal in three to four weeks.[3] In this case the lesions persisted for five months. Recently, a PCR assay utilizing gene 045 was developed to rapidly diagnose cases of Orf.[4]

The family *Poxviridae* is divided into two subfamilies: the poxviruses of vertebrates, *Chordopoxvirinae*, and the poxviruses of insects, *Entomopoxvirane*. *Chordopoxvirinae* comprises eight genera and more poxviruses are being discovered in multiple different species that will be in need of classification.[2] The genome consists of a single linear double-stranded DNA molecule ranging from 130 to 375 kbp depending on genus. Viruses in the *Parapoxvirus* genera include Orf virus, Pseudocowpox virus, Bovine papular stomatitis virus, Ausdyk virus (Camels), and Seal parapoxvirus. The Parapox virion is cocoon shaped and approximately 260 X 160 nm in size compared to other poxvirus virions that are brick shaped and 250 X 200 nm in size. The surface structure of genus *Parapoxvirus* has surface tubules that resemble a ball of yarn.[2]

Orf virus gains access to the host through broken skin and replicates in regenerating epidermal cells. Primary lesions are proliferative and over 4-6 weeks progress from erythematous macule, papule, vesicle and scab. Reinfection lesions have similar

clinical stages, but are generally not proliferative.[1] The character of the lesion in the rumen as only mildly proliferative may indicate it as a reinfection lesion. Viral antigen is present approximately between 3 and 25 days post-infection. Viral antigen localizes in areas of epidermal hyperproliferation, with intensity in degenerating cells, which indicates an *in vivo* cytopathic affect.[1] There is an early neutrophil influx within 48 hours with accumulation of γδ T-cell receptor T-cells, CD4+ T-cells, CD8+ T-cells, B-cells and MHC Class II+ dendritic cells 9-15 days post infection. Preinfection levels return around day 30.[1] The dense accumulation of MHC Class II+ dendritic cells is an unusual feature of lesions. Skin repair, antigen-presentation or virus containment are theories for their presence. Their phenotype is CD1- CD11b- CD11c-, so do not appear related phenotypically to epidermal Langerhans cells that express CD1 or tissue macrophages that express CD11b and/or CD11c.[1]

AFIP Diagnosis: Rumen (per contributor): Rumenitis, proliferative and necrotizing, multifocal, moderate, with epithelial ballooning degeneration and rare intracytoplasmic inclusion bodies, goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides an excellent summary of ovine and caprine parapoxvirus which causes contagious ecthyma/contagious pustular dermatitis in sheep and goats, and orf in humans. Conference participants discussed the unusual location of the proliferative lesion in this case. Lesions typically begin at the commissures of the mouth, then spread to the lips, muzzle, face, eyelids, and feet. Lesions on the limbs typically involve the coronet, interdigital cleft, and bulb of the heels and are less common than lesions on the lips. In severe cases, lesions can occur on the gingiva, dental palate, palate, and tongue. Lesions may also develop on the mammary glands and other areas of sparsely wooled or haired skin. Very rarely, lesions extend into the esophagus, rumen, and omasum. Lesions can also occur in the lower gastrointestinal tract causing an ulcerative gastroenteritis. Additionally, lesions have been reported in the heart and lungs.[5,7]

Lesion development involves progression through the typical poxviral phases, but is much more proliferative:[5]

Macule → papule → vesicle → pustule → crust → scar

The vesicular stage is very brief and the pustules are flat rather than umbilicated. Additionally, the ulcer and crust stage persists and is prominent clinically. A layer of thick brown crust, which may be elevated up to 4mm above the skin surface, is the most significant feature of the gross lesion in contagious ecthyma.[5]

Typical light microscopic findings include marked epidermal hyperplasia with prominent rete ridges; intraepidermal abscesses; swollen and vacuolated keratinocytes in the stratum spinosum (ballooning degeneration); and a thick crust composed of orthokeratotic and parakeratotic hyperkeratosis, proteinaceous fluid, degenerate neutrophils, and bacteria. Newly proliferating keratinocytes in the outer stratum

spinosum are the target cell population for parapoxvirus infections. Basophilic cytoplasm in swollen keratinocytes corresponds to polyribosome proliferation and viral replication. Inclusion bodies are briefly detectable. Basophilic intracytoplasmic inclusion bodies can be seen as early as 31 hours post infection. By 72 hours post infection, keratinocytes may have eosinophilic intracytoplasmic inclusions, pyknotic nuclei, and marked hydropic change. Pseudocarcinomatous hyperplasia is common. Dermal changes include superficial edema, vascular proliferation and dilation, and a perivascular mononuclear infiltrate.[5,6]

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References:

1. Haig D, McInnes C, Deane D, Reid H, Mercer A: The immune and inflammatory response to Orf virus. Comp Immun Microbiol infect Dis 20:197-204, 1997
2. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Poxviridae. In: Veterinary Virology, eds. Murphy FA, Gibbs EPJ, Horzinek MC, and Studdert MJ, 3rd ed., pp. 277-291. Academic Press, London, UK, 1999
3. de la Concha-Bermejillo A, Guo J, Zhang Z, Waldron D: Severe persistent Orf in young goats. J Vet Diagn Invest 15:423-31, 2003
4. Kottaridi C, Nomikou K, Lelli R, Markoulatos P, Mangana O: Laboratory diagnosis of contagious ecthyma: comparison of different PCR protocols with virus isolation in cell culture. J Virol Methods 134:119-124, 2006
5. Yager JA, Scott DW: The skin and appendages. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 630-632. Academic Press, Inc., San Diego, California, 1993
6. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 306-307, Mosby Elsevier, St. Louis, Missouri, 2007
7. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1174-1175, Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 8
CONFERENCE 2 / CASE IV – E27/06 (AFIP 3026008)

Signalment: Juvenile, male, domestic short hair, *Felis silvestris f. catus*, cat

History: A domestic shorthair cat was found dead on the Rügen island of the Baltic Sea in close proximity to places were HPAIV H5N1 infected wild birds, mainly mute and whooper swans and several species of geese and ducks, were detected shortly before.

Gross Pathology: At necropsy the cat showed extensive postmortal loss of the skin, subcutaneous tissue and parts of the skeletal muscles in the cervical and shoulder region due to scavenging. The mucosa of the nasal cavity, pharynx and trachea was diffusely hyperemic. There was a hemohydrothorax of about 20 ml. The lung was severely edematous and showed numerous, sharply demarcated, yellow, nodules. Multifocally in the liver there were few, up to 2 mm in diameter large, sharply demarcated light brown to yellow areas (necrosis). Retropharyngeal and pulmonary lymph nodes were moderately swollen with few ecchymoses. Petechiation or diffuse hemorrhages were found retroperitoneally and intramuscularly in the diaphragm, within the perirenal tissue and the pancreas.

Laboratory Results: M-PCR and H5N1-specific RRT-PCRs of tracheal swabs and liver tissue revealed infection of the cat with H5N1. Molecular pathotyping confirmed the presence of HPAIV H5N1 of the Asian lineage and revealed the closest relationship to a H5N1 HA partial sequence obtained from a dead whooper swan from the island of Rügen. The cat was negative by PCR for the detection of FIV gag-specific and FeLV U3 LTR-specific proviral DNA. In a commercial test (FeLV/FIV Snap®; IDDEX, USA) performed with lung exudate and cardial blood, FeLV-specific antigen or FIV-specific antibodies were also not detected. Bacteriology revealed a minimal growth of *Streptococcus* spp. No relevant bacteria were detected in the liver. Spleen, lung, heart and brain were bacteriologically negative or contained only small to moderate numbers of *E. coli*.
Clinical chemistry of the aqueous humor showed a marked increase of enzyme activitiy of AST, ALT and LDH (Table 1).

Table 1. Postmortem analysis of aqueous humor (anterior chamber, eye).

Parameter	AST (nkat/l)	ALT (nkat/l)	LDH (nkat/l)	ALP (nkat/l)	GGT (nkat/l)
refer. intervals	<650	<970	<120	<2500	<100
E27/06	55440	5120	1148	1250	3

AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phospatase, GGT: gamma glutamyl transferase

Histopathologic Description: Within the liver there were numerous, randomly arranged, sharply demarcated areas of coagulative necrosis with loss of hepatic architecture and cellular detail and replacement by karyorrhectic and cellular debris admixed with abundant fibrin and numerous erythrocytes. Occasionally these foci were surrounded by a small number of lymphocytes. Corresponding to the histological findings strong intracytoplasmic and intranuclear immunohistochemical signals for influenza virus A nucleoprotein antigen were found in degenerating hepatocytes adjacent to hepatocellular necrosis.

Contributor's Morphologic Diagnosis: Liver, necrosis and degeneration, moderate, random, domestic short hair cat (*Felis silvestris f. catus*), cat.

Contributor's Comment: Since its first emergence in 1997 in Hong Kong, highly pathogenic avian influenza virus (HPAIV) of the H5N1 subtype has spread continuously in Southeast Asia. In February 2006, outbreaks of HPAIV H5N1 of Asian lineage had been reported in 17 countries for the first time, including Germany. The H5N1 HPAIV has been shown to have the ability to infect a broad number of avian species and mammals, especially cats and humans. Previously, felids were considered relatively resistant to infection with influenza viruses.[1] In contrast, few recently published reports describe the transmission of highly pathogenic H5N1 to wild and domestic cats. At first, the infection of two leopards and two white tigers with HPAIV H5N1 were reported in a zoo near Bangkok. These animals had been infected by ingestion of H5N1 HPAIV contaminated chicken meat and died with symptoms of a systemic infection especially involving the respiratory tract.[2] Susceptibility of domestic cats to HPAIV H5N1 and its horizontal transmission has been studied by experimental intratracheal inoculation of H5N1 virus (A/Vietnam/1194/04) and feeding of cats with infected chicks.[3] Main lesions in these cats consisted of diffuse alveolar damage as well as necrosis and inflammation in a variety of extra-respiratory tissues, while mortality in these experiments was low or not investigated.[4] In the case presented here, epidemiological data, results of virological investigations and, most strikingly, the intralesional detection of influenza virus NP antigen strongly indicate the lethal course of natural HPAIV H5N1 infection in the cat. According to the marked hepatocellular necrosis that was co-localized with AIV antigen, acute hepatic disease may play a major role in the pathogenesis of the disease. The moderate necrotizing bronchiolitis as result of infection with HPAIV H5N1 in association with marked pulmonary Aelurostrongylosis has certainly contributed to the severity of the clinical course. The cat was found in areas where wild birds were severely affected by an outbreak of HPAI H5N1 of Asian lineage and carcasses of wild swans and geese had been accessible to both avian and mammalian scavengers. It is therefore most likely that the cat contracted the infection from carcasses of infected wild birds. Similar exposure and infection of scavenging mammalian species is assumed in a confirmed H5N1 HPAIV infection of a stone marten found in the same area. Following the complete removal of bird carcasses from the shores, coinciding with the order to keep cats indoors and to take dogs on the leash, no further cases in mammals have been noticed. Although other species like red foxes and raccoon dogs have been included in the ongoing monitoring programs, so far, neither virological nor serological evidence for the involvement of these mammalian species has been obtained. With regard to feline retrovirus infections the animal belonged to the high risk group of straying outdoor cats. FIV and FeLV infections are associated with severe immunodeficiencies which might have favoured infection with HPAIV H5N1. Different lymphoid tissues were investigated for provirus and found negative. Under experimental conditions, cats have been shown to excrete HPAIV H5N1 influenza viruses oronasally and by the fecal route.[4] Here, immunohistochemistry revealed no influenza viral antigen in laryngeal, tonsillar and tracheal tissues or the mucosa of the gastrointestinal tract. Nevertheless, all three cats were diagnosed as HPAIV positive by nasopharyngeal and tracheal swabs and revealed a substantial viral load in these samples. Expectorated bronchial mucous and

cellular debris seem to be responsible for the oronasal excretion as massive virus replication in the upper airways seems unlikely. These observations agree with reports describing the presence of high affinity receptors (alpha 2-3 linked terminal sialic acid residues) for avian H5N1 HPAIV in cells lining the lower respiratory mucosa of cats (pneumocytes type II) and their absence in the mucosa of the upper respiratory tract.[5] Therefore, oropharyngeal swabbing may be the most promising sample for monitoring cats alive for HPAIV H5N1.

AFIP Diagnosis: Liver: Necrosis, random, multifocal, domestic shorthair cat (*Felis silvestris f. catus*), feline.

Conference Comment: The contributor provides a thorough overview of HPAIV H5N1 in felines.

HPAIV is a Type A Orthomyxovirus. Orthomyxoviruses are small-medium sized, enveloped, single stranded, negative sense RNA viruses. In contrast to low pathogenic strains of AI, in which the hemagglutinin glycoprotein can only be cleaved by trypsin or trypsin-like enzymes restricting viral replication to the respiratory and intestinal tracts, HPAI viruses have a hemagglutinin glycoprotein that can be cleaved by a ubiquitous protease found in virtually all organs. Therefore, virulent viruses are capable of replication in all tissues and organs once within the systemic circulation and are not limited to the respiratory and intestinal tracts. Pathogenesis studies in chickens have shown that HPAI viruses replicate in endothelial cells throughout the body with spread to adjacent parenchymal cells in many organs and are capable of extrapulmonary spread. Depending on the virulence of the strain, infected chickens can die peracutely without clinical signs or may present with pulmonary, cardiac, or neurologic signs.[6]

Readers are encouraged to review WSC Conference 18 / Case IV from the 2005-2006 academic year – a case of HPAIV in a chicken.
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References:
1. Paniker CK, Nair CM: Infection with A2 Hong Kong influenza virus in domestic cats. Bull World Health Organ 43:859-862, 1970
2. Keawcharoen JK, Oraveerakul T, Kuiken RA, Fouchier A, Amonsin S, Payungporn S, Noppornpanth S, Wattanodorn A, Theambooniers R, Tantilertcharoen R, Pattanarangsan N, Arya P, Ratanakorn D, Osterhaus AD, Poovorawan Y: Avian influenza H5N1 in tigers and leopards. Emerg Infect Dis 10:2189-2191, 2004
3. Kuiken T, Rimmelzwaan G, van Riel D, van Amerongen G, Baars M, Fouchier R, Osterhaus AD: Avian H5N1 influenza in cats. Science 306:241, 2004
4. Rimmelzwaan GF, van Riel D, Baars M, Bestebroer TM, van Amerongen G, Fouchier RA, Osterhaus AD, Kuiken T: Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of virus spread within and between hosts. Am J Pathol 168:176-183, 2006

5. van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T: H5N1 Virus Attachment to Lower Respiratory Tract. Science 312:399, 2006
6. Brown C: Avian Influenza Virchow's reminder. Am J Pathol 168:6-8, 2006

SLIDE 9
CONFERENCE 3 / CASE I – MVP1-2006 (AFIP 3028509)

Signalment: 5-year-old, female, Suffolk ewe, (*Ovis aries*)

History: Ewe culled due to chronic weight loss and unthriftiness. Euthanized due to poor prognosis.

Gross Pathology: Little to no body fat; irregular thickening of the ileum and distal jejunum; mesenteric lymphadenomegaly; prominent lymphatics in region of distal jejunum and ileum.

Histopathologic Description: There is moderate to marked expansion of the lamina propria and submucosa due to infiltrates of large numbers of macrophages, lymphocytes and lesser numbers of neutrophils. In addition, there is moderate ectasia of lymphatics within the lamina propria. Macrophages contain abundant eosinophilic cytoplasm and staining with the Ziehl-Neelsen technique reveals numerous intracellular acid fast bacteria. Granulomatous infiltrates extend deep through the muscularis mucosa, submucosa and are occasionally seen in the subserosa. In submucosal and subserosal regions small foci of granulomatous infiltrates are associated with lymphatic vessels. Associated lymph nodes are characterized by multifocal infiltrates of macrophages involving the cortex and to a lesser extent the medulla. These macrophages also contain variable numbers of acid fast bacilli.

Contributor's Morphologic Diagnoses: 1. Small intestine: Enteritis, granulomatous, multifocal, moderate, chronic with lymphangiectasia and intralesional intracellular acid fast bacilli.
2. Lymph node: Lymphadenitis, granulomatous, multifocal, moderate with intralesional, intracellular acid fast bacilli.

Contributor's Comment: *Mycobacterium avium* subsp. *paratuberculosis* (Map) is the causative agent of paratuberculosis or Johne's disease. Infection usually occurs soon after birth and clinical disease in ruminants is characterized by chronic, progressive enteritis, often accompanied by protracted diarrhea and weight loss. Paratuberculosis is generally associated with domestic ruminants such as cattle, sheep and goats with sporadic occurrence in free ranging ruminants such as white-tailed deer, Key deer, bighorn sheep, Rocky Mountain goats, tule elk, and bison. Following oral exposure, the organism is generally believed to cross the small intestinal mucosa into the Peyer's patches via M-cells within the follicle associated epithelium.[1] Macrophages taking up Map accumulate in intestinal epithelium, associated lymphoid tissue as well as draining

lymph nodes resulting in granulomatous lesions. Initial lesions generally contain few acid fast bacteria; however, in later stages of lesion development myriads of acid fast bacteria are common. Studies of naturally occurring cases of paratuberculosis in sheep and goats describe different types of lesions represented by two extremes, i.e., paucibacillary (tuberculoid) lesions and multibacillary (lepromatous) lesions. Three classifications of lesions have been suggested that span the spectrum of observed lesions.[2] It has been observed that paucibacillary forms of the disease are associated with a strong cell mediated immune response, while in multibacillary forms of the disease the humoral immune response dominates. The current case represents an example of the multibacillary form of paratuberculosis.

Map is known to exist as 2 phenotypically different strains designated the sheep (S) and cattle (C) strains. Sheep strains are generally more difficult to isolate in culture than cattle strains.[3] Recently, several large genomic deletions were identified in the S strain when compared to the C strain.[4] The cattle strain appears to have a broader host range than the sheep strain as the cattle strain can be frequently isolated from sheep; however, the sheep strain appears to be more host restrictive and uncommonly isolated from cattle. The age of onset of clinical disease tends to be younger in sheep than in cattle. Unlike cattle, chronic weight loss is the primary clinical sign rather than protracted diarrhea. Only 10-20% of clinical cases in sheep present with diarrhea.[4]

AFIP Diagnoses: 1. Small intestine: Enteritis, granulomatous, chronic, multifocal, moderate, with lymphangitis and edema, Suffolk sheep (*Ovis aries*), ovine.
2. Lymph node: Lymphadenitis, granulomatous, chronic, multifocal, moderate.

Conference Comment: The contributor provides a concise summary of the pathogenesis of *Mycobacterium avium* subsp. *paratuberculosis* or Johne's disease in sheep and goats. *Mycobacterium avium* subsp. *paratuberculosis* are acid-fast, weakly Gram-positive, non-spore forming, non-motile, facultative intracellular bacilli. They are slow growing in culture and dependent on mycobactin as a source of iron.[8] The mycolic acid in the cell wall of Mycobacteria makes them acid-fast allowing them to retain carbolfuschin stains after treatment with an acid-alcohol wash. Once Mycobacteria enter macrophages they block phagosome-lysosome fusion by several mechanisms allowing replication within phagosomes.[5]

In cattle, paratuberculosis is characterized by profuse diarrhea, emaciation, and hypoproteinemia in animals over 19 months of age. In sheep and goats, the clinical signs are similar to those seen in cattle with the absence of diarrhea. However, the pygmy goat is unusual in that some develop an explosive diarrhea and die unexpectedly.[6] The moderator emphasized that *Mycobacterium avium* subsp. *paratuberculosis* is one of the top three differentials for chronic wasting in sheep (thin-ewe syndrome). The two other differentials are *Corynebacterium pseudotuberculosis* (caseous lymphadenitis) and malnutrition.

Gross lesions in cattle with Johne's disease include a diffusely thickened intestinal mucosa folded into transverse rugae; mesenteric lymphadenopathy with noncaseating granulomas that contain high numbers of foamy macrophages with many acid-fast bacilli; granulomatous lymphangitis; atrophy of skeletal muscle and fat; and dependent intermandibular edema (bottle jaw). Aortic mineralization, when observed, is specific for Johne's disease in cattle. Hepatic microgranulomas sometimes occur. In contrast, sheep, goats, and deer form tuberculoid (caseating) granulomas with high numbers of epithelioid macrophages and low numbers of acid-fast bacilli. Additionally, granulomas in small ruminants can mineralize while caseation and mineralization is extremely rare in cattle.[6,8] The moderator added that massive serous atrophy of fat and dependent mandibular edema/bottle jaw are often seen in sheep with Johne's disease.

In horses, *Mycobacterium avium-intracellulare complex* (MAIC) can occasionally cause a proliferative enteritis similar to Johne's disease in cattle.[7] *Mycobacterium avium* subsp. *paratuberculosis* has been implicated in some cases of Crohn's disease in humans.[6,8]

Conference attendees briefly reviewed the classification of Mycobacteria. *Mycobacterium avium-intracellulare complex* (MAIC) includes *M. avium*, *M. intracellulare*, *M. scrofulaceum*, and *M. avium* subp. *paratuberculosis*. By convention, the term "tuberculosis" is reserved for infections caused by Mycobacteria in the *M. tuberculosis* complex (MTC) and include *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. "Atypical Mycobacteria" include all Mycobacteria except those in the MAIC or MTC groups, *M. leprae*, and *M. lepraemurium*.[7]

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References:

1. Sigurdardottir OG, Press CM, Evensen O: Uptake of *Mycobacterium avium* subsp. *paratuberculosis* through the distal small intestinal mucosa in goats: An ultrastructural study. Vet Pathol 38:184-189, 2001
2. Perez V, Garcia Marin F, Badiola JJ: Description and classification of different types of lesion associated with natural paratuberculosis infection in sheep. J Comp Pathol 114:107-122, 1996
3. Stehman SM: Paratuberculosis in small ruminants, deer and South American camelids. Vet Clin North Am Food Anim Prac 12(2):441-455, 1996
4. Marsh IB, Bannantine JP, Paustian ML, Tizard ML, Kapur V, Whittington J: Genomic comparison of *Mycobacterium avium* subsp. *paratuberculosis* sheep and cattle strains by microarray hybridization. J Bacteriol 188:2290-2293, 2006
5. McAdam AJ, Sharpe AH: Infectious Diseases. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., p. 381. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
6. Gelberg HB: Alimentary System. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 372-374. Mosby Elsevier, St. Louis, Missouri, 2007

7. Hines ME, Kreeger JM, Herron AJ: Special topic overview. Mycobacterial infections of animals: Pathology and pathogenesis. Laboratory Animal Science 45(4):334-351, 1995
8. Barker IK, Van Dreumel AA, Palmer N: The Alimentary System. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 247-252. Academic Press, Inc., San Diego, California, 1993

SLIDE 10
CONFERENCE 3 / CASE II – IVABS Massey University NZ628-2 (AFIP 3026727)

Signalment: 12-week-old, male, crossbred pig

History: Small piggery with wasting syndrome in weaner pigs first identified in 2003. In previous 3 months the herd has lost approximately 3% of weaners and finishing pigs, mainly 8-12 weeks of age. The tissues presented are from a pig killed in extremis that had gross skin lesions which were representative of other affected animals.

Gross Pathology: The carcass was in poor general condition. All lymph nodes, and particularly those of the superficial inguinal and mandibular lymphocentres, were diffusely enlarged, sometimes nodular and those draining skin and subcutis had reddened subcapsular tissue. The skin was studded with innumerable 0.5-2.0cm roughly circular red to purple macules that often coalesced into plaques, some crusted. Oedema was present in dependent regions and over bony prominences. The lesions were distributed widely over the body but were most extensive over the hind quarters, ventral abdomen and head. The kidneys were diffusely swollen, somewhat pale and the capsular and incised cortical surfaces contained multiple petechial haemorrhages.

Laboratory Results:
Immunohistochemistry: IgG and IgM were demonstrated in glomeruli and renal effusions.

Insitu hybridization: Strong PCV2 DNA signals in histiocytes of follicle centres and splenic periarteriolar sheaths, weak signal in renal tubule epithelium.

Histopathologic Description: 1. Kidney: Most glomeruli in the section are distorted by fibrinous exudate that distends the urinary space or has formed in capillaries. Small numbers of neutrophils, sometimes degenerate are enmeshed in the coagula. Tubules are distended by intralumenal sero-haemorrhagic fluid and proteinaceous casts. The epithelial changes are of irregular distribution throughout the cortex and vary from necrosis (infrequent and often associated with neutrophil infiltrates), to hyaline droplet reabsorption, hyperplasia and hypertrophy, representing the acute to subacute responses to injury. Sparse focal aggregates of lymphocytes and plasma cells are present in the interstitium. Infrequent (one or two in the submitted sections) arcuate and

interlobular arterioles have fibrinoid degeneration of the media accompanied by endothelial cell necrosis, thrombosis and leukocyte infiltration of the vessel wall and perivascular tissue.

2. Spleen: Many of the small arteries and arterioles are obliterated by fibrinoid necrosis, but transmural cellular infiltrates that often include degenerate neutrophils, are recognizable in some of the affected vessels. The periarteriolar sheath lymphoid cells are depleted, and there is a concomitant increase in macrophages, often with bi- and multi-nucleate forms.

Contributor's Morphologic Diagnoses: 1. Kidney: Glomerulonephritis, fibrinous, diffuse and severe with leucocytoclastic vasculitis, porcine (*Sus scrofa*).
2. Spleen: Arteriolitis, fibrinonecrotic, leucocytoclastic, random with periarteriolar lymphoid atrophy and histiocytic infiltration, porcine, (*Sus scrofa*).

Contributor's Comment: The diagnosis of porcine dermatitis and nephropathy syndrome (PDNS) was made in this and other affected pigs on this property. PDNS was first recognised the United Kingdom and has subsequently been reported in most major pig producing countries world-wide.[1,2]

The clinical and pathological features of the disorder are characterized by the appearance of haemorrhagic skin plaques and erosions that are followed by variable signs of pyrexia, anorexia, weight loss, depression and (usually) death.[2] In fatal cases the haemorrhagic renal and cutaneous lesions are the most characteristic features at necropsy, resulting from a necrotising vasculitis with microscopic and immunological features that are consistent with an immune-mediated process, most likely a type III hypersensitivity and/or direct cytotoxic T cell response. The latter is supported by the demonstration of immunoglobulins, complement fragments and CD8+ lymphocytes in the lesions.[3]

Although an inciting antigen has yet to be defined, it is widely suspected that porcine circovirus-2 (PCV2) is implicated.[4] PRRSV, another widely cited causal or contributory agent, does not occur in New Zealand.

AFIP Diagnoses: 1. Kidney: Glomerulonephritis, necrotizing, acute, diffuse, severe, with hemorrhagic and proteinaceous casts, glomerular fibrin thrombi, neutrophilic tubulitis, and tubular degeneration, necrosis, and regeneration, crossbred pig (*Sus scrofa*), porcine.
2. Spleen: Vasculitis, necrotizing, acute, diffuse, severe, with lymphoid depletion, and diffuse moderate granulomatous splenitis.

Conference Comment: PDNS is an emerging disease that primarily affects recently weaned and feeder pigs from 1.5-4 months of age.[2] Although the pathogenesis is not fully understood, both postweaning multisystemic wasting syndrome (PMWS) caused by

porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (porcine arterivirus) have been implicated in the pathogenesis of PDNS.[3,4,5] In both infections, viremia can coexist with the presence of antibodies facilitating immune complex formation. Additionally, both viruses infect monocytes/macrophages and may indirectly affect the efficiency of the mononuclear phagocytic system in removing immune complexes from circulation.[6]

Typical gross lesions range from multifocal dermal petechiation and ecchymoses to dark brown to black thick crusts that are distributed primarily on the hind limbs and perineal area. With time, the skin lesions gradually fade and may leave scars. Kidneys are enlarged, pale, and edematous with multifocal cortical petechiation in acute cases. In chronic cases, the kidneys are finely granular, shrunken, and contracted.[3,4,5,6]

Necrotizing vasculitis is a systemic feature of the disease, showing marked tropism for the skin and kidneys. Histologically, the skin lesions are characterized by marked dermal hemorrhages associated with a severe necrotizing leucocytoclastic vasculitis affecting small caliber blood vessels. Thrombosis and focal ischemic coagulative necrosis may also be present. Histopathological changes in the kidney include an exudative and occasionally necrotizing glomerulonephritis with fibrinoid deposits (immune complexes) in glomeruli that may be accompanied by an interstitial nephritis and fibrosis. Splenic vasculitis, thrombosis, and infarction are also frequently observed.[3,4,5,6]

The differential diagnosis for the skin & renal lesions seen in PDNS includes:

1. Classical swine fever (porcine pestivirus)
2. *Erysipelothrix rhusiopathiae*
3. Salmonellosis
4. *Actinobacillus suis*
5. African swine fever (asfarvirus)

Conference attendees briefly reviewed causes of vasculitis in other species. The diseases that cause vasculitis in animals are summarized in a table below from Pathologic Basis of Veterinary Disease.[7]

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

Contributor: Institute of Veterinary Animal and Biological Sciences, Massey University, Palmerston North, New Zealand, www.massey.ac.nz

References:
1. Thomson JR, Higgins RJ, Smith WJ, Done SH: Porcine dermatitis and nephropathy syndrome. Clinical and pathological features of cases in the United Kingdom. (1993-1998). J Vet Med 49:430-437, 2002
2. Chae C: A review of porcine circovirus 2-associated syndromes and diseases. Vet J 169:326-336, 2005
3. Thibault S, Drolet R, Germain M-C, D'Allaire S, Larochelle R, Magar R: Cutaneous and systemic necrotizing vasculitis in swine. Vet Pathol 35:108-116, 1998
4. Wellenberg GJ, Stockhofe-Zurwieden N, de Jong MF, Boersma WJA, Elbers ARW: Excessive porcine circovirus type 2 antibody titres may trigger the development of porcine dermatitis and nephropathy syndrome: a case-control study. Vet Micro 99:203-214, 2004
5. Cameron R: Diseases of the Skin. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 196-198. Blackwell Publishing, Ames, Iowa, 2006
6. Drolet R, Dee SA: Diseases of the Urinary System. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 203-205. Blackwell Publishing, Ames, Iowa, 2006
7. 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

SLIDE 11
CONFERENCE 3 / CASE III – 8005-1088 (AFIP 2986827)

Signalment: Adult female white tail deer (*Odocoileus virginianus*)

History: Several wild caught adult white tail deer were presented for necropsy following a short history of vague illness of being found dead. Some animals showed bloody diarrhea of very short duration, but many were without premonitory signs. Animals were caught from various regions in Mississippi.
Gross Pathology: This adult female white tail deer in good body condition presented for necropsy within minutes of death. The tail and perineum were soiled with a small amount of frankly bloody fecal paste and dark bloody fluid dripped from the anus. Tissues were dry and tacky. The spiral colon and terminal ileum were dark with bloody

contents and moderate gas; mucosal surfaces had loosely adherent fibrinous plaques. Mesenteric lymph nodes were dark and mushy. The terminal colon had a small amount of red brown fluid and formed no feces.

Laboratory Results: Culture of the spiral colon and ileum yielded heavy growth of *Clostridium perfringens* Type A (negative for enterotoxin and beta-2 toxin by PCR).

Histopathologic Description: Sections of colon showed mucosal necrosis, hemorrhage, inflammation, and exudation with myriad bacterial rods. The mucosa shows areas of collapse due to loss of glands interspersed with glands lined with attenuated epithelial cells. The luminal surface is often covered by a layer of fibrin, hemorrhage, degenerating epithelial and inflammatory cells and myriad chaining bacterial rods. Inflammatory exudate includes many pale swollen neutrophils.

Contributor's Morphologic Diagnosis: Colon: Acute necrotizing and hemorrhagic colitis.

Contributor's Comment: Toxigenic strains of *Clostridium perfringens* are typed according to exotoxin production or, more commonly in contemporary practice, by the presence of toxin genes using PCR. Classical types include type A with alpha toxin; type B with alpha, beta, and epsilon toxins; type C with alpha and beta toxins; type D with alpha and epsilon toxins, and type E with alpha and iota toxins. Clostridial enterotoxin and/or beta-2 toxin can be present or absent in any of the classical clostridial types. *Clostridium perfringens* type A has been associated with severe intraluminal hemorrhage into the small intestine (hemorrhagic bowel syndrome) in mature cattle. As well, *Clostridium perfringens* causes gas gangrene, food poisoning and other forms of necrotizing enteritis. *Clostridium perfringens* alpha toxin is a Zn^{2+} phospholipase that hydrolyses choline-containing phospholipids (phospholipase C) and has sphingomyelinase activity causing a spectrum of membrane damage including hemolysis, myotoxicity, platelet aggregation and necrosis. Why *Clostridium perfringens* proliferates and secretes lethal amounts of toxin in the gut is often speculative but probably includes decreased oxygen concentration and decreased gut motility, two interdependent variables that may be influenced by a variety of factors (feed change, antibiotic administration, ileus, etc.).[1,2,3,5]

AFIP Diagnosis: Colon (per contributor): Colitis, necrotizing, acute, diffuse, severe, with hemorrhage, white tail deer (*Odocoileus virginianus*), cervid.

Conference Comment: The contributor provides a brief summary of the toxins associated with each clostridial type. *Clostridium perfringens* is a Gram-positive, anaerobic bacillus that is a normal inhabitant of the alimentary tract of most species of warm-blooded animals and is ubiquitous in the environment.[1,4] *Clostridium perfringens* type A is the most frequently occurring clostridial species of mammals and birds. Below is a chart of the five types of *C. perfringens*, the toxins they produce, and the most important diseases they cause.[2,3]

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 encephalomalacia - Sheep
E	+	-	-	++	Enterotoxemia - Calves, lambs, guinea pigs, rabbits

Table adapted from Barker et al, 1993 p.237 & Jones et al, 1997 p. 421

Enterotoxin and beta-2 toxin can be produced by all types of *C. perfringens*, but are not used in typing and are, therefore, not included in the chart. *C. perfringens* type A producing beta-2 toxin is associated with necrotic enteritis in piglets and typhlocolitis in horses.[6,7]

Conference attendees considered the contributor's diagnosis of clostridial enterotoxemia. However, the typical myriad clostridial organisms were not present in our H&E or Brown-Brenn stained sections. The moderator also commented that, in his experience, there is typically more hemorrhage associated with enterotoxemia than present in this case. Conference attendees placed diseases that cause crypt necrosis, such as bovine viral diarrhea (bovine pestivirus) and coronavirus, higher in the differential diagnosis.

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References:
1. Timoney JF, Gillespie JH, Scott FW, Barlough JE: Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th ed., pp. 223-229. Cornell University Press, Ithaca, New York, 1988
2. Barker IK, Van Dreumel AA, Palmer Nigel: The Alimentary System. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 237-244. Academic Press, Inc., San Diego, California, 1993
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 420-423. Williams & Wilkins, Baltimore, Maryland, 1997
4. Gelberg HB: Alimentary System. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 365-366, Mosby Elsevier, St. Louis, Missouri, 2007
5. Aschfalk A, Valentin-Weigand P, Müller W, Goethe R: Toxin types of *Clostridium perfringens* isolated from free-ranging, semi-domesticated reindeer in Norway. Vet Rec 151:210-213, 2002
6. Songer JG, Uzal FA: Clostridial enteric infections in pigs. J Vet Diagn Invest 17:528-536, 2005
7. Herholz C, Miserez R, Nicolet J, Frey J, Popoff M, Gibert M, Gerber H, Straub R: Prevalence of _2-toxingenic *Clostridium perfringens* in horses with intestinal disorders. J Clin Microbiol 37(2):358-361, 1999

SLIDE 12
CONFERENCE 3 / CASE IV – CASE 1 (AFIP 3026197)

Signalment: 4-year-old, Female, Holstein Friesian, Dairy cow, bovine

History: The cow came from a herd with problems of chronic mastitis and reduction in milk production. One quarter of the mammary gland was collected at the slaughterhouse and sent for histological and cultural analysis.
Gross Pathology: The mammary gland had an increased consistency and, on cut section, was characterized by diffuse fibrosis, thickening and ectasia of mammary ducts, catarrhal to interstitial inflammation with some areas characterized also by purulent exudate. The supramammary lymph nodes were diffusely and severely hyperplastic.

Laboratory Results: *Prototheca zopfii* was isolated in pure culture from the mammary tissue.

Histopathologic Description: The interlobular septal interstitium of the mammary gland is moderately to severely expanded by fibrosis and variable numbers (different areas) of mature small lymphocytes, macrophages, eosinophils and neutrophils. Occasionally, nodular areas of inflammation with a necrotic center surrounded by neutrophils, macrophages and lymphocytes can be seen. Acini and ducts are

multifocally severely dilated and contain abundant necrotic debris associated with neutrophils, macrophages and poorly stained algal organism free or in the cytoplasm of inflammatory cells. Atrophy of the acinar portion is evident.

In the lumens of the dilated alveoli and ducts, PAS stain reveals the presence of variably abundant free or intracytoplasmic organisms. Organisms are round to oval, non budding, 5-20 micron in diameter with a thick, intensely PAS positive cell wall. Occasional minimal internal septation can be seen.

Contributor's Morphologic Diagnosis: Mammary gland: Severe, diffuse, chronic pyogranulomatous and necrotizing mastitis with acinar atrophy and interstitial fibrosis with intralesional algal structures consistent with *Prototheca* spp.

Contributor's Comment: Protothecosis is an infectious condition caused by achlorophyllic algae of the genus *Prototheca* which affects domestic animals and man. The genus *Prototheca* belongs to the family of *Chlorellaceae*. Prototheca is a unicellular, oval to spherical organism that reproduces asexually by internal septation and irregular cleavage to produce between 2 and 20 sporangiospores within a hyaline sporangium. The sporangiospores are arranged in a characteristic morula configuration and upon rupture of the sporangium, are released to develop into additional endosporulating forms. Sporangiospores measure 3 to 30 µm in diameter and differ from *Chlorella* spp. since they lack chloroplasts and from fungi since they lack glucosamine in the cell wall.[1]

Prototheca spp. are ubiquitous and have been isolated from a variety of environmental sources, including plants, mud, sewage, different water sources and soil.[1,2] Protothecal organisms can also be found in the faeces of various domestic animals or wild animals.[1,2] Occasionally, they have been detected colonizing the human skin, fingernails, respiratory tract, and digestive system.[1] It is presumed that the mechanism leading to protothecosis is the traumatic inoculation of the etiologic agent (cutaneous form) and/or endogenous colonization (gastrointestinal, respiratory or urogenital) followed by bloodstream invasion and algaemia.[1,3] Direct transmission from human to human or animal to human has not been demonstrated. *P.wickerhamii* and *P. zopfii* are the main etiologic agents of protothecosis. *P. wickerhamii* is predominantly responsible for human protothecosis. In man, the microorganism causes a variety of localized to systemic diseases primarily in immunocompromised patients.[1,4,5] The three most common forms of protothecosis described in man are cutaneous, olecranon bursitis and disseminated.[1,4,5]

Infections caused by *P. zopfii* are most frequently observed in domestic animals. *P. zopfii* may cause chronic colitis, dermatitis, ophthalmitis and systemic infections in dogs and mastitis in the bovine species. In dairy cows, protothecosis presents primarily as a severe clinical to subclinical, often therapy-resistant mastitis causing severe economic losses.[6] *P. zopfii* is biochemically and serologically differentiated into three different biotypes. Biotype II isolates are reported as the predominant infectious agents associated with bovine mastitis.[7] Algae seem to colonize the mammary compartment

via the intracanalicular route and poor milking hygiene is considered the main predisposing factor for mammary protothecosis.[6] Algal infection generally triggers a chronic inflammatory response restricted to the mammary gland and regional lymph nodes. Inflammation is predominantly granulomatous with epithelioid and multinucleated giant cells, but it is also associated with necrosis and prominent infiltration of lymphocytes, plasma cells, eosinophils and neutrophils.[8] Algal structures may be also observed in macrophages and less frequently in neutrophils. Organisms are also found within the lumen and between the lining epithelium and basement membrane of the affected alveoli.[9] In macrophages, both sporangiospores and sporangia have been observed, suggesting that, due to their ability to survive and replicate in these cells, the pathogen causes a persistent infection poorly responsive to therapy.

AFIP Diagnosis: Mammary gland: Mastitis, granulomatous and eosinophilic, chronic, diffuse, moderate, with myriad algae, Holstein-Friesian (*Bos taurus*), bovine.

Conference Comment: The moderator provides an excellent overview of *Prototheca*. Tissue reactions induced by *Prototheca* and *Chlorella* can be granulomatous, necrotizing, or a combination of both. When a granulomatous reaction is incited, extracellular organisms are rare. *Prototheca* cannot be differentiated from *Chlorella* on H&E stained sections. However; the green color of the gross lesions, the light microscopic detection of PAS positive starch granules, and the ultrastructural detection of typical chloroplasts in lesions caused by *Chlorella* allow differentiation between the two algal infections.[11,12]
Conference attendees reviewed these other algae and fungi that reproduce by endosporulation:

1. *Chlorella* sp.
2. *Coccidioides immitis*
3. *Rhinosporidium seeberi*
4. *Batrachochytrium dendrobatidis*

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References:
1. Pfaller MA, Diekema DJ: Unusual fungal and pseudofungal infections of humans. J Clin Microbiol 43:1495-1504, 2005
2. Costa EO da, Melville PA, Ribeiro AR, Watanabe ET, Parolari MCFF: Epidemilogic study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis. Mycopathol 137:33-36, 1997
3. Mohabeer AJ, Kaplan PJ, Southern PM Jr, Gander RM: Algaemia due to *Prototheca wickerhamii* in a patient with myasthenia gravis. J Clin Microbiol 35**:** 3305-3307, 1997

4. Torres HA, Bodey GP, Tarrand JJ, Kontoyiannis DP: Protothecosis in patients with cancer: case series and literature review. Clin Microbiol Infect 9:786-792, 2003
5. Pascual JS, Balos LL, Baer AN: Disseminated *Prototheca wickerhamii* infection with arthritis and tenosynovitis. J Rheumatol 31:1861-1865, 2004
6. Buzzini P, Turchetti B, Facelli R, Baudino R, Cavarero F, Mattalia L, Mosso P, Martini A: First large-scale isolation of *Prototheca zopfii* from milk produced by dairy herds in Italy. Mycopathologia158:427-430, 2004
7. Roesler U, Hensel A: Longitudinal analysis of *Prototheca zopfii*-specific immune responses: correlation with disease progression and carriage in dairy cows. J Clin Microbiol 41:1181-1186, 2003
8. Benites NR, Guerra JL, Melville PA, da Costa EO: Aetiology and histopathology of bovine mastitis of spontaneous occurrence. J Vet Med B 49:366-370, 2002
9. Corbellini LG, Driemeier D, Cruz E: Immunohistochemistry combined with periodic acid-schiff for bovine mammary gland with protothecal mastitis. Biotech Histochem 76:85-88, 2001
10. 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
11. Haenichen T, Facher E, Wanner G, Hermanns W: Cutaneous Chlorellosis in a gazelle (*Gazella dorcas*). Vet Pathol 39:386-389, 2002
12. Le Net JL, Ahmed MF, Saint-Martin G, Masson MT, Montois C, Longeart L: Granulomatous enteritis in a dromedary (*Camelus dromedarius*) due to a green algal infection. Vet Pathol 30:370-373, 1993

SLIDE 13
CONFERENCE 4 / CASE I – 06-12468 (AFIP 3026808)

Signalment: Adult, female, mule deer (*Odocoileus hemionus*)

History: Animal was captured by net gun, blindfolded and transported to a staging area where it was anesthetized and received a tonsillar biopsy and a radio collar. The anesthesia was reversed and the animal was then transported back to the original capture site where it was released. Seven days later it was noticed to be severely lame in the hind limbs and was euthanized by gunshot.

Gross Pathology: Prior to necropsy it was noted that the lower hind limbs could be flexed without resistance from the extensor muscles. Deer was in poor body condition with no internal stores of fat. There was marked subcutaneous hemorrhage about the lower aspects of both tibiae. The entirety of both gastrocnemius muscles were swollen, dark red, necrotic, friable and torn from the insertions. Other muscles appeared unremarkable except for the extensor carpi radiali, tongue and deep pectoral muscle, which were streaked with areas of pallor. In addition, the extensor carpi radiali were edematous and tightly swollen within the fascial sheaths.

Histopathologic Description: Approximately 60-80% of the myofibers are degenerative or necrotic in the examined sections. Muscle fibers are characterized by sarcoplasmic hypereosinophilia and flocculation, loss of cross-striations, and nuclear pyknosis, karyorrhexis and karyolysis (necrosis) and varying stages of degeneration (myocyte swelling, pallor). Necrotic myocytes are separated, surrounded and replaced by a low to moderate number of macrophages, lymphocytes and rare neutrophils, hemorrhage and edema. Occasionally macrophages infiltrate individual myofibers. Mineralization is not noted in the examined section. A low number of *Sarcocystis* sp. cysts are present.

Contributor's Morphologic Diagnosis: Skeletal muscle: Degeneration and necrosis, multifocal to coalescing, severe, subacute with lymphohistiocytic myositis and interstitial hemorrhage.

Contributor's Comment: Capture myopathy (exertional rhabdomyolysis) as a disease syndrome is an important cause of morbidity and mortality of handled wild animals. The syndrome is characterized by damage to both skeletal and cardiac musculature and has been seen in a great variety of species including mammals (herbivores and carnivores) and birds. Lesions are associated with extreme exertion and shock related to pursuit, immobilization, restraint, handling and transport. Clinical signs associated with this disease syndrome are varied and include depression, reluctance to stand and ataxia. These signs may be displayed immediately following the triggering episode or may be delayed in their appearance for a period of days or weeks.[1]

The pathophysiology of capture myopathy is reported to be related to both vasogenic-neurological shock and metabolic acidosis.[2] Handling stimulates discharge of the sympathetic nervous system and subsequent release of catecholamines. Exhaustion of the sympathetic nervous system leads to decreased vascular tone, pooling of blood in viscera, decreased venous return and decreased cardiac output. Inadequate nutrient delivery and increased demand for energy caused by exertion rapidly depletes cells of their readily available ATP stores. In order to meet their metabolic requirements cells begin anaerobic metabolism which in turn leads to the accumulation of lactic acid and subsequent metabolic acidosis. This exacerbates the already compromised cardiac output and systemic blood pressure. Decreased function of the sodium-potassium pump due to inadequate ATP levels leads to sodium influx and cellular swelling. Increased intracytoplasmic calcium levels activate numerous enzymes and cause the progression from cellular injury to cell death.[1,2] Release of intracellular potassium can lead to acute cardiac arrest. If animals survive the initial stages, the release of large amounts of myoglobin coupled with hypoxia can lead to renal tubular damage and animals may die subsequent to acute renal failure.

Treatment is problematic in that efforts to administer treatment may induce or compound capture myopathy. Traditional attempts at treatment have involved antioxidant administration and free radical scavengers. Dantrolene sodium has been used to treat humans with malignant hyperthermia and in some cases of capture myopathy. The basis for this is to attempt to prevent calcium release from the

sarcoplasmic reticulum. Its use is hampered by expense and the difficulty in administration of intravenous fluids to wild animals in a field situation. [2]

AFIP Diagnoses: 1. Skeletal muscle: Degeneration and necrosis, multifocal to coalescing, severe, with histiocytic inflammation, satellite cell proliferation, and interstitial hemorrhage, mule deer (*Odocoileus hemionus*), cervid.
2. Skeletal muscle: Sarcocysts, multifocal, few.
Conference Comment: The contributor provides a concise summary of the pathophysiology of capture myopathy. Acquired myopathies generally fall under one of three categories: toxic (e.g., Gossypol, ionophore, and *Cassia* sp.), nutritional (e.g., vitamin E/selenium deficiencies), and exertional (e.g., azoturia, tying-up, porcine stress syndrome, capture myopathy).
Predisposing factors for capture myopathy include species, method of capture, high environmental temperature, physical condition of the animal, and nutritional status of the animal. Species with high metabolic rates are more susceptible to capture myopathy. More aggressive methods of capture cause a greater incidence of capture myopathy. High environmental temperatures do not allow for heat dissipation generated by physical exertion and stress. Deficient levels of vitamin E and selenium predispose to a higher incidence of capture myopathy with more severe clinical signs.[2]

Renal lesions in exertional myopathy are associated with renal ischemia, secondary to shock, and myoglobin. Histomorphologic features include tubular epithelial swelling, degeneration, and necrosis; orange to red granules in tubular epithelial cells; granular, myoglobin, and proteinaceous casts; tubular regeneration; and interstitial edema.[1]

Clinical pathology abnormalities in cases of exertional myopathy include increases in CK, AST, LDH, ALT, BUN, and Cr; hyperphosphatemia; hyperkalemia; myoglobinemia; myoglobinuria; and a stress leukogram.[1,3]

Conference attendees discussed the concepts of monophasic and multiphasic skeletal muscle lesions. Monophasic lesions are all in the same stage of degeneration, regeneration, or necrosis, and imply a single insult (e.g., a single dose of a toxin or a strenuous episode). Multiphasic lesions are in multiple stages of degeneration, regeneration, or necrosis and imply an ongoing insult such as chronic or intermittent toxin exposure or vitamin E/selenium deficiency.

Muscle injuries can also be classified as reversible (metabolic, toxic, nutritional) or irreversible (heat, intense inflammation, infarction). The myofiber basal lamina remains intact and viable satellite cells remain in reversible muscle injury allowing for regeneration. In irreversible muscle injury, large areas of satellite cells are destroyed, and healing occurs by fibrosis. If the insult to muscle disrupts the myofiber basal lamina but does not damage the satellite cells, attempts at regeneration are ineffective resulting in the formation of muscle giant cells.[4]

Attendees also discussed the differential diagnosis for skeletal muscle necrosis in various species to include the following: Exertional rhabdomyolysis, equine polysaccharide storage myopathy (EPSSM), nutritional myopathy (vitamin E/selenium deficiency), ischemic myopathy due to anesthesia, plant toxicity (*Cassia* sp., ionophore toxicity (monensin), clostridial myositis (malignant edema, botulism), malignant hyperthermia-like syndrome, and *Streptococcus*-associated myopathy.
Readers are encouraged to review WSC Conference 7/ Case II from the 2005-2006 academic year – a case of hypovitaminosis E in a brown pelican.

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References:
1. Williams E, Thorne E: Exertional myopathy (capture myopathy). In: Noninfectious Diseases of Wildlife, eds. Fairbrother A, Locke L, Hoff G, 2nd ed., pp 181-193. Iowa State University Press, Ames, Iowa, 1996
2. Woodbury MR: A brief review of capture myopathy and reperfusion Injury. In: The Chemical Immobilization of Wildlife, eds. Cattet M, Shury T, Patenaude R, 2nd ed., pp. E-3-E13. The Canadian Association of Zoo and Wildlife Veterinarians, 2005
3. Bender HS: Muscle. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 260-269. Iowa State Press, Ames, Iowa, 2003
4. Valentine BA, McGavin MD: Skeletal muscle. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 985-989. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 14
CONFERENCE 4 / CASE II – A06-12006-1a (AFIP 3026261)

Signalment: 11-month-old, male, Rocky Mountain elk, *Cervus elaphus nelsoni*

History: Two farm-raised elk on pasture stopped coming to the feeder 3 weeks before presenting for lameness and thickening of the limbs distal to the elbows and hocks. Pulmonary nodules were evident radiographically in both elk and a radiographic diagnosis of hypertrophic osteopathy was made for metacarpal and metatarsal bones. Tuberculosis was suspected and the elk were euthanized.

Gross Pathology: In both elk, the carpal, metacarpal, tarsal, metatarsal bones, and the radii, ulnae, and tibiae had smooth thickening of cortical bone, particularly on dorsal and proximal aspects. Areas of bone lysis, sometimes filled with caseous exudate, were present in the left metatarsus and a distal phalanx of one elk.

Both elk had encapsulated pulmonary nodules that occupied most of the parenchyma in one animal. The nodules contained abundant caseous exudate. Tracheobronchial, retropharyngeal, mediastinal, and prescapular lymph nodes were variably enlarged up to 15 cm in diameter and contained caseous exudate. Caseated nodules up to 4 mm in diameter were also in the myocardium, kidney, and spleen of the elk with lytic and caseated bone lesions; this elk also had lingual ulcers.

Laboratory Results: Clinical pathologic abnormalities included mild anemia and hyperglobulinemia. *Aspergillus fumigatus* was cultured from lung of both elk post mortem. Surveillance testing for chronic wasting disease was negative on formalin-fixed brain. Cultures of lung and lymph node were negative for *Mycobacterium*. Virus isolation from pooled tongue and spleen was negative. Fecal flotation and Baermann funnel technique for lungworms were negative.

Histopathologic Description: Pulmonary granulomas are encapsulated and contain myriad hyphae. Fungal hyphae are 5-8 _m thick with parallel walls, septate, and dichotomously branched. They form palisades at the periphery of granulomas and are more disorganized and fragmented in the center. Fungal hyphae in lung and in other tissues were strongly positive by immunohistochemistry for *Aspergillus* spp. A band of degenerated neutrophils and eosinophils with fewer epithelioid macrophages and rare multinucleated giant cells surrounds the caseous centers of granulomas. Degenerated leukocytes and fungal hyphae are also observed in bronchioles and small bronchi. Some affected airways are ectatic or expand into early (nonencapsulated) granulomas. Interlobular septa are expanded by fibrous connective tissue. Fibrosis of interalveolar septa is evident in some lobules; in these lobules, alveolar spaces are often filled with fibrin that is mixed with neutrophils, macrophages and fibroblasts.

The bony section is prepared from a cross-section of the large metatarsal bone. The periosteum is markedly thickened by parallel and perpendicularly oriented trabeculae of immature bone with abundant osteoblastic activity and much less osteoclastic activity.

Contributor's Morphologic Diagnoses: 1. Granulomatous pneumonia with intralesional fungal hyphae.
2. Periosteal new bone formation (periosteal hyperostosis), metatarsal bone.

Contributor's Comment: Fungal granulomas were also found histologically in tongue, lymph nodes, myocardium, kidney, and bone marrow. Histologic findings in both elk were consistent with pulmonary and systemic aspergillosis and periosteal hyperostosis (hypertrophic osteopathy).

Pulmonary aspergillosis is the most common fungal infection observed in farmed elk in our laboratory. The source of infection in this case was undetermined, but inhalation of spores from contaminated feed was suspected.
Hypertrophic osteopathy has been described in humans, numerous domestic species and in one roe deer from Germany.[1,2,3] The pathogenesis is poorly understood, but hypertrophic osteopathy is usually associated with space-occupying lesions in the

thorax, neoplastic or inflammatory processes in the lung, and neoplasms of abdominal organs, particularly the urinary bladder.[1,2] Hypertrophic osteopathy in our case was most likely the result of numerous pulmonary granulomas.

AFIP Diagnoses: 1. Lung: Granulomas, multifocal and coalescing, with myriad hyphae, Rocky Mountain elk (*Cervus elaphus nelsoni*), cervid.
2. Bone, metatarsus (per contributor): Hyperostosis, periosteal, diffuse, severe.

Conference Comment: *Aspergillus* spp. are opportunistic pathogens that cause serious disease in debilitated or immunocompromised animals or in animals on prolonged antibiotic treatment. Transmission occurs by inhalation of spores resulting in pneumonia. *Aspergillus* commonly invades blood vessels and can spread hematogenously to various organs. The necrotizing vasculitis can lead to thrombosis and infarction. Some species of *Aspergillus*, such as *A. flavus* and *A. parasiticus*, produce highly toxic and carcinogenic aflatoxins. The *Aspergillus* species most commonly associated with disease in animals include *A. fumigatus*, *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus*.[4]

Histologically, the hyphae are 3-6 um wide, parallel walled, regularly septate, with dichotomous acute angle branching. Spores usually do not form in tissues, but can be seen on surfaces exposed to air (air sacs, trachea).[4] Conidial heads or fruiting bodies are composed of a golden-brown dome-shaped terminal vesicle covered by phialides from which chains of conidia are produced.[5]

Conference attendees briefly discussed lesions caused by *Aspergillus* sp. in various species. Aspergillosis is most common and severe in young chicks and turkey poults that become infected by inhaling spores in contaminated bedding resulting in pneumonia/air sacculitis/tracheitis ("brooder pneumonia"). Captive penguins are especially susceptible. In mammals, aspergillosis is seen in most species with mycotic dermatitis, keratitis, and pneumonia being the most common manifestations. However, dissemination to other organs can occur.

Hypertrophic osteopathy occurs in humans and in domestic animals with the dog being most commonly affected. Periosteal new bone formation is usually confined to the diaphyseal region of the distal limbs with the radius, ulna, tibia, and metatarsals most commonly involved. The bones of the upper limbs and phalanges are relatively spared. Lesions typically regress if the inciting cause is removed. Causes of hypertrophic osteopathy in the dog include:[1,6,7]

1. Endocarditis
2. *Dirofilaria immitis*
3. Rhabdomyosarcoma of the urinary bladder
4. Esophageal granulomas and tumors associated with *Spirocerca lupi*
5. *Hepatozoon americanum*

6. Intrathoracic neoplasia or inflammation

Hypertrophic osteopathy is associated with ovarian neoplasms in the horse.[1,6]

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References:

1. Palmer N: Bone and joints. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 116-117. Academic Press, Inc., San Diego, California, 1993
2. Burk RL, Feeney DA: The appendicular skeleton. In: Small Animal Radiology and Ultrasonography: A Diagnostic Atlas and Text, 3rd ed., Saunder, Philadelphia, Pennsylvania, 2003
3. Schulze C, Prejawa T, Paulick D: Fallbericht: Hypertrophe osteopathie beim reh (Capreolus capreolus). Dtsch teirärztl Wschr 112:392-394, 2005
4. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 505-508, 539-542. Williams & Wilkins, Baltimore, Maryland, 1997
5. Rippon JW: Medical Mycology, the Pathogenic Fungi and the Pathogenic Actinomycetes, 3rd ed., pp. 130-131, 637-639. WB Saunders, Philadelphia, Pennsylvania, 1988
6. Weisbrode, SE: Bone and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1084-1085. Mosby Elsevier, St. Louis, Missouri, 2007
7. Panciera RJ, Mathew JS, Ewing SA, Cummings CA, Drost WT, Kocan AA: Skeletal lesions of canine hepatozoonosis caused by *Hepatozoon americanum*. Vet Pathol 37:225-230, 2000

SLIDE 15
CONFERENCE 4 / CASE III – CASE 2 (AFIP 3026185)
Signalment: Age unknown (adult), female, European brown hare, *Lepus europaeus*

History: The hare came from a group of 250 that developed multiple cutaneous tumors out of 1000 farmed game hares.

Gross Pathology: Skin tumors were 1-3 cm in size, often alopecic and ulcerated (Fig. 2) and most commonly located on the ears (Fig. 3) and limbs (Fig. 4). On cut section, lesions had a homogeneous, white surface (Fig. 5). After 4 to 6 weeks the lesions showed progressive regression in most animals. In some cases, the tumors detached from the skin surface leaving bleeding ulcers that evolved into alopecic scars.

Laboratory Results: Electron microscopy from tissue samples identified typical biconcave poxviral particles (Fig. 1). The virus was isolated, injected in embryonated

eggs and produced typical lesions in chicken embryos (white-yellowish pocks). EM from these lesions confirmed the presence of poxviral particles in the embryos.

Histopathologic Description: Epidermis is characterized by erosions, intracellular edema, and serocellular crusts (not in all sections). Dermo-epidermal detachment is also evident (not in all sections). Dermis and adnexa are almost completely substituted by an unencapsulated, poorly demarcated neoplasm extending to the deep borders. The tumor is composed of irregular to interlacing bundles of atypical, variably sized, spindle cells embedded in minimal fibrous stroma containing elevated numbers of capillaries. Cells have generally indistinct cell borders, abundant clear cytoplasm often containing bright, irregular, amorphous to granular, PAS positive (Fig. 6) cytoplasmic eosinophilic poxviral inclusions. Inclusions are occasionally present in the follicular epithelium (not in all sections). Mitoses range from 0-2 per HPF. Among neoplastic cells anisokaryosis and anisocytosis are prominent and bi- to multinucleated giant cells are present.

Contributor's Morphologic Diagnosis: Haired skin, dermis: Dermal fibroma with intracytoplasmic eosinophilic poxviral inclusions, European brown hare, *Lepus europaeus*.

Contributor's Comment: Leporipoxviruses are implicated in the development of Myxomatosis and Shope fibromas in rabbits and fibromatosis in hares.[1] Recently, a form of mucocutaneous dermatitis in Mountain hares (*Lepus timidus*) from Finland, once supposed to have a *Treponema* spp. aetiology seems also to be caused by a leporipoxvirus.[2] The viruses in all these diseases seem to be transmitted by biting insects/arthropods and or direct contact.

Fibromatosis (poxviral hare fibromas) is a disease described in wild and reared game hares.[1,3,4] The disease is caused by a leporipoxvirus which is antigenically related to the Shope fibroma virus of rabbits.[1] Fibromatosis is associated with high morbidity but low mortality and is characterized by single to multiple protruding, dermal tumors mostly located on ears and legs.[3] In 2003, the re-emergence of fibromatosis in farmed game hares has been reported in Italy.[3]

Hare fibromas are grossly and microscopically similar to lesions described in cottontail rabbits with Shope fibroma.[1] Both diseases are characterized by the development of cutaneous, usually benign tumours, characterized by proliferation of dermal fibroblasts, epithelial hyperplasia and intracytoplasmic eosinophilic inclusions within both epithelial and mesenchymal cells.[1] In adult rabbits, the tumors are localized and are characterized by spontaneous regression whereas in neonatal rabbits and immunocompromised adults they may become invasive and may develop into malignant fibrosarcomas.[1] Rabbits bearing Shope fibroma generally develop a cell-mediated immune response and virus-neutralizing antibodies with cytotoxic and cytolytic activity whose kinetics parallel the tumors' development and regression.[5] Extracts of Shope fibroma tumours have been found to contain a second virus, antigenically virtually identical to SFV, but with a different behavior demonstrated *in vitro* and *in vivo*

experiments.[1,6] This virus has been called Malignant rabbit fibroma virus.[1,6] Malignant rabbit fibroma virus represents a lethal tumorigenic rabbit poxvirus derived from a recombination between Shope fibroma virus and Myxomavirus.[1,6] Malignant rabbit fibroma virus induces fibroma-like tumours that disseminate extensively and do not regress. In these lesions inclusion bodies are not detectable. MRV severely reduces both T and B cell function with immunodepression.[6] This feature seems to account for its aggressive behavior.

Leporipoxvirus is a member of the Poxviridae family, which comprises a large family of double-stranded DNA viruses that are able to infect both vertebrates (Chordopoxvirinae) and insects (Entomopoxvirinae). After cell infection, contrary to other DNA viruses, Poxviruses do not enter the nucleus of infected cells but establish a virus factory in the cytoplasm that represents the site of viral transcription and DNA replication. Distinct viral inclusions localized within endoplasmic reticulum start to be evident 6 hours post-infection. With the beginning of virus assembly, endoplasmic reticulum disappears and viral antigens can be found throughout the cytoplasm and the cell membrane.

Poxvirus genome encodes the majority of the enzymes required for its own replication and is mostly autonomous from host functions compared to any other animal virus group. The Poxvirus genome consists of two groups of genes with different locations: the first group consists of genes that are required for viral transcription, genome replication and assembly of progeny virions (essential genes). Essential genes are clustered within the central region of the linear genome. The second group of genes are not essential for growth *in vitro* but are required for the replication of the viruses in their natural hosts since they define the host range, tissue specificity and virulence.[7] This second group includes genes that encode epidermal growth factor like (EGF-like) substances. The EGF-like factors of three poxviruses have been isolated: vaccinia growth factor (VGF), Myxoma virus growth factor MGF) and, Shope fibroma virus growth factor (SFGF).[8] SFGF is a broad-specificity ligand that activates all ErbB-1 containing receptor combinations, VGF binds primarily to ErbB-1 homodimers, and MGF only to heterodimes of ErbB-2 and ErbB-3. The growth factors of the three Poxviruses display unique patterns of specificity to ErbB receptor tyrosine kinases. Although viral growth factors bind to the respective receptor with an affinity that is up to 1000-fold weaker than that of the homologous mammalian ligand, their proliferative signals are more intense than their mammalian counterparts.[8] As an additional mechanism, most poxviruses are able to inhibit apoptosis. Shope fibroma virus has been demonstrated to inhibit apoptosis via binding to the host DNA.[7]

AFIP Diagnosis: Haired skin: Atypical mesenchymal proliferation, dermal, focally extensive, marked, with epithelial ballooning to reticular degeneration, epithelial and mesenchymal eosinophilic cytoplasmic inclusion bodies (hare fibroma), European brown hare (*Lepus europaeus*), lagomorph.

Conference Comment: The contributor provides an excellent overview of hare fibromatosis, Shope fibroma virus of rabbits, and malignant rabbit fibroma virus.

Conference attendees briefly reviewed how to distinguish Shope fibroma virus lesions from those of Myxoma virus. Typical gross findings associated with myxomatosis include multiple, subcutaneous, mucoid to gelatinous masses, especially on the face and around body orifices. Additionally, mucopurulent conjunctivitis and subcutaneous edema are observed.[9,10] Gross findings associated with Shope fibromatosis include circumscribed firm flattened nodules primarily on the legs and feet that may also occur on the muzzle, periorbital, and perineal areas. Metastasis to abdominal viscera and bone marrow may occur in young rabbits. Key histomorphologic features of myxomatosis include proliferation of large, stellate mesenchymal cells ("myxoma" cells) separated by a loose myxomatous matrix. The epithelium overlying the masses may be hyperplastic and/or degenerate (ballooning degeneration). Large eosinophilic intracytoplasmic inclusions may be present in epithelial cells of the epidermis or conjunctiva. Lymphoid depletion of the spleen is also a common finding. Key histomorphologic features of Shope fibromatosis include proliferation of fibroblasts with a mixed inflammatory cell infiltrate. Epithelial hyperplasia is also present with rete pegs projecting into the fibroblastic mass. In contrast to myxomatosis, eosinophilic intracytoplasmic inclusion bodies can be found in the fibroblasts and the epithelial cells of the epidermis overlying the mass.[10]

Attendees also discussed squirrel fibroma virus (Leporipoxvirus) which is also believed to be transmitted by biting arthropods. Gross lesions range from solitary to numerous firm cutaneous nodules over the entire body and/or marked epidermal thickening around the eyes and ears. Internal organs such as the lungs, lymph nodes, liver, and kidney may also be involved. As with rabbit fibroma virus, the lesions often spontaneously regress. The microscopic lesions resemble those of rabbit fibroma virus with proliferation of spindle cells, epithelial hyperplasia, and large prominent eosinophilic intracytoplasmic inclusion bodies in mesenchymal and epithelial cells.[11]

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References:

1. Jones TC, Hunt RD, King NW: Veterinary pathology, 6th ed., pp. 207-209. Williams & Wilkins, Baltimore, Maryland, 1997
2. Saari SA, Rudback E, Niskanen M, Syrjala P, Nylund M, Anttila M: Contagious mucocutaneous dermatitis of the mountain hare (*Lepus timidus*): pathology and cause. J Wildl Dis 41:775-782, 2005
3. Grilli G, Piccirillo A, Pisoni AM, Cerioli M, Gallazzi D, Lavazza A: Re-emergence of fibromatosis in farmed game hares (*Lepus europaeus*) in Italy. Vet Rec 153:152-153, 2003
4. Karstad L, Thorsen J, Davies G, Kaminjolo JS: Poxvirus fibromas on African hares. J Wildl Dis 13:245-247, 1977

5. Singh SB, Smith JW, Rawls WE, Tevethia SS. Demonstration of cytotoxic antibodies in rabbits bearing tumors induced by Shope fibroma virus. Infect Immun 5:352-358, 1972
6. Strayer DS, Skaletsky E, Cabirac GF, Sharp PA, Corbeil LB, Sell S, Leibowitz JL: Malignant rabbit fibroma virus causes secondary immunosuppression in rabbits. J Immunol 130:399-404, 1983
7. Brick DJ, Burke RD, Schiff L, Upton C: Shope fibroma virus RING finger protein N1R binds DNA and inhibits apoptosis. Virology 249:42-51, 1998
8. Tzahar E, Moyer JD, Waterman H, Barbacci EG, Bao J, Levkowitz G, Shelly M, Strano S, Pinkas-Kramarski R, Pierce JH, Andrews GC, Yarden Y: Pathogenic poxviruses reveal viral strategies to exploit the ErbB signalling network. EMBO J 17:5948-5963, 1998
9. Scott DW, Miller WH, Griffin CE: Muller and Kirk's Small Animal Dermatology, 6th ed., pp. 1446-1447. WB Saunders, Philadelphia, Pennsylvania, 2001
10. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, 2nd ed., pp. 252-254. Iowa State University Press, Ames, Iowa, 2001
11. Robinson AJ, Kerr PJ: Poxvirus infections. In: Infectious Diseases of Wild Mammals, eds. Williams ES, Barker IK, 3rd ed., pp.188-190. Iowa State University Press, Ames, Iowa, 2001

SLIDE 16
CONFERENCE 4 / CASE IV – S64564 (AFIP 2812385)

Signalment: Dog, *Canis familiaris*, Beagle, male, 5.5 months of age

History: A male beagle dog arrived with its cohorts from an established vendor and was held in routine quarantine. Standard prophylactic procedures by the vendor included routine vaccination (canine distemper, adenovirus, parainfluenza, parvovirus, Leptospirosis, *Bordetella*, and rabies) and deworming (ivermectin and pyrantel pamoate). After receipt and quarantine for 1 week, this dog and its cohorts received deworming followed several days later by re-immunization. One day following re-immunization, this dog was unexpectedly observed in lateral recumbency. The dog was hypothermic, cyanotic, and dyspneic, with blood in the nose and mouth. The dog died while blood was being drawn for diagnostic assessments (CBC and blood culture).

Gross Pathology: The carcass had blood staining about the muzzle, mouth, and nostrils. The thoracic cavity contained approximately 50 mL of red sanguinous fluid. The pericardium was thickened and reddened, with adhesions to the diaphragm. Thymic lymph nodes were mottled and firm. The trachea contained excess pink froth and the lung lobes had multiple to locally extensive, red to dark, often firm foci.

Laboratory Results: A CBC on the blood from this moribund dog had slightly elevated erythroid parameters (RBC, Hgb, Hct) and leukopenia with a degenerative left shift. Multiple lung and tracheal swabs, lung tissue, and thoracic fluids were submitted for

aerobic and anaerobic bacteriology. *Escherichia coli* (*E. coli*) was isolated from a specimen of lung tissue, from the lung surface, pleural fluid, and trachea, and from blood culture. This *E. coli* isolate was typed as O type 6 with cytotoxic necrotizing factor 1. *Streptococcus* Group G was also isolated from lung surface, pleural fluid, and trachea. Viral isolation and florescent antibody microscopy for adenovirus and parainfluenza virus in lung specimens were negative.

Contributor's Morphologic Diagnosis: Lung, hemorrhagic pneumonia
Contributor's Comment: *Escherichia coli,* of the bacterial family Enterobacteriaceae, is a normal inhabitant of the lower intestinal tract of all warm-blooded animals, is a well-known enteric pathogen, is present in most cases of canine pyometra, and is a common urinary tract pathogen.[3] Recently, this organism has also been reported to be the most commonly isolated bacteria from dogs with lower respiratory tract disease.[1] In contrast, this organism is rarely identified in cases of human pneumonia.[2] The α- and γ-hemolytic Streptococci can be isolated from the lower respiratory tract of normal dogs, which makes their implication as primary respiratory pathogens problematic.[1]

Although *E. coli* O type 6 has been sporadically reported from veterinary diagnostic case materials, this type is most often associated with urogenital tract infections. To our knowledge, this is the first isolation of an *E. coli* O type 6 from a case of fatal hemorrhagic pneumonia in the dog.

AFIP Diagnosis: Lung: Bronchopneumonia, necrotizing, acute, multifocal, severe, with hemorrhage, and myriad bacteria, Beagle (*Canis familiaris*), canine.

Conference Comment: The surface antigens of *E. coli* on which serotypes are based include the following:

1. **O (somatic)** – determined by the sugar side chains on the lipopolysaccharide molecule
2. **K (capsular)** – polysaccharide
3. **H (flagellar)** – proteinaceous
4. **F (fimbrial)** – proteinaceous; adhesive function

E. coli are classified into one of three general categories: (1) gastrointestinal commensals, (2) intestinal pathogenic strains, and (3) extraintestinal pathogenic strains. Most strains of *E. coli* are gastrointestinal commensals and do not typically cause disease in healthy immunocompetent animals. The extraintestinal pathogenic strains are further subdivided based on specific pathogenic mechanisms and include enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroaggregative, and diffusely adherent *E. coli*. The extraintestinal pathogenic *E. coli* have been associated with urinary tract infections, meningitis, septicemia, and pneumonia in humans and animals. In dogs, extraintestinal pathogenic *E. coli* is most

frequently implicated in urinary tract infections and has also been associated with mastitis, pyometra, otitis, prostatitis, skin disease, and cholecystitis.[3,4]

Serotypes O4 and O6 have been isolated from dogs with hemorrhagic pneumonia. Both serotypes have the virulence factors *alpha* hemolysin and cytotoxic necrotizing factor 1 (CNF1) which are frequently identified in infections caused by extraintestinal pathogenic *E. coli*. Cytotoxic necrotizing factor producing strains are also referred to as necrotoxic *E. coli*.[4]

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References:

1. Angus JC, Jang SS, Hirsh DC: Microbiological study of transtracheal aspirates from dogs with suspected lower respiratory tract disease: 264 cases (1989-1995). JAVMA 210: 55-58, 1997
2. Packham DR, Sorrell TC: Pneumonia with bacteremia due to *Escherichia coli*. Aust NZ J Med 11:669-672, 1981
3. Timoney JF, Gillespie JH, Scott FW, Barlough JE: Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th ed., pp. 61-71. Cornell University Press, Ithaca, New York, 1988
4. Handt LK, Stoffregen DA, Prescott JS, Pouch WJ, Ngai DTW, Anderson CA, Gatto NT, DebRoy C, Fairbrother JM, Motzel SL, Klein HJ: Clinical and microbiologic characterization of hemorrhagic pneumonia due to extraintestinal pathogenic *Eschrichia coli* in four young dogs. Comp Med 53(6):663-670, 2003

SLIDE 17
CONFERENCE 5 / CASE I – 5709 (AFIP 3028143)

Signalment: 4-year-old castrated male Romney Marsh sheep (*Ovis aries*)

History: A disease characterized by apathy, loss of weight and jaundice was observed in a herd of 200 Romney Marsh sheep, in a farm in southern Brazil. Twenty affected sheep died approximately 20 days after the first clinical signs. The flock was grazing a pasture severely infested by *Senecio brasiliensis* and *S. cisplatinus* which had evidence of being consumed by the sheep.

Gross Pathology: The sheep was emaciated and slightly icteric. The liver was enlarged and slightly yellowish (Fig. 1) and firm. The gallbladder was distended and the urine had a tan discoloration.

Histopathologic Description: Individual cell necrosis, loss of hepatocytes and vacuolar degeneration, hepatomegalocytosis, biliary ductal proliferation and periportal

fibrosis were observed. Randomly, neutrophilic foci were also found. There was brownish pigment in macrophages and hepatocytes and intranuclear pseudoinclusions. **Contributor's Morphologic Diagnosis:** Liver: Multifocal individual hepatocellular necrosis with loss of hepatocytes, vacuolar hepatocyte degeneration, periportal fibrosis, hepatomegalocytosis, intranuclear pseudoinclusions and bile duct hyperplasia, Romney Marsh sheep.

Contributor's Etiologic Diagnosis: Toxic hepatopathy

Etiology: *Senecio* spp. pyrrolizidine alkaloid toxicosis

Contributor's Comment: *Senecio* poisoning is one of the most important causes of death in cattle in Rio Grande do Sul, southern Brazil, causing 7% of all cattle deaths.[4] Sheep are more resistant than cattle to the toxicosis and frequently are used to control the plant.[1,5] Sheep required more than 2,0 of dried plant/kg of body weight to poison.[1]

The sheep resistance has been associated with the ruminal flora or to the capacity of the liver enzymes to detoxify pyrrolizidine alkaloids.[1] Different clinical pictures can be observed in the intoxication by pyrrolizidine alkaloids in sheep: (1) acute intoxication associated with the consumption of large amounts during a short time leading to liver necrosis; (2) primary chronic intoxication cause by the ingestion of large amounts of plant during long periods, causing diffuse hepatic fibrosis and megalocytosis; and (3) chronic copper intoxication associated with the storage of copper in the affected hepatocytes.[1]

Clinical signs in sheep include weight loss, photosensitization, nervous signs due to hepatic encephalopathy, and in cases of chronic copper poisoning jaundice and haemoglobinuria as a consequence of intravascular haemolysis.[1]

Outbreaks of poisoning by *Senecio* spp. in sheep are rare in Brazil. In Rio Grande do Sul only one outbreak has been reported previously, with some cases complicated by chronic copper intoxication.[2]

In the present outbreak, haemoglobinuria was not observed. Grazing for a long period of time in an area severely invaded by *Senecio* spp. caused primary chronic pyrrolizidine alkaloid intoxication. Nevertheless, the presence of brown yellowish pigment in hepatocytes and Kupffer cells suggests copper accumulation due to the hepatic lesion. Megalocytosis is characteristic of pyrrolizidine alkaloid intoxication. Other lesions are fibrosis and proliferation of bile duct cells. Pseudoinclusions result from the invagination of the plasmatic membrane, which stay sequestrated within the nucleus. Status spongiosus in the white matter of the brain was also observed in the sheep necropsied. This lesion is caused by different substances present in the blood of intoxicated animals, particularly ammonia.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, multifocal, random, moderate, with marked megalocytosis, nodular regeneration, chronic portal hepatitis, and biliary hyperplasia, Romney Marsh sheep (*Ovis aries*), ovine.

Conference Comment: The contributor provides a concise summary of pyrrolizidine alkaloid toxicity in sheep. In addition to the key histomorphologic features of megalocytosis, periportal bridging fibrosis, biliary hyperplasia, and cytoplasmic invaginations into the nucleus, conference attendees also observed oval cell hyperplasia and nodular regeneration.

Pyrrolizidine alkaloids have been found in various species of plants distributed worldwide. In addition to *Senecio*, the genera *Crotolaria*, *Heliotropium*, *Cynoglossum, Amsinckia*, *Echium*, and *Trichodesma* have also been known to cause disease.[6,7]

The most characteristic effect of toxic pyrroles is the induction of nuclear and cytoplasmic gigantism (megalocytosis). This effect is most likely due to an antimitotic effect with continued DNA synthesis as hepatocytes attempt to replace those that have undergone necrosis. Continued nucleoprotein synthesis, coupled with mitotic inhibition, probably accounts for the great increase in size of the nucleus and cytoplasm. The volume of megalocytic cells can range up to 20 times that of normal hepatocytes. Megalocytosis is not pathognomonic for pyrrolizidine alkaloid toxicosis. Other alkylating agents such as nitrosamine and aflatoxins can also result in megalocytosis. Concurrent with the development of megalocytosis, there is proliferation of the bile ducts and fibroplasia. This fibroplasia is generally minimal in sheep, moderate in horses and may be marked in cattle. Additionally, cytoplasmic invaginations into the nucleus are particularly common in chronic pyrrolizidine alkaloid toxicosis although they can occur in any chronically injured liver. Nodular regeneration can be present, but does not always occur due to the antimitotic effects of pyrrolizidines. However, during periods when animals are not grazing on pyrrolizidine alkaloid containing plants, hepatocyte replication can occur. Acidophilic spherical cytosegresomes are also a common finding.[6,7]

Acute poisoning by the pyrrolizidine alkaloids is uncommon due to unpalatability of the plants and results in periacinar necrosis and endothelial damage to the hepatic venules and small hepatic veins. This form of toxicosis is not clearly distinguishable from a variety of other hepatotoxins.[6]

In cattle, chronic pyrrolizidine alkaloidosis produces pronounced hepatic bridging portal fibrosis which infiltrates along the sinusoids to dissect lobules, separate individual cells, and link the walls of efferent veins. This form of fibrosis has been termed veno-occlusive disease.[6]

In sheep, long term consumption of pyrrolizidine containing plants may lead to elevated levels of liver copper followed by the hemolytic crisis of copper toxicity. In pigs, pyrrolizidine alkalosis primarily manifests as pneumonia and renal insufficiency. Pulmonary emphysema is a characteristic finding in pigs and horses. The pulmonary

toxicity of pyrrolizidine alkaloids in rats is well recognized. The primary site of injury in this species appears to be the alveolar septa. The lesions include severe vascular engorgement and edema, and diffuse fibrosis of alveolar and interlobular septa with patchy epithelialization.[6]

Type III or hepatogenous photosensitization may occur in association with pyrrolizidine alkaloid toxicosis secondary to hepatocellular damage and is due to the impaired capacity of the liver to excrete phylloerythrin, a break down product of chlorophyll. Phylloerythrin is carried to the dermis hematogenously where it is deposited and reacts with UV light forming reactive oxygen molecules, including free radicals. Mast cell degranulation and the production of inflammatory mediators cause damage to cell membranes, nucleic acids, proteins, and organelles. This is the most common type of photosensitization and occurs most frequently in herbivores. Lesions occur on areas of the body with nonpigmented skin and hair.[8]

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References:

1. Hooper PT: Pyrrolizidine alkaloids poisoning – pathology with particular reference to differences in animal and plant species. In: Effects of poisonous plants on livestock, eds. Keeler RF, Kampen KRV, James LF pp. 161-176. Academic Press, NY, New York, 1978
2. Ilha MR, Loretti AP, Barros SS, Barros CL: Intoxicação espontânea por *Senecio brasiliensis* (Asteraceae) em ovinos no Rio Grande do Sul. Pesq Vet Bras 21:123-138, 2001
3. Riet-Correa F, Medeiros RMT: Intoxicação por plantas em ruminantes no Brasil e no Uruguai: importância econômica, controle e riscos para a saúde pública. Pesq Vet Bras 21:38-42, 2001
4. Seaman JT: Pyrrolizidine alkaloid poisoning of sheep in New South Wales. Aust Vet Journal 64:164-167, 1987
5. Soares MP, Riet-Correa F, Méndez MC, Rosa FG, Carreira EC: Controle biológico de *Senecio* spp. com pastoreio de ovinos. Anais II Reunión Argentina de Patologia Veterinária. Faculdad de Ciências Veterinárias, Universidad Nacional Del Noroeste, Argentina, 79-80, 2000
6. Kelly WR: The liver and biliary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 326-327, 347-348, 392-395, 398-401. Academic Press, Inc., San Diego, California, 1993
7. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 426-427, 441, 444-446. Mosby Elsevier, St. Louis, Missouri, 2007
8. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1165. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 18
CONFERENCE 5/ CASE II – C05-2714-1 (AFIP 3027385)

Signalment: 8-month-old, intact male, New Zealand White rabbit (*Oryctolagus cuniculus*)

History: Anesthesia had been induced with ketamine/acepromazine and the rabbit was being maintained on 2% isoflurane. During the intraoperative insertion of a large intramedullary nail filling the entire marrow cavity of the proximal half of the bilateral femora, the rabbit became cyanotic and died within 1 minute.

Gross Pathology: The trachea was slightly hyperemic and contained a small amount of foam. All lung lobes were mottled dark red (Figure C05-2714-G1), soft on palpation and floated in formalin. No other overt lesions were apparent.

Histopathologic Description: Throughout all sections of lung examined, numerous alveolar capillaries, pulmonary arterioles and arteries, and less frequently, venules and veins, contain intravascular lipid. The lipid appears as single to multiple, well demarcated, clear, round spaces that often displace circulating erythrocytes and leukocytes to the periphery of the vessel lumen and occasionally occlude vessel lumens. Also associated with the intravascular lipid are aggregates of hematopoietic precursors of the myeloid, erythroid and megakaryocytic series. Some alveolar spaces contain small amounts of eosinophilic proteinaceous material. Bronchial-associated lymphoid tissue is prominent. Fat emboli similar to those described above were also present multifocally in the choroid plexus of the brain and focally in the right ventricle of the heart (not submitted).

Contributor's Morphologic Diagnosis: Lung, pulmonary vascular fat embolism, acute, multifocal, marked.

Contributor's Comment: Fat embolism (FE), whereby fat released by marrow or adipose tissue enters the systemic circulation, occurs in virtually all cases of skeletal trauma such as long bone and pelvic fractures.[5,7,9,11,12] However, the clinical consequence of FE, known as fat embolism syndrome (FES) occurs in less than 10% of cases with an overall mortality rate of 5-15%.[5,7,9,11,12] FES is rare in children and the incidence increases with the number of fractures sustained by an individual.[12] Additional causes of FES include: mechanical disruption of adipocytes such as liposuction and hepatic lipidosis; mechanical disruption of bone marrow such as bone marrow harvest or transplantation; administration of exogenous fat such as total parenteral nutrition or propofol infusion; and, miscellaneous conditions such as burns, acute sickle cell crises and pancreatitis.[5,9]

Regardless of the underlying cause, the pathogenesis of FES involves both mechanical obstruction and biochemical injury.[2,5-7,9,11,12] Fat from marrow is forced under pressure into torn venous sinusoids and mechanically obstructs capillaries in lungs, heart, kidneys, brain and skin. Activated platelets adhere to the surface of marrow fat, exacerbating vascular obstruction and tissue damage. If the amount of liberated fat and subsequent mechanical obstruction within the initial 12 hours after the injury is significant as in the case of this rabbit, fulminant FES characterized by pulmonary hypertension, right heart failure, shock and death ensues.[5,12] Interestingly, the acute lethal intravenous dose of fat in adult humans is 20-50 ml while the volume of femoral marrow fat is 70-100 ml.[5]

The amount of embolized fat often exceeds the fat content of bone. Furthermore, FES can occur in the absence of disrupted depot fat. Experimental *in vitro* and *in vivo* studies have demonstrated the agglutination of circulating chylomicrons and very low-density lipoproteins by C-reactive protein elevated during various infectious, inflammatory and neoplastic processes.[2,5,7,9,11,12]

Biochemical damage is ascribed to conversion of free fatty acids (FFAs) and glycerol from neutral fat and/or triglycerides by lipoprotein lipases which are activated by catecholamines elevated during stress. FFAs, particularly olein, are toxic to endothelial cells with subsequent disruption of vessel walls, vasogenic edema, perivascular bleeding, platelet aggregation and thrombosis. As an aside, the rare occurrence of FES in children is partially explained by FFA composition. Adult bone marrow has a higher fat content than the content of hematopoietic cells and contains more olein than palmitin and stearin while the opposite is true in the bone marrow of children.[12]

Patients are typically asymptomatic for 12-72 hours prior to presenting with the classical clinical triad of respiratory distress, cerebral dysfunction and a pathognomonic petechial rash on the neck, chest, axillae, conjunctiva and oral mucous membranes.[2,5-7,9,11,12] The latter is attributed to embolization of fat, which accumulates in the aortic arch, to nondependent skin via the subclavian and carotid arteries. The neurological manifestations vary widely from lethargy and confusion to seizures and paresis. Minor signs commonly seen include pyrexia, jaundice, retinal infarcts and oliguria or anuria. The delay in onset of clinical signs is attributed to the time required for conversion of neutral fat to FFAs. Consistent clinical pathology findings include unexplained anemia, thrombocytopenia and elevated serum lipase. FFAs bind to albumin and calcium resulting in hypoalbuminemia and hypocalcemia.

As there are no specific therapies for FES; prevention, early diagnosis and symptomatic treatment are crucial. First and foremost in instances of fractures is early immobilization. Surgical fixation typically involves external fixation and/or intramedullary nailing. However, intramedullary nailing as documented in this rabbit is also associated with FE due to marked increases in intramedullary pressure.[2,5,6,11,12] Venting holes distal to the fracture to promote drainage of the medullary cavity during insertion of the nail as well as bone-vacuum cementing techniques to prevent increases in intramedullary pressure have markedly reduced the incidence of FES.[5]

In veterinary medicine, documented cases of FE primarily involve surgical repair of long bone fractures in dogs and a cat or total hip arthroplasty in dogs.[4,11,13,15] FE restricted to glomerular tufts was reported in a diabetic dog following partial cystectomy for transitional cell carcinoma.[1] Reports in large animals are restricted to a 10-month-old Holstein-Friesian cow with Tetralogy of Fallot that died during surgery following costectomy to repair pulmonic stenosis.[14] Recently, FE has been described as a component of the "gas and fat embolic syndrome" found in beaked whales stranded subsequent to regional military sonar exposure.[3] Pulmonary FE was experimentally induced in rats with hepatic lipidosis and core body temperatures of 44°C.[8] This study was conducted to determine the contribution of hepatic lipidosis under elevated ambient temperatures to pulmonary FE. The authors had autopsied a middle-aged, homeless male found dead in a sauna who had pulmonary FE and hepatic lipidosis with no evidence of trauma.

Definitive confirmation of neutral lipids requires special histochemical staining of frozen sections with oil red O or sudan black B which was not performed in this case.

AFIP Diagnosis: Lung: Bone marrow and fibrocartilagenous emboli, numerous, New Zealand White rabbit (*Orytolagus cuniculus*), lagomorph.

Conference Comment: The contributor provides an excellent summary of fat embolism syndrome. Embolism is defined as the occlusion of arteries by lodgement of foreign materials.[16] Types of emboli other than fat emboli include thromboemboli, fibrocartilagenous emboli, bacterial emboli, fungal emboli, parasitic emboli, and neoplastic emboli. Less common sources of emboli include hematopoietic cells from bone marrow, amniotic fluid, agglutinated erythrocytes, or clumps of other cells released after tissue trauma. The significance in all cases, is the potential of emboli to occlude vessels and inhibit blood flow to dependent tissues.[16-20]

The lungs act as a "safety net" that catch emboli before they can reach the brain and other tissues. The most common pulmonary emboli in domestic animals include thromboemboli, septic (bacterial) emboli, fat emboli, and neoplastic emboli. Infarction due to pulmonary emboli is rare due to the lung's dual arterial circulation (pulmonary and bronchial arteries).[18]

Pulmonary thromboemboli typically originate from a thrombus located elsewhere in the venous circulation. Fragments released eventually reach the lungs and lodge in the pulmonary vasculature. Small sterile thromboemboli are clinically and pathologically insignificant due to rapid degradation by the fibrinolytic system. In the dog, causes of pulmonary arterial thrombosis and subsequent pulmonary thromboembolism include *Dirofilaria immitis*, *Angiostrongylus vasorum*, hyperadrenocorticism, hypothyroidism, and hypercoagulable states. Additionally, long-term intravenous catheterization can cause thrombosis of the jugular vein from which fragments can break resulting in pulmonary thromboembolism.[18]

Septic emboli containing bacterial or fungal fragments most often originate from bacterial endocarditis (tricuspid valve) and jugular thrombophlebitis in all species; hepatic abscesses that have eroded into the caudal vena cava in cattle; and septic arthritis and omphalitis in farm animals. Large numbers of septic emboli may result in sudden death due to massive pulmonary edema. Survivors typically develop embolic, suppurative pneumonia that may progress to pulmonary abscesses in addition to pulmonary arteritis and thrombosis.[18]

As pointed out by the contributor, fat emboli can form following bone fractures or surgical/clinical interventions of bone. These are not as significant of a problem in domestic animals as they are in humans.[18]

Pulmonary neoplastic emboli can be numerous and the ultimate cause of death in malignant neoplasia. Hepatic emboli occasionally lodge in the pulmonary vasculature following severe trauma and hepatic rupture. Interestingly, pulmonary vascular brain emboli, reported in cases of severe head injury in humans, have been reported in cattle following pneumatic stunning at slaughter. Although brain emboli are not an important antemortem pulmonary lesion, they may pose a potential public health risk in bovine spongiform encephalopathy (BSE).[18]

Trophoblastic emboli have been reported in the chinchilla.[21] In species with hemochorial placentation (humans, nonhuman primates, rabbits, and rodents), fetal trophoblastic cells are in direct contact with maternal circulation. Trophoblasts erode through the endometrium, migrate into the lumina of maternal blood vessels, through the uterine vessels, and into the mesovarium and mesometrial vessels. Trophoblasts can subsequently dislodge and are carried to small capillary beds such as in the lungs. Domestic animals without hemochorial placentation are not prone to trophoblastic emboli.

Contributor:
http://www.mskcc.org
http://www.mskcc.org/mskcc/html/14131.cfm
http://www.med.cornell.edu;
http://www.med.cornell.edu/research/rea_sup/mouse_phenotyp.html
http://www.med.cornell.edu/research/rea_sup/lab_com_pat.html
http://www.rockefeller.edu

References:
1. Brown TP, Fitzpatrick RK: Glomerular lipid emboli in a diabetic dog. Vet Pathol 23:209-211, 1986
2. Fabian TC: Unraveling the fat embolism syndrome. N Engl J Med 329:961-963, 1993
3. Fernández A, Edwards JF, Rodríguez F, Espinosa De Los Monteros A, Herráez P, Castro P, Jaber JR, Martín V and Arbelo M: "Gas and fat embolic syndrome" involving a mass stranding of beaked whales (family *Ziphiidae*) exposed to anthropogenic sonar signals. Vet Pathol 42:446-457, 2005

4. Furneaux RW: Two cases of fat embolism in the dog. J Am Anim Hosp Assoc 10:45-47, 1974
5. Glover P, Worthley LIG: Fat embolism. Crit Care Resusc 1:276-284, 1999
6. Hofmann S, Huemer G, Salzer M: Pathophysiology and management of the fat embolism syndrome. Anaesthesia 53 (Suppl. 2):35-27, 1998
7. Hulman G: The pathogenesis of fat embolism. J Pathol 176:3-9, 1995
8. Inoue H, Tsuji A, Kudo K, Ikeda N: Pulmonary fat embolism induced by exposure to ambient temperature in rats with a fatty liver. Int J Legal Med 119:275-279, 2005
9. Mellor A and Soni N: Fat embolism. Anaesthesia 56:145-154, 2001
10. Liska WD, Poteet BA: Pulmonary embolism associated with canine total hip replacement. Vet Surg 32:178-186, 2003
11. Müller C, Rahn BA, Pfister U, Meinig RP: The incidence, pathogenesis, diagnosis, and treatment of fat embolism. Orthop Rev 23:107-117, 1994
12. Parisi DM, Koval K, Egol K: Fat embolism syndrome. Am J Orthop 31:507-512, 2002
13. Schwarz T, Crawford PE, Owen MR, Störk, Thompson H: Fatal pulmonary fat embolism during humeral fracture repair in a cat. J Small Anim Pract 42:195-198, 2001
14. Shirota K, Saitoh Y, Muto M, Wakao Y, Une Y, Nomura Y: Crossed fat embolism in a cow with tetralogy of Fallot. J Vet Med Sci 56:969-971, 1994
15. Terrell SP, Chandra AMS, Pablo LS, Lewis DD: Fatal intraoperative pulmonary fat embolism during cemented total hip arthroplasty in a dog. J Am Anim Hosp Assoc 40:345-348, 2004
16. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Patholologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 605-606. Mosby Elsevier, St. Louis, Missouri, 2007
17. Mosier DA: Vascular disorders and thrombosis. In: Patholologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 90-91. Mosby Elsevier, St. Louis, Missouri, 2007
18. L_pez A: Respiratory system. In: Patholologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 500-501. Mosby Elsevier, St. Louis, Missouri, 2007
19. Ackermann MR: Acute inflammation. In: Patholologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 139. Mosby Elsevier, St. Louis, Missouri, 2007
20. Zachary JF: Nervous system. In: Patholologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 926-928. Mosby Elsevier, St. Louis, Missouri, 2007
21. Tvedten HW, Langham RF: Trophoblastic emboli in a chinchilla. J Am Vet Med Assoc 165:828-829, 1974

SLIDE 19
CONFERENCE 5 / CASE III – 97733 (AFIP 2890214)

Signalment: 2-year-old, male, F344 rat, *Rattus norvegicus*

History: Tissue from a rat used in a two year chronic toxicology study.

Gross Pathology: There was generalized lymphadenomegaly and splenomegaly (10x normal size). The liver had a granular appearance. The right kidney was deformed and the opposite renal cortex was mottled. There was generalized pallor and mild icterus.

Histopathologic Description: The spleen is markedly enlarged and white pulp is sparse or absent. Erythrocytes and neoplastic round cells distend the sinusoids. The neoplastic cells have scant amounts of granular (faintly) cytoplasm and round, oval or cleaved open-faced nuclei. Cell margins are irregular but distinct. Most high power fields contain one or two mitotic figures. A few foci of extramedullary hematopoiesis are evident in some sections.

Liver sinusoids and blood vessels are partially filled with the previously mentioned neoplastic cells. In addition, several larger vessels contain cellular emboli. The emboli are composed predominately of a second population of round to spindloid cells with distinct to indistinct cytoplasmic borders, a moderate amount of pale pink cytoplasm and round to elongate open-faced nuclei. Multinucleate giant cells (few) and karyomegaly are evident in some sections. There is moderate to severe bile duct hyperplasia. Minimal to mild oval cell hyperplasia is present in some sections. Randomly distributed throughout the liver, but more prominent in centrilobular areas, are large macrophages and giant cells distended with vacuoles, small acicular clefts, and variable amounts of light brown pigment. Frequently, hepatic parenchyma is replaced by the inflammatory infiltrate.

Contributor's Morphologic Diagnoses: 1. Spleen: Leukemia, mononuclear cell.
2. Liver: Leukemia, mononuclear cell.
3. Liver: Histiocytic sarcoma, metastatic.
4. Liver: Inflammation, granulomatous, multifocal, moderate.
5. Liver, bile duct: Hyperplasia, severe.

Contributor's Comment: Mononuclear cell leukemia is also referred to as Fischer rat leukemia and large granular lymphocytic leukemia. The term mononuclear cell leukemia is used in NTP studies to maintain consistency and avoid confusion. Mononuclear cell leukemia is common in aged Fischer 344 rats. Immune-mediated hemolytic anemia is commonly seen in affected rats. Occasional neoplastic cells contain phagocytized erythrocytes. The neoplastic cells are of uncertain linage but are known to have characteristics of T cells and macrophages. Ultrastructural studies have revealed the cytoplasmic granules to be densely osmophilic membrane-bound lysozomes. The cells stain for napthol AS-D acetate esterase, beta-glucuronidase and acid phosphatase and are positive for OX-8 by immunocytochemistry. Current theory is that the neoplastic cells are effector cells for NK cell activity in the rat. A low incidence

of mononuclear cell leukemia (large granular lymphocyte leukemia) has been reported in Wistar-Furth, Wistar and Sprague-Dawley strains.

Histiocytic sarcoma is relatively common in Sprague-Dawley derived strains but has a low incidence in Fischer, Wistar and Osborne-Mendel rats. In F344 and Sprague-Dawley strains, the liver and lungs are the most commonly and extensively affected organs; however the subcutis is more commonly the primary site of involvement in the Wistar rat. These tumors are frequently associated with hyaline droplet accumulation in the P2 segment of renal proximal convoluted tubules. The hyaline droplets are positive for lysozyme and negative for alpha-2 mu globulin. Tumor cells are positive for lysozyme, alpha-1 trypsin and alpha-1 chymotrypsin by immunocytochemistry.

Foci of granulomatous inflammation are incidental findings in aging rats. Incidence and severity is variable but generally more prevalent in females than males.

AFIP Diagnoses: 1. Liver; spleen: Large granular lymphocytic leukemia, F344 rat (*Rattus norvegicus*), rodent.
2. Liver, veins: Histiocytic sarcoma.
3. Liver: Hepatocellular degeneration and necrosis, multifocal, random, mild, with biliary hyperplasia.
4. Spleen, white pulp: Lymphoid depletion.

Conference Comment: Large granular lymphocytic leukemia (LGL) is a common cause of death in aging F344 rats and occasionally occurs in other strains (Wistar, Wistar-Furth). LGL appears to arise in the spleen and then spreads to other organs such as the lymph nodes, liver, and lungs.[8] Previous studies have shown that splenectomy markedly reduces the incidence of LGL.[2,3]

Typical clinical pathology findings include a marked leukocytosis with leukocyte counts of up to 400,000/ml^3, immune-mediated hemolytic anemia, thrombocytopenia (immune-mediated, DIC), clotting abnormalities, increased conjugated and unconjugated bilirubin, bilirubinuria, urobilinogenuria, hemoglobinuria, and increased liver enzymes.[2,8]

The most consistent gross finding is splenomegaly. Additionally, there may be moderate to marked hepatomegaly with an accentuated lobular pattern. Lymphadenopathy, icterus, and petechial hemorrhages on the lung and lymph nodes may also occur.[2,3,8]

Key histopathologic features include diffuse infiltration of the spleen, lymph nodes, liver, and lungs with malignant lymphocytes. There is marked depletion of splenic lymphoid follicles and diffuse infiltration of leukemic cells in hepatic sinusoids. Centrilobular hepatic degeneration commonly occurs secondary to anemia and neoplastic infiltrates. Erythrophagocytosis occurs in the liver and spleen.[2,3,8]

The fine cellular details of the large granular lymphocytes are not visible in fixed tissue sections, but can be seen in peripheral blood smears or stained impression smears of tissues such as the spleen and include 10-15 um diameter lymphocytes with round to oval, irregular or reniform nuclei; pale cytoplasm; and prominent azurophilic granules. Ultrastructurally, the azurophilic granules appear as electron dense membrane-bound lysosomes. The ultimate cause of death is often attributed to immune mediated hemolytic anemia and centrilobular hepatic degeneration.[2,3,8]

Histiocytic sarcomas most commonly occur in aging Sprague-Dawley rats, but also occur in other strains (Osborne-Mendel, Wistar, Fischer). Grossly, the neoplasm is pale tan and firm and can appear as irregular masses or infiltrate and displace normal tissue in the liver, lymph nodes, lungs, spleen, mediastinum, retroperitoneum, and the subcutis. Histologically, histiocytic sarcomas appear as sheets of elongate palisading fusiform cells to pleomorphic histiocytic cells with abundant cytoplasm that may contain vacuoles or phagocytized erythrocytes. Nuclei are vesiculate with prominent nucleoli. Multinucleated giant cells of the Langhans type are commonly present in tumors with a prominent histiocytic component. Areas of necrosis surrounded by palisading tumor cells are common and characteristic of histiocytic sarcoma. Fibrosis varies from minimal to marked.[7,8]

Contributor: Experimental Pathology Laboratories, Inc.

References:

1. Abbot DP, Prentice DE, Cherry CP: Mononuclear cell leukemia in aged Sprague-Dawley Rats. Vet Pathol 20:434-439, 1983
2. Eustis SL, Boorman GA, Harada H, Popp JA: Liver. In: Pathology of the Fischer Rat, eds. Boorman GA, Eustis SL, Elwell MR, Montgomery CA, MacKenzie WF, pp. 76-77. Academic Press, Inc., San Diego, California, 1990
3. Losco PE, Ward JM: The early stage of large granular lymphocyte leukemia in the F344 Rat. Vet Pathol 21:286-291, 1984
4. Barsoum NJ, Hanna W, Gough AW, Smith GS, Sturgess JM, de la Iglesia FA: Histiocytic sarcoma in Wistar Rats. A light microscopic, immunohistochemical, and ultrastructural study. Arch Pathol Lab Med 108:802-807, 1984
5. Squire RA, Brinkhous KM, Peiper SC, Firminger HI, Mann RB, Strandberg JD: Histiocytic sarcoma with a granuloma-like component occurring in a large colony of Sprague-Dawley Rats. AJP 105:21-30, 1981
6. Stefanski SA, Elwell MR, Stromberg PC: Spleen, lymph nodes, and thymus. In: Pathology of the Fischer Rat, eds. Boorman GA, Eustis SL, Elwell MR, Montgomery CA, MacKenzie WF, pp. 373-379. Academic Press, Inc., San Diego, California, 1990
7. Squire RA: Histiocytic sarcoma, rat. In: Monographs on Pathology of Laboratory Animals, eds. Jones TC, Ward JM, Mohr U, Hunt RD. Springer-Verlag, New York, New York, 1990
8. Percy DH, Barthold SW: Pathology of Lab Animal Rodents, 2nd ed., pp. 158-161. Iowa State University Press, Ames, Iowa, 2001

SLIDE 20
CONFERENCE 5 / CASE IV – 06-1714 (AFIP 3026011)

Signalment: 10-month-old, male, Boxer, canine

History: This 10-month-old Boxer had a three to five day history of neck pain and stiff gait. There were no neurological deficits noted at that time. The dog underwent general anesthesia for cerebrospinal fluid collection. The dog recovered, but one hour after extubation it collapsed. There was no pulse or heart beat. Resuscitative attempts were unsuccessful.

Gross Pathology: The brain and spinal cord were removed by the referring veterinarian. He noted diffuse, subdural hemorrhage around the cervical spinal cord.

Laboratory Results: The spinal fluid had 5,310 white blood cells and 8,370 erythrocytes per milliliter. The protein was 260 mg/dl. Cytologically, there was a neutrophilic pleocytosis and no etiologic agents were observed.

Aerobic culture of the meninges was negative for bacterial growth.

Histopathologic Description: The sections of cervical spinal cord have several small to medium-sized muscular arteries in the leptomeninges with infiltrations of the tunica adventitia, tunica muscularis and tunica intima by neutrophils and a few macrophages. There is deposition of homogenous eosinophilic material and karyorrhectic debris (fibrinoid necrosis) within the tunica media of some affected vessels. There are diffuse infiltrates of neutrophils and fewer macrophages within the surrounding leptomeninges. There is diffuse hemorrhage in the subdural space.

Contributor's Morphologic Diagnoses: 1. Vasculitis, acute to subacute, multifocal, severe with fibrinoid necrosis, severe, meninges, cervical spinal cord.
2. Subdural hemorrhage, acute, diffuse, severe, cervical spinal cord.

Contributor's Comment: The signalment, clinical signs and lesions are consistent with the idiopathic condition known as juvenile polyarteritis syndrome or "beagle pain syndrome." The term beagle pain syndrome was used in the first descriptions of the disease as it was observed in young laboratory beagle dogs that were part of chronic toxicity studies.[1,2] It has subsequently been seen in other breeds.[3]

Young dogs, typically 4-10 months-of-age, are affected. Clinical signs are characterized by acute onset of fever, anorexia and cervical pain. Affected dogs assume a hunched stance and have a reluctance to move. Signs tend to wax and wane.[4] Cerebrospinal fluid analysis reveals moderate to severe neutrophilic pleocytosis and elevations in protein. There is typically a peripheral neutrophilia.[4] Thus far, no etiologic agents have

been identified in affected dogs. Since the disease responds to corticosteroid therapy, an immune-mediated etiology is suspected.[4]

The histologic lesions are those of vasculitis that may involve multiple organs but are seen most consistently in vessels of the cervical spinal cord, mediastinum and heart.[4] Thrombosis and loss of vascular integrity with hemorrhage may be present. [3,4] Presumably, acute subdural hemorrhage with compression of vital portions of the cranial cervical cord was responsible for the acute collapse and death of this dog. Some sections of cranial cervical cord (not submitted) contained evidence of swollen axons in white matter funiculi.

Similarities in clinical signs suggest that the juvenile polyarteritis syndrome and the clinical entity known as steroid responsive suppurative meningitis are related diseases. However, since the latter responds well to corticosteroids there are no good histological studies to confirm this relationship.[3,5] The present case had a clinical diagnosis of steroid responsive suppurative meningitis based on the clinical findings. The histologic lesions presented here are characteristic for the juvenile polyarteritis syndrome and suggest that the two diseases may indeed be the same.

AFIP Diagnosis: Spinal cord and meninges: Vasculitis, necrotizing, multifocal, marked, with neutrophilic and histiocytic meningitis and severe meningeal hemorrhage, Boxer (*Canis familiaris*), canine.

Conference Comment: The contributor provides a concise summary of canine juvenile polyarteritis syndrome or "beagle pain syndrome." The severity of the vasculitis varied between slides. Additionally, some sections of cervical spinal cord contained multifocal neutrophilic and histiocytic peridural steatitis and polyradiculoneuritis.

Lesions similar to canine juvenile polyarteritis syndrome are seen in aging rats with polyarteritis nodosa. This spontaneous disease occurs most commonly in male Sprague-Dawley rats, spontaneous hypertensive rat strains, and rats with late-stage chronic nephropathy. Arterial lesions most commonly occur in the mesentery, pancreas, pancreaticoduodenal artery, and testis. However, lesions may be seen in various other organs except the lung. Histologically, there is fibrinoid degeneration and thickening of the media of affected arteries with neutrophilic and monocytic infiltrates. Lumina of affected vessels vary in size and contour. Thrombosis with recanalization can occur. An immune-mediated pathogenesis is suspected, but has not been confirmed.[6]

Polyarteritis nodosa occasionally occurs in cattle and other domestic species.[7]

Contributor: Arizona Veterinary Diagnostic Laboratory, University of Arizona, Tucson, Arizona, http://home.microvet.arizona.edu/

References:
1. Hayes TJ, Roberts GKS, Halliwell WH: An idiopathic febrile necrotizing arteritis syndrome in the dog: Beagle pain syndrome. Toxicol Pathol 171:29-137,1989
2. Scott-Moncrieff JCR, Snyder PW, Glickman LT, Davis EL, Felsburg PJ: Systemic necrotizing vasculitis in nine young beagles. JAVMA 201:1553-1558,1992
3. Caswell JL, Nykamp SG: Intradural vasculitis and hemorrhage in full sibling Welsh springer spaniels. Can Vet J 44:137-139, 2003
4. Snyder PW, Kazacos EA, Scott-Moncrieff JC, HogenEsch H, Carlton WW, Glickman LT, Felsburg PJ: Pathologic features of naturally occurring juvenile polyarteritis in beagle dogs. Vet Pathol 32:337-345, 1995
5. Webb AA, Taylor SM, Muir GD: Steroid responsive meningitis-arteritis in dogs with noninfectious, nonerosive, idiopathic, immune-mediated polyarthritis. J Vet Intern Med 16:269-273, 2002
6. Percy DH, Barthold SW: Pathology of Lab Animal Rodents, 2nd ed., p. 153. Iowa State University Press, Ames, Iowa, 2001
7. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 1000-1001. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 21
CONFERENCE 6 / CASE I – NADC SCO-01 (AFIP 2787418)

Signalment: Female, 3-year-old bison, 7-10 days prior to estimated parturition
History: This bison cow was anorexic for 5 days and became progressively weaker. The weakness progressed to complete recumbency at which time the animal was euthanized. Blood for analysis was collected immediately. Blood from six "normal" periparturient bison was also collected for comparison.

Gross Pathology: The liver was diffusely firm, pale, and yellow, with rounded edges. Sections collected for microscopic analysis floated in formalin. The pleural surface of the lungs was covered with a fibrinopurulent exudate with multiple fibrinous adhesions to the costal pleura. There were multifocal necrosuppurative lesions of variable size within the right and left cranial lung lobes. There was abundant perirenal and intra-abdominal fat.

Laboratory Results:

	"Normal" Bison		Affected Bison
	Range	Mean±SD	Value
Na	139-147	143.2 ± 2.9	144
K	3.3-4.4	3.92 ± 0.37	3.4
Cl	99-106	102 ± 2.5	104
TCO2	18.7-26.2	21.6 ± 2.7	10.5
Ca	8.2-9.5	8.7 ± 0.4	9.3
Phos	5.3-7.5	6.4 ± 0.7	9.4
Mg	1.54-2.71	1.81 ± 0.45	1.46

Creat	2.3-5.2	3.4 ± 1.0	5.3
BUN	19-46	28 ± 8.5	26
Glucose	52-181	109 ± 42	63
T. Prot	6.9-7.6	7.3 ± 0.3	8.1
Albumin	3.2-3.9	3.7 ± 0.2	3.6
AST	74-594	195 ± 205	246
CK	103-5207	1239 ± 1994	95
Alk. Phos	23-58	44 ± 12	174
GGT	6-29	17 ± 7.0	68
T. Bili.	0.25-0.91	0.58 ± 0.20	0.39
Lip. Ind.	0.0-3.0	1.0 ± 1.4	0.0
Ict. Ind.	1.0-2.0	1.2 ± 0.4	1.0
Anion Gap	21-26	23.5 ± 1.9	33

Histopathologic Description: Diffusely hepatocytes are filled with numerous small vacuoles of variable size and smoothly contoured borders. Hepatocytes in all zones are affected. There are also multifocal small aggregates of neutrophils randomly scattered throughout the parenchyma.

Contributor's Morphologic Diagnoses: 1. Liver: Hepatocellular vacuolar change, diffuse, severe, with mild, multifocal, suppurative hepatitis.
2. Lung (not submitted): Bronchopneumonia, fibrinopurulent with necrosis, multifocal, moderate, with fibrinopurulent pleuritis.
Contributor's Comment: Hepatic lipidosis is a morphologic change characterized by the accumulation of lipid vacuoles within hepatocytes. In cattle, miniature horses, cats, sheep and others, this condition develops in response to a negative energy balance (i.e. energy requirements are not matched by energy intake from feed). Hepatic lipidosis is a common lesion of "fatty liver syndrome" or "fat cow syndrome" described in dairy cattle, and arising as a result of improper feeding during late lactation and the dry period. The syndrome occurs particularly in high yielding dairy cows when overfeeding during the dry period results in overfat cows at calving. A negative energy balance may result from parturition, high milk production, or anorexia from other disease states such as mastitis, displaced abomasum, ketosis, etc. As a result, excessive fat is mobilized from body reservoirs and fatty acids are transported to various organs such as the liver, kidney, and muscle. The ability of the liver to process triglycerides for export as lipoproteins is limited; therefore, the excess is deposited as intracellular droplets of triglycerides.

The elevated GGT, Alk Phos, with decreased TCO_2 and glucose are consistent with that seen in cattle with hepatic lipidosis.

A disease syndrome associated with fat mobilization and hepatic lipidosis has not been identified in bison. The previous year on the same site, 3 bison died in a similar fashion with histories of anorexia of 7-12 days duration at approximately 180 days gestation. Similar gross and microscopic lesions were seen in the livers of all 3 bison. In the current case, the overconditioned nature of the cow, a period of anorexia (likely from the

pneumonia) resulting in a state of negative energy balance, and the resulting lesions, suggest that bison are also susceptible to such a syndrome.

AFIP Diagnosis: Liver: Hepatocellular microvacuolar change, lipid-type, diffuse, severe, bison (*Bison bison*), bovine.

Conference Comment: There are several different mechanisms by which triglycerides can accumulate in the liver. Free fatty acids mobilized from adipose tissue or ingested foods are normally transported to the liver where they are esterified to triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies. Triglycerides are complexed with apoproteins forming lipoproteins that are transported out of the liver into plasma. Defects in any of the steps from fatty acid entry to lipoprotein exit results in excess accumulation of triglycerides in hepatocytes.[4,5]

In addition to the "fat cow syndrome" described by the contributor that occurs in dairy cattle and bison, deficiencies of vitamin B_{12} and cobalt have been associated with fatty liver in sheep (ovine white-liver disease) and goats.[6,7] Hepatocellular lipidosis associated with a negative energy balance secondary to ketosis also occurs in sheep and guinea pigs (pregnancy toxemia).[6,8] Feline fatty liver syndrome is an idiopathic hepatocellular lipidosis that occurs when obese cats are stressed and become anorectic resulting in a negative energy balance and mobilization of fat stores. Affected cats often develop hepatic failure, icterus, and hepatic encephalopathy.[6,7] ALP activity typically increases to a greater magnitude than GGT activity in feline hepatic lipidosis.[9] The hepatic lipidosis that occurs in ponies, miniature horses and donkeys usually occurs in overweight, pregnant, or lactating mares and is associated with stress and/or anorexia. Shetland ponies are predisposed. Affected ponies are hyperlipemic with lipidosis extending to the heart, skeletal muscle, kidneys, and adrenal cortex. Hepatic rupture, renal failure, hepatic encephalopathy, and/or terminal DIC may occur.[6,7] A fatal fasting syndrome of obese macaques has also been described. The syndrome is characterized by acute rapid weight loss in obese macaques which is often attributed to the social stressors following recaging, leading to fatty change in the liver and kidneys. Affected monkeys die unexpectedly or after a very short illness. Interestingly, the syndrome is not associated with liver dysfunction and blood chemistry profiles of affected monkeys do not suggest liver failure. The most common clinical pathologic finding is azotemia.[10,11] Metabolic fatty liver syndromes also occur in chickens and turkeys.[12,13] Hepatic lipidosis can also occur with diabetes mellitus, hypothyroidism, acute pancreatitis (cats), and hepatotoxicity.[6,9]

Conference attendees discussed the clinical pathologic findings associated with the case. The increased anion gap was most likely due to a titration (organic acid excess) acidosis due to ketoacidosis resulting in loss of HCO_3^- (decreased TCO_2) by titration. The increased GGT and ALP indicate cholestasis that occurs secondary to hepatocellular swelling due to lipid accumulation and subsequent compression of bile canaliculi.[9] The total protein was slightly increased with normal albumin levels indicating that the increase was due to increased globulins which can be seen in chronic

infections in ruminants. It is unusual that the CK activity was decreased as this value is usually elevated in downer cows. However, the reference range established by blood samples collected from six “normal” periparturient bison was extremely wide.

In some sections of liver, there was a multifocal, mild, neutrophilic portal hepatitis.

Contributor: National Animal Disease Center, ARS, USDA, 2300 Dayton Avenue, Ames, IA 50010

References:

1. Cebra CK, Garry FB, Getzy DM, Fettman MJ: Hepatic lipidosis in anorectic, lactating Holstein cattle: a retrospective study of serum biochemical abnormalities. J Vet Intern Med 11:231-237, 1997
2. Rukkwamsuk T, Kruip TAM, Wensing T: Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Vet Quart 21:71-77, 1999
3. Palmer MV, Olsen SC: Hepatic lipidosis in pregnant captive American bison (*Bison bison*). J Vet Diag Invest 14(6):512-515, 2002
4. Kumar V, Abbas AK, Fausto N: Pathologic Basis of Disease, 7th ed., pp. 35-36. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
5. Myers RK, McGavin MD: Cellular and Tissue Responses to Injury. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 39-41. Mosby Elsevier, St. Louis, Missouri, 2007
6. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 423-425. Mosby Elsevier, St. Louis, Missouri, 2007
7. Kelley WR: The liver and biliary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 331-336. Academic Press, Inc., San Diego, California, 1993
8. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., pp. 236-237. Iowa State University Press, Ames, Iowa, 2001
9. Bain PJ: Liver. In: Duncan & Prasse’s Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 210-211. Iowa State University Press, Ames, Iowa, 2003
10. Bronson RT, O’Connell M, Klepper-Kilgore N, Chalifoux LV, Sehgal P: Fatal fasting syndrome in obese macaques. Lab Anim Sci 32(2):187-191, 1982
11. Laber-Laird KE, Jokinen MP, Lehner ND: Fatal fatty liver-kidney syndrome in obese monkeys. Lab Anim Sci 37(2):205-209, 1987
12. Hansen RJ, Walzem RL: Avian fatty liver hemorrhagic syndrome: a comparative review. Adv Vet Sci Comp Med 37:451-468, 1993
13. Whitehead CC, Siller WG: Experimentally induced fatty liver and kidney syndrome in the young turkey. Res Vet Sci 34(1):73-76, 1983

SLIDE 22

CONFERENCE 6 / CASE II – 06-61-2 (AFIP 3006324)

Signalment: 9-year-old, neutered male, German Shepherd Dog, canine

History: A conjunctival mass was removed from the dog's left eye two months before referral and was diagnosed as a melanocytic neoplasm. Thoracic radiographs were taken to check for metastasis. A large thoracic mass was identified radiographically in all views. On the lateral view, the mass was located in the cranioventral thorax and was approximately the size of the cardiac silhouette. On ventrodorsal view, the entire cranial thorax was radiopaque and normal architecture obscured. An ultrasound-guided percutaneous fine needle aspirate was performed. A cavernous, mixed echogenicity mass was located around rib 3-4 and copious amounts of mucoid, viscous serosanguineous fluid was aspirated. The dog was clinically normal at this time. The dog was referred for further work-up and possible surgical intervention. Additional imaging and a repeat fine-needle aspirate were performed along with a needle biopsy of the mass. Surgical resection of the mass was deemed impractical. The dog was euthanized and a necropsy performed.

Gross Pathology: At necropsy, an approximately 15 cm diameter, irregularly round, firm, multilobular mass was identified in the right cranial lung lobe (digital images). The mass is white to tan on cross section with a cavitated center. The right middle lung lobe is multifocally atelectatic. The tracheobronchial lymph nodes are moderately enlarged, firm and mottled red on cross section. There is approximately 5-10 ml of clear fluid in the pericardial sac. The heart is mildly rounded with a flaccid left ventricle. The spleen is enlarged and oozes blood on cross section (congestion). There is a 1 cm soft tan mass in the subcutis of the left flank (lipoma, confirmed with histopathology). Physiological atrophy of prostate is noted (neutered male).

Laboratory Results: FNA cytology of thoracic mass: Multiple slides are examined and are moderately cellular with a prominent, dense, eosinophilic, stippled background and windrowing of erythrocytes with occasional aggregates of amorphous basophilic material suggestive of protein. A moderate amount of blood is also present. Nucleated cells are predominantly a population of polygonal to spindle cells that exhibit moderate anisocytosis and anisokaryosis. Cells are medium to occasionally large with a moderate amount of deeply basophilic cytoplasm and frequent intracytoplasmic vacuoles. Occasional cells contain intracytoplasmic, homogenous, eosinophilic material. Nuclei are round to oval with stippled chromatin and prominent nucleoli. A rare mitotic figure is observed.

Contributor's Morphologic Diagnosis: Lung: Chondrosarcoma, German Shepherd Dog, canine.

Contributor's Comment: This appears to be a primary chondrosarcoma of the lung due to the size of the lung mass and failure to identify any primary bony lesion clinically or during gross necropsy. During histological examination of tissues collected at necropsy, a microscopic focus of apparent metastasis of the pulmonary

chondrosarcoma was found in extradural fibro-adipose tissue at the lumbar level of the spinal column. No compression of the spinal cord at this level was noted. The tracheobronchial lymph nodes, noted to be enlarged during gross necropsy, did not show evidence of metastasis, but did exhibit draining hemorrhage and edema.

Primary lung tumors in dogs are rare and usually originate from epithelial tissue.[1] Primary chondrosarcomas are extremely rare in both dogs and humans, but have been occasionally reported.[1,2,3] Chondrosarcomas can arise from existing normal cartilage and perichondrium. In dogs, more common sites of occurrence are the pelvis, nasal cavity, sternum and ribs and less commonly in long bones.[4] Chondrosarcomas are less likely to metastasize and do so later in the course of disease than osteosarcomas.[5] Chondrosarcomas tend to occur in older large breed dogs and are the most frequent bone tumor in sheep.[5]

Cytology findings typical of a chondrosarcoma include oval to fusiform or spindle shaped cells with abundant basophilic cytoplasm that often contains vacuoles and eosinophilic granules. Often lakes of eosinophilic smooth to slightly granular material may be present in the background and occasionally cells are noted to be embedded. This material is thought to represent matrix material or “chondroid”. Cells generally exfoliate in lower numbers (compared to epithelial lesions) as individual cells or small aggregates. These finding are not always specific for a chondrosarcoma. Other sarcomas including osteosarcoma, myxosarcoma, and fibrosarcomas can have similar cytological appearances. Definitive diagnosis of sarcomas often requires histopathological examination of tissues.[6] A staining procedure using alkaline phosphatase on cytological specimens has been recently described to help differentiate osteosarcomas from other Vimentin-positive tumors in animals.[7]

AFIP Diagnosis: Lung: Chondrosarcoma, German Shepherd Dog, canine.

Conference Comment: The contributor provides an excellent synopsis of chondrosarcomas in the dog as well as the typical cytological findings associated with the neoplasm.

Primary lung tumors are uncommon to extremely rare in domestic animals depending on the species. One exception is ovine pulmonary carcinoma (pulmonary adenomatosis), a transmissible, Type B/D retrovirus-induced neoplasia of ovine lungs. Neoplastic cuboidal to columnar epithelial cells tend to infiltrate the cranioventral lung lobes and line airways and alveoli forming papillary or acinar structures. The neoplasm is considered a bronchioalveolar carcinoma since neoplastic cells originate from type II pneumocytes and Clara cells.[1,8,9] Granular cell tumors are the most frequently reported primary tumor of horses and usually present as a unilateral, firm, coarsely nodular, whitish mass causing obstruction of a major airway or several major airways. The neoplasm occurs most frequently in the right lung lobe. Histologically, neoplastic cells are round to polygonal with abundant, coarsely granular, eosinophilic cytoplasm. The cytoplasmic granules are PAS positive and diastase resistant. Ultrastructurally, the

cells contain packed lysosomes and phagosomes (myelin bodies). The granular cells are thought to be derived from Schwann cells.[1,10] Primary lung tumors, largely carcinomas, occur most frequently in aged dogs and cats.[1,9]

Secondary neoplasms in the lung are relatively common compared to primary neoplasms and can be of epithelial or mesenchymal origin as well. Common metastatic neoplasms of epithelial origin include mammary, thyroid, and uterine carcinomas. Metastatic neoplasms of mesenchymal origin include osteosarcoma, hemangiosarcoma, melanoma (dogs), lymphoma (cows, pigs, dogs, cats), and vaccine associated fibrosarcomas (cats).[9]

The gross and microscopic appearance of metastatic tumors can be difficult or impossible to distinguish from those of a primary tumor. Therefore, a thorough attempt to exclude the possibility of metastasis from a distant site must be made prior to diagnosing a primary lung tumor.

The most common types of benign and malignant pulmonary neoplasms in domestic animals are listed in the table below from Pathologic Basis of Veterinary Disease:[9]

Classification of Pulmonary Neoplasms

PRIMARY EPITHELIAL ORIGIN
Benign
Papillary adenoma
Bronchiolar-alveolar adenoma

Malignant
Adenocarcinoma (acinar or papillar)
Squamous cell carcinoma
Adenosquamous carcinoma
Bronchiolar-alveolar carcinoma
Small cell and large cell carcinomas
Anaplastic (undifferentiated) carcinoma
Carcinoid tumor (neuroendocrine)
Ovine (retroviral) pulmonary carcinoma

PRIMARY MESENCHYMAL ORIGIN
Benign
Hemangioma

Malignant
Osteosarcoma, chondrosarcoma
Hemangiosarcoma
Malignant histiocytosis
Lymphomatoid granulomatosis
Granular cell tumor
Mesothelioma

SECONDARY (METASTATIC) LUNG TUMORS
Any malignant tumor metastatic from another body location (e.g., osteosarcoma in dogs, uterine carcinoma in cows, malignant melanoma in horses)

Contributor: Comparative Pathology, AFRL/HEDV, 2509 Kennedy Circle, Brooks AFB, TX

References:

1. Dungworth DL, Hauser B, Hahn FF, Wilson DW, Haenichen T, and Harkema JR: Histological Classification of Tumors of the Respiratory System of Domestic Animals, Second Series, vol. VI, pp. 21, 25-26, 33-34. Armed Forces Institute of Pathology, Washington, D.C., 1999
2. Weller RE, Dagle GE, Perry RL, Park JF: Primary pulmonary chondrosarcoma in a dog. Cornell Vet 82(4):447-52, 1992
3. Colby TV, Koss MN, Travis WD: Tumors of the Lower Respiratory Tract, Atlas of Tumor Pathology, 3rd series, Fascicle 13, pp. 384-385. Armed Forces Institute of Pathology, Washington, D.C., 1994
4. Palmer N: Bones and Joints. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 132. Academic Press, Inc., San Diego, CA, 1993
5. Slayter MV, Boosinger TR, Pool RR, Dämmrich K, Misdorp W, and Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, Second Series, vol. I, pp. 11, 13-14, 34-35. Armed Forces Institute of Pathology, Washington, D.C., 1994
6. Cowell RL, Tyler RD, and Meinkoth JH: Diagnostic Cytology and Hematology of the Dog and Cat, 2nd ed., pp. 57, 112, 122-123. Mosby, Inc., St. Louis, Missouri, 1999
7. Barger A, Graca R, Bailey K, Messick J, de Lorimier LP, Fan T, Hoffmann W: Use of alkaline phosphatase staining to differentiate canine osteosarcoma from other vimentin-positive tumors. Vet Pathol 42(2):161-165, 2005
8. Hecht SJ, Stedman KE, Carlson JO, DeMartini JC: Distribution of endogenous and type B and type D sheep retrovirus sequences in ungulates and other mammals. Proc Natl Acad Sci USA 93(8):3297-3302, 1996
9. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 550-553. Mosby Elsevier, St. Louis, Missouri, 2007
10. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 692-693. Academic Press, Inc., San Diego, California, 1993

SLIDE 23
CONFERENCE 6 / CASE III – AFIP case 2 (AFIP 3026267)

Signalment: 8-month-old, intact male, domestic short-haired cat (*Felis catus*)

History: (From referring veterinarian) Patient is from a multiple (7) cat household. Two of the seven cats exhibited acute onset lethargy, vomiting and pyrexia. No response to antibiotics. Both patients died.

Gross Pathology: An intact male gray cat with white paws weighing 10 pounds 6 ounces is presented for necropsy. There are moderate numbers of fleas present on the carcass with a few ticks identified. Oral and scleral mucous membranes are yellow in color with some edema. Bilaterally, lungs are overinflated, dark red to dark pink in color with thin clear fluid and foam exuding out of airways on cut surface. Multifocal hemorrhages are noted on the epicardial surface. The spleen is enlarged. The stomach is empty, and the small intestine contains minimal yellow content and some tapeworms.

Histopathologic Description: Spleen and lung: The red pulp of the spleen contains a disseminated population of large schizont-laden macrophages. Similarly, numerous schizont-laden macrophages partially or completely occlude trabecular vessels. The white pulp is moderately atrophied. The open alveolar architecture of the lung is extensively obscured secondary to alveolar filling by hemorrhage, edema and scattered fibrin deposition. Alveoli contain numerous large foamy macrophages, some exhibiting erythrophagocytosis and others expanded by schizonts. Alveolar septa are expanded by edema. The septal capillaries along with large pulmonary vessels (both arteries and veins) are partially to completely occluded by numerous schizont-laden macrophages.

Contributor's Morphologic Diagnoses: 1. Lung: Severe, regionally extensive, pulmonary hemorrhage and edema with intralveolar and intravascular, intrahistiocytic schizonts consistent with *Cytauxzoon felis*.
2. Spleen: Intravascular and intrahistiocytic (red pulp) schizonts consistent with *Cytauxzoon felis* and mild to moderate, diffuse, lymphoid atrophy.

Contributor's Comment: *Cytauxzoon felis* is a protozoan hemoparasite associated with severe clinical disease and high mortality in domestic cats. Organisms in the genus *Cytauxzoon* are closely related to the genera *Babesia* and *Theileria*.[1] Moreover, piroplasms of small *Babesia* species are morphologically indistinguishable from *C. felis*.[1]

The reservoir for *C. felis* appears to be the bobcat, where *Cytauxzoon* infection is usually asymptomatic but results in a long-lasting erythroparasitemia. The parasite is transferred form the wild bobcat to domesticated cats by tick vectors. Research has shown *Dermacentor variabilis* to be a competent vector.[2] Whereas the intraerythrocytic piroplasms seem to do little harm, the schizogonous phase of the organism is responsible for the marked clinical signs of disease.[1] Schizont-laden macrophages become greatly enlarged and are typically found within the lumen of blood vessels that become nearly or totally occluded.[1] Vascular obstruction and damage is thought to be one of the major pathophysiologic mechanisms in this disease.[3] Concomitantly, gross lesions of infection are associated with vascular lesions and include: marked pulmonary congestion and edema, widespread petechial and ecchymotic hemorrhages, abdominal vein distension, and body cavity effusions. Figure 2 highlights gross lesions of Cytauxzoonosis including pulmonary congestion/edema, pulmonary hemorrhages and pleural effusions.

Diagnosis of Cytauxzoonosis is usually made by identification of the organism in blood smears. Since the schizogonous phase is responsible for clinical disease, and since this phase precedes detectable erythroparasitemia, some cats may not have circulating piroplasms at the time of initial presentation.[1] Therefore, negative smears should be temporally repeated if clinical suspicion of disease remains strong. On the other hand, false diagnosis of Cytauxzoonosis is not uncommon secondary to misidentification of stain precipitates and Howell-Jolly bodies (Figure 1) as true organisms.[1]

Lastly, cats infected with *Cytauxzoon felis* have invariably been given a grave prognosis. However, recently, there have been some reports of cats surviving natural infection without treatment.[4] The current hypothesis is that recovery is linked to the existence of a less virulent strain of *C. felis*.[4]

AFIP Diagnoses: 1. Lung, vessels: Intrahistiocytic schizonts, myriad, with diffuse hemorrhage and edema, domestic short hair (*Felis catus*), feline.
2. Spleen, vessels and red pulp: Intrahistiocytic schizonts, myriad.
3. Spleen, white pulp: Lymphoid depletion, diffuse, moderate.

Conference Comment: The contributor provides an excellent synopsis of Cytauxzoonosis.

As pointed out by the contributor, the signet ring-shaped erythrocytic piroplasms of *C. felis* closely resemble the small form of *Babesia* and some *Theileria* organisms. In contrast to *C. felis* with an erythrocytic phase and a schizogenous phase in macrophages, *Babesia* only infects erythrocytes. *Theileria* organisms also have erythrocytic and non-erythrocytic phases; however, the schizogenous phase occurs in lymphocytes rather than in macrophages.[1]

In addition to the 3 intracellular protozoal parasites mentioned above, intracellular rickettsial organisms (*Anaplasma*) and epicellular mycoplasma organisms (*Mycoplasma haemofelis*, *M. suis*) are known to occur in or on erythrocytes. All of these erythrocyte infectious agents cause mild to severe hemolytic anemia depending on the pathogenicity of the organism and host susceptibility. Additionally, distemper virus inclusions may be seen in canine erythrocytes.[5]

Many species of *Babesia* infect animals worldwide and vary markedly in size from large and easy to visualize (*B. canis*) to small and difficult to see (*B. gibsoni*, *B. felis*). Large babesial species typically occur in pairs and are pear-shaped whereas small babesial organisms are more round.[5]

Theilerial organisms appear similar to babesial organisms on stained blood films. The theilerial organisms present in the United States are usually nonpathogenic and are most commonly observed in deer blood.[5]

Anaplasma organisms are round to oval basophilic inclusions in ruminant erythrocytes and must be differentiated from Howell-Jolly bodies. *Anaplasma* organisms are generally not perfect spheres and are smaller than Howell-Jolly bodies.[5]

Mycoplasma haemofelis (formerly *Haemobartonella felis*) appears as small blue-staining, variably sized (0.5 – 1.5 um in diameter) cocci, rings, or rods on feline erythrocytes. *M. haemofelis* occurs in cyclic parasitemias and is not always visible in blood smears. *M. haemocanis* (formerly *Haemobartonella canis*) forms chains of organisms on the surface of erythrocytes. *Mycoplasma suis* (formerly *Eperythrozoon suis*) species appear as small delicate basophilic rings on or between erythrocytes in sheep, pigs, cattle, and llamas. While mycoplasma infections can cause significant anemia in pigs and sheep (especially young animals), they usually do not in cattle and llamas.[5] Additionally, most hemotropic mycoplasma subspecies cause disease in immunocompromised animals or animals with concurrent disease. The exception is *M. haemofelis* which causes acute hemolytic anemia in immunocompetent cats.[6]

Although *Bartonella henselae* infects erythrocytes, this small Gram-negative bacteria is rarely seen in blood films.[5]

Distemper viral inclusions appear as variably sized, round, oval, or irregular, blue-gray inclusions that most often occur in polychromatophilic cells during the viremic phase of infection. If the Diff-Quik stain is used, distemper inclusions typically stain red.[5]

Common avian blood parasites include *Hemoproteus*, *Plasmodium*, and *Leukocytozoon*. Only the gametocytes of *Hemoproteus* are found in peripheral blood and vary in size from small, developing ring forms to elongate, crescent-shaped, mature gametocytes that partially encircle the erythrocyte nucleus. *Hemoproteus* gametocytes contain refractile, yellow to brown pigment granules (iron pigment). In contrast to *Hemoproteus*, schizonts and trophozoites as well as gametocytes can be found in erythrocytes, thrombocytes, and leukocytes in infections with *Plasmodium*. The gametocytes of *Plasmodium* also contain iron pigment granules. Like *Hemoproteus*, only the gametocytes of *Leukocytozoon* are seen in peripheral blood. The gametocytes are large and grossly distort infected cells making cell identification difficult. Some parasitologists believe that erythrocytes rather than leukocytes serve as the host cell for *Leukocytozoon*. The gametocytes of *Leukocytozoon* do not contain refractile pigment granules.[7]
Hemogregarines are the most common reptilian hemoparasites and include *Hemogregarina*, *Hepatozoon*, and *Karyolysus*. The sausage-shaped gametocytes are found within the cytoplasm of erythrocytes. Typically only one gametocyte is found per erythrocyte; however, in heavy infections up to two gametocytes may be found in erythrocytes.[8]

Erythrocyte parasites must be differentiated from precipitated stains, refractile drying or fixation artifacts, poorly staining Howell-Jolly bodies, basophilic stippling, and platelets overlying erythrocytes.[5]

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References:

1. Meinkoth JH, Kocan AA: Feline cytauxzoonosis. Vet Clin Small Anim 35:89-101, 2005
2. Blouin EF, Kocan AA, Glenn BL, Kocan KM: Transmission of *Cytauxzoon felis* from bobcats (*Felis rufus*) to domestic cats by *Dermacentor variabilis*. J Wildl Dis 20:241-242, 1984
3. Wagner JE, Ferris DH, Kier AB, Wightman SR, Maring E, Morehouse LG, Hansen RD: Experimentally induced cytauxzoonosis-like disease in domestic cats. Vet Parasitol 6:305-311, 1980
4. Meinkoth J, Kocan AA, Whitworth L, Murphy G, Fox JC, Woods JP: Cats surviving natural infection with *Cytauxzoon felis*: 18 cases (1997-1998). J Vet Intern Med 14:521-525, 2000
5. Harvey JW: Atlas of Veterinary Hematology Blood and Bone Marrow of Domestic Animals, pp. 40-44. W.B. Saunders Company, Philadelphia, Pennsylvania, 2001
6. Fry MM, McGavin: Bone marrow, blood cells, and lymphatic system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 786-787. Mosby Elsevier, St. Louis, Missouri, 2007
7. Campbell TW: Hematology of Birds. In: Veterinary Hematology and Clinical Chemistry, ed. Thrall MA, pp. 245-249. Lippincott, Williams & Wilkins, Baltimore, Maryland, 2004
8. Campbell TW: Hematology of Reptiles. In: Veterinary Hematology and Clinical Chemistry, ed. Thrall MA, pp. 271-274. Lippincott, Williams & Wilkins, Baltimore, Maryland, 2004

SLIDE 24
CONFERENCE 6 / CASE IV – 06-12133 (AFIP 3027434)

Signalment: 8-year-old, 41.5kg (91lb), spayed female, Dalmatian dog (*Canis familiaris*)

History: Approximately two weeks prior to necropsy, the dog presented to the referring veterinarian after falling down the stairs. The dog was slightly lame in the hind limbs and treated with Adequan® (polysulfated glycosaminoglycan) injections and pain management and sent home. Over a week and a half later the dog presented to the University of Illinois Veterinary Teaching Hospital for acute lameness and swelling of the left hind limb. On physical examination the left hind limb was cool to the touch with cyanotic nail beds. Proprioceptive and nociceptive deficits were also detected. Evaluation of the right limb also revealed similar, but less severe findings. A complete blood cell count, serum chemistry and urinalysis were performed. Abdominal ultrasonography with Doppler evaluation revealed minimal flow in the distal aorta. The dog was subsequently euthanized due to progressive clinical signs.

Gross Pathology: On gross examination the dog was obese with plentiful subcutaneous and visceral adipose tissue stores (Figure A). The abdominal aorta distal to the renal arteries was completely occluded by a column of a dark red to tan dull and friable material that was tenuosly adhered to the vascular endothelium (thrombus). The thrombus extended through the external iliac arteries into the femoral arteries occluding the lumina along the entire length of the vessels (Figures B and C). The endothelial surface adjacent to the ostia of major branches of the aorta was often thickened, firm, roughened and slightly raised by irregular plaques composed of a dull yellow tan grumous to granular core with a pale yellow to white firm fibrous cap.

The coronary arteries were also segmentally affected by similar plaques (Figures D and E).

The thyroid glands were symmetrically shrunken measuring approximately 0.3 x 0.3 x 1 cm and diffusely pale tan.

Laboratory Results: The CBC revealed a mild anemia with a hematocrit of 31.1% (35-52%), red blood cell count of $4.36x10^6/\_l$ (5.50 to $8.50x10^6/\_l$) and a hemoglobin concentration of 11.1 g/dl (12.0 to 18.0). The dog also had a moderate leukocytosis with a white blood cell count of $29.1x10^3/\_l$ (6.00 to $17.00x10^3/\_l$) and a mature neutrophilia $27.7x10^3/\_l$ (3.00 to $11.50x10^3/\_l$) and mild lymphopenia $0.819x10^3/\_l$ (1.00 to $4.80x10^3/\_l$).

Serum chemistry revealed minimal to mild elevations in serum calcium 12.0mg/dl (7.9 to 11.5mg/dl), alkaline phosphatase 178 U/l (12 to 110 U/l), and corticosteroid induced alkaline phosphatase (cALP) 87 U/l (0 to 40 U/l). Serum triglyceride levels were moderately increased at 235 mg/dL (25 to 145 mg/dL) and total serum cholesterol was severely increased at 1416 mg/dl (109 to 315 mg/dl). Bicarbonate levels (TCO2) were also moderately decreased at 13.3 mmol/l (17.0 to 29.0 mmol/l) with a normal anion gap.

The urinalysis was unremarkable with a specific gravity of 1.025.

A thyroid profile revealed a severely decreased total T4 of less than 6.4 nmol/L (15.0 to 48.0 nmol/L), a severely decreased free T4 of 1 pmol/L (6 to 42 pmol/L) and a markedly increased thyroxin stimulating hormone (TSH) concentration of 2 ng/ml (0.03ng to 1.00 ng/ml)..

Histopathologic Description: The tunica intima and media are eccentrically thickened, moderately to markedly expanded and partially effaced by variable amounts of foamy vacuolated macrophages (foam cells) admixed with lipid droplets and acicular clear spaces (cholesterol clefts) that separate the smooth muscle myofibers. In affected areas the myofibers are occasionally fragmented and degenerative with foamy pale eosinophilic cytoplasm and pyknotic or karyorrhectic nuclei. The tunica media is occasionally mildly infiltrated by lymphocytes and plasma cells admixed with an

amorphous to granular deeply basophilic material (mineral). The internal elastic lamina and endothelium are segmentally disrupted and confluent with a fibrillar to amorphous pale eosinophilic material (fibrin) that fills and occludes the lumen entrapping numerous leukocytes, erythrocytes, hematoidin crystals and much cellular debris in vaguely laminated sheets (lines of Zhan).

Contributor's Morphologic Diagnosis: Marked chronic atherosclerosis with severe acute thrombosis, femoral artery, dog

Contributor's Comment: Atherosclerosis, while a leading cause of morbidity and mortality in man, is uncommon in domestic species. Atherosclerosis is characterized by intimal thickening due to lipid accumulation with variable amounts of inflammatory infiltrate (macrophages, lymphocytes and plasma cells), fibrosis, proteoglycan matrix and mineralization.[4] These aggregates form atheromatous plaques that protrude into the lumen and weaken the tunica media. Elastic arteries are often affected and serious sequela include thrombosis and aneurism; however canine atherosclerosis is uncommonly associated with thrombosis and extensive atheromatous plaque formation.[1] In this case segmental disruption of the internal elastic lamina is visualized using Verhoeff's Elastic stain (Figure F). In man atherosclerosis is currently considered to be a chronic inflammatory response to endothelial injury.[4] Unlike the disease in man, the role of chronic inflammation in the development of canine atherosclerosis remains unclear.[1]

In the dog a predisposition for spontaneous atherosclerosis has been associated with hypothyroidism. In concordance with this observation, atherosclerosis can be experimentally induced in thryoidectomized dogs fed large quantities of cholesterol or cholic acids.[1] Hypothyroidism is a commonly diagnosed condition of canines with idiopathic thymic atrophy or lymphocytic thyroiditis as the leading causes of this disorder. An immune mediated pathogenesis for lymphocytic thyroiditis has been postulated due to the similarities to Hashimoto's thyroiditis in man, however a definitive molecular pathogenesis has yet to be determined.[2] Current research has demonstrated an association between a rare DLA class II haplotype and lymphocytic thyroiditis in Doberman Pinschers and several other breeds.[3]

In this case, the thyroid gland was evaluated histologically and was found to be almost entirely effaced by a lymphoplasmacytic infiltrate that surrounded, separated and individualized the few remaining follicles (Figures G and H). The remaining follicles were often small shrunken with a pale amphophilic finely stippled colloid. The microscopic findings in the thyroid were consistent with lymphocytic thyroiditis.

AFIP Diagnosis: Large muscular artery: Atherosclerosis, chronic, multifocally extensive, severe, with thrombosis, Dalmation (*Canis familiaris*), canine.

Conference Comment: The contributor provides a nice summary of the components of a classic atherosclerotic lesion, possible sequela, and its association with hypothyroidism in the dog.

Conference attendees began by discussing the thyroid profile. A normal total T4 generally rules out hypothyroidism. Although the presence of anti-T4 autoantibodies in 10% of cases of hypothyroidism may increase the total T4 concentration into or above the reference interval, and hypothyroidism will not be detected. A decreased total T4 alone does not confirm hypothyroidism. Nonthyroidal illness may decrease the serum total T4 concentration (euthyroid sick syndrome). Hypothyroidism should be confirmed or excluded by determining TSH and fT4 concentrations. The direct dialysis fT4 assay has the highest single-test diagnostic sensitivity, specificity, and accuracy in detecting thyroid disease. Measurement of endogenous TSH concentration detects a lack of negative feedback on the pituitary gland and hypothalamus. Serum TSH levels are increased in approximately 75% of dogs with primary hypothyroidism.[5]

Generalized vascular degenerative diseases in animals are classified into three different groups: atherosclerosis, arteriosclerosis, and arterial medial calcification.[6] Pigs, rabbits, and chickens develop atherosclerosis when fed high-cholesterol diets, whereas dogs, cats, cows, goats, and rats are resistant. Atherosclerosis naturally develops in aged pigs and birds, and also occurs in dogs with hypothyroidism or diabetes mellitus.[6,7] Atherosclerosis is infrequently seen in dogs with hypothyroidism. When it is seen, it is most likely associated with an increased proportion of cholesterol-rich VLDL.[7] Grossly, arteries of the heart, mesentery, and kidney are prominent, yellow-white, and cordlike.[6] Arteriosclerosis literally means hardening of the arteries and is further defined as "chronic arterial change consisting of hardening, loss of elasticity, and luminal narrowing resulting usually from proliferative and degenerative, rather than inflammatory, changes of the media and intima" in Pathology of Domestic Animals.[7] Arteriosclerosis occurs commonly in many aged animal species and rarely causes clinical signs. The aorta is most frequently affected, but other elastic and muscular arteries may be involved. Grossly, the lesions appear as raised, firm, white plaques. Histologically, the intima is thickened by accumulation of mucopolysaccharides, proliferation of smooth muscle cells in the tunica media, and fibrous tissue infiltration in to the intima. Additionally, the internal elastic lamina is frequently split and fragmented. Arterial medical calcification involves muscular and elastic arteries and is commonly seen in animals with concurrent endocardial mineralization. Causes of arterial medial calcification include vitamin D toxicosis, renal insufficiency, calcinogenic plant toxicosis, and severe debilitation (Johne's disease). Spontaneous medial calcification occurs in rabbits, aged guinea pigs, and rats with chronic renal disease. Grossly, affected arteries appear as solid, dense, pipelike structures with multiple white mineralized foci in the tunica intima and media. Histologically, prominent basophilic granular mineral deposits to complete rings of mineralization are visible in the tunica media.[6,7]

Other lesions associated with hypothyroidism in the dog include hepatomegaly, glomerular and corneal lipidosis, bilaterally symmetrical alopecia, hyperkeratosis, hyperpigmentation, and myxedema.[8,9]

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References:

1. Fox PR, Petrie JP, Hohenhaus AE: Peripheral vascular disease. In: Textbook of Veterinary Internal Medicine, eds. Ettinger SJ, Feldman EC, 6th ed., vol. 2, pp. 1156 - 1157. Elsevier Saunders, St. Louis, Missouri, 2005
2. Scott-Moncrieff JCR, Guptill-Yoran L: Hypothyroidism. In: Textbook of Veterinary Internal Medicine, eds. Ettinger SJ, Feldman EC, 6th ed., vol. 2, pp. 1535 -1544. Elsevier Saunders, St. Louis, Missouri, 2005
3. Kennedy LJ, Quarmby S, Happ GM, Barnes A, Ramsey IK, Dixon RM, Catchpole B, Rusbridge C, Graham PA, Hillbertz NS, Roethel C, Dodds WJ, Carmicheal NG, Ollier WER: Association of canine hypothryoidism with a common major histocompatibility complex DLA class II allele. Tissue Antigens 68:82-86, 2006
4. Schoen FJ: Blood vessels. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 516-525. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
5. Ferguson DC, Hoenig M: Endocrine system. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 283-289. Iowa State University Press, Ames, Iowa, 2003
6. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 599-600. Mosby Elsevier, St. Louis, Missouri, 2007
7. Robinson WF, Maxie MG: The cardiovascular system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 54-56. Academic Press, Inc., San Diego, California, 1993
8. La Perle KMD, Capen CC: Endocrine system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 720-722. Mosby Elsevier, St. Louis, Missouri, 2007
9. Capen CC: The endocrine glands. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, p. 314. Academic Press, Inc., San Diego, California, 1993

SLIDE 25
CONFERENCE 7 / CASE I – S9700095 A1 (AFIP 3024109)

Signalment:

S9700002B	Holstein cow, 2-year-old
S9700002D	Holstein cow, 3-year-old
S9700095A	Holstein cow, 4-year-old

History: The three animals were part of a group of approximately 20 cows which were corralled for hoof trimming treatment for lameness (a routine weekly procedure), on two

1000-cow drylot Holstein dairies in southern California. Both herds were involved by endemic papillomatous digital dermatitis (PDD; digital dermatitis; footwarts). Gross lesions of PDD were selected for transmission studies because of their high activity. They were surgically excised from the feet of the three cows and portions were placed in formalin.

Gross Pathology: The lesions were singular, oval to circular, 2 to 3 cm across, slightly raised and well-demarcated. Each lesion involved the skin of a heel of a hind leg adjacent to the interdigital cleft (S9700002B and D) or pastern (S9700095A). Their surfaces were non-crusted except around the edges, moist with serous exudates, red-blue, raw-appearing, very painful to touch and prone to bleed, and tufted by grey/yellow/black filiform papillae of varying lengths (approximately 1 to 20 mm). No digital swelling or interdigital lesions were present.

Laboratory Results: Darkfield microscopy of saline suspensions of homogenized superficial scrapings of the lesions revealed profuse numbers of singular and matted, highly motile, slender, spiral organisms (SSO) admixed with small numbers of non motile, long, straight filaments with pointed ends. The motility, which was characterized by vigorous axial spinning and end-to-end flexing, was best observed in specimens stored in plain vials on wet ice and examined within 4 hours of biopsy. Motility diminished to zero by 8 hours post collection. Gram stain revealed numerous Gram-negative filaments and rods and sparse Gram-positive diphtheroids. Victoria Blue and Steiner silver stains revealed profuse SSO's resembling spirochetes (Fig. 1).

Aerobic and anaerobic bacteriologic cultures were performed on similar gross lesions on other cows in other dairies. Three novel molecular phylotypes of spirochetes were isolated and identified most closely resembling, *Treponema denticola* (Type 1), *phagendinis* (Type 2) and *medium/vincentii* (Type 3). Other anaerobes regularly isolated were *Porphyromonas levii* and an unidentified Gram negative filamentous rod. Aerobic growth was inconsistent and slight. A *Mycoplasma* sp. of an unidentified serotype was regularly isolated.

Histopathologic Description: Findings were similar in each biopsy. Epidermis and dermis is well represented. Junctional borders with normal skin are not present. Skin is raised and convex due to a combination of the bulk-effects of a markedly thickened epidermis and increased vascularity of the deep dermis. Stratum corneum is, for the most part, transformed into a thick ragged, papillated, discontinuous layer of hyperkeratosis. The hyperkeratosis is extensively parakerotic and focally orthokeratotic. The parakeratosis is poorly cornified in some areas and has multifocal microcavities containing fibrin, proteinaceous fluid, red cells and/or neutrophils. The continuity of the stratum corneum is interrupted by ulceration of the tips of multiple dermal papillae. It is also focally to extensively involved by dense mats of pale, basophilic fibrillary material. In one biopsy, stratum corneum is mostly absent, not through artifact but by failure of the stratum spinosum to cornify. Stratum granulosum is absent except for beneath the focal areas of orthokeratotic stratum corneum. Stratum spinosum is markedly thickened by excessive numbers of hypertrophic keratinocytes (acanthosis). These are

architecturally arranged in a broad band of uniformly thick (3-4 mm), interconnected columns, The columns are interdigitated by long, thin dermal papillae. In sections perpendicular to the surface of the lesion, the columns tended perpendicular also. Some columns are continuous with the papillae. Borders of the columns are spongiotic and lined by primitive-appearing basal cells, some of which are in mitosis. No breach of basement membrane is seen. Superficial stratum spinosum is focally to extensively involved by neutrophil infiltration and presence of the basophilic fibrillary material, often outlining keratinocytes. Dermal papillae are hyperemic, infiltrated by small numbers of neutrophils and plasma cells. Superficially they are involved by hemorrhage, capillary thrombosis, suppuration and invasion by the basophilic fibrillary material. The deep dermis is involved by increased numbers of arterioles, most of which are hypertrophied, slight to moderate diffuse to perivascular infiltrations of neutrophils and plasma cells and the presence atretic hair follicles. No granulation tissue, fibromatous change or inclusion bodies are present. Specially stained preparations reveal that the basophilic fibrillary material consisted almost entirely of dense mats of long (10-15 um) slender spiral organisms. These were blue with Giemsa, red with Gram's method, black with Steiner silver, grey with Methenamine silver and pale blue with PAS.

Figures 2-5 illustrate colonization of the stratum corneum and invasion of the stratum spinosum by spirochetes by use of Steiner silver stain, electron microscopy and immunohistochemistry in PDD lesions of similar histologic character to those supplied in this case. Ultrastructurally, the slender spiral organisms were identified as spirochetes by the presence of a periplasmic membrane enclosing axial filaments at each extremity and an axial protoplasmic body.

Contributor's Morphologic Diagnosis: Skin: Chronic-active, diffuse ulceroproliferative dermatitis with papillary epidermal hyperplasia, parakeratotic hyperkeratosis, ulceration of dermal papillae and superficial colonization and invasion by spirochete-dominant bacterial mat, heel, hind leg, Holstein cow, bovine.

Contributor's Comment: Papillomatous digital dermatitis (PDD), also known as "footwarts" or "hairy heel warts" in the US, is a world-wide, contagious, painful, wart-like disease of the feet of cattle. It was first reported in 1974 in Italy by Cheli and Mortellaro.[1] In Europe it is commonly known as Mortellaro's disease or digital dermatitis (DD). In North America, the disease was first reported in 1980 in New York where it was known as interdigital papillomatosis (IP).[2] We reported a similar disease in California in 1992 associated with invasive spirochetes.[3] It is now generally believed that all three entities are the same disease. [4,5] This is supported by a comparative histopathologic and immunohistochemical study of lesions of PDD and DD from 18 countries which found that the pathologic character and immunoreactivity of the spirochetes in the three entities were identical.[6]

The gross appearance of PDD varies markedly. In our studies, in which we observed the spontaneous progression/remission of natural and experimental lesions, the appearance depended on the wetness/dryness of the foot environment, whether or not the lesion was recently treated by antibacterials/footbaths, age of the lesion and/or

anatomic location.[5,7,8] The sections in this case had gross features of high activity lesions, namely, moist, red/grey raw-appearing surfaces. These lesions were not photographed but are typically represented in photographs of lesions in other cows that had wet foot environments (Figs. 6-9). Typically, these lesions have ulceration and dense mats of invasive spirochetes. However, if the foot environment becomes dry or if footbaths are introduced, lesion surfaces become water resistant, brown, rubbery and non-painful in a matter of a few days.[3,5] They still appear grossly "warty" but the histologic diagnosis become presumptive because the spirochetes disintegrate, the ulcers heal and the stratum corneum becomes intact.[7] Papillomatous change (Figs. 8 and 9) is probably an indication of maturity but its relationship to age is not clear-cut because papillae were observed to occur in only 40% of large lesions.[5] My estimate of the ages of the lesions in figures 6-9 are: 3-4 weeks (Fig. 6), 2-3 months (Fig. 7 and 8) and 3-6 months (Fig.9). The anatomic site seems to influence the gross appearance of PDD because papillae have not been observed in lesions involving the interdigital skin.[5,9]

The sections nicely illustrate the diagnostic criteria of PDD: parakeratotic hyperkeratosis, epidermal acanthotic hyperplasia, ulceration of tips of dermal papillae; and, colonization of the stratum corneum and invasion of the stratum spinosum and dermal papillae by spirochete-dominant mats.[9]

PDD differs pathologically from other ulcerative/proliferative conditions of the bovine foot. Interdigital necrobacillosis (Footrot) is characterized by deep fissuring and caseation necrosis of dermis with extensive cellulitis.[10] Bovine papillomavirus-induced lesions rarely involve the feet of cattle, the granular layer is prominent and cytoplasm of keratinocytes in the stratum spinosum is basophilic.[11] Traumatic laceration of skin, or deep ulceration from any cause, heals by granulation tissue formation, a rare finding in PDD. Granulomatous inflammation, or presence of fungal elements, foreign bodies, *Dermatophilus*, parasites or inclusion bodies have not been observed.[9]

The etiology of PDD is unknown because Koch's postulates have not been demonstrated. However, several lines of evidence have shed light on its pathogenesis. A viral association was investigated by electron microscopy, immunohistochemistry for generic papillomavirus, DNA probes for bovine papillomavirus types 1-6 and inoculation of laboratory animals.[12,13] No virus was detected. The marked sensitivity of the lesion to parenteral antibiotics (without manipulating the lesion) in association with the consistent finding of invasive spirochetes provided evidence that bacteria may play a major role.[3,5,9] Sequential ultrastructural examination of experimental lesions revealed that spirochetal invasion was both primary and dominant; other bacteria rarely invaded viable tissue, even in full-blown lesions. No viral particles were observed.[14] Isolation of spirochetes and detection of significant increases in humoral antibody responses to PDD-associated *Treponema* spp. in cows with natural PDD indicated that the spirochetes isolated were invasive, not mere commensals colonizing non-viable parakeratotic debris.[15,16] Experimental proof that hydropic maceration of digital skin (by constant moisture for 7-14 days) was an essential preqequisite for successful transmission indicated that the pathogenesis of PDD was multifactorial with infectious

and environmental factors.[8] Finally, the histologic similarity of PDD to yaws, a papillomatous ulcerative condition of the feet of people living in the tropics caused by *Treponema pallidum* subsp*. pertenue*, also adds support to the notion that *Treponema* sp. may play a major role in the genesis of PDD.[17]

AFIP Diagnosis: Glabrous skin: Epidermal hyperplasia, papillated, diffuse, marked with orthokeratotic and parakeratotic hyperkeratosis, superficial necrosis, mild chronic-active dermatitis, and intracorneal bacteria, Holstein (*Bos taurus*), bovine.

Conference Comment: The contributor provides a thorough overview of papillomatous digital dermatitis as well as how to differentiate PDD from other ulcerative/proliferative conditions of the bovine foot. Below is a table from Pathologic Basis of Veterinary Disease summarizing the digital infections of ruminants.[18]

Digital Infections of Ruminants

Species	Disorder	Predisposing Factors	Bacteria Involved	Severity	Contagious
Sheep	Contagious foot rot, virulent form	Moisture & trauma	*Dichelobacter nodosus* plus *Fusobacterium necrophorum* and other bacteria	Severe; virulent strains of *D. nodosus* produce more proteolytic enzymes	Yes
Sheep	Contagious foot rot, benign form (foot scald)	Moisture & trauma	*Dichelobacter nodosus* *Fusobacterium necrophorum*	Mild; less virulent strains of *D. nodosus* produce fewer proteolytic enzymes and are less pathogenic	Yes
Sheep	Necrobacillosis of the foot I. Ovine interdigital Dermatitis		*Fusobacterium necrophorum* Other bacteria, but no *Dichelobacter nodosus*	Clinically similar to benign foot rot	No
	II. Foot abscesses A. Heel abscesses (infective bulbar necrosis) B. Toe abscesses (lamellar abscesses)	Wet seasons Heavy adult sheep	*Fusobacterium necrophorum* *Arcanobacterium pyogenes*	Can cause severe lameness with permanent foot deformity	No
Cattle	Foot rot	Trauma & moisture	*Dichelobacter nodosus* *Fusobacterium necrophorum* Other bacteria	Mild; similar to benign foot rot in sheep	Yes
Cattle	Necrobacillosis of the foot (foul-in-the-foot)	Trauma	*Fusobacterium Necrophorum* *Bacteroides melaninogenicus*	Can be severe with cellulitis involving tendons, joints, and bone	No
Cattle	Papillomatous digital dermatitis (foot warts; hairy heel warts)	Prolonged wet conditions	Probably *Treponema* sp. spirochete	Moderate to severe lameness	Yes

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References:

1. Cheli R, Mortellaro C: La dermatite digitale del bovino. Proc 8th Int Conf Bov Lameness, pp. 208-213. Milan, 1974
2. Rebhun WC, Payne RM, King JM, Wolfe M, Begg SN: Interdigital papillomatosis in dairy cattle. J Amer Vet Med Assoc 177(5):437-440, 1980
3. Read DH, Walker RL, Castro AE, Sundberg JP, Thurmond MC: An invasive spirochete associated with interdigital papillomatosis of dairy cattle. Vet Rec 130:59-60, 1992
4. Gourreau JM, Scott DW, Rosseau JF: Digital dermatitis of cattle. Le Point Vet 24:49-57, 1992
5. Read DH, Walker RL: Papillomatous digital dermatitis (footwarts) in California dairy cattle: clinical and gross pathologic findings. J Vet Diagn Invest 10:67-76, 1998
6. Read DH, Walker RL: Comparison of papillomatous digital dermatitis and digital dermatitis of cattle by histopathology and immunohistochemistry. Proc 10th Int Symp Lameness in Ruminants, p. 268, 1998
7. Read DH: Unpublished data, 1998
8. Read DH, Walker RL: Experimental transmission of papillomatous digital dermatitis (footwarts) in dairy cattle. Proc 10th Int Symp Lameness in Ruminants, p. 270, 1998
9. Read DH, Walker RL: Papillomatous digital dermatitis and associated lesions of dairy cattle in California: Pathologic findings. Proc 8th Int Conf Bov Lameness, pp. 156-158, 1994
10. Edmonson AJ: Interdigital necrobacillosis (footrot) of cattle. In: Large Animal Internal Medicine, ed. Smith BP, 3rd ed., pp. 1127-1129. Mosby, St. Louis, Missouri, 2002
11. Goldschmidt MH, Dunstan RW, Stannard AA, von Tscharner C, Walder EJ, Yager JA: Histologic Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals, Second Series, vol. III, p. 19. Armed Forces Institute of Pathology, Washington, D.C., 1998
12. Read DH, Walker RL, Van Ranst M, Nordhausen RW: Studies on the etiology of papillomatous digital dermatitis (footwarts) of dairy cattle. 38th Ann Meeting Amer Assn Vet Lab Diag, p. 68, 1995
13. Read DH, Walker RL: Research update: Etiology of papillomatous digital dermatitis (footwarts) in dairy cattle. Proc Wild West Vet Conf, pp. 105-109, 1997 14. Read DH, Nordhausen RW, Walker RL: Pathogenesis of experimental papillomatous digital dermatitis (footwarts) in cattle: Bacterial morphotypes associated with early lesions development. Proc 10th Int Symp Lameness in Ruminants, p. 271, 1998
15. Walker RW, Read DH, Loretz KJ, Nordhausen RW: Spirochetes isolated from dairy cattle with papillomatous digital dermatitis and interdigital dermatitis. Vet Micro 47:343-355, 1995

16. Walker RL, Read DH, Loretz KJ, Hird DW, Berry SL: Humoral response of dairy cattle to spirochetes isolated from papillomatous digital dermatitis lesions. AJVR 58:744-748, 1997
17. Englekens HJH, Judanso J, Oranje AP, Vuzevski VD, Niemel PLA, van der Sluis JJ, Stolz E: Endemic treponematosis, Part 1. Yaws. Int J Dermatol 30:77-83, 1991
18. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1190. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 26
CONFERENCE 7 / CASE II – 06-1098 (AFIP 3027583)

Signalment: Female mountain goat

History: An old, adult, female mountain goat was found dead near a hot springs in central Idaho.

Gross Pathology: The submitter reported an emaciated, partially scavenged carcass with severe dental disease. Two "chicken egg-sized cysts" were present in the lungs. The gross specimen submitted was a piece of lung with a discrete, 5 x 4.5 x 4.5 cm cyst filled with clear fluid and having a finely granular lining (figs. 1 and 2)

Histopathologic Description: In a section of lung, alveoli are compressed by a cyst that is composed of a peripheral rim of collagenous tissue (host tissue) lined by a prominent, slightly lamellar layer of finely granular, lightly basophilic, acellular material (fig. 3). Internally, the cyst is further lined by a thin, eosinophilic layer with numerous, round to ovoid, lightly eosinophilic structures (calcareous corpuscles, (fig 4). From this extends a discontinuous layer of numerous, spherical, thin-walled structures (brood capsules) up to 460 _m diameter and containing groupings of 2-14 protoscolices with prominent refractive hooks and containing calcareous corpuscles (fig. 5).

Contributor's Morphologic Diagnosis: Pulmonary unilocular hydatid cyst (typical of *Echinococcus granulosus*)

Contributor's Comment: The hydatid cyst found in the aged mountain goat was thought to be an incidental finding unrelated to the cause of death (likely due to emaciation related to poor dental condition).

The cestode genus *Echinococcus* includes 2 major species, *E. granulosus* and *E. multilocularis.* Both species are parasites of canids (dog, coyote, wolf and dingo), although there is a lion-adapted strain in Africa.[1] The parasite has world wide distribution, and the range of *E. granulosus* and *E. multilocularis* overlap in North America.[2] The intermediate hosts for the larval stages of *Echinococcus* include sheep, swine, cattle, wild cervids (primarily moose and caribou) and other species such as

kangaroos in Australia and a variety of prey species in Africa. The sylvatic cycle between wild canids and wild cervids can overlap into a pastoral cycle between domestic dogs (and occasionally cats) and domestic ungulates. Man may become infected as an intermediate host primarily through contact with domestic dogs. Cystic echinococcosis in people in North America occurs throughout Canada and Alaska due to the practice of feeding wild cervid entrails to domestic dogs. A nidus of infection in the 1970s occurred among sheepherders in the western US (primarily California and Utah) presumably because of similar practices. A few cases continue to be diagnosed among Native Americans in New Mexico.[3] Recombinant vaccines have been developed for *E. granulosus* intermediate stages in cattle and sheep.[4]

The intermediate or larval stage of *E. granulosus* is called the unilocular hydatid cyst. This stage begins as a small cyst and slowly grows, usually reaching a size of a few centimeters in cattle and sheep.[2] In longer lived hosts, such as human beings (and, in this case, an aged mountain goat), cysts may grow to several centimeters. Generally, unilocular hydatid cysts do not cause clinical disease, although they may inhibit organ function by compression in some human patients. This is in contrast to the alveolar hydatid cyst, intermediate stage of *E. multilocularis*, which grows by infiltration of surrounding tissue and is often fatal.

Unilocular hydatid cyst has a typical gross and histological appearance as seen in this case. The smooth wall of the cyst is made up of a layer of host connective tissue adjacent to the hyalinized cyst wall. Numerous small scolices, termed protoscolices are clustered in brood capsules. The “hydatid sand” is made up of scolices that break free and float in the hydatid fluid. Calcareous corpuscles, characteristic of cestodes, are present in the cyst lining.[5]

Cystic echinococcosis has not previously been reported in wild ungulates in Idaho; this parasite may have arrived with re-introduced wolf populations from Canada.

AFIP Diagnosis: Lung: Hydatid cyst, with mild interstitial fibrosis, mountain goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides a concise summary of Echinococcosis. While both *E. granulosus* and *E. multilocularis* form hydatid cysts composed of a bladder with myriad protoscolices that are often clustered into brood capsules, *E. granulosus* forms unilocular hydatid cysts and *E. multilocularis* forms multilocular hydatid cysts. Other cystic larval cestodes include the following:[5]

1. **Cysticercoids** – very small larvae, with a tiny bladder and a scolex that is surrounded by parenchymous arms
2. **Cysticercus** – bladder with a single inverted neck and scolex; scolex may be armed, and always has four suckers
3. **Coenurus** – resembles Cysticercus but has more than one scolex

Solid-bodied cestode larvae include the following:[5]

1. **Plerocercoid** – lacks suckers
2. **Tetrathyridium** – has suckers

Below is a useful chart from Veterinary Pathology summarizing the features of some important tapeworms.[6]

Features of some important tapeworms

Name of adult tapeworm	*Definitive host*	*Intermediate stage Name/type of larval cestode*	*Intermediate hosts*	*Anatomic Site*
Taenia saginata	Humans	Cysticercus (bovis)	Cattle	Heart, skeletal muscle
T. solium	Humans	Cysticercus (cellulosae)	Swine	Muscle, heart, viscera
Echinococcus granulosus	Humans, dog, fox, wolf, jackal	Echinococcus (granulosus)	Humans, cattle, swine, sheep, deer, horse, moose, etc.	Liver, lungs, other viscera
E. multilocularis	Humans, dog, fox, wolf, jackal	Echinococcus (multilocularis)	Humans, cattle, swine, sheep, deer, horse, moose, etc.	Liver, lungs, other viscera
Taenia hydatigena	Dog	Cysticercus (tenuicollis)	Squirrels, cattle, wild ruminants, sheep, goats, swine	Peritoneal cavity
T. ovis	Dog, fox, wolf, coyote	Cysticercus (ovis)	Sheep, goats	Muscles
T. pisiformis	Dog, cat, fox, wolf	Cysticercus (pisiformis)	Rabbit, squirrel, other small rodents	Liver capsule, peritoneum
T. taeniaeformis (syn.: *T. crassicollis*)	Cat, dog, fox	Cysticercus (fasciolaris)	Rats, mice, rabbits	Liver
T. (*Multiceps*) *multiceps*	Dog	Coenurus (cerebralis)	Sheep, goats	Brain, spinal cord
T. serialis	Dog, other carnivores	Coenurus (serialis)	Rabbit	Subcutis
Diphyllobothrium latum	Humans, bear, dog, cat, pig, fox	Procercoid and plerocercoid	Microcrustacea, freshwater fish	Muscles
Spirometra (*Diphyllobothrium*) *mansonoides*	Dog, cat	Procercoid and plerocercoid (sparganum)	Snakes, humans, monkeys, dog	Peritoneal cavity
Mesocestoides corti, *M. lineatus*	Dog, cat, wild carnivore, humans	Tetrathridium	Mites and wild rodents, dogs, cat, other mammals, reptiles	Peritoneal and pleural cavities, liver, lung, other organs
Dipylidium caninum	Dog, cat	Cysticercoid	Dog flea, biting lice	
Moniezia expansa	Sheep, goats, cattle	Cysticercoid	Mites: *Galumna*, *Scherloribates*, *Scutovertex minutus*	
M. benedeni	Sheep, goats, cattle	Cysticercoid		
Anoplocephala magna	Equines	Cysticercoid	Mites of family *Oribatidae*	
A. perfoliata	Equines	Cysticercoid	*Oribatidae*	Intestinal tract
Paranoplocephala mammillana	Equines	Cysticercoid	*Oribatidae*	
Thysanosoma actinioides	Sheep, cattle, goats, deer	Cysticercoid	*Oribatidae*	
Spirometra mansonoides	Cat	Sparganum	Snakes, rodents	Connective tissue

Dr. Chris Gardiner, AFIP consultant in veterinary parasitology, adds, “This is a good case. All the characteristics we would want to see of the organism are present. The laminated cyst wall is well represented, the germinal membrane, the protoscolices, the calcareous corpuscles, the hooklets are all present…another interesting fact is that the hooklets are acid fast and sometimes when the cysts are old and ruptured you can find the hooklets out in the tissue and when you do an acid fast stain they are nice and red!”

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References:

1. Samuel WM, Pybus MJ, Kocan AA: Parasitic Diseases of Wild Mammals, 2nd ed., pp. 174-177. Iowa State University Press, Ames, Iowa, 2001
2. Bowman DD: Georgi’s Parasitology for Veterinarians, 7th ed., pp. 134-138. WB Saunders, Philadelphia, Pennsylvania, 1999
3. Moro P, Schantz PM: Cystic echinococcosis in the Americas. Parasitol Int 55: S181-S186, 2006
4. Lightowlers MW, Gauci CG: Vaccines against cysticercosis and hydatidosis. Vet Parasitol 101:337-352, 2001
5. Gardiner CH, Poynton SL: An Atlas of Metazoan Parasites in Animal Tissues, Armed Forces Institute of Pathology, Washington, D.C., pp. 50, 53, 1999
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 654. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 27
CONFERENCE 7 / CASE III – A06-18116 (AFIP 3030466)

Signalment: 3-month-old, female, pug-cross, *Canis lupus familiaris*

History: Depression, nasal discharge and dyspnea

Gross Pathology: An 11-week-old female Pug puppy with a history of depression, nasal discharge, and dyspnea was submitted for necropsy in good body condition with a moderate amount of cloudy white, partially crusting exudate along the nares and the eyelid conjunctiva. Autolysis was minimal. The lungs were diffusely heavy and wet, and remained inflated. All four foot pads were markedly thickened with crusts.

Laboratory Results:
Fluorescent Antibody Tests:
1. Canine Distemper: Positive
2. PI3: Negative

Histopathologic Description: The following tissues were examined microscopically: cerebrum, hippocampus, cerebellum with brain stem, nasal mucosa and turbinates, trachea, lung, liver, kidneys, urinary bladder, spleen, mesenteric lymph node, heart, jejunum, stomach, and footpads (all four feet).

Foot pads (all four feet): There is marked compact orthokeratotic hyperkeratosis. Multifocally and predominantly within the superficial layers of the stratum spinosum, numerous keratinocytes exhibit varying degrees of ballooning degeneration/spongiosis, with 1-3 round to ovoid, 2-4 um-wide brightly eosinophilic intracytoplasmic viral inclusion bodies. Rarely keratinocytic syncytial cells with multiple nuclei are present (Note: Not observed in all slides submitted). Occasionally, similar viral inclusion bodies are present in basal cells. There is mild to moderate irregular acanthosis with thickened rete ridges extending into the superficial dermis. Within the superficial dermis, there is a mild perivascular inflammatory cellular infiltrate chiefly consisting of lymphocytes and macrophages.

Contributor's Morphologic Diagnoses:
Distemper virus infection, with:
- Necrotizing encephalitis, multifocal, severe, with gitter cells and I/C viral inclusion bodies.
- Necrotizing bronchopneumonia, lymphohistiocytic, subacute, severe, multifocal, with I/C viral inclusion bodies.
- Mild multifocal necrotizing enteritis with I/C viral inclusion bodies.
- Orthokeratotic hyperkeratosis of foot pads, moderate, quadrilateral, with keratinocytic ballooning degeneration, rare syncytia and I/C viral inclusion bodies
- Nasal thrush

Contributor's Comment: Canine Distemper is a disease of certain species of terrestrial carnivores. In recent years the host range of this disease appears to have widened which includes wild felids like cheetahs and lions. Similarly, the disease outbreaks have been reported in seals, weasels and ferrets. In a typical disease outbreak, the virus spreads through aerosol and localizes in lymphoid organs (tonsils, bronchial lymph nodes, thymus, spleen and retropharyngeal lymph nodes). Within a week followed by aerosol exposure, viremia is established and mononuclear cells of blood can carry virus into multiple organs. Poor humoral response by the host predisposes for severe multisystemic disease. Mild or inapparent disease and recovery is seen in animals with adequate humoral response.[1]

Clinical signs and organs affected depend on strain of the virus, environmental conditions, age and immune status of the animal. It is interesting to note that puppies with vesicular and pustular dermatitis rarely develop CNS lesions. On the contrary, dogs with nasal and digital hyperkeratosis usually have neurologic lesions as it has been observed in the present case submission.[1]

Digital hyperkeratosis (hard pad disease) is an uncommon manifestation of the disease process. It is characterized by severe orthokeratotic hyperkeratosis with eosinophilic

intracytoplasmic viral inclusion bodies in footpad epithelium.[1] The disease mechanism of hyperkeratosis in CDV is poorly understood. However, a recent study involving naturally infected dogs with CDV indicated that selective infection of keratinocytes in the stratum spinosum might be the key event in development of hard pad disease. Furthermore, this report also indicated that presence of CDV specific-inclusion bodies and ballooning degeneration in footpad epidermis may not be present in hard pad disease.[2] Conversely, in dogs with experimental inoculation of virulent CDV strains can exhibit CDV antigen and viral specific mRNA in all layers of footpad epidermis.[3] Findings in another experimental study concluded that accelerated proliferation of keratinocytes in hard pad disease may be due to interference of CDV in reducing translocation of p65 (a component of nuclear factor (NF)-kappa B transcription factor) into the nucleus.[4]

AFIP Diagnosis: Footpad: Epidermal hyperplasia, diffuse, moderate, with orthokeratotic and parakeratotic hyperkeratosis, rare syncytia and numerous epithelial eosinophilic intracytoplasmic inclusion bodies, Pug (*Canis familiaris*), canine.

Conference Comment: Canine distemper virus (CDV) is a member of the genus Morbillivirus in the Paramyxoviridae family. This family is composed of large (100-300 nm), pleomorphic, single-stranded RNA viruses. Members of the genus Morbillivirus include measles virus, rinderpest virus, peste-des-petits-ruminants virus, phocine morbillivirus, and cetacean morbillivirus.[5,6,7]

Canine morbillivirus infects a wide range of species including Canidae (e.g., dog, dingo, fox, coyote, wolf, jackal), Procyonidae (e.g., raccoon, coati, kinkajou, panda), Mustelidae (e.g., ferret, mink, badger, weasel, otter), Viveridae (Binturong), marine mammals, and wild Felidae.[5,6,7]

CDV is pantropic, preferentially infecting lymphoid, epithelial, and nervous cells, and can cause a variety of clinical signs in dogs with an inadequate antibody response. In addition to digital hyperkeratosis, CDV can target the lungs directly causing a viral pneumonia or indirectly render the lungs susceptible to secondary bacterial infections (e.g. *Bordetella bronchiseptica*, mycoplasma) due to its immunosuppressive effects.[6,8] Viral infection of ameloblasts during enamel formation results in segmental enamel hypoplasia.[6,9] Dogs that survive the disease may develop late complications such as demyelinating encephalomyelitis and hyperkeratosis of the footpads (“hard pad” disease) and nose. Other complications include systemic toxoplasmosis and sarcocystosis.[6]

Microscopically, eosinophilic intranuclear and/or intracytoplamic inclusion bodies can be seen in epithelial cells of many tissues, but are most prominent in epithelium of the urinary bladder, renal pelvis, stomach, and lung.[6]

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References:

1. Greene CE, Appel MJ: Canine Distemper. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed., pp. 25-41. Saunders Elsevier, St. Louis, Missouri, 2006
2. Koutinas AF, Baumgartner W, Tontis D, Polizopoulou Z, Saridomichelakis MN, Lekkas S: Histopathology and immunohistochemistry of canine distemper virus-induced footpad hyperkeratosis (Hard Pad disease) in dogs with natural canine distemper. Vet Pathol 41:2-9, 2004
3. Grone A, Doherr MG, Zurbriggen A: Canine distemper virus infection of canine footpad epidermis. Vet Dermatol 15:159-167, 2004
4. Friess M, Engelhardt P, Dobbelaere D, Zurbriggen A, Grone A: Reduced nuclear translocation of nuclear factor (NF)-kappaB p65 in the footpad epidermis of dogs infected with distemper virus. J Comp Pathol 132:82-89, 2005
5. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 617-622. Academic Press, Inc., San Diego, California, 1993
6. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 541-542. Mosby Elsevier, St. Louis, Missouri, 2007
7. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 310-315. Williams & Wilkins, Baltimore, Maryland, 1997
8. 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. 822. Mosby Elsevier, St. Louis, Missouri, 2007
9. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 312. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 28
CONFERENCE 7 / CASE IV – S0507399 (AFIP 3024118)

Signalment: 12-year-old, female, Paso Fino, *Equus caballus*, domestic horse

History: Horse was euthanized for chronic laminitis.

Gross Pathology: A 0.8 cm diameter, fluctuant, tan, exophytic mass, with multifocal hemorrhagic areas was present within the anterior pituitary gland.

Histopathologic Description: Within the pars distalis is an expansile, nonencapsulated, well-demarcated, ovoid mass, which compresses the adjacent normal parenchyma. The neoplastic cells are arranged in a sheet of closely-packed, polygonal cells supported by scant fibrovascular stroma. Cells have abundant cytoplasm, distinct cell borders and contain large numbers of densely packed eosinophilic granules. Nuclei are singular, round to ovoid, usually with coarsely clumped chromatin. Occasional

agranular cells of uncertain histogenesis are present. No mitotic activity, vascular/lymphatic invasion or local metastases are present.

Contributor's Morphologic Diagnosis: Pituitary gland, pars distalis: Acidophil adenoma

Contributor's Comment: Acidophil adenomas are benign neoplasms derived from granulated acidophils within the pars distalis of the pituitary gland and have previously been described in the rat, dog, sheep, cat, rabbit, and goat.[1-7] Acidophils can be divided functionally into somatotrophs (which produce growth hormone) and mammotrophs (which produce prolactin).[8,9] Here, we report an acidophil adenoma in a Paso Fino horse.

In this case, the mare had grossly evident mammary enlargement in the absence of recent pregnancy or parturition, suggesting an endocrinopathy. Teat enlargement was reported in New Zealand White rabbits with prolactin-secreting acidophil adenomas.[6] Udder enlargement and inappropriate lactation associated with acidophil adenoma was also reported in two goats. Interestingly, both goats also had concurrent pheochromocytoma and cystic endometrial hyperplasia.[7] In cats, acidophil adenomas are often associated with hypersecretion of somatotropin, resulting in acromegaly.[4,5] In the dog, hypersecrection of somatotropin resulting in diabetes mellitus was reported.[2]

AFIP Diagnosis: Pituitary gland, pars distalis: Adenoma, Paso Fino (*Equus caballus*), equine.

Conference Comment: This case is unusual in that the adenoma is within the pars distalis. Most equine pituitary gland adenomas occur in the pars intermedia of older female horses. They are often large neoplasms that extend out of the fossa hypophysialis and severely compress the overlying hypothalamus. The clinical syndrome in horses with adenomas of the pars intermedia is distinctly different from that of Cushing's disease seen in dogs, cats, and humans, and is characterized by polyuria, polydipsia, polyphagia, muscle weakness, somnolence, intermittent hyperpyrexia, generalized hyperhidrosis, and hirsutism. Horses with larger neoplasms often have insulin-resistant hyperglycemia most likely due to down regulation of insulin receptors on target cells secondary to chronic excessive intake of feed and hyperinsulinemia. The hypothalamus is the primary homeostatic regulatory center for body temperature, appetite, and cyclic shedding of hair. The clinical syndrome is considered to be primarily due to deranged hypothalamic function secondary to compression by the neoplasm. Additionally, some adenomas of the pars intermedia are endocrinologically active.

In the dog, functional pituitary adenomas usually arise in the pars distalis, but can also arise in the pars intermedia, and are most likely derived from ACTH-secreting corticotrophs. Adult to aged Boxers, Boston Terriers, and Dachshunds are predisposed. Bilateral adrenocortical hyperplasia and hyperfunction result in pituitary

origin Cushing's disease. Larger tumors may compress the posterior pituitary and infundibular stalk leading to diabetes insipidus.[10,12]

Growth hormone secreting acidophil adenomas have been most frequently described in cats and have also been reported in sheep. In adults, whose epiphyses have closed, the bones grow heavier and thicker, producing large hands, feet, and skull bones (acromegaly). Acromegaly results from the production of somatomedins which stimulate cartilage formation. Excessive growth hormone secretion also leads to diabetes mellitus due to interference with tissue glucose uptake and insulin resistance.[12]

Prolactin-producing pituitary adenomas in rabbits result in hormone-responsive dysplastic changes in the mammary glands. Microscopically, dilated cystic ducts are lined by flattened to cuboidal epithelium with papillary projections into cystic areas.[13]

Pituitary adenomas commonly occur in aged male and female rats, especially in certain strains (e.g. Sprague-Dawley, Wistar). Prolactin-producing tumors are the most common type and most frequently arise in the pars distalis. Attempts have been made to correlate prolactin-producing pituitary tumors with an increased incidence of mammary fibroadenomas, but this had not been resolved to date.[13]
Histologically, pituitary adenomas are composed of polygonal to spindle cells arranged in solidly cellular or sinusoidal patterns. Neoplastic cells have round to oval, vesiculate nuclei, one to two small nucleoli, and a moderate to abundant amount of granular cytoplasm. The mitotic rate is low. Neoplastic cells may be immunoreactive for ACTH, thyroid stimulating hormone (TSH), luteinizing hormone (LH), _-endorphin, and _-lipoproteins. In horses, cells stain strongly for (POMC), _- and _-melanocortin, _-lipoprotein and _-endorphin with a weak reactivity for ACTH and are presumably melanotrophs not corticotrophs.[10,11,12]

Pituitary carcinomas are similar to pituitary adenomas; however, in contrast to pituitary adenomas, pituitary gland carcinomas are rare and exhibit extensive invasion along the base of the brain into the sphenoid bone, vascular invasion and/or metastasis. The mitotic index and cellular atypia are often greater in pituitary carcinomas than adenomas.[10,11]

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References:

1. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, 2nd ed., p. 162. Iowa State University Press, Ames, Iowa, 2001
2. Van Keulen LJM, Wesdorp JL, Kooistra HS: Diabetes mellitus in a dog with a growth hormone-producing acidophilic adenoma of the adenohypophysis. Vet Pathol 33:451-453, 1996
3. Olson DP, Ohlson DL, Davis SL, Laurence KA: Acidophil adenoma in the pituitary gland of a sheep. Vet Pathol 18(1):132-135, 1981

4. Lichtensteiger CA, Wortman JA, Eigenmann JE: Functional pituitary acidophil adenoma in a cat with diabetes mellitus and acromegalic features. Vet Pathol 23:518-521, 1986
5. Peterson ME, Taylor RS, Greco DS, Nelson RW, Randolph JF, Foodman MS, Moroff SD, Morrison SA, Lothrop CD: Acromegaly in 14 cats. J Vet Intern Med 4(4):192-201, 1990
6. Lipman NS, Zhi-Bo Z, Andrutis KA, Hurley RJ, Fox JG, White HJ: Prolactin-secreting pituitary adenomas with mammary dysplasia in New Zealand white rabbits. Lab Anim Sci 44(2):114-120, 1994
7. Miller CC, Williamson LH, Miller-Liebl DM, Thompson FN: Lactation associated with acidophilic pituitary adenoma, pheochromocytoma, and cystic endometrial hyperplasia in two goats. J Amer Vet Med Assoc 210:378-381, 1997
8. Capen CC: Tumors of the endocrine glands. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 620-622. Iowa State Press, Ames, Iowa, 2001
9. Capen CC: The endocrine glands. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 273, 282-283. Academic Press, Inc., San Diego, California, 1993
10. La Perle KMD, Capen CC: Endocrine system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 709-712. Mosby Elsevier, St. Louis, Missouri, 2007
11. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Histological Classification of Tumors of the Nervous System of Domestic Animals, Second Series, vol. V, p. 33. Armed Forces Institute of Pathology, Washington, D.C., 1999
12. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 1230-1231. Williams & Wilkins, Baltimore, Maryland, 1997
13. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., pp. 162-165, 303-304. Iowa State University Press, Ames, Iowa, 2001

SLIDE 29
CONFERENCE 8 / CASE I – H-7645 (AFIP 3031560)

Signalment: ~30-year-old, male, rhesus macaque (*Macaca mulatta*), nonhuman primate

History: Submitted for diagnostic workup were heart and lung tissues. Per the clinician, the animal was found lethargic with labored breathing and swelling of the ventral neck. Auscultation revealed harsh lung sounds and audible wheezing. There was a foul odor from the oral cavity with mucus and purulent material. Symptomatic treatment was unsuccessful and the monkey was euthanized.

Gross Pathology: At necropsy, the clinician found a pocket of purulent material in the ventral neck. The lungs were congested and most lobes had fibrous adhesions to the thoracic wall. The dorsal surfaces of the lungs contained several yellow/tan, 0.5-1.5 cm

oval and slightly depressed foci that on sectioning were cavitated and gritty. Similar areas were found within the lung parenchyma. A small lung lobe was atelectatic, dark red, and firm. Coronary disease was suspected in the heart.

Histopathologic Description: Lung sections are characterized by dilated, chronically inflamed airways; the presence of parasites; and, pleural inflammation and fibrosis. Affected airways vary from 3-5 mm in width/length with lumens up to 2000 _ in diameter; have thickened walls (up to 500 _) with mixed infiltrates of mononuclear cells (lymphocytes, macrophages, plasma cells), segmented cells (eosinophils, neutrophils), and/or macrophages containing golden-brown, anisotropic pigment; and, often contain the profile of a parasite (with jointed appendages) consistent with lung mites (presumably genus *Pneumonyssus*). Focally, this process involves the pleural surface with chronic inflammation and fibrosis. Peripheral to the latter, the lung is congested, edematous, and occasional fibrin thrombi are observed within blood vessels.

Contributor's Morphologic Diagnosis: Lung: Bronchiolitis/bronchiectasis, chronic-active, multifocal, severe with lung mites and pleural inflammation and fibrosis.

Contributor's Comment: Gross and microscopic lesions are consistent with the presence of lung mites (pulmonary acariasis). Although not confirmed in this case, lung mite infection in the rhesus and other macaques is usually attributed to *Pneumonyssus simicola*.[1] In addition to the severe chronic-active inflammatory process associated with the mites, adjacent lung tissues had areas of acute, severe pneumonia and vasculitis and sections of ventricular myocardium had mild cardiomyopathy (slides not submitted). Taken together, the lung lesions suggest a severe lung infection with secondary pneumonia and vascular problems. The role, if any, of the heart lesion could not be determined.

To our knowledge, lung mites are considered an uncommon finding in today's rhesus monkey colonies. Reported to occur in up to 100% of wild or imported rhesus monkeys, import restrictions and the routine use of anthelminthics (e.g., ivermectin) in colony health programs probably explain the lack of reports in recent years.[1] Also, the fact that the infection is usually subclinical and not a significant factor in colony management may account for the low reporting.

While subclinical disease is most common, secondary and severe infections can occur.[1] However, severely infected monkeys may not be detected until necropsy or may only develop coughing and dyspnea.[1,2] Exceptions to the latter scenarios in the rhesus are complications such as pneumothorax and pulmonary arteritis.[1] In other macaques, lung mite-induced dyspnea and pneumatocele have been reported in the pig-tailed macaque (*Macaca nemestrina*).[3,4] Massive infections leading to death have also been reported in the pig-tailed monkey, the douc langur (*Pygathrix nemaeus nemaeus*), and proboscis monkey (*Nasalis larvatus orintalis*).[1] Although we had access to only limited tissues from this animal, we believe this case may represent the exceptional scenario where lung mite infection and associated complications lead to death.

AFIP Diagnosis: Lung: Bronchiolitis, granulomatous and eosinophilic, multifocal, marked, with bronchiolectasis, abundant anisotropic yellow-brown mite pigment/excrement, and adult mites, etiology consistent with *Pneumonyssus simicola*, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: Conference participants agreed that the lung mites in this case were most likely *Pneumonyssus simicola*. Several species of mites in the genus *Pneumonyssus* infect the respiratory system of Old World primates. *Pneumonyssus simicola* is recognized as the genus and species found in rhesus monkeys.[1]

The exact life cycle is unknown. It is believed that the entire life cycle can be completed in the lungs as all stages of *P. simicola* (adults, eggs, larvae) can be found there. Transmission is most likely by direct contact. Mites feed on host erythrocytes, lymph, and pulmonary epithelial cells. Infection rarely causes clinical disease in immunocompetent animals. Secondary infections may occur due to altered bronchiolar epithelium and impaired mucociliary clearance.[1] As pointed out by the contributor, clinical signs vary from none to clinically significant infections with heavy infestations resulting in coughing, sneezing, and dyspnea.

Typical gross findings include discrete, 1-7 mm in diameter, yellow or gray cystic foci ("mite houses") present throughout the lung parenchyma.[1]

Typical light microscopic findings include arthropods with a chitinized cuticle, jointed appendages, striated musculature, a body cavity, digestive tract, and reproductive structures; golden brown refractile mite pigment; granulomatous bronchiolitis and peribronchiolitis; alveolar emphysema; bronchiolar smooth muscle hyperplasia; and interstitial fibrosis.[1,5]

Conference participants briefly reviewed other parasites of the respiratory system to include those listed below.[1,6,7,8, 9]

NASAL PASSAGE/SINUS

1. *Oestrus ovis* (nasal bot) – sheep
2. *Linguatula serrata* (pentastome) – dogs
3. *Pneumonyssus caninum* (arthropod) – dogs
4. *Anatrichosoma* sp. (nematode) – nonhuman primates
5. *Halicephalobus deletrix* (nematode) – horses
6. *Syngamus laryngeus* (nematode) – cat, cattle
7. *Cephenemyia* sp. (arthropod) – wild cervids
8. *Rhinophagia* sp. (arthropod) – Old World monkeys

COMMON LUNGWORMS

1. *Metastrongylus* sp. – (bronchi, bronchioles) swine
2. *Protostrongylus rufescens* – (bronchioles) sheep, goats

3. *Muellerius capillaris* – (alveoli) sheep, goats
4. *Filaroides osleri* – (trachea, bronchi) dogs
5. *Filaroides milksi/hirthi* – (bronchi, bronchioles, alveoli) dogs
6. *Capillaria aerophila* – (trachea, bronchi) dogs, cats, foxes
7. *Syngamus trachea* – (trachea) birds
8. *Angiostrongylus vasorum* **–** (pulmonary arteries) dogs, foxes
9. *Angiostrongylus cantonensis* – (pulmonary arteries and capillaries) rats
10. *Dictyocaulus filaria* – (bronchi, bronchioles) sheep, goats
11. *Dictyocaulus viviparus* – (bronchi, bronchioles) cattle
12. *Dictyocaulus arnfieldi* – (bronchi, bronchioles) horses, donkeys
13. *Aelurostrongylus abstrusus* – (bronchioles, alveolar ducts) cats
14. *Otostrongylus circumlitis* – (bronchi, bronchioles) pinnipeds
15. *Parafilaroides decorus* – (bronchi, bronchioles) pinnipeds

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References:

1. Osborn KG, Lowenstine LJ: Respiratory Diseases. In: Nonhuman Primates in Biomedical Research Diseases, eds. Bennett BT, Abee CR, Henrickson R, pp. 302-303. Academic Press, San Diego, California, 1998
2. Hiraoka E, Sato T, Shirai W, Kimura J, Nogami S, Itou M, Shimizu K: A case of pulmonary acariasis in lung of Japanese macaque. J Vet Med Sci 63**:**87-89, 2001
3. Kim JCS: Diagnostic exercise: macaque with dyspnea. Lab Anim Sci 38**:**77-78, 1988
4. Gillett CS, Ringler DH, Pond CL: Pneumatocele in a pig-tailed macaque (*Macaca nemestrina*). Lab Anim Sci 34**:**91-93, 1984
5. Gardiner CH, Poynton SL: An Atlas of Metazoan Parasites in Animal Tissues, pp. 56-58. Armed Forces Institute of Pathology, Armed Registry of Pathology, Washington, D.C., 1999
6. L_pez Alfonso: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 481-483, 491. Mosby Elsevier, St. Louis, Missouri, 2007
7. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 897. Mosby Elsevier, St. Louis, Missouri, 2007
8. Foreyt WJ: Veterinary Parasitology Reference Manual, 4th ed., pp.107, 141, 172. Washington State University College of Veterinary Medicine, Pullman, Washington, 1997
9. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p.632. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 30
CONFERENCE 8 / CASE II – 05-0314 (AFIP 3028754)

Signalment: 4-week-old Labrador Retriever, canine

History: This puppy was unable to nurse, unable to defecate and had difficulty in breathing for 2 days. The clinical signs progressed to generalized flaccid paresis. The puppy was treated by antibiotics and prednisone with no improvement and then died one week after the appearance of the clinical signs. All other puppies in litter were normal.

Gross Pathology: No significant lesions were described by the referring veterinarian who performed the necropsy; however, the submitted formalin-fixed muscles had patchy white streaks.

Laboratory Results:
1. Immunohistochemistry for *Toxoplasma* spp. on muscle sections was positive.
2. Pure culture of hemolytic *E.coli* was isolated from the spleen and lung of this puppy.

Histopathologic Description: Affecting 80% of the submitted skeletal muscle sections, there was myofibrillar necrosis and fibrosis with a massive multifocal to coalescing infiltration of lymphocytes, histiocytes, plasma cells, and fewer neutrophils. Myriads of protozoal zoites (2-3 microns with no visible cyst wall, and present in aggregates of 4 to >100 zoites) were present amid the inflammatory cells. Both the liver and heart had random multifocal areas of necrosis characterized by cellular loss and aggregation of neutrophils. No significant lesions were present in the sections examined from lung, thymus, lymph node, kidney, spleen, and gastrointestinal tract. Brain and spinal cord were not available for examination.

Contributor's Morphologic Diagnosis: Skeletal muscles: Myositis, lymphohistiocytic, necrotizing, with muscle regeneration and myriad tachyzoites and protozoal cysts.

Contributor's Comment: The differential diagnoses for protozoal skeletal myositis in dogs include *Neospora caninum*, *Toxoplasma gondii*, and *Sarcocystis canis*. The immunohistochemistry was positive when muscle sections from the current case were stained for *Toxoplasma* sp. Toxoplasmosis caused by *Toxoplasma gondii* is one of the most common protozoal diseases affecting domestic animals. Felids are the only definitive host. Felids and other mammals act as intermediate hosts. Infective stages for both the final and intermediate hosts are the oocysts, which are produced only by felids, tachyzoites and tissue cysts are present in different organs of the intermediate host particularly the skeletal muscles.[1] The infection is maintained between intermediate hosts by facultative homoxenous transmission without the need of oocyst production. Transplacental transmission is important in cats, sheep, and goats; however, the importance of transplacental infection in dogs is still unknown. Other minor modes of transmission include transfusion of fluids or transplantation of organs. Disseminated toxoplasmosis is a rare primary disease in adult dogs and is commonly seen in puppies and immunosuppressed adults and is characterized by lymphoplasmacytic to histiocytic inflammation virtually in any organ but particularly in brain, skeletal muscles, heart, and liver.[2] In addition to toxoplasmosis, the submitted puppy had a colisepticemia.

AFIP Diagnosis: Skeletal muscle: Myositis, pyogranulomatous, diffuse, marked, with myocyte degeneration, necrosis, regeneration, and numerous protozoal cysts, Labrador Retriever (*Canis familiaris*), canine.

Conference Comment: Most infections with *Toxoplasma* go unrecognized. Clinical manifestations of disease occur most frequently in young animals or the aged. In dogs, toxoplasmosis is often triggered by immunosuppression, such as that caused by canine distemper virus. Necrosis is the predominant histologic lesion. In disseminated infections lesions include multifocal necrotizing interstitial pneumonia, focal necrotizing hepatitis, myocarditis, splenitis, myositis, encephalitis, and ophthalmitis.[3,4]

Toxoplasma gondii can infect a wide variety of animals. New World monkeys and Australian marsupials are the most susceptible, whereas Old World monkeys, rats, cattle, and horses are highly resistant.[5] The most common expressions of *Toxoplasma* in cats are pneumonia, encephalitis, and pancreatitis. In sheep and goats, toxoplasmosis most commonly causes necrotizing cotyledonary placentitis, with characteristic 1-2 mm diameter white foci of inflammation, necrosis, and mineralization.[4,6] In humans, toxoplasmosis is a common complication in immunosuppressed patients and can cause disseminated and often fatal parasitemia in the human fetus.[2]

Conference participants discussed *Neospora caninum* as a differential diagnosis for this lesion. *Neospora* is nearly identical in appearance to *Toxoplasma* and induces similar lesions, particularly in the central nervous system (CNS). *Neospora* has a thicker cyst wall and cysts are restricted to the CNS whereas *Toxoplasma* cysts are thin-walled and can be found in multiple tissues. Although there are morphologic differences between the two protozoa, differentiation by light microscopy is unreliable and electron microscopy or immunohistochemistry are required. Ultrastructurally, *Toxoplasma* tachyzoites are within parasitophorous vacuoles and have 4-6 rhoptries whereas *Neospora* tachyzoites do not develop within parasitophorous vacuoles and have numerous rhoptries.[4]

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References:
1. Dubey JP: Toxoplasmosis in Cats and Dogs. 30th World Congress of the World Small Animal Veterinary Association. Available at: http://www.vin.com/proceedings/Proceedings.plx?CID=WSAVA2005&PID=10951&O=Generic

2. Lappin MR: Protozoal and miscellaneous infections. In: Textbook of Veterinary Internal Medicine, eds. Ettinger SJ, Feldman EC, 6th ed., pp. 639-642. Elsevier Saunders, St. Louis, Missouri, 2005
3. L_pez A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 546. Mosby Elsevier, St. Louis, Missouri, 2007
4. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 555-567. Williams & Wilkins, Baltimore, Maryland, 1997
5. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 894-896. Mosby Elsevier, St. Louis, Missouri, 2007
6. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1300. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 31
CONFERENCE 8 / CASE III – 04-0147-1 (AFIP 2936168)

Signalment: 8-month-old, male, Bengali, cat

History: This animal with growth delay (1.8 Kg on presentation time) presented with sudden prostration and anorexia. Clinical neurological exam revealed a severe proprioceptive deficiency with suspicion of central nervous system or cervical cranial affection. The cat was euthanized four days later and necropsy performed.
Gross Pathology: Brain: Severe dilatation of lateral ventricles (stars) and dilatation of the fourth ventricle (arrow) associated with thickening of meninges in the ventral surfaces of the brain (arrow head).

Laboratory Results: Magnetic Resonance imaging analysis: Severe hydrocephalus.
CSF analysis: Pleocytosis with predominance of polynuclear neutrophils.
Serological exam for FIP: Positive at 1/16000.
Hemogram: Leucocytosis (70% polynuclear neutrophils; 30% lymphocytes).

Contributor's Morphologic Diagnosis: Cortex sections at the level of lateral ventricles: Severe pyogranulomatous periventriculitis characterized by a partial effacement of ependymal lining by a heavy infiltrate of histiocytes and lymphocytes associated with reactive astrocytes ("gemistocytes"). This lesion was closely associated with perivascular cuffing in the adjacent neuroparenchyma.

Slides are from various localizations: Size of the ventricular cavities varies with slides. Furthermore, some slides present edematous choroid plexus fragments with granulomatous infiltrate.

Contributor's Comment: Feline infectious peritonitis (FIP), first described in 1963 as a chronic fibrinous peritonitis, was identified in domestic as well as in wild *felidae* all over

the world. This disease is due to a coronavirus that affects most frequently cats younger than 3 years of age.[1] Recent evidence supports the idea that FIP virus has evolved as a mutation of FECV (Feline Enteric Coronavirus), a common and not serious disease in the cat.[1,3]

FIP has been subdivided into two forms:
- the classical effusive form with peritoneal and/or pleural fluid effusions
- the dry form characterized by pyogranulomatous lesions in various visceral organs.

Clinical signs of the effusive form are chronic fever, anorexia, weight loss, depression and abdominal distention. The dry form is more chronic and frequently induces ocular signs and 25% of cats develop a neurologic form of the disease with spastic paresis, ataxia, nystagmus and balance loss.[2,4]

Gross lesions of FIP in the CNS may be subtle with thickening and opacity of meninges in the ventral surfaces of the brain and ventricular dilatation, usually of the fourth ventricle and less frequently lateral ventricles. Histopathologic lesions consist of meningitis, ependymitis, periventriculitis, choroiditis with dense infiltrate of lymphocytes, plasma cells, neutrophils and macrophages. Meningitis is generally more severe on the ventrocaudal surfaces of the brain and the inflammation may extend into cranial nerve roots as well as in the neuropil with perivascular cuffs and glial nodule formation. In the periventricular area inflammation is accompanied by reactive astrocytes and accumulation of cellular debris may cause obstruction of the cerebrospinal fluid flow and then hydrocephalus.[2,4] FIP meningoencephalitis is one of the most common inflammatory disorders of the CNS in the cat.[4]

Physiopathology :
Once ingested or inhaled, FIP virus replicates in macrophages that travel to regional lymph nodes; a viremia results of active replication allowing virus-laden macrophages to be deposited in endothelium of blood vessels. If the cat cannot develop a cell mediated immune response, the effusive form develops accompanied by pyogranulomatous vasculitis. A partial cell mediated immune response can also occur allowing a slower viral replication and formation of classical granulomas observed in the dry form of the disease.[1]

The way of entry of the virus in the CNS is probably hematogenous via macrophages. There is no evidence of replication of the virus in vascular basement membranes or in the cells of the neuropil.[2] Mechanisms of the disease are primarily immune mediated, involving humoral and cell immunity with massive complement activation.[3,2] Apparently some antibody production occurs locally in the CNS in response to locally replicating virus.[2]

Remarks on FIP diagnosis:
Pre-mortem diagnosis is based on the leucocytosis with neutrophilia, mild to moderate non regenerative anaemia, and increased plasma protein concentration. CSF examination reveals elevated protein concentration and extensive pleocytosis.

Serological analysis can be made (ELISA or IFA) but doesn't permit to distinguish safely virulent or avirulent strains. Similarly the use of RT-PCR for coronavirus doesn't allow distinguishing FIP from FECV. The combination of clinical signs, historical factors and laboratory values is important.[1]

Generally, post-mortem histopathological analysis allows one to obtain a conclusive etiological diagnostic.

AFIP Diagnosis: Brain, cerebrum: Ventriculitis and periventriculitis, granulomatous, diffuse, moderate, with lymphocytic perivasculitis, Bengali (*Felis domesticus*), feline.

Conference Comment: The contributor provides an excellent overview of feline infectious peritonitis virus. In addition to the slide variability addressed by the contributor, there was also more neutrophilic inflammation in some sections. The inflammatory process in cases of FIP meningoencephalitis is focused on the inner and outer surfaces of the CNS. Recognition of this surface-related pattern is useful in differentiating FIP from other forms of encephalomyelitis in the cat.[4] Recent literature suggests that activated monocytes play a central role in the development of FIP vasculitis.[5]

Conference participants discussed the definition of vasculitis and what histomorphologic features must be present to diagnose a vasculitis. Many participants felt that there must be fibrinoid necrosis of vessel walls, inflammation, and/or apoptotic or necrotic cellular debris within a vessel wall to call a vasculitis. Evidence of damage to the vessel wall such as hemorrhage, fibrin, edema, and thrombi around and within the affected vessel lend support to the diagnosis of a vasculitis. Jubb & Kennedy states that "vasculitis is characterized by the presence of inflammatory cells within and around blood vessel walls with concomitant vessel wall damage as indicated by fibrin deposition, collagen degeneration, and necrosis of endothelial and smooth muscle cells."

Other Coronaviruses include those listed below.[7,8,9]

Bovine coronavirus (winter dysentery)	Bovine	Gastroenteritis, coronavirus implicated
Canine coronavirus	Canine	Enteritis
Feline coronavirus (FIP)	Feline	Peritonitis, pneumonia, meningoencephalitis, panophthalmitis; granulomatous vasculitis
Feline enteric coronavirus	Feline	Diarrhea in kittens
Mouse hepatitis virus (MHV)	Mouse	Hepatic necrosis, enteritis, encephalomyelitis; syncytia formation
Porcine transmissible gastroenteritis (TGE)	Porcine	Gastroenteritis
Porcine hemagglutinating encephalomyelitis virus	Porcine	Vomiting, wasting and encephalomyelitis (usually no diarrhea)
Porcine epidemic diarrhea	Porcine	Gastroenteritis (western Europe, similar to

		TGE)
Rat coronavirus	Rat	Rhinitis, tracheitis, pneumonitis in young
Rat sialodacryoadenitis virus	Rat	Sialodacryoadenitis, porphyrin released from damaged harderian gland, squamous metaplasia of ducts
Avian infectious bronchitis	Chickens	Tracheobronchitis, nephritis
Bluecomb (turkeys)	Turkeys	Enteritis, cyanosis of the comb
Rabbit coronavirus	Rabbits	Enteritis, myocarditis
SARS virus	Humans	Severe Acute Respiratory Syndrome
Epizootic catarrhal enteritis (ECE)	Ferrets	Profuse, green mucoid diarrhea in adults; thought to be a coronavirus

Contributor: Ecole Nationale Veterinaire d'Alfort, Pathology Department anapath@vet-alfort.fr.

References:

1. Andrew SE: Feline infectious peritonitis. Vet Clin North Am Small Anim Pract 30:987-1000, 2000
2. Foley JE, Leutenegger C: A review of Coronavirus infection in the central nervous system of cats and mice. J Vet Intern Med 15:438-444, 2001
3. Storts RW, Montgomery DL: The nervous system. In: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 435-436. Mosby, Inc., St. Louis, Missouri, 2001
4. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, p. 119. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
5. Kipar A, May H, Menger S, Weber M, Leukert W, Reinacher M: Morphologic features and development of granulomatous vasculitis in feline infectious peritonitis. Vet Pathol 42:321-320, 2005
6. Robinson WF, Maxie MG: The cardiovascular system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., pp. 51-53. Academic Press, Inc., San Diego, California, 1993
7. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 350-356. Williams & Wilkins, Baltimore, MD, 1997
8. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Coronaviridae. In: Veterinary Virology, 3rd ed., pp. 495-508. Academic Press, San Diego, California, 1999
9. Hoefer HL: Gastrointestinal diseases part I general gastrointestinal disorders. In: Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery, eds. Quesenberry KE, Carpenter JW, 2nd ed., pp. 30-31. Saunders, St. Louis, Missouri, 2004

SLIDE 32
CONFERENCE 8 / CASE IV – 06-A-025 (AFIP 3031275)

Signalment: 6-year/308-day-old adult female, rhesus macaque, *Macaca mulatta*, nonhuman primate

History: This animal experienced a three kg weight loss since September 2005. She was removed from an outdoor corral for an unthrifty appearance, diarrhea and difficulty ambulating in January 2006. Physical examination revealed cachexia, abdominal distension, mild kyphosis, atrophy of the pelvic limb musculature and contracture of the joints. There was minimal response to supportive and therapeutic care. Humane euthanasia was performed and she was submitted for necropsy.

Gross Pathology: The animal was cachexic and there were no visible stores of subcutaneous or visceral adipose tissue. The mesenteric lymph nodes were moderately enlarged. The mucosa of the small intestine was raised, thickened, rugose and exhibited a terry cloth appearance (Fig. 1). The cecum and colon were markedly dilated, flaccid and reddened and contained abundant green fluid feces (Fig. 2). The large intestinal mucosa was diffusely reddened with thickened mucosal folds. There was mild kyphosis and the medial condyles of both femurs were moderately eroded.

Laboratory Results: *Campylobacter coli* was isolated on one of two fecal cultures performed in January 2006. One of two fecal parasitology screens demonstrated flagellates. The remaining screen was negative.

Histopathologic Description: The lamina propria is diffusely expanded with an abundant amount of amorphous, acellular, pale eosinophilic material that widely separates the villous stromal cells and the intestinal glands. Villi are mildly and multifocally blunted and fused. Multiple lacteals are moderately dilated. There are scattered aggregates of low numbers of neutrophils in the villous tips.

Contributor's Morphologic Diagnoses:
1. Small intestine: Amyloid deposition, lamina propria, diffuse, severe with multifocal mild villous blunting and fusion, rhesus macaque (*Macaca mulatta*).
2. Mesentery: Atrophy, adipose tissue, diffuse, severe.

Contributor's Comment: The profound weight loss in this animal is attributed to severe amyloid enteropathy. The deposits stained light orange with the Congo red method and demonstrated green birefringence under polarized light (Fig 3). There were abundant deposits of amyloid in the lamina propria of the small intestine with minimal to moderate involvement of the stomach, cecum, colon, liver, spleen, adrenal gland, mesenteric lymph nodes, mammary gland, and renal medulla. A moderate, chronic-active, proliferative typhlocolitis was present. The amyloid deposits in the small intestine with consequent malabsorption of nutrients and protein loss and the typhlocolitis account for the emaciated body condition and diarrhea noted clinically. Underlying conditions that presumably led to systemic amyloidosis include typhlocolitis, osteoarthritis and spondyloarthropathy.

Amyloidosis is a heterogeneous group of diseases characterized by the extracellular deposition of insoluble protein in various tissues with consequent compromise of normal function. Ultrastructurally, the deposits are composed of protein fibrils assembled in antiparallel _-pleated sheets. The deposition of amyloid protein A (AA) occurs in

reactive or secondary amyloidosis and is associated with infectious and noninfectious chronic inflammatory conditions. Its precursor, serum amyloid A (SAA) is an acute phase protein produced primarily by the liver.[1,11] Functions ascribed to this protein include a role in cholesterol transport and metabolism as well as both anti- and proinflammatory activities.[13]
The pathogenesis of reactive systemic amyloidosis is poorly understood. SAA is produced under the control of cytokines such as interleukin-1, interleukin-6 as well as tumor necrosis factor-_ released during inflammation.[14] Increased levels of SAA are common with chronic inflammation but amyloid deposition usually does not occur. In individuals that do develop amyloidosis there is limited or defective proteolysis of SAA with formation and deposition of insoluble AA protein. Proposed mechanisms include failure of degradation due to excessive levels of SAA relative to enzyme, an intrinsic proteolytic enzyme defect or a structural anomaly in the SAA molecule making it resistant to degradation.[1] Pressure atrophy of surrounding tissue occurs with progressive accumulation of amyloid.

Reactive systemic amyloidosis is not an uncommon disease in rhesus macaques and has been reported in several species of nonhuman primates including common marmosets, squirrel monkeys, pigtail macaques, Celebes macaque, cynomolgus macaques, a stumptailed macaque, baboons, a mangabey and chimpanzees.[2-10,12,13] Underlying conditions include chronic enterocolitis, osteoarthritis, chronic vascular catheterization, and retroperitoneal fibromatosis associated with Type D retroviral infection.[3-5,8,10,12] The gastrointestinal tract, liver, adrenal gland, renal medulla and spleen are sites often affected. Lymph nodes, thyroid gland and gallbladder may also be affected.

Clinical signs are related to the site affected and the amount of amyloid deposited. They include weight loss, diarrhea refractory to treatment, hepatomegaly and splenomegaly. A protein losing enteropathy may be seen with enteric amyloidosis. Laboratory findings may include elevated levels of SAA, hypoproteinemia, hypoalbuminemia, hypergammaglobulinemia, and elevated liver enzymes with hepatic involvement.

Although gross lesions are often absent, the liver and/or spleen may be extraordinarily enlarged, pale, waxy and firm. Prominent splenic nodules may be observed on sectioned surface with white pulp involvement. The intestinal mucosa may be thickened and either pale or hyperemic.

Histologically, amyloid is an acellular, homogeneous to finely fibrillar, lightly eosinophilic extracellular material. AA amyloid deposits stain pale orange with the Congo red histochemical method and demonstrate green birefringence under polarized light. Typically, these deposits do not stain as prominently in nonhuman primates when compared to those in canine tissues.[3] In nonhuman primates, AA amyloid is most often deposited in the space of Disse in the liver, the lamina propria of the gastrointestinal tract, the corticomedullary junction of the adrenal gland, either the red or white pulp of the spleen and the renal medullary interstitium. The small intestine is the segment of the gastrointestinal tract most often and severely affected. Other than marmosets, the

renal glomeruli are rarely involved.[2-10,13] Progressive amyloid deposition results in atrophy of adjacent tissues.

AFIP Diagnosis: Small intestine, lamina propria: Amyloidosis, diffuse, marked, with moderate villar blunting and fusion, and lymphangiectasia, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: The contributor provides an excellent overview of reactive systemic amyloidosis in nonhuman primates.

Amyloidosis has been classified several different ways to include the following:

1. Primary versus secondary
2. Systemic (generalized) versus localized
3. Combination of the above

Systemic amyloidosis is also divided into 1) primary amyloidosis (immunocyte dyscrasia) and 2) secondary amyloidosis (reactive systemic amyloidosis).[15]

Reactive systemic amyloidosis is the most common form of amyloidosis in animals with amyloid being deposited in the kidney, liver, spleen, and lymph nodes. The spleen is the most frequent site of amyloid deposition in systemic reactive amyloidosis and occurs in the periarteriolar lymphoid sheaths and red pulp. The most functionally important deposition of amyloid occurs in the renal glomeruli of aged dogs resulting in proteinuria.[15]

AL amyloid consists of immunoglobulin light chains, is monoclonal, and secreted by plasma cells in immunocyte dyscrasias. This is the most common form of amyloidosis in humans, but does not commonly occur in animals.[15]

Localized amyloidosis involves a single organ or tissue and occurs in the nasal vestibule or rostral portion of the nasal septum and turbinates of horses and in the pancreatic islets of cats.[15]

-Amyloidosis involves the extracellular accumulation of amyloid- protein (A_) and is characteristic of Alzheimer's disease in humans. Amyloid- _ protein has also been identified in the brains of aged dogs with highest concentration in the frontal cortex.[15]

Contributor: Oregon National Primate Research Center, http://onprc.ohsu.edu

References:

1. Abbas AK: Diseases of immunity. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 258-267. Elsevier Saunders, Philadelphia, Pennsylvania, 2005

2. Banks KL, Bullock BC: Naturally occurring secondary amyloidosis of a squirrel monkey (*Saimiri sciureus*). J Am Vet Med Assoc 151(7)**:**839-842, 1967
3. Blanchard JL, Baskin GB, Watson EA: Generalized amyloidosis in rhesus monkeys. Vet Pathol 23**:**425-430, 1986
4. Doepel FM, Ringler DH, Petkus AR: Secondary amyloidosis in rhesus monkeys with chronic indwelling venous catheters. Lab Anim Sci 34(5)**:**494-496, 1984
5. Ellsworth L, Farley S, DiGiacomo RF, Tsai CC: Factors associated with intestinal amyloidosis in pigtailed macaques (*Macaca nemestrina*). Lab Anim Sci 42(4)**:**352-355, 1992
6. Hubbard GB, Lee DR, Steele KE, Lee S, Binhazim AA, Brasky KM: Spontaneous amyloidosis in twelve chimpanzees, *Pan troglodytes*. J Med Primatol 30**:**260-267, 2001
7. Hukkanen RR, Liggitt HD, Anderson DM, Kelley ST: Detection of systemic amyloidosis in the pig-tailed macaque (*Macaca nemestrina*). Comp Med 56(2):119-127
8. Jennings VM, Dillehay DL, Webb SK: Diagnostic Exercise: Hepatomegaly in an aged cynomolgus (*Macaca fascicularis*) monkey. Lab Anim Sci 45(6):680-682, 1995
9. Ludlage E, Murphy CL, Davern SM et al: Systemic AA amyloidosis in the common marmoset. Vet Pathol 42**:**117-124, 2005
10. McClure HM: Nonhuman primate models for human disease. Adv Vet Sci Comp Med 28**:**267-304, 1984
11. Pepys MB: Amyloidosis. Annu Rev Med 57**:**223-241, 2006
12. Slattum MM, Rosenkranz SL, DiGiacomo RF et al: Amyloidosis in pigtailed macaques (*Macaca nemestrina*): Epidemiologic aspects. Lab Anim Sci 39(6):560-566, 1989
13. Slattum MM, Tsai CC, DiGiacomo RF, Giddens WE: Amyloidosis in pigtailed macaques (*Macaca nemestrina*): Pathologic aspects. Lab Anim Sci 39(6):567-570, 1989
14. Urieli-Shoval S, Linke RP, Matzner Y: Expression and function of serum amyloid A, a major acute-phase protein. Curr Opin Hematol 7**:**64-69, 2000
15. Myers RK, McGavin MD: Cellular and tissue responses to injury. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 45-46. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 33
CONFERENCE 9 / CASE I – 03 R 732 (AFIP 2944354)

Signalment: 1-year-old, female, Prkdc.Scid mouse

History: The mouse was inoculated subcutaneously in its left hind footpad with 10 million stationary phase *Leishmania amazonensis* promastigotes (MHOM/BR/00/LTB0016). Approximately 10 months post-infection, while the left hind footpad only displayed minimal swelling, there was macroscopic evidence of pronounced, soft and non-ulcerated tumefactions (~ 0.25 to 1cm in length and/or

thickness) at the level of the ipsilateral and contralateral limbs, and on the head. At that time point, the mouse was sacrificed and its tissues processed for histology.

Gross Pathology: The swellings of the limbs and head consisted of white, soft, subcutaneous nodules that appeared like adipose tissue.

Contributor's Morphologic Diagnoses: 1. Dermatitis and panniculitis, pyogranulomatous, diffuse, chronic, severe.
2. Rhinitis, granulomatous, diffuse, chronic, moderate.

Contributor's Comment: *Leishmania amazonensis*, a member of the *L. mexicana* complex, is prevalent in South America. Transmission occurs through the bite of phlebotomine sand flies of the genus *Lutzomyia* with *Lu. flaviscutellata* being considered as the main sand fly vector. The reservoir includes forest rodents and marsupials. Human infection with *L. amazonensis* is not frequent and estimated to represent approximately 3% of the cases in the Amazon region.[1] Infection of the host with *L. amazonensis* usually results in localized to diffuse cutaneous lesions that sometimes spread to mucous membranes and occasionally to viscera.[1] Mouse models of *L. amazonensis* infection are considered as good models of diffuse cutaneous leishmaniasis and mucocutaneous leishmaniasis. After infection with *L. amazonensis*, most inbred strains of mice develop chronic cutaneous lesions that are characterized by a diffuse infiltrate of heavily parasitized macrophages, rare lymphocytes and scattered areas of necrosis.[2] In the macrophages, *L. amazonensis* amastigotes reside within large parasitophorous vacuoles, which result from the fusion of the phagosome with late endosomes/lysosomes.[2,3] The amastigotes are usually arranged along the periphery of the parasitophorous vacuole, and it is thought that the proteophosphoglycans that they secrete are responsible for the characteristic enlargement of the parasitophorous vacuole.[2]
While inflammation, and in particular $CD4^+$ T cells, have been shown to participate in the pathology and the development of cutaneous metastases after *L. amazonensis* infection, metastases have recently been reported in SCID mice, indicating that functional T and B cells are not necessary for the spread of the infection to distant cutaneous sites.[4] This is consistent with what is observed in patients co-infected with *Leishmania* spp. and HIV, who can develop atypical disseminated cutaneous lesions.

AFIP Diagnosis: Head, multiple cross sections: Dermatitis and panniculitis, histiocytic and neutrophilic, chronic, diffuse, severe, with mild rhinitis and myriad intrahistiocytic protozoal amastigotes, Prkdc.Scid mouse (*Mus musculus*), rodent.

Conference Comment: *Leishmania* sp. are protozoans in the order Kinetoplastida, family Trypanosomatidae.[5] Leishmaniasis is endemic in Mediterranean countries, and in some parts of Africa, India, and Central and South America. Leishmaniasis occurs rarely in animals in the United States except in endemic areas in Oklahoma, Texas, and

Ohio. It has also been reported in Foxhounds in the eastern coastal states. The disease occurs in 3 forms – cutaneous, mucocutaneous, and visceral. Amastigotes are most commonly identified within macrophages, but can occasionally be found within other leukocytes, endothelial cells, or fibroblasts. Additionally, free organisms may be found within the interstitium of necrotic areas.[5,6,7]

Two forms are involved in the life cycle of *Leishmania*: the promastigote, which develops extracellulary in the sandfly vector, and the amastigotes, which multiply intracellularly in host macrophages. Promastigotes released into the host dermis by infected sandflies are phagocytosed by macrophages. The acidity within phagolysosomes induces them to transform into amastigotes. The amastigotes are protected from the intravacuolar acid by a proton-transporting ATPase, which maintains the intracellular parasite pH at 6.5. The amastigotes proliferate within macrophages causing them to rupture and release progeny amastigotes that infect additional cells. Additionally, *Leishmania* organisms possess two surface glycoconjugates, which appear to be important in their virulence – lipophosphoglycans and gp63. Lipophosphoglycan is a glycolipid that forms a sense glycocalyx causing C3b deposition on the parasite surface (complement activation). On the other hand, lipophosphoglycan inhibits complement action by preventing insertion of the membrane attack complex (C5-C9) into the parasite membrane. The C3b binds to Mac-1 and CR1 on macrophages resulting in phagocytosis of the organisms. Lipophosphoglycans protect amastigotes once within macrophages by inhibiting lysosomal enzymes and scavenging oxygen free radicals. The second glycoconjugate, gp63, is a zinc-dependent metalloproteinase that cleaves complement and some lysosomal enzymes in addition to binding fibronectin receptors on macrophages facilitating adhesion of promastigotes.[8]

Humoral immune responses are non-protective and detrimental. T_H2 cytokines (e.g. IL-4, IL-13, IL-10) prevent effective killing of *Leishmania* by inhibiting activation of macrophages. Massive antibody production leads to organ damage by immune complex deposition. A cell-mediated (T_H1) immune response is protective and genetically predetermined.[8,9]

Many conference participants considered *Histoplasma* as a differential for this case. *Histoplasma* organisms are also typically found in macrophages, are similar in size to *Leishmania* and may illicit an intense histiocytic to granulomatous response; however, the yeast lack a kinetoplast and stain with PAS and GMS stains. *Leishmani*a amastigotes contain a round, eccentric nucleus with a rod-shaped kinetoplast that lies perpendicular to the nucleus. In Giemsa stained sections, amastigotes have pale blue cytoplasm, a red nucleus, and purple kinetoplasts.[5]

Other differentials include:

Trypanosoma cruzi – kinetoplast is parallel to the nucleus
Toxoplasma gondii – 2-5 um tachyzoites, no kinetoplast
Neospora caninum – 4-7 um tachyzoites, no kinetoplast
Sporothrix schenkii – 4-10 um, oval to cigar shaped, no kinetoplast

Blastomyces dermatitidis – 10-20 um, broad-based budding, no kinetoplast

Contributor: http://www.vetmed.iastate.edu/departments/vetpath/

References:

1. Barral A, Pedral-Sampaio D, Grimaldi G Jr, Momen H, McMahon-Pratt D, Ribeiro de Jesus A, Almeida R, Badaro R, Barral-Netto M, Carvalho E, Johnson W Jr.: Leishmaniasis in Bahia, Brazil: evidence that *Leishmania amazonensis* produces a wide spectrum of clinical disease. Am J Trop Med Hyg 44:536-546, 1991
2. Lemos de Souza V, Ascencao Souza J, Correia Silva TM, Sampaio Tavares Veras P, Rodrigues de-Freitas LA: Different *Leishmania* species determine distinct profiles of immune and histopathological responses in CBA mice. Microbes Infect 2(15):1807-1815, 2000
3. Courret N, Frehel C, Gouhier N, Pouchelet M, Prina E, Roux P, Antoine JC: Biogenesis of *Leishmania*-harbouring parasitophorous vacuoles following phagocytosis of the metacyclic promastigote or amastigote stages of the parasites. J Cell Sci 115(Pt 11):2303-2316, 2002
4. Vanloubbeeck Y, Ackermann MR, Jones DE: Late cutaneous metastases in C3H SCID mice infected with *Leishmania amazonensis*. J Parasitol 91(1):226-228, 2005
5. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 550. Williams & Wilkins, Baltimore, Maryland, 1997
6. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1205. Mosby Elsevier, St. Louis, Missouri, 2007
7. Eddlestone SM: Visceral leishmaniasis in a dog from Maryland. J Am Vet Med Assoc 217(11):1686-1688, 2000
8. McAdam AJ, Sharpe AH: Infectious diseases. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 403-404. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
9. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Diseases of the dermis. In: Skin Diseases of the Dog and Cat Clinical and Histopathologic Diagnosis, 2nd ed., pp. 312-316. Blackwell Publishing Professional, Ames, Iowa, 2005

SLIDE 34
CONFERENCE 9 / CASE II – 9457B (AFIP 3032023)

Signalment: 4-week-old, female, Saanen goat (*Capra hircus*), caprine

History: On a Saanen goat farm outside Melbourne Australia with 20 milking goats, four 6 week old female kids became anorexic, lethargic, tachypneic and dyspneic over 2 to 3 days. One goat was killed and examined and three other kids were treated with 8mg/kg Engemycin 100 (oxytetracycline 100mg/mL) (Intervet) for 10 days and

recovered and returned to the herd. The following week, another kid developed multi-focal arthritis and with treatment recovered.

Gross Pathology: At necropsy this goat had a marked diffuse mucopurulent pleuritis and pericarditis and no other significant gross pathological findings.

Laboratory Results: From the pleural swab, on horse blood agar, chocolate blood agar and CNA plates a pure colony of small slimy green mucoid alpha haemolytic colonies grew within 48 hours. Other bacteria were not isolated. Isolates were positive for casein reduction, sensitivity to digitonin, reduction of Tetrazolium chloride, glucose metabolism and arginine metabolism and liquefaction of coagulated serum. Bacterial DNA was extracted from two isolates and a 1400 base pair region of the 16S ribosomal gene was amplified by PCR using universal bacterial primers. Polymerase chain reaction amplicons were purified using Magnasil magnetic beads (Promega) following the supplied protocol. The PCR amplicons were sequenced using the Big Dye Terminator V3.1 sequencing kit (Applied Biosystems) with one of the 16S PCR primers and also an internal primer to obtain a continuous sequence of 800 base pairs on an ABI Prism 310 genetic analyser. Sequence reactions were cleaned and purified using Magnasil Green (Promega) and were analysed on the Ribosomal Database Project website (http://rdp.cme.msu.edu) maintained at the Michigan State University, USA. 16S RNA sequence analysis of both isolates was consistent with members of the *Mycoplasma mycoides* cluster. The sequence of one isolate was most consistent with *M. mycoides* subsp *mycoides* large colony (LC) type Y goat strain with 95.9% sequence homology. The second isolate was most consistent with *M. mycoides* subsp *mycoides* LC type Y goat strain with 85.7% homology.[1]

Histopathologic Description: The pleura and interlobular connective tissue septa of the lung were thickened by edema fluid, fibrin and moderate numbers of loosely packed neutrophils. Macrophages, lymphocytes, neutrophils and necrotic cells partially distended lymphatics and thin walled blood vessels. The lung parenchyma was collapsed and consolidated. Individual lobules had bronchial and bronchiolar intraluminal plugs of neutrophils and debris which less frequently extended into alveoli, which had thickened septal walls. The pericardium was markedly and diffusely thickened by fibrin and edema fluid, together with moderate numbers of lymphocytes. Also in the pericardium there were neutrophils and fragmented karyorrhectic debris with multi-focal areas of necrosis.[1]

Contributor's Morphologic Diagnosis: Subacute diffuse moderate suppurative bronchopneumonia and pleuritis.

Contributor's Comment: The isolation of a *Mycoplasma* spp. from a goat lung has always been a dilemma as it is important to distinguish *Mycoplasma capricolum* subsp. *capripneumoniae*, the cause of contagious caprine pleuropneumonia, from other *Mycoplasma* spp. Contagious caprine pleuropneumonia, a condition with high morbidity and mortality that causes severe fibrinous pleuropneumonia has never been reported in Australia.[2] The Mycoides cluster is a group of closely related mycoplasmas consisting

of several ruminant pathogens. The cluster is divided into two subgroups, Mycoides subgroup, that includes *M. mycoides* subsp. *mycoides* large colony (LC); *M. mycoides* subsp. *mycoides* small colony (SC); and *M. mycoides* subsp. *capri* and the capricolum subgroup, that includes *M. capricolum* subsp. *capricolum; M. capricolum* subsp. *capripneumoinae and M.* sp. Group 7 of Leach.[2,3] *M. mycoides* subsp. *mycoides* small colony (SC) is the cause of contagious bovine pleuropneumonia, a severe fibrinous pleuropneumonia of cattle that results in pronounced interlobular edema and intralymphatic thrombosis, this agent is also exotic to Australia.[5] *Mycoplasmas* have previously been isolated from Australian goats both with and without clinical disease.[4] Mycoplasmal pneumonia needs to be differentiated from other causes of bacterial bronchopneumonia in goats, including *Pasteurella multocida* and *Mannheima haemolytica*.[6]

Contagious caprine pleuropneumonia is an exotic disease in most countries and until recently the classification and diagnosis of *M. capricolum* subsp. *capripneumoniae* and the distinction from other pathogenic Mycoplasmas of goats was difficult due to the limited number of biochemical and physiological properties that could be used to differentiate the species. The development and recent advances in molecular techniques and sequence analysis has provided a useful complement or alternative to conventional methods for disease diagnosis and phylogenetic studies.[3] The histological findings of this case are similar to a previous Australian case of *M. mycoides* subsp. *mycoides* LC.[4] However, while *M. capricolum* subsp. *capripneumoniae* causes only pleuropneumonia, *M. mycoides* subsp. *mycoides* LC can result in septicemia, mastitis, keratitis, arthritis or genital lesions.[2] Recently, *M. mycoides* subsp. *mycoides* large colony (LC) was isolated from an Australian goat with mastitis (unpublished). *M. mycoides* subsp. *mycoides* LC, has been isolated from the ears of clinically normal Australian goats and ear mites may aid the spread of the organism.[7]

AFIP Diagnosis: Lung: Bronchopneumonia, chronic-active, multifocal, moderate, with marked fibrinous pleuritis, Saanen goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides a concise and thorough overview of *Mycoplasma* spp. in goats and emphasizes the importance of distinguishing *M. capricolum* subsp. *capripneumoniae*, the cause of contagious caprine pleuropneumonia (CCPP), from other *Mycoplasma* spp.

Mycoplasmas are the smallest prokaryotes capable of self-replication and lack cell walls resulting in extreme pleomorphism. Most pathogenic mycoplasmas are host and site specific. Additionally most pathogenic mycoplasmas parasitize joints and mucous membranes and are almost always associated with respiratory, urogenital, mammary, or ocular infections.[6,8]

Infections with *M. capricolum* subsp. *capripneumoniae* are limited to the thoracic cavity and always result in only pleuropneumonia while infection with the other

mycoplasmas results in polysystemic infections. Other mycoplasmas can cause histopathologic pulmonary lesions similar to *M. capricolum* subsp. *capripneumoniae* (*Mycoplasma* biotype F38) in goats, but are not considered to cause CCPP. The pathogenesis of CCPP is thought to involve a cross-reaction between IgG antibodies against mycoplasmal antigens and ciliary proteins causing inflammation and ciliary disfunction.[9] CCPP is the caprine counterpart of contagious bovine pleuropneumonia. In contrast to contagious bovine pleuropneumonia, prominent interstitial edema and formation of pulmonary sequestra are not prominent features of CCPP.[6,9, 10]

Some diseases of veterinary significance caused by common *Mycoplasma* spp. infections in livestock are listed in the table below:[8,9]

***MYCOPLASMA* SPP.**	**DISEASE**	**SPECIES AFFECTED**
Mycoplasma mycoides subsp. *mycoides* SC	Contagious bovine pleuropneumonia	Cattle
Mycoplasma bovis	Mastitis, arthritis, pneumonia	Cattle
Mycoplasma mycoides subsp. *mycoides* LC	Pneumonia, arthritis, mastitis, septicemia	Goats, sheep
Mycoplasma capricolum subsp. *capripneumoniae*	Contagious caprine pleuropnemonia	Goats
Mycoplasma agalactiae	Mastitis (contagious agalactia), arthritis, pneumonia, kertoconjunctivits, vulvovaginitis	Goats, sheep
Mycoplasma capricolum subsp. *capricolum*	Septicemia, mastitis, polyarthritis, pneumonia	Goats, sheep
Mycoplasma mycoides subsp. *capri*	Septicemia, pleuropneumonia, arthritis, mastitis	Goats
Mycoplasma hyopneumoniae	Enzootic pneumonia of swine	Pigs
Mycoplasma hyorhinis	Pneumonia, arthritis, polyserositis	Pigs
Mycoplasma hyosynoviae	Polyarthritis	Pigs

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References:

1. Williamson MM, Pettifer JK, McCoy RJ, Taylor T, Kennedy J, Ross AD: Pleuropneumonia and pericarditis in a goat with isolation of *Mycoplasma mycoides* subspecies *mycoides* large colony. Aust Vet J (in press)
2. Lefevre P-C, Thiaucourt F: Contagious caprine pleuropneumonia. In: Infectious Diseases of Livestock, eds. Coetzer JAW, Tustin RC, 2nd ed., pp. 2060-2065. Oxford University Press, South Africa, Cape Town, South Africa, 2004
3. Pettersson B, Leitner T, Ronaghi M, Bölske G, Uhlén M, Johansson KE: Phylogeny of the *Mycoplasma mycoides* cluster as determined by sequence analysis of the 16S rRNA genes from the two rRNA operons. J Bacteriol 178(14):4131-4142, 1996
4. Cottew GS, Lloyd LC: An outbreak of pleurisy and pneumonia in goats in

Australia attributed to a *Mycoplasma* species. J Comp Path 75**:**363-377, 1965
5. Thiaucourt F, van der Lugt JJ, Provost A: Contagious bovine pleuropneumonia. In: Infectious Diseases of Livestock, eds. Coetzer JAW, Tustin RC, 2nd ed., pp. 2045-59. Oxford University Press, South Africa, Cape Town, South Africa, 2004
6. Dungworth DL: The Respiratory System. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 539-699. Academic Press, Inc., San Deigo, California, 1993
7. Cottrew GS, Yeats FR: Mycoplasmas and mites in the ears of clinically normal goats. Aust Vet J 59**:**77-81, 1982
8. Coetzer JAW, Tustin RC: Infectious Diseases of Livestock, 2nd ed., p2043. Oxford University Press, South Africa, Cape Town, South Africa, 2004
9. L_pez A: Respiratory system. In: Pathologic Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 532-533. Mosby Elsevier, St. Louis, Missouri, 2007
10. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 371-384. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 35
CONFERENCE 9 / CASE III – AFIP case 1 (AFIP 3026272)

Signalment: 1-year-old, intact male, domestic short-haired cat (*Felis catus*)

History: Owner has lost 5-6 cats over the past 6 months. The cats lose weight despite normal eating habits and activity. The owner finds the cats dead without other signs. Owner is concerned about possible toxicity.

Gross Pathology: The patient is in poor nutritional condition. Mucous membranes and subcutaneous tissues are mildly pale and icteric. The spleen is enlarged 3-5 times normal with widely scattered, 2-3 mm white foci throughout the splenic capsule and cut surface. Mesenteric and submandibular lymph nodes are 2-5 times expected size; their cut surfaces bulge slightly and have scattered, discrete white foci similar to that seen in the spleen. The lungs fail to collapse entirely, are mildly firm, and diffusely reddened. Sections of spleen and lymph node are collected fresh and submitted for bacterial culture.

Histopathologic Description: Spleen: The spleen exhibits multifocal, individual to coalescing foci of inflammation and necrosis particularly centered upon and effacing the white pulp. The foci are relatively abruptly demarcated from adjacent red pulp. The necroinflammatory foci consist of an admixture of intact and degenerate neutrophils and macrophages admixed with conspicuous cellular debris and fibrin.

Contributor's Morphologic Diagnosis: Spleen: Acute, multifocal, severe, necrosuppurative splenitis.

Contributor's Comment: Tularemia is caused by a small pleomorphic, strictly aerobic, Gram-negative coccobacillus *Francisella tularensis*. Humans and animals become infected by either direct contact with infected animals (usually lagomorphs or rodents) or by arthropod bites, particularly fleas, flies and ticks that have fed on infected animals. Ticks can maintain infection throughout their life cycle.[1]

After *F. tularensis* enters the host, the organism multiplies and disseminates by invading vascular endothelium or by spreading along superficial or deep lymphatics.[2] If bacteremia develops, the organisms are removed by the mononuclear-phagocytic system; however, *Francisella* can survive and multiply within macrophages (facultative intracellular pathogen).[1,2] Ensuing lesions are characteristic (Figures 1-4 Case 028A), but not specific, and consist of yellow/white foci of necrosis within the spleen, liver and lungs. Lymph nodes are often extremely enlarged and contain foci of necrosis and other lymphoid tissue (Peyer's patches, tonsils) can also be involved. Clinical signs can range from asymptomatic infection to fulminant fatal disease; salient features include fever, lethargy, depression, lymphadenopathy, hepatosplenomegaly, oral or lingual ulcers, and icterus.[2]

The diagnosis of tularemia in cats can be challenging because neither the clinical signs, gross, nor microscopic lesions are specific. In cats, important etiological differentials include: plague (*Yersinia pestis*), pseudotuberculosis (*Yersinia pseudotuberculosis*), and feline infectious peritonitis virus (feline coronavirus) infection.[3] Confirmatory diagnosis can be achieved through serology, culture, PCR, fluorescent-antibody or immunohistochemical staining methods on infected tissues.[1,2,3]

AFIP Diagnosis: Spleen: Splenitis, necrotizing, acute, multifocal to coalescing, severe, with lymphoid depletion and fibrin thrombi, cat (*Felis catus*), feline.

Conference Comment: Tularemia (deer fly fever, rabbit fever) is a zoonotic disease with worldwide distribution, affecting more than 100 species of wild and domestic mammals, birds, fish, and reptiles. The primary reservoir in the U.S. is the wild rabbit. There are two main biovars:[1,3,4]

1. *F. tularensis* subsp. *tularensis* (type A) is highly virulent; associated with a tick-rabbit cycle; occurs only in North America; produces classic disease in humans
2. *F. tularensis* subsp. *holarctica* (Type B) is less virulent; associated with rodents, ticks, mosquitoes, mud, and water; and occurs throughout the Northern Hemisphere

Both strains have been isolated from cats in the United States. Ticks and the deerfly (*Chrysops discalis*) are important vectors in North America.
The most common modes of transmission of *F. tularensis* to humans are via an arthropod bite or direct contact with infected tissues. Cat-associated cases usually involve people being scratched or bitten by cats that have a history of hunting or eating wild animals, especially rabbits. The infectious dose for humans is as few as 10 to 50

organisms inhaled as an aerosol or injected intradermally. Therefore, isolation of *F. tularensis* and necropsies of animals with suspected tularemia should be performed with adequate biosafety equipment.[1,2,4]

The differential diagnosis considered in this case included infections caused by *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Toxoplasma gondii*, feline infectious peritonitis virus and feline virulent systemic calicivirus.

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References:

1. Feldman KA: Zoonosis update: Tularemia. J Am Vet Med Assoc 222:725-730, 2003
2. Woods JP, Panciera RJ, Morton RJ, Lehenbauer TW: Feline tularemia. Compend Contin Educ Prac Vet 20:442-457, 1998
3. Debay BM, Andrews GA, Bergstrom CC, Cox L: Immunohistochemical demonstration of *Francisella tularensis* in lesions of cats with tularemia. J Vet Diagn Invest 14:162-164, 2002
4. Kaufmann AF: Tularemia. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd ed., pp. 300-302. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998

SLIDE 36
CONFERENCE 9 / CASE IV – 050780-18 (AFIP 3027308)

Signalment: Adult, female, strain 13 breeder guinea pig, approximately 7-8 months old

History:* This adult female strain 13 breeder guinea pig was born at the USAMRIID colony in November 2004 and paired with boar #FA61. On July 3, 2005 the animal presented clinically for listlessness. During physical examination the attending veterinarian noted pain upon abdominal palpation, distended abdomen, bloody vaginal discharge, and pododermatitis of the forefeet. The clinical differential diagnosis was dystocia. Because of progressively worse clinical signs, the animal was euthanized with carbon dioxide on the same day and immediately necropsied.

Gross Pathology: This adult female guinea pig was in good flesh and had abundant subcutaneous and cavitary fat. Externally, the abdomen was slightly distended, and there was mild excoriation of the dorsal surface of both front feet. Frank blood stained the perineum and external genitalia (vaginal labia). On internal examination, the right horn of the uterus was markedly enlarged approximately five times normal size (2 cm in diameter) as compared to the left uterine horn. There was multifocal coalescing hemorrhage and diffuse congestion of the right uterine horn visible from the serosal surface. Filling the lumen of the right uterine horn and attached to the wall of the uterus was a hemorrhagic mass of dense fibrovascular tissue admixed with hemorrhagic clots.

The left uterine horn was slightly congested and enlarged. Recognizable fetuses were not observed in the lumen of either uterine horn. The mesenteric lymph nodes were enlarged approximately three times normal size. The spleen was slightly enlarged and congested.

Gross Diagnoses:
1. Uterus, right horn: Endometritis, hemorrhagic, with retained placenta.
2. Uterus, left horn: Congestion, diffuse, mild.
3. Lymph nodes, mesenteric: Lymphadenopathy, moderate.
4. Spleen: Congestion, diffuse, acute, mild.

Laboratory Results: *Proteus mirabilis* and *Citrobacter freundii* were cultured from the uterine contents. *Proteus mirabilis* and *Escherichia coli* were cultured from the heart blood.

Histopathologic Description: Diffusely and transmurally, the uterine wall is expanded up to twice normal thickness by abundant hemorrhage, fibrin, and edema. The endometrial and myometrial vasculature is markedly congested, and occasionally endometrial vessels are occluded by fibrin thrombi. There is multifocally extensive attenuation, erosion, and ulceration of the mucosal epithelium with loss of endometrial glands, and replacement by hemorrhage, fibrin, and necrotic cellular debris admixed with low numbers of degenerate heterophils. Multifocally, remaining endometrial glands are mildly ectatic, lined by degenerate and necrotic glandular epithelial cells, and contain few degenerate heterophils and necrotic cellular debris. Multifocally, the mucosal and glandular epithelium is infiltrated and disrupted by small colonies of bacilli; the bacteria measure approximately 1-2 µm in length.

Partially filling the enlarged uterine lumen, and multifocally attached to the endometrium in some histologic sections, there is a dense, highly cellular fibrovascular mass (retained placenta) covered by a single layer of columnar epithelial cells (yolk sac epithelium). The retained placenta is composed of loosely arranged fibroblasts in a collagenous matrix separated by hemorrhage, fibrin and edema; multifocally degenerate and necrotic yolk sac epithelial cells that are occasionally disrupted and replaced by small colonies of bacilli; variably sized and congested yolk sac vessels; and a labyrinth of anastomosing columns of polygonal cells with large amounts of microvacuolated cytoplasm and prominent oval nuclei (syncytiotrophoblasts) separated by vascular channels and capillaries.

Tissue Gram stains of serial sections of the uterus by the Lillietwort method demonstrates numerous, small to medium Gram-negative bacilli within the uterine lumen, endometrium, and necrotic retained placenta; few, scattered Gram-positive cocci are also present.

Contributor's Morphologic Diagnoses: 1. Uterus, right horn; and placenta: Endometritis and placentitis, necrotizing and hemorrhagic, subacute, diffuse, moderate

to marked, with congestion, edema, retained placental tissue, and many small to medium Gram-negative bacilli.
2. Uterus, left horn: Endometritis, suppurative, subacute, diffuse, mild to moderate, with congestion, small fragment of retained placenta, and small to medium Gram-negative bacilli (slides not submitted).
3. Kidney: Pyelonephritis, chronic-active, multifocal, mild to moderate, with tubular degeneration, mineralization, necrosis, and dilatation, neutrophilic tubulitis, and scattered cellular and hyaline casts (slides not submitted).
4. Urinary bladder: Cystitis, heterophilic, chronic-active, multifocal, minimal to mild, with scattered small bacilli (slides not submitted).

Contributor's Comment:** The clinical signs, necropsy lesions, histopathologic findings, and tissue Gram stains indicate bacterial endometritis and retained placenta as the cause of the pain and listlessness in this adult female breeder guinea pig. The gross findings of an intraluminal hemorrhagic mass in the uterus, confirmed histologically as a necrotic placenta, suggests pregnancy and abortion during the period just before euthanasia, although no fetuses were identified grossly or histologically in the uterus, and neither aborted fetuses nor dead pups were observed in the cage. We cannot explain the absence of dead pups or fetuses, although we speculate the aborted young may have been consumed by other adult animals in the cage; guinea pigs, both males and females, are known to consume the placenta after expulsion, and we suppose that any aborted fetuses may have met the same fate.[5]

The underlying pathogenesis for the retained placenta and bacterial endometritis is uncertain in this case. The animal had concurrent chronic-active pyelonephritis and chronic-active cystitis with intralesional bacteria (slides not submitted), indicating an ascending urinary tract infection. The bacterial infection in the uterus and placenta may have originated from a chronic, subclinical, ascending urinary tract infection, causing abortion and retention of the placenta. Alternatively, the retained placenta, and subsequent secondary bacterial infection of the reproductive tract may have resulted from uterine inertia as a result of dystocia. Primiparous guinea pig sows not bred before 7 to 8 months are at significant risk for dystocia because the pelvic symphysis may not separate adequately during parturition, resulting in dystocia and uterine inertia.[5] Unfortunately, the clinical history in this case was incomplete, and we are uncertain if this older female guinea pig was a primiparous or multiparous breeder.

The microbial culture results from the uterine contents and heart blood, and the histomorphology of the infectious organisms in the uterus and retained placenta indicate a mixed bacterial infection. The isolation of *Proteus mirabilis* from both the uterine contents and heart blood suggests this bacterium may have been the primary offending pathogen, although we cannot exclude contribution of the other isolated bacteria.

A wide variety of causes for dystocia, abortion, and stillbirths are described for the guinea pig. Reported causes of abortion and stillbirths include nutritional deficiencies, pregnancy toxemia, *Bordetella, Salmonella, Streptococcus*, cytomegalovirus infections, asphyxia at birth, toxoplasmosis, erysipelas, and dystocia.[2] In addition to delayed

breeding of primiparous sows after 6 months of age, other reported non-infectious causes of dystocia in the guinea pig include obesity and large fetuses.[5]

Proteus organisms are Gram-negative rods, 0.5 µm wide by 1.0 to 3.0 µm long, and are easily demonstrable in the feces of animals, but are rarely found in large numbers except when the normal intestinal microflora is deranged. *Proteus* sp., as well as other endogenous bacteria of the bowel and skin such as *E. coli*, staphylococci, streptococci, *Enterobacter*, and *Pseudomonas* are frequently involved in urinary tract infections.[4] These organisms establish infection in the lower urinary tract (i.e. urethra and urinary bladder) and often ascend to the renal pelvis and parenchyma causing pyelonephritis. Like animals, 95 percent of human cases of acute pyelonephritis are secondary to ascending bacterial infection emanating from a primary infection in the urinary bladder.[1] We are uncertain if *Proteus* sp. was the cause of the ascending urinary tract infection in this guinea pig, as the urine was not cultured.

The guinea pig placenta shares multiple similarities to that of humans and is considered a favorite among pharmacologists and toxicologists when studying placental pathology. Unlike other laboratory rodents, the guinea pig has a rather long gestation period (up to 70 days); it is hardy, patient, and easily bred; it has an endocrine pregnancy control similar to that of the human; and it has a discoidal, hemomonochorial placenta with a fetal/maternal transport barrier which is very similar to that of the human placenta.[3]

* Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

**Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by the U.S. Army.

AFIP Diagnosis: Uterus: Metritis, subacute, diffuse, mild with hemorrhage, congestion, edema, colonies of bacilli, and retained placenta, strain 13 breeder guinea pig (*Cavia porcellus*), rodent.

Conference Comment: The pregnant uterus and its contents, the placenta, and developing embryo or fetus are more prone to infection than the non-gravid uterus. Reasons for this include the following:

1. The gravid uterus is under the influence of persistent rather than cyclic progesterone stimulation.
2. The chorionic epithelium of the placenta secretes substances that predispose the gravid uterus to certain types of infection.

3. The placenta and embryo/fetus are immunologically-privileged sites and are not protected from infection by the maternal immune system.

There are two basic sources of infection of the gravid uterus: the maternal blood (hematogenous route) and the maternal cervix and vagina (ascending infection). Certain infections persist in a latent state and become activated during pregnancy and invade the gravid uterus and conceptus resulting in abortion and stillbirth. Microbiologic agents associated with abortion and stillbirth in domestic species include bacteria, fungi, protozoa, rickettsia, chlamydia, and viruses.[6]

Additionally, nonspecific endometritis is common in the postpartum uterus. This is especially common as a complication of abnormal deliveries, such as abortion, retained placenta, dystocia, twinning, and traumatic injuries of the reproductive tract. In these cases, delayed involution of the uterus, coupled with the accumulation of necrotic placental and endometrial debris in the presence of an open cervix promotes establishment of infection that may progress to pyometra. Bacteria isolated from nonspecific endometritis in domestic animal species include: *E. coli*, *Proteus*, *Actinomyces pyogenes*, _-hemolytic streptococci, *Klebsiella*, *Clostridium*, *Fusobacterium*, and *Bacteroides*.[6]
The pathogenesis of retained placentas is multifactorial and may involve infectious diseases of the placenta, abnormal gestation periods, hormonal imbalances, and mechanical factors.[6]

As pointed out by the contributor, guinea pigs have a discoidal, labyrinthine, hemo-monochorial placenta that represents the chorioallantoic main placenta. Additionally, guinea pigs have a separate subplacenta and yolk sac placenta. The subplacenta is a specialized segment of the chorioallantoic placenta that connects the main placenta with the junctional zone and serves as a source of trophoblast invasion into the endometrium. Throughout pregnancy subplacental syncytiotrophoblasts produce large amounts of glycoprotein secretory granules that are secreted into the maternal blood lacunae where they accumulate and are released during degeneration of the subplacenta. The secretion of the granules may assist in separation of the placenta or in postpartum removal of cellular debris and wound healing. The yolk sac placenta participates in the selective absorption and transfer of maternal immunoglobulins for fetal immunoprotection and occurs in mice, rats, rabbits, and guinea pigs.[3]

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References:
1. D'Agati VD, Jennette JC, Silva FG: Infectious tubulointerstitial nephritis. In: Atlas of Nontumor Pathology: Non-Neoplastic Kidney Diseases, p. 549. American Registry of Pathology in collaboration with the Armed Forces Institute of Pathology, Washington, DC, 2005

2. Harkness JE, Wagner JE: The Biology and Medicine of Rabbits and Rodents, p. 153. Williams & Wilkins, Baltimore, Maryland, 1995
3. Kaufman P: Guinea Pig (*Cavia porcellus*), July 2004 http://medicine.ucsd.edu/cpa/guinea.htm
4. Maxie MG, Prescott JF: The urinary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th edition, vol. 2, p. 511. Academic Press, San Diego, California, 1993
5. Schaeffer DO: Disease problems of guinea pigs and chinchillas part A. In: Ferrets, Rabbits, and Rodents, eds. Hillyer EV, Quesenberry KE, p. 265. WB Saunders Company, Philadelphia, Pennsylvania, 1997
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 1173-1187. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 37
CONFERENCE 10 / CASE I – AVC F-4686-06 (AFIP 3031128)

Signalment: 12-year-old, spayed, female, domestic short-haired cat (*Felis domesticus*), feline.

History: Owners had noticed a hard mass in the dorsal sacral area which had been present for approximately 2 months.

Gross Pathology: Surgical excision was attempted. The mass peeled out of a hard capsule in 5 pieces, several of which were sent for histologic examination.

Laboratory Results: Radiographs revealed a multilobulated mass which invaded and replaced most of a coccygeal vertebra. Tail and anal tone were apparently normal.

Histopathologic Description: The submitted tissue consisted of a densely cellular tumor composed of short, interlacing bundles and clusters of plump, spindloid to polygonal neoplastic stromal cells. These cells have relatively uniform, oval nuclei with coarsely stippled chromatin, small nucleoli and small amounts of poorly-defined cytoplasm. Mitotic figures are infrequent (1 per 5 HPF). Interspersed uniformly throughout the tumor are many, large, multinucleated, neoplastic giant cells. These cells generally have 5-25 uniform nuclei and variable amounts of eosinophilic cytoplasm. These cellular infiltrates are supported by small to moderate amounts of uniformly distributed, dense, hyalinized, coarse collagenous stroma which contains frequent small foci of fine, mineral deposition. In some areas, small deposits of pale, acellular, pink matrix (interpreted as osteoid) are also scattered amongst stromal cells. A fibrous capsule (likely the periosteum) lines the periphery of the tissue. There are multifocal, small aggregates of mineralized bone (likely remnants of the coccygeal vertebrae) beneath this capsule.

Contributor's Morphologic Diagnosis: Giant cell tumor of bone, coccygeal vertebra.

Contributor's Comment: Giant cell tumors of bone are well-described but rare in domestic animals. Most reports involved sporadic tumors in dogs and cats. In humans, most giant cell tumors are regarded as low grade malignancies and local recurrence is not uncommon. Wide tissue resection tended to produce the lowest rate of recurrence. A small percentage of tumors may metastasize, typically to the lung. Interestingly in humans, most cases of metastasis of giant cell tumors occurred after attempts at surgical excision, likely due to local tissue damage promoting access to systemic circulation.[1]
Giant cell tumors are typically expansile, osteolytic lesions often involving long bones in humans and animals but which have also been reported in vertebral sites. Because few cases have been reported the biologic behavior of giant cell tumors of bone in animals has not been well-defined. However, those tumors described tended to be locally aggressive with rare incidences of distant metastasize. Retrovirus infection has been suggested as a primary etiology in cats in some cases.[2]

AFIP Diagnosis: Vertebra, coccygeal (per contributor): Giant cell tumor of bone, domestic shorthair (*Felis domesticus*), feline.

Conference Comment: This case sparked much discussion among conference participants and other consulted pathologists in reference to the differentiation of giant cell type osteosarcomas from giant cell tumors of bone since both contain multinucleate giant cells. Some participants favored a diagnosis of giant cell type osteosarcoma.

As stated by the contributor, giant cell tumor of bone is very rare and has been reported in dogs, cats, and cattle. The cases that have been described tend to behave like giant cell tumors in humans and occur most commonly in the epiphyses of long bones; however, involvement of the axial skeleton and metacarpal bones has been recorded in dogs and cats. The tumor tends to destroy cortical bone as it expands, but always tends to be at least partially encapsulated by a thin shell of bone (as in this case) which gives the tumor a characteristic "soap-bubble" appearance on radiographs. Giant cell tumors are locally aggressive, but usually do not metastasize.[3]

Two cell types are present in giant cell tumors. Mononuclear stromal cells are most numerous and have a histiocytic or fibroblastic appearance. Multinucleate giant cells compose up to 35% of the cell population and resemble osteoclasts. There may be some collagen or osteoid in the tumor, but this is not a prominent feature in contrast to osteosarcomas in which the production of osteoid is characteristic and usually prominent. When present in giant cell tumor of bone, osteoid is very sparse. Hemorrhages and cavernous vascular spaces commonly occur.[2,3]

Giant cells occur in many bone lesions, but in giant cell tumor they are an integral part of the neoplasm. The giant cells are often very large, are scattered uniformly throughout the tumor and their nuclei resemble those of the mononuclear cells. The

cytoplasmic borders of the giant cells are often indistinct. In contrast, in the giant cell variant of osteosarcoma, giant cells are increased in regions rather than distributed uniformly throughout the tumor. Additionally, the mononuclear cells in osteosarcoma have darker, angular nuclei or some other distinguishing shape clearly different from the multinucleate cells. Opinions differ as to whether the giant cells in giant cell tumor of bone are formed by coalescence of stromal cells or by amitotic division or nuclear segmentation without cytoplasmic separation. There is some controversy regarding the cell of origin. According to Jubb, Kennedy, and Palmer, the tumor giant cells have histochemical and ultrastructural characteristics of osteoclasts suggesting giant cell tumors probably arise from osteoclast stem cells of the bone marrow. Meuten's Tumors in Domestic Animals states that the histiogenesis of giant cell tumor is uncertain, but immunohistochemical staining suggests that the mononuclear cells are of histiocytic origin and that the giant cells arise from their fusion.[2,3,4]

In a study performed by Josten and Rudolph, multinucleate giant cells present in various neoplasms were classified as neoplastic or reactive (non-neoplastic). The nuclei of neoplastic giant cells were immunohistochemically positive for MIB1 (Ki67) while the cytoplasm was immunohistochemically negative for tartrate resistant acid phosphatase (TRAP). The nuclei were polymorphic and atypical mitoses were observed. In contrast to neoplastic giant cells, osteoclast-like giant cells were negative for MIB1 and positive for TRAP. Osteoclast-like giant cell nuclei were homogenous. Other non-neoplastic giant cells (e.g. foreign body cells, Langhans-giant cells) were negative for both MIB1 and TRAP.[5]

This case was reviewed in consultation with Dr. Roy Pool who preferred the diagnosis of atypical giant cell tumor of bone. In his opinion, this case represents one end of a spectrum between a low grade fibrosarcoma of bone with some tumor giant cells present and a giant cell tumor of bone with less than normal numbers of the multinucleate cells that characterize this tumor.

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References:

1. Rosai J: Ackerman's Surgical Pathology, 8th ed., vol. 2, pp. 1957-1962. Mosby-Year Book, Inc., St. Louis, Missouri, 1996
2. Thompson KG, Pool RR: Tumors of bones. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 294-296. Iowa State Press, Ames, Iowa, 2002
3. Palmer N: Bones and joints. In: The Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 135-136. Academic Press, San Diego, California, 1993
4. Slayter MV, Boosinger TR, Pool RR, Dämmrich, Misorp W, Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, Second Series, vol. I, p. 12, Armed Forces Institute of Pathology, Washington, D.C., 1994
5. Josten M, Rudolph R: Methods for differentiaton of giant cells in canine and feline neoplasia in paraffin sections. Zentralbl Veterinarmed A 44(3):159-166, 1997

SLIDE 38
CONFERENCE 10 / CASE II – 06-29801 (AFIP 3031049)

Signalment: 8-week-old female wirehaired Dachshund (*Canis familiaris*), canine

History: This pup was the first born and runt of a litter. Beginning at the age of 4 weeks, she developed motor problems and an intention tremor that progressed from walking only a few steps at a time to complete inability to walk by the time of euthanasia at 8 weeks of age. No information was supplied regarding the health status of this pup's littermates.

Gross Pathology: All long bones and ribs were fragile and easily fractured on manipulation. Fractures with callus formation were present on multiple ribs, thoracic vertebrae 5 and 6, mid-shaft right humerus, and left carpus.

Histopathologic Description: Histologic lesions were similar in all long bones and ribs examined but varied in severity among anatomic sites. Primary spongiosa was present in the epiphysis and metaphysis and was histologically normal, but secondary spongiosa was sparse or absent in both sites. Peri-trabecular fibroplasia was present in the epiphysis. There was a paucity of trabeculae in the metaphysis, and those present were thin and frequently fractured, with surfaces covered by a thin and often discontinuous layer of osteoid. The cortex was very thin and consisted mainly of woven bone with loose vascular stroma in wide spaces between trabeculae. Moderate subperiosteal fibrosis was present. Few osteocytes and osteoclasts were present.

Contributor's Morphologic Diagnosis: Osteopenia with failure of development of secondary spongiosa, consistent with osteogenesis imperfecta.

Contributor's Comment: Osteogenesis imperfecta (OI) is a hereditary osteopenic condition described in humans, dogs, cats, cattle, and sheep that results from a qualitative and/or quantitative defect in collagen I production.[1,2,3,4,5] Although growth plate organization and mineralization of primary spongiosa occurs in normal sequence and pattern, osteoid deposition on primary spongiosa of long bones is impaired, leading to defective endochondral ossification through failure of development of secondary spongiosa.[1,2] In humans, the phenotypic expression of OI is quite variable and has been classified into multiple groups based on clinical and prognostic features.[2] In most severely affected cases, pathologic fractures with callus formation may develop *in utero*. Animals with OI typically appear normal at birth but have variably severe locomotor difficulty that progresses over the first few weeks of life. Contribution of collagen I to other tissues, such as teeth (dentin) and tendons, may also predispose to additional lesions, including abnormal tooth production (dentinogenesis imperfecta) and joint laxity.[1,2] Affected humans frequently have blue discoloration of sclera due to diminished collagen content.

Both autosomal dominant and recessive forms of OI have been identified in humans. Most human cases of OI result from mutations in either the COL1A1 or COL1A2 gene, resulting in glycine substitution in the procollagen molecule that leads to abnormal formation of the collagen I triple helix, or mutations resulting in a premature stop codon. Evidence of similar mutations in dogs with OI is limited, but this latter mutation has been identified in the COL1A2 gene of one Beagle pup with OI.[6] No consistent mutation was identified in several related Dachshund pups with clinical OI.[3]

AFIP Diagnosis: Long bone: Osteopenia, diffuse, marked, with failure to develop secondary spongiosa, Dachshund (*Canis familiaris*), canine.

Conference Comment: Osteogenesis imperfecta (OI) is an osteopenic disease that has been described in calves, lambs, puppies, domestic cats, mice, and tigers involving bone, dentin, tendons, and sclera which are composed primarily of type I collagen. Interestingly, other type I collagen-rich tissues, such as the skin, are rarely affected. Typical clinical findings include bone fractures, joint laxity (tendon hypoplasia), defective dentin (fragile/fractured teeth with translucent pink discoloration), and scleral thinning (blue discoloration). Animals born alive often cannot stand.[1,3,7,8]

The pathogenesis involves a defect in osteoblastic/odontoblastic production of type I collagen. In some cases, decreased synthesis of noncollagenous proteins is involved (e.g. osteonectin). As pointed out by the contributor, in humans, and most likely in animals, OI is primarily due to mutations in one or both genes that code for type I collagen. Defects in COL1A1 or COL1A2 genes have been found in mice and Golden Retrievers. In cases in which mutations are not found in these genes, alterations in genes for enzymes responsible for posttranslational modification of collagen should be evaluated.[3,7]

The primary histomorphologic lesion in OI is osteopenia. Growth plates are not affected as cartilage is composed primarily of type II collagen. There is deficient deposition of osteoid on cartilage spicules and the chondro-osseous complex persists into the metaphysis. The metaphysis may have multiple growth arrest lines due to formation of transverse trabeculae. Trabeculae are not modeled (retention of cartilage cores), but are resorbed at the metaphyseal-diaphyseal junction. The marrow cavity contains loose mesenchymal tissue. In severe cases, there is much less trabecular bone than normal. In some cases, the amount and histologic appearance of bone is normal, but there is evidence of fracture disease. In these cases, bone fragility is most likely due to errors in helix-formation or cross-linking of tropocollagen molecules. Osteoblasts can appear normal or small. Additionally, there is a delay in compaction of cortical bone in which the cortices are composed of spicules of woven bone with large vascular spaces. Dentin is dysplastic and thin (dentinogenesis imperfecta).[1,7]

The moderator stressed that teeth should always be collected in suspected cases of OI as the qualitative and quantitative changes present in the dentin are specific for the

disease; whereas the bony changes are secondary and can be non-specific lesions due to a variety of causes (e.g. osteopenia due to disuse, nutritional causes, etc.). Histologically, dental tubules are short, tortuous, and sometimes absent.

The differential diagnosis for multiple fractures in young dogs includes trauma, nutritional or renal secondary hyperparathyroidism, and OI.

OI is best defined in cattle and has been reported in Charolais and Hostein-Friesians in Denmark as well as in Holsteins in the U.S. and Australia. Several chemical defects occur in the bone tissue of calves with OI including the following:

1. Decreased apatite crystal size (American, Australian)
2. Reduced amount of type I collagen
3. Levels of osteonectin decreased to less than 10% of normal (American)
4. Decreased bone acidic plasma proteins (American, Australian)
5. Decreased bone proteoglycans (American, Australian)
6. Decreased bone sialoprotein (American)
7. Decreased phosphophoryn – dentin-specific protein
8. Markedly deficient levels of an osteonectin-like protein of dentin

Calves with notable bone fragility can have bone that is histologically normal without obvious reduction in bone mass. These calves most likely become osteopenic secondary to disuse due to bone pain from fractures.[1,7,8,9]
OI in lambs is similar to calves in that joint laxity, reduced tendon size and increased fragility of long bones are present; however, teeth and collagen and osteonectin levels are normal.[1]

Fragilitas ossium (*fro*), an autosomal recessive mutation in the mouse, is similar to the severe form of OI.[1]
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References:
1. Palmer N: Bones and joints. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 42-44. Academic Press, Inc., San Diego, California, 1993
2. Rauch F, Glorieux FH: Osteogenesis imperfecta. Lancet 363:1377-1385, 2004
3. Seeliger F, Leeb T, Peters M, Brügmann M, Fehr M, Hewicker-Trautwein M: Osteogenesis imperfecta in two litters of Dachshunds. Vet Pathol 40:530-539, 2003
4. Campbell BG, Wootton JAM, MacLeod JN, Minor RR: Clinical signs and diagnosis of osteogenesis imperfecta in three dogs. J Vet Am Vet Med Assoc 211:183-187, 1997
5. Agerholm JS, Lund AM, Block B, Reibel J, Basse A, Arnbjerg J: Osteogenesis imperfecta in Holstein-Friesian calves. Zentralbl Veterinarmed A 41:128-138, 1994
6. Campbell BG, Wootton JAM, MacLeod MH, Minor RR: Canine COL1A2 mutation resulting in C-terminal truncation of pro-_2(I) and severe osteogenesis imperfecta. J Bone Miner Res 16:1147-1153, 2001

7. Weisbrode, SE: Bones and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1065-1066. Mosby Elsevier, St. Louis, Missouri, 2007
8. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 909-910. Williams & Wilkins, Baltimore, Maryland, 1997
9. Denholm LJ, Cole WG: Heritable bone fragility, bone laxity and dysplastic dentin in Friesan calves: a bovine syndrome of osteogenesis imperfecta. Aust Vet J 60(1):9-17

SLIDE 39
CONFERENCE 10 / CASE III – N312/99 (AFIP 2810381)

Signalment: 10-year-old, male, Springer Spaniel, canine

History: An immovable, smooth contoured, hard mass on the right dorsal cranium increased in size over a 6 month period with the growth rate accelerating over the final 6 weeks. The overlying skin was non-adherent. The animal was euthanized.

Gross Pathology: Carcass of moderate nutritional body condition. A large (~7x6 cm), multinodular, firm to hard mass extended from the dorsal cranium to the right of midline. On sectioning the mass contained multiple cavitations and gritty foci and extended into the caudal cranial cavity compressing the caudal cerebrum, the cerebellum, and brainstem. The mass extended caudally as far as the atlas. Multiple subpleural hard nodules, 1-5 mm in diameter were detected. Prostatic enlargement was apparent.

Histopathologic Description: Extending to the margins of the section are multiple irregular nodules containing varying amounts of intermixed cartilaginous and osseous tissue. Nodules are separated by and blend with surrounding dense fibrous stroma. Densely packed spindle-shaped cells at the periphery of the nodules contrast with plumper ovoid cells surrounded by basophilic cartilaginous or paler staining interconnecting spicules of osteoid matrix in more central locations. Atypical mitotic figures and nuclear hyperchromasia are noted. One to two mitotic figures per hpf are observed in peripheral areas. Mineralization of matrix extends out from the center of nodules. Necrosis of cells within lacunae and multinucleate giant cells (osteoclasts/chondroclasts) are present in some areas.

The lung lesions have a similar microscopic appearance (not included).

Contributor's Morphologic Diagnosis: Skull: Multilobular tumor of bone.

Contributor's Comment: Multilobular tumors of bone are infrequently reported, locally aggressive neoplasms usually involving the membranous bones of the canine skull. They are usually found as solitary masses in mature medium to large breed dogs. Synonyms of the lesion are chondroma rodens, calcifying aponeurotic fibroma, juvenile aponeurotic fibroma and cartilage analog fibromatosis. These tumors can produce

clinical signs as a result of their compression of adjacent brain of lacrimal duct. They are most commonly found on the temporo-occiptal area and zygomatic process of the skull. Locally aggressive growth and recurrence following surgery is the typical pattern of behavior with pulmonary metastases occurring late in the clinical course.

AFIP Diagnosis: Bone, skull (per contributor): Osteosarcoma, chondroblastic, Springer Spaniel (*Canis familiaris*), canine.

Conference Comment: Although conference participants carefully considered the contributor's diagnosis of multilobular tumor of bone, they felt that the histomorphology is consistent with a chondroblastic osteosarcoma. The characteristic pattern of multilobular tumor of bone is not present in this case, i.e., the repetitious tri-laminar appearance of multiple lobules of centrally located cartilage or bone surrounded by plump mesenchymal cells that are further bounded by interlobular fibrous septa.[2,3,4] Osteosarcomas are the most common primary bone neoplasm in dogs comprising 80-85% of canine bone tumors and occur most commonly in older, male, large-breed dogs. Metastasis is common, primarily to the lung and lymph nodes. Osteosarcomas of the canine axial skeleton metastasize less readily. Common primary sites in the dog include the distal radius, proximal humerus, distal femur, and the distal tibia. The front limbs are affected twice as often as the hind limbs. Osteosarcomas can also occur in other bones such as the ribs, vertebrae, and skull, as in this case. Rarely, extraskeletal osteosarcomas may arise in soft tissue. Most osteosarcomas originate centrally from the medulla and display more malignant behavior than osteosarcomas of periosteal origin. Osteosarcomas of periosteal origin include periosteal osteosarcomas that behave like central osteosarcomas and parosteal (juxtacortical) osteosarcomas with a high degree of structural differentiation, slower growth, and a better prognosis than central osteosarcoma.[2,3,5,6]

No specific cause of osteosarcoma has been established; however, several associations have been made. Osteosarcomas have been associated with bone infarctions, previous fractures, and the use of metallic fixation devices in domestic animals. Osteosarcomas of viral origin have been reported in mice.[2,3,5]

Grossly, osteosarcomas have a gray-white appearance and lyse and replace normal bone extending into adjacent soft tissues, but do not penetrate articular cartilage and invade into joint spaces. Areas of infarction may be present characterized by large pale areas surrounded by a zone of hyperemia. Cortical bone is usually destroyed with varying amounts of reactive periosteal bone formation. Pathologic fractures are not uncommon.[2,3]

Osteosarcomas are malignant neoplasms in which neoplastic osteoblasts form osteoid, bone, or both and can be classified based on the matrix produced, the predominant cell type involved, radiographic appearance (lytic, sclerotic, or mixed) and origin (central, juxtacortical, periosteal).[3]

Classification based on matrix produced:[2,3]

1. Simple – produce osteoid and bone
2. Compound – produce osteoid, bone and cartilage
3. Pleomorphic – anaplastic with only small islands of osteoid

Classification based on cell type and activity:[2,3,5]

1. Osteoblastic – anaplastic osteoblasts and plump to spindle-shaped osteogenic precursor cells with angular borders; eccentrically located, hyperchromatic nuclei; dark staining cytoplasm
2. Chondroblastic – neoplastic bone and cartilage produced
3. Fibroblastic – spindle cell population in early lesions resembles fibrosarcoma, later tumor cells form tumor bone; better prognosis than other types
4. Poorly differentiated – malignant cells produce small amounts of osteoid and occasionally spicules of tumor bone; malignant mesenchymal cells vary from small, reticular-appearing cells to large, pleomorphic, sarcoma cells; highly aggressive; lytic lesions lead to pathologic fracture

Giant cell type osteosarcomas resemble osteoblastic osteosarcomas, but contain large areas in which giant cells predominate and must be differentiated from malignant giant cell tumor of bone.[5]

Telangiectatic osteosarcomas are uncommon and are composed of osteoblasts, osteoid, and large-cystic, blood-filled cavities lined by malignant osteoblasts rather than endothelium. The tumor metastasizes easily and is highly fatal.[2,3,4]

Osteosarcomas are rare in animals other than the dog and cat. In the cat, osteosarcomas account for 70% of feline bone tumors with the hind limbs most commonly affected. As in the dog, feline osteosarcomas are commonly of medullary origin. In contrast to dogs; however, metastasis occurs less frequently. Additionally, feline axial osteosarcomas are more likely to metastasize than are those arising in long bones. In horses, sheep and cattle, most osteosarcomas involve the head, especially the mandible. Osteosarcomas are rare in pigs, but have been occasionally seen in extremely young animals.[2,5,6]

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References:

1. Weisbrode SE, Doige CE: Bone and joints. In: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 523-524. Mosby Inc., St. Louis, Missouri, 2001
2. Palmer N: Bones and joints. In: The Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 130-132. Academic Press, San Diego, California, 1993

3. Weisbrode, SE: Bones and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1086-1091. Mosby Elsevier, St. Louis, Missouri, 2007
4. Slayter MV, Boosinger TR, Pool RR, Dämmrich, Misorp W, Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, Second Series, vol. I, pp. 13, 39-40, 9-11, 27-32, Armed Forces Institute of Pathology, Washington, D.C., 1994
5. Thompson KG, Pool RR: Tumors of bones. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 263-283. Iowa State Press, Ames, Iowa, 2002
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 940-941. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 40
CONFERENCE 10 / CASE IV – 06-1264 (AFIP 3026963)
Signalment: Seven-year-old, intact, male, Bassett Hound

History: The dog was reported to have a skin mass and protrusion of the penis for an "extended period of time". The dog was euthanatized by the local humane society because of alleged neglect by the owner.

Gross Pathology: Findings pertinent to the submitted tissue: Approximately four inches of the penis protruded from the prepuce and was firm and dark red with ulceration and congestion of the tip. The bulbus glandis was one and a half inches in diameter and congested. Maggots were present on the penis. A one inch diameter semi firm subcutaneous mass was present on the left shoulder which on cross section was tan to dark brown with gray layers of soft material. Within the caudal abdominal cavity, there was a 12 cm, lobulated, rubbery to hard, round, irregular, white gritty mass involving the pelvis, all sacral vertebral bodies, the bodies of the proximal caudal vertebrae, and the bodies of the caudal lumbar vertebrae with sparing of the intervertebral discs. On cut section, the mass was hard and gritty. A 6 cm mass was present in the right perianal area as was a 5 cm diameter mediastinal lymph node with both consisting of the same material as the larger mass. The mediastinal mass was obstructing lymphatic drainage. Approximately twenty-five percent of the lung tissue involving all lobes was replaced by multifocal firm to hard white nodules ranging in size from 3-5 cm that were gritty on cut surface. The remaining lung was mottled dark red.

Histopathologic Description: Vertebrae: Portions of annulus fibrosis are present with most of the vertebral bodies on either side of the annulus replaced by a mass composed of anastamosing stratified squamous epithelial cells with large coalescing areas of necrotic (coagulation) epithelium ("ghost" cells) and extensive woven bone formation. The limited amount of pre-existing bone present has undergone marked osteolysis at the interface with the infiltrating mass.

Contributor's Morphologic Diagnosis: Vertebral bodies: Metastatic (malignant) pilomatricoma with bone lysis and reactive bone formation.

Contributor's Comment: The subcutaneous shoulder mass was a malignant pilomatricoma and was presumed the primary. The masses in the pelvis, vertebrae, lung and mediastinal lymph nodes were the same processes and presumed metastatic sites. This mass likely impinged on lumbar and sacral nerves causing damage and impaired neurologic function leading to the paraphimosis.

Pilomatricoma, also referred to as Malherbe's epithelioma or calcifying epthelioma, is a benign tumor of the hair follicle showing matrical differentiation.[1] Pilomatricomas are most frequently diagnosed in the dog, accounting for between 1 and 3 per cent of all dog skin tumors and are rare in other domestic animals.[2,3] According to Goldschmidt and Hendrick, these tumors typically arise on the neck, thorax, back, and tail, and take the form of a solitary, well-marginated, firm, and freely moveable mass affecting the dermis and hypodermis with alopecia and ulceration of the overlying skin.[1] On cut surface, the tumors consist of lobulated gray-white, chalky tissue with occasional areas of mineralization. Histologically, the lobules are characterized by zones of two different cells types: basophilic cells resembling hair matrix cells at the periphery and necrotic, keratinized 'ghost cells' centrally. Calcification and osseous metaplasia are frequently seen within the ghost cell region. Malignant pilomatricoma, or pilomatrix carcinoma, is extremely rare, and has been described only in dog and man. Histological features are the same as those of the benign variant, but the basal cells are invasive into the adjacent tissue and lymphatic invasion may be seen at the tumor's margins.[1] Published cases of malignant pilomatricoma in dogs have described metastases to lymph nodes, lung, and, in two cases, bone.[2,4] Both cases of bone metastases involved the thoracic vertebrae and resulted in neurological deficits in the limbs. The primary tumors in these cases were likely cutaneous nodules removed approximately one year prior to presentation of metastases. However, only one of these cutaneous tumors was examined histopathogically and diagnosed as pilomatricoma. No skin tumors were present in either dog at the time of death.

Skeletal metastasis in dog

Cooley and Waters conducted a study of 19 dogs that showed skeletal metastases as the initial clinical manifestation of metastatic carcinoma.[5] They found that the most common sites for metastasis to the skeleton were the axial skeleton and proximal long bones. Only 4 of the 36 skeletal carcinoma sites in these dogs occurred distal to the elbow. The primary sites most frequently identified in this study were mammary gland, prostate, and bladder.

Factors in bone metastasis

Blood flow is a major determinant of the site of skeletal metastasis.[6] In both humans and dogs, skeletal metastases show a predilection for the most heavily vascularized areas of the skeleton – the vertebral column, ribs, and proximal ends of long bones.[5,6] However, it is clear in human medicine that bone is a favored site of metastasis for certain solid tumors, including breast and prostate carcinoma, suggesting that there are more specialized processes at work than random hematogenous seeding.[6] Tumor cell metastasis is a complex, multi-step process involving interactions between the tumor cells and the microenvironment of the host tissue. There are several features of bone that make it a ready site for tumor metastases. Bone is a major storehouse for growth

regulatory factors including transforming growth factor _, bone morphogenic proteins, platelet-derived growth factor, and many others that may enhance the survival rate of certain tumors and facilitate their aggressive behavior in this site. Local bone resorption spurred by skeletal mesatsasis may work to release these factors from the bone, resulting in a continuous cycle of tumor growth stimulation and osteoclastic bone destruction. In addition, a number of chemoattractant factors produced by bone marrow stromal cells and osteblasts, such as monocyte chemoattractant protein 1 and stromal cell derived factor-1, may play a key role in directing cancer cells to their metastatic destination. Recognizing the importance of these complex interactions has led to the concept of therapeutic interventions aimed at blocking the expression of growth factors that are critical to the metastatic process.[7]

Also critical to the migration of cancer cells from primary to metastatic sites are the interactions between tumor cells and vascular endothelia. Inflammatory cytokines, adhesion molecules, and chemotactic factors produced by endothelial cells may influence the passage of tumor cells through the vasculature as well as their eventual arrest at the metastatic site.[8] Cancer types showing a propensity for bone metastasis may express adhesion factors specific to bone marrow-derived endothelial cells, as was suggested by studies of prostatic carcinoma and bone-homing myeloma.

<u>Mechanisms of heterotopic ossicifaction in pilomatricomas</u>
Because the ghost cell regions of pilomatricomas are necrotic, the mineralization frequently associated with these tumors is presumed to be dystrophic. The mechanism of heterotopic bone formation within carcinomas is not understood. Studies have speculated that stimuli generated by malignant epithelial cells induce pluripotent mesenchymal cells to become osteoblasts, which go on to produce metaplastic bone.[9] A case study by Kypson et al. of osseus metaplasia in a rectal adenocarcinoma found overexpression of bone morphogenic protein 2 (BMP-2), a known inducer of osteoblastic differentiation, within the tumor cells.[9] BMP-2 secretion likely results from random activation of the BMP gene in certain cells. Although heterotopic ossification in rectal carcinoma is rare, this finding offers an intriguing glimpse into the complex interactions occurring between epithelial cancers and their surrounding stroma. Stromal changes, which were once believed to be largely reactive in nature, may play an important part in carcinoma progression.[10]

A 2006 study by Rifas proposed that T-cell cytokines released at local sites of inflammation may induce differentiation of local mesenchymal stromal cells into osteoblasts and thereby play a major role in heteoptropic ossification in chronic inflammatory diseases.[11] The study demonstrated that activated T-cell conditioned medium effectively induced BMP-2 and alkaline phosphatase production in human mesenchymal stromal cells in culture.

AFIP Diagnosis: Bone, vertebral body: Malignant pilomatricoma, metastatic, Bassett Hound (*Canis familiaris*), canine.

Conference Comment: The contributor provides an excellent and thorough overview of malignant pilomatricomas to include histomorphologic and gross appearance, skeletal metastasis in the dog, factors in bone metastasis, and mechanisms of heterotopic ossification in pilomatricomas.

The mechanisms of heterotopic ossification in pilomatricomas generated discussion in conference regarding epithelial to mesenchymal transitions. Some believe that members of the transforming growth factor (TGF-beta) family of growth factors can initiate and maintain epithelial to mesenchymal transitions while others believe that there is no convincing evidence that epithelial cells are able to convert to mesenchymal cells *in vivo*.[12,13]

Readers are encouraged to read references 12 and 13 for further information on this controversial topic.

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References:

1. Goldschmidt MH, Hendrick, MJ: Tumors of the skin and soft tissues. In: Tumors of Domestic Animals, ed. Meuten DJ, 4th ed., pp. 61-63. Iowa State Press, Ames, Iowa, 2002
2. Rodriguez F, Herraez E, Rodriguez JC, Gomez-Villamandos A, Espinosa de los Monteros A: Metastatic pilomatrixoma associated with neurological signs in a dog. Vet Rec 137:247-248, 1995
3. Madewell BR, Theilen GH: Tumors and tumor-like conditions of epithelial origin. In: Veterinary Cancer Medicine, eds. Thelien GH, Madewell BR, 2nd ed., p. 257. Lea & Febiger, Philadelphia, Pennsylvania, 1987
4. Van Ham L, van Bree T, Maenhout T, Tshamala M, Broekart D, Hoorens J, Mattheeuws D: Metastatic pilomatrixoma presenting as paraplegia in a dog. J Small Anim Pract 32:27-30, 1990
5. Cooley DM, Waters DJ: Skeletal metastasis as the initial clinical manifestation of metastatic carcinoma in 19 dogs. J Vet Intern Med 4:288-293, 1998
6. Mundy GR: Mechanisms of bone metastasis. Cancer Oct15;80(8 Suppl):1546-1556, 1997
7. Roodman GD: Role of stromal-derived cytokines and growth factors in bone metastasis. Cancer Feb 1;97(3 Suppl):733-738, 2003
8. Choong PFM: The molecular basis of skeletal metastasis. Clin Orthop Relat Res Oct.;(415 Suppl)S19-31, 2003
9. Kypson AP, Morphew E, Jones R, Gottrfried MR, Seigler HF: Heterotropic ossification in rectal cancer: rare finding with a novel proposed mechanism. J Surg Oncol 82:132-136, 2003
10. Magliocco AM: Commentary. J Surg Oncol 82:137, 2003
11. Rifas L: T-cell cytokine induction of BMP-2 regulates human mesenchymal stromal cell differentiation and mineralization. J Cell Biochem Jul1;98(4):706-14, 2006

12. Zavaldi J, Bottinger EP: TGF-beta and epithelial-to-mesenchymal transitions. Oncogene Aug 29;24(37):5764-5774, 2005
13. Tarin D: The fallacy of epithelial to mesenchymal transition in neoplasia. Cancer Res 65(14):5996-6001, 2005

SLIDE 41
CONFERENCE 11 / CASE I – 05RD1701 (AFIP 3028106)

Signalment: Mack: 11-year-old, male, neutered, Jack Russell Terrier, *Canis familiaris*, canine

History: The following is a chronological outline of ophthalmic tissue submissions to the Comparative Ocular Pathology Laboratory of Wisconsin (C.O.P.L.O.W.) at the University of Wisconsin-Madison, School of Veterinary Medicine from the previously described canine patient. The slides that we have submitted to you are from the left eye enucleation that was performed on October 3, 2005.

May 11, 2005: Mack had a 1-month history of having a prominent nodule in/on the medial aspect of the superior cornea of the left eye, which was non-responsive to steroid treatment. The submitting DVM surgically removed and submitted the nodule to C.O.P.L.O.W.

July 6, 2005: A second nodule appeared in/on the medial aspect of the inferior cornea, near the limbus, of the left eye and a keratectomy was performed and the sample was submitted to C.O.P.L.O.W. There was no regrowth of the first nodule at the previous excision site. A lesion in the right eye was reported at this time as having regressed with topical steroids.

October 3, 2005: The left eye was enucleated and submitted to C.O.P.L.O.W. at this time there was a larger corneal mass in/on the lateral aspect of the eye. This is the specimen submitted for the AFIP WSC (05RD1707). At this time there was also neovascularization and corneal edema reported in the medial aspect of the right eye.

December 12, 2005: A keratectomy was performed on the right eye to remove a lesion that was non-responsive to steroids/cyclosporine and the sample was submitted to C.O.P.L.O.W. The submitting DVM was at this time initiating treatment with an oral synthetic retinoid. The diagnosis in the second eye was the same as the first.

Gross Pathology: The tissue submitted is the formalin-fixed left globe and lid. The globe is distorted by an exophytic solid tan mass involving the axial cornea and extending peripherally. Gross digital images have been provided.

Histopathologic Description: Histologically, the mass is made up of an intense cellular infiltrate. Superficially, the cellular infiltrate consists of a fairly monomorphic

population of lymphocytes with large nuclear to cytoplasmic ratios. In several areas, they exist as solid sheets. In other areas, they exist as aggregated clusters within the epithelium and abut on the posterior aspect of the epithelium. At the margins, there is granulation tissue and in some areas, a histiocytic inflammatory infiltrate. The rest of the cornea shows superficial corneal stromal fibrosis and vascular infiltrate but no clear evidence of neoplasia. Although there are tumor cells at the margins of the cornea, the globe was trimmed (lids removed) at the C.O.P.L.O.W. Remaining structures of the globe are within normal limits. Histologically within the lids, a severe lymphoplasmacytic inflammatory infiltrate subtends and sparsely invades the conjunctival epithelium (lids were sectioned separately and are not submitted for AFIP WSC).

The neoplastic cells within this sample are a CD3 positive, T-Cell lineage (a digital image has been provided of this special staining). There are also few, scattered HM57, B-Cells seen within the neoplastic cell population (picture not provided).

Contributor's Morphologic Diagnoses:
1. Corneal epitheliotrophic lymphoma (mycosis fungoides)
2. Lymphoplasmacytic conjunctivitis

Contributor's Comment: The nodular tissue sample removed from the left eye on May 11, 2005 was diagnosed as corneal lymphoma, with cells staining positive for both CD3 (T-cells) and HM57 (B-Cells). The corneal tissue from the keratectomy performed on July 11th, 2005, from a different corneal location in/on the left eye, was diagnosed as an epitheliotrophic lymphoma (mycosis fungoides) with dirty margins. IHC cell marker staining was not performed on this sample. The left eye was then enucleated on October 3, 2005, after Mack presented with a third and larger corneal lesion. This enucleation is the sample that has been submitted to the AFIP WSC (05RD1707). The keratectomy sample that was performed on the right eye on December 12, 2005 was diagnosed as an epitheliotrophic corneal T-cell lymphoma (mycosis fungoides).

AFIP Diagnoses:
1. Eye, limbus: Epitheliotropic lymphoma, Jack Russell Terrier (*Canis familiaris*), canine.
2. Eye, cornea: Keratitis, chronic-active, diffuse, moderate.

Conference Comment: This case was unique in that the cornea is an unusual location for epitheliotropic lymphoma. The moderator emphasized that tumors do not occur in the normal cornea as tissues with no mitotic activity cannot give rise to neoplasms. There must be pre-existing corneal disease, such as cutaneous metaplasia that occurs secondary to repeated trauma, to allow for tumor formation at this site.

Conference participants briefly reviewed the differential diagnosis for limbal masses in the dog to include nodular granulomatous episcleritis and amelanotic conjunctival melanoma. All other neoplasms at this site are rare in the moderator's experience.

Immunohistochemical staining performed at the AFIP revealed diffuse, strong, cytoplasmic reactivity of neoplastic cells with CD3 consistent with T cell origin.

Cutaneous epitheliotropic lymphoma (mycosis fungoides) is an uncommon, slowly progressive disease characterized by neoplastic infiltration of the epidermis and adnexal structures. In dogs, cutaneous epitheliotropic lymphoma cells are usually CD8+. These T cells display _1 and _2 integrins that help them localize to the epithelium. Epitheliotropic lymphoma is a disease of aged dogs affecting dogs older than 10 years of age and can mimic virtually any inflammatory disease of the skin. In dogs, the course of the disease varies from a few months to 2 years. Eventually, the lesions may extend to lymph nodes and, rarely, to other organs. Chemotherapeutic protocols useful for other lymphomas are of no value. Sézary syndrome rarely occurs with any cutaneous clinical presentation; circulating tumor cells (large convoluted T lymphocytes) are required for this diagnosis. [1,2,3,4,5]

Clinically, epitheliotropic lymphoma may present as generalized pruritic erythema and scaling (exfoliative erythroderma); mucocutaneous ulceration; solitary or multiple plaques or nodules; or infiltrative or ulcerative oral mucosal disease. Many consider the various clinical syndromes to be temporal stages of a progressive disease.[1,2]

The key histomorphologic feature of epitheliotropic lymphoma is the tropism of neoplastic lymphocytes for the epidermal or mucosal epithelium and adnexal structures, especially the follicular wall. The intraepithelial neoplastic lymphocytes are either diffusely distributed within the epithelium or form discrete aggregates referred to as Pautrier's microabscesses or microaggregates which are pathognomonic for epitheliotropic lymphoma. In some cases, complete obliteration of hair follicles and adnexal glands may occur. Infiltration of apocrine glands can be striking in some cases and is highly diagnostic for epitheliotropic lymphoma since inflammatory infiltrates do not generally occur in this area. Neoplastic cells are pleomorphic often with a cerebriform nucleus (mycosis cell). Mycosis cells appear in the epithelium as individual cells with a clear halo of spongiosis.[2,4,5]

Cutaneous epitheliotropic lymphoma occurs less commonly in cats than in dogs. The clinical presentations are similar to those seen in dogs; however, feline lesions most frequently affect the face, eyelids, mucocutaneous junctions, elbows, and the trunk. Cutaneous epitheliotropic lymphoma also occurs in cattle and, rarely, in horses.[2,3]

Tumors with epithelial tropism include the following:
1. Melanoma
2. Epitheliotropic lymphoma
3. Histiocytoma

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References:
1. Donaldson D, Day MJ: Epitheliotrophic lymphoma (mycosis fungoides) presenting as blepharoconjunctivitis in an Irish setter. Journal of Small Animal Practice 41:317-320, 2000
2. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat Clinical and Histopathologic Diagnosis, 2nd ed., pp. 876-882. Blackwell Publishing Professional, Ames, Iowa, 2005
3. Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF: Histological Classification of Hematopoietic Tumors of Domestic Animals, Second Series, vol. III, pp. 42-43. Armed Forces Institute of Pathology, Washington, D.C., 2002
4. Yager JA, Scott DW: The skin and appendages. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 731-732. Academic Press, Inc., San Diego, California, 1993
5. Jacobs RM, Messick JB, Valli VE: Tumors of the hemolymphatic system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 140-141. Iowa State Press Ames, Iowa, 2002

SLIDE 42
CONFERENCE 11 / CASE II – 05-25602 (AFIP 3024121)

Signalment: Tissue from a 7-year-old, neutered male, domestic short-hair cat, KitCat

History: The left eye of this cat was eventually enucleated after a 19 month history of a progressive corneal lesion. When first presented, the cat had a non-healing corneal ulcer. The lesion appeared to bother the cat, and he would rub at it. There was no response to various antimicrobials, lysine or vidarbine 3%. Over time the left cornea became a mottled yellow-pink pale and granular over its entire surface. When the cat began to develop a similar lesion on the right eye, the left eye was enucleated for diagnostic purposes.

Gross Pathology: None.

Histopathologic Description: The eye is characterized by very severe corneal epithelial and stromal inflammation. The corneal epithelium varies in width and is disorganized. Individual keratinocytes have become rounded and detached, especially in the basal layer. Squamous metaplasia is observed in the outer layers and eosinophils are consistently present in the epithelium. A segment of corneal ulceration is associated with degenerate eosinophils. The irregular epithelium contains several facet-like indentations. The corneal stroma is edematous superficially, with the added presence of an inflammatory infiltrate in the superficial and middle layers, particularly the central cornea. Numerous eosinophils are present, mixed with macrophages, plasma cells and lymphocytes in the central cornea. Inflammation extends across the width of the stroma, with segmental accumulation of cells on the corneal side of Descemet's membrane. Melanosis and vascularization extend from the periphery to the

center of the cornea and affect the middle to deep portions of the stroma. There is focal conjunctival ulceration, associated with mixed eosinophilic and neutrophilic infiltrates.

Contributor's Morphologic Diagnosis: OS: Eosinophilic keratoconjunctivitis, severe, with corneal ulceration, keratinocyte necrosis, melanosis and vascular proliferation.

Contributor's Comment: The case demonstrates several types of changes that interfere with corneal transparency. Any interference with corneal stromal architecture, including stromal edema, stimulates vascular growth, promotes melanosis or alters the epithelial architecture and disrupts transparency.[1] The normal cornea is transparent because of non-keratinized epithelium, an even, lamellar arrangement of dehydrated stromal collagen fibers, as maintained by functional corneal endothelium, and an absence of blood vessels and pigmentation. The pink to red lesion described grossly is consistent with stromal neovascularization and commonly occurs in eosinophilic keratitis.

Proliferative or eosinophilic keratitis/keratoconjunctivitis is a progressive corneal disease with superficial neovascularity beginning near the limbus, and stromal edema at the leading edge of the lesion. Ulceration is often present and considerable areas of the cornea may stain with fluorescene.[2] The disease progresses to become an irregular mottled mass that is often gritty. Bilateral lesions occur in about a quarter of the cases. Neutered males and domestic short-hairs are favored. Pathology is characterized by a chronic inflammatory response and vascularization. Inflammation is dominated by eosinophils, mast cells and plasma cells superficially and lymphocytes in the deeper stroma. Predominantly observed in the US and UK, this disease has also been recently reported from Europe.[3]

The etiology of this disease is unknown with certainty. There is no association with eosinophilic granuloma complex, and variable indication in 30% to 76%(PCR) of cats that feline herpesvirus 1 (FHV1) plays a role in its pathogenesis.[4,5] Response to corticosteroids is generally favorable but with a high recurrence rate.[6] Topical antivirals administered to this cat are inconsistently effective. Eosinophilic conjunctivitis may occur without keratitis in cats, but the inability to detect FHV1 suggests that it may be a different disease.[7] This inflammatory infiltrate is unique to suspected herpetic keratitis in this species. Eosinophilic keratitis also occurs in horses, also not in conjunction with dermal eosinophilic granuloma.[8,9] In that species it has been speculated to be a result of *Thelazia*, *Habronema* or *Onchocerca* infections. In humans, a similar disease, vernal conjunctivitis, is an extremely severe proliferative conjunctivitis that rarely is associated with keratitis, and has a seasonal occurrence.[10] Vernal conjunctivitis and eosinophilic responses in the conjunctiva may be linked to atopy, and humans with vernal conjunctivitis frequently have elevated IgE in tears.[11]

Other ocular manifestations attributed to feline herpesvirus 1 infection include chronic conjunctivitis, synblepharon, keratoconjunctivitis sicca, stromal keratitis and corneal sequestrum.[12] Concurrent respiratory disease may or may not be present. FHV1 is the

only documented viral cause of corneal ulceration. Keratitis probably results from viral reactivation, and may affect the epithelium or stroma.

AFIP Diagnosis: Eye: Keratitis, eosinophilic and lymphoplasmacytic, chronic, diffuse, marked, with edema and superficial eosinophilic coagula, domestic short hair (*Felis domesticus*), feline.

Conference Comment: The contributor provides an excellent overview of the key histomorphologic features and potential etiologies of feline and equine eosinophilic keratitis/keratoconjunctivitis.
The characteristic gross appearance is a white, granular, proliferative lesion that extends inwardly along the corneal surface from the medial or lateral limbus. With time, the entire cornea may be involved. Similar lesions may be present in the adjacent conjunctiva and third eyelid. In some cases, the lesions are exclusively conjunctival. Eosinophilic keratitis typically begins as a unilateral disease that eventually involves both eyes.[4,13]

As discussed by the contributor, the superficial stroma is infiltrated by a mixed population of inflammatory cells to include eosinophils, plasma cells, mast cells, and macrophages. The percentage of each type of cell may vary depending on duration; however, eosinophils are always present and are a requirement for the diagnosis. The granular gross appearance is caused by the degranulation of eosinophils which creates a thick refractile eosinophilic coagulum along the surface of the lesion.[13]

The diagnosis is usually made on the basis of clinical appearance and the presence of eosinophils in a superficial corneal scraping. The moderator stressed that only a few eosinophils with compatible clinical signs are sufficient to make the diagnosis.[13]

There was variability among slides with some sections having multifocal corneal ulcerations. Corneal ulceration is not a typical finding but is reported in 13 to 24% of cases.[1] Some slides had sparse superficial eosinophilic coagulum.

Contributor: Veterinary Medical Diagnostic Laboratory and Department of Veterinary Pathobiology, University of Missouri www.cvm.missouri.edu/vpbio/index.html

References:
1. Moore,PA: Feline corneal disease. Clin Tech Small Anim Pract 20:83-93, 2005
2. Hodges A: Eosinophilic keratitis and keratoconjunctivitis in a 7-year-old domestic shorthaired cat. Can Vet J 46:1034-1035, 2005
3. Spiess BM, Leber A, von Beust BR, Hauser B: [Chronic eosinophilic keratitis in the cat]. Schweiz Arch Tierheilk 133:133-118, 1991
4. Morgan RV, Abrams KL, Kern TJ: Feline eosinophilic conjunctivitis: retrospective study of 54 cases. Vet Comparative Ophthalmol 6:131-134, 1996
5. Nasisse MP, Glover TL, Moore CP, Weigler BJ: Detection of feline herpesvirus 1

DNA in corneas of cats with eosinophilic keratitis or corneal sequestration. Am J Vet Res 59:856-858, 1998
6. Paulsen ME, Lavach JD, Severin GA, Eichenbaum JD: Feline eosinophilic keratitis: a review of 15 clinical cases. J Amer Anim Hosp Assoc 23:63-69, 1987
7. Allgoewer I, Schaffer, EH, Stockhaus C, Vogtlin A: Feline eosinophilic conjunctivitis. Vet Ophthalm 4:69-74, 2001
8. Yamagata M, Wilkie DA, Gilger BC: Eosinophilic conjunctivitis in seven horses, J Amer Vet Med Assoc 209:1283-1286, 1996
9. Ramsey DT, Whiteley HE, Gerding PA Jr, Valdez RA: Eosinophilic keratoconjunctivitis in a horse. J Amer Vet Med Assoc 205:1308-1311, 1994
10. Chamblass SL, Trocme S: Developments in ocular allergy. Curr Opin Allergy Clin Immunol 4:431-434, 2004
11. Zavaro A, Baryishak YR, Smara Z, Sompolinsky D: Extrinsic and idiopathic vernal keratoconjunctivitis? Two cases with dissimilar immunopathology. Brit J Ophthalmol 67:742-745, 1983
12. Andrew SE: Ocular manifestations of feline herpesvirus. J Feline Medicine and Surg 3:9-16, 2001
13. Wilcock BP: Eye, eyelids, conjunctiva, and orbit. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp.1385-1386. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 43
CONFERENCE 11 / CASE III – Ex57J (AFIP 3031286)

Signalment: An enucleated globe from a mature female neutered DSH

History: Exophthalmos, corneal ulceration and ocular pain.

Gross Pathology: The anterior chamber is filled with gelatinous material. There is a central corneal ulcer.

Histopathologic Description: Over the central cornea the epithelium is absent or markedly attenuated. Primarily in the stroma subjacent to the ulcer and to a lesser degree peripherally there is neovascularization with microhemorrhages, mild neutrophilic infiltration and a small amount of necrotic cellular debris. Hemorrhage, vascular hyperplasia and mild lymphoplasmacytic and neutrophilic infiltration are present at the limbus and extend into the conjunctiva. The anterior chamber contains a large amount of proteinaceous material admixed with RBC, a small amount of neutrophils and occasional macrophages. The anterior aspect of the iris is covered by a thin layer of spindle cells (preiridal fibrovascular membrane, PIFM). In the central iris the PIFM extends into the anterior chamber as a very loose spindle cell proliferation admixed with blood and pigment-laden macrophages, some of which stain positively for iron (Fig. 1). Peripherally, the iris is flattened against the cornea by the PIFM with closure of the filtration angle. There is edema of the iridal stroma and ciliary body with

scattered hemorrhages and mild multifocal lymphocytic and lesser neutrophilic infiltration. There is profound retinal atrophy.
Throughout most of its length, the retina is converted into a thin paucicellular band and remaining nuclei are either of Mueller cells or residual outer nuclear layer neurons. There is marked retinal edema with formation of cystic spaces (microcystoid retinal degeneration), retinal and subretinal hemorrhages and mutifocal retinal detachment. Scattered pigmented macrophages are present in the retina and in the subretinal spaces where some show erythrophagocytosis. The retinal pigment epithelium (RPE) is difficult to identify in many areas but occasional hypertrophic RPE cells are seen in areas of retinal detachment. Multifocally in the choroid and retina, arterioles show narrowing of the lumen and marked thickening of the wall by homogenous eosinophilic and PAS-positive extracellular material with loss of underlying structural detail (Figs. 2 and 3). The optic disk (not present in the submitted slide) was depressed (Fig. 4). In the optic nerve there was widespread axonal loss and gliosis. The lens could not be adequately evaluated for technical reasons.

Contributor's Morphologic Diagnosis: Hypertensive choroidal and retinal vasculopathy with diffuse retinal atrophy, multifocal retinal detachment, multifocal hemorrhages, mild lymphocytic and neutrophilic anterior uveitis and ulcerative keratitis.

Contributor's Comment: The degenerative changes in choroidal and retinal arterioles are typical of hypertension. Severe systemic hypertension causes damage to endothelial cells leading to arteriolar dilatation, discontinuity of the endothelial layer, increased permeability and insudation of plasma proteins into the vascular wall.[2] In other cases there may be medial hypertrophy with adventitial fibrosis ('onion-skinning').[6,7] The morphology of affected vessels is seen to advantage in PAS stain and has been referred to as fibrinoid necrosis or hyaline arteriolosclerosis.[2,6,7] Veins are usually unaffected.[2] Arterial retinal vessels are arterioles rather than arteries as, unlike arteries, they lack an internal elastic lamina and a continuous muscular coat.[2] Changes considered secondary to vascular damage include foci of retinal necrosis, exudative retinal separation and intraretinal hemosiderin deposition.[7]

In this eye there is profound retinal atrophy attributable to hypertensive vascular degeneration probably compounded by secondary glaucoma. Hypertension leads to multifocal retinal necrosis which involves the outer retinal layers, including the RPE.[2,4,7] Glaucomatous retinal atrophy is limited to loss of ganglion cells and nerve fiber layer with sustained moderate elevation of intraocular pressure but involves all retinal layers with extremely elevated pressure.[5] Staining of the retina with GFAP showed markedly increased staining in Mueller cells with the retina virtually uniformly GFAP-positive between the inner and outer limiting membranes (Fig. 5). In the normal retina positive staining is present in astrocytes at the vitreo-retinal border (nerve fiber layer) and multifocally within Mueller cells (Fig. 6).[1] Mueller cells increase GFAP expression in response to focal or generalized retinal injury.[1]
The vertebrate retina is inverted when compared to the retina of lower organisms (e.g. cephalopods). In the inverted retina the outer segments of the metabolically active photoreceptors are apposed to the RPE. Photoreceptors have a very high energy

demand. Feline photoreceptors require 3 to 4 times more oxygen than other retinal and CNS neurons for glucose metabolism in the light-adapted state and twice as much in dark-adapted conditions. In order not to compromise vision, vessels are excluded from the outer half of the retina. This results in the paradox that the most energy-dependant part of the CNS is the only region that lacks intrinsic blood vessels. The energy needs of the outer retina are supplied by diffusion of glucose and oxygen from the capillaries of the choroid, collectively termed the choriocapillaris. The chorocapillaris is a thin layer of capillaries separated from the RPE by a basement membrane complex (Bruch's membrane), which is poorly developed in carnivores. The choroid has an extremely high rate of blood flow and its highly fenestrated capillaries are more permeable than those of any other tissue in the body. The outer retina is exposed to near arterial levels of oxygen.[2]

Autoregulation refers to the intrinsic ability of a tissue to maintain its blood flow during changes in perfusion pressure. It operates by altering vascular resistance mostly by modifying the size of the lumen of precapillary arterioles. Excessive perfusion pressure may lead to failure of autoregulation.[2,4] In response to increase in blood pressure retinal arterioles undergo vasoconstriction leading to hyperplasia and hypertrophy of their smooth muscle cells. With sustained vasoconstriction damage to the smooth muscle and endothelial cells ensues and manifests as the vascular changes described above. This is accompanied by leakage of blood and serum into the surrounding retinal tissue leading to edema, hemorrhage and retinal detachment - the typical ophthalmoscopic and macroscopic findings in affected cats.[2,4,7] Although the choroid is not an autoregulatory vascular bed, hypertension-induced injury to this arterial system may cause occlusion of the choriocapillaris leading to necrosis and atrophy of the RPE and outer retinal ischemia.[4]

Enucleated eyes may have other lesions that probably occur secondary to chronic retinal detachment and chronic intraocular hemorrhage. PIFM with its resultant hyphema and neovascular glaucoma are the most notable and were present in this case.[7]
In a minority of slides a moderate amount of neutrophils were present, predominantly within the exudate in the anterior chamber where they formed small groups or a single larger collection. The presence of neutrophils raised the possibility of sepsis. However, since the extent of neutrophilic infiltration was maximal in these sections and was very low in multiple sections from other levels of the globe, and there was no macroscopic or microscopic evidence of a perforation, we consider bacterial infection unlikely.

Systemic hypertension (SHT), generally defined as systolic pressure ≥160-170mmHg is increasingly recognized in older cats.[4] It is most commonly associated with chronic renal failure but the cause and effect relationship between the two remains uncertain.[3,4,7] Many cats with SHT develop hypertensive retinopathy, and ocular lesions are the most commonly detected complication of SHT in cats.[4] Other causes of SHT include hyperthyroidism, which appears to be less commonly associated with ocular signs, diabetes mellitus, chronic anemia and high-salt diet. Primary SHT is relatively rare.[3,4]

Other causes of retinal degeneration in cats include glaucoma, nutritional deficiencies (Feline Central Retinal Degeneration caused by taurine deficiency), hereditary retinal atrophy (best studied in Abyssinian cats), inflammation, toxins (e.g. fluoroquinolone) and senile change.[7] With the exception of glaucoma, these conditions usually manifest initially as degeneration of the photoreceptor outer segments and RPE.[7]

AFIP Diagnoses:
1. Eye, retina and uvea: Vascular fibrinoid change, multifocal, with fibrin, hemorrhage, edema, mild lymphocytic uveitis, retinal atrophy, and preiridal fibrovascular membrane, domestic short hair (*Felis domesticus*), feline.
2. Eye, cornea: Corneal ulcer.

Conference Comment: The contributor provides an excellent summary of the histopathologic changes observed in as well as the pathophysiology of feline hypertensive retinopathy.

Hypertensive retinopathy secondary to systemic hypertension is an increasingly frequent cause of retinal and choroidal lesions and blindness in cats over 10 years of age. It is reported as a complication in 80-100% of cats with systemic hypertension. The three most common causes of hypertension are chronic renal failure, diabetes mellitus, and hyperthyroidism.[4,8]

Typical gross findings include intravitreal and intra- and subretinal hemorrhages, hyphema, retinal edema, and retinal detachment.[3,7]
Conference participants discussed the two most common causes of preiridal fibrovascular membrane (PIFM) formation to include retinal detachment and intraocular neoplasms. PIFMs are also formed following uveitis. PIFMs are simply a layer of granulation tissue on the anterior surface of the iris that forms by budding and migration of capillaries from the iris stroma and recruitment of fibroblasts in response to cytokine mediators of wound healing, e.g., VEGF. If the PIFM migrates across the anterior face of the lens causing pupillary block or across the filtration angle creating a peripheral anterior synechia, glaucoma results. In cases of retinal detachment, separation of the retina from the choroid results in retinal ischemia. VEGF is released into the vitreous and the iris responds with PIFM formation. Ocular tumors that require a stroma, such as iridociliary adenomas, also produce VEGF. Like immature granulation tissue elsewhere in the body, PIFMs are susceptible to hemorrhage and are a frequent cause of hyphema.[9]

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References:
1. Chong NH, Alexander RA, Barnett KC, Bird AC, Luthert PJ: An immunohistochemical study of an autosomal dominant feline rod/cone dysplasia

(Rdy cats). Exp Eye Res 68:51-57, 1999
2. Crispin SM, Mould JR: Systemic hypertensive disease and the feline fundus. Vet Ophthalmol 4:131-140, 2001
3. Komaromy AM, Andrew SE, Denis HM, Brooks DE, Gelatt KN: Hypertensive retinopathy and choroidopathy in a cat. Vet Ophthalmol 7:3-9, 2004
4. Maggio F, DeFrancesco TC, Atkins CE, Pizzirani S, Gilger BC, Davidson MG: Ocular lesions associated with systemic hypertension in cats: 69 cases (1985-1998). J Am Vet Med Assoc 217:695-702, 2000
5. Martin CL: The pathology of glaucoma. In: Comparative Ophthalmic Pathology, ed. Peiffer RL, p. 165. Charles C Thomas, Springfield, Illinois, 1983
6. Schoen FJ, Cotran RS: Blood vessels. In: Robbins Pathologic Basis of Disease, eds. Cotran RS, Kumar V, Collins T, 6th ed., pp. 514-515. WB Saunders and Co., Philadelphia, Pennsylvania, 1999
7. Wilcok BP: The eye and ear. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol.1, p. 506. Academic Press, Inc., San Diego, California, 1993
8. Glaze MB, Gelatt KN: Feline Ophthalmology. In: Veterinary Ophthalmology, ed. Gelatt KN, 3rd ed., pp. 1039-1049. Lippincott Williams & Wilkins, Baltimore, Maryland, 1999
9. Wilcock BP: Eye, eyelids, conjunctiva, and orbit. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp.1369-1370. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 44
CONFERENCE 11 / CASE IV – 05 M 5661 (AFIP 3026800)

Signalment: Domestic short haired cat, 9-year-old, male, castrated (*Felis domesticus*)

History: The cat had been boarded at a veterinary clinic while the owners were away. Upon returning home, the cat collapsed and presented to the hospital in severe respiratory distress. The cat was anemic, had bilateral mydriasis with retinal hemorrhage and bilateral detached retinas. Radiographically, there was mild cardiomegaly and mild rounding of the cardiac silhouette. The cat failed to respond to treatment and the owners elected euthanasia. Prior to this episode, the cat had been apparently healthy.

Gross Pathology: There was a small amount of clear yellow-tinged free fluid within the thorax, and the lungs were diffusely edematous.

Laboratory Results:
WBC: 5.19 (normal 5.5-19.5)
RBC: 1.53 (normal 5.00-10.00)
Hemoglobin: 3.0 (normal 8.0-15.0)
Hematocrit: 11.3 (normal 30.0-45.0)
MCV: 74.0 (normal 39.0-55.0)
MCH: 19.8 (normal 12.5-17.5)

MCHC: 26.7 (normal 30.0-36.0)
Platelet: 43 (normal 300-800)
Band neutrophil: 0.77 (normal 0.0-0.3)
Lymphocyte: 1.29 (normal 1.5-7.0)

Chemistry:
Sodium: 153 (normal 155-165)
Chloride: 119 (normal 123-131)
Bicarbonate: 5.4 (normal 17.0-24.0)
Phosphorus: 10.8 (normal 4.0-7.6)
Magnesium: 3.62 (normal 1.95-3.04)
BUN: 40 (normal 15-35)
Glucose: 275 (normal 70-135)
Total protein: 5.7 (normal 6.1-8.0)
ALT: 185 (normal 20-125)
Total bilirubin: 0.66 (normal 0.01-0.50)
Hemolytic index: 104.0 (normal 0-50)
Anion gap: 33 (normal 12-16)

PT/PTT
PTT: 41.0 (normal 8.9-18.7)

Histopathologic Description: There are multifocal to coalescing intravascular cellular accumulations of bland spindle cells that partially or completely occlude approximately 75-85% of myocardial arterioles. The cells are arranged in tight to loose whorls and nests within vascular lumens. These cells have plump fusiform to oval nuclei with finely stippled and basophilic chromatin, basophilic nucleoli and scant eosinophilic cytoplasm. Nuclei of the cells lack atypia and mitotic figures are rare. Within affected vessels, there are multifocal fibrin thrombi as well as free erythrocytes within small slit-like vascular channels. Affected vessels often are thickened by proliferative adventitial fibroblasts, and are surrounded by mild accumulations of mucinous edema. In some sections, there is mild to moderate multifocal subendocardial and myocardial hemorrhage with mild lymphocytic myocarditis. Multifocal myofibers are swollen, hypereosinophilic and have varying degrees of cross-striation loss (myodegeneration).

Similar intravascular proliferative lesions were also present within the following organs (not submitted): meninges, cerebrum, cerebellum, hippocampus, bone marrow, liver, spleen, lung, pancreas, stomach, small and large intestine, kidney, and choroid of the eye.

Contributor's Morphologic Diagnoses:
1. Heart: Angioendotheliomatosis, reactive, multifocal, marked with multifocal fibrin thrombi, part of Feline Systemic Reactive Angioendotheliomatosis (FSRA) syndrome
2. Brain, bone marrow, liver, spleen, pancreas, small and large intestine, eye and kidney (not submitted): Feline systemic reactive angioendotheliomatosis (FSRA)

Contributor's Comment: The intravascular lesions present in this case closely resemble those described by Rothwell et al in 1985, Straumann et al in 1993, Dunn et al in 1997, and most recently thoroughly reviewed by Fuji et al in 2005.[1,2,3,4] This is a rare idiopathic reactive and proliferative lesion of vascular endothelial cells and pericytes. Immunohistochemical stains in our case were consistent with other previous reports and confirmed the origin of the proliferative cells in that vWF (factor VIII) and vimentin were consistently positive in all the proliferative lesions while cytokeratin, CD18, CD79 and CD3 were negative. No organisms were seen by silver staining (Warthin-Starry). Ultrastructural examination using electron microscopy by others has shown that the endothelial cells are intermixed with pericyte-like cells.[1,3]

Systemic reactive and proliferative intravascular disorders in the feline are extremely rare. Some naming confusion exists with these conditions, but briefly, they are divided into two categories. These are intravascular angiotropic lymphoma and a variant of reactive angioendotheliomatosis (this case). One case of intravascular lymphoma has been reported in the cat, and in that case the intravascular neoplastic round cells had immunohistochemical staining properties of T lymphocytes.[5] In the present case (and in 12 other reported cases), the intravascular globoid cellular proliferations consisted of plump fusiform endothelial cells and immunohistochemically were not marked for T lymphocytes (CD3), B lymphocytes (CD79), and were positive for endothelial cells (vWF/Factor VIII).[1,2,3,4]

This unique and recently named condition in cats bears some semblance to several human disorders, but is in other ways distinctly different.[4] In humans, several cutaneous disorders characterized by proliferative and mixed endothelial cell and pericyte intravascular lesions have been described. Although various descriptive names have been given to these disorders (reactive angioendotheliomatosis (RAE), diffuse dermal angiomatosis, acroangiodermatitis (pseudo-Kaposi's sarcoma), reactive intravascular histiocytosis, glomeruloid reactive angioendotheliomatosis, angiopericytomatosis), all are thought to be variants of cutaneous angiomatoses. In humans, these conditions are thought to be caused by occlusion or inflammation of vascular lumina by a variety of causes such as arteriosclerosis, infections (*Bartonella hensalae*, human immunodeficiency virus), valvular cardiac disease, cholesterol emboli, monoclonal gammopathies, hemolymphoproliferative diseases, immune complex deposition in hypersensitivity reactions and many others, none of which were identified in our case or in previous cases.[6] In humans, these conditions are known to affect only the skin; multiple organ system lesions have not been described as in the present case and previous feline cases.[1,2,3,4] Additionally, this unique condition has not been identified in other domestic or wild animal species.

For a thorough review of comparative pathology, please see reference 4 (Fuji et al).

AFIP Diagnosis: Heart: Reactive angioendotheliomatosis, with fibrin thrombi, domestic short hair (*Felis domesticus*), feline.

Conference Comment: The contributor provides a thorough overview of feline systemic reactive and proliferative intravascular disorders and compares and contrasts them with similar human angioproliferative disorders.

As pointed out by the contributor, in humans reactive (benign) angioendotheliomatosis is usually limited to the skin and may resolve spontaneously. In contrast, the disease in cats is multisystemic (commonly involving the heart and brain) and fatal.[1,2,3,4,6]
Contributor: Iowa State University College of Veterinary Medicine, Department of Veterinary Pathology, http://www.vetmed.iastate.edu/departments/vetpath/

References:
1. Rothwell TL, Xu FN, Wills EJ, Middleton DJ, Bow JL, Smith JS, Davies JS: Unusual multisystemic vascular lesions in a cat. Vet Pathol 22(5):510-512, 1985
2. Straumann KU, Ossent P, Lott-Stolz G: Generalized intravascular proliferation in two cats: endotheliosis or intravascular pseudoangiosarcoma? J Comp Pathol 109(1):99-102, 1993
3. Dunn KA, Smith KC, Blunden AS: Fatal multisystemic intravascular lesions in a cat. Vet Rec 140:128-129, 1997
4. Fuji RN, Patton KM, Steinbach TJ, Schulman FY, Bradley GA, Brown TT, Wilson EA, Summers BA: Feline systemic reactive angioendotheliomatosis: eight cases and literature review. Vet Pathol 42(5):608-617, 2005
5. Lapointe JM, Higgins RJ, Kortz GD, Bailey CS, Moore PF: Intravascular malignant T-cell lymphoma (malignant angioendotheliomatosis) in a cat. Vet Pathol 34(3):247-250, 1997
6. Rongioletti F, Rebora A: Cutaneous reactive angiomatoses: patterns and classification of reactive vascular proliferation. J Am Acad Dermatol 49:887-896, 2003

SLIDE 45
CONFERENCE 12 / CASE I – 03-0212 (AFIP 2986822)

Signalment: 9-month-old female African Grey parrot (*Psittacus erithacus*)

History: This parrot was found dead in a commercial aviary and was submitted for postmortem examination.

Gross Pathology: At necropsy, general body condition was poor; the serosal membranes including air sacs were turbid, wet and covered by few fibrin tags. The liver was enlarged, markedly firm, and had patchy multifocal pale firm areas (Fig.1). The spleen was enlarged and markedly congested.

Laboratory Results: PCR for *Chlamydophila psittaci* was positive on liver and spleen.

Histopathologic Description: Microscopically, there was multifocal hepatocellular individualization, loss and necrosis. Necrotic hepatocytes had pyknotic nuclei and hypereosinophilic cytoplasm and contained myriad intracytoplasmic basophilic inclusions or clusters of organisms (cocci, 1 micron in diameter and stained red against a green background with PVK (Pierce-van der Kamp) stain (Figs 2 and 3). Similar organisms were present in the cytoplasm of the splenic reticuloendothelial cells. Serous membranes were edematous and infiltrated with high numbers of histiocytes (containing similar organisms) and fewer heterophils. Based on the aforementioned lesions and the presence of typical organisms, a tentative diagnosis of avian chlamydiosis was made and confirmed by identification of *Chlamydophila psittaci* (formerly *Chlamydia psittac*i) using PCR.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, lymphocytic and plasmacytic, necrotizing, subacute with myriad intrahepatocellular organisms typical of *Chlamydophila* spp.

Contributor's Comment: *Chlamydophila psittaci* (*C. psittaci)* is an obligate intracellular bacterium that affects a wide variety of birds including psittacines, turkeys, waterfowl, and many species of pet birds causing lesions that range from mild sinusitis and conjunctivitis to severe necrotizing multisystemic disease.[1,2] Chickens are relatively resistant and the incidence of epidemics in commercial breeds is rare.[2] Transmission of chlamydiae is by inhalation and ingestion of contaminated materials. *C. psittaci* is a serious zoonotic pathogen that infects humans by inhalation of contaminated materials causing severe pneumonia (Ornithosis). Avian chlamydiosis is an immediately notifiable disease in many countries and many laboratories require that the handling of infected materials and postmortem be done in a biosafety level III lab. Differential diagnosis for necrotizing hepatitis in parrots should include bacterial septicemia and the infection with psittacid herpesvirus (Pacheco's disease), avian polyomavirus, adenovirus, avian circovirus (Psittacine beak and feather disease virus), and avian reovirus.[1]

AFIP Diagnosis: Liver: Hepatitis, necrotizing, random, moderate, with intrahepatocellular bacteria, African Grey parrot (*Psittacus erithacus*), avian.

Conference Comment: The family Chlamydiaceae has been reclassified into two genera, *Chlamydia* and *Chlamydophila*. Under the new classification, *Chlamydia* includes three species (*C. trachomatis*, *C. muridarum*, and *C. suis*) and *Chlamydophila* includes six species (*C. psittaci*, *C. abortus*, *C. felis*, *C. caviae*, *C. pneumoniae*, and *C. pecorum*).[2]

All chlamydiae are Gram-negative; however, Gram stains are of no practical value in identifying chlamydiae. In some cases, the organisms may be seen in Giminez, Machiavello, Giemsa, or Castañeda stained impression smears of liver, spleen, or air

sacs. Electron microscopy is a good method for definitive diagnosis of *Chlamydophila*. There are three morphologically distinct forms:[1,2,3]

1. Elementary body (EB) – infectious form; 0.2-0.3 um diameter characterized by a highly electron dense nucleoid at the periphery of the EB clearly separated from an electron dense cytoplasm
2. Reticulate body (RB) – intracellular, metabolically active form; 0.5-2.0 um diameter; lacy or reticular nucleus, "hour-glass" profiles when undergoing binary fission
3. Intermediate body (IB) – 0.3-1.0 um diameter; central electron dense core with radially arranged individual nucleoid fibers surrounding the core; cytoplasmic granules tightly packed at the periphery of the IB separated from the core by a translucent zone

Typical gross findings in birds include hepatomegaly, splenomegaly, fibrinous air sacculitis, pericarditis, and peritonitis.[2]
Typical light microscopic findings in birds include multifocal hepatic necrosis, lymphoplasmacytic portal hepatitis, intrahepatocellular bacteria, multifocal splenic necrosis, splenic histiocytosis with intrahistiocytic bacteria, splenic reticuloendothelial cell hyperplasia, and a fibrinous air sacculitis with heterophils and macrophages.[1,3]

Chlamydophila psittaci affects a wide range of hosts. A modified chart of diseases caused by *C. psittaci* in various species is included below.[3]

Disease caused by *C. psittaci*

Psittacosis (ornithosis)	Humans, birds
Sporadic bovine encephalitis	Cattle
Polyarthritis	Cattle, sheep, horses
Enzootic bovine abortion	Cattle
Enzootic ovine abortion	Sheep
Abortion	Horses, swine
Pneumonia	Cattle, sheep, goats, horses, dogs, rabbits
Conjunctivitis	Sheep, cats, guinea pigs, hamsters
Enteritis	Cattle, pigs, muskrats, snowshoe hares

There was some variation among slides with some sections containing a focus of nodular histiocytic inflammation.

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References:

1. Schmidt RE, Reavill DR, Phalen DN: Pathology of Pet and Aviary Birds, pp. 68-82. Iowa State Press, Ames, Iowa, 2003

2. Anderson AA, Vanrompay D: Avian Chlamydiosis (psittacosis, ornithosis). In: Diseases of Poultry, eds. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, 11th ed., pp. 863-879. Iowa State Press, Ames, Iowa, 2003
3. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 402-410. Williams & Wilkins, Baltimore, Maryland, 1997

SLIDE 46
CONFERENCE 12 / CASE II – XN3076 (AFIP 2988331)

Signalment: 3-year-old, male, red-tailed boa constrictor, snake, *Boa constrictor*

History: Two captive bred 3-year-old boa constrictors (*Boa constrictor*) were purchased by a reptile collector in the United Kingdom in October 2004, housed together in a vivarium and fed killed thawed mice, rats and day-old chicks. The male of the pair had radiologically confirmed spondylosis involving vertebral segments over a length of 25 cm at mid-body level. This snake exhibited regurgitation and developed stomatitis one week after purchase. It was treated with 10 mg/kg enrofloxacin (Baytril 2.5% Oral Solution, Bayer) administered by daily gavage for one week and appeared to recover. It developed anorexia in early February 2005 and was found dead on 9 March 2005, four months after purchase.

Gross Pathology: At postmortem examination, the male boa constrictor was 1.6 m long, weighed 2.9 kg and had adequate reserves of body fat. The lungs contained frothy greenish brown fluid and there was oedema around the lungs and heart.

Laboratory Results: Bacteriology: Profuse growths of *Salmonella enterica* serovar San Diego were recovered from the lungs and intestine at postmortem examination.

Histopathologic Description: Histologically, the snake had a proliferative pneumonia, with papillary expansion of interconnecting trabeculae lined by ciliated or non-ciliated columnar epithelial cells, supported by fibrovascular connective tissue. There were mild multifocal infiltrates of lymphocytes and plasma cells in the interstitial tissue and mild individual degeneration of epithelial cells. Large numbers of small bacilliform bacteria were present in the lumen of the lung, but there were few inflammatory cells in the luminal exudate. Numerous single or occasionally multiple, ovoid, eosinophilic cytoplasmic inclusion bodies, 1 to 5 mm in diameter, were detected in epithelial cells in the lungs. Similar inclusion bodies were also detected in the kidneys, pancreas, stomach and intestine. Pigment deposits and interstitial fibrosis were evident in the kidneys. There was mild individual degeneration of renal tubular epithelial cells and occasional sloughing of cells into tubule lumina. Diffuse vacuolation of hepatocytes, with ballooning degeneration and moderate numbers of inclusion bodies, was evident in the liver. Inclusion bodies were also detected in neurons and glial cells in the brain in association with mild meningoencephalitis. There was lymphoid depletion in the spleen, with fibrosis and numerous inclusion bodies in lymphoreticular cells.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, proliferative, diffuse, severe, with eosinophilic cytoplasmic inclusion bodies, snake, *Boa constrictor*.

Contributor's Comment: Boid inclusion body disease (IBD) is an important transmissible disease of captive snakes that occurs worldwide.[1,2,3] The disease occurs primarily in boids and pythons (Family *Boidae*), including the boa constrictor (*Boa constrictor*). It has also been diagnosed in colubrids (Family *Colubridae*) and viperids (Family *Viperidae*), but appears to be less common in these groups of snakes.[2,4] Boid IBD is characterised clinically by anorexia, regurgitation, weight loss, lethargy and neurological signs, including disorientation, incoordination, head tilting, "star gazing", tremors, convulsions and flaccid paralysis.[1,2,3] Affected snakes usually die or are euthanased after a prolonged clinical course. The disease appears to be immunosuppressive, permitting the development of secondary disease, such as bacterial pneumonia and stomatitis. Histopathological changes in affected snakes include demyelinating encephalomyelitis, interstitial pneumonia, hepatopathy with vacuolation and ballooning degeneration of hepatocytes, pancreatic atrophy and nephrosis.[1,2,3] Lymphoid depletion is also evident. Eosinophilic inclusion bodies are present in the cytoplasm of epithelial cells in the lungs, gastrointestinal tract, kidneys and pancreas, as well as in hepatocytes, neuroglial cells in the brain and lymphoreticular cells in the spleen.[1,2,3]

The aetiology of boid IBD is currently a matter of controversy. Retroviruses have been isolated in cell culture and detected by electron microscopy in tissues from affected snakes.[1,5] However, these may be endogenous retroviruses that are not aetiologically associated with boid IBD.[6] Ophidian paramyxoviruses (OPMV), of which more than 18 types have been recognized, are associated with necrotising or proliferative interstitial pneumonia, meningoencephalitis and mortality in viperids and colubrids.[7,8,9] Eosinophilic inclusion bodies, along with occasional multinucleate syncytia, are usually produced in the cytoplasm of infected cells. The degree to which boids are susceptible to OPMV is uncertain. OPMV type 7 has been isolated from a reticulated python (*Python reticulatus*) with respiratory disease in the UK.[10] High antibody titres against OPMV were detected by haemagglutination inhibition in serum from an unaffected reticulated python following an outbreak of disease in viperids in the USA.[9] *In situ* hybridisation was positive for paramyxovirus sequences in the brain of a Boelen's python (*Morelia boeleni*) with meningoencephalitis and eosinophilic cytoplasmic inclusions in glial cells in the brain but not in other tissues.[11] There is currently insufficient evidence to implicate OPMV in the aetiology of boid IBD, despite the pathological similarities. The cytoplasmic inclusion bodies in boid IBD are widely distributed in epithelial, nervous and lymphoreticular tissues.[1] Infection with OPMV may produce inclusion bodies in the lungs, brain, liver and kidneys, although they are less numerous, multinucleate syncytia may be present and there is usually a more pronounced suppurative and necrotising pneumonia.[8,9,11]

The proliferative pneumonia in the affected snake may be attributable to boid IBD, since there were only mild lymphoplasmacytic inflammatory infiltrates in the lungs, limited

necrosis, minimal exudation of heterophils and no evidence of multinucleate syncytia. A profuse growth of *Salmonella enterica* serovar San Diego was obtained from the lungs at postmortem examination and large numbers of bacteria were present in the pulmonary exudate. The *Salmonella* isolate may have been an opportunistic colonist of the lungs in an immunocompromised snake secondary to boid IBD, possibly related to regurgitation and inhalation of gastrointestinal contents.

AFIP Diagnosis: Lung: Bronchointerstitial pneumonia, proliferative, heterophilic and lymphoplasmacytic, diffuse, moderate, with edema, fibrin, and hemorrhage, numerous epithelial eosinophilic intracytoplasmic inclusion bodies, Gram negative bacilli and Gram-positive cocci, red-tailed boa constrictor (*Boa constrictor*), reptile**.**

Conference Comment: The contributor provides a thorough overview of boid inclusion body disease (IBD) and compares and contrasts it with ophidian paramyxoviruses (OPMV). The controversial association of a type C retrovirus and IBD was discussed during conference. Ultrastructurally, the inclusions appear as electron dense structures that may vary in size and shape. The inclusions may represent previral material or some type of storage material from a dysfunctional cell. In one study, an antigenically distinct 68-kilodalton protein was isolated and characterized from nonviral inclusions in IBD-infected Boa Constrictors.[12]

As pointed out by the contributor, IBD affects both boids and pythons. Boas may be inapparent carriers. However, the severity of the disease is significantly worse in pythons, which have a rapid clinical course that progresses to a fatal CNS disturbance. There is no treatment for IBD and infected snakes die. Therefore, boas and pythons should not be mixed in the same collection. The snake mite, *Ophionyssus natricis*, is suspected as a vector associated with the spread of disease. Other modes of transmission include direct contact and venereal spread.[13]

Gross lesions are frequently limited to changes associated with secondary bacterial infections, such as pneumonia, stomatitis, and bacterial granulomas within the liver and kidneys. In some species, such as Boa Constrictors, fibrous changes and splenic atrophy may be observed.[12]
Some conference participants favored OPMV. The moderator preferred a diagnosis of IBD since the inclusions were widespread in other organs and there was a lack of a pronounced suppurative and necrotizing pneumonia as pointed out by the contributor. Additionally, in his experience, it is uncommon to see so many inclusions with OPMV infections. He also added that the syncytial cells seen in OPMV infections are typically striking and the inclusions are more pleomorphic similar to those seen with canine distemper virus.

Gram stains performed at the AFIP revealed myriad Gram-negative bacilli as well as chains and pairs of Gram-positive cocci within the pulmonary exudate and were most likely opportunistic pathogens in this debilitated snake.

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References:

1. Schumacher J, Jacobson ER, Homer BL, Gaskin JM: Inclusion body disease in boid snakes. J Zoo Wildl Med 25:511-524, 1994
2. Carlisle-Nowak MS, Sullivan N, Carrigan M, Knight C, Ryan C, Jacobson ER: Inclusion body disease in two captive Australian pythons (*Morelia spilota variegata* and *Morelia spilota spilota*). Aust Vet J 76:98-100, 1998
3. Orós J, Tucker S, Jacobson ER: Inclusion body disease in two captive boas in the Canary islands. Vet Rec 143:283-285, 1998
4. Raymond JT, Garner MM, Nordhausen RW, Jacobson ER: A disease resembling inclusion body disease of boid snakes in captive palm vipers (*Bothriechis marchi*). J Vet Diagn Invest 13:82-86, 2001
5. Jacobson ER, Orós J, Tucker SJ, Pollock DP, Kelley KL, Munn RJ, Lock BA, Mergia A, Yamamoto JK: Partial characterization of retroviruses from boid snakes with inclusion body disease. Am J Vet Res 62:217-224, 2001
6. Huder JB, Boni J, Hatt JM, Soldati G, Lutz H, Schupbach J: Identification and characterization of two closely related unclassifiable endogenous retroviruses in pythons (*Python molurus* and *Python curtus*). J Virol 76:7607-7615, 2002
7. Franke J, Essbauer S, Ahne W, Blahak S: Identification and molecular characterization of 18 paramyxoviruses isolated from snakes. Virus Res 80:67-74, 2001
8. Jacobson E, Gaskin JM, Page D, Iverson WO, Johnson JW: Illness associated with paramyxo-like virus infection in a zoologic collection of snakes. J Am Vet Med Assoc 179:1227-1230, 1981
9. Jacobson ER, Gaskin JM, Wells S, Bowler K, Schumacher J: Epizootic of ophidian paramyxovirus in a zoological collection. Pathological, microbiological, and serological findings. J Zoo Wildl Med 23:318-327, 1992
10. Manvell RJ, Drury SE, Geach M, Lewis JC: Isolation of ophidian paramyxovirus type 7 from a reticulated python in the UK. Vet Rec 147:696, 2000
11. West G, Garner M, Raymond J, Latimer KS, Nordhausen R: Meningoencephalitis in a Boelen's python (*Morelia boeleni*) associated with paramyxovirus infection. J Zoo Wildl Med 32:360-365, 2001
12. Schumacher J: Inclusion body disease virus. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 836-840. Saunders Elsevier, St. Louis, Missouri, 2006
13. Bennet RA, Mehler SJ: Neurology. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 248-249. Saunders Elsevier, St. Louis, Missouri, 2006

SLIDE 47
CONFERENCE 12 / CASE III – S 2674/00 R (AFIP 2788661)

Signalment: 1-year-old, male, Chinchilla (*Chinchilla lanigera f. domestica*)

History: The chinchilla had been kept as a single animal by a private owner. The animal suffered four days from neurological signs characterized by seizures, biting into the cage wire and problems swallowing. Therapy with antibiotics and electrolytes did not improve the condition. The animal was euthanized.

Gross Pathology: Except for a moderate unilateral purulent rhinitis no gross lesions were detected.

Contributor's Morphologic Diagnosis: Chinchilla, brain: Multifocal acute, moderate, lymphocytic meningitis and severe, acute, diffuse encephalitis with intraneuronal eosinophilic inclusion bodies.

Contributor's Comment: The histological findings in the brain of this case were indicative of a virus infection consisting of a lymphocytic leptomeningitis and an acute encephaltis with perivascular cuffing and extensive neuronal damage. The distribution of lesions was bilateral in the cerebral hemispheres, basal ganglia and hippocampus, whereas the rhinencephalon was affected more unilaterally. Other parts of the brain including the trigeminal ganglia and the optic chiasm showed only minor or no lesions. Predominantly in neurons, intranuclear inclusion bodies were present either as eosinophilic inclusions surrounded by a clear halo and marked margination of chromatin along the nuclear membrane or homogenous amphophilic inclusions occupying the whole nucleus.

Ultrastructural examination of brain tissue post-fixed with glutaraldehyde revealed enveloped virus particles of 120-140 nm in diameter with morphology consistent with a herpes virus. Immunohistochemistry was performed using polyclonal antibodies for human herpes simplex virus 1 and 2 resulting in extensive labeling of viral antigen. Native brain samples were used for virological culture in vero cells. A rapidly growing virus causing a severe cytopathogenic effect was isolated. Two genome fragments encoding for replicative polymerase and glycoprotein B were amplified by PCR and subsequently sequenced. The comparison with known sequences of other herpesviruses resulted in a 100% and 99.5% homology with the human herpes simplex virus type 1 (HSV1) and type 2 (HSV2), respectively.
Histological examination of other tissues revealed a unilateral purulent rhinitis corresponding with the predominant unilateral affection of the rhinencephalon. In the cutaneous mucous membrane of the nasal vestibulum single erosive to ulcerative lesions with intranuclear inclusion bodies in epithelial cells were present. Additionally, occasional circumscribed necrosis was found in the adrenal glands. Only in the nasal mucosa was viral antigen demonstrated by immunohistochemistry.

The findings in the nasal cavity and the unilaterally predominant affection of the rhinencephalon are suggestive of a rhinogenic infection with HSV1. The mode of infection was probably close contact to a person with herpes labialis. Reports about virus infections in chinchillas are extremely rare. One case is described in Canada with a circumscribed brain stem encephalitis associated with necrotic foci in adrenal glands and spleen. Ultrastructurally, a herpes-like virus was detected, but the agent was not

identified virologically. In other rodents, like rabbits, HSV1 infection has been well documented after spontaneous or experimental infections.

AFIP Diagnosis: Cerebrum: Meningoencephalitis, neutrophilic, lymphoplasmacytic and histiocytic, subacute, focally extensive, marked, with neuronal necrosis, gliosis, and eosinophilic intranuclear inclusion bodies, chinchilla (*Chinchilla lanigera f. domestica*), rodent.

Conference Comment: *Herpes simplex* is a double-stranded DNA enveloped virus, with an icosahedral capsid in the alphaherpesvirus subfamily. There are two serotypes, *H. simplex* virus type 1 associated with oral and conjunctival infections and encephalomyelitis in adults and *H. simplex* virus type 2 associated with genital and neonatal infections. Humans are the natural or reservoir host for the virus.[5]

Domestic rabbits have been used as an animal model for *Herpes simplex* encephalitis for decades. Naturally occurring cases of herpes encephalitis have also been observed in pet rabbits and were most likely acquired from contact with human shedders of *Herpes simplex* virus. Key histomorphologic lesions include a nonsuppurative meningoencephalitis with necrosis of neurons and prominent intranuclear inclusion bodies in neurons and astroglial cells.[1,4]

Human-to-monkey and monkey-to-monkey transmission have also been described. Lesions may be local or generalized. Oral vesicles and ulcers, conjunctivitis, encephalitis, and death may occur. Owl monkeys, tree shrews, lemurs, maromosets, and tamarins are susceptible to generalized disease. Infections in gorillas, chimpanzees, gibbons and cebus monkeys are usually confined to the skin, oral cavity, external genitalia, and conjunctiva; however, fatal encephalitis may also develop in gibbons. Oral, lingual, labial, or genital vesicles and ulcers associated with conjunctivitis and keratitis are seen. Necrotizing meningoencephalitis may occur and focal necrosis can be found in the visceral organs. Histologically, multinucleated giant cells and intranuclear inclusion bodies can be seen adjacent to necrotic foci. *H. simplex* infection in owl monkeys, marmosets, and tamarins cannot be differentiated from herpesvirus T (*H. tamarinus*) infection grossly or microscopically. Squirrel monkeys are the natural host for herpesvirus T and a definitive diagnosis can only be made by virus isolation and identification, Immunohistochemistry, or molecular techniques. Although outbreaks of *H. simplex* infections can be devastating in owl monkeys, *H. simplex* infection is not common in any of the primate species.[5,6,7]

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References:

1. Weissenböck H, Hainfellner JH, Berger J, Kasper I, Budka H: Naturally occurring herpes simplex encephalitis in a domestic rabbit (*Oryctologaus cuniculus*). Vet Pathol 34:44-47, 1997
2. Goudas P, Gilroy JS: Spontaneous herpes-like viral infection in a chinchilla (*Chinchilla laniger*). J Wildlife Dis 6:175-179, 1970
3. Granzow H, Klupp BG, Fuchs W, Veits J, Osterrieder N, Mettenleiter TC: Egress of alphaherpesviruses: comparative ultrastructural study. J Virol 75:3675-3684, 2001
4. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., p. 254. Iowa State University Press, Ames, Iowa, 2001
5. Hunt RD: *Herpesvirus simplex* infection. In: Monographs on Pathology of Laboratory Animals Nonhuman Primates I, eds. Jones TC, Mohr U, Hunt RD, pp. 82-86. Springer-Verlag, Berlin, Germany, 1993
6. Mansfield K, King N: Viral diseases. In: Nonhuman Primates in Biomedical Research Diseases, eds. Bennet BT, Abee CR, Henrickson R, pp.10-11. Academic Press, San Diego, California, 1998
7. Mätz-Rensing K, Jentsch KD, Rensing S, Langenhuyzen S, Verschoor E, Niphuis H, Kaup FJ: Fatal *Herpes simplex* infection in a group of common marmosets (*Callithrix jacchus*). Vet Pathol 40:405-411, 2003

SLIDE 48
CONFERENCE 12 / CASE IV – Sn090/05 (AFIP 3026208)

Signalment: 2-year-old, Crucian carp, male, fish, cyprinid

History: In spring 2005 a local retailer, specialized in original Japanese koi, received a charge of two-year-old Crucian carps from a German wholesaler. Among these fishes he noticed one animal with a bilateral symmetrical swelling anterior to the dorsal fin. During the following months the fish was reared in one large glass aquarium together with all other fish of this shipment. Obviously the swelling was well tolerated by the fish although a slight increase in size was noticed. Due to the curious and unshaped appearance of the animal in contrast to the other fish the dealer refused to sell this fish. In autumn the bilateral swellings reached a size of 2.0 x 1.0 x 1.0 cm each. The skin became susceptible to superficial damages and intermittent small quantities of suppurative fluid derived from circumscribed ulcers at the top of the swellings. By reason of animal welfare the fish was euthanized and subjected to necropsy.

Gross Pathology: The main gross finding was a bilateral symmetrical swelling of the anterior part of the musculus laterodorsalis (each 2.0 x 1.0 x 1.0 cm in size, figure 1 and 2). The section revealed a cream-colored, pasty compound resembling pus (figure 3). A macroscopically visible demarcation of connective tissue or any inflammatory reaction was missing. The other organs were macroscopically without any pathological findings.

Histopathologic Description: The cream-colored, pasty compound turned out to be a mixture of necrotic debris and developmental stages of parasites within the muscular

and connective tissue (figure 4: Native spores, X400; figure 5: Giemsa stain, X400). Intracellular stages of the parasites were hardly observed. Host reaction was limited to few lymphocytes and macrophages. At the border area eosinophilic degeneration of few muscle fibers was seen. The epidermis next to the focal ulceration was characterized by moderate mononuclear infiltrations and moderate to profound intracellular edema. Numerous parasitic spores were observed penetrating all dermal and epidermal layers.

Contributor's Morphologic Diagnoses:
1. Back muscles (Musculus laterodorsalis): Necrosis, focal, mild, with massive accumulation of myxosporidian spores in the connective tissue, consistent with *Myxobolus lentisuturalis*
2. Skin: Ulceration, focal, mild, with numerous myxosporidian spores, consistent with *Myxobolus lentisuturalis* (missing in slides)

Contributor's Comment: Up to now more than four hundred species of the genus *Myxobolus* are known to parasitize fish. In contrast, species with intramuscular (intracellular, histozoic) developmental stages are rare in cyprinid fishes.[1,2] Depending on spore morphology and tissue localisation we assume a severe infection with *Myxobolus lentisuturalis* as described in *Carassius gibelio* by Dykova et al. from Lake Bao'an in Hubei Province, China.[1] This publication stated a close phylogenetic relation of *M. lentisuturalis* to *M. xiaoi* and a still unclassified species from *Catostomus commersoni* based on SSU rDNA sequence data whereas spore morphology and gross lesions resemble these of *M. carassii* and *M. kubanicus*. Mature spores of the myxosporidian genus *Myxobolus* are ellipsoidal, ovoid or rounded in valvular view and biconvex in sutural view. The two pyriform polar capsules with convergent anterior points contain an invaginated and coiled polar filament. Beside the binucleated sporoplasm two residual nuclei of the capsulogenic cells may be present.

Development of myxosporidians comprise an oligochaete and a vertebrate host.[3] Various actinosporeans, formerly supposed to be parasites of oligochaetes, are the infective stages to fish. The most common oligochaete seems to be *Tubifex tubifex*, however *Lumbriculus* spp. or *Branchiura* spp. can serve as hosts as well. For example, observations of the life cycle of *Myxobolus cerebralis* revealed the intrinsic function of actinosporeans as vehicles for the transmission between hosts. Infection of the fish host occurs by actinosporean perorally or percutaneously.[4] During clonal reproduction in the vertebrate host the sarcoplasm of infected cells is replaced by large masses of plasmodial stages, followed by enlargement of the host cells. Destruction of adjoining tissues due to pressure atrophy may be seen aside. Dispersal of mature spores occurs after rupture of the host cell wall into the connective tissue and epithelial structures like the mucous cells of the intestine and the upper layers of the skin. Thus a release of infective stages is given during the lifetime of the host. Infection of oligochaetes occurs by oral infection with mature spores. After release of the sporoplasma further asexual and sexual development takes place in the gut epithelium.[2]

As a result of evolutionary coexistence of myxozoans and their hosts there are only few humoral, cell or tissue responses of the host to histozoic plasmodia during asexual development. Normally the release of mature spores initiates a granulomatous inflammation including melanomacrophages and the replacement of parasite lesions by granulation tissue. Severe alterations of earlier stages are only seen in atypical tissues or hosts and are followed by elimination of the parasites before maturation. In addition, alteration of host tissue may be seen as pressure atrophy of surrounding tissues and reactive hypertrophy or hyperplasia respectively of infected internal and external organs.[5] In this case of *M. lentisuturalis* asexual division and development to mature spores occurs intracellularly in the muscle fibres of the musculus laterodorsalis. After degeneration of the host cell mature spores are released in the connective tissue of myosepta. Compared to other species of the genus *Myxobolus* only few cellular reactions caused by *M. lentisuturalis* are observed and despite wide dispersal of spores into other organs there is a lack of demarcation.[4]

In general, the genus *Myxobolus* is a member of the taxon Myxozoa. Nowadays myxozoans are classified as metazoans nested within the genus *Cnidaria*. Their main morphological features are the polar capsules, resembling nematocysts of cnidarians and the existence of desmosomes, tight junctions and collagen production demonstrated in different developmental stages. Present works concluded that the most reliable pattern to distinguish between special taxa of myxozoans is not spore morphology but rather development and tissue location. Molecular characterization becomes more popular although the data set might be confusing depending on molecular overlap and individual variability between the 18S rDNA of different species.[4]

AFIP Diagnosis: Skeletal muscle: Myositis, necrotizing, subacute, multifocal, severe, with myriad myxosporidian spores, Crucian carp (*Carassius carassius*), cyprinid.

Conference Comment: The contributor provides a thorough overview of *Myxobolus* to include characteristic histomorphologic features, life cycle, and recent reclassification as metazoans.

Myxozoans parasitize invertebrates (primarily annelids) and poikolothermic invertebrates with the vast majority infecting fish. The Myxozoa that infect fish are obligate parasites of either tissues (histozoic forms that reside in intercellular spaces, intracellulary, or in blood vessels) or organ cavities (coelozoic forms that reside primarily in the gall bladder, swim bladder, or urinary bladder). Most are intercellular parasites that are typically site and species specific. Key characteristics include a multicellular spore and the presence of one to six (usually two) polar capsules each of which contains a polar filament. Spores with polar capsules are pathognomonic for myxozoan infection. Polar capsules can be seen in fresh wet mounts but are more easily seen in Giemsa or Wright's stained smears. Spores are refractile and difficult to see in hematoxylin and eosin stained sections, but polar capsules stain intensely with Giemsa or toluidine blue.[6]

Most myxozoan infections of fish incite only a moderate host reaction; however, heavy infections can result in serious mechanical damage from pseudocysts or tissue necrosis and inflammation from trophozoite feeding. Young fish are usually most seriously affected and histozoic forms usually cause more serious disease. In many cases, tissue damage is most severe after death of the host when enzymes released by the parasites cause massive muscle liquefaction.[6]

Gross myxozoan lesions can look similar to other diseases that cause focal masses including microsporidians, *Ichthyopthirius multifilis*, lymphocystis (iridovirus), and dermal metacercariae. Internal lesions may resemble focal granulomas and neoplasia. These can be easily differentiated by histopathology or by examining wet mounts.[6]

Other important fish diseases in which myxozoans are known or suspected to be involved include proliferative kidney disease, proliferative gill disease, and whirling disease/black tail (*Myxobolus cerebralis*).[6]
Some conference participants considered microsporidians as a differential for this case. In contrast to myxozoans, all microsporidians are intracellular parasites that form a characteristic thick-walled spore which contains a sporoplasm. Some microsporidians induce the formation of a markedly hypertrophied cell that, together with the parasite, forms a xenoma or xenoparasitic complex. Xenomas appear as whitish, cyst-like structures, up to several millimeters in diameter. Some species may form large pseudotumors comprised of many individual xenomas. The presence of spores that are small (2 to 10 um), egg-shaped to elliptical, with a prominent posterior vacuole is diagnostic for microsporidia. Spores have a polar tube (typically not seen with routine light microscopy) and, unlike the Myxozoa, have no polar capsule. Microsporidian spores are Gram-positive.

Conference participants discussed the reclassification of myxozoans as metazoans. The Myxozoa were grouped with the protistan taxa until the early 1900s. As early as 1899, Stolc claimed that myxozoans are not protists and should be included with the Metazoa since their spores are multicellular. In 1938, Weill suggested myxozoans are cnidarians since the polar capsules of myxozoans showed identical discharge properties to nematocysts. In 1995, Siddall et al. used molecular and morphological data to show that myxozoans nested within the cnidaria and were the first to note desmosomes, tight junctions, and collagen production. Siddall et al. also provided ultrastructural characterization of the development of myxozoan polar capsules finding it to be indistinguishable from the development of cnidarian nematocysts.[4]

There was some variation among slides with some sections containing scaled skin.

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology.

Contributor: http://www.vetmed.uni-leipzig.de/ik/wpathologie

References:
1. Dykova I, Fiala I, Nie P: *Myxobolus lentisuturalis* sp n. (Myxozoa: Myxobolidae), a new muscle-infecting species from the Prussian carp, *Carassius gibelio* from China. Folia Parasitol 49:253-8, 2002
2. Longshaw M, Frear PA, Feist SW: Descriptions, development and pathogenicity of myxozoan (Myxozoa: Myxosporea) parasites of juvenile cyprinids (Pisces: Cyprinidae). J Fish Dis 28:489-508, 2005
3. Gardiner CH, Fayer R, Dubey JP: An atlas of Protozoan Parasites in Animal Tissues, 2nd ed., pp. 14-15. Armed Forces Institute of Pathology, Washington, D.C., 1998
4. Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallet SL, Lester RJG, Longshaw M, Palenzeula O, Siddall ME, Xiao C: Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48:395-413, 2001
5. Lom J, Dyková I: Protozoan Parasites of Fishes, pp. 159-215. Elsevier, London, England, 1992
6. Noga EJ: Fish Disease Diagnosis and Treatment, pp.173-193. Iowa State University Press, Ames, Iowa, 2000

SLIDE 49
CONFERENCE 13 / CASE I – A22 512297 (AFIP 2812387)

Signalment: Rat, *Rattus rattus*, ACI/Seg, male, 10 months of age at study initiation

History: The ACI-rat strain is an established animal model that develops malformations of the urogenital system.[2] The ACI/Seg substrain has a propensity to develop adenocarcinoma of the prostate.[1,4] ACI/Seg male rats (Harlan Sprague-Dawley) were evaluated during an approximately 22-month study to determine the background incidence of prostatic changes; none of these rats were exposed to any xenobiotics. One group of male rats was castrated at approximately 8 months of age, 2 months prior to study start and a second group served as controls. An unexpected finding was that approximately 10-30% of the rats (from both the surgically treated and control groups) developed unilateral facial (periorbital) swelling with or without exophthalmia and/or corneal opacity. Affected rats developed facial swelling as early as Study Week 8 and subsequently died or were sacrificed beginning in Study Week 18. This change continued to develop in select rats throughout the duration of the study.

Gross Pathology: The facial lesion was generally localized adjacent to the eye/orbit or the cervical areas and described as an irregular/nodular mass which contained yellow, caseous to granular material. Some facial lesions were locally extensive, involving the skin, bone, skeletal muscle, and/or nerves in the affected area, as well as the Harderian gland and orbit.

Laboratory Results: Bacteriological culture of the affected tissue(s) generally yielded pure isolates of coagulase positive *Staphylococcus aureus*.

Contributor's Morphologic Diagnosis: Facial area, soft and osseous tissues- pyogranulomatous inflammation.

Contributor's Comment: Pyogranulomatous inflammation involving soft and osseous tissues of the facial area of rats is an unusual change in our experience. Based on available data, it seems that the oral cavity is the likely source of the organism. However, none of these rats had any experimental manipulations of the oral cavity (i.e. no oral gavage) and there was no history of trauma to the oral cavity.

We are unaware of similar occurrences being reported in rats, although an outbreak of botryomycosis was reported in a colony of urokinase-type plasminogen activator (uPA) deficient mice.[3] The majority of lesions occurred in the head and neck region, and *Stapylococcus aureus* was isolated from all mice sampled for bacterial culture.

AFIP Diagnosis: Head, cross section, at the level of the olfactory bulb: Myositis, cellulitis, pharyngitis, osteomyelitis, and meningitis, pyogranulomatous and necrotizing, multifocal to coalescing, severe, with Splendore-Hoeppli material and large colonies of cocci, ACI/seg rat (*Rattus rattus*), rodent.

Conference Comment: Botryomycosis is a chronic granulomatous disease caused by non-filamentous non-branching bacteria surrounded by eosinophilic, acellular material that forms radiating clubs (Splendore-Hoeppli material) and affects the skin, and rarely, the viscera. The term "botryomycosis" is technically incorrect as it implies that the disease is caused by a fungus; however, the term has persisted since the lesions closely resemble fungal granulomas. Synonyms include bacterial pseudomycosis, bacterial pseudomycetoma, and bacterial pseudogranulomas. Bacterial pseudomycetomas are primarily caused by staphylococcal bacteria (usually coagulase-positive *Staphylococcus aureus*) as in this case; however, other causes alone or in conjunction with *S. aureus* include *Streptococcus* sp., *Proteus* sp., *Pseudomonas* sp., and *Actinobacillus* sp. Bacterial pseudomycetomas occur in cattle, sheep, horses, dogs, cats, pigs, and rodents among other species.[5-9]

The pathogenesis is unclear, but most likely results from an imbalance between host resistance and organism virulence. The host can isolate and contain the infection but cannot eliminate it. Infections are usually initiated by local trauma to the skin while some infections are associated with a foreign body which provides a nidus for granule formation. There is a possible genetic predilection, as pointed out by the contributor; certain strains of mice are more susceptible than others. Immunodeficiency may also play a role. Bacterial pseudomycetomas have been reported in humans with HIV infection and chronic granulomatous disease.[5,10]

Typical gross findings include firm nodules with draining fistulous tracts that exude purulent exudate that may contain small white granules (grains). Infection involves the

deep dermis and subcutis, and occasionally extends to the muscle, adjacent bone, and rarely the viscera.[5,6,8,9]

Typical light microscopic findings include nodular to diffuse pyogranulomatous dermatitis and panniculitis. The pyogranulomas are characterized by a central core of compact bacterial colonies (tissue granules or grains) surrounded by amorphous eosinophilic material that often forms radiating clubs (Splendore-Hoeppli material) further bounded by many neutrophils, epithelioid macrophages, lymphocytes, plasma cells, multinucleate histiocytic giant cells, and variable amounts of fibrosis. Splendore-Hoeppli material is thought to represent a localized antigen-antibody reaction.[5,6,8,9,11]

The differential diagnosis includes eumycotic mycetoma, actinomycosis, nocardiosis, systemic mycosis, dermatophytic pseudomycetoma, foreign body reactions, opportunistic fungi and algae, and chronic bacterial abscesses.[5,7,9]

Botryomycotic mastitis occurs in cattle, pigs, and sheep. *Actinobacillus lignieresii* (wooden tongue) is a form of botryomycosis in cattle. The neck and pectoral region ("breast boils") are common sites of botryomycosis in horses and the stub of the spermatic cord in geldings is a common site. In pigs, castration wounds are also a common site. In rats and mice, botryomycosis typically results in swelling of the head, abscessation, and visceral lesions. In nude mice, periorbital abscesses and nasal furunculosis result due to sparse protective pelage, distorted vibrissae and hair shaft growth, and impaired T-cell function. B6 mice are especially prone to ulcerative dermatitis associated with *S. aureus*. Pulmonary botryomycosis is a rare condition in horses, guinea pigs, and cattle. There is a single report of mammary botryomycosis in an African elephant.[6,8,11,12,13]

Gram stains performed at the AFIP revealed myriad Gram-positive cocci.

Contributor: Merck Research Laboratories, Department of Safety and Assessment, West Point, PA

References:

1. Isaacs JT: The aging ACI/Seg versus Copenhagen male rat as a model system for the study of prostatic carcinogenesis. Cancer Res 44:5785-5796, 1984
2. Kneidl M, Shibasaki Y, Komitoski D: Trisomy 8 and urogenital malformation in the ACI-rat. Hereditas 123:97-101, 1995
3. Shapiro RL, Duquette JG, Nunes I, Roses DF, Harris MN, Wilson EL, Rifkin DB: Urokinase-type plasminogen activator deficient mice are predisposed to Staphylococcal botryomycosis, pleuritis, and effacement of lymphoid follicles. Am J Pathol 150:359-369, 1997
4. Ward JM, Reznik G, Stinson SF, Lattuada CP, Longfellow DG, Cameron TP: Histogenesis and morphology of naturally occurring prostatic carcinoma in the ACI/segHapBR rat. Lab Invest 45:517-522, 1980
5. Scott DW, Miller WH, Griffin CE: Muller & Kirk's Small Animal Dermatology, 6th ed., pp. 311-312. W.B. Saunders Company, Philadelphia, Pennsylvania, 2001

6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p.430. Williams & Wilkins, Baltimore, Maryland, 1997
7. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1187-1188. St. Louis, Missouri, 2007
8. Yager JA, Scott DW: The skin and appendages. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 657. Academic Press Inc., San Diego, California, 1993
9. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp.275-276. Blackwell Publishing Professional, Ames, Iowa, 2005
10. Scott DW, Miller WH: Equine Dermatology, pp. 245-249. Saunders, St. Louis, Missouri, 2003
11. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., pp. 64-65. Iowa State University Press, Ames, Iowa, 2001
12. Carlton WW, Hunt RD: Bacterial diseases. In: Pathology of Laboratory Animals, eds. Benirschke K, Garner FM, Jones TC, vol. 2, pp. 1451-1453. Springer-Verlag, New York, New York, 1978
13. Sleeman JM, Clyde VL, Finnegan MV, Ramsay EC, Shires MG: Mammary botryomycosis in an African elephant (*Loxodonta Africana*). Vet Rec 152:54-55, 2003

SLIDE 50
CONFERENCE 13 / CASE II – H-7663 (AFIP 3031561)

Signalment: 17-year-old, female, rhesus macaque, *Macaca mulatta*

History: Presented 3 days prior to death with 2-day-old infant, moderate weight loss, and post-partum bleeding, non-responsive to supportive therapy, found non-responsive in cage with vomit, CPR attempts unsuccessful.

Gross Pathology: Multifocal aspiration pneumonia; dilatation of esophagus, stomach, and jejunum; mid-jejunal constrictive mass; fatty liver

Histopathologic Description: Jejunal mass – The ring shaped fibrous constriction observed histologically is evident on low magnification examination of the microscope slide. The mucosal tissue is expanded forming elongated crypts and cystic glands. The lamina propria is invaded by cells proliferating as tubes and cords. A prominent fibrous connective tissue stroma is present supporting the invading epithelium. The neoplastic cells vary from tall columnar to short cuboidal and have variable amounts of cytoplasm. Crypts in the mucosa vary from having a single layer of columnar epithelium to having highly stacked pseudostratified columnar cells. As the cells invade atypia increases with an increase in nuclear to cytoplasmic ratio, increased anisokaryosis, and the presence of obvious small angular amphophilic single or double nucleoli. Mitotic index is variable from 1-5 per 40X field with abnormal mitoses being observed.

Contributor's Morphologic Diagnosis: Jejunum, adenocarcinoma, locally invasive with stricture

Contributor's Comment: Small intestinal neoplasia has been reported in rhesus macaques as a report on a series of necropsies and in a case report.[1,2] The colonic adenocarcinoma of the aging rhesus macaque is better known and published.[3,4] The incidence of intestinal neoplasia in rhesus has a strong affinity to the cecocolic junction, with virtually all of the cases reported in the literature occurring at this site and are associated with clinical histories including chronic diarrhea.[3,4] Small intestinal tumors such as this case are not adequately represented in the literature to determine if this is a typical presentation and location. This animal had an unremarkable medical history, had been a good producing female in the breeding colony and her weight had fluctuated between 8 and 10.5 kg. The weight loss terminally wasn't appreciated until the prolonged post-partum bleeding was noted and she was brought out of the group housing area she had been in. Rapid decline with terminal aspiration resulted in death. Post-mortem examination indicated that the partial obstruction of the intestinal tumor caused proximal dilatation of the gastrointestinal tract with ileus and a third space fluid accumulation phenomenon. Histologic examination revealed a moderately well differentiated locally invasive neoplasm that had extended to the intestinal serosal; although, no distant metastasis was noted.

AFIP Diagnosis: Jejunum: Adenocarcinoma, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: Intestinal adenocarcinoma is rare in most animal species except cotton-top tamarins and New Zealand, Australian and European sheep. Cotton-top tamarins are a model for ulcerative colitis and associated carcinoma in humans.[5,6]

Adenocarcinomas are divided into four histomorphologic types. More that one type is typically present and classification is based on the predominant cell type:

1. Acinar (Tubular): Irregularly branching tubules lined by flattened to columnar cells, embedded on a fibrous stroma, that infiltrate the submucosa and muscularis.
2. Papillary: Papillary projections lined by multiple layers of anaplastic columnar cells with little stroma; mitotic index tends to be high.
3. Mucinous: Acinar or irregular crypts, filled or distended with mucin, which replace at least 50% of the tumor. Mucin may be intracellular and extracellular.
4. Signet-ring: Isolated cells or nests of cells that contain intracellular mucin that displaces the nucleus to the periphery.

Some classifications include a solid type composed of solid sheets of large, anaplastic, or pleomorphic cells with little stroma and no evidence of squamous or glandular differentiation. Desmoplasia is a prominent feature of all types except for papillary

adenocarcinomas. Generally, all types, with the exception of the papillary type, metastasize widely via the lymphatics to the regional lymph nodes.[5,7,8]

Grossly, adenocarcinomas appear as nodular or annular, firm, gray-white stenotic areas that are frequently transmural. There is often intestinal dilation/muscular hypertrophy proximal to the stenosis or tumor obstruction. Although adenocarcinomas typically do not project into the intestinal lumen, papillary types do form intraluminal masses.[5]

Most primary neoplasms of the intestines in the dog are adenocarcinomas and are more common in the large than the small intestine. The duodenum and colorectum are the most common sites. Some investigators have reported a breed predisposition in Boxers, Collies, and German Shepherd Dogs.[5,7,8]

Adenocarcinoma is the third most common intestinal neoplasm in cats after lymphoma and mast cell tumors. The jejunum is the most common site of intestinal adenocarcinoma in cats and Siamese are predisposed. Stromal osteochondroid metaplasia is a common feature of feline intestinal adenocarcinomas.[5,7,8]

The incidence of intestinal adenocarcinoma in cattle and sheep varies by region. In some countries, intestinal adenocarcinoma of cattle is rare and the jejunum is most commonly affected. In other countries, bovine intestinal adenocarcinoma is more common and is associated with ingestion of bracken fern in animals with bovine papillomavirus-4 infection. These lesions are multiple and range from adenomas to carcinomas throughout all levels of the small intestine. Metastasis to regional lymph nodes, omentum, and liver most commonly occurs with rare metastasis to extra-abdominal lymph nodes and lung. In countries in which ovine adenocarcinoma is common, ingestion of bracken fern and herbicide use have been incriminated as possible etiologies. Unlike cattle in high prevalence areas, lesions in sheep are mid-jejunal and solitary with marked desmoplasia. Metastasis occurs via lymphatics to the draining lymph nodes and diaphragm, and rarely hematogenously to the lung and liver. In pregnant ewes, the altered position of the uterus allows transcoelomic metastasis to the reproductive organs.[5,7,8]

Intestinal adenocarcinoma in horses is rare, but involves the cecum and large colon 3 times more often than the small intestine. There is often marked desmoplasia in which cartilaginous and osseous metaplasia can occur. As in felines, intestinal lymphoma is more common in horses.[5,7,8]

Intestinal adenocarcinoma is extremely rare in pigs and is most commonly seen in the jejunum. Desmoplasia and inflammatory cells, predominantly eosinophils, are characteristic. Infiltration into the mesentery and regional lymph node metastasis are common.[5,7,8]

Adenocarcinoma of the colon is uncommon in most nonhuman primates; however, the laboratory maintained cotton-top tamarin has a high incidence of colonic adenocarcinoma that arises in animals with chronic idiopathic colitis resembling

ulcerative colitis in humans. These adenocarcinomas are typically mucinous and highly invasive. A novel *Helicobacter* sp. has been isolated from the colon of cotton-top tamarins that may be involved in the pathogenesis of ulcerative colitis and colonic adenocarcinoma.[6,9]

Contributor: The University of Texas M. D. Anderson Cancer Center, Department of Veterinary Sciences, Bastrop, Texas, www.mdanderson.org

References:

1. Seibold HR, Wolf RH: Neoplasms and proliferative lesions in 1065 nonhuman primate necropsies. Lab Anim Sci 23:533-539, 1973
2. Fanton JW, Hubbard GB, Witt WM, Wood DH: Adenocarcinoma of the small intestine in two rhesus monkeys. J Am Vet Med Assoc 185:1377-1378, 1984
3. Uno H, Alsum P, Zimbric ML, Houser WD, Thomson JA, Kemnitz JW. Colon cancer in aged captive rhesus monkeys (*Macaca mulatta*). Am J Primatol 44:19-27, 1998
4. O'Sullivan MG, Carlson CS: Colonic adenocarcinoma in rhesus macaques. J Comp Path 124:212-215, 2001
5. Barker IK, Van Dreumel AA, Palmer N: The alimentary sytem. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 135-138. Academic Press, Inc., San Diego, California, 1993
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 1081-1085. Williams & Wilkins, Baltimore, Maryland, 1997
7. 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, Second Series, vol. X, pp. 89-93. Armed Forces Institute of Pathology, Washington, D.C., 2003
8. Head KW, Else RW, Dubielzig RR: Tumors of the alimentary tract. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 461-467. Iowa State Press, Ames, Iowa, 2002
9. Saunders KE, Shen Z, Dewhirst FE, Paster BJ, Dangler CA, Fox JG: Novel intestinal *Helicobacter* species isolated from cotton-top tamarins (*Sanguinus oedipus*) with chronic colitis. J Clin Microbiol 37(1):146-151, 1999

SLIDE 51
CONFERENCE 13 / CASE III – R06-0251 (AFIP 3027375)

Signalment: 2-year-old, intact male, Hartley guinea pig (*Cavia porcellus*).

History: The animal had been maintained for hands-on training. Enrichment treats including rolled oats, dehydrated vegetables (Veggie-Bites) and supplements high in vitamins C and D_3 (Supreme Mini-Treats, PRIMA-Treats, Fruit Crunchies and Turf Foraging Crumbles) were administered frequently. There was a history of repeated colonic impaction starting 2 months prior to death which intermittently resolved with supportive therapy including a laxative (Laxatone) and electrolyte replenisher (Prang).

The animal was found dead in his cage 3 weeks after the last bout of colonic impaction had resolved.

Gross Pathology: The thoracic cavity contained 15 ml of serosanguineous and fibrinous pleural effusion and the lungs were mildly atelectatic (Figure R06-0251-G1). The pericardial sac contained 10 ml of hemorrhagic effusion (Figure R06-0251-G2). A pale tan, soft to firm, beaded to verrucous mass was present on the epicardium of the dorsal aspect of the right heart (Figure R06-0251-G3). The liver was mottled dark red (Figure R06-0251-G4). Bilaterally, the kidneys were mottled pale tan to dark brown with a pitted capsular surface (post-formalin fixation, Figures R06-0251-G5 and R06-0251-G6).

Histopathologic Description: Slide consists of a sagittal section of heart including bilateral atria, ventricles, atrioventricular valves and the interventricular septum. Randomly distributed throughout the myocardium are multifocal, small areas of metastatic mineralization (confirmed by von Kossa histochemical staining) and fibrosis (confirmed by Masson's trichrome histochemical staining) associated with myofiber degeneration, atrophy and loss. Infrequent capillaries and small arterioles throughout the myocardium are also mineralized. Some slides also include sections of pulmonary artery and/or aorta. These great vessels have multifocal areas of mineralization affecting the internal elastic lamina, subintima and/or the tunica media including individual elastin fibers. The epicardium is variably thickened by moderate amounts of collagen, fibrin and fibroblasts as well as scattered lymphocytes, histiocytes and fewer heterophils. Epicardial neovascularization, particularly at the base of the heart is prominent. In multifocal areas, there is also hyperplasia of mesothelial cells on the epicardial surface. In some slides, the section includes the epicardial mass noted grossly. Histologically, this mass is composed of organizing fibrin within which are scattered fibroblasts, endothelial cells and aggregates of erythrocytes.

Mineralization was also documented in the trachea, lung, kidney, stomach, colon, seminal vesicles, liver, spleen and various vessels within skeletal muscle, mesentery/peritoneum, lung, tongue, stomach and choroid plexus. Myofiber degeneration, atrophy and fibrosis were also noted in skeletal muscle from the hindlimb.

Contributor's Morphologic Diagnoses:
1. Heart, myofiber and vascular mineralization, chronic, multifocal, marked with multifocal degeneration, atrophy, loss and fibrosis.
2. Great vessels, intimal and medial mineralization, multifocal, marked.
3. Epicardium, epicarditis, fibrinous to fibrosing, chronic, multifocal to widespread, marked with lymphohistiocytic inflammation and mesothelial hyperplasia.
4. Pericardium, hemopericardium with cardiac tamponade (gross).

Contributor's Comment: The systemic mineralization present in numerous tissues is consistent with metastatic or soft tissue calcification of guinea pigs. It is most common in guinea pigs older than 1 year of age and affects males more often than females.[1-3,5,6,8,9] Although the condition is typically asymptomatic, the clinical signs exhibited

include weight loss, muscle and joint stiffness, renal failure and sudden death.[2,3,5,6] The condition was previously referred to as 'wrist stiffness syndrome' since mineralization was often restricted to soft tissues around the elbows.[4,5,7] However, mineralization can also be more widespread as in this case.[3,6,8,9] This syndrome is attributed to a dietary imbalance of magnesium, calcium and phosphorus rather than a deficiency of a single mineral.[1-9] Excessive phosphorus and calcium impede absorption and utilization of magnesium. The condition can be minimized or prevented by feeding diets that contain 0.3-0.4% magnesium, 0.9-1.1% calcium, 0.6-0.7% phosphorus, 0.4-1.4% potassium, ≤ 6 IU of vitamin D/g and a calcium:phosphorus ratio of 1.5:1.[9] The imbalance in this guinea pig was likely due to excessive dietary enrichment with treats high in vitamin D_3 leading to hypercalcemia.

No evidence of colonic impaction was noted at necropsy. Historical reports of colonic impaction in this guinea pig could be attributed to metastatic mineralization of the colon. However, published reports documenting an association between these two conditions in humans or animals were not found.

The cardiac and skeletal muscle lesions are also likely consequences of the metastatic mineralization. However, guinea pigs also develop vitamin E/selenium responsive myopathies as well as a poorly understood syndrome known as 'myocardial and skeletal muscle degeneration with mineralization'.[6] Contributing factors for the latter condition have not been clearly identified; although, vitamin E and selenium levels are typically within normal limits and genetic factors are usually implicated. Animals are typically asymptomatic without gross lesions; histologic lesions include degeneration of cardiac and skeletal myofibers with variable mineralization and mononuclear infiltration.

The cause of death in this case is ascribed to the extensive myocardial lesions resulting in acute congestive heart failure characterized by pleural effusion. The guinea pig's condition was also exacerbated by hemopericardium and cardiac tamponade which were likely secondary to vascular rupture of a mineralized vessel into the pericardium.

We cannot completely rule out concurrent renal failure in this case since renal failure can be a complicating condition of metastatic mineralization. Pre-existing underlying renal disease independent of metastatic mineralization cannot be discounted either since aging guinea pigs also develop segmental nephrosclerosis.

AFIP Diagnoses: 1. Heart: Epicarditis, proliferative, chronic, diffuse, moderate, with multifocal myocardial fibrosis and mineralization, Hartley guinea pig (*Cavia porcellus*), rodent.
2. Heart, great vessels: Mineralization, intramural, multifocal, moderate.
Conference Comment: The contributor provides a thorough overview of metastatic or soft tissue calcification of guinea pigs as well as touching on the differentials of vitamin E/selenium deficiency, and the "myocardial and skeletal muscle degeneration with mineralization" syndrome. As pointed out by the contributor, concurrent renal failure

could not be completely ruled out as a potential cause of the metastatic mineralization in this case.

Metastatic calcification occurs in otherwise normal tissue and almost always results from hypercalcemia secondary to a disturbance in calcium metabolism. The pathogenesis of metastatic calcification involves the entry of large amounts of calcium ions into cells where they precipitate on organelles, especially mitochondria. The four causes of metastatic calcification in order of their importance in veterinary medicine are listed below:

1. Renal failure – retention of phosphates induces secondary renal hyperparathyroidism and hypercalcemia. Calcium is deposited in the kidneys, alveolar septa, gastric mucosa, and pleura.
2. Vitamin D toxicosis – ingestion of calcinogenic plants (*Cestrum diurnum*, *Tricetum flavescens*, *Solanum malacoxylon*) by herbivores results in severe soft tissue mineralization primarily involving the aorta, heart (endocardium of right and left atria and left ventricle), and lungs. Acute vitamin D toxicosis in dogs and cats is commonly caused by ingestion of cholecalciferol containing rodenticides. The intestinal mucosa, vessel walls, lung, and kidneys are mineralized.
3. Parathormone (PTH) and PTH-related protein – primary hyperthyroidism is rare. Elevated levels of PTH-related protein and hypercalcemia can be associated with canine lymphoma and adenocarcinoma of the apocrine gland of the anal sac. Mineralization occurs in the intestinal mucosa, vessel walls, lung, and kidneys.
4. Destruction of bone by primary or metastatic neoplasms.[10,11,12]

Metastatic calcification may occur widely throughout the body; however, it principally occurs in the interstitial tissues of the gastric mucosa, kidneys, lungs, systemic arteries, and pulmonary veins. All of these tissues lose acid and therefore have an internal alkaline compartment predisposing them to metastatic calcification. The deposition of mineral salts usually does not result in clinical dysfunction; however, on occasion, massive involvement of the lungs or kidneys can cause respiratory deficits and renal damage respectively.[10,11]

Contributor:
http://www.mskcc.org
http://www.mskcc.org/mskcc/html/14131.cfm
http://www.med.cornell.edu;
http://www.med.cornell.edu/research/rea_sup/mouse_phenotyp.html
http://www.med.cornell.edu/research/rea_sup/lab_com_pat.html
http://www.rockefeller.edu

References:
1. Galloway JH, Glover D, Fox WC: Relationship of diet and age to metastatic calcification in guinea pigs. Lab Anim Care 14:6-12, 1964

2. Harkness JE, Murray KA and Wagner JE: Biology and diseases of guinea pigs. In: Laboratory Animal Medicine, eds. Fox JG, Anderson LC, Loew FM, Quimby FW, 2nd ed., p. 235. Academic Press, San Diego, California, 2002
3. Hrapkiewicz K, Medina L, Holmes DD: Clinical Laboratory Animal Medicine, 2nd ed., p.114. Iowa State University Press, Ames, Iowa, 1998
4. Maynard LA, Boggs D, Fisk G, Seguin D: Dietary mineral interrelations as a cause of soft tissue calcification in guinea pigs. J Nutr 64:85-97, 1958
5. Navia JM, Hunt CE: Nutrition, nutritional diseases, and nutrition research applications. In: Biology of the Guinea Pig, eds. Wagner JE, Manning PJ, pp. 256-257. Academic Press, New York, New York, 1976
6. Percy DH, Barthold SW: Pathology of Laboratory Rodents and Rabbits, 2nd ed., pp. 233-236. Iowa State University Press, Ames, Iowa, 2001
7. Smith SE, Williams MA, Bauer AC, Maynard LA: The wrist stiffness syndrome in guinea pigs. J Nutr 38**:**87-96, 1949
8. Sparschu GL, Christie RJ: Metastatic calcification in a guinea pig colony: A pathological survey. Lab Anim Care 18**:**520-526, 1968
9. www.merckvetmanual.com/mvm/htm/bc/171520.htm: Guinea pigs: Nutritional and metabolic diseases. In: The Merck Veterinary Manual, ed. Kahn CM, 9th ed., 2006
10. Myers RK, McGavin MD: Cellular and tissue responses to injury. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 47-49. Mosby Elsevier, St. Louis, Missouri, 2007
11. Kumar V, Abbas AK, Fausto N: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 41-42. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
12. Newman SJ, Confer AW, P anciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 642-643. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 52
CONFERENCE 13 / CASE IV – 9550 (AFIP 3026801)

Signalment: 10- to 12-week-old, female, F344 rat (*Rattus norvegicus*)

History: The rat arrived at our research institution at six to eight weeks of age and was group housed in standard rat caging. Four weeks after arrival, a large tumor was noted by caretakers. Physical exam revealed a large, firm, multilobulated tumor, firmly attached to the body wall on the left caudal thorax. She was in otherwise normal body condition and was demonstrating no other clinical signs. Due to the extent of the tumor, the rat was humanely euthanized.

Gross Pathology: A firm, well-demarcated, unencapsulated multilobular mass was present, arising from the left thoracic wall. The mass was 5.5 cm in maximal dimension and encompassed the left caudal ribs, invading the thoracic cavity (Figures 9550-G1, G2, G3: R denotes rib cage; thoracic side of mass shown). No other gross pathologic changes were observed.

Histopathologic Description: There is a multilobular well-demarcated and infiltrative mass that has effaced the majority of the left caudal and lateral rib cage (Figures 9550-HE-1-5) and extends into the pleural cavity. The mass is characterized by large irregular nodules and sheets of highly pleomorphic cells supported by a fine vascular stroma. Neoplastic cells vary markedly in size and shape from elongate to round or angular and contain one or more round to elongate markedly pleomorphic euchromatic nuclei with one or more prominent nucleoli and variable amounts of finely stippled to deeply eosinophilic cytoplasm. Large multinucleated giant cells and mitotic figures are common. Occasionally, large vacuolated cells ("spider" cells) may also be present. Neoplastic cells are red with Masson's Trichrome-stained sections of tumor (9550-MaTri-1), and fibrillar cross-striations are observed in phosphotungstic acid hematoxylin (PTAH)–stained sections of tumor (9550-PTAH-1,2,3).

Immunohistochemistry (See figure panel): The neoplastic cells are uniformly negative for Cytokeratin 7 and GFAP. The neoplastic cells are uniformly positive for Desmin. However, the neoplastic cells are variably positive for alpha smooth muscle actin. In some regions of the tumor there is strong positive cytoplasmic staining and in other areas the neoplastic cells are negative.

Although sections of this tumor were evaluated by electron microscopy (not shown), the images obtained were suboptimal due to preservation of the tissues in Bouins fixative. Regardless, we were able to visualize filament structures and Z-lines, further supporting the diagnosis of rhabdomyosarcoma.

Contributor's Morphologic Diagnosis: Thorax: Rib: Rhabdomyosarcoma, pleomorphic, Fischer 344 rat (*Rattus norvigicius*).

Contributor's Comment: Rhabdomyosarcoma is an uncommon malignant neoplasm that can arise in skeletal muscle or from nests of primitive mesenchymal cells located in tissues that lack striated muscle. Based on the human classification scheme, tumors can be categorized into one of the following types: embryonal (juvenile), botryoid, alveolar and pleomorphic (adult). These four types of tumors differ in gross presentation, histological appearance, prognosis, and age of onset.[1,2]

Rhabdomyosarcomas are often difficult to diagnose due to the variable and pleomorphic nature of the neoplastic cells. The presence of elongated cells containing visible striations ("strap cells") are often helpful, but striated cells are commonly absent or difficult to see in these tumors. Special stains, including phosphotungstic acid-hematoxylin (PTAH) and immunohistochemical staining, as well as electron microscopy, are useful and often necessary to perform in order to conclusively diagnose rhabdomyosarcoma.[1-4] Immunohistochemistry results are somewhat dependent upon the tumor type, as some cells vary in staining based on differentiation. The following table, extracted from Cooper, et. al., demonstrates the typical staining patterns of rhabdomyosarcomas:

Antibody	Result
Vimentin	- or weak +
Cytokeratin	-
Desmin	+
Muscle-specific actin	+
Smooth-muscle actin	-
Sarcomeric actin	+
Myoglobin	+

The immunohistochemistry results for alpha SMA in this rat are interesting. Most rhabdomyosarcomas are negative; however, it has been reported that some human and domestic animal rhabdomyosarcomas occasionally express smooth muscle actin.[5]

Electron microscopy is another useful tool for diagnosing rhabdomyosarcoma. Though there is some disagreement about the specific criteria used to diagnose the tumor, general components of the sarcomere should be recognized. Z lines, A—H—M band complexes or cross sections of thick and thin filaments in a hexagonal array are examples of these ultrastructural characteristics.[3]

Rhabdomyosarcomas occur in a variety of anatomical locations in humans and animals. The heart and lower extremities are the two most commonly affected regions in animals, though many other areas have been reported, including the ear, kidney, lung, urinary bladder, and uterus.[2] Rhabdomyosarcomas have been most commonly diagnosed in the dog; in this species, laryngeal rhabdomyosarcoma and botryoid rhabdomyosarcoma of the urinary bladder are recognized as distinct clinicopathologic entities.[5] The embryonal form is the most common form reported in the dog, while alveolar seems to be the least common, as with humans. In rats, spontaneous primary rhabdomyosarcoma has been reported in the mandible, ear pinna, left forelimb and axillary region, left hind limb, ventral abdominal wall, and the thoracic and abdominal cavities. In addition, metastatic lesions have been found in the mediastinum, lungs, diaphragm, and the base of the right ear.[2,4,6-9]

As with other species, rhabdomyosarcomas in rats are rare; in Fischer 344 rats specifically, the incidence is less than 1%.[10] Case reports have described tumors occurring in a wide age range, though it is thought that rhabdomyosarcoma is predominantly a disease of older rats.[2,6] This is in contrast to other species, where rhabdomyosarcoma is more frequently diagnosed in younger animals. It has been suggested that rhabdomyosarcomas of young rats are a distinct entity from that in the older animals, with the disease in young rats being characterized by a low incidence and the presence of highly differentiated tumors.[6] Consistent with case reports in young rats, this tumor demonstrates a high degree of cellular differentiation.

In humans, rhabdomyosarcoma is the most common soft tissue sarcoma in children.[6] Primary thoracic rhabdomyosarcomas are rare but can develop within the lung,

mediastinum, heart, and chest wall. Males are most commonly affected, and presentation generally occurs either during childhood or the 5th to 7th decades of life. In children with chest wall tumors, involvement of the ribs is rare, compared to other primary sarcomas of the chest wall, though focal invasion is common.[11] The tumor in this rat is similar to thoracic wall rhabdomyosarcoma in children in that it is a focally invasive tumor that encompasses, but does not invade, the ribs.

AFIP Diagnosis: Skeletal muscle: Rhabdomyosarcoma, Fischer 344 rat (*Rattus norvigicius*), rodent.

Conference Comment: The contributor provides a thorough summary of rhabdomyosarcomas to include the four types of rhabdomyosarcoma, the histomorphologic appearance, useful special stains, immunohistochemistry, and ultrastructural characteristics.

As pointed out by the contributor, rhabdomyosarcomas occur infrequently in many animal species. The histomorphologic appearance varies from well-differentiated neoplasms that resemble immature striated muscle to highly undifferentiated forms with pleomorphic cells that frequently contain giant, bizarre, multiple nuclei. There may be small or abundant amounts of cytoplasm that may be extensively vacuolated (spider cells). Rhabdomyosarcomas are prone to outgrow their blood supply which leads to deep necrosis.[11,12,13]

Botryoid rhabdomyosarcoma of the urinary bladder is an uncommon, distinct entity of the dog. It presents as a botryoid mass typically at the trigone area of the bladder in juvenile large-breed dogs, especially Saint Bernards. In cats, rhabdomyosarcoma is a rare variant of postvaccinal sarcomas.[11,12,13]

The benign form, rhabdomyoma, is virtually unknown in animals. Nearly all of the benign muscle tumors seen in animals are congenital rhabdomyomas of the heart and been reported in neonatal or juvenile pigs, cows, sheep, and dogs suggesting the tumors arise in utero. Less common sites of origin include the larynx, tongue, and dermis. The tumor is composed of large pleomorphic mononuclear or binucleated cells with abundant eosinophilic granular cytoplasm. Mitotic figures are rare; however, cytoplasmic cross-striations are frequently found. Many cells contain abundant cytoplasmic glycogen demonstrable by PAS staining.[11,12,13]

Contributor: Section of Comparative Medicine, School of Medicine, Yale University New Haven, CT, http://info.med.yale.edu/compmed/compmed/index.htm

References:

1. Cooper BJ, Valentine BA: Tumors of muscle. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 319-363, Iowa State Press, Ames, Iowa, 2002
2. Radi ZA: Auricular rhabdomyosarcoma in a rat. J Vet Med A Physiol Pathol Clin

Med 53(5):246-248, 2006
3. McDonald MM, Hamilton BF: Skeletal muscle. In: Pathology of the Fischer Rat: Reference and Atlas, eds. Boorman GA, Eustis SL, Elwell MR, Montgomery Jr. CA, MacKenzie WF, pp. 198-199. Academic Press, Inc., San Diego, California, 1990
4. Brockus CW, Rakich PM, King CS, Broderson JR: Rhabdomyosarcoma associated with incisor malocclusion in a laboratory rat. Contemp Top Lab Anim Sci 38(6):42-43, 1999
5. Conner MW: Spontaneous rhabdomyosarcoma in a young Sprague-Dawley rat. Vet Pathol 31(2):252-254, 1994
6. Glaister JR: Rhabdomyosarcoma in a young rat. Lab Anim 15(2):145-146, 1981
7. Kerry PJ, Evans JG, Pearson EC, Coleman H: Identification of a spontaneous pleomorphic rhabdomyosarcoma in the thoracic and abdominal cavities of a female Wistar rat. Vet Pathol 32(1):76-78, 1995
8. Minato Y, Takada H, Yamanaka H, Wada I, Takeshita M, Okaniwa A: Spontaneous rhabdomyosarcoma in a young rat. Nippon Juigaku Zasshi 45(6):837-842, 1983
9. Boorman GA, Everitt JI: Neoplastic disease. In: The Laboratory Rat, eds. Suckow MA, Weisbroth SH, Franklin CL, pp. 479-511. Elsevier, Burlington, Massachusetts, 2006
10. Gladish GW, Sabloff BM, Munden RF, Truong MT, Erasmus JJ, Chasen MH: Primary thoracic sarcomas. Radiographics 22(3):621-37, 2002
11. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 896. Williams & Wilkins, Baltimore, Maryland, 1997
12. Hendrick MJ, Mahaffey EA, Moore FM, Vos JH, Walder EJ: Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals, Second Series, vol. 2, pp. 21-22. Armed Forces Institute of Pathology, Washington, D.C., 1998
13. Hulland, TJ: Muscle and tendon. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 261-262. Academic Press, Inc., San Diego, California, 1993

SLIDE 53
CONFERENCE 14 / CASE I – 05-625 (AFIP 3026821)

Signalment: 1.5-year-old Tennessee Walking Horse gelding

History: This horse was recumbent and unable to rise three days after introduction of a new feed mix. Three of the group of five horses refused the feed. The two horses that ate it (including this one) were “wobbly” on their feet for two days. This horse ate double rations and progressed from recumbency to a moribund state. Treatment was unsuccessful and the horse was euthanized.

Gross Pathology: There were 720ml of serosanguinous pericardial effusion. The heart weighed 2.37kg (0.7% of body weight). There were multifocal to coalescing

irregular areas of myocardial pallor. The myocardium of the apex, subendocardium, and papillary muscles were most severely affected.

Laboratory Results: Feed samples were positive for monensin at 396 ppm.

Histopathologic Description: Heart (left ventricular free wall): There are multifocal to coalescing patchy areas of necrosis characterized by loss of striations, granular pale eosinophilic cytoplasm, and mineralization. There are increased numbers of plump nuclei in the interstitium (fibroblasts, macrophages, and hypertrophic endothelial cells).

Contributor's Morphologic Diagnosis: Subacute multifocal to coalescing myocardial necrosis

Contributor's Comment: Ionophores such as monensin, salinomycin, lasalocid, laidlomycin, and narasin are used as growth promoters in ruminants and coccidiostats in poultry. In this case, monensin was introduced into equine sweet feed in a mixing error at a local feed mill. Several horses on multiple different farms died or showed clinical signs. Exposed horses were evaluated by bloodwork, electrocardiogram (ECG), and echocardiogram. Numerous horses had elevated creatine kinase (CK), the cardiac isoform of CK (CK-MB), and/or aspartate transaminase (AST). Horses are exquisitely susceptible to monensin toxicity. This is thought to be due to the low catalytic activity of equine hepatic cytochrome P450.[1] The species susceptibilities are as follows:[2]

Species	Monensin LD50 (mg/kg)
Horses	2-3
Dogs	5-8
Pigs	5-8
Sheep and Goats	10-12
Cattle	20-34
Poultry	90-200

Ionophores inhibit sodium and potassium ion transport across mitochondrial and other cell membranes. This leads to mitochondrial failure, depletion of ATP, failure of calcium transport out of cell, and persistent contraction. Highly metabolically active cells, such as papillary muscle myocardiocytes, are most severely affected. In some cases skeletal muscles are also affected, while in others there are no gross or microscopic lesions.[1] Although skeletal muscles were affected in this horse, the lesions only involved the large pelvic limb adductors, and this was presumed to be due to recumbency.

AFIP Diagnosis: Heart, myocardium: Degeneration and necrosis, multifocal, moderate, with mineralization, Tennessee Walking Horse (*Equus caballus*), equine.

Conference Comment: The contributor provides a concise summary of ionophores, differences in species susceptibility, and the pathogenesis involved in ionophore toxicity.

Gross lesions associated with monensin toxicity vary depending on dose and duration of exposure and may not be detectable in acute cases. Ill-defined pale streaks may be visible in cardiac and skeletal muscle. As the disease progresses, these areas of pallor become more prominent and affected skeletal muscles atrophy. Myocardial lesions in monensin toxicity are irreparable, especially in growing animals, and the probability of lasting cardiac malfunction is high.[2]

Ultrastructural characteristics include marked swelling and disintegration of mitochondria, multifocal necrosis with type 1 fiber preference, vacuolization of sarcoplasmic reticulum, lipid accumulation, and formation of myeloid bodies.[2,3]
The differential diagnosis for the cardiac muscle lesions in this horse includes ionophore (monensin) toxicity, vitamin E/selenium deficiency, exertional rhabdomyolysis, and ingestion of *Cassia* sp. plants. Because the histologic lesions in monensin toxicity differ very little from nutritional or exertional myopathy, clinical history and feed analysis are critical for definitive diagnosis. Simultaneous onset of pronounced clinical signs in multiple, especially adult, animals suggests exposure to a toxic agent. Vitamin E/selenium deficiency most often occurs in young foals and commonly involves the masticatory muscles and tongue, and concurrent steatitis is often present. Nutritional myopathy occurs sporadically in older horses, and steatitis is usually absent. Myocardial lesions may be present in foals and adult horses with nutritional myopathy. The myocardium is infrequently involved in exertional rhabdomyolysis, and there is usually significant damage to the renal proximal convoluted tubules secondary to myoglobinuric nephrosis and ischemia. The myocardium is also not extensively involved in cases of *Cassia* sp. toxicity and necrosis of pancreatic acinar cells also occurs.[2,4]

Readers are encouraged to review WSC 4 / Case I, 2006-2007 to compare and contrast this case with a case of capture (exertional) myopathy.

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References:
1. Peek SF, Marques FD, Morgan J, Steinberg H, Zoromski DW, McGuirk S: Atypical acute monensin toxicoses and delayed cardiomyopathy in Belgian draft horses. J Vet Intern Med 18:761-764, 2004
2. Hulland TJ: Muscle and tendon. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 234, 237-240. Academic Press, Inc., San Diego, California, 1993
3. Cheville NF: Ultrastructural Pathology: An Introduction to Interpretation, p. 811. Iowa State University Press, Ames, Iowa, 1994

4. Cullen JM: Liver, biliary system, and exocrine pancreas. In: Pathologic Basis of Veterinary Disease, eds. McGavin, MD, Zachary JF, 4th ed., p. 457. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 54
CONFERENCE 14 / CASE II – 03-11548 (AFIP 3027582)
Signalment: 1-year-old, female German Shorthair Pointer

History: The dog was bitten by a rattlesnake while hiking with its owners. It was treated with steroids by a local veterinarian but died the next day.

Gross Pathology: The subcutis of the muzzle, lips and intermandibular space were severely expanded by thin, red-tinged fluid, especially on the left side. Within the oral cavity, on the ventral buccal mucosa of the left side were a pair of 2mm, round, red puncture wounds that were 1.3 cm apart (presumptive snake bite wound). The right ventricular myocardium had numerous, pale, linear streaks.

Other gross lesions included pansystemic petechiation, serosanguinous pleural effusion, suffusive pulmonary hemorrhage and severe melena. The kidneys were grossly normal.

Histopathologic Description: Within a single section of kidney, many glomerular capillaries contain fibrin thrombi. In several glomeruli, capillaries are widely dilated and distended by fibrin and red blood cells (microaneurysm formation). PTAH stain confirmed fibrin within dilated glomerular capillaries. Occasionally, microaneurysms obliterate glomerular structures. Hyaline casts and bright red, granular casts are present within proximal convoluted tubules and within collecting ducts. Renal tubular epithelium is degenerate and necrotic in these areas. Rare basement membranes are mineralized.

Other histologic lesions seen in this case were 1) Skeletal muscle hemorrhage and necrosis (associated with the snake bite), 2) Pulmonary hemorrhage and edema and 3) Acute, severe, multifocal myocardial degeneration and necrosis with mineralization

Contributor's Morphologic Diagnoses:
1. Diffuse, acute, moderate hemoglobin nephrosis
2. Mesangiolysis with glomerular capillary thrombosis and microaneurysm formation

Contributor's Comment: Pit vipers are the largest group of venomous snakes in the United States, and account for the vast majority of venomous snake bites in the US.[1] The common pit vipers of the western US are rattlesnakes (*Crotalus* and *Sistrurus* spp.); in the location where this bite took place, the most likely species was the Northern Pacific rattle snake (*C. viridis oreganus*). Pit viper venom does not vary in toxicity throughout the year, although snakes produce more venom in the hot, summer months

and adults within a species can have higher levels of toxic components.[1] Individual snakes can also control the amount of venom delivered in each strike; initial defensive strikes may be non-envenomating, whereas agonal bites often deliver the entire venom load. Beside these snake-related factors, severity of envenomation is also affected by host factors, including body mass, location of the bite, post bite excitability and premedications such as NSAIDS that can predispose the victim to more severe clotting defects.

Pit viper venom is composed of several enzymatic and non-enzymatic components that affect the variable clinical signs of pain, swelling, ecchymoses, weakness, nausea, shock and grossly visible tissue necrosis. Trypsin-like enzymes can mediate tissue destruction, while hyaluronidase, also called “spreading factor”, decreases connective tissue viscosity, allowing penetration of other toxic components. Phospholipases A and B mediate membrane breakdown and release of a variety of endogenous proinflammatory products. Systemic hemorrhage may be mediated by venom components that either inhibit platelet aggregation or platelet-collagen interactions, inducing fibrinogenolysis through plasminogen activation or by direct toxic action on blood vessels. Thrombin-like enzymes can mediate increased clotting; this action, along with venom-induced thrombocytopenia and fibrinogenolysis often results in a syndrome resembling disseminated intravascular coagulation. In addition, direct cardiotoxic effects have been attributed to snake venom. Some venoms have primarily neurotoxic effects; in these cases, life-threatening envenomation can occur with little local tissue reaction.

Toxic glomerular vasculopathy associated with snake envenomation has been called mesangiolysis, capillary ballooning or glomerular microaneurysm formation. Mesangiolysis refers to direct injury to mesangial cells and matrix, reducing support for glomerular capillaries and leading to capillary dilation. Eventually endothelial damage occurs leading to coalescence of capillary lumina and microaneurysm formation.[2] Direct injury to mesangial cells by snake venom has been demonstrated experimentally using venom from the pit viper *Bothrops moojeni*.[3] Phospholipase A and other proteolytic enzymes are likely responsible for severe mesangiolysis seen in some snake bite cases. It is unclear whether glomerular fibrin deposits are secondary to abnormal vascular flow through the dilated glomerular capillaries, or are secondary to clotting abnormalities. Experimental renal lesions of envenomation are transitory and mesangiolysis is considered to be a reversible change, or the lesion may resolve to proliferative glomerulonephritis or glomerulosclerosis.

Although snake bite cases are most often not fatal, the bite in the oral cavity from a snake likely in its death throes probably delivered a large amount of venom into the systemic circulation of this dog. The cause of death was considered to be a combination of acute myocardial necrosis, acute renal failure and a DIC type syndrome associated with snake envenomation.

AFIP Diagnosis: Kidney: Glomerular aneurysms and fibrin thrombi, multifocal, with moderate, acute tubular degeneration and necrosis, and cellular and granular casts, German Shorthair Pointer (*Canis familiaris*), canine.

Conference Comment: The contributor provides a thorough overview of snake envenomation to include snake and host related factors affecting the severity of envenomation, the components of pit viper venom, the typical light microscopic findings associated with toxic glomerular vasculopathy, and the pathogenesis involved in the development of the clinical signs associated with snake bite envenomation.

Clinical pathological findings may include the following: echinocytosis, leukocytosis, hemolytic anemia, thrombocytopenia, hyperfibrinogenemia, prolonged clotting times, elevated fibrin split products, and elevated creatinine kinase. In a retrospective study performed by Hackett et. al involving 100 client owned dogs, echinocytosis was the most common hematological change in prairie rattlesnake envenomation in dogs and was evident before substantial swelling took place. Possible mechanisms for the echinocytosis include depletion of ATP from cell membrane cation pumps by ATPase enzymes and alteration of erythrocyte membrane composition by phospholipases, both of which are present in rattlesnake venom. In a study performed by Walton et. al, the addition of ethylenediaminetetraacetic acid prevented the formation of echinocytes in vitro, suggesting a change in calcium or a metalloprotein participated in echinocyte formation. In cases of severe envenomation (e.g., large venom dose in a small dog), spherocytosis may be observed.[1,4,5,6]

Snake bites are common in the dog, horse, and to a lesser degree cats and are most commonly inflicted on the head or legs. The differential diagnosis for snake bites includes trauma, angioedema (e.g., insect bites and stings), other animal bites, draining abscesses, and penetrating wounds.[1,7]

Conference participants briefly reviewed the intrinsic and extrinsic pathways of the coagulation cascade. Many agents or conditions can initiate coagulation by causing widespread endothelial damage exposing thrombogenic subendothelial collagen or by directly activating the coagulation cascade via the intrinsic or extrinsic pathway. Exposure of thrombogenic subendothelial collagen will cause the expression of tissue factor (factor III, thromboplastin) leading to activation of the extrinsic pathway. Although the coagulation cascade is divided into intrinsic and extrinsic pathways for ease of understanding and for in vitro testing, this division does not exist in vivo. The tissue factor-activated factor VII complex is a potent activator of factor IX and factor X.[9] Included below is a table of agents or conditions known to induce DIC in animals from Jubb and Kennedy. Many of the agents listed in the table may initiate DIC by more than one route.[8]

Agents or Conditions Known to Induce Disseminated Intravascular Coagulation in Animals

Bacteria	Gram negative (endotoxin)
	Gram positive
Helminths	*Dirofilaria immitis*

Protozoa	*Theileria* sp.
	Sarcocystis sp.
	Babesia sp.
Rickettsia	*Rickettsia rickettsii*
Viruses	African swine fever
	Hog cholera
	Bluetongue
	Epizootic hemorrhagic disease of deer
	Infectious canine hepatitis
	Feline infectious peritonitis
	Aleutian disease of mink
Neoplasia	Carcinoma
	Leukemia
	Hemangiosarcoma
Other	Aflatoxicosis
	Antigen-antibody complexes (incompatible blood transfusion)
	Gastric dilation (volvulus)
	Heat stroke
	Hyperlipemia in ponies
	Hyperosmolality
	Immunologic endothelial injury
	Ingestion of red maple leaves
	Proteolytic enzymes (pancreatitis, snake bite)
	Shock, vascular stasis, prolonged anesthesia, acidosis
	Tissue necrosis (hepatic, pneumonia, postsurgery, burns)

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References:

1. Peterson ME. Snake bite: North American pit vipers. In: Small Animal Toxicology, eds. Peterson ME, Talcott PA, 2nd ed., pp. 1017-1083. Elsevier Saunders, St. Louis, Missouri, 2006
2. Morita T, Yamamoto T, Churg J: Mesangiolysis: an update. Am J Kidney Dis 31:559-573, 1998
3. Boer-Lima PA, Gontijo JA, Da Cruz-Höfling MA: *Bothrops moojeni* snake venom-induced renal glomeruli changes in rat. Am J Trop Med Hyg 67:217-222, 2002
4. Hackett TB, Wingfield WE, Mazzaferro EM, Benedetti JS: Clinical findings associated with prairie rattlesnake bites in dogs: 100 cases (1989-1998). J Am Vet Med Assoc 220:1675-1680, 2002
5. Willey JR, Schaer M: Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) envenomation of dogs: 31 cases (1982-2002). J Am Anim Hosp Assoc 41:22-33, 2005
6. Walton RM, Brown DE, Hamar DW, Meador VP, Horn JW, Thrall MA: Mechanisms of echinocytosis induced by *Crotalus atrox* venom. Vet Pathol 34:442-449, 1997
7. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin, MD, Zachary JF, 4th ed., p. 1168. Mosby Elsevier, St. Louis, Missouri, 2007

8. Robinson WF, Maxie MG: The cardiovascular system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 62-64. Academic Press, Inc., San Diego, California, 1993
9. Topper MJ, Welles EG: Hemostasis. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse KW, 4th ed., pp. 117-119. Iowa State Press, Ames, Iowa, 2003

SLIDE 55
CONFERENCE 14 / CASE III – D0604909 (AFIP 3027589)

Signalment: Adult, Angus cow, Bovidae, subfamily Bovinae, *Bos taurus*

History: An 880 lb cow on free range pasture started to show clinical signs of lethargy, dehydration, weight loss and alopecia which started one week prior to submission. Eight cows died within a week, all showing similar clinical signs. All cows that showed clinical signs died. Only cows that had been in the pasture the previous year died. None of the heifers on the same pasture or bulls on adjacent pasture died.

Gross Pathology: This 880 lb Angus cow was thin and had large confluent regions of alopecia and crusting on the head, neck, axillary region, and ventrum with scattered patchy foci on the skin of the trunk. There was consolidation of the anterioventral portion of the right lung. The kidneys were pale and swollen and the liver was uniformly red/brown and swollen. There was generalized enlargement of the lymph nodes which were uniformly tan and wet.

Laboratory Results: *Pasteurella mannheimia* was isolated from the consolidated region of the right lung.

Histopathologic Description: Adrenal gland: Diffuse dense lymphohistiocytic, plasmacytic and lesser granulocytic infiltrates extend from the capsular surface through the cortex and medulla with widely scattered single cell necrosis.
Kidney: Severe multifocal to coalescing regions of interstitial inflammation that is most prominent in the cortex but extends multifocally into the medulla. Inflammation separates and entraps tubules and glomeruli and is comprised of mostly lymphocytes with lesser numbers of eosinophils, macrophages, and multinucleated giant cells. Occasional entrapped tubules are mildly distended with luminal necrotic cell debris.

Skin: There is parakeratotic hyperkeratosis with occasional subcorneal pustules, mild acanthosis and spongiosis and light to moderate diffuse inflammatory cell infiltrates that extend from the superficial through the papillary dermis. The infiltrate is most prominent in the superficial dermis subtending the epidermis and around adnexa with some exocytosis into the follicular epithelium. Inflammatory cells are comprised of mostly lymphocytes with lesser numbers of macrophages, many pigment-laden, and

eosinophils and plasma cells. There is superficial dermal edema and mild exocytosis of lymphocytes into the epidermis.

Contributor's Morphologic Diagnoses: Vetch toxicosis with:
1. Interstitial nephritis, severe, multifocal-coalescing, lymphohistiocytic, eosinophilic and granulomatous.
2. Adrenalitis, severe, diffuse, lymphogranulocytic.
3. Dermatitis, moderate, diffuse, lymphohistiocytic, eosinophilic with hyperkeratotic parakeratosis and subcorneal pustules.

Contributor's Comment: Vetch poisoning is a generalized disease characterized by multiorgan infiltration with lymphocytes, plasma cells, eosinophils, and multinucleated giant cells reported in cattle and horses.[1,2,3,4,5] Clinical signs include dermatitis, alopecia, diarrhea, weight loss, organ failure and death. Purple vetch (*Vicia benghalensis*), a hybrid variety (*Vicia villosa* subspecies *dasycarpa*), and hairy vetch (*Vicia villosa*) have been associated with vetch poisoning.[1,2,3,4] Reports from Oklahoma, Missouri, Kansas, Georgia, New York, California, Australia and Argentina suggest geographic distribution is extensive.[1] The cause(s) or factors associated with vetch toxicosis are unknown. Although vetch is widely used as forage for livestock, morbidity is typically low but has been reported as high as 68%.[1,5] Additionally, vetch-like disease has been described in cattle that have had similar clinical and pathologic signs/lesions but have not been exposed to vetch. Association with grass silage preserved with a formalin/sulphuric acid commercial additive, citrinin and citrus pulp have been described in these cases.[6,7,8]

In this case, the cows were on pasture that contained large stands of purple vetch (*Vicia benghalensis*). Only cows that had been on the pasture the previous year were affected suggesting these animals were previously sensitized to antigen associated with the vetch. The factor that previous exposure to the antigen is required to elicit clinical signs/lesions and the characteristic of the inflammatory response suggest the host response is likely a type-IV hypersensitivity reaction but neither the cutaneous hypersensitivity nor the lymphocyte blastogenesis tests have supported this theory thus far.[1]

AFIP Diagnoses:
1. Kidney: Nephritis, interstitial, granulomatous and eosinophilic, multifocal to coalescing, moderate, with mild tubular degeneration, necrosis, and regeneration, Angus (*Bos taurus*), bovine.
2. Adrenal gland: Adrenalitis, granulomatous and eosinophilic, multifocal to coalescing, severe.
3. Haired skin: Dermatitis, lymphoplasmacytic, histiocytic, and eosinophilic, diffuse, moderate, with acanthosis, orthokeratotic hyperkeratosis, spongiosis, and superficial dermal edema.

Conference Comment: Hairy vetch is a legume that is cultivated and used as pasture, hay, and silage in most of the United States and in other countries. Vetch toxicosis occurs in cattle and to a lesser extent in horses after consumption of vetch-containing pastures. Vetch toxicosis is most commonly seen as a syndrome characterized by dermatitis, conjunctivitis, diarrhea, and multisystemic granulomatous and eosinophilic disease.[1,9]

The toxic principle in vetch seeds is prussic acid; however, the cause of the granulomatous inflammation remains unclear. One proposed pathogenesis involves a type IV hypersensitivity reaction secondary to ingestion of constituents of the plant that are absorbed and act as antigens that sensitize lymphocytes and evoke a multisystemic granulomatous response upon repeat exposure. Alternatively, vetch lectin may directly activate T lymphocytes to initiate the cellular response.[1,9,10]

Three syndromes associated with consumption of hairy vetch have been reported in cattle:

1. Acute nervous derangement and death after ingestion of seeds
2. Subcutaneous swellings if the head, neck, and body, herpetiform eruptions in the oral mucous membranes, purulent nasal discharge, rales, cough, and congestion associated with consumption of hairy vetch pasture
3. Dermatitis, conjunctivitis, diarrhea, and extensive infiltration of various organs by monocytes, lymphoplasmacytic cells, occasional multinucleated giant cells, and often eosinophils also associated with consumption of hairy vetch pasture

Vetch-associated disease is more prevalent and severe in cattle over 3 years of age with Holstein and Angus breeds being disproportionately affected.[1]
Initial lesions in cattle include a rough coat with papules and crusts on the skin of the udder, teats, escutcheon, and neck progressing to involvement of the trunk, face, and limbs. The skin becomes alopecic and lichenified. Marked pruritis leads to excoriations secondary to self-induced trauma. Grossly, yellow nodular infiltrates of mononuclear leukocytes disrupt the architecture of a wide range of organs but are most severe in myocardium, kidney, lymph nodes, thyroid gland, and adrenal glands. In cattle, other species of *Vicia* and additional compounds such as diureidoisobutane, citrus pulp, and citrinin are capable of inducing disease indistinguishable from hairy vetch as pointed out by the contributor.[7,9,10]

Hairy vetch toxicosis in horses is similar to cattle; however, marked pruritis, diarrhea, and large numbers of eosinophils in the inflammatory infiltrate do not occur. Additionally, conditions very similar to vetch toxicosis have been reported in horses with no exposure to vetch. These cases have been referred to as “equine sarcoidosis,” “idiopathic granulomatous disease involving the skin,” “systemic granulomatous disease,” or “generalized granulomatous disease.” Scaling, crusting, and alopecia of the face or limbs are seen that progresses to a generalized exfoliative dermatitis. Organ involvement is variable.[9,11]

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References:
1. Panciera RJ, Mosier DA, Ritchey JW: Hairy vetch (*Vicia villosa* Roth) poisoning in cattle: update and experimental induction of disease. J Vet Diagn Invest 4:318-325, 1992
2. Woods LW, Johnson B, Hietala SK, Galey FD, Gillen D: Systemic granulomatous disease in a horse grazing pasture containing vetch (*Vicia* sp). J Vet Diagn Invest 4:356-360, 1992
3. Burroughs GW, Neser JA, Kellerman TS, Van Niekerk FA: Suspected hybrid vetch (*Vicia villosa* crossed with *Vicia dasycarpa*) poisoning of cattle in the republic of South Africa. J South Afr Vet Assoc 54:75-79, 1983
4. Johnson B, Moore J, Woods LW, Galey FD: Systemic granulomatous disease in cattle in California associated with grazing hairy vetch (*Vicia villosa).* J Vet Diagn Invest 4:360-362, 1992
5. Odriozola E, Paloma E, Lopez T, Campero C**:** An outbreak of *Vicia villosa* (hairy vetch) poisoning in grazing Aberdeen Angus bulls in Argentina. Vet Hum Toxicol 33:278-280, 1991
6. Thomas GW: Pyrexia with dermatitis in dairy cows. Vet Rec 102:368, 1978
7. Griffiths IB, Done SH: Citrinin as a possible cause of the pruritis, pyrexia, haemorrhagic syndrome in cattle. Vet Rec 129:113-117, 1991
8. Saunders GK, Blodgett DJ, Hutchins TA, Prater RM, Robertson JL, Friday PA, Scarratt WK: Suspected citrus pulp toxicosis in dairy cattle. J Vet Diagn Invest 12:269-271, 2000
9. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin, MD, Zachary JF, 4th ed., pp. 1169-1170. Mosby Elsevier, St. Louis, Missouri, 2007
10. Yager JA, Scott DW: The skin and appendages. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 591. Academic Press, Inc., San Diego, California, 1993
11. Scott DW, Miller WH: Equine Dermatology, p. 615. Saunders, St. Louis, Missouri, 2003

SLIDE 56
CONFERENCE 14 / CASE IV – S01262 (AFIP 3038505)

Signalment: 8-year-old, male, mixed breed dog

History: The dog was presented to the local veterinarian with lethargy, anorexia, vomiting, bloody diarrhea, jaundice and seizures. Dogs die within a few days of liver and kidney failure and disseminated intravascular coagulation.

Gross Pathology: Severe diffuse jaundice. The liver was enlarged, pale, and yellow with multifocal petechial hemorrhages. Small amounts of blood-stained contents were found in the gastrointestinal tract, especially in the small intestine. In the respiratory

tract, multifocal hemorrhages in the laryngeal mucosa were present. No other significant findings on post mortem examination.

Laboratory Results: A profile of the blood clinical chemistry parameters of the dog:

Parameter	Result	Lower normal limit	Upper normal limit
ALT (U/L)	691	0	60
ALP (U/L0	1113	0	150
AST (U/L)	203	0	50
GGT (U/L)	33.0	0.0	6.0
Amylase (U/L)	267	200	1480
Total Bilirubin (mg/dL)	11.7	0.1	0.5
Creatinine (mg/dL)	0.8	0.5	1.5
Urea (mg/dL)	5.4	10	28
Total protein (g/dL)	7.1	5.4	7.5
Albumin (g/dL)	3.6	2.6	4.0
Cholesterol (mg/dL)	195	135	280
Triglycerides (mg/dL)	121	50	100
Calcium (mg/dL)	11.7	9.0	11.7
Phosphorus (mg/dL)	6.4	2.5	6.2

Contributor's Morphologic Diagnosis: Liver: Hepatic degeneration and necrosis, hydropic and fatty, with bile duct proliferation, marked bridging fibrosis and mild histiocytic lymphocytic infiltration.

Contributor's Comment: Aflatoxicosis in dogs is a condition caused by the contamination of dog food by the fungal species *Aspergillus* with the production of aflatoxins. The first report of pathology in dogs due to aflatoxicosis was made in the United Sates in 1952; however, at that stage the etiology was unknown and the disease was referred to as "hepatitis X".[1] Aflatoxin production may occur both in the field and in storage when environmental conditions are favorable as occurs in warm, temperate, subtropical or tropical climates.[3] Dog food manufacturers regularly test their food products; however, small amounts may reach the final product undetected. The toxin produced by the fungus is usually aflatoxin B1.

Aflatoxins are hepatotoxic.[4] Hepatotoxicity is the result of widespread and nonspecific interactions between aflatoxins and/or their activated metabolites and various cell proteins. This interaction results in the disruption of basic metabolic processes and protein synthesis causing cell death.[3]

Besides being potent toxic compounds they are also carcinogenic, mutagenic and immunosuppressive agents. Aflatoxins are rapidly absorbed from the small intestine, metabolized in the liver to an epoxide active form by cytochrome P450 and conjugated with glutathione. The epoxide form results in oxidative damage via free-radical formation. Additionally, the epoxides may undergo further reactions including conjugation to glutathione, conversion to dihydrodiols and subsequently binding to macromolecules or to DNA, resulting in disruption of DNA and carcinogenesis.

The necrotizing effects and fatty changes seen in intoxicated animals are probably related to the inhibition of protein and RNA synthesis by the toxin. The bile duct hyperplasia seen in intoxication with aflatoxin may represent an attempt to regenerate parenchyma when the parenchymal cells have lost their ability to regenerate.[4] Anisocytosis, karyomegaly, binucleation and multinucleation probably represent the direct of effects of aflatoxin on the hepatocytes, as aflatoxin B1 is known to interfere with mitosis.[2]

Aflatoxin B1 is toxic to a wide range of species although there are significant differences in their sensitivity. Pigs are more sensitive that cattle which are more sensitive than sheep. Among laboratory animals, rats, guinea pigs and rabbits are significantly more sensitive than mice.[3] Dogs are considered to be quite sensitive with other susceptible species such as the duck, rabbit, cat and pig.[5] Dogs may be fatally intoxicated by a dose rate of less than 1.0 mg/kg bodyweight.[4]
Aflatoxicosis among dogs was identified in Israel in December 2005 due to the contamination of dog food from a specific supplier. Despite the warning by the manufacturer and the recall of the particular batch, some retailers apparently continued to sell the food with the resulting widespread toxicity.

In this outbreak, signs of toxicity covered a wide spectrum from acute to chronic. The aflatoxin dose and exposure duration determine the time of onset of clinical signs and their manifestations. Acute intoxication results from exposure to high concentrations of aflatoxin while chronic intoxication, which is more common, occurs following exposure to lower doses for a more prolonged period of time. A diagnosis of aflatoxicosis relies on the demonstration of aflatoxins in the feed and the occurrence of characteristic hepatic lesions. There is no specific treatment for affected animals.

AFIP Diagnosis: Liver: Hepatocellular vacuolar degeneration and necrosis, diffuse, severe, with biliary hyperplasia, and numerous pigment-laden macrophages, mixed breed (*Canis familiaris*), canine.

Conference Comment: The contributor provides a thorough overview of aflatoxicosis. Aflatoxins are a group of bishydrofurans produced by several strains of fungi, primarily *Aspergillus flavus*, *A. parasiticus*, and *Penicillium puberlum*. At least 13 aflatoxins have been identified with B_1, B_2, G_1, and G_2 being most common.[3,4]

In addition to hepatotoxic, carcinogenic, teratogenic, and immunosuppressive effects, aflatoxins also have anticoagulative effects due to decreased hepatic synthesis of coagulation factors, prothrombin, and fibrinogen. In acute aflatoxicosis with severe hepatic necrosis, disseminated intravascular coagulation (DIC) can cause coagulopathy which can lead to extensive hemorrhage and anemia.[1]

As the level of aflatoxin increases, the liver may show all or none of the following changes: enlargement, pallor, bile staining, increased firmness due to fibrosis, and

nodular regenerative hyperplasia. In severe cases, edema of the gallbladder and bile-tinged ascites may be observed. In cases of acute fulminating liver necrosis, after consumption of very high concentrations of aflatoxin, widespread hemorrhage and massive hepatic necrosis are observed.[3,4]

Typical light microscopic findings in cases of acute or subchronic aflatoxicosis in most species include hepatocyte degeneration, necrosis, hepatocellular vacuolation, anisocytosis, anisokaryosis, megalocytosis, bile duct or oval cell proliferation, cytosegresome formation and nodular regeneration that may progress to cirrhosis or cancer.[3,4]

Marked bridging fibrosis was not observed in the sections of liver received. Additionally, some slides had a section of essentially normal small intestine.

Contributor: Kimron Veterinary Institute, Department of Pathology, P.O. Box 12, 50250, Bet Dagan, Israel

References:

1. Bastianello SS, Nesbit JW, Williams MC, Lange AL: Pathological findings in a natural outbreak of aflatoxicosis in dogs. Onderstepoort J Vet Res 54:635-640, 1987
2. Chaffee VW, Edds GT, Himes JA, Neal FC: Aflatoxicosis in dogs. Am J Vet Res 30(10):1737-1749, 1969
3. Haschek WM, Voss KA, Beasley VR: Mycotoxins. In: Handbook of Toxicologic Pathology, eds. Haschek WM, Rousseaux CG, Wallig MA, pp. 650-656. Academic Press, San Diego, California, 2002
4. Kelly, WR: The liver and biliary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 319-406. Academic Press, Inc., San Diego, California, 1993
5. Ketterer P J, Williams ES, Blaney BJ, Connole MD: Canine aflatoxicosis. Aust Vet J 51:355-357, 1975

SLIDE 57
CONFERENCE 15 / CASE I – 06-2418 (AFIP 3031120)

Signalment: Term fetus, male, Angus, bovid, *Bos taurus*

History: Abortion. No information regarding vaccination of the dam or other abortions in the group available.

Gross Pathology: Field necropsy performed by submitting veterinarian. No gross pathologic lesions described.

Laboratory Results:

Bacterial culture - stomach content, lung, liver, placenta: No significant bacteria isolated.

Serology, dam – negative for *Leptospira* (*L. pomona, L. grippotyphosa, L. hardjo, L. ictohemorrhagica, L. canicola*), negative for *Neospora caninum*. IBR positive at 1:128, BVD-1 positive at 1:64, BVD-2 positive at 1:256

Fetal fluid immunoglobulin: 23 mg/dl

PCR: Positive for IBR, negative for BVD

Fetal liver selenium: 1.58 µg/g weight (normal 1.5-3.5)

Viral isolation: Lung, kidney, thymus: No viruses isolated

Histopathologic Description: Fixed tissues submitted and examined were cerebrum, small intestine, skeletal muscle, heart, spleen, kidney, liver, lung, thymus, eyelid, colon, and placenta. Tissues were moderately autolyzed.

Cerebral sections contain numerous small perivascular cuffs of lymphocytes, with scattered lymphocytes within the parenchyma. Inflammation is associated with areas of parenchymal rarefaction and prominent neuronal necrosis, with suspect areas of gliosis. Neuronal nuclei are often markedly enlarged with marginated chromatin, and often contain large round to oval amphophilic inclusions. Mild lymphocytic infiltration is present within the meninges.

Other lesions detected are thymic and splenic lymphoid depletion, placental vasculitis and vascular necrosis, multifocal renal necrosis, and a focal crypt abscess in the small intestine. Bile is prominent within canaliculi, but no convincing necrosis is detected in the liver.

Contributor's Morphologic Diagnosis: Cerebrum: Necrotizing nonsuppurative encephalitis with intranuclear inclusions (herpesvirus infection).

Contributor's Comment: Findings are indicative of abortion and encephalitis due to herpesvirus. Two alphaherpesviruses, BHV-1 and BHV-5, have been associated with encephalitis and meningoencephalitis in calves and adult cattle.[1-6] Both are neurotropic but, although BHV-1 can be isolated from brain tissue of cattle with the respiratory form of disease, encephalitis due to BHV-1 is uncommon.[1,2,5] Encephalitis due to herpesvirus infection in cattle is most often due to BHV-5.[1-7] To our knowledge, encephalitis in an aborted fetus infected with BHV has not been reported.

BHV-1 subtypes include subtype 1 (BHV-1.1, which is primarily associated with respiratory disease) and subtypes 2 (BHV-1.2a and BHV-1.2b), which are associated with genital disease.[7] The virus previously classified as BHV-1 subtype 3 (BHV-1.3) is now classified as BHV-5. Experimental infection of calves with neurovirulent BHV-5

resulted in neuronal infection and encephalitis.[3] BHV-5 meningoencephalitis is endemic in South America but occurs only sporadically elsewhere.[2-4]

Both BHV-1.1 and BHV-1.2a are capable of causing abortion.[7] Foci of necrosis and leukocytic infiltration of multiple organs, but especially of liver, are characteristic of BHV abortion.[7] In this case, characteristic hepatic necrosis was not detected.
Intranuclear inclusions within neurons infected with either BHV-1 or BHV-5 encephalitis have been reported, but are uncommon.[2,3,4,7] Viral inclusions are reported to be transient, appearing for approximately 2-3 days after infection, which likely explains their absence in most field cases.[7]
There is evidence that central nervous system infection occurs due to spread of virus from nasal mucosa via the trigeminal nerve to the trigeminal ganglion or via the olfactory nerves to the olfactory cortex.[4,6] Central nervous system infection due to viremia has also been suspected.[6] Viral transport by circulating leukocytes leads to placental and fetal infection.[6]

Differentiation of BHV-1 and BHV-5 can be difficult.[4] Although the PCR primers utilized in this case are thought to be specific for BHV-1, viral isolation was attempted in order to further characterize the virus. Unfortunately, no unfixed brain tissue was available for viral studies, and no virus was isolated from other tissues. Immunohistochemistry for BHV was also positive in this case (courtesy of Dr. Fabio Del Piero, New Bolton Center, University of Pennsylvania), but further studies are needed to characterize the virus in this case.

AFIP Diagnosis: Brain, cerebrum: Encephalitis, lymphoplasmacytic and necrotizing, multifocal, moderate, with glial and neuronal intranuclear inclusion bodies, Angus (*Bos taurus*), bovine.

Conference Comment: The contributor provides a concise summary of bovine herpesvirus 1 (BHV-1) and bovine herpesvirus 5 (BHV-5). Other important bovine herpesviruses include bovine herpesvirus 2 (pseudo-lumpy skin disease, bovine herpes mammilitis) and bovine herpesvirus 4 (bovine herpes mammary pustular dermatitis). Bovine herpesvirus 2 is dermatotropic and can cause generalized disease (pseudo-lumpy skin disease) or a localized infection of the teat. Localized infection occurs most commonly in dairy cattle secondary to trauma. Decreased milk production and secondary bacterial mastitis are common sequelae. Lesions develop on the teats and skin of the udder. Suckling calves develop lesions on the muzzle. Bovine herpesvirus 4 causes similar but milder disease that the localized form of bovine herpesvirus 2.[8]
Conference participants briefly reviewed other encephalitic herpes viruses that occur in various species to include malignant catarrhal fever (ovine herpesvirus 2, alcelaphine herpesvirus 1), equine herpesvirus 1, and pseudorabies (porcine herpesvirus 1). Encephalitic herpesviruses cause cell injury by inducing 1) neuronal and glial necrosis, 2) endothelial cell necrosis, and 3) secondary effects of inflammation, cytokines, and chemokines.[9]

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References:

1. Roels S, Charlier G, Letellier C, Meyer G, Schynts F, Kerkhofs P, Thiry E, Vanopdenbosch E: Natural case of bovine herpesvirus 1 meningoencephalitis in an adult cow. Vet Rec 146:586-588, 2000
2. Ely RW, d'Offay JM, Ruefer AH, Cash CY: Bovine herpesviral encephalitis: a retrospective study on archived formalin-fixed, paraffin-embedded brain tissue. J Vet Diagn Invest 8:487-492, 1996
3. Belknap EB, Collins JK, Ayers VK, Schultheiss PC: Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. Vet Pathol 31:358-365, 1994
4. Perez SE, Bretschneider G, Leunda MR, Osorio, FA, Floes, EF, Odeón AC: Primary infection, latency, and reactivation of bovine herpesvirus type 5 in the bovine nervous system. Vet Pathol 39:437-444, 2002
5. Penny CD, Howie F, Nettleton PF, Sargison ND, Schock A: Upper respiratory disease and encephalitis in neonatal beef calves caused by bovine herpesvirus type 1. Vet Rec 151:89-91, 2002
6. Radostits OM, Gay CC, Blood DC, Hinchcliff KW: Veterinary Medicine A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th ed., pp. 1173-1184. WB Saunders, Philadelphia, Pennsylvania, 2000
7. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 556-558. Academic Press, Inc., San Diego, California, 1993
8. Hargis AM, Ginn PE: The integument: In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1176-1177. Mosby Elsevier, St. Louis, Missouri, 2007
9. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 885-887. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 58
CONFERENCE 15 / CASE II – 1051/05 (AFIP 3026718)

Signalment: 3-year-old neutered-female domestic short hair cat, feline

History: The cat showed altered behaviour for one week with apathy and anorexia. The clinical examination revealed anisocoria whereas the pupillary reflex was normal. Radiographs were also unremarkable. Soon after the clinical examination the cat died. The carcass was submitted for necropsy by the clinical veterinarian in a frozen

condition. The cat lived with two dogs and a rabbit in a household and had free outdoor access. More than one year prior to death the cat had a bite wound in an unknown body region.

Gross Pathology: On gross examination of the brain, there was a round dark grey mass of about 3 cm in diameter without a distinct border in the caudal part of the right cerebral hemisphere. The meninges adhered on this spot. A brain tumor or encephalitis was suspected initially. The bones of the skull, the internal and middle ear as well as the nasal cavity showed no alteration.

Laboratory Results: Detection of FeLV p27-antigen (Witness FeLV®-Test, Synbiotocs Corp., Lyon, France) and evidence of antibodies against FIV gp40-antigen (Witness FIV®-Test, Synbiotocs Corp., Lyon, France) in samples of full blood were negative.

Histopathologic Description: Brain, cerebrum (right hemisphere): Unfortunately the carcass was frozen, so freezing and thawing artefacts are obvious. Throughout the whole slide there are parallel clefts dissecting the neuropil. They are free of any material and show no accompanying cellular reaction.

Within the cerebral cortex there is a focus of marked cellular degradation (necrosis) and debris. There are abundant intralesional septate fungal hyphae with yellow-brown coloured walls. The dichotomous branching hyphae have a diameter of about 5-7 µm and show occasional vesicular swellings. There is a dominant infiltration of macrophages and neutrophilic granulocytes, a moderate amount of plasma cells and lymphocytes and some giant cells which sometimes contain intracytoplasmatic melanized fungi.

There are also some slides from the periphery which do not show the central necrotic area. In some slides submeningeal clotted vessels with intraluminal hyphae can be seen. Vessels with a marked lymphocytic perivascular infiltration are surrounding the necrotic lesion.

The meninges overlying the necrosis revealed a moderate to marked infiltration with lymphocytes and macrophages.

Contributor's Morphologic Diagnoses:
1. Cerebrum: Encephalitis, pyogranulomatous and necrotising, subacute, focally extensive, severe with intralesional brown-pigmented fungal hyphae (cerebral phaeohyphomycosis).
2. Cerebrum: Meningitis, granulomatous, subacute, focal, moderate to severe.

Contributor's Comment: Phaeohyphomycosis, an uncommon opportunistic infection of human beings and a wide variety of animals (e.g. amphibians, birds, and mammals), is caused by dematiaceous (naturally pigmented) fungi.[1-4] In cats about 15 genera of pigmented fungi have been recognized as agents of feline phaeohyphomycosis, including the species of *Cladophyalophora bantiana*, *Exophiala jeanselmei*, and

Fonsecaea pedrosoi.[5-8] Most of them are known as plant pathogens, soil saprophytes or laboratory and environment contaminants.[3,9] The infection occurs mainly after inhalation of spores or through traumatic fungal implantation of fungi from contaminated soil, thorns or wood splinters as well as the introduction through bite wounds.[6] In domestic animals, cerebral phaeohyphomycosis is rarely seen and has been reported in humans, dogs and cats.[10]

The brown or black pigmentation of these fungi can always be seen in cultured fungal cells but may not be visible or only faintly in tissue sections. The hyphae have a width of about 2-6 µm, vary in length in tissue sections and are septated, branched or unbranched. There are different histomorphologic shapes from vesicular-like swellings, bizarre shapes to fragmented hyphae. Fungal cells can be found in the pus of abscesses or in granulomas as well as intracellularly.[3]

Dematiaceous fungi may cause phaeohyphomycosis, chromoblastomycosis or eumycotic mycetomas.[3,11,12] These forms have to be differentiated by their tissue appearance. Eumycotic mycetomas are characterized by black grains or granules of pigmented fungi, whereas chromoblastomycosis reveals spherical fungal cells (sclerotic bodies) in tissue sections.

Frequently phaeohyphomycosis occurs in cutaneous or subcutaneous locations after the infection with for example *Phialophora verrucosa*, *Fonsecaea pedrosoi* or *Exophiala jeanselmei*. *Cladophialophora bantiana* seems to be a neurotropic agent causing cerebral phaeohyphomycosis predominantly after inhalation.[5,7,8,13]

Unfortunately, a mycological determination of the fungal species was not carried out in this presented case. So it is not possible to confirm *Cladophialophora bantiana* as etiologic agent although some hints point it out. Although considered an infectious disease of immunocompromised patients, phaeohyphomycosis is often found in 'healthy' individuals with no obvious immunosuppression.[5,6,8,14]

AFIP Diagnosis: Brain, cerebrum: Meningoencephalitis, necrotizing, pyogranulomatous, focally extensive, severe, with fibrinoid vasculitis, and numerous dematiaceous hyphae, domestic short hair (*Felis domesticus*), feline.

Conference Comment: The contributor provides a thorough concise summary of phaeohyphomycosis. *Ochroconis gallopavum* (formerly *Dactylaria constricta* var. *gallopava*) causes cerebral phaeohyphomycosis in various avian species and was recently reported to have caused fatal systemic phaeohyphomycosis in a dog.[2,15] With sparsely pigmented fungi, special stains such as Fontana-Masson may be helpful in identifying melanin pigment; however, culture is required for definitive diagnosis.[4] There is variation between slides with fibrinoid vasculitis and fibrin thrombi present in some sections.

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References:

1. Bube A, Burkhardt E, Weiß E: Spontaneous chromomycosis in the marine toad (*Bufo marinus*). J Comp Path 106:73-77, 1992
2. Salkin IF, Dixon DM, Kemna ME, Danneman PJ, Griffith JW: Fatal encephalitis caused by *Dactylaria constricta* var. *gallopava* in a snowy owl chick (*Nyctea scandiaca*). J Clin Microbiol 28(12):2845-2847, 1992
3. Salfelder K, de Liscano TR, Sauerteig E: Phaeohyphomycosis. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 56-59. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990
4. Foil CS: Phaeohyphomycosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd edition, pp. 426-428. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998
5. Beccati M, Vercelli A, Peano A, Gallo MG. Phaeohyphomycosis by *Phialophora verrucosa*: first European case in a cat. Vet Rec 157:93-94, 2005
6. Abramo F, Bastelli F, Nardoni S, Mancianti F: Feline cutaneous phaeohyphomycosis due to *Cladophyalophora bantiana*. J Fel Med Surg 4:157-163, 2002
7. Helms SR, McLeod CG: Systemic *Exophiala jeanselmei* infection in a cat. JAVMA 217(12):1858-1861, 2000
8. Fondati A, Gallo MG, Romano E, Fondevila D: A case of feline phaeohyphomycosis due to *Fonsecaea pedrosoi*. Vet Dermatol 12:297-301, 2001
9. Dixon DM, Shadomy HJ, Shadomy S: Dematiaceous fungal pathogens isolated from nature. Mycopathologia 70(3):153-161, 1980
10. Boulijhad M, Lindeman CJ, Hayden DW: Pyogranulomatous meningoencephalitis associated with dematiaceous fungal (*Cladophialophora bantiana*) infection in a domestic cat. J Vet Diagn Invest 14:70-72, 2002
11. Salfelder K, de Liscano TR, Sauerteig E: Chromoblastomycosis. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 145-150. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990
12. Salfelder K, de Liscano TR, Sauerteig E: Mycetomas. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 151-159. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990
13. Dixon DM, Merz WG, Elliott HL, Macleay S: Experimental central nervous system phaeohyphomycosis following intranasal inoculation of *Xylohypha bantiana* in cortisone-treated mice. Mycopathologia 100:145-153, 1987
14. Mariani CL, Platt SR, Scase TJ, Howerth EW, Chrisman CL, Clemmons RM: Cerebral phaeohyphomycosis caused by *Cladosporium spp*. in two domestic shorthair cats. J Am Anim Hosp Assoc 38:225-230, 2002
15. Singh K, Flood J, Welsh RD, Wyckoff JH, Snider TA, Sutton DA: Fatal systemic phaeohyphomycosis caused by *Ochroconis gallopavum* in a dog (Canis *familiaris*). Vet Pathol 43:988-992, 2006

SLIDE 59
CONFERENCE 15 / CASE III – 05-575 (AFIP 3026925)

Signalment: 5-month-old, intact male, Greater Swiss Mountain Dog

History: This dog had a 2 week history of progressive hindlimb paraparesis consistent with a T3-L3 spinal cord lesion.

Gross Pathology: There was a focal yellow-tan subdural mass (1.7 x 1.2 x 0.5cm) in the spinal cord at L2 with minimal adjacent hemorrhage and spinal cord compression (Figure 1, Figure 2). All other organs appeared grossly normal.

Histopathologic Description: Within the dura and severely compressing and replacing the spinal cord is an expansile, well-demarcated neoplastic cellular proliferation arranged in bundles and small lobules separated by variably dense fibrous connective tissue. There are two distinct neoplastic cell populations. One population consists of interlacing fascicles of spindleloid cells with indistinct cell borders. Nuclei are elongate-ovoid, with finely stippled chromatin, and 1-2 nucleoli. The second population of cells forms tubules and acini, and occasionally form structures resembling glomeruli. Cells are columnar with ovoid-polygonal nuclei and finely stippled chromatin. Mitoses are variable and range from 0-2 per 5 high-power fields. Few dilated myelin sheaths with swollen axons (spheroids) are present in the adjacent white matter.

Contributor's Morphologic Diagnosis: Thoracolumbar spinal cord tumor of young, large breed dogs (Nephroblastoma)

Contributor's Comment: Thoracolumbar spinal cord tumor of young dogs (spinal cord nephroblastoma) occurs most often in dogs between six months and three years of age with clinical signs of pelvic limb paresis and ataxia, consistent with a compressive lesion between T10-L2 spinal cord segments. This intradural, extramedullary mass is thought to arise from residual embryonic tissue within the dura from fetal development.[1] The histologic appearance is variable, but contains distinct cell populations including a spindleloid cell component and an epithelial component forming tubules and glomeruloid-like structures.[2,3,4] Differential diagnoses for thoracolumbar spinal cord tumors include primitive neuroectodermal tumors, poorly differentiated astrocytomas, and ependymomas.[5,9]
Immunohistochemical staining properties of these tumors support a renal origin. The neural markers GFAP and NSE are negative, but tumors cells demonstrate positive immunoreactivity for both cytokeratin and vimentin.[5,6] This immunoreactivity pattern is similar to renal nephroblastomas. Glomeruloid structures within the tumor are strongly labeled with the Wilms' tumor protein 1 (WT1) antibody.[7] WT1 is inactivated in childhood Wilms' tumors (nephroblastoma), and the properties of WT1 in humans are identified as a transcriptional target implicated in renal differentiation. About 10% of

sporadic Wilms' tumors have inactivating mutations in WT1, and many of these same tumors often contain B-catenin mutations as well.[8]

There is only one case report documenting a spinal cord nephroblastoma metastasis.[9] A 2-year-old intact female Basset Hound was found to have two spinal cord masses at T11-T12 and L4-L6. The mass at T11-T12 was composed of embryonic blastemal cells, and an epithelial component of tubules and glomeruloid structures. The L4-L6 mass was composed of sheets of undifferentiated blastemal cells with no glomerular component and a higher mitotic index. Because the more distal tumor was much less differentiated, it was thought to represent a metastasis of the T11-T12 mass. It was hypothesized that the spread occurred through the subarachnoid space, or, less likely, occurred within the parenchymal blood vessels. A second case report details an extradural spinal cord metastasis of a renal nephroblastoma, and other sites of metastases included adrenal glands, hepatic and mediastinal lymph nodes, and bone marrow.[10]

In the case submitted, no other lesions were observed grossly or histologically, so the diagnosis of primary thoracolumbar spinal cord tumor was given.

AFIP Diagnosis: Spinal cord and dura mater: Thoracolumbar spinal cord tumor of young dogs (nephroblastoma), Greater Swiss Mountain Dog (*Canis familiaris*), canine.

Conference Comment: The contributor provides a thorough overview of spinal nephroblastomas in the dog. Nephroblastoma is the most common primary renal tumor of swine and chickens. Nephroblastomas occur less frequently in calves and dogs, and are very uncommon in other species. They are most often seen in young animals and sometimes in fetuses. Nephroblastomas are true embryonal tumors that arise from metanephric blastema.[11,12]

Grossly, nephroblastomas in the kidney are typically encapsulated white to tan lobulated, meaty to firm with spongy and cystic areas, often with foci of hemorrhage and necrosis. They are usually located in the cortex and extend through the capsule. They are usually unilateral and located at one pole of the kidney, although they can be bilateral and extrarenal. They can be very large (up to 34 kg in swine) resulting in abdominal enlargement. In contrast to spinal cord nephroblastomas in the dog in which metastasis is rare, the tumors in the kidney have widespread metastasis to the lung and liver in over half the canine cases. Metastasis is rare in pigs and calves.[11,13]

The characteristic histologic features include (1) an epithelial component that varies from glandular structures to normal tubules to glomeruloid structures that lack capillaries to islands and serpentine patterns (2) a mesenchymal component that may be arranged in undifferentiated lobules or streams of mesenchymal cells or differentiated into fibrous, mucoid or adipose tissue, or smooth/skeletal muscle, cartilage or bone and (3) blastemal cells found in clumps or dispersed between the epithelial and mesenchymal tissues. All three components are found in varying amounts and stages of

differentiation resulting in tremendous variation in appearance. Regardless of the predominant element, the tumor has an embryonic appearance. The epithelial component can occasionally become squamous and keratinize. The proliferation rate and malignant potential of each element may vary even within a single tumor. Tubular and glomerular differentiation is associated with a good prognosis while an anaplastic sarcomatous appearance is associated with a greater likelihood of metastasis and a poor prognosis.[11,13]

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References:

1. Macri NP, Alstine WN, Coolman RA: Canine spinal nephroblastoma. J Am Anim Hosp Assoc 33:302-306, 1997
2. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Histological Classification of Tumors of the Nervous System of Domestic Animals, Second Series, vol. V, pp. 26-27. The Armed Forces Institute of Pathology, Washington, D.C., 1999
3. Koestner A, Higgins RJ: Tumors of the nervous system. In: Tumors in Domestic Animals, ed. Meuten DJ**,** 4th ed., pp. 716-717. Iowa State Press, Ames, Iowa, 2002
4. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, pp. 386-390. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
5. Baumgärtner W, Peixoto PV: Immunohistochemical demonstration of keratin in canine neuroepithelioma. Vet Pathol 24:500-503, 1987
6. Summers BA, deLahunta A, McEntee M, Kuhajda FP: A novel intradural extramedullary spinal cord tumor in young dogs. Acta Neuropathol 75:402-410, 1988
7. Pearson GR, Gregory SP, Charles AK: Immunohistochemical demonstration of Wilms tumour gene product WT1 in a canine “neuroepithelioma” providing evidence for its classification as an extrarenal nephroblastoma. J Comp Path 116:321-327, 1997
8. Rivera MN, Haber DA: Wilms’ tumour: connecting tumorigenesis and organ development in the kidney. Nat Rev Cancer 5:699-712, 2005
9. Terrell SP, Platt SR, Chrisman CL, Homer BL, de Lahunta A, Summers BA: Possible intraspinal metastasis of a canine spinal cord nephroblastoma. Vet Pathol 37:94-97, 2000
10. Gasser AM, Bush WW, Smith S, Walton R: Extradural spinal, bone marrow, and renal nephroblastoma. J Am Anim Hosp Assoc 39:80-85, 2003
11. Maxie MG: The urinary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 520-521. Academic Press, Inc., San Diego, California, 1993
12. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 680. Mosby Elsevier, St. Louis, Missouri, 2007
13. Meuten DJ: Tumors of the urinary system. In: Tumors in Domestic Animals, ed. Meuten DJ**,** 4th ed., pp. 518-519. Iowa State Press, Ames, Iowa, 2002

SLIDE 60
CONFERENCE 15 / CASE IV – Massey IVABS 30060-99 (AFIP 2788405)

Signalment: 18-month-old male New Zealand Huntaway dog
History: The dog exhibited progressively worsening ataxia for a month. The owner had noted a high stepping, prancing gait affecting the forelegs and that the dog had difficulty jumping into a utility vehicle. In addition, the dog had started defecating in its kennel.

Upon clinical examination ataxia and hypermetria were noted in all 4 limbs, but there were no tremors or cranial nerve deficits. No abnormalities were observed on fundic examination. Postural reactions (conscious proprioception, wheelbarrowing, hemistand and hemiwalk) were also normal as were spinal reflexes in the forelimbs. A crossed extensor response was seen when the hindlimb withdrawal reflexes were being assessed. The patella, cranial tibial and gastrocnemius reflexes were exaggerated in both hindlimbs and clonus was observed on the right side.

Gross Pathology: The only abnormality noted at necropsy was an enlarged and congested liver.

Laboratory Results: Lysosomal hydrolase activities: A deficiency of sulphamidase activity was identified in the affected liver. Alpha-mannosidase is significantly elevated in the liver from the affected dog compared with the control.

Glycosaminoglycan analysis: GAGs from the affected and control dog livers were compared with each other and with those from two human patients and a mouse with mucopolysaccharidosis-IIIA. Complex banding patterns were different between samples from controls (human and dog) and affected subjects. A similar pattern of banding was evident between human and mouse mucopolysaccharidosis-IIIA GAGs and those of the affected dog, particularly at the low molecular weight oligosaccharide region of the gel.

Histopathologic Description: In the section provided, many neurons are distended with fine granular material which varies from eosinophilic to slightly basophilic. This storage material was variably PAS-positive, stained moderately with Luxol fast blue, lightly with Sudan black and slightly with alcian blue in some severely affected neurons. It gave a yellow autofluorescence on fluorescence microscopy. In some areas axonal spheroids are prominent. A moderate number of highly vacuolated macrophages are present in the meninges and perivascular spaces. There is a variable local loss of Purkinje cells in some cerebellar folia of the cerebellum, accompanied by thinning of the molecular layer in the same areas.

Electron microscopy: The storage material in neurons of the cerebral cortex was in the form of membrane-bound accumulations of membranous whorls. In occasional neurons there were also membranous stacks of the type known as 'zebra bodies' and these predominated in some cells. The fine vacuoles noted at the light-microscopic level in hepatocytes were seen as empty membrane-bound vesicles.
Contributor's Morphologic Diagnosis: Brain, cerebellum and medulla oblongata: Neuronal cytoplasmic accumulation of granular storage product, diffuse moderate. Histiocyte cytoplasmic vacuolation of meninges and perivascular spaces. Huntaway. Canine.

Aetiology: Mucopolysaccharidosis IIIA (Sanfilippo syndrome)

Contributor's Comment: A lysosomal storage disease was suspected and confirmed by histopathology and electron microscopy, which revealed widespread neuronal lesions of a lysosomal storage disease that were interpreted as indicative of one of the forms of mucopolysaccharidosis-III. This was on the basis of apparent ganglioside accumulation (indicated by membranous whorls/stacks as seen in electron micrographs) and vacuolated macrophages in perivascular spaces and leptomeninges. In other tissues examined from this dog (notably liver) there was also cytoplasmic vacuolation of fibroblasts and hepatocytes. The absence of notable skeletal or soft-connective-tissue lesions supported this diagnosis, rather than one of the other forms of mucopolysaccharidosis.

Most of the non-degraded heparan sulphate is excreted in urine and primary stigmata associated with its accumulation in tissues are limited to the macrophages in perivascular spaces and meninges, and vesicles in hepatocytes and connective tissue cells. Paradoxically, the major accumulated material in neurons is comprised of gangliosides, which as polar lipids, help form the membranous whorls and stacks noted on electron microscopy.

There are 4 phenotypically similar forms of MPS-III (A-D) resulting from deficiencies of 1 or the other of 4 enzymes particular to the sequential catabolism of heparan sulphate. Enzyme analyses undertaken were for potential diagnosis of MPS-III A,B,C and multiple sulphatase deficiency. The lack of sulphamidase in the affected liver indicated a probable diagnosis of MPS-IIIA. The presence of the same amount of total sulphatase in the control and the affected dog liver indicated that the sulphamidase-deficiency was not the result of a multiple sulphatase deficiency. The elevated α-mannosidase activity is consistent with a lysosomal storage disorder.

The mucopolysaccharidosis-IIIA (Sanfilippo syndrome) is an autosomal recessive inherited lysosomal storage disease of humans that has also been diagnosed in wire-haired Dachshund dogs and mice. A similar genetic cause is assumed for the disease in this Huntaway dog.

AFIP Diagnosis: Brain, cerebellum and brain stem: Neuronal and axonal degeneration, multifocal, moderate, with spongiosis, spheroids and abundant neuronal cytoplasmic eosinophilic granular material, New Zealand Huntaway dog (*Canis familiaris*), canine.

Conference Comment: Lysosomal storage diseases occur secondary to dysfunction of lysosome-mediated degradation of products (substrates) of normal cellular metabolism. These substrates cannot be degraded by lysosomes, accumulate, and eventually result in the death of affected cells. Lysosomal storage diseases that affect the central nervous system result in the accumulation of substrate in neurons and myelinating cells. When these cells die, the accumulated substrate is released into adjacent tissue. Macrophages phagocytose the unprocessed substrate; however, macrophages have the same genetic defect and accumulate the substrate in their lysosomes leading to their eventual death. With few exceptions, these are inherited autosomal recessive diseases.[4]

Originally, lysosomal storage diseases were thought to develop exclusively due to mutations resulting in a reduction in lysosomal enzyme synthesis; however, it is now clear that there are other defects including the following:

1. Synthesis of catalytically inactive enzymes that resemble normal active enzymes
2. Defects in posttranslational processing of the enzyme resulting in its being misdirected to sites other than lysosomes
3. Lack of enzyme activator (enzyme that normally increases the rate of an enzyme-catalyzed reaction) or protector protein (facilitate repair and refolding of stress-damaged proteins)
4. Lack of substrate activator protein required to assist with hydrolysis of substrate
5. Lack of transport protein required for elimination of digested material from lysosomes

Therefore, lysosomal storage diseases can result due to defects in any protein that is essential for normal lysosome function. Cell swelling and vacuolation occur due to accumulation of unprocessed substrate in lysosomes. Differences in size and appearance of cells (neurons versus hepatocytes) are therefore dependent on the availability of the substrate (carbohydrate or lipid) in a particular organ system. For example, many lipids and glycolipids are unique to the central nervous system (CNS). Therefore, when there is a lysosomal defect in the CNS, neural cells often accumulate lipids and glycolipids.[4]

The following chart summarizing inherited metabolic diseases affecting the nervous system of domestic animals was kindly provided by the moderator.[4-7]

Table Inherited metabolic diseases affecting the nervous system of domestic animals

Disease name	Stored material	Defective enzyme/gene	Morphology Histochemistry*	Neurons	Myelin	Other tissues/cells	S

Ceroid-lipofuscinosis	Ceroid/lipofuscin	Numerous	Yellow-brown cytoplasmic granules (F,L,P,S)	X		axonal spheroids, neuronal necrosis, Wallerian degeneration, gliosis, macrophages	ca bo
Fucosidosis	Fucose containing glycolipids, glycoproteins, poly-/ oligo-saccharides	Alpha-L-fucosidase	Clear fine vacuoles (P)	X		glia, macrophages, lymphocytes, peripheral nerves, kidney, pancreas, lymph nodes, lung	ca Sp
Galactosylceramide lipidosis (Globoid-cell leukodystrophy, Krabbe's-like disease)	Galactosylceramide (galactocerebroside and galactosylsphingosine (psychosine)	Galactosylceramidase (galactocerebroside _-galactosidase)	Demyelination with accumulation of macrophages filled with PAS positive material		X		ca Hi Ca po He ho Po Se Dl (D tw
Galactosialidosis	Glycolipids	Beta-galactosidase	Clear vacuoles (F, L, P)	X		hepatocytes, macrophages	ca
Glucocerebrosidosis (Gaucher's disease)	Glucocerebroside	Acid beta-glucosidase (glucocerebrosidase)	Clear to weakly eosinophilic small vacuoles (P)	X	X	macrophages in liver and lymph nodes, axonal spheroids	ca ov po
Glycogen storage disease IA (Von Gierke)	Glycogen	Glucose-6-phosphatase	Swollen hepatocytes (described as vacuoles, but looks like glycogen?)			liver, neurologic signs are due to hypoglycemia	ca
Glycogen storage disease II (Pompe's)	Glycogen	Acid alpha-glucosidase	Swollen cells	X		muscle (skeletal, cardiac, and smooth)	ca fe ov bo Br qu
Glycogen storage disease III (Cori's)	Glycogen	Amylo-1,6-glucosidase	Enlarged foamy? (P)	X		hepatocytes, muscle, glia	ca Sl
Glycogen storage disease Type IV	Abnormally branched glycogen (alpha-1,4-D-glucan)	Branching enzyme	Pale blue granules (I, P)	X		muscle	fe fo
GM1 gangliosidosis	Gangliosides	Beta-galactosidase	Clear to pale pink granular material in vacuoles (L, P, S)	X	X	hepatocytes, macrophages, renal tubular cells, pancreatic exocrine cells	ca Er Sp W Hu do fe Ko bo Fr (S Co
Mucolipidosis (I-cell disease)	Mucopolysaccharides, lipids, glycoproteins	N-acetylglucosamine-1-phosphotransferase	Clear vacuoles	X (rare)		bones, cartilage, skin	fe
α-mannosidosis	Glycoprotein-derived mannose-rich oligosaccharides	Alpha mannosidase	Clear vacuoles	X	loss	glia, fibroblasts, endothelial	bo M G

						and glandular epithelial cells, hepatocytes, macrophages	(F Dl
β-mannosidosis	Glycoprotein-derived mannose-rich oligosaccharides	Beta mannosidase	Clear vacuoles	X	loss	glia, fibroblasts, endothelial and glandular epithelial cells, macrophages	ca bo
MPS I (Hurler's, Scheie, and Hurler/Scheie disease)	Heparan and dermatan sulfate	Alpha-L-iduronidase	Clear vacuoles Skeletal abnormalities predominate in most MPS syndromes	X		hepatocytes, macrophages, glia, chondrocytes, smooth muscle, fibroblasts, leukocytes	fe ca R
MPS II (Hunter syndrome)	Heparan and dermatan sulfate	Iduronate-2-sulfatase	Clear vacuoles (P)	X		epithelial and endothelial cells, macrophages	ca R
MPS IIIA (Sanfilippo A)	Heparin sulfate gangliosides	Heparan N-sulfatase	Clear vacuoles (F, L, P, S)	X		hepatocytes, macrophages	ca D Ze
MPS IIIB (Sanfilippo B)	Heparin sulfate	Alpha-N-acetylglucosaminidase	Clear vacuoles (P, T)			monocytes, hepatocytes	ca er
MPS IIID (Sanfilippo D)	Heparin sulfate gangliosides	N-acetylglucosamine 6-sulfatase	Clear vacuoles	X		muscle, fibroblasts, chondrocytes, others	ca
MPS VI (Maroteaux-Lamy)	Dermatan sulfate	N-acetylglucosamine 4-sulfatase (arylsulfatase B)	Clear vacuoles	X		hepatocytes, macrophages, wbc	fe D ca Pi Sc Cc Ba
MPS VII (Sly)	Chondroitin and dermatan sulfate	beta-glucuronidase	Clear vacuoles	X		hepatocytes, fibroblasts, chondrocytes, RPE, keratocytes, synovial cells, macrophages	ca Sl br fe
Sphingomyelinosis (Niemann-Pick A & B)	Unesterified cholesterol (ganglioside)	Acid sphingomyelinase	Clear vacuoles (P variable)	X		macrophages, spheroids	fe ca pc
Sphingomyelinosis (Niemann-Pick C)	Unesterified cholesterol (ganglioside)	NPC1	Clear vacuoles (P variable)	X		macrophages, spheroids	Fe Si (M Bc

*Histochemistry refers to the histochemical stains that stain the stored material assuming ideal conditions of tissue preservation, fixation, and processing. Most are only reliably positive on frozen sections of unfixed tissues.

(F = autofluorescent, I = Lugol's iodine, L = luxol fast blue, P = PAS, S = Sudanophilic, T = toluidine blue)

Cerebellar atrophy of varying degrees was present in some slides.

Readers are encouraged to read reference 3 for a comprehensive overview of lysosomal storage diseases of animals.

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References:
1. Jolly RD, Allan FJ, Collett MG, Rozaklis T, Muller VJ, Hopwood JJ: Mucopolysaccharidosis IIIA (Sanfilippo syndrome) in a New Zealand Huntaway dog with ataxia. N Z Vet J 48:144-148, 2000
2. Neufeld EF, Muenzer J. The mucopolysaccharidoses. *In*: The Metabolic Basis of Inherited Disease, eds. Scriver R, Beudet AL, Sly WS, Valle D, 6th edition, vol. 2, pp. 1565-1587. McGraw-Hill, New York, New York, 1989
3. Jolly RD, Walkley SU: Lysosomal storage diseases of animals: an essay in comparative pathology. Vet Pathol 34:527-548, 1997
4. Zachary J: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 928-930. Mosby Elsevier, St. Louis, Missouri, 2007
5. Haskins M, Giger U: Lysosomal storage diseases. In Press, 2007
6. Braund K: Clinical Neurology in Small Animals: Localization, Diagnosis, and Treatment, VIN
7. Summers BA, Cummings JF, de Lahunta A: Degenerative diseases of the central nervous system. In: Veterinary Neuropathology, pp. 214-236. Mosby-Year Book, Inc., St. Louis, Missouri, 1995

SLIDE 61
CONFERENCE 16 / CASE I – CASE 1 771-2 NIEHS (AFIP 2799444)

Signalment: 2-year-old female Harlan Sprague-Dawley rat

History: High-dose animal from a 2-year carcinogenicity/toxicity study.

Gross Pathology: Livers from the treated rats were enlarged and had granular surfaces and multicentric nodules and masses randomly scattered within the parenchyma.

Histopathologic Description: Cholangiocarcinomas were variably sized, irregular, non-circumscribed proliferations of dense fibrous connective tissue stroma containing dysplastic, atypical biliary epithelium which formed numerous irregular ducts and duct-like structures that were lined by pleomorphic neoplastic epithelial cells. Small islands of dysplastic neoplastic cells were also interspersed among the ducts. Within the ducts, the neoplastic cells were flattened in some areas and cuboidal to columnar in others. In some ducts, there was segmental loss of epithelium. Bizarre epithelial cells were frequently present. Component neoplastic cells had scant to moderate amounts of basophilic cytoplasm and prominent markedly pleomorphic vesicular to hyperchromatic nuclei with one or more prominent nucleoli. Mitoses were sometimes numerous. Ducts often contained mucinous material, degenerate epithelial and inflammatory cells, and cell debris. Low numbers of mast cells, lymphocytes and neutrophils were scattered throughout the stroma. Additional alterations include nodular regenerative hepatocellular hyperplasia, hepatocellular hypertrophy and bile duct hyperplasia.

Contributor's Morphologic Diagnosis: Liver: Cholangiocarcinoma, multiple.

Contributor's Comment: The terminology for benign and malignant lesions of the biliary epithelium is often confusing reflecting the uncertainty surrounding the biology of the so-called more benign appearing or preneoplastic lesions. The latter have been called cholangiofibrosis, adenofibrosis, cholangiofibroma, cystic cholangioma, cholangiohepatitis, toxic hepatitis and toxic cholangitis. Cholangiofibrosis, cholangiofibroma, cystic cholangioma and cholangiocarcinoma are closely related lesions that are distinguished on the basis of the degree of proliferation and anaplasia of the biliary epithelium, evidence of invasion, and the quantity of fibrous connective tissue stroma. In this study, transplantation of the cells from the proliferative lesions grew and metastasized confirming the malignancy of these lesions.

AFIP Diagnosis: Liver: Cholangiocarcinoma, Harlan Sprague-Dawley rat (*Rattus norvegicus*), rodent.

Conference Comment: As pointed out by the contributor, the terminology for benign and malignant lesions of the biliary epithelium is often confusing. Furthermore, the distinction between cholangiofibrosis, cholangiofibroma, and cholangiocarcinoma has not been clearly defined. Morphologic features such as intrahepatic infiltration and microinvasion (disruption of basement membranes of glandular formations), absence of histological evidence of regression, piling up of epithelial cells lining glandular lumens, formation of branched or anastomosing glands, nuclear hyperchromasia and cellular dysplasia, increased mitotic figures and compression of the surrounding hepatic parenchyma support the potential malignancy of proliferative cholangial lesions.[1]

There was much discussion about whether the lesion in this case was more consistent with cholangiofibrosis or cholangiocarcinoma. The gross appearance of cholangiofibrosis varies from multifocal microscopic foci to grossly visible firm, pearly white areas up to 5 cm in diameter. Lesions on the surface are depressed. Characteristic light microscopic findings of cholangiofibrosis include atypical glandular structures line by hyperbasophilic, sometimes dysplastic epithelium that ranges from flattened to large cuboidal cells with goblet cells and occasional Paneth cells. The glands often appear crescent-shaped due to tall columnar epithelium on one side of the gland and attenuated epithelium on the other side. Mitotic figures and necrotic cells are often present within the epithelium. The lumen is usually filled with mucin/necrotic debris. Mucin production is usually pronounced. The glandular structures are embedded within dense connective tissue with sclerosis in the more central areas of the lesion. Multifocal areas of cholangiofibrosis often coalesce.[2]

Cholangiocarcinomas are usually firm, white to grey masses with irregular borders and may protrude from the surface of the liver. They may have a spongy texture and exude clear to yellow fluid from cut surfaces in cystic areas. Microscopically, these tumors

may have glandular, solid, or papillary patterns. They are comprised primarily of cuboidal to columnar cells with basophilic cytoplasm and prominent hyperchromatic nuclei. Cellular atypia and a high mitotic index are common. The epithelium lining dilated glands is occasionally piled up. Mucin production is highly variable. Abundant scirrhous stroma is often present. Cholangiocarcinomas usually exhibit microinvasion and can invade surrounding tissues, blood vessels,and may metastasize.

After much discussion, a diagnosis of cholangiocarcinoma was made due to the presence of glandular structures lined by one to multiple cell layers; prominent hyperchromatic nuclei; a high mitotic index; cellular atypia; and reported metastasis.

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References:

1. Maronpot RR, Giles HD, Dykes DJ, Irwin RD: Furan-induced hepatic cholangiocarcinomas in Fischer 344 Rats. Toxicol Pathol 19:561-570, 1991
2. Goodman DG, Maronpot RR, Newberne PM, Popp JA, Squire RA: Proliferative and selected other lesions of the liver of rats, GI-5. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington D.C., 1994

SLIDE 62
CONFERENCE 16 / CASE II – 6-2156-A (AFIP 3030596)

Signalment: 13-week-old female pig, Landrace

History: Gilts found acutely dead, had appeared healthy, introduced to barn 2 weeks previously, recent increase in mortality within the barn, suspect circovirus.

Gross Pathology: Bilaterally involving the ventral half of all lung lobes, cranial mediastinum and entire pericardium, the serosal surfaces are diffusely overlaid by abundant amounts of fibrin. The lungs are heavy, dark red and wet and the regional lymph nodes are moderately enlarged and glistening. Throughout the abdominal cavity overlying the serosal surface of multiple loops of bowel and the entire splenic capsule, there is moderate fibrin deposition.

Laboratory Results: *Hemophilus parasuis* type 4 recovered from lung and mediastinal lymph node, PCR negative for swine flu and positive for porcine circovirus 2. Egg inoculation was negative for influenza virus.

Histopathologic Description: Lung: Diffusely overlying and multifocally extending below the visceral pleura, there are multifocal to coalescing accumulations of predominantly neutrophils with fewer macrophages and lymphocytes interspersed within variable amounts of fibrinoserous exudate and acute hemorrhage. The aforementioned

inflammatory infiltrate multifocally expands and occludes alveolar and bronchiolar spaces and interlobular septa.

Contributor's Morphologic Diagnosis: Lung: Pleuropneumonia, marked, diffuse, fibrinosuppurative, necrotizing, subacute.

Contributor's Comment: The polyserositis noted in this case would have been sufficiently severe to have contributed significantly to antemortem morbidity and the loss of this animal. Special culture recovered *Hemophilus parasuis* type 4 from the lung and mediastinal lymph node and this pathogen was considered significant. In a previous case series, swine infected with *H. parasuis* featured dual infections and the most prevalent combination was with Circovirus 2. The contribution of Circovirus 2 in predisposing or exacerbating this infection is unknown.

Infection with *H. parasuis* typically presents with fibrinous polyserositis, polyarthritis, and meningitis. These bacteria are a commensal of the upper respiratory system (nasal cavity and trachea) with invasion into the lungs and development of clinical disease often associated with some stressor. The history of recent introduction to a new herd as in this case is a common factor in the development of clinical disease. Additional contributory factors include concurrent infections with swine influenza virus, pseudorabies, PRRSV, suboptimal environmental conditions or inclement weather.

AFIP Diagnosis: Lung: Pleuropneumonia, fibrinohemorrhagic and suppurative, diffuse, severe, Landrace pig (*Sus scrofa*), porcine.

Conference Comment: As pointed out by the contributor, *Haemophilus parasuis*, the cause of Glasser's disease, results in severe serofibrinous to fibrinopurulent meningitis, polyserositis, and/or polyarthritis in young (5-12 weeks) pigs following a stressful episode. Polyarthritis is usually most severe in the carpal and tarsal joints and the atlanto-occipital joint. The primary differential diagnosis for fibrinous serositis in pigs includes *Mycoplasma hyorhinus*, *Streptococcus suis* type II (zoonotic), and septicemic salmonellosis, and septicemic *E. coli*. Like *H. parasuis*, *M. hyorhinus* also causes polyarthritis; however, meningitis is not usually a feature of mycoplasmal infection. If meningitis is present, it is mild with lymphocytic inflammation. In addition to purulent meningitis and polyarthritis, *S. suis* type II can also cause endocarditis.[3-6]

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References:

1. Oliveira S, Pijoan C: *Haemophilus parasuis*: new trends on diagnosis, epidemiology and control. Vet Microbiol 103:1-12, 2004

2. Blanco I, Galina-Pantoja L, Oliveira S, Pijoan C, Sanchez C, Canals A: Comparison between *Haemophilus parasuis* infection in colostrums-deprived and sow-reared piglets. Vet Microbiol 103:21-27, 2004
3. Palmer N: Bones and joints. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp.169-170. Academic Press, Inc., San Diego, California, 1993
4. Barker IK: The peritoneum and retroperiotneum. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, p. 434. Academic Press, Inc., San Diego, California, 1993
5. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 377-378. Mosby Elsevier, St. Louis, Missouri, 2007
6. Rapp-Gabrielson VJ, Oliveira SR, Pijoan C: *Haemophilus parasuis*. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp. 681-690. Blackwell Publishing Professional, Ames, Iowa, 2006

SLIDE 63
CONFERENCE 16 / CASE III – PG06-2481 (AFIP 3026809)

Signalment: 8-month-old female Yucatan mini-pig
History: The pig was naïve without prior medical treatment. It was euthanized after a sudden onset of cyanosis of extremities, lethargy and inappetence.

Gross Pathology: The pulmonary artery was markedly dilated, approximately 7 cm in diameter. The lumen of the pulmonary artery immediately proximal to the pulmonic valve was filled with yellow-white chalky vegetations. Smaller vegetations of similar consistency were also on the aortic valve leaflets. The rest of the heart appeared normal. There were small petechial hemorrhages in the eyelids bilaterally. The brain was not examined. The lungs, kidney and spleen are grossly normal.

Histopathologic Description: Pulmonic valves and pulmonary arteries had fibrinosuppurative inflammatory infiltrates, mineral deposits, and large colonies of bacteria extending into the lumen from the intimal surface. The intimal aspect of the lesion had proliferating capillaries with plump endothelial cells and fibroblasts. Aortic valves had similar but smaller lesions. Pulmonary thrombi were present in large and small pulmonary arteries as well as alveolar capillaries. Evidence of early organization was present with fibroblasts and endothelialization of thrombi surfaces. The heart had foci of myocardial necrosis and suppurative inflammation. Acute microthrombi were also present in glomerular capillaries. The liver had centrilobular necrosis and congestion.

Contributor's Morphologic Diagnosis: Pulmonary valve/artery: Vegetative endocarditis, subacute, severe with mineralization and large colonies of intralesional bacteria; etiology *Streptococcus suis*.

Contributor's Comment: Gram stain of affected tissue shows that bacterial colonies contain Gram-positive cocci. Foci of necrosuppurative myocarditis are present on some submission slides.

Endocarditis is usually bacterial but parasitic and fungal etiologies have been reported. Valves are most commonly affected beginning in the area of apposition of valve leaflets. The AV-valves are most commonly affected. Experimentally, the lesion has been reproduced with a single injection of bacteria intravenously. In swine, the most common cause is *Streptococcus suis*. *Erysipelas rhusiopathiae* is also a common etiologic agent in pigs.[1]

Streptococcus suis has over 30 serotypes. *S. suis* type 1 generally causes disease in suckling piglets with septicemia, synovitis and meningitis. Type 2 causes disease in weaner and feed pigs causing bacteremia, synovitis, arthritis and meningitis; acute and chronic disease can occur. In addition, endocarditis, myocarditis and pericarditis can occur.[4] *S. suis* is carried by healthy pigs in the tonsils, nasal cavity, genital and alimentary tract.[2,3,4] Spread is via aerosols and close contact.[2] It also has been isolated from wild boars, horses, dogs, cats and birds.[2] *S. suis* is a zoonotic agent causing meningitis, septicemia and other inflammatory diseases in recent outbreaks in China.[2,4]

AFIP Diagnosis: Heart, pulmonary artery (per contributor): Endocarditis, valvulitis, and arteritis, fibrinosuppurative and granulomatous, chronic, diffuse, severe, with mineralization and colonies of Gram-positive cocci, Yucatan mini-pig, porcine.

Conference Comment: *Streptococcus suis* type II is carried in the palatine tonsils of pigs and infection is probably by the respiratory route. In addition to endocarditis, *S. suis* type II can also cause purulent meningitis, polyserositis, arthritis, and possibly pneumonia in pigs.[3,4]

As pointed out by the contributor, *S. suis* is a zoonotic agent causing meningitis, septicemia, septic shock, and residual deafness in man. Recently, scientists confirmed the first human case of *S. suis* meningitis in North America. Lack of reports of this disease in humans in the U.S. is most likely due to misidentification of the organism. *S. suis* infections in humans is most frequently observed in intensive pig farming areas or where people live or work in close contact with pigs (e.g., butchers, veterinarians, pig farmers).[5,6,7]

The pathogenesis of endocarditis involves the components of Virchow's triad of thrombogenesis – turbulence of blood flow, endothelial injury and hypercoagulability. Turbulent intracardiac blood flow associated with congenital anomalies or intracardiac devices contribute to initiation of the lesion. Endothelial disruption of the valves allows bacteria to adhere, proliferate, and initiate an inflammatory reaction with subsequent

fibrin deposition. In addition, preexisting extracardiac infections are often present in affected animals, such as gingivitis or dermatitis, which result in bacteremia.[8]

In cattle, valvular endocarditis is most frequently caused by *Actinomyces pyogenes*, and less commonly, streptococci of enteric origin. Horses rarely develop bacterial endocarditis, but it has been observed in association with *Streptococcus equi*, *Actinobacillus equi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida parapsilosis*. Endocarditis is also rarely observed in dogs and cats, but can be associated with a variety of organisms including *Streptococcus* sp., *Erysipelothrix rhusiopathiae*, and *E. coli*.[1,8]

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References:

1. Robinson W, Maxie M: Endocarditis. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 24-26. Academic Press, Inc., San Diego, California, 1993
2. Huang Y, Teng L, Ho S, Hsueh P: *Streptococcus suis* infection. J Microbiol Immunol Infect 38:306-313, 2005
3. Madsen L, Svensmark B, Elvestad K, Aalbaek B, Jensen H: *Streptococcus suis* Serotype 2 infection in pigs: new diagnostic and pathogenic aspects. J Comp Pathol 126**:**57-65, 2002
4. Palmer N: Infectious arthritis. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 167-168. Academic Press, Inc., San Diego, California, 1993
5. López A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 540. Mosby Elsevier, St. Louis, Missouri, 2007
6. http://www.promedmail.org *Streptococcus suis*, porcine, human – USA (NY)
7. Higgins R, Gottschalk M: Streptococcal diseases. In: Diseases of Swine, eds. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, 9th ed., pp.769-782. Blackwell Publishing Professional, Ames, Iowa, 2006
8. Van Vleet JF, Ferrans VJ: Cardiovascular system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 579-580. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 64
CONFERENCE 16 / CASE IV – S1552/05 (AFIP 3032267)

Signalment: 3-year-old, female, German Improved Fawn breed, goat (*Capra hircus*)

History: The goat belonged to a flock of three goats and five sheep. Over a period of one week the animal was febrile and showed central nervous signs including temporary

blindness and ataxia. Therapy with vitamin B1 and antibiotics was not successful. The goat was euthanized due to the poor prognosis.

Gross Pathology: The goat had a body weight of 26 kg, and was in good nutritional condition. The superficial cervical lymph nodes were markedly enlarged. The lung exhibited multifocal pale firm areas, and a moderate alveolar edema and emphysema. The spleen was slightly enlarged. The liver and the kidneys were mottled and showed multifocal pale foci with a diameter up to 5 mm (Fig. 1).

Laboratory Results: Antigen of rabies virus could not be detected by immunofluorescence microscopy.
In liver samples a severe deficiency of copper (0.74 mg/kg wet weight; reference value: 10-120 mg/kg wet weight), and vitamin E (2.34 mg/kg wet weight; reference: >10 mg/kg wet weight), and a mild deficiency of selenium (0.213 mg/kg wet weight; reference value: 0.25-1.5 mg/kg wet weight) were detected.

Samples from the brain and the kidney were tested by PCR with primers designated for the glycoprotein B region of OvHV-2 and CpHV-2. The reaction product was cloned and sequenced. Comparing the nucleotide sequences to other herpesviruses similarities of 99.49% for OvHV-2 and 86.22% for CpHV-2 were observed.

Histopathologic Description: The renal cortex and medulla displays a multifocal interstitial nephritis characterized by infiltration of lymphocytes, macrophages and plasma cells. Occasionally, necrosis of single cells is observed. The inflammatory lesions are mainly associated with blood vessels. Numerous arteries and arterioles show lymphohistiocytic vasculitis with fibrinoid necrosis of the vascular wall. Some vessels exhibit destruction of endothelial cells and thrombi are present. The epithelium of the renal pelvis is moderately hyperplastic and focally there is a moderate lymphohistiocytic subepithelial infiltration.

In the superficial cervical lymph nodes, lung, spleen, liver, brain and spinal cord a severe vasculitis and perivasculitis of similar quality are found. Additionally, the lung shows a moderate granulomatous pneumonia with nematodes.

Contributor's Morphologic Diagnoses:
1. Kidney: Interstitial nephritis, vasculitis and perivasculitis, lymphoplasmacytic and histiocytic, multifocal, subacute, severe, with fibrinoid necrosis of vessel walls and thrombi, German Improved Fawn breed, goat.
2. Renal pelvis: Pyelitis, lymphohistiocytic, focal, subacute, mild to moderate, with hyperplasia of epithelium, German Improved Fawn breed, goat.

Contributor's Comment: Malignant catarrhal fever (MCF) is a fatal lymphoproliferative disease of ruminant species including domestic cattle and wild living ruminants. The disease is caused by a group of closely related gammaherpesviruses collectively referred to as malignant catarrhal fever (MCF) viruses. Epidemiologically, two primary forms of MCF in cattle have been described. Wildebeest-derived (WD)-MCF, caused by

alcelaphine herpesvirus type 1 (AlHV-1), primarily seen in Africa, and the sheep-associated form (SA-MCF) due to ovine herpesvirus type 2 (OvHV-2) infection, which is found in North America, Europe, and Australia.[1] In goats, a different gammaherpesvirus, termed caprine herpesvirus-2 (CpHV-2), has been identified.[3] This virus caused MCF in white-tailed deer and in sika deer in the USA.[2,4] However, infection associated lesions or disease have not been observed in goats. Interestingly, various ruminant species may be infected by MCF-viruses, but only few develop clinical disease. In goats, which can be infected by OvHV-2 and CpHV-2, lesions have not been described so far.[1,2]

Domestic sheep represent the primary reservoir for OvHV-2. Clinical signs of MCF are not observed in domestic sheep or goats under natural conditions.[1] Experimentally, MCF-like disease was induced in sheep after aerosol inoculation with OvHV-2.[7] MCF was also described in Barbary sheep and pigs and OvHV-2 was identified by PCR as the causative agent.[6,7]

Goetze described four clinical forms of MCF in cattle, termed “peracute”, “head-and-eye”, “intestinal”, and “mild”.[8] The most frequent signs in cattle include high fever, corneal opacity, and lymph node enlargement. Neurological signs may be observed but are typically not that pronounced until the terminal stages of the disease. The animals are typically lethargic and appear obtunded.[1] Head and eyes were not infected in the goat presented here.
Macroscopically, the present case showed enlargement of lymph nodes and visceral organs with multifocally distributed tiny white spots in liver and kidney (Fig. 1). In spontaneous cases of bovine MCF, the kidneys show similar lesions. Many bovine cases present generalized lymph node enlargement, which is particularly marked in the head and neck region, and visceral nodes.[8]

Histopathologically, in cattle a lymphohistiocytic vasculitis with fibrinoid necrosis is characteristic and almost pathognomonic for MCF.[1] The vasculitis in bovine is segmental and irregular in distribution, most readily seen in medium-sized arteries invariably accompanied by perivascular and intramural infiltration of mononuclear cells. There is frequently a striking degeneration, often fibrinoid in character, of infiltrated connective tissues and smooth muscle elements of vessel walls. Vascular lesions appear to have predilection sites including kidney, brain and meninges, hepatic triads, lung, capsule of lymph nodes and adrenal gland and the rete mirabile.[8] Until now, a disease pattern similar to that in bovine has not been described in goats.

Though the pathology of MCF is well documented, the pathogenesis is still poorly understood. It is assumed that disease is caused by a lymphoproliferative disorder caused by a dysregulation of T-lymphocytes.[1] In addition, there is evidence that MCF is an immunopathological condition. The essential defect in MCF pathogenesis appears to be an immune dysregulation attributable to a dysfunction of Natural Killer cells and uncontrolled proliferation of lymphoblastoid elements in many tissues. Whether MCF-lesions are due to a type III or Arthus-like hypersensitivity reaction is still discussed controversially.[8]

Most episodes of spontaneous SA-MCF appear to be a sequel of close contact between cattle and infected sheep actively shedding the agent.[9] The goat presented was kept together with sheep. Confirmation of the diagnosis was based on histological demonstration of generalized lymphoid vasculitis in multiple organs, including the brain. Infection with viruses inducing MCF can be most easily demonstrated by PCR in post mortem tissues.[1] Infection of goats with OvHV-2 or CpHV-2 has been demonstrated previously.[9] Both viruses have the ability to infect other ruminants and to cause MCF.[2,4,6,7] In this goat, an infection with OvHV-2 could be confirmed by PCR and discriminated by phylogenetic analysis.

AFIP Diagnosis: Kidney: Vasculitis and perivasculitis, lymphohistiocytic, diffuse, marked, with intimal and medial fibrinoid necrosis, hemorrhage, and few fibrin thrombi, German Improved Fawn breed goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides a thorough overview of malignant catarrhal fever. Other gammaherpesviruses include the following:[10-12]

1. Herpesvirus saimiri (Saimiriine herpesvirus 2) – carried by squirrel monkeys, causes lymphoma in owl monkeys, tamarins and marmosets
2. Herpesvirus ateles (Atleine herpesvirus 2) – carried by spider monkeys, causes lymphoma in owl monkeys, tamarins and marmosets
3. Marmoset lymphosarcoma virus – outbreak of spontaneous fatal lymphoproliferative disease in captive marmosets due to a novel lymphocryptovirus 4. Herpesvirus sylvilagus (Leporid herpesvirus 1) – lymphoma and infectious mononucleosis-like syndrome in cotton-tail rabbits
5. Epstein-Barr virus (human herpesvirus 4) – Infectious mononucleosis in humans; associated with Bukitt's lymphoma, nasopharyngeal carcinoma, and Hodgkin's disease

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References:

1. Callan RJ, Van Metre DC: Viral diseases of the ruminant nervous system. Vet Clin North Am Food Anim Pract 20:327-362, 2004
2. Keel MK, Patterson JG, Noon TH, Bradley GA, Collins JK: Caprine herpesvirus-2 in association with naturally occurring malignant catarrhal fever in captive sika deer (*Cervus nippon*). J Vet Diagn Invest 15:179-183, 2003
3. Li H, Keller J, Knowles DP, Crawford TB: Recognition of another member of the malignant catarrhal fever virus group: an endemic gammaherpesvirus in domestic goats. J Gen Virol 82:227-232, 2001

4. Li H, Wunschmann A, Keller J, Hall DG, Crawford TB: Caprine herpesvirus-2-associated malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). J Vet Diagn Invest 15:46-49, 2003
5. Li H, O'Toole D, Kim O, Oaks JL, Crawford TB: Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2. J Vet Diagn Invest 17:171-175, 2005
6. Yeruham I, David D, Brenner J, Goshen T, Perl S: Malignant catarrhal fever in a Barbary sheep (*Ammotragus lervia*). Vet Rec 155:463-465, 2004
7. Loken T, Aleksandersen M, Reid H, Pow I: Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. Vet Rec 143:464-467, 1998
8. Plowright W: Malignant catarrhal fever virus. In: Virus Infections of Ruminants, eds. Dinter Z, Morein B, pp. 123-150. Elsevier Science Publishers B.V., Amsterdam, The Netherlands, 1990
9. Metzler AE: The malignant catarrhal fever complex. Comp Immunol Microbiol Infect Dis 14:107-124, 1991
10. Mansfield K, King N: Viral diseases. In: Nonhuman Primates in Biomedical Research Diseases, eds. Bennet BT, Abee CR, Henrickson R, pp.14-16. Academic Press, San Diego, California, 1998
11. Ramer JC, Garber RL, Steele KE, Boyson JF, O'Rourke C, Thomson JA: Fatal lymphoproliferative disease associated with a novel gammaherpesvirus in a captive population of common marmosets. Comp Med 50:59-68, 2000
12. Percy DH, Barthold SW: Pathology of Laboratory Rodents & Rabbits, 2nd ed., p. 254. Blackwell Publishing Professional, Ames, Iowa, 2001
13. McAdam AJ, Sharpe AH: Infectious diseases. In: Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 369-371. Elsevier Saunders, Philadelphia, Pennsylvania, 2005

SLIDE 65
CONFERENCE 17 / CASE I – NOVARTIS CASE 1 (AFIP 2985458)

Signalment: 2-year-old, male, rat, IGS Wistar Hannover Rat, Crl: WI (G1x/BRL/Han) IGS BR (*Rattus norvegicus*)

History: This rat was part of a standard 2 year carcinogenicity study.

Gross Pathology: At scheduled sacrifice, there was a firm mass at the proximal tail that measured 30x20x20mm.

Histopathologic Description: Histologically, the tumor is composed of an expansile, unencapsulated mass of physaliphorous (foamy, 'bubble cells') cells with clear, vacuolated cytoplasm, distinct plasma membranes, round to oval euchromatic nuclei and central nucleoli, growing in nests or cords and divided into lobules by a thin fibrous stroma. Mitotic figures are rare or not present. The tumor cells have locally invaded and replaced adjacent bone, muscle and neurovascular bundles. At multiple sites, the

tumor has small foci of necrosis, mixed cell inflammation and islands of bone that are entrapped by the tumor cells. This bone consists of well-differentiated osteocytes with variable numbers of osteoclasts, some of which have assumed giant, bizarre morphology. No metastases were noted.

Contributor's Morphologic Diagnosis: Tail: Chordoma, IGS Wistar Hannover, rat.

Contributor's Comment: Chordomas are believed to arise from residual notochordal tissue in the axial skeleton, and have a predilection form the proximal and distal extremities. They are most common in the lumbosacral spinal cord of Fischer 344 rats.[1] Chordomas have been reported in humans, rats, mice, dogs, cats, ferrets and mink.

Chordoma can be confirmed with histochemistry (fat negative, PAS positive) and immunohistochemistry (S-100, keratin, and neuron-specific enolase positive), which differentiate it from liposarcoma and chondrosarcoma.[2]

Tissue sections and records of 56 rats with chordoma were identified in the National Toxicology Program's (NTP) data base of approximately 115,000 Fischer 344 rats.[2] Tumors were examined to determine morphological characteristics, incidence, and aspects of biological behavior. Chordomas occurred in aged rats, originated predominantly in lumbosacral vertebrae, were highly malignant, occurred three times more often in male versus female rats, and commonly produced bilateral posterior paresis, paralysis, and/or distention of the colon and rectum.

AFIP Diagnosis: Tail: Chordoma, IGS Wistar Hannover rat (*Rattus norvegicus*), rodent.

Conference Comment: Chordomas are usually composed of three concentrically arranged components in domestic animals: lobules of closely packed vacuolated (physaliferous) cells at the periphery, cartilage in between, and well-differentiated trabecular bone in the center. Physaliferous cells are pathognomonic for chordomas and may be surrounded by a mucinous matrix. The mitotic rate, as in this case, is low.[3,4]

As pointed out by the contributor, immunohistochemical stains such as S-100, keratin, and neuron-specific enolase help to differentiate chordoma from liposarcoma and chondrosarcoma. Additionally, neoplastic cells express vimentin.[3,4,7,8]

Chordomas are the most frequently reported musculoskeletal neoplasm of ferrets and are rarely reported in other species. Chordomas can arise anywhere along the axial skeleton; however, predilection sites differ among species. In the ferret, chordomas are typically located distal to the last caudal vertebra expanding the tip of the tail forming a multilobulated club-shaped mass. Chordomas located on the tail of ferrets are slow-growing and rarely, if ever, metastasize. Cervical chordomas, on the other hand, can

induce osteolysis as well as compression of the spinal cord and adjacent tissues. Rarely, chordomas occur in the thoracic vertebra of ferrets. Cutaneous metastasis and neurological signs may also occur in cervical chordomas. In other species, chordomas are more commonly located in the sacrococcygeal region.[3,4,5,6,7]

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References:

1. Leinninger JR, Riley MGI: Bones, joints, and synovia. In: Pathology of the Fischer Rat, eds. Boorman GA, Eustis SL, Elwell MR, Mongomery CA, Jr., Mackenzie WF, pp. 209-226. Academic Press, San Diego, California, 1990
2. Stefanski SA, Elwell MR, Mitsumori K, Yoshitomi K, Dittrich K, Giles HD: Chordomas in Fischer 344 rats. Vet Pathol 25:42-47, 1988
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, Second Series, vol. V, p.36. Armed Forces Institute of Pathology, Washington D.C., 1999
4. Li X, Fox JG: Neoplastic diseases. In: Biology and Diseases of the Ferret, ed. Fox JG, 2nd ed., pp. 432-435. Lippincott Williams & Wilkins, Baltimore, Maryland, 1998
5. Pye GW, Bennett RA, Roberts GD, Terrell SP: Thoracic vertebral chordoma in a domestic ferret (*Mustela putorius furo*). J Zoo Wildl Med 31:107-111, 2000
6. Munday JS, Brown CA, Richey LJ: Suspected metastatic coccygeal chordoma in a ferret (*Mustela putorius furo*). J Vet Diagn Invest 16:454-458, 2004
7. Williams BH, Eighmy JJ, Berbert MH, Dunn DG: Cervical chordoma in two ferrets. Vet Pathol 30:204-206, 1993
8. Dunn DG, Harris RK, Meis JM, Sweet DE: A histomorphologic and immunohistochemical study of chordoma in twenty ferrets (*Mustela putorius furo*). Vet Pathol 28:467-473, 1991

SLIDE 66
CONFERENCE 17 / CASE II – LMU Neuropath 214/04 (AFIP 3031290)

Signalment: German Fleckvieh (*Bos taurus taurus*), 7-month-old, female, bovine

History: Sudden onset of ataxia and paraparesis, progressing to paraplegia within hours. Further signs included unilateral hypopyon and fever. The animal was euthanized after unsuccessful symptomatic treatment.

Gross Pathology: Brain (not provided): On cut surface there were multiple, well circumscribed brownish-red foci of a few millimeters diameter confined to the watershed area of the telencephalon.

Spinal cord: On transverse slices, bilaterally asymmetric, non-contiguous brownish-red discoloration of the thoracolumbar gray matter with occasional softening and cavitation could be seen at multiple segments.

Histopathologic Description: Slices of the brain, spinal cord and spinal nerve roots reveal a severe multifocal thrombotic necrotizing vasculitis of small parenchymal blood vessels with associated edema, microinfarcts and rarefaction of the adjacent neuroparenchyma. Vasculitis is characterized by fibrinoid necrosis, perivascular fibrin deposition and massive transmural, mixed-cellular inflammatory infiltrations, comprising mainly polymorphonuclear leukocytes and macrophages as well as many eosinophils. These lesions are far most severe in the ventral column of the spinal cord, the adjacent ventral funiculus and some ventral nerve roots. Within the affected spinal gray matter, there are numerous eosinophilic nerve cell necroses, with or without neuronophagia, multiple axonal spheroids, and activated microglial cells. Affected parts of the spinal white matter tracts display signs of bystander demyelination and clusters of multiple eosinophilic spheroids. Large thrombi can also be found in branches of the ventral spinal artery and in the main trunk of the ventral spinal vein. Some of these thrombi present with an endothelial cell lining at the surface and already have been incorporated into the vessel wall. Others undergo puriform lysis. Some sections contain intralesional Gram-negative coccoid bacteria.

Contributor's Morphologic Diagnosis: Spinal cord, vasculitic radiculomyelitis, necrotizing, thrombotic, subacute, severe, multifocal, with Gram-negative coccoid bacteria

Contributor's Comment: Histopathologic patterns of this case strongly resemble those of thrombotic meningoencephalitis (TME) caused by *Histophilus somni* (former *Haemophilus somnus*) infection. *Histophilus somni* is a Gram-negative inhabitant of the bovine nasopharynx and urogenital tract. Under circumstances that compromise local resistance and the immune system in general, *H. somni* acts as an opportunistic pathogen causing severe localized or systemic infections. Thereby, infection occurs mostly around yearling age and in winter time, or is associated with transportation and/or crowding stress.

Septicemic courses present with meningoencephalitis, pneumonia, pleuritis, myocarditis, infertility, abortion, and arthritis. The initial lesion is a widespread necrotizing vasculitis with extensive thrombus formation. Although incompletely understood, *H. somni*-mediated vascular damage induces endothelial cell apoptosis and immunopathic mechanisms.[3]

Therefore, *H. somni* is equipped with virulence factors that mediate adherence to endothelial cells, interference with phagocytosis, and resistance to killing by complement. Evasion from host's immune defense is achieved by lipo-oligosaccharide (LOS) phase variation and sialylation, and employment of immunoglobulin binding proteins.[2] Just recently a group of high molecular weight binding proteins (HMWBP), encoded by one single open reading frame, have been identified that are capable of

binding bovine IgG2 Fc-segments. They are antigenic and have been protective to calves in an immunization /challenge experiment.[4] Once uptaken by neutrophils and macrophages, *H. somni* survives innate bactericidal immune responses by inhibition of O_2^- production and scavenging H_2O_2 in presence of carbohydrate energy sources.[2]

HMWBP also mediate adhesion to bovine endothelial cells.[4] These tackled cells undergo LOS-induced activation of caspases 1, 3 and 8 and upregulate IL-1 transcription.[3] Unfortunately, recruitment of immune cells, complement and coagulation cascades through endothelial signals result in fulminant destruction of the blood vessels rather than elimination of these bacteria.

In the CNS, the vasculitis predominantly is seen in watershed areas. In early stages (vasculitic encephalomyelopathy), brain and spinal cord homeostasis is affected by reduced perfusion and blood-brain-barrier break down. With influx of polymorphonuclear inflammatory cells and bleeding, release of enzymes, cytokines/chemokines and reactive oxygen species aggravate cell and tissue destruction (bystander effect) as a vicious cycle.

Other specific agents causing similar pictures are *Salmonella* spp. and Zygomycetes.[1] The latter could be excluded by absence of fungal hyphae in additional stains (periodic acid Schiff).

AFIP Diagnosis: Spinal cord: Vasculitis, leucocytoclastic, multifocal, with thrombosis, axonal degeneration, and Gram-negative bacteria, German Fleckvieh (*Bos taurus*), bovine.

Conference Comment: The contributor provides a concise overview of the pathogenesis and histologic lesions associated with *Histophilus somni*, the cause of thrombotic meningoencephalitis (TME). Grossly, random red-brown necrohemorrhagic foci (infarcts) are visible most frequently in the cerebrum at the cortical gray matter-white matter interface. The brain may be swollen with flattened gyri due to edema. Fibrinopurulent meningitis is common with cloudiness of the CSF. The lesions are visible externally as well as on cut surfaces and may be seen in the spinal cord as in this case.[5,6] As pointed out by the contributor, the lesions in this case are most severe in the gray matter, but do extend into the adjacent white matter.

In addition to TME, pneumonia, pleuritis, myocarditis, infertility, abortion, and arthritis, *H. somnus* has also been implicated as a cause of necrotizing laryngitis (calf diphtheria) in conjunction with *Fusobacterium necrophorum*.[7]

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References:

1. AFIP WSC 20/2005-2006, Case III – 37236 (AFIP 2996898)
2. Howard MD, Boone JH, Buechner-Maxwell V, Schurig GG, Inzana TJ: Inhibition of bovine macrophage and polymorphonuclear leukocyte superoxide anion production by *Haemophilus somnus*. Microb Pathog 37:263-271, 2004
3. Sylte MJ, Kuckleburg CJ, Atapattu D, Leite FP, McClenahan D, Inzana TJ, Czuprynski CJ: Signaling through interleukin-1 type 1 receptor diminishes *Haemophilus somnus* lipooligosaccharide-mediated apoptosis of endothelial cells. Microb Pathog 39:121-130, 2005
4. Tagawa Y, Sanders JD, Uchida I, Bastida-Corcuera FD, Kawashima K, Corbeil LB: Genetic and functional analysis of *Haemophilus somnus* high molecular weight-immunoglobulin binding proteins. Microb Pathog 39:159-170, 2005
5. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 880-881. Mosby Elsevier, St. Louis, Missouri, 2007
6. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, pp.143-144. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
7. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, p. 568. Academic Press, Inc., San Diego, California, 1993

SLIDE 67
CONFERENCE 17 / CASE III – 2006904019 (AFIP 3031788)

Signalment: 5-year-old, neutered male, domestic ferret, *Mustela putorius furo*

History: The animal had diarrhea for a long time and visited a veterinary hospital due to persistent diarrhea lasting more than 10 days before death. This animal received antibiotics and prednisolone but did not respond to therapy. It went into a coma and died.

Gross Pathology: The animal was necropsied by a veterinary practitioner. Two small whitish nodules were detected on each cut surface of the spleen and liver. The right adrenal was markedly enlarged (11x7x7 mm) and adhered to the liver.

Histopathologic Description: The structure of the cortical and medullary tissue of the right adrenal gland was within almost normal range except for slight proliferation of spindle cells in the medulla. No hyperplastic focus or neoplastic changes were seen in the submitted sample. A multilocular cyst lined by flattened or cuboidal monolayered epithelium occupied more than half of right adrenal gland (Fig. 1). These cysts were filled with eosinophilic mucoproteinaceous fluid containing cell debris and calcified granules. There were also many tubular structures lined by cuboidal epithelial cells adjacent to these cysts. These tubular structures were similar in appearance to the bile ducts and some of the tubules contained small amounts of eosinophilic fluid and

connected to the cysts. The cysts and tubules were located in the cortex and medulla. In some areas, cysts and tubular structures were surrounded by small amounts of connective tissue and metaplastic bone. Clusters of hepatocytes were scattered near the cysts (Fig. 2). Although the morphological characteristics of the hepatocytes were very similar to those of cortical cells, the hepatocytes had darker cytoplasm and wider intercellular spaces in contrast to the cortical cells. Adjacent to the right adrenal, similar tubular structures and multiloculated cyst filled with eosinophilic mucoproteinaceous material were also observed in the liver. This area was well demarcated from surrounding liver tissue and contained cluster of adrenocortical and medullary cells_(Fig. 3).

Immunohistochemically, epithelial cells lined the tubular structures and cysts were strongly positive for cytokeratin 7 (DAKO), weakly positive for cytokeratin AE1/AE3 (DAKO) and negative for cytokeratin 14 (Biomeda).

Contributor's Morphologic Diagnoses: 1. Adrenal gland: Ectopic hepatic tissue with cystic dilatation of bile ducts filled with eosinophilic mucoproteinaceous fluid
2. Liver: Ectopic adrenal tissue
3. Small intestine and visceral lymph nodes: Malignant lymphoma (not submitted)

Contributor's Comment: Solitary or multiple cysts and tubular structures lined by monolayered epithelial cells are rarely seen in the surgically resected adrenal gland tissue from pet ferrets. We have examined a total of 792 surgically resected adrenal glands and epithelial-lined cysts or tubular structures were detected in 20 cases (2.5%). They consisted of 16 out of 108 right adrenals (14.8%) and 11 out of 684 left ones (1.6%). Seven cases had bilateral lesions. All ferrets were gonadectomized at an early age.

Almost all adrenal glands were surgically resected, because proliferative changes were suspected in the adrenal cortices by the onset of clinical signs such as alopecia, enlargement of prostatic gland or swelling of vulva. Nodular or diffuse hyperplasia, adenoma or carcinoma of cortical cells was detected in the majority of the affected adrenal glands. In many cases, moderate to severe enlargement of the spleen with enhanced extramedullary hematopoiesis was also recognized at laparotomy. Clinical signs and enhanced extramedullary hematopoiesis of the spleen were attributable to the imbalance of steroid hormones.
Remnant of the mesonephric duct or ovarian tissue was considered for the genesis of cysts and tubular structures, although the pathologic significance was not clear. Ectopic bile cyst was also considered, because small cluster of hepatic cells was observed near the cysts in two cases. However, hepatocytes and adrenocortical cells took very similar morphologic characteristics and immunohistochemical staining for hepatic mitochondria (hepatocyte paraffin-1, DAKO) to differentiate the hepatic cells from cortical cells was not available for the ferret tissue.

From the changes of this case, it is confirmed that multilocular cysts or tubular structures lined by monolayered epithelial cells are derived from bile cysts from ectopic hepatic tissue. Results of immunohistochemical staining of epithelial cells for various cytokeratins are also identical to those of bile duct epithelium.

AFIP Diagnosis: Adrenal gland: Cysts, multiple, ferret (*Mustela putorius furo*), mustelid.

Conference Comment: Biliary cysts commonly occur in ferret adrenal glands and are considered an incidental finding. They are most often encountered in the right adrenal gland which may share a common capsule with the caudate lobe of the liver. Cysts are filled with a hard translucent, waxy material, which appears eosinophilic on H&E. In some cases, this material may be the only submission in surgical biopsies of ferret adrenal glands.

A recent study in Japan identified epithelial-lined cysts as incidental findings in 0.03% of adrenal glands of ferrets (11/440).[1] In this study, the right adrenal gland was twice as likely to contain these structures as the left. Positive immunohistochemical staining for cytokeratin 7, 14 and 19, AE1/AE2 in the epithelial cells lining these cysts strongly suggest that the cysts are biliary in origin.

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References:
1. Narama I, Nakahara Y, Ano N, Nomura K, Ozaki K: Adrenal epithelial-lined cysts in ferrets. Proceedings of the 23rd Annual Meeting of the European Society of Veterinary Pathology, 2006, http://www.esvp.eu/

SLIDE 68
CONFERENCE 17 / CASE IV – MK062234 (AFIP 3026799)

Signalment: Five-year-old male rhesus macaque

History: An approximately five-year old male rhesus macaque was inoculated intravenously with 5 $X10^3$ $TCID_{50}$ SHIV DH12R obtained by mixing virus obtained from five previously SHIV infected rhesus macaques. Plasma viral loads decreased and remained at low levels until euthanasia. CD4 and CD8 levels remained between 500-1500 cells/*u*l over the course of 180 weeks. The animal was euthanized on 12/15/2005 due to scrotal edema and overall decline in condition.

Gross Pathology: At necropsy the macaque had moderate subcutaneous edema and moderate ascites with approximately 150 ml of clear fluid (total protein of 1.4 g/dl) in the abdominal cavity. The urinary bladder contained a moderate amount of urine (SG 1.010, 2+ protein). Both kidneys were severely enlarged, pale and tan. The right kidney weighed 64.9 g and measured 6.8 X 4.5 X 3.0 cm and the left weighed 67.4 g and measured 7.0 X 4.0 X 2.8 cm. The average kidney weight for a normal adult male rhesus is 13.7 g for the left kidney and 12.9 g for the right with each measuring approximately 3.5 X 2.5 X 1.5 cm.[1] The ascites and subcutaneous edema are consistent with hypoproteinemia and hypoalbuminemia likely secondary to renal disease.

Laboratory Results: 12/7/2005:

BUN	67 mg/dl (9-23)
CREAT	1.5 mg/ml (0.7-1.3)
PHOS	3.5 mg/dl (0.9-8.0)
ALB	0.9 g/dl (3.3-4.7)
TP	3.1 g/dl (6.0-7.8)

Histopathologic Description: Sections of kidney revealed bilateral severe glomerulonephritis characterized by the following glomerular changes: enlarged glomeruli with thickened capillary basement membranes (wire loops), increased numbers of parietal and visceral epithelial cells, increased mesangial matrix and adhesions to Bowman's capsule. Within glomeruli there were small numbers of necrotic cells and associated neutrophils. Prominent dilated tubules were present containing proteinaceous fluid. There was a moderate lymphoplasmacytic interstitial nephritis and multifocal lymphoid aggregates with associated tubular loss and tubular regeneration.

Other findings in this case included mild plasmacytic colitis, mild nonsuppurative pulmonary perivasculitis, mild lymphoid hyperplasia of lymph nodes and spleen, and mild hemosiderosis of the spleen and liver.

Electron microscopy (EM) of the kidney revealed, dense deposits primarily in the basement membrane, with lesser amounts on the epithelial side of the basement membrane. These dense deposits formed humps and the basement membrane was markedly thickened and wrinkled.

Frozen kidney sections were not available and immunohistochemistry performed on paraffin sections were negative for IgA, IgM and IgG.

Contributor's Morphologic Diagnoses: 1. Glomerulonephritis, membrano-proliferative, diffuse, severe, bilateral.
2. Interstitial nephritis, lymphoplasmacytic, multifocal, moderate, bilateral.

Contributor's Comment: Morphological findings associated with membranoproliferative glomerulonephritis include proliferation of parietal epithelial cells with possible crescent formation, mesangial cell proliferation, leukocyte infiltration, thickening of basement membranes and adhesions to Bowman's capsule. The thickened basement membrane occurs when the immune system, with an adequate number of T-cells, is overstimulated, and antibodies react with antigens located in and around the basement membrane. Additional pathological processes affecting the glomerulus include necrosis, apoptosis and activation of glomerular endothelial cells. Complement activation and has been experimentally shown to cause apoptosis.[2] Silver stains can distinguish the splitting of the basement membrane due to the mesangium expanding into the capillary loops.[3] Definitive diagnosis of glomerulonephritis is by renal biopsy and electron microscopy to localize the immune complexes in and around the basement membrane. Immunofluorescent labeling is needed to further characterize the immune complexes as IgG, IgM or C3 which typically has a linear pattern.

Based on ultrastructure and immunofluorescence membranoproliferative glomerulonephritis is classified into three morphologic types. In type I, immune deposits are primarily subendothelial with granular deposition of C3 and IgG. In type II, immune deposits are primarily within the glomerular basement membrane with granular deposition of C3 and IgG usually absent. In type III, the immune deposits are both subepithelial and subendothelial. In all three types there is often a double contour of the basement membrane caused by duplication of the basement membrane with interposition of mesangial cell processes giving a "tram-track" appearance.[3]

Glomerulonephritis has been reported in humans, monkeys, dogs, cats, mice, rats and white perch.[4,5,6,7,8,9,10] Membranoproliferative glomerulonephritis has been reported in approximately 10% of HIV infected individuals.[11] Immunofluorescence of the glomeruli may be positive for IgA, IgG, IgM, C3 and C1q. IgM deposits were most commonly seen in patients with HIV who had clinical signs of nephrotic syndrome and renal insufficiency. EM in these cases showed deposits in the subendothelial, subepithelial side of the basement membrane and within the mesangium.[12] SIV has a similar genetic structure as HIV-1 and infects macaques causing similar adverse health effects including the loss of $CD4^+$ T cells, thus making an excellent model for studying AIDS.[13] Immune mediated glomerulonephritis is an infrequently reported finding in macaques.[5] It is unclear in the present case if the finding of glomerulonephritis is incidental to the experimental infection with SHIV. Only one case has been reported in the literature of an SIV infected macaque which developed immune mediated glomerulonephritis.[14] The possibility that long term antigenic stimulation from chronic low level virus infection may have predisposed this animal to immune mediated disease can not be ruled out.

An important differential diagnosis for glomerular disease associated with infection with SIV or HIV is focal segmental glomerulosclerosis (FSGS).[15] FSGS is characterized by proteinuria, microhematuria, and mesangial expansion. By EM there is podocyte damage, foot process effacement, immune deposits in the filter slits and endothelial tubuloreticular inclusions. These immune deposits are a passive response to accumulation of clumps in the bloodstream that are trapped. By immunofluorescence

there is IgM and C3 in the mesangium and/or sclerotic areas. With disease progression the sclerosis increases along with the amount of mesangial matrix.[3] The pathology of SIV associated FSGS is similar to that seen with HIV with increased mesangial matrix, collapsing glomeruli capillaries, immunoglobulin deposits, increased macrophages and tubuloreticular inclusions.[16] FSGS has been reported in approximately 60% of people infected with HIV that have had renal insufficiency and occasionally in monkeys infected with SIV. The pathogenesis of FSGS is poorly understood.[11,13]

Additional differential diagnoses include minimal change disease (lipoid nephrosis), diabetic nephropathy, renal amyloidosis, lupus nephropathy and poststreptococcal glomerulonephritis.[3] Congo red staining in this case was negative of amyloid deposition.

AFIP Diagnosis: Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, with tubular degeneration, regeneration, protein casts, and multifocal, moderate lymphoplasmacytic interstitial nephritis, rhesus macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: The contributor provides a complete and thorough overview of glomerulonephritis to include different types of glomerulonephritis, histopathologic and ultrastructural characteristics of each, as well as differential diagnoses. Glomerulonephritis, usually of immune origin, is a common cause of renal disease in domestic animals, and frequently precedes end-stage kidneys and renal failure, especially in dogs and cats. A list of causes of immune-mediated glomerulonephritis in domestic animals is summarized below.[17,18,19]

Diseases with Immune-Complex Glomerulonephritis

HORSES
Equine infectious anemia
Streptococcus sp.

CATTLE
Bovine viral diarrhea
Trypanosomiasis

SHEEP
Hereditary hypocomplementemia in Finnish Landrace lambs

PIGS
Hog cholera
African swine fever

DOGS
Infectious canine hepatitis
Chronic hepatitis
Chronic bacterial diseases
Endometritis (pyometra)
Pyoderma
Prostatitis

Dirofilariasis
Borreliosis (Lyme disease)
Systemic lupus erythematosus
Polyarteritis
Autoimmune hemolytic anemia
Immune-mediated polyarthritis
Neoplasia – mastocytoma
Hereditary C3 deficiency
Leishmaniasis

CATS
Feline leukemia virus infection
Feline infectious peritonitis
Feline immunodeficiency virus
Progressive polyarthritis
Neoplasia
Progressive membranous glomerulonephritis

MINK
Aleutian disease

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References:

1. Hill LR, Hess KR, Stephens LC, Price RE, Gray KN: Correlations of kidney weight and volume and selected skeletal parameters to sex in the adult rhesus monkey (*Macaca mulatta*) J Med Primatol 30:56-60, 2001
2. Chadban SJ, Atkins RC: Glomerulonephritis. Lancet 365:1797-1806, 2005
3. Alpers CE: The kidney. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 955-992. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
4. Wen YK, Chen ML: Crescentic glomerulonephritis associated with rifampicin in a patient co-infected with tuberculosis and human immunodeficiency virus. Clin Nephrol 65:284-289, 2006
5. Boyce JT, Giddens WE, Seifert R: Spontaneous mesangioproliferative glomerulonephritis in pigtailed macaques (*Macaca nemestrina*) Vet Pathol 18 (Suppl 6):82-88, 1981
6. Grauer GF: Canine glomerulonephritis: new thoughts on proteinuria and treatment. J Small Anim Pract 46:469-78, 2005
7. Inoue K, Kami-ie J, Ohtake S, Wakui S, Machida S, Shirota K: Atypical membranoproliferative glomerulonephritis in a cat. Vet Pathol 38:468-70, 2001
8. de Lema GP, Maier H, Franz TJ, Escribese M, Chilla S, Segerer S, Camarasa N, Schmid H, Banas B, Kalaydjiev S, Busch DH, Pfeffer K, Mampaso F, Schlondorff D, Luckow B: Chemokine receptor Ccr2 deficiency reduces renal disease and prolongs survival in MRL/lpr lupus-prone mice. J Am Soc Nephrol 16:3592-3601, 2005
9. Manabe N, Furuya Y, Nagano N, Yagi M, Kuramitsu K, Miyamoto H: Immunohistochemical quantitation for extracellular matrix proteins in rats with glomerulonephritis induced by monoclonal anti-Thy-1.1 antibody. Nephron 71:79-86,

1995
10. Ferguson HW, Moccia RD, Down NE: An epizootic of diffuse glomerulonephritis in white perch (*Roccus americanus*) from heavily polluted industrial basin in Lake Ontario. Vet Pathol 19:638-645, 1982
11. D'Agati V, Appel GB: Renal pathology of human immunodeficiency virus infection. Semin Nephrol 18:406-421, 1998
12. Haas M, Kaul S, Eustace JA: HIV-associated immune complex glomerulonephritis with "lupus-like" features: A clinicopathologic study of 14 cases. Kidney Int 67:1381-1390, 2005
13. Gattone VH, Tian C, Zhuge W, Sahni M, Narayan O, Stephens, E: SIV-associated nephropathy in rhesus macaques infected with lymphocyte-tropic $SIV_{mac}239$. AIDS Res Hum Retroviruses 14:1163-1180, 1998
14. Borda JT, Pauley DR, MacKey JJ, Alvarez X, Simon MA, Klumpp SA: Immunoglobulin-A nephropathy with crescentic glomerulonephritis in a pigtailed macaque (*Macaca nemestrina*). Vet Pathol 41:44-49, 2004
15. Alpers CE, Tsai CC, Hudkins KL, Cui Y, Kuller L, Benveniste RE, Ward JM, Morton WR: Focal segmental glomerulosclerosis in primates infected with a simian immunodeficiency virus. AIDS Res Hum Retroviruses 13: 413-424, 1997
16. Stephens EB, Tian C, Dalton SB, Gattone VH: Simian-Human immunodeficeincy virus-associated nephropathy in macaques. AIDS Res Hum Retroviruses 16:1295-1306, 2000
17. Maxie MG: The urinary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 475-484. Academic Press, Inc., San Diego, California, 1993
18. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 628-638. Mosby Elsevier, St. Louis, Missouri, 2007
19. Brown TT, Suter MM, Slauson DO: Immunopathology. In: Mechanisms of Disease a Textbook of Comparative General Pathology, eds. Slauson DO, Cooper BJ, 3rd ed., pp. 278-281.Mosby, Inc., St. Louis, Missouri, 2002

SLIDE 69
CONFERENCE 18 / CASE I – 419101 (AFIP 2938297)

Signalment: 10-year-old, intact female, cocker spaniel, *Canis familiaris*, dog

History: Not available.

Gross Pathology: A 2.0 x 1.5 x 0.5 cm ellipse of haired skin has been removed from the lateral thorax caudal to the left humerus. The ellipse of haired skin has a firm, well circumscribed, expansile mass in the hypodermis.

Histopathologic Description: Within the hypodermis, there is an unencapsulated, expansile, well circumscribed and minimally invasive nodular mass that abuts and

compresses preexistent hypodermal connective tissue and underlying skeletal muscle bundles. The mass is densely cellular and composed of tightly packed pleomorphic polygonal mononuclear cells that are arranged in confluent small clusters and supported by a delicate pervasive fibrovascular stroma. Individual neoplastic cells have a round to oval, occasionally indented or reniform euchromatic nucleus, single to frequently, multiple prominent nucleoli, and an abundant, variably eosinophilic, vesicular cytoplasm. Many neoplastic cells contain empty, sharply defined, variably sized cytoplasmic vacuoles. There are frequent binucleated and multinucleated cells, and there are a moderate number of cells that display anisokaryosis. Karyomegalic nuclei and nuclear "molding" are often seen in many microscopic fields. Mitoses average 1 to 3 per HPF. The neoplastic cells exhibit intense, diffuse cytoplasmic immunopositivity for vimentin; they are not labeled by an antibody to pancytokeratin.

Contributor's Morphologic Diagnosis: Haired skin with attached skeletal muscle: Liposarcoma, pleomorphic

Contributor's Comment: Liposarcomas are relatively rare mesenchymal neoplasms that are typically found in the subcutis of domestic animals. In dogs, liposarcomas are usually cutaneous but can be found in other anatomic locations, including the abdominal cavity and other extracutaneous sites.[1] There is no breed or sex predilection reported for liposarcomas, and they most often affect older dogs. Metastasis of liposarcomas is reported as infrequent; when it occurs, metastatic foci usually are observed in the lung, liver or bone.[1,2] There are three distinct subtypes of liposarcomas that occur in dogs, namely well-differentiated, pleomorphic and myxoid.[1,2] Diagnosis is usually straightforward based on these subtypes, although the pleomorphic variant can sometimes be confused with malignant fibrous histiocytoma.[2] In human medicine, liposarcomas typically involve the retroperitoneum, central body sites or thigh, occur in middle-aged to older people and have been reported to metastasize to the lungs, liver and bone.[3]

AFIP Diagnosis: Haired skin and subcutis: Liposarcoma, pleomorphic, Cocker Spaniel (*Canis familiaris*), canine.

Conference Comment: Liposarcomas are malignant tumors of lipocytes with variable pleomorphism and little or no collagenous stroma. Ultrastructural evaluation suggests that liposarcomas arise from precursor cells of white or unilocular adipose tissue. The etiology of canine liposarcomas is unknown; however, some strains of feline sarcoma virus have been associated with the development of liposarcomas in kittens.[4,5]

As mentioned by the contributor, there are three distinct subtypes of liposarcomas that occur in dogs. Additionally, a fourth subtype, atypical lipoma, is included in Skin Diseases of the Dog and Cat. Atypical lipomas are composed primarily of well-differentiated lipocytes admixed with low numbers of smaller individual or clustered lipoblasts with large, centrally located nuclei and few cytoplasmic vacuoles. Mild

pleomorphism, nuclear hyperchromatism, and rare mitotic figures are occasionally observed within lipoblasts. The presence of lipoblasts with occasional atypia is considered an indicator of early transition to a more aggressive behavior and, in the authors' opinion, atypical lipomas are best considered low-grade liposarcomas.[5]

Well-differentiated liposarcomas are the most common histopathologic subtype of liposarcoma in which the majority of cells resemble normal adipocytes with a single clear fat vacuole and a peripheral nucleus. Other cells have variable numbers of intracytoplasmic lipid droplets or resemble lipoblasts. Mitotic activity is low; however, most of the mitoses are atypical.[2,4,5]

Myxoid liposarcomas are characterized by scattered lipoblasts, lipocytes, spindle cells, and stellate cells within a "bubbly" mucinous stroma. Occasional multinucleated cells may be present.[2,4,5]

Pleomorphic liposarcomas are composed of anaplastic cells with large bizarre multinucleated giant cells. The cells have abundant eosinophilic glassy or foamy cytoplasm. Distinct lipid vacuoles are only present in a few cells. There is marked variability in nuclear size, shape, and chromatin pattern. Nuclear hyperchromatism is common and nucleoli are prominent. Many mitoses are present, and atypical mitoses are common.[2,4,5]

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References:

1. Baez JL, Hendrick MJ, Shofer FS, Goldkamp C, Sorenmo KU: Liposarcomas in dogs: 56 cases (1989-2000). J Am Vet Med Assoc 224 (6):887-891, 2004
2. Hendrick M: Mesenchymal tumors. In: Tumors in Domestic Animals, ed. Meuten D, 4th ed., pp. 97-99. Blackwell Publishing, Ames, Iowa, 2002
3. Kempson RL, Fletcher CDM, Evans HL, Hendrickson MR, Sibley RK: Lipomatous tumors. In: Atlas of Tumor Pathology: Tumors of the Soft Tissues, eds. Kempson RL, Fletcher CDM, Evans HL, Hendrickson MR, Sibley RK, pp. 187-238. American Registry of Pathology, Armed Forces Institute of Pathology, Washington, D.C., 2001
4. Hendrick MJ, Mahaffey EA, Moore FM, Vos JH, Walder EJ: Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals, Second Series, vol. 2, pp.19-20. Armed Forces Institute of Pathology, Washington, D.C., 1998
5. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat Clinical and Histopathologic Diagnosis, 2nd ed., pp.772-776. Blackwell Science Ltd, Oxford, United Kingdom, 2005

SLIDE 70
CONFERENCE 18 / CASE II – 06-8313 (AFIP 3026722)

Signalment: 3-year-old, female spayed, Bassett Hound, *Canis lupus familiaris*

History: The owner noticed multiple ulcerated nodules on the bridge of the nose in the last 24 to 48 hours that increased in size. Biopsy specimens from the margins of four lesions were submitted for evaluation.

Histopathologic Description: All four biopsy specimens had similar lesions. The epidermis was moderately acanthotic with decreased pigmentation. The superficial dermis was edematous with severely dilated vessels lined by swollen endothelial cells. Leukocytes were marginated in several vessels. Perivascular cuffs of predominantly eosinophils ranged from one to a few cells in thickness. Dermal macrophages laden with melanin indicated mild pigmentary incontinence. At the outer margin of the biopsy specimen, the inferior segment and isthmus portion of several hair follicles were extensively destroyed by infiltrates of eosinophils. Along the lesion margin some follicles were completely effaced. Fragments of hair shafts, keratin debris, and adnexal structures were often surrounded by eosinophilic exudate. Deep dermal fibrosis, edema, and perivascular infiltrates of inflammatory cells were noted. Small aggregates of degranulated eosinophils were present around a few collagen fibers in one section. Flame figures were not present. Special stains revealed no bacteria or fungi.

Contributor's Morphologic Diagnosis: Subacute eosinophilic folliculitis and furunculosis.

Contributor's Comment: The clinical and histopathological findings are consistent with canine eosinophilic furunculosis of the face.[2,5] Although the face and especially the muzzle is the most common site for lesion development, lesions can also occur on other parts of the body. The disease is usually seen in young, active dogs with or without access to the outdoors. Lesions are often multifocal and, as in this case, they develop rapidly and frequently ulcerate. Most cases are diagnosed during the summer months. This case was received in late February.

Hypersensitivity reactions to envenomation by arthropods or stinging insects such as wasps, bees, and hornets are the most commonly listed causes of this lesion.[2,5] *Arthropoda* is the largest animal phylum and includes a diverse group of taxa such as insects, crustaceans, spiders, scorpions, and centipedes. Without definitive knowledge of the causative agent, it is difficult to propose a pathogenesis. Regardless of whether it is multiple wasp, bee, or spider stings/bites, the role of insect or spider may be four fold: (1) insect parts and secretions act as irritants; (2) initiation of immediate and delayed hypersensitivity reactions; (3) cytotoxic and other effects of the venom components e.g. histamine and mellitin; and (4) vectors for secondary invaders.[4] Once recruited by activated mast cells, eosinophils appear to play a significant role with infiltration and degranulation in the hair follicle sheaths with resultant destruction of the follicle. Eosinophil derived major basic protein and eosinophil cationic proteins are toxic to epithelial cells.[1] They also have the ability to amplify the hypersensitivity reaction. Eosinophil destruction of hair follicles is not unique to insect envenomation. It may be

seen with dermatophyte infections, immune-mediated disease (pemphigus foliaceus), idiopathic skin disease (sterile eosinophilic pustulosis) and even in transplant rejection.[2,3]

Kerion and sterile eosinophilic pustulosis should be considered in a list of differential diagnoses for eosinophilic folliculitis and furunculosis.[2] Kerion because of the low numbers of dermatophyte hyphae and spores can present a diagnostic challenge. The spores and hyphae can often be seen in H&E stained sections. Examination of multiple sections and fungal stains are helpful in establishing or ruling out the diagnosis. Sterile eosinophilic pustulosis has subcorneal pustules and less severe eosinophilic folliculitis and furunculosis.

AFIP Diagnosis: Haired skin: Dermatitis, folliculitis and furunculosis, eosinophilic, subacute, focally extensive, marked, with mucin and mild epidermal hyperplasia, Bassett Hound (*Canis familiaris*), canine.

Conference Comment: The contributor provides a concise overview of canine eosinophilic furunculosis of the face. Grossly, the lesions appear as severely erythematous or hemorrhagic pustules, papules as well as edematous nodules and plaques that frequently ulcerate forming a hemorrhagic crust. The lesions are most often localized to the dorsal and lateral muzzle and periorbital areas. Peripheral blood eosinophilia is present in most cases. Key histomorphologic features include intense, predominantly eosinophilic, destructive folliculocentric dermatitis; severe dermal edema/mucin deposition; and variable, sometimes severe, hemorrhage. Explosive follicular rupture is characteristic when present. Flame figures – brightly eosinophilic, granular to amorphous material bordering collagen fibers and somewhat obscuring fiber detail – may be present. The contributor explains how to differentiate kerion and sterile eosinophilic pustulosis from eosinophilic furunculosis of the face. Additionally, pemphigus foliaceus should feature more acantholysis and typically develops gradually from subtle initial lesions.[2,5,6]

Canine eosinophilic furunculosis of the face exhibits very similar clinical and histopathologic lesions as feline mosquito bite hypersensitivity. The syndrome is characterized by lesions that develop in the summer and regress during fall predominantly occurring in outdoor cats. Lesions vary from a partially symmetrical, erythematous facial eruption with papules and crusting to bilaterally symmetric facial swelling, alopecia, erosion, ulceration, and fistulation. The planum nasale, periorbital region, and pinnae may be affected. Histologically, there is severe eosinophilic dermatitis with degranulated eosinophils forming flame figures. The epidermis is acanthotic with variable erosion, ulceration, and exudation. The intact epidermis and superficial hair follicles are spongiotic and acanthotic. Differential diagnoses include feline herpesvirus ulcerative dermatitis, eosinophilic plaque, and eosinophilic indolent ulcer. If herpesviral inclusions cannot be identified, herpesvirus ulcerative dermatitis may be indistinguishable from mosquito bite hypersensitivity. Eosinophilic plaques typically display more profound spongiosis and mucinosis of the intact epidermis and

superficial hair follicles while indolent ulcers have eosinophilic granulation in or subjacent to the ulcerated epithelium.[2]

Insect hypersensitivity is the most common allergic skin disease of the horse caused by hypersensitivity to the bites of *Culicoides* (gnats), *Simulium* (black flies), *Stomoxys calcitrans* (stable fly), and, possibly, *Haematobia irritans* (horn fly). Histologically, a superficial and deep perivascular to interstitial eosinophilic dermatitis with focal areas of infiltrative to necrotizing eosinophilic mural folliculitis is seen. Focal eosinophilic granulomas may be present. Variable epidermal hyperplasia, hyperkeratosis, spongiosis, erosion, ulceration, dermal edema, and fibrosis may be seen. Hypersensitivity is also thought to be involved in the pathogenesis of cutaneous habronemiasis, which is believed to be a reaction to the larval stages of *Habronema muscae*, *H. majus*, and *Draschia megastoma*.[7]

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References:

1. Abbas AK: Disease of immunity. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 206-209. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
2. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp. 13-18, 122-126, 417-419, 440-442, 450-453. Blackwell Science Ltd, Oxford, United Kingdom, 2005
3. Le Moine AL, Surquin M, Demoor FX, Noel JC, Nahori M-A, Pretolani M, Flamand V, Braun MY, Goldman M, Abramowicz D: IL-5 mediates eosinophilic rejection of MHC class II-disparate skin allografts in mice. J Immunol 163:3778-3784, 1999
4. Murphy GF, Sellheyer K, Mihm MC Jr.: The Skin. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 1268-1269. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
5. Scott DW, Miller WH Jr., Griffin CE: Muller and Kirk's Small Animal Dermatology, 6th ed., pp. 641-642. W. B. Saunders Co., Philadelphia, Pennsylvania, 2001
6. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1234-1236. Mosby Elsevier, St. Louis, Missouri, 2007
7. Scott DW, Miller WH: Equine Dermatology, pp. 458-466. Saunders, St. Louis, Missouri, 2003

SLIDE 71
CONFERNECE 18 / CASE III – B4903/06 (AFIP 3031852)

Signalment: 10-year-old, male, Tollare, canine, dog

History: Large tumors in the skin/subcutis adjacent to the tuber ischii of the pelvic bones on both sides could be palpated. The tumors had developed during 1 month. One was sent for microscopic examination.

Gross Pathology: One ulcerated, firm tumor, 3 cm in diameter, was submitted.

Histopathologic Description: An inflammatory reaction in the deep dermis and subcutis with severe diffuse cell infiltrates is present. A diffuse granulomatous inflammation is mostly seen, with multiple granulomas, sometimes discrete but often confluent. The granulomas are characterized by central collagen necrosis with macrophages in a radiated palisading pattern around collagen fibers. Marked infiltrates of plasma cells are found throughout the lesion. The plasma cells are predominantly seen in the periphery of the granulomas, perivascularly and diffusely in the adjacent connective tissue. A mild mixture of lymphocytes and neutrophils is noted focally in the plasma cell infiltrates.

In some sections the epidermis/dermis is present. The overlying dermis and epidermis show mild epidermal hyperplasia and a diffuse to perivascular infiltration of mostly plasma cells in the dermis.

Contributor's Morphologic Diagnosis: Granuloma, palisading, skin, dog.

Contributor's Comment: Palisading granulomas are described in the literature as solitary nodules, occasionally seen in dogs, usually located over pressure points.[1] It is macroscopically seen as a discrete nodule of the dermis or tongue. The localization in dermis is noted at the zygomatic arch, hip or lips, which has lead to suggest blunt trauma as the cause. The present case developed bilateral lesions. One lesion was surgically removed, but the other one decreased in size after treatment with corticosteroids, systemically.

There are no case reports of palisading granuloma in the dog, except for the description in the book Skin Diseases of the Dog and Cat.[1] Granulomas with a similar morphology have been reported in man as cutaneous extravascular necrotizing granuloma.[2,3] In these cases, it was associated with systemic immunoreactive or autoimmune disease.

The present case, with bilateral lesions and the good response to corticosteroids may indicate that this canine palisading granuloma was associated with an immunoreactive disease.

The microscopic differential diagnoses for this type of granuloma are reported to be sterile granuloma and pyogranuloma syndrome, cutaneous sarcoidosis and reactive fibrohistocytic nodules. The typical palisading arrangement of the macrophages adjacent to the necrotic collagen, found in the present case, is not found in these three syndromes. The clinical features also differ; however, the reported solitary lesion of palisading granuloma can be questioned.

AFIP Diagnosis: Haired skin and subcutis: Panniculitis, granulomatous and palisading, marked, with lymphoplasmacytic inflammation, Tollare (*Canis familiaris*), canine.

Conference Comment: Palisading granulomas typically present as solitary nodules composed of distinct, discrete granulomas centered on supposed ischemically altered, degenerate or devitalized collagen fibers. Histologically, the nodules are composed of a central core of brightly eosinophilic, fragmented, smudged and occasionally mineralized (basophilic) collagen fibers admixed with few neutrophils surrounded by a zone of pale, eosinophilic, amorphous material further bounded by epithelioid macrophages that palisade along the fibers. The palisading pattern can be striking in some lesions creating a "starburst" pattern. Lymphoplasmacytic inflammation variably separates the granulomas. Additionally, necrotic foci may obliterate parts of the lesion and mucin may be present in some cases. The lesions may be present in the dermis, panniculus and subcutis.[1]

The moderator emphasized that this case was atypical in the degree of lymphoplasmacytic inflammation present. Although lymphoplasmacytic inflammation can be seen in palisading granulomas, it is usually not this severe. Additionally, the moderator also emphasized that this case is not an extravascular necrotizing granuloma which is multicentric, neutrophilic and leucocytoclastic.

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References:

1. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp. 337-340. Blackwell Science Ltd, Oxford, United Kingdom, 2005
2. Lee MW, Jang KA, Lim YS, Choi JH, Sung KJ, Moon KC, Koh JK: Cutaneous extravascular necrotizing granuloma (Churg Strauss granuloma). Clin Exp Dermatol 24:193-195, 1999
3. Obermoser G, Zelger B, Zangerle R, Sepp N: Extravascular necrotizing palisaded granulomas as the presenting skin sign of systemic lupus erythematosus. Br J Dermatol 147:371-374, 2002

SLIDE 72
CONFERENCE 18 / CASE IV – 05-2007 (AFIP 2984011)

Signalment: 2-year-old Quarter Horse stallion

History: This horse was reported to have extremely pliable skin over the lumbar region and withers.

Histopathologic Description: The epidermis in some sections has mild orthokeratotic hyperkeratosis. The superficial dermis is infiltrated by minimal numbers of mast cells. Within the mid to deep dermis, collagen bundles are short, comma-shaped and arranged in clusters frequently separated by clear spaces. In the deep dermis, collagen bundles are small, packeted and separated by clear spaces. Hair follicles and other adnexal structures are within normal limits.

Contributor's Morphologic Diagnosis: Collagen dysplasia, compatible with hereditary equine regional dermal asthenia (HERDA).

Contributor's Comment: Hereditary equine regional dermal asthenia (HERDA), previously known as 'hyperelastosis cutis' is a hereditary collagen dysplasia affecting horses primarily of the Quarter Horse breed, although similar conditions have been reported in a few other breeds and in some cross bred horses.[1] A recent report indicates autosomal recessive as the most likely mode of inheritance.[2] Diagnosis of HERDA is made based upon a combination of clinical findings, histopathology and other ancillary tests, such as special stains and electron microscopy.

Horses often present at an early age with complaints of seromas or hematomas, non-healing wounds, sloughed skin or easily tented skin. A distinct regional distribution over the dorsum is characteristic. Areas affected in decreasing frequency are 1) withers and croup, 2) gluteal region, 3) neck, 4) lateral thorax and abdomen, 5) distal legs, 6) face and 7) coronary band.[1] Owners may report the onset of signs as coincident with saddle training.

Histopathology of skin biopsies is not pathognomonic, but is characteristic and suggestive if combined with clinical history. Collagen bundles in the mid to deep dermis are small, thin and often arranged in small packets separated by clear spaces. A clear cleft may be present in the deep dermis. Early reports indicated that trichrome stains would demonstrate abnormal collagen fibers with red cores; this has been found to be an inconsistent finding.[1,3] Elastin abnormalities are also variable and non-diagnostic. Electron microscopy shows random orientation of collagen fibers, although this change is non-specific and may be seen in other skin conditions.[1] Immunohistochemistry for types I and III collagens has failed to show a difference between affected and normal horses.

HERDA is one member of a heterogeneous group of inherited collagen dysplasias of man and animals.[4] Ehlers-Danlos syndromes in humans include defects in various structural components of different collagen types or in enzymes which process those proteins to form collagen fibrils. Autosomal dominant, Ehlers-Danlos type syndromes have been described in the dog and cat. 'Dermatosparaxis' (literally 'torn skin') is a disease described in cattle, sheep and cats. It is an autosomal recessive genetic disease resulting from a defect in procollagen peptidase. Procollagen accumulates and cannot be packeted into normal collagen fibrils. The mutation responsible for HERDA has not been identified, nor is the type of collagen affected in HERDA known.

HERDA seems to be unique in having a distinct regional distribution of affected tissue. While dorsal midline lesions may be predisposed by rubbing from saddles, several reports have noted normal wound healing after castration of affected males and lack of lesions in the girth.[1,3] These facts suggest there is an actual differential expression of this defect in horses.
Veterinary pathologists need to be aware of this clinical condition in horses, and should be prepared to corroborate clinical findings with compatible histologic lesions.

AFIP Diagnosis: Haired skin: Collagen dysplasia, diffuse, Quarter Horse (*Equus caballus*), equine.

Conference Comment: The contributor provides an excellent summary of hereditary equine regional dermal asthenia (HERDA). Collagen dysplasia (hyperelastosis cutis, dermatosparaxis, cutaneous asthenia) occurs in most domestic animals and is characterized by hyperextensible, loose skin that tears easily. Histologic features vary among the different types of collagen dysplasia syndromes, and in some can be histologically normal. Electron microscopy or biochemical analyses are sometimes necessary to reach a definitive diagnosis.[5]
An important histologic feature of cutaneous dermal asthenia is the subfollicular artifactual split that occurs during procurement or processing. As pointed out by the moderator, this is a useable artifact (after Stannard). Additionally, the dermis ends abruptly and granulation tissue is often present at the deep margin of biopsies of tears or lacerations indicating prior healing attempts where the skin was pulled up and snapped back.[6]

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References:
1. White SD, Affolter VK, Bannasch DL, Schultheiss PC, Hamar DW, Chapman PL, Naydan D, Spier SJ, Rosychuk RAW, Rees C, Veneklasen GO, Martin A, Bevier D, Jackson HA, Bettenay S, Matousek J, Campbell KL, Ihrke P: Hereditary equine regional dermal asthenia ('hyperelastosis cutis') in 50 horses: clinical, histological, immunohistochemical and ultrastructural findings. Vet Dermatol 15:207-217, 2004
2. Tryon RC, White SD, Famula TR, Schultheiss PC, Hamar DW, Bannasch DL: Inheritance of hereditary equine regional dermal asthenia in Quarter Horses. Am J Vet Res 66:437-442, 2005
3. Borges AS, Conceicao LG, Alves ALG, Fabris VE, Pessoa MA: Hereditary equine regional dermal asthenia in three related Quarter horses in Brazil. Vet Dermatol 16:125-130, 2005
4. Yager JA, Scott DW: Skin and appendages. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, vol. 1, 4th ed., pp. 564-567. Academic Press, Inc., San Diego, California, 1993

5. Hargis AM, Ginn PE: The integument. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1160-1161. Mosby Elsevier, St. Louis, Missouri, 2007
6. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp. 386-390. Blackwell Science Ltd, Oxford, United Kingdom, 2005

SLIDE 73
CONFERENCE 19 / CASE I – 06049-A WFUHS (AFIP 3026269)

Signalment: 12+year-old, female, cynomolgus monkey (*Macaca fascicularis*)

History: The monkey was in quarantine after importation from Indonesia. This animal appeared to be in good health upon arrival. A tuberculin test had been performed three times during the quarantine; the first test showed a complete negative result; the second showed a questionable reaction at 48 hours observation, but then was considered negative at 72 hours observation; and the third showed marked eyelid swelling at 24 and 48 hours observation. The monkey had lost 0.2 kg of its body weight since arrival. The monkey was euthanized due to the suspicion that it had tuberculosis.

Gross Pathology: The monkey was in thin body condition and the eyelid at the tuberculin site was edematous. Numerous 1-3 mm diameter, rounded, punctate, tan, raised nodules (tubercles) were scattered on the spleen capsule and within the parenchyma. Similar tubercles were also present on the capsule and within the liver parenchyma, but were smaller in size, about 1-1.5 mm in diameter. The mesenteric lymph nodes were diffusely enlarged and prominent. No lung lesions were noted grossly.

Laboratory Results: Culture for acid fast bacteria identification is pending.

Histopathologic Description: Lung: There are multifocal, confluent granulomatous foci containing large numbers of epithelioid macrophages admixed with multinucleated giant cells, fewer lymphocytes, plasma cells, and some neutrophils with central necrosis, compressing adjacent alveoli. Adjacent peribronchial connective tissue is markedly expanded by moderate to large numbers of similar inflammatory cells. A bronchus is segmentally eroded by the granulomas, and the remaining epithelial cells are hyperplastic and reactive (not present in some slides). The tunica intima of a medium artery is focally eroded by loose mesenchyme, interspersed with small to moderate numbers of lymphocytes and plasma cells with few small, reactive vessels (varies between slides). Some lymphatic vessels multifocally are markedly expanded by clusters of macrophages and multinucleated giant cells. The alveolar walls adjacent to the granulomas are often thickened by prominent type 2 pneumocytes. The remaining alveoli occasionally contain small to moderate numbers of alveolar macrophages or pale eosinophilic liquid material (edema). There is a focal extensive

thickening of the pleura by collagenous connective tissue, and the pleura is multifocally expanded by similar granulomas.

Spleen: Replacing the parenchyma multifocally there are discrete, irregular, variably sized granulomatous foci, up to 1.5 mm in diameter. The periarteriolar lymphoid sheaths (PALS) are reduced in number and size (lymphoid depletion). Within the center of some lymphoid follicles, there are small to moderate amounts of amorphous deeply eosinophilic material (amyloid, presumed).

Granulomas were also present in liver, pancreatic lymph node, mesenteric lymph node, and tracheobronchial lymph node. Moderate numbers of nematodes (Strongylid and filarid) were present in small intestine and colon; cysts of *Sarcocystis* sp. were found in the tongue (Tissues are not submitted).

The eyelid lesions demonstrated that the connective tissue and muscle within the dermis were multifocally dissociated, fragmented, and expanded by fibrinous material (edema and inflammation) and large aggregates of lymphocytes, plasma cells, macrophages and fewer neutrophils, particularly surrounding blood vessels (perivascular), severe in a focally extensive area (Type IV hypersensitivity reaction, tissue is not submitted).

Acid fast staining was performed on the lung, spleen, and liver. There were myriads of acid fast bacteria intercellular and intracellular of phagocytic cells within the focus of necrosis in the lung, but acid fast bacteria were rarely present in the spleen and liver.

Contributor's Morphologic Diagnoses:
1. Bronchopneumonia, necrotizing, granulomatous, severe, focally extensive, with multifocal granulomatous lymphatic emboli and myriad intralesional acid fast bacteria, consistent with *Mycobacterium tuberculosis*.
2. Splenitis, necrotizing, granulomatous, moderate to severe, multifocal with mild amyloidosis and lymphoid depletion.

Contributor's Comment: *Mycobacterium tuberculosis* is the principal cause of tuberculosis in humans and nonhuman primates. It is occasionally encountered as a cause of tuberculosis in dogs, but cattle and cats are relatively resistant. Guinea pigs and hamsters are highly susceptible, but rabbits and birds are resistant.[1]

M. tuberculosis is an obligatory aerobic, intracellular pathogen, which has a predilection for the lung tissue. The tubercle bacilli enter the body via the respiratory route; the bacilli may massively disseminate lymphohaematogenously (miliary tuberculosis) from a pulmonary or extrapulmonary focus via embolisation to the vascular beds of various organs. Organs with high blood flow, e.g. spleen, liver, lungs, bone marrow, kidneys, and adrenals are frequently affected; regional lymph nodes are commonly affected through the lymphatic system.[2] The granulomas may also rupture into airways, allowing the mycobacteria to be released and transmitted to other hosts via aerosols.

The early event following inhalation of *M. tuberculosis* is engulfment by alveolar macrophages (phagocytosis). It is well known that mycobacteria have the ability to survive in phagosomes by inhibiting phagolysosomal fusion. Early studies showed that sulphatides (cell wall component), derivatives of multinucleated trehalose-2 sulfate, a lysosomotropic polyanionic glycolipid, have a major role in the inhibition of phagolysosomal fusion. More recent studies proposed several mechanisms for the fusion inhibition, including high ammonia production by the mycobacteria, glycosylated phosphatidylinositol lipoarabinomannan (ManLAM) lipid rich cell component, and mycobacterial protein kinase G.[3, 4, 5]

Within 2-6 weeks cell mediated immunity (CMI) develops, and there is an influx of lymphocytes and activated macrophages into the lesion resulting in granuloma formation. The exponential growth of the bacilli is checked and dead macrophages form a region of caseous necrosis. The bacilli are contained in the caseous centers of the granulomas.[2] The bacilli may remain indefinitely within the granuloma, become re-activated later or may get discharged into the airways after erosion of airways. If the bacteria in the lesion are eventually overcome, the tubercle is reduced to a small mass of fibrous and hyaline scar tissue. When miliary tuberculosis occurs in human beings, the lesions appear alike and are termed “soft” or “exudative” and the lesions often reveal acid fast bacilli. Acid fast bacilli are less abundant in more chronic “hard” tubercles.[6]

Nonhuman primates (NHP) acquire classic tuberculosis infection by contact with other nonhuman primates or humans through inhalation or the digestive route. These infected animals can become reservoirs, causing outbreak of disease. Clinical diagnosis of tuberculosis in NHP can be difficult because infected monkeys may only show mild behavioral changes like anorexia and lethargy. Occasionally, infected monkeys may suddenly die while appearing in good body condition. Tuberculosis in cynomolgus monkeys has many comparable gross and histopathology changes consistent with different stages in human infection; which makes the cynomolgus monkey an animal model for human tuberculosis.[7]

Detection of tuberculosis in the NHP has relied on tuberculin skin response, serological testing, histopathology, microscopy and culture identification. Among these, the most frequently used methods are culture identification and the tuberculin skin test (also known as Purified Protein Derivative), the latter being a routine test in quarantine and preventive medicine protocols. However, the PPD test is not adequately sensitive or specific in many species and the rate of false negatives is high.

Tuberculin is the protein lipopolysaccharide component of *M. tuberculosis* that is commonly used in diagnosis of subclinical tuberculosis infection. The edema at the site of tuberculin injection is the result of a type IV hypersensitivity (cell mediated or delayed type hypersensitivity) reaction, that usually appears in 8-12 hours, and reaches a peak in 24-72 hours. On intradermal injection of tuberculin in an animal previously exposed to the mycobacteria, the memory Th1 cells interact with the antigen on the surface of antigen-presenting cells and are activated (undergo blast transformation and

proliferation). These changes are accompanied by the secretion of the Th1 type cytokines (IL-12, IFN-_, IL-2, TNF-_) which attract mononuclear cells (T cells and macrophages) to the area.[8]

AFIP Diagnoses: 1. Lung: Granulomas, multifocal to coalescing, severe, cynomolgus monkey (*Macaca fascicularis*), nonhuman primate.
2. Spleen: Splenitis, granulomatous, multifocal to coalescing, moderate.
3. Spleen: Lymphoid depletion, diffuse, mild.
4. Pancreas: No significant lesions.

Conference Comment: The contributor provides an excellent and thorough overview of *Mycobacterium tuberculosis* to include species susceptibility and pathogenesis. Four species of *Mycobacterium* are considered causes of "classic" tuberculosis – *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti* (*M. tuberculosis* complex). Though there is some species predilection with each, all three can infect a wide range of species, and especially immunocompromised animals. The most common agents of tuberculosis in primates are *Mycobacterium tuberculosis* and *M. bovis*. Organisms in the *M. avium-intracellulare* group are frequently isolated in macaques with mycobacterial infections in which tubercle formation is not a feature. *M. avium-intracellulare* infections in nonhuman primates resemble Johne's disease primarily affecting the intestinal mucosa and mesenteric lymph nodes.[1,9,10]
There is slide variability with some slides containing sections of pleura attached to myocardium.

Contributor: Department of Pathology, Section on Comparative Medicine, Wake Forest University School of Medicine

References:
1. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 489-498. Williams & Wilkins, Baltimore, Maryland, 1997
2. Raja A: Immunology of tuberculosis. Indian J Med Res 120:213-232, 2004
3. Nguyen L, Pieters J: The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. Trends in Cell Biology 5:269-276, 2005
4. Grosset J: Guest commentary: *Mycobacterium tuberculosis* in the extracellular compartment: an underestimated adversary. Antimicrob Agents Chemother 47:833-836, 2003
5. Vergne I, Chua J, Singh SB, Deretic V: Cell biology of *Mycobacterium tuberculosis* phagosome. Annu Rev Cell Dev Biol 20:367-394, 2004
6. Sharma SK, Mohan A, Sharma A, Mitra DK: Miliary tuberculosis: new insights into an old disease. http://infection.thelancet.com 5:415-430, 2005
7. Capuano SV, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, Bissel S, Fuhrman C, Klein E, Flynn JL: Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infect Immun 71:5831-5844, 2003

8. Abbas AK: Diseases of immunity. In: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 215-218. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
9. Hines ME, Kreeger JM, Herron AJ: Special topic overview mycobacterial infections of animals: pathology and pathogenesis. Lab Ani Sci 45:334-351, 1995
10. King NW: Tuberculosis. In: Monographs on Pathology of Laboratory Animals Nonhuman Primates, eds. Jones TC, Mohr U, Hunt RD, vol. 1, pp. 141-148. Springer-Verlag, Berlin, Germany, 1993

SLIDE 74
CONFERENCE 19 / CASE II – G 7282 (AFIP 3034591)

Signalment: 5-year-old, intact female, rhesus macaque (*Macaca mulatta*), non-human primate

History: This monkey was inoculated with simian immunodeficiency virus (SIV_{mac}) on 10/02/04 via the tonsillar route. Eighty-five weeks after the tonsillar challenge the animal showed a deteriorating general condition and reduced appetite. The monkey was euthanized on 26/11/05 due to a poor prognosis.
Gross Pathology: At necropsy the rhesus macaque was in moderate nutritional condition. Severe ascites with amber fluid was observed in the abdominal cavity. The liver showed moderate hepatomegaly with blunt margins and multiple hemorrhagic foci throughout the parenchyma. Further findings included moderate hypertrophy of the left ventricle, generalized hyperplasia of the lymph nodes and severe follicular hyperplasia of the spleen.

Histopathologic Description: At microscopic examination the liver parenchyma reveals multiple coalescing foci of hepatocellular necrosis with multifocal hemorrhages and a sparse inflammatory reaction, characterized by few neutrophils in areas of necrosis and a mild periportal mononuclear cell infiltrate. Numerous intranuclear basophilic round to oval shaped inclusion bodies are present within the hepatocytes.

Immunohistochemical staining for SV40 performed on sections of formalin-fixed liver was negative.

Transmission electron microscopy of liver samples revealed adenovirus like particles in the nucleus of hepatocytes.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal to coalescing, severe, with intranuclear basophilic inclusion bodies, rhesus macaque (*Macaca mulatta*), non-human primate.

Contributor's Comment: The family *Adenoviridae* is subdivided into four genera: *Mastadenovirus* affecting mammals, *Aviadenovirus* which infect birds, *Atadenovirus* with a broad host range from several vertebrate classes including reptiles, birds and

mammals and *Siadenovirus* which has been isolated from amphibians (frog) and birds (turkey pheasant, chicken). Adenoviruses are non enveloped, double-stranded DNA viruses that consist of a capsid, fibers, a core, and associated proteins. The icosahedral capsid has a diameter of 80-110 nm. Viral replication is located within the nucleus of host cells and results in characteristic inclusion bodies visible by light microscopy.[1,2]

The recognized diseases associated with adenovirus infection in humans predominantly involve the respiratory tract (pneumonia), the GI tract (gastroenteritis), the eye (keratoconjunctivitis) and the genitourinary tract (cystitis, urethritis, cervicitis). Virus may be introduced through contact, respiratory droplets or ingestion. Immunocompromised patients are especially susceptible to adenovirus infections and opportunistic adenovirus induced disease with high case-fatality rates is a frequent finding in organ transplant recipients and AIDS patients.[3]

Experimentally, several adenoviruses have been shown to cause malignant neoplasms in newborn hamsters and other laboratory animals and to transform cells in tissue culture. However, a causal relationship to spontaneous neoplasms has not been established yet.[1]

At least 27 serotypes of adenoviruses have been isolated from nonhuman primate species, including macaques (*Macaca* spp.), African green monkeys (*Cercopithecus aethiops*), baboons (*Pan* spp.), squirrel monkeys (*Saimiri sciureus*) and cotton top tamarins (*Saguinus oedipus*). However in immunocompetent animals, infection with adenovirus most often leads to subclinical disease and reports of adenoviral pneumonia, gastroenteritis and conjuctivitis are few.[4]

In immunocompromised monkeys simian adenovirus is an uncommon, but significant potential opportunistic pathogen and has been associated with segmental enteritis involving the ileum and necrotizing pancreatitis in SIV infected rhesus macaques.[4,5,6] Necrotizing hepatitis is an extremely rare manifestation of adenovirus infection in nonhuman primates. Individual cases have been described in rhesus monkeys (*Macaca mulatta*, African green monkeys (*Cercopithecus aethiops*) and chimpanzees (*Pan troglodytes*) that all showed evidence of immunosuppression.[7]

In the present case etiological diagnosis of adenoviral hepatitis was based on transmission electron microscopy with evidence of paracrystalline arrays of adenovirus in the nucleus of hepatocytes (Fig. 1).

Other causes of necrotizing hepatitis in rhesus monkeys include hepatitis A and hepatitis B, SV40 and herpesvirus infection. In domestic animals adenoviruses have been recovered from cattle, sheep, pigs, horses, mice and dogs and are most often associated with pneumonia and enteritis. However, with the exception of infectious canine hepatitis, most are not serious causes of disease in animals other than those that are immunocompromised.[1]

AFIP Diagnoses: 1. Liver: Hepatitis, necrotizing, multifocal, random, marked, with fibrin, hemorrhage, edema, and eosinophilic to basophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), nonhuman primate.
2. Liver: Hepatitis, portal, lymphocytic, multifocal, mild.
3. Gallbladder, lamina propria: Edema, diffuse, marked.

Conference Comment: The contributor provides a thorough overview of adenoviruses. As mentioned by the contributor, adenoviruses are currently classified into four genera. Below is a comparative list of adenoviruses and some of the diseases they cause:[1,2,4,8,9,10]

Genus Aviadenovirus (Group 1 avian adenoviruses)**:**

- Fowl, goose, duck, pigeon, turkey adenovirus: Inclusion Body Hepatitis (IBH); hydropericardium syndrome; respiratory disease; necrotizing pancreatitis and gizzard erosions
- Quail Bronchitis (avian adenovirus Type 1)

Genus Siadenovirus:

- Marble spleen disease (MSD) (Adenovirus Type 2): Pheasants; splenic necrosis, respiratory edema, congestion and asphyxia
- Hemorrhagic enteritis (HE) (Adenovirus Type 2): Young turkeys; bloody droppings, death
- Avian adenovirus splenomegaly virus (AASV)
- Frog Adenovirus

Genus Atadenovirus:

- Egg drop syndrome (subgroup 3 avian adenovirus): Laying hens, viral replication in pouch shell gland epithelium; intranuclear inclusion bodies
- Ovine, bovine, duck, possum adenoviruses
- Adenoviral Hemorrhagic Disease in California mule deer and black-tailed deer: Vasculitis with endothelial intranuclear inclusion bodies, pulmonary edema, hemorrhagic enteropathy; produces similar lesions to Bluetongue virus and Epizootic Hemorrhagic Disease (EHD) (orbiviruses)
- Reptilian Adenoviruses
    - Bearded dragon, snake, chameleon, gecko

Genus Mastadenovirus:

- Human adenovirus: Respiratory disease, enteritis; keratoconjunctivitis
- Simian adenovirus (27 different viruses): Mostly subclinical; some secondary to immunosuppression, mild to moderately severe respiratory and enteric disease, keratitis/conjunctivitis
- Canine adenovirus 1: Infectious canine hepatitis
- Canine adenovirus 2: Necrotizing bronchiolitis and alveolar epithelialization; most cases secondary to immunosuppression

- Equine adenovirus: Mild respiratory disease except in CID Arabian foals where adenoviral infection leads to severe bronchiolitis, atelectasis, and pancreatitis
- Bovine adenovirus: Respiratory tract disease, pyrexia, KCS, colic, associated with respiratory and enteric disease in calves but not considered the primary pathogen in either syndrome
- Ovine adenovirus: Respiratory tract disease, conjunctivitis, enteritis
- Porcine adenovirus: Widespread, mostly subclinical, pneumonia, enteritis associated with encephalitis and diarrhea
- Murine adenovirus: Oncogenic in newborns, experimentally induce CNS lesions
- Guinea Pig Adenovirus: Pneumonitis
- Adenovirus can experimentally cause tumors in hamsters and rats
- Wildlife: Brown bear, coyotes, foxes, wolves, skunks and raccoons are also susceptible to CAV-1.

Note: Several viral isolates share the same name with isolates from other genera and are only differentiated by letter designators. For a complete table of the adenoviruses, readers are encouraged to visit the ICTV website listed in reference 8.

Contributor: German Primate Center, Department of Infectious Pathology, Kellnerweg 4, 37077 Göttingen, Germany, http://www.dpz.gwdg.de

References:

1. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 241-248. Williams & Wilkins, Baltimore, Maryland, 1997
2. Davison AJ, Benko M, Harrach B: Genetic content and evolution of adenoviruses. J Gen Virol 84:2895-2908, 2003
3. Carrigan DR: Adenovirus infections in immunocompromised patients. Am J Med 102:71-74, 1997
4. Mansfield K, King N: Viral diseases. In: Nonhuman primates in biomedical research diseases, eds. Bennett BT, Abee CR, Henrickson R, pp. 16-19. Academic Press, San Diego, California, 1998
5. Chandler FW, McClure HM: Adenoviral pancreatitis in rhesus monkeys: current knowledge. Vet Pathol 19:171-180, 1982
6. Baskin GB, Murphey-Corb M, Watson EA, Martin LN: Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 25:456-467, 1988
7. Davis KJ, Hubbard GB, Soike KF, Butler TM, Lipscomb TP: Fatal necrotizing adenoviral hepatitis in a chimpanzee (*Pan troglodytes*) with disseminated cytomegalovirus infection. Vet Pathol 29:547-549, 1992
8. International Committee on Taxonomy of Viruses database [database online] http://www.ncbi.nlm.nih.gov/ICTVdb/index.htm, 2006
9. Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ: Veterinary Virology, 3rd ed., pp.327-334. Academic Press, San Diego, California, 1999

10. Woods LW, Hanley RS, Chiu PH, Lehmkuhl HD, Nordhausen RW, Stillian MH, Swift PK: Lesions and transmission of experimental adenovirus hemorrhagic disease in black-tailed deer fawn. Vet Pathol 36:100-110, 1999

SLIDE 75
CONFERENCE / CASE III – S0511870-1 (AFIP 3044695)

Signalment: 1-year-old, female, black face, *Ovis aries*, sheep

History: CNS disease of 2 days duration, affecting approximately 1% of the flock.

Gross Pathology: Bilateral and focal symmetrical areas of malacia in basal ganglia, thalamus and cerebellar peduncles

Laboratory Results: *Clostridium perfringens* type D isolated from small intestine. *Clostridium perfringens* alpha and epsilon toxin detected in small intestinal contents by capture ELISA.

Histopathologic Description: Corpus striatum: At the subgross level there are several foci of pallor (rarefaction) with loss of tissue architecture (degeneration) within the ventral aspect of the internal capsule (the internal capsule is the more acidophilic white matter track that crosses the section). In these areas there is multifocal perivascular proteinaceous edema surrounding veins and a few arterioles. The endothelium of these vessels shows hypertrophy and there is an increased number of neutrophils within the lumen and a few of these cells are occasionally seen in the perivascular space together with a few lymphocytes. Diffusely in the areas of rarefaction, there is vacuolation of the neuropil (spongiosis) and dilated axon sheaths with or without swollen axons (spheroids). There is also gliosis, mostly represented by large and vesicular astrocytes, some of which have a moderate amount of acidophilic cytoplasm (gemistocytes). Gitter cells are not a prominent feature in this section. The gray matter surrounding the internal capsule (basal ganglion) looks mostly unaffected.

Contributor's Morphologic Diagnosis: Corpus striatum (internal capsule): Degeneration of white matter, focally extensive, bilateral with proteinaceous perivascular edema

Contributor's Comment: *C. perfringens* type D produces a peracute, acute or chronic neurological condition in sheep, characterized by sudden death or neurological signs including blindness, opisthotonos, convulsions, bleating and recumbency. Diarrhea is occasionally observed, although this is not a common clinical sign in sheep.[4] Small intestinal changes, if present, consist of hyperemic intestinal mucosa with slightly-to-markedly red fluid contents. Colitis may occur, but is not a consistent finding in sheep enterotoxemia. Several other gross findings, such as excess pericardial pleural and/or abdominal fluids with or without fibrin strands, serosal petechiation, and lung edema,

suggest, but are not specific for, type D enterotoxemia. Pathognomonic gross changes in sheep are in the brain, and consist of herniation of the cerebellar vermis (cerebellar coning) and/or focal symmetrical encephalomalacia (FSE). While cerebellar coning can be found in peracute and acute cases, FSE is seen only in chronic cases and is characterized by dark, hemorrhagic foci in corpus striatum, thalamus, midbrain, and cerebellar white matter cores.[1,2,3]

Microscopic changes in the brain of sheep are unique and pathognomonic, although they are not present in all cases.[2,5,6] The most consistent change is perivascular proteinaceous edema (microangiopathy), consisting of acidophilic accumulations of protein surrounding small and medium sized arteries and veins.[2] This lesion is very prominent in the sections of the submitted case. Perivascular edema can already be seen a few hours after onset of clinical signs. To the authors' knowledge, there are no other conditions of sheep that produce this highly proteinaceous perivascular edema of the brain in sheep and this change is therefore diagnostic for type D enterotoxemia. In chronic disease, a lesion characterized by degeneration and/or necrosis of white matter can be observed.[3] This lesion is the histological counterpart of the gross change of FSE and it is characterized by degeneration of white matter, hemorrhage, and astrocyte and axonal swelling as seen in the accompanying section. Perivascular edema and degeneration and necrosis of brain parenchyma are always bilateral and symmetrical and they have been described most frequently in corpus striatum, internal capsule, thalamus, mid brain, cerebellar peduncles, and cerebellar white matter cores.[1,2] These areas are not exclusively affected, and lesions can sometimes be seen in other parts of brain, such as cortex and hippocampus.[2] Because histological lesions are observed in most, but not all cases of ovine enterotoxemia, these changes are a very useful indicator of enterotoxemia, but absence of these lesion does not therefore preclude a diagnosis of this disease in sheep. The most widely-accepted criterion in establishing a definitive diagnosis of type D enterotoxemia in both sheep and goats is detection of epsilon toxin in intestinal contents.[6] The currently submitted case tested positive for epsilon toxin in intestinal content thus confirming a diagnosis of enterotoxemia.

AFIP Diagnosis: Brain, white matter: Necrosis and loss, multifocal, with edema, sheep (*Ovis aries*), ovine.

Conference Comment: The contributor provides an excellent summary of focal symmetric encephalomalacia in sheep caused by *Clostridium perfringens* type D to include clinical findings and pathognomonic gross and light microscopic findings.

Clostridium perfringens is an anaerobic, spore-forming, Gram-positive bacillus that causes disease through the elaboration of toxins within the gastrointestinal tract. High starch diets facilitate clostridial overgrowth. *C. perfringens* type D produces alpha and epsilon toxin. Epsilon toxin is secreted as an inactive prototoxin in the gut, with activation through cleavage by trypsin. Epsilon toxin binds endothelial cell surface receptors resulting in opening of tight junctions, disturbed transport processes, and

increased vascular permeability resulting in vasogenic edema, swelling of astrocytic foot processes, hypoxia, ischemia, and necrosis. Additionally, some of the effects of epsilon can be mediated by the adenyl cyclase/cAMP system.[7,8,10]

Enterotoxemia caused by *C. perfringens* type D is a common disease of sheep and goats, and has been reported in calves. In sheep, the disease most frequently occurs in fattening lambs and is known as "overeating disease", or as "pulpy kidney disease" (due to the rapid postmortem autolysis of the kidneys in some animals dying from the condition). In sheep, the disease is predominately characterized by central nervous system (CNS) signs and lesions, as was observed in this case. In the gastrointestinal tract, there is peritoneal hemorrhage, mucosal congestion of the intestines, superficial desquamation of the intestinal epithelium, and numerous bacilli in the intestinal contents. In goats, the disease primarily occurs in the gastrointestinal tract, and CNS signs and lesions occur less frequently than in sheep. Additionally, lesions in the gastrointestinal tract of goats more commonly affect the colon, rather than the small intestine.[4,7,8,9]

There was some variation between slides with neutrophils present in some sections.

Contributor: California Animal Health and Food Safety Laboratory, San Bernardino Branch, School of Veterinary Medicine, UC Davis, http://cahfs.ucdavis.edu

References:

1. Buxton D, Morgan KT: Studies of the lesions produced in the brains of colostrum deprived lambs by *Clostridium welchii* (*Clostridium perfringens*) type D toxin. J Comp Path 86:435-447, 1976
2. Buxton D, Linklater KA, Dyson DA: Pulpy kidney disease and its diagnosis by histological examination. Vet Rec 102:241, 1978
3. Hartley WJ: A focal symmetrical encephalomalacia of lambs. N Z Vet J 4:129-135, 1956
4. Lewis CJ: Clostridial diseases. In: Diseases of sheep, eds. Martin WB, Aitken ID, 3rd ed., pp. 131-143. Blackwell Science Ltd, Malden, Massachusetts, 2000
5. Uzal FA, Kelly WR, Morris WE, Bermudez J, Bias_n M: The pathology of peracute experimental *Clostridium perfringens* type D enterotoxemia in sheep. J Vet Diagn Invest 16:403-411, 2004
6. Uzal FA: Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. Anaerobe 10:135-143, 2004
7. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, pp. 269-270. Mosby-Year Book Inc., St. Louis, Missouri, 1995
8. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 422. Williams & Wilkins, Baltimore, Maryland, 1997
9. Barker IK, Van Dreumel AA, Palmer N: The alimentary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 241-244. Academic Press, Inc., San Diego, California, 1993

10. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 917-918. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 76
CONFERENCE 19 / CASE IV – 06-0111 (AFIP 3028791)

Signalment: Adult male vasectomized common marmoset (*Callithrix jacchus*) that was pair housed with a female marmoset

History: The marmoset had its tail bitten by another marmoset several weeks ago followed by surgical amputation. Started on IM enrofloxacin on 21 March 06. On the morning of 22 March 06, animal presented as weak with opisthotonos & hypothermia & was given SQ saline & IV cefazolin. The monkey developed seizures & was subsequently euthanized. Septic shock was suspected.

Gross Pathology: There is adequate body fat & normal hydration status. Both inguinal lymph nodes are enlarged & SQ edema of left inner thigh. The tail is amputated 1/3 from tip & over 1/2 of the remaining tail is red & black. There are sutures at the amputation site. The liver is diffusely pale with an accentuated lobular pattern. Lungs do not collapse & are congested. All other organs – NSGL.

Histopathologic Description: This is a section of tail. There is diffuse to multifocal, depending on the section, epidermal and superficial dermal coagulative necrosis with numerous neutrophils and abundant large colonies of coccobacilli. In some sections, the necrosis also involves the panniculus. There is multifocal edema and often vessels are partially or completely blocked by fibrin thrombi. The neutrophils often extend into the panniculus and underlying skeletal muscle bundles. In some sections, there is scattered myocyte degeneration and necrosis. With the Brown and Hopps staining method (not included), the coccobacilli are Gram-negative.

Contributor's Morphologic Diagnosis: Tail: Necrosis, epidermal and dermal, with edema, fibrin thrombi, moderate suppurative dermatitis, panniculitis, and myositis, and numerous coccobacilli.

Other histopathologic findings included diffuse pulmonary interstitial edema and congestion, diffuse thymic involution, and moderate hepatic hemosiderosis and lipidosis.

Contributor's Comment: The history, gross findings, and histopathologic findings are all consistent with bacterial sepsis as the cause of this marmoset's morbundity. Sepsis may be caused by infection with Gram-negative bacteria and subsequent endotoxin production (lipopolysaccharide or LPS), Gram-positive bacteria, fungi, or viruses.[1] In this case, the most likely cause is Gram-negative bacterial endotoxin.

Sepsis is a generalized inflammatory response that results from triggering of the body's defense mechanisms (cytokine release; neutrophils, monocyte, and endothelial activation; neuroendocrine reflexes; and complement, coagulation, and fibrinolytic system activation) by microbial invasion, extensive tissue injury, or ischemia/reperfusion injury.[1]

The pathogenesis of Gram-negative sepsis involves the innate immune system. Local phagocytic cells (primarily macrophages and neutrophils) recognize microbes via several types of membrane receptors, including different types of Toll-like receptors (TLRs). Specifically, TLR-4 recognizes Gram-negative bacterial LPS. The "lipid A" portion of LPS is recognized by and bound to a circulating acute-phase protein, LPS-binding protein (LPB), which greatly enhances the binding of LPS to the CD14 receptor on the phagocytic cells. Once LPS is bound to CD14, LPB dissociates and the LPS-CD14 complex physically associates with TLR-4 and an extracellular accessory protein, MD2. Ligand binding causes recruitment of cytoplasmic signaling molecules MyD88 (an adaptor protein) and IL-1 Receptor Associated Kinase (IRAK) into the complex. IRAK undergoes autophosphorylation, dissociates from MyD88, and subsequently activates TNF-Receptor Associated Factor-6 (TRAF-6). TRAF-6 then activates the I-_B Kinase cascade, leading to NF-_B transcription factor activation. In some cell types, the MAP Kinase cascade is also activated, leading to AP-1 transcription factor activation. This signal transduction and transcription factor up-regulation results in leukocyte activation, cytokine/mediator synthesis and secretion, and general immune stimulation.[2,3]

The effects of LPS and the secondarily induced effector molecules (especially TNF, IL-1, IL-6, IL-8, IL-12, NO, and PAF) varies depending on the level of LPS and the number of phagocytes activated. At low doses, LPS activates local phagocytes, endothelial cells, and complement to enhance the local acute inflammatory response and improve infection clearance. At moderate levels, more systemic effects occur (fever, hepatic acute-phase protein production), in addition to the local vascular effects. With high levels, septic shock develops, resulting in systemic vasodilation, decreased cardiac output, widespread endothelial injury and activation, and disseminated intravascular coagulopathy (DIC).[4]

AFIP Diagnosis: Tail, transverse section: Dermatitis and cellulitis, neutrophilic and necrotizing, diffuse, moderate, with fibrin, edema, hemorrhage, thrombosis, and myriad intra-epidermal colonies of bacilli, common marmoset (*Callithrix jacchus*), nonhuman primate.

Conference Comment: The contributor provides a concise and thorough overview of the pathogenesis of Gram-negative sepsis.

Gram-positive bacteria can trigger sepsis and septic shock by producing and releasing exotoxins that act as "superantigens" and by the release of cell membrane fragments

(e.g. peptidoglycans, teichoic acids, and lipoteichoic acids). Superantigens are polyclonal T-lymphocyte activators that induce systemic inflammatory cytokine cascades similar to those occurring downstream in septic shock. Superantigens bind to class II MHC molecules and V_ domains of the T-lymphocyte antigen receptor (TCR). This binding occurs outside of the normal antigen binding site and activates all T lymphocytes expressing the same V_ domains irrespective of their antigen specificity resulting in the activation of numerous T lymphocytes and the elaboration of cytokines. Superantigens are subclassified as either exogenous or endogenous. Exogenous superantigens are produced by bacteria and include some enterotoxins, toxic shock syndrome toxin (TSST1), and exfoliating toxin. Endogenous superantigens are specific cell-membrane molecules produced during viral infections.[1,4,5]

Contributor: USAMRICD, Comparative Pathology Branch
http://usamricd.apgea.army.mil/

References:
1. Tsiotou AG, Sakorafas GH, Anagnostopoulos G, Bramis J: Septic shock: current pathogenetic concepts from a clinical perspective. Med Sci Monit 11:76-85, 2005
2. Abbas AK: Diseases of immunity. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 194-205. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
3. Slauson DO, Cooper BJ: Mechanisms of Disease A Textbook of Comparative General Pathology, 3rd ed., pp. 193-195. Mosby, Inc., St. Louis, Missouri, 2002
4. Mitchell RN: Hemodynamic disorders, thromboembolic disease, and shock. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 139-144. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
5. Snyder PW: Diseases of immunity. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 226. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 77
CONFERENCE 20 / CASE I – 04-2782 (AFIP 2984018)

Signalment: Male, Labrador Retriever, 9 yrs, canine

History: The dog had an eleven month history of dermatitis. The footpads were hyperkeratotic, inflamed, and painful with multiple pustules. There was severe hyperpigmentation on the ventral abdomen and an overall greasy haircoat. Multiple areas of crusting, alopecia, and erythema, with multiple bacterial agents were seen by cytology. The infections were resistant to treatment and topical treatment was finally discontinued due to the pain associated with the treatments. Originally he responded to parental amino acid infusions. He was euthanized at the owner's request.

Gross Pathology: The body was in poor post-mortem condition with advanced autolysis and a body condition score of approximately 1.5/5. The skin showed

multifocal, widespread alopecia with erosion, ulceration, greasy covering and crust formation. In the lungs mild, acute diffuse, alveolar edema and emphysema as well as mild, acute congestion was present. The liver was approximately normal size with diffusely dispersed small nodules (approximately 0.5x0.5x0.5 cm to 1.0x1.0x1.0 cm) and of firm consistency. In the cortices of both kidneys a mild, grey, radial striation was present.

Laboratory Results: Multiple skin cultures revealing *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus/intermedius, Beta streptococcus* and others.

Significant lab data included: ALT 188 IU/L (reference range 10-55), ALP 307 IU/L (reference range 15-120), Bile Acids 98 Umol/L (reference range 5-20)

Histopathologic Description: The specimen is lip. The skin surface has diffuse marked acanthosis, parakeratotic hyperkeratosis and hydropic degeneration of the stratum spinosum with focal epidermal ulceration, suppuration and intralesional coccoid bacteria. Also in the skin, there is moderate to marked widespread pigmentary incontinence, mild dermal edema, mild dermal fibrosis, and mild superficial perivascular lymphoplasmacytic dermatitis. The stratum basale has marked hyperplasia and mild interstitial edema with occasional apoptotic cells. The mucosa and submucosa of the lip have similar changes (minus the hyperkeratosis) but they are less severe.

Contributor's Morphologic Diagnosis: Skin of Lip: Diffuse marked acanthosis, parakeratotic hyperkeratosis and hydropic degeneration of the stratum spinosum with focal epidermal ulceration and suppuration with intralesional coccoid bacteria.

Contributor's Comment: The histopathologic lesions in the skin are characteristic of hepatocutaneous syndrome. Hepatocutaneous syndrome is a generalized metabolic disorder that results in very characteristic "red, white and blue" skin lesions histopathologically formed by a hyperplastic basal cell layer, intracellular edema and necrosis of keratinocytes in the stratum spinosum, and marked parakeratotic hyperkeratosis.[1-5] In addition, there may be macules and papules as well as melanin incontinence.[1,3,5] These cases are presented clinically for crusty skin lesions on footpads, mucocutaneous junctions, ears, periorbital region and pressure points.[1-5] Pruritis may or may not be present as the result of secondary bacterial infections.[1-5] Anorexia, weight loss and lethargy may also be present.[1-5]

The underlying cause of the skin lesions in canines is most often idiopathic hepatopathy, although a similar disease in humans is most often the result of a glucagon-secreting tumor.[1-5] In most cases of hepatocutaneous syndrome the liver is small with nodular lesions surrounded by the collapse of the adjacent parenchyma.[1-5] The liver lesions in this case were characterized by periportal bridging fibrosis with biliary hyperplasia causing pseudolobulation of the liver. The pathogenesis of the skin lesions is unknown but may be related to hepatic dysfunction leading to elevated glucagon levels (due to decreased hepatic metabolism), decreased levels of amino acids (from increased gluconeogenesis), or disturbance of zinc metabolism (possibly a

result of malabsorption).[1-5] Administration of amino acid supplements, high-quality protein diets or zinc may be helpful, however long-term prognosis is poor.[1,2,4,5]

AFIP Diagnosis: Mucocutaneous junction, lip: Hyperkeratosis, parakeratotic, diffuse, marked, with acanthosis, edema of the stratum spinosum, basal cell hyperplasia, pigmentary incontinence, moderate diffuse lymphoplasmacytic dermatitis, focal ulcer with pyogranulomatous inflammation, and surface bacteria, Labrador Retriever (*Canis familiaris*), canine.

Conference Comment: The contributor provides a concise summary of hepatocutaneous syndrome in the dog to include typical gross and histopathologic findings as well as potential causes and associated poor prognosis. Synonyms for the syndrome include metabolic epidermal necrosis (MEN), superficial necrolytic dermatitis (SND), and necrolytic migratory erythema (NME) in humans.[1]

As pointed out by the contributor, most cases of hepatocutaneous syndrome in the dog are associated with a hepatopathy. Hepatocutaneous syndrome can also be associated with diabetes mellitus and, less commonly, with a glucagon secreting tumor. The most common clinical dermatologic lesion is severe hyperkeratosis and deep fissuring of the footpads. The typical light microscopic finding in the liver is severe vacuolar degeneration with diffuse parenchymal collapse, condensation of reticulin, and nodular regeneration. Abdominal ultrasound of the liver reveals the unique and pathognomonic "honeycomb" pattern seen with SND.[1,2,3,7]

Hepatocutaneous syndrome has also been reported in cats and the black rhinoceros. Of the few cases of hepatocutaneous syndrome reported in cats, one was associated with pancreatic carcinoma, another with thymic amyloidosis, and four cases were associated with hepatopathy. Hepatocutaneous syndrome occurs in up to 50% of captive black rhinoceroses and is not associated with underlying metabolic disease. Interestingly, in the black rhinoceros, stressful events and/or dietary insufficiency leading to disruption of metabolic homeostasis is suspected as the basis for these skin lesions.[1,4,6,7]

The differential diagnosis includes other parakeratotic diseases such as zinc-responsive dermatosis, thallium toxicosis, lethal acrodermatitis of Bull Terriers, *Sarcoptes scabei*, and generic dog food dermatosis. The clinical differential diagnosis includes pemphigus foliaceous, demodicosis, dermatophytosis, bacterial folliculitis, toxic epidermal necrolysis, systemic lupus erythematous, and contact-irritant dermatitis. Most of these can be ruled out by appropriate historical, physical examination, clinical laboratory, and histopathologic findings.[1]

Contributor: http://vet.osu.edu/biosciences.htm

References:

1. Byrne KP: Metabolic epidermal necrosis-hepatocutaneous syndrome. Vet Clin North Am:Sm Anim Pract 29(6):1337-1335, 1999
2. Ghisleni G, Nageli-Schenker C, Caniatti M: Symmetrical crusting rash in a dog. Vet Clin Pathol 32(1):19-21, 2003
3. Jacobson LS, Kirberger RM, Nesbit JW: Hepatic ultrasonography and pathological findings in dogs with hepatocutaneous syndrome: new concepts. J Vet Intern Med 9(6):399-404, 1995
4. Kimmel SE, Christiansen W, Byrne KP: Clinicopathological, ultrasonographic, and histopathologic findings of superficial necrolytic dermatitis with hepatopathy in a cat. J Am Anim Hosp Assoc 39:23-27, 2003. Available at http://www.jaaha.org.proxy.lib.ohio-state.edu
5. Turek MM: Cutaneous paraneoplastic syndromes in dogs and cats: a review of the literature. Vet Dermatol 14(6):279-296, 2003. Available at http://www.blackwell-synergy.com
6. Munson L, Koehler JW, Wilkinson JE, Miller RE: Vesicular and ulcerative dermatopathy resembling superficial necrolytic dermatitis in captive black rhinoceroses (*Diceros bicornis*). Vet Pathol 35:31-42, 1998
7. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp. 86-91. Blackwell Publishing Professional, Ames, Iowa, 2005

SLIDE 78
CONFERENCE 20 / CASE II – 05.16743 (AFIP 3026815)

Signalment: 10-year-old, spayed female, mixed breed dog, *Canis familiaris*

History: The animal presented with a 3-4 week history of abnormal mentation, circling to the right, inappropriate urination/defecation, lethargy and weight loss. On neurological exam, the animal exhibited difficulty navigating in low light intensity and had decreased pupillary light reflexes.

Gross Pathology: A large, yellow-grey, moderately firm to soft, expansile, well demarcated mass measuring 1.6x 2x 3.3 cm is present within the third ventricle with extension into both the mesencephalic aqueduct and the fourth ventricle. Multifocally, small red-brown areas of hemorrhage are also noticed within the mass. The mass extended dorso-laterally into the adjacent neural parenchyma with compression and partial effacement of the corpus callosum, thalamus and midbrain. There is mild bilateral dilation (hydrocephalus) of the lateral ventricles.

Laboratory Results: Magnetic resonance imaging (MRI) of the brain revealed a large, irregular, well demarcated, midline contrast-enhancing mass arising from the third ventricle with mass effect on the surrounding parenchyma and bilateral ventriculomegaly.

Histopathologic Description: Depending upon the section examined, the unencapsulated intraventricular neoplasm is mostly well demarcated, expands the third ventricle, mesencephalic aqueduct and fourth ventricle and occasionally extends into adjacent neural parenchyma. The neoplasm is moderately to densely cellular and well vascularized. Neoplastic cells are arranged in sheets, clusters or fascicles and are moderately pleomorphic with indistinct cell borders and fibrillar eosinophilic cytoplasm. Nuclei are round to oval, with a hyperchromatic to coarsely granular chromatic pattern and prominent nucleolus in some. Mitotic figures are 2 per 10 40X objective fields. Pseudorosettes are common, as are foci of necrosis with peripheral palisades of neoplastic cells. Acute and chronic hemorrhage and perivascular aggregates of lymphocytes are also present.

Contributor's Morphologic Diagnosis: Brain: Ependymoma

Contributor's Comment: On the basis of histological features of prominent perivascular pseudorosettes, ependymoma, papillary meningioma and paraganglioma were considered as differential diagnoses. Immunohistochemical stains were performed for Vimentin, Pankeratin, GFAP and S100 (TUFTS-New England Medical Center). The neoplastic cells were negative for Pankeratin, showed mild variable cytoplasmic positive staining for S100 and diffuse, intense cytoplasmic staining for Vimentin. There was mild to moderate, variable (involving one-third of the mass) cytoplasmic positive staining for GFAP. The features of the neoplasm were most consistent with anaplastic ependymoma.

Ependymoma is an uncommon neoplasm originating from the ependymal lining of the ventricles of the brain and central canal of the spinal cord. In animals, intracranial ependymomas involving the lateral ventricles and less commonly the third and fourth ventricles have been reported in rats, cats, dogs, non-human primates, cattle, horses, deer, and fish. Rare cases of intraspinal ependymoma have also been described in animals.[2,3,4] In humans, this neoplasm is more common in the spinal canal and fourth ventricle rather than the lateral ventricles. Rare instances of ectopic ependymomas involving the presacral or postsacral soft tissue as well as intracranial tumors far removed from the ventricular system are documented in humans.[1] These tumors are usually slow-growing, can be well demarcated and confined within the ventricular system or in anaplastic variants can be highly infiltrative into the surrounding parenchyma and metastasize via the cerebrospinal fluid. Additionally, these tumors often cause concomitant compression of the adjacent neuropil and secondary hydrocephalus. Histologically, these tumors are characterized by distinct ependymal rosettes and perivascular pseudorossettes. Malignant forms exhibit marked necrosis, hemorrhage, focal infiltrative growth, together with moderate cellular atypia and marked mitotic activity. Ultrastructurally, ependymal cells have intercellular tight junctions or microvilli that project into the lumen or interdigitate between cells. Some of the ependymal cells may be ciliated and contain cytoskeletal ciliary basal bodies (blepharoplasts). In some tumors, blepharoplasts can be visualized with phosphotungstic acid hematoxylin (PTAH). Immunohistochemically, ependymomas are variably stained for vimentin, GFAP, and cytokeratin. Canine ependymomas are

frequently GFAP negative whereas the neoplasm in humans, cats and horses usually exhibits positive staining for GFAP. Mild to moderate positive staining is noted with vimentin and cytokeratin in all species.[1,2,3,4]

AFIP Diagnosis: Brain, at the level of the hippocampus: Ependymoma, mixed breed (*Canis familiaris*), canine.

Conference Comment: The contributor provides a thorough overview of ependymomas to include behavior, histomorphologic features, and ultrastructural features. Grossly, ependymomas are usually large expansile intraventricular masses with generally well-demarcated margins. The neoplasm is gray to red, if hemorrhagic. More aggressive tumors infiltrate into normal tissue at its margins. As pointed out by the contributor, secondary obstructive hydrocephalus is common. Canine ependymomas have a smooth texture on cut surface while feline ependymomas are more granular.[3,5,6,7]

The differential diagnosis considered included astrocytoma, primitive neuroectodermal tumor, papillary meningioma, choroid plexus tumor and paraganglioma. Astrocytomas usually blend with the adjacent neuropil and do not form rosettes or pseudorosettes. Primitive neuroectodermal tumors are composed of small, primitive-appearing, round to carrot-shaped cells and usually have a high mitotic index. This neoplasm was not papillary, ruling out papillary meningioma. Choroid plexus tumors stain positively for cytokeratin and have a fibrovascular stroma verses the GFAP-positive glial processes that abut the vasculature in ependymomas. Paragangliomas display neuroendocrine packeting, which was not present in this case.[3,7]

Contributor: Cummings School of Veterinary Medicine at Tufts University, Section of Pathology, http://vet.tufts.edu/

References:

1. Burger PC, Scheithauer BW: Tumors of the Central Nervous System, 3rd series, fascicle 10, pp. 120-133. Armed Forces Institute of Pathology, Washington, D.C., 1994
2. Carrigan MJ, Higgins RJ, Carlson GP, Naydan DK: Equine papillary ependymoma**.** Vet Pathol 33:77-80, 1996
3. Koestner A, Higgins RJ: Tumors in Domestic animals, ed. Meuten DJ, 4th ed., pp. 707-709. Iowa State Press, Ames, Iowa, 2002
4. Michimae Y, Morita T, Sunagawa Y, Sawada M, Okamoto Y, Shimada A: Anaplastic ependymoma in the spinal cord of a maltese dog. J Vet Med Sci 66(9): 1155-1158 2004
5. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, p. 375. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
6. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 950. Mosby Elsevier, St. Louis, Missouri, 2007
7. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Histological Classification of Tumors of the Nervous System of Domestic Animals, 2nd

series, vol. V, pp. 17-19, 22, 25. Armed Forces Institute of Pathology, Washington, D.C., 1999

SLIDE 79
CONFERENCE 20 / CASE III – 05N658C (AFIP 3026835)
Signalment: 12-year-old male Avian Sulphur-crested Cockatoo (*Cacatua galerita*)

History: Not acting normal the last few days, staying "fluffed". Died suddenly this a.m.

Gross Pathology: A 12-year-old male cockatoo weighs 553.8 g and is in good nutritional body condition. There is edematous fluid within the subcutis over the ventral coelomic cavity and within the coelom (approximately 3 mls of clear fluid). A small amount of clear fluid is also within the pericardial sac and the lungs are edematous. There is blunting of the choanal papillae. The spleen is markedly enlarged (approximately 1 cm in diameter).

Histopathologic Description: Sections of brain, lung, intestines, liver, kidney, proventriculus, pancreas, small intestines, crop, heart, spleen, skeletal muscle, testis, adrenal gland and ventriculus are examined.

In sections of lung, tertiary or parabronchi as well as air capillaries contain homogenous eosinophilic material (serofibrinous exudate). Air capillary walls are markedly thickened and there is multifocal necrosis. Frequently groups of intracellular organisms, either banana shaped merozoites or small aggregates forming cysts, are in air capillary endothelial cells throughout the parenchyma. Mild perivascular infiltrates of lymphocytes and plasma cells are also noted.
In liver sections there is a moderate increase in lymphocytes and plasma cells throughout sinusoids. Kupffer cells are also increased and usually contain phagocytized cellular debris. There is mild scattered hepatocellular degeneration.

In the spleen there is a marked increase in lymphocytes and plasma cells throughout the parenchyma and numerous histiocytes contain phagocytized necrotic cellular debris. There is also widespread necrosis with karyorrhexis.
Significant lesions are not observed in other tissues examined.

Contributor's Morphologic Diagnoses: 1. Lung, interstitial pneumonia with serofibrinous exudation and intracellular protozoal merozoites
2. Liver, moderate, diffuse, lymphoplasmacytic hepatitis
3. Spleen, moderate, diffuse, lymphoplasmacytic and histiocytic splenitis with necrosis

Contributor's Comment: Pulmonary Sarcocystosis is a hyperacute disease and birds are usually found dead without any prior clinical signs. Due to the acute nature of the disease, affected birds are usually in good nutritional body condition. The most consistent gross lesion on necropsy is pulmonary edema. Liver and spleen can also be

enlarged. Microscopically, lungs are congested and there is fibrin deposition, edema and hemorrhage. Lymphocytes and plasma cells accumulate around blood vessels and bronchi. Aggregates of organisms (small elliptical or crescent shaped) are in pulmonary endothelial cells.[1] Often organisms conform to the shape of the vessel. Light microscopy alone cannot accurately identify the organism. Differentials include *Neospora* and *Toxoplasma*. Immunohistochemistry and electron microscopy can be used to establish a specific disease agent. A definitive diagnosis of *Sarcocystis falcatula*-like pneumonia is not established in this case but that is considered the most likely based on lesions seen. Recent work suggests that substrains of *S. falcatula* may exist.[2,3]

Sarcocystis spp. are obligate two-life cycle coccidia. In North America the Virginia opossum (*Didelphis virginiana*) is listed as the definitive host. Intermediate hosts include various orders of birds. Old World psittacines develop more severe disease than New World psittacines and this is thought to be due to possible co-habiting with the definitive host.

Sarcocysts are ingested by the definitive host. Sexual reproduction occurs and infective sporulated sporocysts are excreted. The intermediate host ingests sporocysts and sporozoites are released and invade the host's gut. Asexual reproduction occurs in endothelial cells and parenchymal cells in various organs. Merozoites are produced which form sarcocysts in skeletal muscle.[4]

AFIP Diagnosis: Lung: Pneumonia, interstitial, histiocytic and plasmacytic, diffuse, mild to moderate, with edema, necrosis, and intraendothelial sarcocysts, Sulphur-crested Cockatoo (*Cacatua galerita*), avian.

Conference Comment: The contributor provides an excellent overview of pulmonary sarcocystosis. Although sarcocysts also occur in skeletal muscle and the heart, the primary target in birds is the lung. Affected birds die acutely, although the presence of lymphocytes and plasma cells surrounding blood vessels and bronchi suggests the infection is subacute. As pointed out by the contributor, the opossum is the definitive host.[1,2]

While *T. gondii* should be considered, *S. falcatula* schizonts, unlike those of *T. gondii*, are located in endothelial cells and follow the shape of capillaries appearing tortuous with high variability in their size as seen in this case. Additionally, toxoplasmosis occurs infrequently in birds, is generally associated with more necrosis than seen in this case, has intrahistiocytic zoites, and has a wider tissue tropism. Ultrastructurally, *Sarcocystis* spp., unlike *T. gondii*, are not located within a parasitophorous vacuole and merozoites lack rhoptries.

Atoxoplasma sp., another protozoa that infects birds, is very difficult to see on H&E stained sections. Tiny merozoite-like stages of *Atoxoplasma* sp. have been found in the

intestine and other tissues of birds. Some appear to be intracellular and others extracellular.

Natural infection with *Neospora caninum* has not been reported in birds.[2,5]

This case was reviewed in consultation with the AFIP Department of Infectious Diseases and Dr. Chris Gardiner, AFIP consultant in veterinary parasitology.

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References:
1. Schmidt RE, Reavill DR, Phalen DN: Pathology of Pet and Aviary Birds, pp.29-30. Iowa State Press, Ames, Iowa, 2003
2. Dubey JP, Garner MM, Stetter MD, Marsh AE, Barr BC: Acute *Sarcocystis falcatula*-like infection in a carmine bee-eater (*Merops nubicus*) and immunohistochemical cross reactivity between *Sarcocystis falcatula* and *Sarcocystis neurona*. J Parasitol 87:824-832, 2001
3. Suedmeyer WK, Bermudez AJ, Barr BC, Marsh AE: Acute pulmonary *Sarcocystis falcatula*-like infection in three Victoria Crowned Pigeons (*Goura victoria*) housed indoors. J Zoo Wildl Med 32:252-256, 2001
4. Smith JH, Meier JL, Neill PJG, Box ED: Pathogenesis of *Sarcocystis falcatula* in the Budgerigar II. Pulmonary Pathology. Lab Invest 56:72-84, 1987
5. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites in Animal Tissues, 2nd ed., p. 34. Armed Forces Institute of Pathology, Washington, D.C., 1998

SLIDE 80
CONFERENCE 20 / CASE IV – X4233 AVC Pathology (AFIP 2943289)

Signalment: Adult female moose (*Alces alces*)

History: On February 14, 2003, in western Newfoundland, Canada, this free-ranging, wild moose was seen dragging a hind leg through the snow and was not very alert. The investigating conservation officer euthanized the animal with a gunshot behind its ears and submitted its head to the provincial Animal Health Division for examination.

Gross Pathology: The entire brain is received for histological examination. A relatively well demarcated, expansile, oval mass (4.6 cm rostral-caudal dimension; 3.2 cm dorsal-ventral dimension; and 4.2 cm lateral dimension) is replacing much of the diencephalon, primarily centered on the thalamic region. The mass extends across the interthalamic adhesion to affect both sides of the brain. Bilaterally, the rostral and dorsal aspects of the mass are compressing the interventricular foramen; the rostral aspect of the mass is compressing the third ventricle; and the ventral aspect of the

mass is obliterating the mesencephalic aqueduct, restricting and preventing the normal flow of cerebrospinal fluid. The result is a moderate increase in accumulation of cerebrospinal fluid, dilating the lateral ventricles (i.e., moderate hydrocephalus). There is also mild compression atrophy of the cerebrum overlying the lateral ventricles. Irregular areas of necrosis are present within the mass.

Laboratory Results: Immunohistochemistry:

1. Glial Fibrillary Acid Protein (Avidin biotin complex – peroxidase) – the cytoplasm of neoplastic cells stains strongly for Glial Fibrillary Acid Protein.
2. Vimentin (Avidin biotin complex – peroxidase) – the cytoplasm of neoplastic cells stains moderately for vimentin.

The immunohistochemistry results support the diagnosis that the neoplastic cells are of astrocyte origin.

Histopathologic Description: In this section of brainstem, a nonencapsulated, well delineated thalamic mass is compressing the adjacent surrounding neuropil. The mass consists of sheets of round cells supported by a delicate fibrovascular stroma. The cells have variably well defined cell borders; a small to moderate amount of finely granular, acidophilic cytoplasm that in some cells contains large discrete clear vacuoles; and a single round or oval or indented nucleus with three to five fold anisokaryosis, a finely granular chromatin pattern and multiple prominent nucleoli. A few binucleate and trinucleate cells are admixed with other cells. Mitotic figures are very common (at least one mitosis and often multiple mitoses are observed in a single random high power field). Several areas of necrosis are randomly distributed throughout the mass. Microhemorrhages and areas of locally extensive hemorrhage are also randomly scattered throughout the mass (this finding is likely related to the gunshot trauma associated with euthanasia). There is artefactual vacuolization of the adjacent unaffected neuropil due to post mortem autolysis.

Contributor's Morphologic Diagnosis: Astrocytoma, high-grade (glioblastoma multiforme) with secondary moderate hydrocephalus

Contributor's Comment: The tumor is diagnosed as an astrocytoma based on criteria previously described in the literature.[1,2] These include the anatomical location of the tumor (i.e., thalamic region), the morphology of the neoplastic cells, and the immunohistochemical detection of glial fibrillary acid protein (GFAP) and vimentin in the neoplastic cells' cytoplasm. Several classification schemes have been proposed for astrocytomas.[1,2,3] The astrocytoma in this case is considered high-grade because it is characterized by features of anaplasia such as increased cellularity, cellular pleomorphism, nuclear atypia, high mitotic index and necrosis. However, the additional features of vascular proliferation and pseudopalisading of neoplastic cells around necrotic areas described in some high-grade astrocytomas are absent in this tumor.

Astrocytomas are among the most common primary central nervous system tumors in aged dogs and cats, but they are rare in other domestic animal species.[1,2,3] These neoplasms have been reported in free-ranging Cervidae, including white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*).[4,5,6] However, the literature is sparse on cases of neoplasia in wild moose, and, to our knowledge, this is the first report of a primary brain tumor identified in this species.

AFIP Diagnosis: Brain: Astrocytoma, high-grade (glioblastoma multiforme), moose (*Alces alces*), artiodactyl.

Conference Comment: Glioblastoma multiforme or high-grade astrocytoma is the most malignant variant of astrocytoma characterized by anaplastic features, vascular proliferation and/or necrosis. In the dog, brachycephalic breeds are predisposed, in particular, the Boxer and Boston Terrier.[2,7]

Grossly, high-grade astrocytomas affect the convexities of the cerebral hemispheres, the temporal and piriform lobes, the thalamus-hypothalamus, the midbrain, and less commonly, the cerebellum and spinal cord. They are often well-demarcated, gray-white, with variable amounts of necrosis and hemorrhage. Edema of the surrounding neuropil is often present.[1,2,7]

Typical light microscopic findings include a hypercellular mass of small, round, fusiform to anaplastic cells with hyperchromatic nuclei, glomeruloid vascular proliferation, pseudopalisading of neoplastic cells around necrotic areas, scattered multinucleate cells, and mitoses. Areas of well-differentiated astrocytoma are often present within high-grade astrocytomas. A rare giant cell variant consists predominantly of bizarre giant cells and multinucleated cells. High-grade astrocytomas are variably immunoreactive for GFAP, vimentin, and cytokeratin.[1,2]

The differential diagnosis includes low-grade (well-differentiated) astrocytoma, medium-grade astrocytoma, gliomatosis cerebri, anaplastic oligodendroglioma, mixed gliomas (oligoastrocytoma), and primitive neuroectodermal tumor (PNET). The lower grade astrocytomas do not have necrosis. Gliomatosis cerebri is a diffuse glioma composed of elongate neoplastic cells that infiltrate diffusely but do not form a discrete mass. Because of postmortem autolysis and vacuolization, some conference participants also considered oligodendroglioma. Oligodendrogliomas are composed of neoplastic oligodendroglial cells with central, uniform, round nuclei and a halo of clear to lightly stained cytoplasm and have a background of branching capillaries forming a delicate “chicken-wire” pattern. Mixed gliomas (oligoastrocytomas) are a mixture of neoplastic astrocytes and oligodendrocytes and are not diffusely GFAP-positive. Primitive neuroectodermal tumors are composed of densely packed round to polygonal cells with often hyperchromatic carrot-shaped nuclei, rosettes, and pseudorosettes.[1,2,3]

Although glomeruloid vascular proliferation and palisading of neoplastic cells around areas of necrosis were not seen in this case, necrosis was present and is consistent with high-grade astrocytoma.

This case was reviewed in consultation with the AFIP Department of Neuropathology.

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References:
1. Koestner A, Higgins RJ: Tumors of the nervous system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 699–703. Iowa State Press, Ames, Iowa, 2002
2. Summers BA, Cummings JF, de Lahunta A: Veterinary neuropathology, pp. 362-370. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
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, 2nd series, vol. V, pp. 17-21, 25. Armed Forces Institute of Pathology, Washington, D.C., 1999
4. Holscher MA, Page DL, Netsky MG, Powell HS: Mixed glioma and rhabdomyosarcoma in brain of a wild deer. Vet Pathol 14:643-647, 1977
5. Howard DR, Krehbiel JD, Fay LD, Stuht JN, Whitenack DL: Visual defects in white-tailed deer from Michigan: six case reports. J Wildl Dis 12:143-147, 1976
6. Snyder SP, Davies RB, Stevens D: Brain tumors in two free-ranging elk in Colorado. J Wildl Dis 17(1):101-104, 1981
7. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p.949. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 81
CONFERENCE 21 / CASE I – C31715-04 (AFIP 2988635)

Signalment: 3-year-old, male, castrated, Cocker Spaniel, *Canis domesticus*, dog

History: The dog was presented to the referring veterinarian with a 3 week history of coughing. The dog was placed on Amoxil and prednisone. Two days later the dog began vomiting. Thoracic radiographs were performed at this time and revealed what was interpreted as peri-hilar pulmonary edema and right atrial enlargement. The dog was then treated with furosemide. Prednisone was discontinued after 4 days of treatment due to the development of polyuria and polydipsia. The dog then began seizuring, became very dehydrated and was referred to the Atlantic Veterinary College 6 days after initial presentation. On arrival, the dog was lethargic, dehydrated and a freely moveable, mid-abdominal mass was palpated. The dog was rehydrated with IV lactated ringers. Exploratory laparotomy revealed a 5 cm long segment of proximal jejunum where the intestinal wall was firm, pale yellow-tan and approximately 8-18 mm

thick. There was a poorly defined approximately 3 cm in greatest diameter, mottled tan and red, firm, thickened area in the adjacent mesentery. The nearby lleocecal lymph node was moderately enlarged measuring 6 cm in greatest diameter. These tissues were surgically excised and submitted for histopathology. The dog never completely recovered from anesthesia and remained depressed with no papillary light reflex, no menace response, and was unable to stand or walk. The owners elected euthanasia 2 days later and the dog was submitted for necropsy.

Gross Pathology: The dog was in fair body condition with small visceral and subcutaneous fat stores. The lungs were diffusely dark red, rubbery, failed to collapse, and contained many scattered, poorly-defined, pale, whitish, firm foci. There were two, 5 mm in greatest diameter, slightly raised, discrete, pale, yellow nodules visible on the epicardial surface of the right atrium. The right ventricular free wall was slightly thickened and measured 5 mm thick (compared to the left which was 1 cm thick). The site of tissue resection and skin incisions were unremarkable. Otherwise, the carcass, including the nasal passages, was grossly unremarkable.

Laboratory Results: Fungal culture of lung tissue contained a heavy growth of yeast identified as *Cryptococcus neoformans*.

Histopathologic Description: The pulmonary parenchyma contains numerous, multifocal to coalescing, nodular, interstitial aggregates of large, epithelioid macrophages interspersed with fewer lymphocytes, plasma cells, rare multinucleated foreign body type giant cells and large numbers of uninucleate yeasts. The latter organisms have 2.5-8 µm, pale, eosinophilic to slightly blue-grey, round to oval, thin-walled yeast bodies surrounded by a thick clear capsule. Mucicarmine staining reveals a thick, intensely carminophilic capsule which often has a slightly spiny outer surface surrounding the cell body of these organisms.

The thickened segment of small intestine is characterized by similar moderate inflammatory infiltrates consisting largely of epithelioid macrophages and myriads of the previously described yeasts. These foci extend in to the surrounding mesentery and largely effaced the enlarged ileocecal lymph node. The leptomeninges of the cerebrum, cerebellum and brain stem are multifocally, mildly to moderately, thickened due to similar granulomatous infiltrates accompanied by numerous yeasts. Within the cerebral cortex and the hippocampus there are patchy, poorly-defined areas where the parenchyma is mildly hypercellular, mildly vacuolated and sometimes congested. Blood vessels within these areas are often lined by plump, reactive endothelial cells. Also in these areas, neuronal cell bodies are frequently hypereosinophilic, angular, shrunken and often have pyknotic nuclei.

Multifocal, variably-sized nodular discrete granulomatous foci accompanied with numerous yeasts are also present in sections of the pancreas, spleen, cortex of the right kidney and the wall of the right atrium. In the liver, similar infiltrates often moderately to markedly expand most portal areas and are frequently scattered randomly throughout the lobules. Smaller granulomatous foci containing yeasts are

also scattered within the myocardium of the left and right ventricular free walls and the thyroid glands.

Contributor's Morphologic Diagnoses: 1. Granulomatous interstitial pneumonia, multifocal, severe, with myriads of intralesional yeast
2. Granulomatous transmural enteritis and lymphadenitis, locally extensive, severe, with many intralesional yeast
3. Granulomatous meningitis, multifocal, moderate, with intralesional yeasts
4. Neuronal necrosis, multifocal, moderate, acute, cerebral cortex and hippocampus
5. Granulomatous splenitis, hepatitis, thyroiditis, pancreatitis, myocarditis and nephritis, multifocal, mild to moderate, with numerous intralesional yeasts

Contributor's Comment: The postmortem findings in this case are consistent with widely disseminated cryptococcosis. *Cryptococcus neoformans* occurs worldwide in temperate, as well as tropical climates.[1] This opportunistic pathogen infects a wide variety of wild and domestic animals (most commonly cats and dogs), as well as humans. The organism is saprophytic in soil and is environmentally often associated with avian habitats or areas heavily contaminated with pigeon droppings.[2] Infection occurs most commonly via inhalation of yeasts from the environment and is not considered contagious. Nasal and/or pulmonary infection may become disseminated via direct extension and/or hematogenous routes.[3] Recovery or localization of infection is dependent on a good cell mediated immune response. Most cases of disseminated human cryptococcosis are associated with concurrent immunosuppressive disease processes (e.g. AIDS) or treatments (e.g. chemotherapy or prolonged use of glucocorticoids).[4] However, immunosuppression has not been documented in most affected cats and dogs. There was no evidence of an underlying immunosuppressive disease process nor was there a history of prolonged steroid use in this case.

The cause of coughing (the initial presenting complaint) in this dog was severe, fungal pneumonia. The right atrial enlargement noted by the referring veterinarian and mild right ventricular hypertrophy found at necropsy were likely secondary to pulmonary hypertension due to severe pneumonia. The initial seizure activity described was due to fungal meningitis (*C. neoformans* is often referred to as neurotropic). The lack of full recovery from anesthesia and postsurgical neurological clinical signs which necessitated euthanasia were due to more acute areas of neuronal necrosis in the cerebral cortex and hippocampus. This finding may have been due to hypoxia secondary to prolonged seizure activity or possibly was associated with poor oxygen exchange through the abnormal lung during surgery.

AFIP Diagnosis: Lung: Pneumonia, granulomatous, multifocal to coalescing, moderate, with edema and myriad intralesional yeasts, Cocker Spaniel (*Canis familiaris*), canine.

Conference Comment: *Cryptococcus neoformans* is a saprophytic fungus that causes disease in a wide variety of animals, but most frequently in cats, dogs, horses and humans. As pointed out by the contributor, immunosuppression has not been documented in most affected cats and dogs. Lesions can occur in any organ, but are most common in the nasal cavity and central nervous system, followed by the integumentary system and eyes. *Cryptococcus neoformans* is a cause of mastitis in cattle. Cryptococcosis is the most frequent systemic mycosis in cats, and often begins in the nasal cavity. Dissemination to brain, eyes, skin, subcutis, and lymph nodes is common. Pulmonary involvement is rare in cats.[2,3,5,6]

C. neoformans is the species that most commonly causes disease and is environmentally associated with avian habitats, especially pigeon droppings. *C. gattii* is also pathogenic, typically occurs in tropical and subtropical climates and is generally associated with bark and leaf litter of certain eucalyptus trees. Following an outbreak of cryptococcosis on Vancouver Island, British Columbia that affected humans, dogs, Dall's porpoises and other mammals, it was found that *C. gattii* was the cause and was present on the bark of non-eucalypts in the area including alder, bitter cherry, cedar, Douglas fir and Garry oak. Changing climatic conditions, possibly caused by global warming, may have been involved in the spread of *C. gattii* to new ecological niches.[6,7]

Gross lesions of *Cryptococcus neoformans* are often gelatinous due to the yeast's mucopolysaccharide capsule. The capsule hinders phagocytosis and is a major diagnostic feature of the organism. However, unencapsulated mutants do exist. Histologically, the yeasts are round, 5-20 µm in diameter, reproduce by narrow-based budding and are usually surrounded by a 2-8 µm mucopolysaccharide capsule that stains with mucicarmine, PAS, and Alcian blue. Masses of organisms surrounded by clear capsules have a "soap bubble" appearance. The immune response varies from sparse to a prominent granulomatous depending on the presence of a capsule and the host's immune status.[2,3,5,6,7]

Other fungi that cause granulomatous pneumonia include *Blastomyces dermatitidis*, *Coccidioides immitis* and *Histoplasma capsulatum*. *Blastomyces*, *Coccidioides*, and *Histoplasma* are unencapsulated, unlike *Cryptococcus*. *Blastomyces* reproduces by broad-based budding, while *Cryptococcus* and *Histoplasma* reproduce by narrow-based budding. *Coccidioides* reproduces by endosporulation. Mature sporangia of *Coccidioides* are 10-80um in diameter with a double-contoured highly refractile wall and are filled with 2-5um diameter endospores. *Histoplasma* is much smaller (5-6um diameter) than *Cryptococcus* and is located intracellularly within macrophages.[5]

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References:

1. Chandler FW, Watts JC: Pathologic Diagnosis of Fungal Infections, pp. 161-175. ASCP Press, Chicago, Illinois, 1987

2. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 667-668. Academic Press, Inc., San Diego, California, 1993
3. Taboada J, Grooters AM: Systemic mycoses. In: Textbook of Veterinary Internal Medicine Diseases of the Dog and Cat, eds. Ettinger SJ, Feldman EC, 6th ed., vol. 1, pp. 671-685. Elsevier Saunders, St. Louis, Missouri, 2005
4. Jacobs GJ, Medleau L: Cryptococcosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd ed., pp. 383-390. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998
5. L_pez A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 543-545, 548. Mosby Elsevier, St. Louis, Missouri, 2007
6. Malik R, Krockenberger M, O'Brien CR, Martin P, Wigney D, Medleau L: Cryptococcosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed., pp. 584-598. Saunders Elsevier, St. Louis, Missouri, 2006
7. Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, MacDougall L, Boekhout T, Kwon-Chung KJ, and Meyer W: A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island, (British Columbia, Canada) Proc Natl Acad Sci USA 101:17258-17263, 2004

SLIDE 82
CONFERENCE 21 / CASE II – P04-4470 (AFIP 2956312)

Signalment: 12-year-old, male neutered, Cocker Spaniel, *Canis familiaris*, pet dog

History: 12-year-old neutered male Cocker Spaniel pet dog weighing 27.4 kg, with signs of diabetes mellitus; hyperglycemia (16.8 mmol/l) and polyuria/polydipsia. There was a firm mass in the caudal part of the pancreas. The dog was also affected by an axillary and inguinal alopecic hyperkeratotic dermatosis. The partially excised pancreas containing the nodular mass was sent in for histological evaluation. No skin biopsies were available.

Gross Pathology: Tissue section approximately 5 x 1 cm, pale, firm, multinodular

Histopathologic Description: Pancreas: The pancreatic parenchyma is partly effaced by a multinodular non-encapsulated partially circumscribed focally infiltrating hypercellular mass consisting of moderately pleomorphic neoplastic cells arranged in small multi-layered trabeculae and islands separated and supported by well vascularized delicate fibrous septa.

The polygonal tumor cells have round to oval vesicular nuclei, which vary moderately in size with finely stippled chromatin containing a single central conspicuous medium-sized nucleolus and regularly displaying mitotic figures (ranging from 2-4 per HPF).

Tumor cells have abundant pale basophilic, finely granular cytoplasm with mainly indistinct cell borders.

Extensive tumorous vascular invasion can be observed within lymphatics and blood vessels in the pre-existing pancreatic hilar stroma and in the extra-pancreatic mesenteric adipose tissues.

Some portions of the tumor display a moderate amount of fibrovascular stroma with extensive central tumorous necrosis and hemorrhage with the presence of iron pigment and/or ceroid bearing macrophages.

The interlobular pancreatic septa are slightly edematous containing congested blood vessels with moderate neutrophilic leucocytosis.

In the intact pancreatic parenchyma, there is diffuse prominent cytoplasmic medio- to macro-vacuolation of the exocrine acinar cells and intercalated ductular cells, and also to a lesser extent affecting the endocrine islet cells. Multifocally there are poorly delineated areas in which the aforementioned cytoplasmic vacuolation is more pronounced with a tendency of loss of acinar detail.

Immunohistochemistry: Tumor cells stain strongly positive for glucagon and show no positivity for insulin, ACTH, gastrin or somatostatin. Pancreatic islets of Langerhans stain slightly positive for insulin.

Contributor's Morphologic Diagnoses: 1. Pancreas: Well-differentiated pancreatic glucagon-producing endocrine carcinoma, (Malignant pancreatic _-cell tumor, malignant glucagonoma), with vascular invasion and dissemination, canine, *Canis familiaris*, Cocker Spaniel.
2. Pancreas: Pancreatic endocrine and exocrine and ductular vacuolar fatty degeneration consistent with paraneoplastic hyperglycemia and diabetes mellitus type II, canine, *Canis familiaris*, Cocker Spaniel.

Contributor's Comment: Several types of pancreatic endocrine tumors in animals and man are recognized. In dogs they include insulinoma (_-cells), glucagonoma (_-cells) and gastrinoma (gastrin-producing non-_-cell tumor). Some islet cell tumors show mixed immunohistochemical reactivity, including somatostatin (_-cells). Additionally, in humans, VIPomas (which produce vasoactive intestinal peptide) are seen. In this case, the tumor cells only showed immunohistochemical positivity for glucagon. The preexisting pancreatic islets showed only faint positivity for insulin (periphery of islets) when compared to the strongly staining positive control of a normal dog. The preexisting pancreatic islets did not stain for glucagon.

This dog was also affected by an axillary and inguinal alopecic hyperkeratotic dermatosis as can be seen in conjunction with liver disease and with pancreatic disease; this is known as hepatocutaneous syndrome or superficial necrolytic dermatitis (SND). SND in canids and in humans (necrolytic migratory erythema (NME)) is a

paraneoplastic syndrome associated with functional pancreatic endocrine tumors that secrete glucagon (glucagonoma) resulting in hyperglycemia. The pathogenesis of SND is still obscure. Persistent gluconeogenesis, due to hyperglucagonemia, is associated with hypoaminoacidemia and seems to be a likely pathogenic factor. In the cat, paraneoplastic flank alopecia is associated with pancreatic neoplasms.

AFIP Diagnoses: 1. Pancreas: Islet cell carcinoma, Cocker Spaniel (*Canis familiaris*), canine.
2. Pancreas, acini, islets, ducts: Vacuolation, cytoplasmic, multifocal, moderate.

Conference Comment: Almost all islet cell tumors in dogs are malignant and generally microscopic metastasis has occurred by the time of surgical diagnosis. Carcinomas of the pancreatic islets commonly occur in the duodenal (right) lobe of the pancreas. Metastasis most commonly occurs to the liver, mesentery, omentum, and regional lymph nodes. In contrast, islet cell tumors in humans and ferrets are usually benign. Islet cell carcinomas tend to be larger than adenomas, invade into and through the fibrous capsule of the pancreas, and are multilobular. Mitotic figures are usually infrequent.[12,13]

Glucagonomas in humans cause the glucagonoma syndrome, which is characterized by necrolytic migratory erythema, glossitis, stomatitis, anemia, weight loss, mild diabetes mellitus, hypoaminoacidemia, deep vein thrombosis and depression. In dogs, glucagonomas have been associated with superficial necrolytic dermatitis, lethargy, anorexia, hyperglycemia and hypoaminoacidemia. The large majority of cases of canine superficial necrolytic dermatitis are associated with severe vacuolar hepatopathy rather than glucagonoma.[13,14,15]
Other neoplasms of islet cell origin which can be differentiated on the basis of clinical signs and/or immunohistochemical stains include insulinoma and gastrinoma. Typical clinical findings associated with insulinoma include marked hypoglycemia, recurrent disorientation or seizures associated with exercise, stress or fasting. Animals recover with administration of glucose. Gastrinomas secrete excess gastrin producing Zollinger-Ellison syndrome, which is characterized by gastric hypersecretion resulting in gastric hyperacidity and gastric and duodenal ulceration.[12, 15]

The cytoplasmic vacuolation of multiple pancreatic cell types in this case was probably caused by hyperglycemia. This finding suggested that the islet cell carcinoma might be a glucagonoma.

Readers are encouraged to review WSC 20 / Case I, 2006-2007 for a summary of superficial necrolytic dermatitis.

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References:

1. Allenspach K, Arnold P, Glaus T, Hauser B, Wolff C, Eberle C, Komminoth P: Glucagon-producing neuroendocrine tumour associated with hypoaminoacidaemia and skin lesions. J Small Anim Pract 41:402-406, 2000
2. Byrne KP: Metabolic epidermal necrosis-hepatocutaneous syndrome. Vet Clin North Am Small Anim Pract 29(6):1337-1355, 1999
3. Fukushima U, Sato M, Okano S, Aramaki Y, Ito H, Yoshioka K, Oyamada T, Uechi M: A case of gastrinoma in a Shih-Tzu Dog. J Vet Med Sci 66:311-313, 2004
4. Gross TL, Song MD, Havel PJ, Ihrke PJ: Superficial necrolytic dermatitis (necrolytic migratory erythema) in dogs. Vet Pathol 30:75-81, 1993
5. Hawkins KL, Summers BA, Kuhajda FP, Smith CA: Immunocytochemistry of normal pancreatic islets and spontaneous islet cell tumors in dogs. Vet Pathol 24:170-179, 1987
6. Kasper CS, McMurry K: Necrolytic migratory erythema without glucagonoma versus canine superficial necrolytic dermatitis: is hepatic impairment a clue to pathogenesis? J Am Acad Dermatol 25:534-541, 1991
7. Turek MM: Invited review cutaneous paraneoplastic syndromes in dogs and cats: a review of the literature. Vet Dermatol 14:279-296, 2003
8. Miller WH, Scott DW, Buerger RG, Shanley KJ, Paradis M, McMurdy MA, Angarano DW: Necrolytic migratory erythema in dogs: a hepatocutaneous syndrome. J Am Anim Hosp Assoc 26:573-581, 1990
9. Ramos-Vara JA, Miller MA, Preziosi D: Glucagonoma in a jaguar (*Panthera onca*) J Zoo Wildl Med 31:563-565, 2000
10. Robben JH, Van Garderen E, Mol JA, Wolfswinkel J, Rijnberk A: Locally produced growth hormone in canine insulinomas. Mol Cell Endocrinol 29:187-195, 2002
11. Solcia E, Klöppel G, Sobin LH: World Health Organization International Histological Classification of Tumours Histological Typing of Endocrine Tumours, 2nd ed., pp. 48-56. Springer-Verlag, Berlin, Germany, 2000
12. Capen CC: Tumors of the endocrine glands. In: Tumors of Domestic Animals, ed. Meuten DJ, 4th ed., pp. 684-690. Iowa State Press, Ames, Iowa, 2002
13. La Perle KMD, Capen CC: Endocrine system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 738-739. Mosby Elsevier, St. Louis, Missouri, 2007
14. Jubb KVF: The pancreas. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 423-424. Academic Press, Inc., San Diego, California, 1993
15. Ward CR, Washabau RJ: Gastrointestinal endocrine disease. In: Textbook of Veterinary Internal Medicine Diseases of the Dog and Cat, eds. Ettinger SJ, Feldman EC, 6th ed., vol. 2, pp. 1627-1631. Elsevier Saunders, St. Louis, Missouri, 2005

SLIDE 83
CONFERENCE 21 / CASE III – N05-238 (AFIP 2984014)

Signalment: 11-year-old, female spayed, domestic short-haired feline (*Felis domesticus*)

History: The patient was an indoor cat which resided in a household of four cats. All four cats began sneezing acutely. By the following evening, the patient was coughing and began open mouth breathing with her neck extended. On the third day, the patient was inappetant, lethargic, and was drooling excessively in addition to the open mouth breathing. The patient was taken to the veterinarian where she was sedated with telazol for radiographs. After sedation, the patient went into extreme respiratory distress and was taken to NCSU-CVM Veterinary Teaching Hospital for emergency treatment. The patient was placed in an oxygen cage for observation and to decrease stress and was noted to be unable to expand her chest (working clinical diagnosis of diaphragm paralysis secondary to the zolazepam). Intubation and hand ventilation was initiated. The zolazepam portion of the telazol was reversed with flumenzanil and the patient regained the ability to move her chest; however, the severe dyspnea was still present. The patient was current on rabies vaccination only. FeLV and FIV status are not known.

Gross Pathology: Within the left cranial and caudal lung lobes and the right cranial and middle lung lobes, there were multifocal, poorly demarcated, dark pink to purple, slightly collapsed, semi-firm to rubbery regions, interpreted as pneumonia. There was a moderate amount of yellow mucoid material within the trachea and large airways. Cytology of the tracheal material revealed moderate numbers of neutrophils and macrophages within a proteinaceous and mucinous background. Involving approximately 50-60% of the mucosal surface of the esophagus, there were numerous multifocal to coalescing, tan, 2-4 mm diameter, ulcers.

Histopathologic Description: There is diffuse acute interstitial pneumonia characterized by alveolar septal necrosis and fragmentation, and mild expansion of the septa with fibrin, edema, and small numbers of macrophages and neutrophils. There is intra-alveolar accumulation of edema, fibrin with scattered hyaline membrane formation, alveolar macrophages, small to moderate numbers of neutrophils, and small amounts of hemorrhage. Throughout all sections of lung examined, there are multifocal to coalescing regions of marked necrosis of the bronchi and bronchioles with often complete loss of the epithelium and infiltration of the surrounding alveoli and septa with moderate numbers of macrophages and neutrophils. The bronchial and bronchiolar necrosis extends into the surrounding tissue with necrosis of adjacent bronchial glands and pulmonary parenchyma. Within remaining bronchiolar epithelial cells and bronchial glandular epithelial cells, there are scattered eosinophilic intranuclear inclusions surrounded by a clear nuclear halo and marginated chromatin (herpesviral inclusions). ESOPHAGUS: Multifocally, there is ulceration of the esophageal mucosa with necrosis of the underlying submucosa and infiltration by moderate numbers of neutrophils. Within the epithelium adjacent to the regions of ulceration, there are scattered eosinophilic intranuclear inclusions surrounded by a clear nuclear halo and marginated chromatin (herpesviral inclusions).

Contributor's Morphologic Diagnoses:
1. LUNGS, Multiple lobes:

a. Multifocal to coalescing, severe, acute necrotizing and suppurative bronchiolitis and bronchopneumonia with intraepithelial herpetic intranuclear inclusions.
b. Diffuse, moderate to severe, acute interstitial pneumonia.
2. ESOPHAGUS: Multifocal to coalescing, marked, subacute, ulcerative and neutrophilic esophagitis with intraepithelial herpetic intranuclear inclusions.

Contributor's Comment: The primary lesion in this patient is a herpesvirus induced severe necrotizing pneumonia, bronchitis and bronchiolitis with intraepithelial intranuclear inclusions consistent with a fulminant fatal herpesviral infection. Similar inclusions are identified within the esophageal mucosa adjacent to regions of ulceration.

Feline viral rhinotracheitis caused by feline herpesvirus-1 (FHV-1) is primarily an infection of the upper respiratory system in cats. Infected cats exhibit sneezing, coughing, oral respiration, and salivation, similar to clinical signs identified in the presented case. Infected cats usually recover in 7-14 days, although there can be high mortality in kittens, debilitated, or immunosuppressed cats. Lesions are typically limited to the upper respiratory tract including nasal cavity, pharynx, soft palate, tonsils, and conjunctiva. Rare infections proceed to a fulminant fatal pneumonia characterized by a severe necrotizing bronchitis and bronchiolitis with an interstitial pneumonia.[1]

The ulcerative esophagitis in this patient is thought to be related to the herpesviral infection due to the presence of intranuclear inclusions with a similar histomorphology. Ulcerative esophagitis in cats is most commonly associated with calicivirus infection. Esophageal ulcers are not specifically mentioned in association with feline herpesviral infections, although oral ulcers and pharyngitis have been reported.

Although FeLV and FIV status in this patient are not known, we suspect some underlying cause of immunosuppression.
We did consider a possible severe fatal feline caliciviral infection in this case. Feline caliciviral pneumonia is generally less severe than herpesviral pneumonia and often requires a secondary bacterial infection to become significant. Also, the presence of the typical herpesviral intranuclear inclusion bodies within both the lungs and esophagus make a diagnosis of herpesviral pneumonia and esophagitis most likely in this case.[2] Virulent systemic feline calicivirus infection typically includes cutaneous ulceration of the pinnae, footpads, nares, and skin, along with subcutaneous edema, alopecia, and other systemic lesions such as bronchopneumonia and hepatic necrosis.[3] Cutaneous ulcerations and marked subcutaneous edema were not identified in this patient.

AFIP Diagnoses: 1. Lung: Pneumonia, bronchointerstitial, necrotizing, diffuse, severe, with fibrin, edema, syncytia, and epithelial intranuclear inclusion bodies, etiology consistent with feline herpesvirus, domestic shorthair (*Felis domesticus*), feline.
2. Esophagus: Esophagitis, necro-ulcerative, multifocal, marked, with fibrin, edema, and intraepithelial inclusion bodies.

Conference Comment: Feline herpesvirus 1 (FHV-1) is a double-stranded DNA alpha-herpesvirus that causes feline viral rhinotracheitis. All species of felidae are believed to be susceptible. Infection with FHV-1 is naturally acquired through oral, nasal, or conjunctival routes by either direct contact or from aerosolized oronasal secretions of virus-shedding infected cats. After an incubation of 24-48 hours, the onset of typical clinical signs of serous to mucopurulent nasal and conjunctival discharge occurs, accompanied by fever, sneezing, coughing, oral respiration, profuse salivation and corneal ulcers. While oral ulceration may also be present in FHV-1, this lesion is more typical of feline calicivirus infections. Skin ulcers and dermatitis syndrome in domestic cats and cheetahs, and nervous signs have been described, but are likely rare sequels to infection.[1,4,5]

Viral replication occurs primarily in the epithelium of the nasal cavity, oropharynx, conjunctiva, tonsils, and, to a lesser extent, the trachea. Shedding of viral particles may begin as early as 24 hours post infection and may last as long as one to three weeks, though most active viral replication and cell necrosis occur between two to seven days post infection. During this time, herpesviral intranuclear inclusions are most often present in infected epithelial cells and occasionally within endothelial cells. Inclusions are rarely detected beyond seven days after infection and cannot be relied on for diagnosis. Because viral replication is normally restricted to areas of lower body temperature, such as the upper respiratory passages, viremia is rare, and resolution of disease normally takes about two to three weeks. FHV-1 remains latent in carriers in the trigeminal ganglia.[1,4,5]

Uncommonly, generalized disease may follow initial upper respiratory tract infection in debilitated or immunocompromised animals and in neonatal kittens. In these cases, viremia may be present. Mortality due to FHV-1 is rare in domestic cats; however, when fulminating cases of viral infection occur, there is often widespread necrotizing bronchitis, bronchiolitis, and interstitial pneumonia with edema. Viral infection may predispose to fatal secondary bacterial bronchopneumonia with bacteria such as *Pasteurella multocida*, *Bordetella bronchiseptica*, *Streptococcus* sp., and *Mycoplasma felis*.[1,4,5]

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References:

1. Dungworth DL: The respiratory system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 558-559. Academic Press, Inc., San Diego, California, 1993
2. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 274. Williams & Wilkins, Baltimore, Maryland, 1997
3. Pesavento PA, MacLachlan NJ, Dillard-Telm L, Grant CK, Hurley KF: Pathologic, immunohistochemical, and electron microscopic findings in naturally occurring virulent systemic feline calicivirus infection in cats. Vet Pathol 41:257-263, 2004

4. L_pez A: Respiratory system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 483. Mosby Elsevier, St. Louis, Missouri, 2007
5. Gaskell RM, Dawson S, Radford A: Feline respiratory disease. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 3rd ed., pp. 145-154. Saunders Elsevier, St. Louis, Missouri, 2006

SLIDE 84
CASE IV – C30551-06 (AFIP 3034589)

Signalment: 1.5-year-old, male/neutered, Doberman Pinscher, canine

History: The patient presented with a 3 month history of lethargy, shifting leg lameness, and waxing and waning fever. The lameness was getting progressively worse, and the dog was very depressed and anorexic at the time of referral. Vaccinations were current. The dog was given a monthly heartworm preventative and was routinely treated for the control of fleas and ticks.

Gross Pathology: The dog was humanely killed. The dog had significant loss of muscle mass and was emaciated, evidenced by minimal body fat stores. Peripheral lymph nodes including mandibular, prescapular, axillary, cranial mediastinal, and popliteal were moderately enlarged and soft. The liver was massively enlarged, extending approximately 6 cm caudal to the costal arch, and had a pale reticulated lobular pattern and friable consistency. The spleen was similarly massively enlarged and had a meaty consistency exuding very little blood on the cut surface. Numerous small thin-walled tortuous blood vessels extended from the portal vein to the left renal vein and caudal vena cava, consistent with acquired portosystemic shunts. Both kidneys had a slightly pitted capsular surface. Numerous approximately 1 mm diameter red foci were scattered throughout the cortex. The ribs folded rather than breaking. The femoral bone marrow had replacement of marrow by firm white tissue that sank in formalin. This change was prevalent throughout the diaphysis of the bone.

Laboratory Results: The CBC revealed a hyperproteinemia (8.3), a leukopenia (5.3) characterized by a neutropenia (1007) and monocytopenia (106), and a profound non-regenerative anemia (RBC 1.77, Hgb 3.8, HCT 11.4) with an increased number of nucleated RBC (128). Abnormalities in the serum chemistry included a low carbon dioxide (18.7), mild hypercalcemia (11.3), hypoalbuminemia (1.7), hyperglobulinemia (4.8), low ALT (8), and high cholesterol (436). Evaluation of the blood smear revealed variably sized blast cells that had round, centrally located nuclei, indistinct nucleoli, and deeply basophilic cytoplasm. Several of the blast cells had cytoplasmic projections or blebs. The morphologic features of these blast cells were suggestive of a megakaryocytic lineage. The platelet count was within the reference interval; however, several giant, atypical platelets (macrothrombocytes) were observed. Bone marrow aspiration was performed but was not diagnostic; only peripheral blood contamination was obtained.

Histopathologic Description: The bone marrow is diffusely filled with a population of neoplastic cells interspersed with abundant fibrous tissue and intermittent bony trabeculae with evidence of marked osteolysis. The neoplastic cells are pleomorphic polygonal cells with prominent anisocytosis and anisokaryosis and variable chromatin patterns and amounts of cytoplasm. Larger cells have large, lobulated and sometimes ring-shaped nuclei or multiple small nuclei, often with dense ropey chromatin, and abundant eosinophilic cytoplasm with distinct cytoplasmic margins. Smaller blast-type cells also occur and blend with a background of fibroblasts and intervening fibrillary collagen. Bony trabeculae are often scalloped and marginated by osteoclasts in Howship's lacunae.
Similar large multi-nucleated cells resembling megakaryocytes, as well as smaller blast type cells, fill some capsular and medullary sinuses and often efface the architecture of lymph nodes. The spleen is similarly filled with neoplastic megakaryocytes and immature precursors intermingled with small residual populations of lymphocytes. Virtually no distinction of red and white pulp can be seen. The sinusoids of the liver are diffusely filled with neoplastic megakaryocytes. Atrophy of hepatic cords is often prominent in centrilobular regions. Alveolar septal capillaries are prominently thickened by intraluminal neoplastic megakaryocytes. Small numbers of similar cells occur in pulmonary vessels and some pulmonary arteries have prominent medial hypertrophy and tortuosity. Glomeruli throughout the kidney have global thickening of glomerular capillary loops and moderate hypercellularity. This correlates with frequent intraluminal hyaline casts in tubules.

Contributor's Morphologic Diagnosis: Megakaryocytic leukemia with myelofibrosis and metastatic sites in spleen, liver and lymph nodes

Contributor's Comment: Acute myeloid leukemias (AML) are neoplastic myeloproliferative disorders (MPD) that arise from hematopoietic precursors, including granulocytic, monocytic, erythrocytic, and megakaryocytic cell lines.[1] Megakaryocytic leukemia is a rare subtype of AML, both in humans and animals, and is designated as AML-M7. While chronic leukemias are characterized by infiltration of the bone marrow with more mature neoplastic hematopoietic cells, AMLs, in contrast, are characterized by large numbers ($\geq$20%) of blast cells in the bone marrow.[2] Megakaryoblasts are pleomorphic, and can potentially resemble lymphoblasts or myeloblasts. Thus, a diagnosis of AML-M7 based on morphology alone can only be accomplished if the blasts demonstrate some degree of differentiation. Cytoplasmic blebs or platelet shedding help identify megakaryoblasts. When the megakaryoblasts are poorly differentiated, it is difficult to distinguish AML- M7 from acute myeloid leukemia, acute lymphoid leukemias, and pure erythroid leukemia simply from evaluation of blood smears or bone marrow aspirates.[2] Thus, cytochemical, ultrastructural, and immunophenotypic features may be required for a definitive diagnosis. Immunophenotyping is a very useful diagnostic tool for determination of cell origin in poorly differentiated leukemias. In dogs with AML-M7, the blasts will be positive for CD41 (GPIIb/IIIa), CD61 (GPIIIa), and factor VIII-related antigen.[2]

Myelofibrosis develops in some cases of AML-M7 in dogs, similar to human patients with megakaryocytic leukemia.[1] A critical role for megakaryocytes in the pathogenesis of myelofibrosis has been recognized in human patients with idiopathic myelofibrosis (IMF). IMF is a clonal hematopoietic disorder characterized by atypical megakaryocytes, severe myelofibrosis, and splenic extramedullary hematopoiesis.[1] Megakaryocytic overproduction of fibrogenic cytokines, particularly platelet derived growth factor (PDGF) and transforming growth factor-_ (TGF-_), are thought to be responsible for the myelofibrosis observed in IMF and other disorders of the megakaryocytic lineage, including AML-M7 and MDS with prominent dysmegakaryopoiesis.[3] It has been demonstrated that the PDGF contained within the platelet _ granules are capable of inducing fibroblast proliferation.[3] The release of PDGF from megakaryocytes, however, is not thought to be completely responsible for the observed myelofibrotic stroma observed in megakaryocytic disorders. PDGF does not have angiogenic properties or the capability of inducing gene transcription of laminin, fibronectin, or the collagens. Thus, other growth factors must be involved; TGF-_ is probably the most important additional growth factor involved.[3] TGF-_, also stored in platelet _ granules, regulates the synthesis of the extracellular matrix in myelofibrotic disorders.[3] It accomplishes this by increasing the expression of genes for fibronectin, collagens type I, III, and IV production, in addition to chondroitin/dermatan sulphate proteoglycans. TGF-_ is also pro-angiogenic and decreases the production of collegenase-like enzymes that are responsible for degrading extracellular matrices. Ultimately, these properties of TGF-_ result in an increase in extracellular matrix.

AFIP Diagnoses: 1. Bone marrow: Acute megakaryoblastic leukemia (AML M7) with myelofibrosis, Doberman Pinscher (*Canis familiaris*), canine.
2. Lymph node and liver: Acute megakaryoblastic leukemia, metastatic.

Conference Comment: The contributor provides a thorough overview of megakaryocytic leukemia, a rare subtype of acute myeloid leukemia. This condition is designated acute megakaryoblastic leukemia (AML M7) in the WHO classification. CD61 immunohistochemical staining performed at the AFIP revealed multifocal cytoplasmic immunoreactivity of the neoplastic megakaryocytes supporting the diagnosis of megakaryoblastic leukemia.[5]

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References:

1. Blue JT: Myelodysplastic syndromes and myelofibrosis. In: Schalm's Veterinary Hematology, eds. Feldman BF, Zinkl JG, Jain NC, 5th ed., pp. 682-688. Lippincott Williams & Wilkins, Baltimore, Maryland, 2000
2. Grindem CB: Acute myeloid leukemia. In: Schalm's Veterinary Hematology, eds. Feldman BF, Zinkl JG, Jain NC, 5th ed., pp. 717-726. Lippincott Williams & Wilkins, Baltimore, Maryland, 2000

3. Ledieu D, Palazzi X, Marchal T, Fournel-Fleury C: Acute megakaryoblastic leukemia with erythrophagocytosis and thrombosis in a dog. Vet Clin Pathol 34:52-55, 2005
4. Reilly JT: Pathogenesis of idiopathic myelofibrosis: role of growth factors. J Clin Pathol 45:461-464, 1992
5. Valli VE, Jacobs RM, Parodi AL, Vernau W, Moore PF: World Health Organization Histological Classification of Hematopoietic Tumors of Domestic Animals, Second Series, vol. VIII, p. 55. Armed Forces Institute of Pathology, Washington, D.C., 2002

SLIDE 85
CONFERENCE 22 / CASE I – A906 (AFIP 2935882)

Signalment: 23-year-old, female, Indian rhesus macaque (*Macaca mulatta*)

History: This macaque, born at the Tulane National Primate Research Center was part of the breeding colony from 1980 to 2002. A906 delivered her 13th infant in 2002, after which she was transferred to an aging project. In November 2003, A906 was found dead.

Gross Pathology: External exam - The animal was emaciated and dehydrated, with severe alopecia. The body weight at the date of the death was 6.5 kg.

Digestive system - The entire small intestine was necrotic with bloody fluid feces. The colon had two firm infiltrative masses, one of 2X2 cm located at 15 cm from the ileocecal junction and a second one of 1X1 cm located at 5 cm from the ileocecal junction.

Reproductive system - The left ovary was atrophic. The right ovary was enlarged, and was totally replaced by a multilocular cyst measuring 3X2.5 cm and lined by a thin wall. In some of the compartments of the cyst a sebum-like material was visible through the thin wall.

Contributor's Morphologic Diagnosis: Mature benign teratoma of ovary (Dermoid cyst).

Contributor's Comment: The cause of death established at necropsy was colon carcinoma with intestinal infarction. The ovarian teratoma presented here was an incidental finding.

On histologic examination, the cyst wall was composed of stratified epithelium with rare underlying sebaceous glands and hair follicles. Numerous cells situated in the cyst wall were producing melanin. A heterogeneous collection of structures from other germ layers were also identified, such as bone, cartilage, smooth muscle, adipose tissue, thyroid tissue and glandular structures. All these tissues lacked morphologic features of anaplasia. The lumen of the cyst contained amorphous material and necrotic cellular

debris. As in most cases of teratoma in rhesus monkeys, this lesion was unilateral.[1] Ovarian teratomas have also been previously reported in an orangutan and an African green monkey.[2] While teratomas are often detected in the young and cause impaired fertility, the onset of neoplasia in this case was unknown and the reproductive history of this animal suggests no impairment in fertility.[3]

AFIP Diagnosis: Ovary: Teratoma, Indian macaque (*Macaca mulatta*), nonhuman primate.

Conference Comment: Teratoma is a germ cell neoplasm that arises from totipotential primordial germ cells and has disorganized elements of at least two of three embryonic germ layers (endoderm, mesoderm, ectoderm). Endoderm includes simple, stratified columnar, and cuboidal, ciliated or non-ciliated epithelium; goblet cells; and sebaceous or sweat glands. Mesoderm includes muscle, cartilage, bone, and blood vessels. Ectoderm includes keratinizing squamous epithelium, hair, teeth, and nervous tissue.[4,7]

Teratomas are classified as immature or mature. Immature (teratocarcinoma) contain less-differentiated embryonal tissues with some differentiated structures. Mature teratomas are composed of well-differentiated tissues. Most are well-differentiated and benign; however, malignant variants can occur and have been described in the bitch and mare. Teratomas can be solid or cystic. Cystic teratomas are often referred to as dermoid cysts and consist primarily of a cyst lined by skin.[4,5,6,7,9] Teratomas in the dog and cat are usually ovarian and almost always benign. Teratomas in the horse are usually testicular and often occur in cryptorchid testicles. Benign and malignant teratomas occur in mice and occur spontaneously in certain strains such as B6C3F1LT/SV, CD1, and C3H mice. Adrenal and uterine teratomas have been reported in ferrets. Other species in which teratomas have been reported include, but are not limited to, cattle, swine, and sheep.[5,6,7,10]

Other germ cell tumors include dysgerminoma, choriocarcinoma, embryonal carcinoma, and yolk sac carcinoma. Dysgerminomas are the least differentiated and resemble seminomas with solid sheets of round cells with vesiculate centrally placed nuclei and amphophilic cytoplasm. Choriocarcinomas undergo trophoblastic differentiation with large pleomorphic trophoblastic giant cells, syncytiotrophoblasts, cytotrophoblasts, and prominent blood-filled spaces. Embryonal carcinomas are rare and may contain multinucleated giant cells resembling syncytiotrophoblastic cells; however, cytotrophoblastic cells are absent. Yolk sac carcinomas differentiate into mesoblast and yolk sac endoderm composed of nests and ribbons of neoplastic epithelium in periodic acid-Schiff (PAS) positive material. Its characteristic histologic feature is a glomerulus-like structure composed of a central blood vessel enveloped by germ cells within a space lined by germ cells (Schiller-Duval body).[5,7,8]

Contributor: http://www.tpc.tulane.edu/

References:
1. Chalifoux LV: Ovarian Teratoma, *Macaca mulatta*. In: Nonhuman Primates II, eds. Jones TC, Mohr U, Hunt RD, pp. 151-154. Springer-Verlag, Berlin, Germany, 1993
2. Baskin GB, Soike K, Jirge SK, Wolf RW: Ovarian teratoma in an African Green Monkey (*Cercopithecus aethiops*). Vet Pathol 19(2):219-221, 1982
3. Crum CP: The female genital tract. In: Robbins Pathologic Basis of Disease, eds. Cotran RS, Kumar V, Collins T, 6th ed., pp. 1073-1075. W.B. Saunders Company, Philadelphia, Pennsylvania, 1999
4. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1280. Mosby Elsevier, St. Louis, Missouri, 2007
5. 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, Second Series, vol. IV, p. 18. Armed Forces Institute of Pathology, Washington D.C., 1998
6. Kennedy PC, Miller RB: The female genital system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 368-369. Academic Press, Inc., San Diego, California, 1993
7. Alison RH, Morgan KT: Teratoma, ovary, mouse. In: Monographs on Pathology of Laboratroy Animals Genital System, eds. Jones TC, Mohr U, Hunt RD, pp. 46-52. Springer-Verlag, Berlin, Germany, 1987
8. Crum CP: The female genital tract. In: Robbin and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 1099-1102. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
9. MacLachlan NJ, Kennedy PC: Tumors of the genital systems. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 554, 565-566. Iowa State Press, Ames, Iowa, 2002
10. Beach JE, Greenwood B: Spontaneous neoplasia in the ferret (*Mustela putorius furo*). J Comp Pathol 108(2):133-147, 1993

SLIDE 86
CONFERENCE 22 / CASE II – 756 (AFIP 2789028)

Signalment: Three late term caprine, *Capra* sp., fetuses with placenta

History: In February of 2001, a newly established goat herd, assembled from multiple sources, experienced an abortion rate of over 10%.

Gross Pathology: No lesions were observed in the fetuses. The placenta exhibited a thick creamy reddish-brown exudate between cotyledons.

Histopathologic Description: There was moderate to marked suppurative inflammation of the intercotyledonary chorionic membrane, with moderate multifocal villar necrosis. Cotyledonary inflammation was most developed at the base of villi and

was characterized by a superficial multifocal mixed cellular inflammatory infiltrate in which plasma cells predominated. Intracytoplasmic, blue foamy organisms were found in trophoblast cells throughout the arcade and were most numerous at the base of villi.

Contributor's Morphologic Diagnosis: Placentitis, necrotizing, subacute, multifocal, suppurative, plasmacytic with large numbers of foamy blue intracellular organisms, etiology consistent with *Coxiella burnetii.*

Contributor's Comment: Lesions typical of *Coxiella burnetii* were restricted to the placenta. Trophoblast cells were distended with intracytoplasmic, foamy blue organisms characteristic of *C. burnetii*. This was confirmed by PCR analysis of placental tissue. Since the affected goat herd was assembled from numerous sources, it is probable that both reservoir and naïve animals were purchased. Exposure of the naïve animals to organisms shed by the reservoir animals and/or organisms in the new farm environment likely preceded the abortion storm.

Coxiella burnetti is a rickettsial, obligate intracellular parasite with worldwide distribution. The organism is resilient in the environment and persists indefinitely in animal reservoirs such as dairy cows, sheep, goats and wildlife. Reservoirs shed the organism in milk, fetal fluids, urine and feces. The organism rarely causes systemic disease in infected animals other than abortion and stillbirth. *C. burnetii* is the cause of Q fever in humans, a zoonosis. Inhalation of contaminated dust or ingestion of infected milk can lead to a clinical syndrome in man characterized by fever with or without pneumonia and hepatitis.

AFIP Diagnosis: Chorioallantois: Placentitis, subacute, diffuse, moderate, with multifocal necrosis and myriad intratrophoblastic organisms, etiology consistent with *Coxiella burnetti*, goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides a general background overview of *Coxiella burnetti*.
Typical light microscopic findings include an acute, diffuse, suppurative placentitis with extensive necrosis of cotyledonary villi and intercotyledonary epithelium. The placentitis is most severe in the intercotyledonary areas. A lymphoplasmacytic infiltrate is usually present in the chorioallantoic interstitium. As pointed out by the contributor, hypertrophic trophoblasts containing the organisms have a characteristic foamy appearance with multiple unstained vacuoles within a finely granular blue cytoplasm. Vasculitis is not usually a feature.[1,3]

Grossly, the intercotyledonary chorioallantois is thick, leathery, yellow, and covered with surface exudates. Cotyledons may have multiple areas of gray discoloration in areas of inflammation and necrosis. Gross fetal lesions are nonspecific.[1,3]

The differential diagnosis for a necrotizing placentitis associated with intra-trophoblastic organisms includes:[1,3]

1. *Brucella ovis* – vasculitis common
2. *Chlamydophila abortus* – vasculitis common, more severe cotyledonary inflammation, coccoid organisms
3. *Toxoplasma gondii* – primarily affects cotyledons

As pointed out by the contributor, *C. burnetii* is a zoonosis causing Q fever in man. The organism is extraordinarily virulent in man and a single organism can result in disease. Infections occur primarily in abbatoir employees and personnel working in research centers. Clinical syndromes associated with Q fever include a self-limited febrile illness, pneumonia, hepatitis, endocarditis, osteomyelitis, and neurologic manifestations. The majority of infections are mild self-limited febrile illnesses.[2]

Contributor: BCMAF Animal Health Centre, Abbotsford, BC, Canada

References:

1. Kennedy PC, Miller RB: The Female Genital System. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 417-419. Academic Press, Inc., San Diego, California, 1993
2. Marrie, TJ: Q fever – A review. Can Vet J 31:555-563, 1990
3. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1296. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 87
CONFERENCE 22 / CASE III – 4075-10-02 (AFIP 2940308)

Signalment: Fetus, gestational age about 7 months, female, Ayrshire, bovine

History: The fetus was stillborn. Conception was by embryo transfer. The heifer had retained placenta but showed no other clinical signs at the time of abortion. The farm had had several abortions during the previous 2 months. The farm was free of bovine leucosis (BVL), infectious bovine rhinotracheitis (IBR) and bovine virus diarrhea (BVD).

Gross Pathology: The fetus had moderate interstitial oedema of the lungs. The liver was swollen and brick-red. The cotyledons were necrotic and oedematous. Intercotyledonary areas were thickened and leathery. The amnion was markedly thickened and oedematous with a leathery aspect.

Laboratory Results: Bacteriology and PCR: *Ureaplasma diversum* was isolated from the placenta and abomasal contents and identified by nested PCR. No other specific bacteria were isolated. Mycology: No fungal growth.

Histopathologic Description: Necrotizing placentitis with multifocal mineralization was present in the chorioallantoic placenta. In the liver there was diffuse moderate centrilobular degeneration. In the lung there was marked multifocal necrotizing alveolitis.

Contributor's Morphologic Diagnosis: Placenta, allantoamnion, amnionitis, necrotizing, diffuse, chronic, severe with vasculitis and mineralization.
Contributor's Comment: The macroscopic and histological findings are characteristic but not pathognomonic of ureaplasma infection.[1,2,3] Definitive diagnosis requires isolation and identification of the bacterium. The strain was identified by nested polymerase chain reaction.[4] *Ureaplasma diversum* can be spread via artificial insemination and embryo transfer as well as via natural breeding. The infection is often subclinical in chronically infected herds.[5] In central Finland there appeared to have been an epidemic of virulent *U. diversum* infection during 2002-2003. Numerous farms in that area experienced problems ranging from early embryonic death and vulvitis to abortions in the 2nd and last trimester. *U. diversum* has recently been reported as an important pathogen causing reproductive problems in Brazil.[6]

AFIP Diagnosis: Placenta: Placentitis, necrotizing, subacute, diffuse, severe, with vasculitis, thrombosis, and mineralization, Ayrshire (*Bos taurus*), bovine.

Conference Comment: *Ureaplasma diversum* is an important cause of reproductive failure and abortion in cattle. *U. diversum* belongs to the family Mycoplasmataceae, the smallest free-living parasites capable of autonomous growth. It is frequently present on the mucous membranes of the nasal passages, vulva, and vagina of the cow, the sheath of bulls, and in semen and embryo transfer fluids.[5,7]

Virulent strains of *U. diversum* can cause abortion, embryonic death, birth of dead or weak calves, and vulvitis. Grossly, the amnion is typically the most severely affected with patchy thickening and yellow discoloration, fibrosis, edema, inflammation, necrosis, and mineralization. The fetus is usually well preserved.[5,7]

Histologically, fibrosis and interstitial necrosis of the placenta are extensive; there is often a mononuclear inflammatory infiltrate. A mild arteritis is typically present. Erosive conjunctivitis with prominent goblet cell formation is present in the fetus as well as a nonsuppurative pneumonia with prominent peribronchiolar lymphoid tissue.

Some slides contained vessels partially occluded by fibrin thrombi.

Contributor: National Veterinary and Food Research Institute (EELA), http://www.eela.fi

References:
1. Murray RD: Concurrent conjunctivitis and placentitis in aborted bovine fetuses. J Vet Med A 38:660-667, 1991
2. Miller RB, Ruhnke HL, Doig PA, Poitras BJ, Palmer NC: The effects of *Ureaplasma diversum* inoculated into the amniotic cavity of cows. Theriogenology 20:367-374, 1983
3. Ruhnke HL, Palmer NC, Doig PA, Miller RB: Bovine abortion and neonatal death associated with *Ureaplasma diversum*. Theriogenology 21:295-301, 1984
4. Cardoso MV, Blanchard A, Ferris S, Verlegia R, Timenetsky J, Florio Da Cunha RA: Detection of *Ureaplasma diversum* in cattle using newly developed PCR-based detection assay. Vet Microbiol 72:241-250, 2000
5. Kennedy PC, Miller RB: The female genital system. In: Pathology of Domestic animals, eds. Jubb KVF, Kennedy PC, Palmer NC, 4th edition, vol. 3, pp. 408-409. Academic Press, Inc., San Diego, California, 1993
6. Cardoso MV, Scarcelli E, Grasso LMPS, Teixeira SR, Genovez ME: *Ureaplasma diversum* and reproductive disorder in Brazilian cows and heifers; first report. Anim Reprod Sci 63:137-143, 2000
7. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 1297. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 88
CONFERENCE 22 / CASE IV – AFIP #1 (AFIP 2983846)

Signalment: Adult Holstein cow

History: Placenta from an adult, multiparous Holstein cow is presented for examination after abortion of an approximately 120-day-gestation fetus.

Gross Pathology: Tissue representing approximately _ of a bovine placenta is examined. The tissue is diffusely edematous. The chorionic surface is covered by variably sized (0.5 to 3.0 cm diameter), individual and coalescing raised, firm, ovoid, dark red plaques thickening intercotyledonary spaces. A few grossly normal cotyledons are present.

Histopathologic Description: Dark red plaques consist of multifocal, raised, well demarcated primitive placental villus structures that replace and widen intercotyledonary stroma. Structures are composed of variably sized, thin walled vessels aligned perpendicularly to the chorionic surface, supported within a dense fibrous stroma. These vessels extend to the tissue surface which is occasionally covered by thin layers of mineralized material. In many of the plaques, vessels are separated by vertically linear pools of free erythrocytes. Surrounding stroma is edematous. Moderate numbers of plasma cells are among villous structures in some sections.

Contributor's Morphologic Diagnosis: Adventitial placentation, multifocal, placenta

Contributor's Comment: Adventitial placentation is the development of additional sites of placentation between adjacent placentomes, as the result of inadequate development of existing placentomes. In ruminants, development of such structures is a mechanism used for compensation of inadequate placentation.[1]

The condition generally results from an insufficient number of caruncles resulting either from congenital disorders of endometrial organization (too few caruncles) or more commonly by the loss of caruncles due to inflammation/scarring following repeated episodes of endometritis.[2] Primitive villus attachments between the chorion and endometrium attempt to compensate for inadequate placentation.[1]

Cows normally have 75-120 caruncles in the uterus, and ewes and goats have 40-125. Not all caruncles are utilized in a normal single pregnancy.[2] When adventitial placentation becomes diffuse, pregnancy cannot proceed beyond midterm. Hydrallantois is a common complication.[1]
In most cases, the compensatory effort leads to placental insufficiency and abortion. However, one case of successful live birth of a healthy cloned calf found to have been supported by an atypical placenta affected with severe adventitial placentation is reported.[3]

AFIP Diagnosis: Chorioallantois: Adventitial placentation, multifocal, with edema and minimal lymphoplasmacytic placentitis, Holstein (*Bos taurus*), bovine.

Conference Comment: The contributor provides a concise summary of the causes of adventitial placentation. As mentioned by the contributor, hydrallantois is a common complication that is characterized by excessive fluid in the allantoic sac. The quantity of fluid present may exceed 150 liters verses a normal 15-20 liters of fetal fluid present between the amnion and allantoic sacs. Common sequelae to hydrallantois, if the fetus is not aborted early, include dystocia, uterine atony with retained fetal membranes, and metritis. Additionally, adventitial placentation is often seen in cloned fetuses and is also thought to be an age-associated change as it is often seen in cows with a higher parity.[1,2,4]

Grossly, additional sites of placentation are visible in intercotyledonary areas and appear as red plaques, sometimes with villi that extend from cotyledons. Corresponding changes are visible in the endometrium.[4]

Contributor: Utah Veterinary Diagnostic Laboratory (UVDL), Utah State University, ADVS Dept., Logan, UT 84322, www.usu.edu/uvdl

References:

1. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 1178-1179. Williams & Wilkins, Baltimore, Maryland, 1997
2. Kennedy PC, Miller RB: The female genital system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 390-391. Academic Press, Inc., San Diego, California, 1993
3. Hill JR, Edwards JF, Sawyer N, Blackwell C, Cibelli JB: Placental anomalies in a viable cloned calf. Cloning 3(2):83-88, 2001
4. Foster RA: Female reproductive system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p.1301. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 89
CONFERENCE 23 / CASE I – Vet. Path. ZH S03-003.1 (AFIP 2936459)
Signalment: 32-year-old, female, captive black rhinoceros (*Diceros bicornis*), perissodactyl

History: From the age of 15 years, this rhinoceros from the zoological garden, Zürich, showed intermittent reluctance to eat fibrous foods; it hypersalivated and ejected wads which the animal had chewed partially. The diet was Lucerne and grass hay (diet A). From the age of 30 years on, quidding was noticed constantly and the animal lost weight. Green meal pellets were added to the diet (diet B) to improve the fiber utilization. In February 2001, increasing foetor ex ore led to an examination of the oral cavity. Large amounts of dental plaque and calculus were removed. An abscess was discovered in the upper mandible between P1 and P2 and treated. After that, the animal showed an improvement in its food intake. The animal died at the age of 32 years in January 2003. Minor bleeding from the mouth was observed prior to death.

Gross Pathology: The animal was emaciated. There was a fistula in the tongue that extended from the dorsal surface to the base. The teeth were covered with thick dental plaque and calculus. The liver was firm, light brown and showed markedly sharp edges. The duodenal mucosa was a rusty brown.

Laboratory Results: Serology revealed a mean transferrin saturation of approximately 90% (Reference value 28%), and mean ferritin was 6046 ng/ml (Reference value 133 ng/ml).[1]

Contributor's Morphologic Diagnoses: 1. Liver: Hepatitis, lymphoplasmacytic, chronic, moderate, with severe fibrosis, biliary duct proliferation, multifocal to coalescing necrosis, hemorrhage and massive deposition of hemosiderin (Hemochromatosis).
2. Duodenum: Hemosiderin deposition in the submucosa, lamina propria and tips of villi, severe, diffuse (Hemosiderosis).

Contributor's Comment: Iron storage disease was suspected because of the high transferrin saturation and the high level of ferritin in the blood serum. The iron content

of diet A and diet B were analyzed and it turned out that the addition of green meal pellets had increased the iron concentration from approximately 270 to approximately 590 mg/kg. Not only had the iron content been increased but also the minor concentration of tannins in the diet had provided more absorbable iron.[4]

Hemosiderosis is defined as a systemic overload of iron resulting in excessive deposition of hemosiderin in different organs or tissues. In contrast, hemochromatosis is also a deposition of hemosiderin but it is always combined with a morphological or functional disturbance of the organ, tissue or cell.[5]

Iron is essential for all living organisms, but it also may act as a potent toxin. Evolutionary mechanisms have therefore resulted in the adaptation of specific proteins for the uptake, transport (transferrin), utilization (hemoglobin), and storage of iron. Regulation of the uptake is essential because the mechanisms for excretion are limited. Iron is absorbed predominately in the duodenum and proximal jejunum. Here, the absorbed iron is bound to ferritin in the intestinal epithelial cells. The absorption rate and the release of iron-loaded ferritin into the blood are dependent on the plasma iron level. The remaining iron is lost when the epithelial cells are sloughed into the intestinal lumen. Most of the plasma iron is bound to transferrin.[3]

These mechanisms are referred to as "mucosal block" or, in human medicine, as feedback. This feedback is the most important limiting step in iron absorption and is designed to prevent iron uptake exceeding the range to which a species has adapted during its evolution. This species-specific range is the iron concentration in the animal's natural diet.

Chronically excessive dietary iron will lead to iron storage. The lack of dietary tannins in captivity could be responsible for excessive iron disease. Supportive for this hypothesis is the fact that not only black rhinoceroses suffer from iron storage disease, but also many different captive mammalian herbivores and birds that naturally consume large amounts of tannin. However, the analysis of postmortem examinations of exotic animals from different zoos suggested that it was not the content of tannins per se but rather the species' adaptation to a low iron natural diet that dispose them to excessive iron storage when they receive diets with high-iron content in captivity.[1,2]

In contrast to these species, sera from captive foregut-fermenting browsers showed no elevation of transferrin saturation or ferritin levels. A possible explanation could be that the ingesta remain in the forestomachs longer whereby the tannins have more time to bind to the salivary and microbal proteins instead of the dietary iron. Furthermore, the anaerobic conditions of the forestomachs may reduce more normally unavailable Fe^{3+} ions to the more readily available Fe^{2+} ions. Thus, for such animals an effective iron absorption mechanism is unnecessary and the maintenance of controlled absorption for higher available iron ranges via feedback is important. There was no evidence of iron excess in the African white rhino (*Ceratotherium simum*) and the Indian rhinoceros (*Rhinoceros unicornis*). These grazers naturally consume large amounts of grass with a much higher concentration of iron and almost no tannins.[1,2]

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, periportal to midzonal, chronic, diffuse, marked, with moderate biliary duct proliferation, fibrosis, and hemosiderosis, black rhinoceros (*Diceros bicornis*), perissodactyl.

Conference Comment: The contributor provides an excellent summary of the pathogenesis of hemochromatosis and defines the difference between hemosiderosis and hemochromatosis. Diffuse hemosiderosis is common in all species and when present is suggestive of excessive hemolytic activity relative to the reutilization rate of iron. Hemosiderosis is seen in hemolytic anemias; anemia of copper deficiency; cachexia; severe chronic passive congestion of the liver, lungs, or spleen and in areas of hemorrhage. Additionally, hemosiderin is normally present in the early neonatal period when fetal hemoglobin is being replaced by mature hemoglobin. The ferric iron component of hemosiderin can be demonstrated by staining with Prussian blue. Hemosiderin has a chemical structure identical to ferritin. Hemosiderin must be distinguished from lipofuscin, hematin, and bile pigments.[6,7]

As pointed out by the contributor, tannins chelate iron forming a non-absorbable complex; therefore, decreased tannins in the diet increase the bioavailability of iron. Increased bioavailability of iron results in the increased accumulation of iron in tissues in animals' whose natural diet has high concentrations of tannins, phytates, fiber, polyphenolics, phosphates, and other compounds that chelate iron into insoluble complexes that normally pass through the gastrointestinal tract unabsorbed.[1,2]

Hemochromatosis has also been reported in humans, hyraxes, lemurs, simians, prosimians, various avian species (mynahs, birds of paradise, toucans), gorillas, tapirs, pikas, pinnipeds, cheetahs, snow leopards, siamangs, callithrichids, cattle (hereditary hemochromatosis of Salers cattle), and sheep.[1,2,6,8]

Conference participants reviewed iron parameters useful in the diagnosis of hemochromatosis such as serum iron concentration, total iron binding capacity (TIBC), ferritin levels, transferrin levels, and percent saturation of transferrin.[8]

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References:

1. Paglia D, Dennis P: Role of chronic iron overload in multiple disorders of captive black rhinoceroses (*Diceros bicornis*). In: Proc Am Assoc Zoo Vet, pp. 163-170. Columbus, 1999
2. Clauss M, Lechner-Dol M, Hänichen T, Hatt J-M: Excessive iron storage in captive mammalian herbivores – a hypothesis for its evolutionary etiopathology. In: Proc

European Association of Zoo and Wildlife Veterinarians, 4th scientific meeting, pp. 123-131. Heidelberg, Germany, 2002
3. Lowenstine LJ, Munson L: Iron Overload in the Animal Kingdom. In: Zoo and Wild Animal Medicine, ed. Fowler ME, Miller RE, 4th ed., pp. 260-268. W.B. Saunders Company, Philadelphia, Pennsylvania, 1999
4. Hatt J-M, Wenker C, Castell J, Clauss M: Dietary and veterinary management of a lingual abscess in a geriatric captive black rhino (*Diceros bicornis*) with iron storage disease. In: Proc European Association of Zoo and Wildlife Veterinarians, 5th scientific meeting, Ebeltoft, Denmark, 2004
5. Crawford JM: Liver and biliary tract. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp. 908-910. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
6. Kelly WR: The liver and biliary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, p. 329. Academic Press, Inc., San Diego, California, 1993
7. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp.69-70. Williams & Wilkins, Baltimore, Maryland, 1997
8. Brockus CW, Andreasen CB: Erythrocytes. In: Duncan & Prasse's Veterinary Laboratory Medicine Clinical Pathology, eds. Latimer KS, Mahaffey EA, Prasse, KW, 4th ed., pp. 4-5. Iowa State Press, Ames, Iowa, 1994

SLIDE 90
CONFERENCE 23 / CASE II – S2671-02 (AFIP 2890238)

Signalment: Seven-week-old, female, Arabian crossbred, equine

History: This 7 week-old Anglo Arabian filly died while suffering from chronic respiratory disease and osteomyelitis. Radiography and ultrasound examination prior to death indicated what appeared to be multiple pulmonary abscessation and osteomyelitis of the distal left metacarpal bone. *Nocardia asteroides* had been isolated from a needle aspirate of the exudate from the suspected osteomyelitis lesion before death.

Gross Pathology: Severe, bilateral, subacute, diffuse fibrinonecrotic broncho-pneumonia involving the cranial and cardiac lobes. There appeared to be aplasia of the thymus as well as severe generalized hypoplasia of lymph nodes. The spleen was small (± 21x4x1,5cm) with inconspicuous lymphoid tissue. There was a moderate fibrinopurulent sialoadenitis of the mandibular salivary glands as well as a necropurulent osteomyelitis of the distal palmar surface of the left metacarpal bone.

Laboratory Results: Haematology (absolute counts):

WBC	6,500/ul (N 5,400–14,300)
Neutrophils	6,150/ul (N 2,260-8,580)
Band cells	7/ul (N 0-100)
Lymphocytes	30/ul (N 1,500–7,700)

There was a low absolute lymphocyte count of 30/ul compared to the normal range of 1,500–7,700/ul. However, the total white blood cell count was at the lower margin of the normal range.

Microbiology: A pathogenic smooth isolate of *Escherichia coli* and *Klebsiella pneumoniae* were isolated from several organs, including the lungs. *Nocardia asteroides* was isolated from the osteomyelitis lesion.

Genetic Analysis: DNA extracted from wax-embedded blocks of spleen tissue of this foal displayed the 158 base pair alleles characteristic of the severe combined immunodeficiency disease (SCID) genetic mutation using PCR amplification with specific DNA primers. DNA extracted from blood samples collected from the sire and dam of the foal were heterozygous for the 158 and 163 base pair alleles typical of carriers of severe combined immunodeficiency disease.
Contributor's Morphologic Diagnoses: 1. Pancreas: Moderate multifocal to coalescing, subacute, necropurulent pancreatitis associated with the presence of numerous large, amphophilic, intranuclear inclusion bodies, morphologically compatible with adenovirus infection.
2. Spleen: Severe diffuse lymphoid hypoplasia with an almost total absence of lymphoid tissue in the periarteriolar lymphoid follicles and sinusoids.

Contributor's Comment: Inclusion bodies appeared to be present within the nuclei of the pancreatic acinar and duct cells only. The presence of adenoviral inclusion bodies within the pancreas and their absence from the lung appears to be unusual when compared to previous reports of their presence within the lungs and pancreas. There was moderate necrosis and infiltration of neutrophils as well as scattered colonies of coccoid to bacilliform bacteria within the pancreas.[1]

On transmission electron-microscopy of uranyl acetate- and lead citrate-stained sections of pancreas, characteristic adenovirus particles in crystalline array could be identified within the intranuclear inclusion bodies at 19000x magnification.
The breed and the history of death from multiple infections at the early age of 7 weeks, in conjunction with severe lymphopaenia, lymphoid hypoplasia and positive PCR results are consistent with a diagnosis of SCID in Arabian and related horse breeds[1,2,3,4,5,6,7]

The disease has been shown to be caused by an autosomal recessive trait expressed as severe B and T lymphocyte dysfunction at the level of the prolymphocytic bone marrow-derived and thymus dependent stem cells. The basic genetic lesion, induced by a recessive gene mutation, comprises a five base pair deletion in the gene responsible for B and T cell lymphopoiesis. This deletion has been shown to cause an inactivation of the gene for DNA-dependent kinase, catalytic subunit DNA-PK, which is required for the coding sequences of immu-noglobulin and T cell antigen receptors during B and T cell lymphopoiesis.[1,4,5,6,7]

The disease is believed to occur in approximately 0.2-2% of Arabian-bred foals of both sexes, but the incidence of phenotypically normal heterozygotes will be much higher. Carriers pass the gene on to 50% of their offspring, and affected foals are born from 25% of successful matings between carriers.[1,4,5,7,8]

A specific test for the SCID mutation provides the ability to screen Arab horses used for breeding. DNA is extracted from appropriate samples (whole blood sample in EDTA or sodium citrate, hairs with roots or any other tissue) collected from the horse in question. Appropriately labeled DNA primers (forward primer 5'-AAG TTG GTC TTG TCA TTG AGC-3'; reverse primer 5'-TTT GTG ATG ATG TCA TCC CAG-3') that flank the gene region affected by the mutation are used in a PCR to amplify the affected region from the DNA sample. The fragment sizes can be determined using a genetic analyzer. The fragment size generated by the wild type allele is 163 base pairs and the fragment generated by the SCID allele is 158 base pairs.[5,6,7]

Affected foals are normal at birth, but at 10 days of age frequently develop pneumonia and diarrhoea as a result of bacterial and adenovirus infections, as typified in this case. There is usually a severe lymphopaenia, as well as hypoplasia of all lymphoid tissue including thymus, spleen, and lymph nodes, with a marked paucity of tissue- and circulating lymphocytes, as demonstrated in this case. The total white cell count is usually within the normal range as a result of compensation by increased neutrophils or immature leucocytes. Despite intensive veterinary care, foals rarely survive for longer than 3 months.[1]
Although there were numerous adenovirus inclusions in the pancreas, no inclusions could be seen within the lungs. The identity of the adenovirus inclusions could be established by their characteristic morphology of crystalline array by transmission electron microscopy.[2]

AFIP Diagnoses: 1. Pancreas: Pancreatitis, necrotizing, multifocal to coalescing, marked, with intraepithelial basophilic intranuclear inclusion bodies, etiology consistent with adenovirus, Arabian crossbred (*Equus caballus*), equine**.**
2. Spleen, white pulp: Lymphoid hypoplasia, diffuse, severe.

Conference Comment: The contributor provides a thorough overview of severe combined immunodeficiency (SCID) in Arabian foals. As stated by the contributor, SCID in Arabian foals involves failure of maturation of both B- and T-lymphocytes. Neutrophils, macrophages, natural killer (NK) cells and the complement system function normally. In addition to adenovirus, SCID foals often succumb to secondary infections with *Pneumocystis carinii*, *Rhodococcus equi*, and *Cryptosporidium parvum*, or a variety of common equine bacterial pathogens.[10]

SCID has also been reported in dogs, mice and humans. Two molecular mechanisms account for SCID in dogs. X-linked SCID has been described in Bassett Hounds and a Cardigan Welsh Corgi resulting from a mutation in the gene encoding the _-chain of the

IL-2 receptor. The same _-chain is also a component of the IL-4, IL-7, IL-9, IL-15, and IL-21 receptors. T-cell numbers may be nearly normal in affected dogs and their ability to produce IL-2 is not impaired. However, the defect in the _-chain renders T cells unable to bind and respond to IL-2 resulting in T cells that are unresponsive to mitogenic stimuli, increased susceptibility to infection, thymic dysplasia, hypogammaglobulinemia, and a failure to grow normally. Jack Russell Terriers have a mutation within the DNA-PKcs gene. A spontaneous mutation in DNA-PKcs of BALB/C mice results in SCID, as well as experimentally induced mutations in recombinase-activating genes 1 and 2 (RAG1 and RAG2). The most common form of human SCID is X-linked with a mutation in the common _-chain subunit of several cytokine receptors resulting in a profound defect in the earliest stages of lymphocyte development. Other forms show autosomal recessive inheritance and are usually due to an adenosine deaminase deficiency which may cause impaired DNA synthesis in lymphocytes.[1,10,11] There was some discussion as to whether the bacteria present are ante- or post-mortem. The location (intravascular), morphology (short rods), and lack of inflammation are supportive of post-mortem bacterial overgrowth. As stated previously, in SCID foals, neutrophils, macrophages, NK cells and the complement system function normally. However, the failure of maturation of B and T cells leads to ineffective cell mediated and humoral immunity and foals often succumb to a variety of common equine bacterial pathogens.[12]

Readers are encouraged to review reference 9 – Animal models molecular pathology of severe combined immunodeficiency in mice, horses, and dogs.

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References:

1. Valli VEO: The hematopoietic system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 3, pp. 211-214. Academic Press, Inc., San Diego, California, 1993
2. Ghadially FN: Ultrastructural Pathology of the Cell and Matrix, 3rd ed., vol. 1, pp. 132-133. Butterworths, London, United Kingdom, 1988
3. Jain NC: Essentials of Veterinary Hematology, p. 21. Lea & Febiger, Philadelphia, Pennsylvania, 1993
4. Perryman LE, Torbeck RL: Combined immunodeficiency of Arabian horses: confirmation of autosomal recessive mode of inheritance. J Am Vet Med Assoc 176:1250-1251, 1980
5. Shin EK, Perryman LE, and Meek K: A kinase-negative mutation of DNA-PK_{CS} in equine SCID results in defective coding and signal joint formation. J Immunol 158:3565-3569, 1997
6. Shin EK, Rijkers T, Pastink A, Meek K: Analysis of TCR_B rearrangements substantiate a profound deficit in recombination signal sequence joining in SCID foals: implications for the role of DNA-dependent protein kinase in V(D)J recombination. J Immunol 164(3):1416-1424, 2000

7. Swinburne J, Lockhart L, Scott M, Burns MM: Estimation of the prevalence of severe combined immunodeficiency disease in UK Arab horses as determined by a DNA-based test. Vet Rec 145:22-23, 1999
8. Bernoco D, Bailey E: Frequency of the SCID gene among Arabian horses in the USA. Animal Genetics 29:41-42, 1998
9. Perryman LE: Animal models molecular pathology of severe combined immunodeficiency in mice, horses, and dogs. Vet Pathol 41:95-100, 2004
10. Brown TT, Suter MM, Slauson DO: Immunopathology. In: Mechanisms of Disease a Textbook of Comparative General Pathology, eds. Slauson DO, Cooper BJ, 3rd ed., pp. 288-289. Mosby, St. Louis, Missouri, 2002
11. Abbas AK: Diseases of immunity. In: Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, 7th ed., pp.243-244. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
12. Snyder PW: Diseases of immunity. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 240-242. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 91
CONFERENCE 23 / CASE III – 062172 (AFIP 3026810)

Signalment: Field vole (*Microtus arvalis*), male, one-year-old

History: Animal captured near Lausanne (Switzerland) for breeding research, then submitted and euthanatized for routine sanitary control of the colony to the plateform of veterinary diagnosis of the University of Geneva (Switzerland). Graciously sent to the Department of Pathology of the Nantes Veterinary School for unreservedly pedagogic use by Dr. Laurence Fiette.

Gross Pathology: Infestation by fleas (*Nosophyllus* sp.). Liver: Multiple multivesicular cysts invading the parenchyma, a few mm in diameter and containing whitish solid material.

Laboratory Results: No significant results from the routine sanitary control (annual FELASA list of agents).

Histopathologic Description: The liver is totally invaded by alveolar structures composed of numerous cysts of irregular shape, a few millimeters in diameter. The cysts contain numerous invaginated heads (protoscolices) and an eosinophilic material. The wall of these cysts has two layers. The external layer is acellular and fibrous. The inner layer is the germinal membrane producing the protoscolices. The presence of these cysts elicits a fibrous reaction of the liver tissue and a minimal mononuclear inflammatory reaction.

These structures are characteristic of the metacestode stage of *Echinococcus multilocularis*.

Contributor's Morphologic Diagnosis: Alveolar echinococcosis.

Other names of the disease: Alveolar hydatid disease, multilocular echinococcosis or multivesicular hydatidosis.

Contributor's Comment: Voles - *Microtus arvalis,* (field vole) and *Arvicola terrestris* (water vole) - and other Arvicolidae as well as small mammals (lemmings, shrews, mice) are natural intermediate hosts for *E. multilocularis* (Plathelminthes, Cestodes, Taeniidae). Intermediate hosts become contaminated by ingestion of eggs released from the definitive hosts to the environment. Foxes, and in some endemic areas, other species of wild carnivores (coyotes, wolves and raccoon dogs) are the definitive hosts in the sylvatic cycle. Dogs and cats are also definitive hosts in synanthropic or domestic cycles.

M. arvalis is a very sensitive intermediate host, in which cysts are fertile and contain numerous protoscolices. The metacestodes develop primarily in the liver, like in this case, but can be observed in other organs, especially the brain and lungs.

Besides rodents, humans and a number of mammals, may also acquire *E. multilocularis* and become aberrant hosts which do not play a role in the transmission cycle. Cysts have been reported in dogs, cats, domestic and wild pigs, horses, and primates.[1]

The metacestode stage is characterized by an alveolar structure, composed of numerous small vesicles. This stage has a characteristic exogenous tumor-like proliferation, which leads to infiltration of the affected organs and, in progressive cases, to severe disease and even death.

In man, *E. multilocularis infection* is considered as the most severe helminthic infection, lethal without treatment.

This zoonosis is known in Northern America and Northern and Central Eurasia.

The spatial distribution of infected rodents is heterogeneous. The average prevalence of *E. multilocularis* metacestodes in rodents is generally low (<1%) in areas of endemic infection in central Europe. But they may be higher locally, in "hot spots" of intensive transmission. For example, up to 21 and 39% of *Arvicola terrestris* were found to be positive in high-endemicity areas.[2,3]

Following the successful oral vaccination campaigns against rabies in Europe, the fox population densities have increased from 1985 onwards in many countries. Moreover, red foxes are now more and more frequent in urban areas not only in Great Britain as known since the 1930s, but in other countries including 20 of the 30 largest Swiss

towns. Their number tends to increase as reported in Zurich, Switzerland (20-fold from 1985 to 1997).[1,3]

Strong evidence for the existence of an urban cycle has been provided from many European cities. *E. multilocularis* prevalences in Zurich of 47% (61/129) in foxes from the city and of 67% (82/123) in foxes from the adjacent rural area are reported. Furthermore, metacestodes of *E. multilocularis* were found in 14% (19/135) of water voles (*A. terrestris*) in a Zurich city park, and two animals harbored protoscolices of the parasite. Therefore the establishment of urban cycles of *E. multilocularis* and potential risk for urban residents and pet animals has become an important issue.[2,4]

In this animal, metacestodes of *T. taeniaeformis* (*Strobilocercus fasciolaris*) were also found. This co-infection has already been reported in rare cases.[4,5]

AFIP Diagnosis: Liver: Hydatid cyst, field vole (*Microtus arvalis*), rodent.

Conference Comment: The contributor provides an excellent overview of *E. multilocularis*. Additional species of *Echinococcus* include *E. granulosus*, *E. oligarthus*, and *E. vogeli*. The latter two involve sylvatic cycles in Central and South America, with felids and canids as definitive hosts, respectively, and rodents as intermediate hosts. *E. vogeli* may infect humans.[6]

Readers are encouraged to compare this case to WSC 7 / Case 2, 2006-2007 – *Echinococcus granulosus* in a mountain goat. In contrast to *E. multilocularis*, *E. granulosus* forms a unilocular hydatid cyst and is usually not invasive.

Skeletal muscle and esophagus were present in some sections.

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology.

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References:

1. Deplazes P, Eckert J: Veterinary aspects of alveolar echinococcosis—a zoonosis of public health significance. Vet Parasitol 98:65-87, 2001
2. Gottstein B, Saucy F, Deplazes P, Reichen J, Demierre G, Busato A, Zuercher C, Pugin P: Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? Emerg Infect Dis 7:408-412, 2001
3. Eckert J, Deplazes P: Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 17:107-135, 2004

4. Hofer S, Gloor S, Müller U, Mathis A, Hegglin D, Deplazes P: High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. Parasitol 120:135-142, 2000
5. Pétavy AF, Tenora F, Deblock S: Co-occurrence of metacestode of *Echinococcus multilocularis* and *Taenia taeniaeformis* (Cestoda) in *Arvicola terrestris* (Rodentia) in France. Folia parasitol 50:157-158, 2003
6. Barker IK, Van Dreumel AA, Palmer N: The alimentary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 290-292. Academic Press, Inc., San Diego, California, 1993

SLIDE 92
CONFERENCE 23 / CASE IV – 051203 (AFIP 2983846)

Signalment: 6-year-old, male, Bernese Mountain Dog, canine

History: Responsive anemia, splenomegaly.

Gross Pathology: Splenomegaly with infarction.

Histopathologic Description: Spleen: The red pulp is diffusely expanded by variably differentiated histiocytes with moderate to marked hemophagocytosis. The cells have abundant, pale eosinophilic, often coarsely vacuolated cytoplasm and oval and occasionally indented nuclei. The cytoplasm of histiocytes frequently contains hemosiderin and occasionally red blood cell precursors and granulocytes. Mitoses are 1-3 per HPF. There is mild anisocytosis and anisokaryosis. Marked extramedullary hematopoiesis is present. Extensive areas of red pulp infarction associated with thrombosis are present within some sections.

Immunohistochemistry: The neoplastic cells express CD11d, CD18 and CD45 antigens.

Contributor's Morphologic Diagnosis: Hemophagocytic histiocytic sarcoma, spleen, Bernese Mountain Dog, canine

Contributor's Comment: Histiocytic disorders of dogs include histiocytoma, localized histiocytic sarcoma (HS), disseminated HS (equivalent to malignant histocytosis), and the reactive histiocytoses: cutaneous histiocytosis and systemic histiocytosis. A common element to these diseases is proliferation of dendritic cells (DC) of either Langerhans cell (epithelial DC) or interstitial DC lineage.[1]

Hemophagocytic HS is a distinctive clinical and pathologic entity, marked by an aggressive clinical course dominated by splenomegaly, regenerative anemia, thrombocytopenia, hypoalbuminemia, and hypocholesterolemia. Hemophagocytic histiocytic sarcomas of the spleen (and bone marrow) arise from splenic red pulp (and

bone marrow) macrophages. Hemophagocytic HS is prevalent in the same breeds affected by localized and disseminated HS, which include Bernese Mountain Dog, Golden Retriever, Rottweiler and Labrador Retriever. Hemophagocytic HS initially involve spleen and bone marrow simultaneously and later spread to liver and lungs often via insidious intravascular invasion with minimal mass formation. The splenic lesions consist of diffuse splenomegaly often with additional, ill-defined masses and infarction. Neoplastic histiocytes dominantly expressed MHC class II and the leuko-integrin CD11d/CD18; expression of CD11c/CD18 and CD1c is far less prevalent. In contrast, localized and disseminated HS as previously reported dominantly expressed CD1c, CD11c, and MHC class II and lacked expression of CD11d, which supported their origin from interstitial DC.[2,3]

The clinical presentation of hemophagocytic HS is often confused with immune-mediated hemolytic anemia (IMHA) or more specifically with Evan's syndrome, because thrombocytopenia usually occurs concurrently. The direct anti-globulin (Coombs) test is negative in dogs with hemophagocytic HS. In contrast, dogs with IMHA typically have higher serum bilirubin concentrations. Additionally, hypoalbuminemia and hypocholesterolemia were common in dogs with hemophagocytic HS, which also helped to differentiate it from IMHA.[2]

Hemophagocytic HS of dogs shares clinicopathologic and morphologic features with a rare subtype of malignant histiocytosis of humans, also referred to as histiocytic medullary reticulosis. In histiocytic medullary reticulosis, anemia, thrombocytopenia, and hyperbilirubinemia occur in association with diffuse hepatosplenomegaly and bone marrow infiltration. Cytologically atypical, hemophagocytic histiocytes expand the splenic red pulp and invade the hepatic sinusoids without significant mass formation.[4]

AFIP Diagnosis: Spleen: Hemophagocytic histiocytic sarcoma, Bernese Mountain Dog (*Canis familiaris*), canine.

Conference Comment: The contributor provides an excellent and thorough summary of hemophagocytic histiocytic sarcoma to include clinical presentation, associated clinical pathologic findings, breeds affected, biological behavior, and immunohistochemical findings.

As previously stated by the contributor, histiocytic disorders in the dog include histiocytoma, localized histiocytic sarcoma (HS), disseminated HS (equivalent to malignant histocytosis), and the reactive histiocytoses: cutaneous histiocytosis and systemic histiocytosis. Histiocytoma is a localized tumor of epidermal Langerhans cells. Localized and disseminated HS likely arise from interstitial dendritic cells prevalent in almost all organs and tissues with the exception of the brain. The reactive histiocytoses are complex disorders likely associated with disordered immune regulation characterized by infiltration or proliferation of lymphocytes and perivascular interstitial dendritic cells of the dermis and subcutis. As previously stated by the contributor,

hemophagocytic HS is a proliferative disease of splenic red pulp and bone marrow macrophages; it is not a proliferative disorder of interstitial dendritic cells, as are most histiocytic sarcomas in the dog.[2,3]

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References:
1. Moore PF: Canine Histiocytosis - http://www.histiocytosis.ucdavis.edu/
2. Moore PF, Affolter VK, Vernau W: Canine hemophagocytic histiocytic sarcoma: a proliferative disorder of CD11d+ macrophages. Vet Pathol 43:632-645, 2006
3. Affolter VK, Moore PF: Localized and disseminated histiocytic sarcoma of dendritic cell origin in dogs. Vet Pathol 39:74-83, 2002
4. Warnke RA, Kim H, Dorfman RF: Malignant histiocytosis (histiocytic medullary reticulosis). I. Clinicopatholigic study of 29 cases. Cancer 35:215-230, 1975

SLIDE 93
CONFERENCE 24 / CASE I – 06-1650 (AFIP 3027077)

Signalment: One-year-6-month old gelding donkey

History: Generalized nodular dermatitis that was present for 2 months. The donkey was clinically normal. Skin biopsies were taken at time of castration.
Gross Pathology: None provided other than the history of generalized nodular dermatitis.

Histopathologic Description: Specimen is skin with numerous protozoan parasitic cysts in the dermis. These cysts contain many bradyzoites within a parasitophorous vacuolar membrane. The parasitophorous vacuoles appear to be surrounded by a layer of hypertrophied fibrocytes with an outer layer of collagen. Many of the cysts are degenerate/necrotic and associated with marked eosinophilic and granulomatous (epithelioid macrophages and multinucleated giant cells) inflammation that occasionally forms eosinophilic granulomas with central eosinophilic “abscesses". There is a widespread multifocal mild lymphoplasmacytic and eosinophilic superficial perivascular dermatitis with mild widespread acanthosis of the overlying epidermis.

Contributor’s Morphologic Diagnosis: Multifocal marked eosinophilic granulomatous nodular dermatitis with intralesional protozoal cysts consistent with *Besnoitia bennetti.*

Contributor’s Comment: Species of the genus *Besnoitia* are parasites of a wide range of domestic and wild species, including cattle, goats, reindeer, horses, donkeys, opossums, rabbits, rodents, and lizards.[1] *Besnoitia* species are unlike other members of the coccidian family Sacrocystidae (i.e. *Sarcocystis, Toxoplasma,* and *Neospora*) in

that the cysts containing bradyzoites are found mainly within fibroblasts in the skin, subcutaneous tissues, and fascia.[2] *Besnoitia besnoiti*, which infects cattle as the intermediate host, causes significant economic losses in countries outside the U.S. due to condemnation of hides at slaughter. The less common *B. bennetti* affects equids.[1] Over the past ten years, cases of *B. bennetti* have been infrequently reported in donkeys in the United States.[1,3]

Clinical signs of *B. bennetti* in donkeys are common with other forms of besnoitiosis, and include varying intensities of alopecia, lichenification, hyperpigmentation, exudative crusts, anorexia, and lethargy.[3] In donkeys, areas of lichenification and raised dermal nodules were most commonly seen on the head, base of the ears, withers, inner aspects of the hind limbs, and the perineal and perivulvar regions.[1,3] Cysts in the ocular sclera, submucosa of the inner lip, eyelids, and external nares were observed in some affected donkeys.[1] Pruritis was associated with the skin lesions in two published cases.[1,3] Clinical differentials for dermatitis in donkeys include chronic bacterial dermatitis, dermatophytosis, autoimmune dermatoses, sarcoidosis, parasitic dermatitis (i.e. pediculosis), unusual manifestation of multi-systemic disease, and nutritional deficiencies.[3]

Tissue cysts containing *B. bennetti* bradyzoites can reach up to 650 um in diameter and are visible to the naked eye as white to glistening white nodules embedded in the host tissue.[1,3] The thick walls of the cysts are comprised of three layers recognizable by light microscopy. The outermost layer consists of hyaline connective tissue, the middle layer encloses the nucleus and cytoplasmic organelles of the host fibroblast, and the thin, innermost layer represents the parasitophorous vacuolar membrane surrounding myriads of bradyzoites. The bradyzoites vary in size and shape and can be seen by TEM to contain a conoid, micronemes, rhoptries, a nucleus, amylopectine, a mitochondrium, and dense granules. The characteristics of these structures can be used to identify the organism to the genus level, while immunohistochemistry is useful in identifying the species.

Inflammatory responses associated with the cysts can be seen histologically as perivascular to interstitial accumulations of lymphocytes and eosinophils. Ruptured cysts incite a granulomatous response. A study of a *B. bennetti* outbreak in a heard of donkeys reported that degenerating cysts were most common in animals undergoing trimethoprim-sulfamethoxazole treatment.[1] In a study by Davis et al, post-treatment skin biopsies showed the presence of cysts without histological evidence of inflammation, suggesting that chronic infection with *Besnoita* sp. may result in a subclincally affected state in some animals.[3]

Like other coccidian parasites, the *Besnoitia* life cycle involves both a definitive and intermediate host. Domestic cats have been recognized as the definitive host for the three *Besnoita* species whose life cycles are known.[1] Transmission to the intermediate host involves ingestion of sporulated oocysts in feed or water contaminated by feces of the definitive host. However, only the tissue cyst and bradyzoite states of the *B. bennetti* life cycle have been described. Also, results of studies in donkeys suggest that

domestic cats are unlikely to play a role in transmitting the disease. The presence of extracellular bradyzoites in the dermal crusts of infected donkeys lends support to the proposal of an arthropod vector.

AFIP Diagnosis: Haired skin and subcutis: Dermatitis, granulomatous and eosinophilic, multifocal, moderate, with protozoal cysts etiology consistent with *Besnoitia* sp., donkey (*Equus asinus*), equine.

Conference Comment: The contributor provides an excellent summary of *Besnoitia bennetti* in equids. Jubb, Kennedy, and Palmer includes 4 layers that comprise the mature cyst wall: 1) the outermost layer of compressed dermal collagen, 2) a very thin homogenous intermediate zone, 3) the host cell with peripheralized nuclei, and 4) the innermost parasitophorous vacuole filled with crescentic bradyzoites.[4]

Other species of *Besnoitia* and their intermediate hosts are listed below:[4,5,6]
B. besnoiti – cattle[1,4]
B. caprae – goats[1]
B. tarandi – caribou, reindeer, mule deer, musk ox[1,6]
B. wallacei, *B. akodoni* – rodents[1,4,5]
B. jellisoni – deer mice, kangaroo rat, opossum[6]
B. oryctofelisi – rabbits[1]
B. darlingi – opossum, lizard[1,5]

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology.

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References:
1. Dubey JP, Sreekumar C, Donovan T, Rozmanec M, Rosenthal BM, Vianna MCB, Davis WP, Belden JS: Redescription of *Besnoitia bennetti* (Protazoa: Apicomplexa) from the donkey (*Equus asinus*). Int J Parasitol 35:659-672, 2005
2. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW: Veterinary Parasitology, p. 234. Blackwell Publishing Professional, New York, New York, 1987
3. Davis WP, Peters DF, Dunstan RW: Besnoitiosis in a miniature donkey. Vet Dermatol 8:139-143, 1997
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, pp. 709-710. Elsevier Saunders, Philadelphia, Pennsylvania, 2007

5. Levine ND: Veterinary Protozoology, pp. 256-259. Iowa State University Press, Ames, Iowa, 1985
6. Gardiner CH, Fayer R, Dubey JP: An Atlas of Protozoan Parasites, 2nd ed., pp. 49-52. Armed Forces Institute of Pathology, Washington, D.C., 1998

SLIDE 94
CONFERENCE 24 / CASE II – GUVS 2 (AFIP 3032057)

Signalment: 6-year-old, female, Mediterranean spur-thighed tortoise, chelonian, *Testudo graeca*

History: An adult captive bred Mediterranean spur-thighed tortoise (*Testudo graeca*) had a history of urolithiasis five years previously, at which time a urolith of undetermined composition had been removed from the urinary bladder by coeliotomy. After an uneventful recovery, the tortoise remained healthy until April 2006, when it became depressed, lethargic and anorexic over a one month period. On clinical examination in May 2006, it also appeared to be lame in the right forelimb. Haematological examination revealed anaemia and leucopaenia. There were markedly increased plasma concentrations of uric acid and urea, along with hyponatraemia, hypochloraemia and hyperkalaemia. Consistent hyperuricaemia was demonstrated on repeat biochemical examination during the next few days. Radiographic findings were unremarkable. The tortoise was given daily supportive fluid therapy (Critical Care, Leith Petwerks, 5 g powder in 20 ml water), along with enrofloxacin (Baytril 2.5% Oral Solution, Bayer, 10 mg/kg). Despite treatment, it became progressively more lethargic and was euthanased one week later by intracoelomic injection of barbiturate solution.

Gross Pathology: At postmortem examination, the tortoise weighed 521 g and the carapace was 17 cm long and 11 cm wide. There was little body fat. Both kidneys were firm and pale. Extensive pale yellow granular deposits were visible on the capsules and cut surfaces of the kidneys. There were pale yellow granular deposits in multiple appendicular joints, including the right and left coxofemoral, stifle, elbow, carpal and tarsal joints.

Laboratory Results:

Haematology (Reference ranges):
Packed cell volume: 19% (26-43%)
Total leucocyte count: 4.1 x 10^9/L (6.0-48.0 x 10^9/L)
Differential leucocyte count (Reference ranges not available):
Heterophils: 1.6 x 10^9/L
Lymphocytes: 1.2 x 10^9/L
Monocytes: 1.0 x 10^9/L

Eosinophils: 0.0 x 10^9/L
Basophils: 0.4 x 10^9/L

Biochemistry (Reference ranges):
Sodium: 89 mmol/L (111-154 mmol/L)
Potassium: 12.6 mmol/L (2.2-6.1 mmol/L)
Chloride: 79 mmol/L (83-120 mmol/L)
Calcium: 5.0 mmol/L (2.0-3.5 mmol/L)
Phosphate: 0.9 mmol/L (0.5-3.5 mmol/L)
Urea: 129.2 mmol/L (0.2-16.0 mmol/L)
Uric acid: 3252 µmol/L (35-707 µmol/L)
Creatinine: 31 µmol/L (9-218 µmol/L)
Cholesterol: 2.4 mmol/L (0.7-6.1 mmol/L)
Total protein: 33 g/L (13-61 g/L)
Albumin: 11 g/L (5-28 g/L)
Globulin: 22 g/L (Reference range not available)
Aspartate aminotransferase: 327 U/L (18-534 U/L)

Histopathologic Description: The kidney has severe, generalised, urate nephrosis, with deposition of lightly basophilic, radiating, fibrillary, crystalline urates (gouty tophi) in renal tubules and the renal interstitium. There is necrosis of renal tubular epithelial cells and destruction of tubules. Interstitial oedema and fibroplasia are evident. Infiltrates of heterophils are present, especially in areas of necrosis. There are also mild, multifocal interstitial infiltrates of lymphocytes. Barbiturate change is evident at the periphery of the kidney.

Contributor's Morphologic Diagnosis: Kidney: Nephrosis, generalised, severe, with urate deposits (gouty tophi), Mediterranean spur-thighed tortoise, *Testudo graeca*

Contributor's Comment: This tortoise had bilateral urate nephrosis (renal gout) and generalised urate arthritis (articular gout). The renal gout was characterised by deposition of urate crystals (gouty tophi) in the kidney, accompanied by nephrosis and interstitial nephritis. Causes of renal disease in tortoises include dehydration, abnormal diets, viral, bacterial or parasitic infection and nephrotoxins such as aminoglycosides.[1] Many cases of renal gout are idiopathic. Obstruction of the lower urinary tract in tortoises has been associated with urolithiasis, particularly deposition of urate crystals, and coelomic masses such as neoplasia.[1,2] The cause of the renal gout in this tortoise was unknown, but the history of urolithiasis may be significant, possibly indicating a predisposition to deposition of urate crystals in the urinary tract.

Clinical manifestations of renal disease in this case were depression, lethargy and anorexia, accompanied by anaemia, leucopaenia and elevated plasma concentrations of uric acid and urea. Concentrations of urea and creatinine are not reliable indices of renal disease in tortoises, whereas uric acid concentrations greater than 1000 µmol/l are usually due to renal insufficiency.[1] Elevated uric acid concentrations may also occur

in tortoises with dehydration and hepatic disease.[1] Uric acid concentrations greater than 1500 µmol/l are associated with deposition of urate crystals in tissues.

The articular gout in this tortoise was associated with granulomatous inflammation and joint erosions in multiple appendicular joints. Arthritis in tortoises may be caused by deposition of urate crystals (articular gout) or other mineral salts (pseudogout) in or around joints, bacterial infection (septic arthritis) or degenerative joint disease related to trauma or aging. Visceral, articular and periarticular gout are common manifestations of renal disease, but may also be caused by prerenal factors, such as dehydration.[1,3]

AFIP Diagnosis: Kidney: Nephritis, tubulo-interstitial, chronic, diffuse, mild, with marked interstitial fibrosis, tubular loss, and numerous urate tophi (gout), Mediterranean spur-thighed tortoise (*Testudo graeca*), chelonian.

Conference Comment: Gout is the deposition of sodium urate crystals or urates in tissue and occurs in humans, birds, and reptiles (species that lack the enzyme uricase). There are no convincing reports of gout in either dogs or cats. Even in Dalmatian dogs, with their high serum uric acid concentrations, do not appear to develop gout. As previously stated by the contributor, causes of gout include impaired excretion by the kidneys (severe renal disease, postrenal obstruction, dehydration, nephrotoxic drugs) or overproduction of uric acid (high-protein diets) leading to elevated plasma uric acid concentration (hyperuricemia) resulting in the precipitation of urates on many visceral and/or articular surfaces. Additionally, vitamin A deficiency and excess dietary calcium may result in gout.[4,5,6,7,9]
There are two forms of gout in birds and reptiles: visceral and articular. Visceral gout is more common and presents grossly as a thin layer of gray granules or white/gray chalky patches on the visceral serosae, especially the parietal pericardium and the kidneys. The articular form is rare and is characterized by swollen joints with white chalky deposits in and around joints. The joints of the extremities are most commonly affected.[4,6,8]

Gout is a disease of purine (adenine, guanine) metabolism. Uric acid and urates are the end products of purine metabolism and are eliminated as semisolid urates in birds and reptiles. The degradation of purines to uric acid is outlined in the figure below.[4,8]

Adenine > hypoxanthine > xanthine > uric acid
Guanine > xanthine > uric acid

Both of these pathways require xanthine oxidase to form xanthine from hypoxanthine and uric acid from xanthine.[8]

Microscopically, aggregates of acicular birefringent urate crystals (tophi) or the spaces left after the crystals dissolve during preparation of paraffin embedded histologic sections are pathognomonic of gout and are usually surrounded by numerous

neutrophils, macrophages, and giant cells.[4] Since formalin fixation leaches out most of the water-soluble urate deposits, collection of tissues in absolute ethyl alcohol is preferable.

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.[3,6,8]

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References:

1. McArthur S: Problem-solving approach to common diseases of terrestrial and semi-aquatic chelonians. In: Medicine and Surgery of Tortoises and Turtles, eds. McArthur S, Wilkinson R, Meyer J, pp. 361-366. Blackwell Publishing, Oxford, United Kingdom, 2004
2. Homer BL, Berry KH, Brown MB, Ellis G, Jacobson ER: Pathology of diseases in wild desert tortoises from California. J Wildl Dis 34(3):508-523, 1998
3. Casimire-Etzioni AL, Wellehan JFX, Embury JE, Terrell SP, Raskin RE: Synovial fluid from an African spur-thighed tortoise (*Geochelone sulcata*). Vet Clin Pathol 33(1):43-46, 2004
4. 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
5. 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
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 60-61. Williams & Wilkins, Baltimore, Maryland, 1997
7. Donoghue S: Nutrition. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., p. 281. Saunders Elsevier, St. Louis, Missouri, 2006
8. Mader DR: Gout. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., pp. 793-800. Saunders Elsevier, St. Louis, Missouri, 2006
9. 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

SLIDE 95
CONFERENCE 24 / CASE III – 06-0150-2 (AFIP 3050733)

Signalment: Adult, male, Northern water snake (*Nerodia sipedom*)

History: Refused food one week before death.

Gross Pathology: The small and large intestines are mildly distended to 10 mm in diameter, and the lumen is filled with necrotic debris. The serosal blood vessels are congested. The liver has multiple 2 mm in diameter white plaques. There are many up to 5 x 2 x 2 mm dark brown to black nodules within the hepatic and renal parenchyma and overlying the capsules.

Gross morphologic diagnoses:
1. Necrotizing enteritis, colitis, gastritis and hepatitis.
2. Pentastome parasites within liver and kidney.

Laboratory Results: Heart blood culture: *Providencia ruttgeri*
Cytology (stomach, fecal material), fecal screening: Amoebic trophozoites; minimal histiocytic inflammation the gastric sample and none in feces

Histopathologic Description: Intestine: Diffusely and transmurally there is necrosis of all sections on the slide. The lumen is filled with necrotic debris and the mucosa is replaced by eosinophilic and karyorrhectic debris mixed with cross sections of cestodes with calcareous corpuscles, and many bacteria. The submucosa, tunica muscularis and serosa contain numerous up to 30 um in diameter amoebic trophozoites, eosinophilic and karyorrhectic debris and some viable macrophages and fewer heterophils.

Contributor's Morphologic Diagnosis: Small intestine and large intestine: Enteritis and colitis, necrotizing, transmural, subacute, marked with many amoebic trophozoites and some cestodes.

Contributor's Comment: Amoebiasis in reptiles, caused by *Entamoeba invadens*, is an important disease in captive snakes, lizards, and chelonians. The disease is characterized by ulcerative colitis and hepatitis, and is one of the most common gastrointestinal diseases in snakes.[2] *E. invadens* is morphologically, or biologically similar to *E. histolytica* in humans, but the two species are distinguished by the host species and temperature tolerance.[5] *E. invadens* prefers an optimum temperature of 80-84.2 degrees F and cannot be transmitted to warm-blooded animals.[4] The biological host for *E. invadens* is thought to be the herbivorous turtles, in which a symbiotic relationship without any pathogenicity may be observed. In the intestine of turtles, amoebic protozoa take nourishment from ingested plants to form cysts, and complete their life cycle without being pathogenic to the host. However, in the intestine of the other carnivorous reptiles, the amoeba is unable to take the specific nourishments it requires, and has to invade the intestinal mucosa to survive, resulting in harmful infection of the host.[5] The immune status of the host also plays a role in protozoal pathogenicity.[2]

This protozoa moves and feeds by forming pseudopodia, changing shape while in the trophozoite state. The cyst is a resting stage in which a wall is produced by the trophozoite to encapsulate and protect the parasite while it is in the abiotic environment.[2]

The characteristic microscopic lesions induced by *E. invadens* are severe intestinal erosion, ulceration, and inflammation often with a fibrinonecrotic pseudomembrane. The ileum and colon are the most severely affected intestinal segments. Hepatic necrosis can also be present.[1]

This water snake had classic lesions of amoebiasis, including enteritis and gastritis. The presence of organisms and necrotic lesions within other organs (liver, kidney, pancreas, spleen, epididymis, stomach) was a result of blood invasion and spread.

There are several genera of cestode parasites which affect reptiles. *Acanthotaenia, Crepitdobothrium, Ophiotaenia, Spirometra, Bothriocephalus, and Bothridium* are described affecting snakes. *Ophiotaenia* is the most common in North American snakes and is acquired by ingestion of infected frogs.[3]

This snake was also infected with pentastomes, a relatively common snake lung parasite which can be found in a nymph stage within other tissues. This parasite has an indirect life cycle that involves fish, amphibians or mammals. Heavy infestations in snakes may cause respiratory problems. No adult pentastomes were seen in the lungs of this individual, but hyperplasia of the pulmonary smooth muscle could be related to the previous presence of pentastomes within the lumen. Parasites were present in the liver and kidneys.

A pure culture of *Providencia ruttgeri*, an opportunistic organism in reptiles, grew from heart blood culture. Bacterial septicemia is a common finding in snakes infected with *Entamoeba invadens* as erosion of the intestinal mucosal barrier allows passage of bacteria from the gut lumen into the bloodstream.

No bacteria were isolated from the liver, and no *Salmonella* or *Shigella* grew from intestinal culture.

AFIP Diagnoses: 1. Intestine: Enteritis, necrotizing, transmural, acute to subacute, diffuse, severe, with fibrin, edema, and numerous amoebic trophozoites, Northern water snake (*Nerodia sipedom*), reptile.
2. Intestine: Intramural cestodes, few.
3. Intestine: Intramural pentastomes, few.

Conference Comment: The contributor provides a thorough summary of *Entamoeba invadens* as well as providing additional information about cestodes that parasitize reptiles and pentastomes in snakes.

Entamoeba histolytica causes amebic dysentery in humans and nonhuman primates (especially Old World), and rarely infects other species (dogs, cats, pigs, cattle). Many infections are asymptomatic. Microscopically, flask-shaped ulcers in the colon, with a narrow neck through the mucosa and a broad base in the submucosa are characteristic. Trophozoites may disseminate to other organs, especially the liver and brain, where they form amebic abscesses. A distinctive feature of the necrotizing lesions is the almost complete lack of a cellular inflammatory response.[6,7,8,9]

Amebic trophozoites and cysts are PAS and GMS positive.[5,8]

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References:

1. Frye FL: Applied clinical nonhemic parasitology of reptiles. In: Biomedical and surgical aspects of captive reptile husbandry, ed. Frye LF, 2nd ed., vol. 1, pp. 284-285. Krieger, Malabar, Florida, 1991
2. Greiner EC, Mader DR: Parasitology. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., p. 347. Saunders Elsevier, St. Louis, Missouri, 2006
3. Hernandez-Divers SJ: Appendix B Reptile parasites – summary table. In: Reptile Medicine and Surgery, ed. Mader DR, 2nd ed., p.1166. Saunders Elsevier, St. Louis, Missouri, 2006
4. Kohler G: Diseases of amphibians and reptiles, pp. 116-119. Krieger, Malabar, Florida, 2006
5. Kojimoto A, Uchida K, Horii Y, Okumura S, Yamaguchi R, Tateyama S: Amebiasis in four ball pythons, *Python reginus*. J Vet Med Sci 63:1365-1368, 2001
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., p. 578. Williams & Wilkins, Baltimore, Maryland, 1997
7. 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.839-840. Elsevier Saunders, Philadelphia, Pennsylvania, 2005
8. Brown CB, Baker DC, Barker IK: Alimentary system. In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, p. 277. Elsevier Saunders, Philadelphia, Pennsylvania, 2007
9. Gelberg HB: Alimentary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 384. Mosby Elsevier, St. Louis, Missouri, 2007

SLIDE 96
CONFERENCE 24 / CASE IV – 040166 43 (AFIP 3027300)

Signalment: Three-year-old, female, Nubian goat (*Capra hircus*)

History: This animal was part of a herd of goats used for the production of antibodies to various antigens. The goats are housed outdoors on pastures and have free access to open sheds for shelter.

This goat arrived at USAMRIID on 30 December 2003 and was placed in a separate pasture away from the resident herd for a 30-day quarantine period. It appeared to be healthy during a physical exam 6 days after its arrival. However, 25 days later, the goat was noted to be vocalizing and having difficulty walking. When it did walk, it consistently circled to the left. Physical examination at this time revealed that its body temperature, pulse, and respiration rate were unremarkable. The goat was placed in an indoor stall and treatment with an anti-inflammatory drug (flunixin meglumine) and antibiotics (enrofloxacin and ampicillin) was begun.

Although the animal ate and drank normally, there was no improvement in its locomotion. Two days after initial clinical presentation, the goat also had left horizontal nystagmus. The following day, it was noted to be repeatedly rubbing an area of alopecia and scaliness on the right side of the neck and shoulder.

On the fourth day after clinical presentation, the goat was recumbent and unable to rise. Euthanasia was then performed and the carcass was submitted for a complete necropsy

Gross Pathology: The only lesion noted grossly was a large area of alopecia over the right shoulder; within this area there was cutaneous ulceration covered by a scab.

Laboratory Results: Hematology and serum chemistry results from blood samples collected on the first day of clinical signs and just before euthanasia were unremarkable. Serology results on samples collected on the day of euthanasia were negative for antibodies against caprine Arthritis-Encephalitis virus and pseudorabies virus.

Histopathologic Description: Spinal cord (transverse and sagittal sections): Multifocally within the white matter there are degenerate axons (characterized by axonal swelling and increased eosinophilia) and spongiform change caused by axonal loss and myelin sheath ectasia, often accompanied by astrocytic hypertrophy and hyperplasia. Multifocal necrosis and microcavitation of the white matter is also present and is accompanied by infiltrates of low to moderate numbers of macrophages with abundant foamy cytoplasm (gitter cells); these lesions are more abundant and prominent in slides from histology block 42. Multifocal perivascular infiltrates of lymphocytes, plasma cells, and fewer macrophages are present in the meninges and white matter – with occasional extension into adjacent gray matter; numbers of such inflammatory cells vary from low to moderate to numerous.

Contributor's Morphologic Diagnosis: Spinal cord: Multifocal axonal degeneration and loss, moderate, with astrocytosis, white matter necrosis and histiocytic inflammation, and nonsuppurative perivascular meningomyelitis

Contributor's Comment: In addition to the lesions in the spinal cord, multiple foci of white matter necrosis with cavitation, gitter cell infiltration, and nonsuppurative perivascular inflammation are also present within the brain. Within the white matter of the cerebrum, a single nematode parasite was found. The morphology of this nematode is characteristic of a metastrongyle and is consistent with *Parelaphostrongylus tenuis* (*P. tenuis*).[1]

The normal hosts of *P. tenuis* are white-tailed deer (*Odocoileus virginianus*). Adult worms reside in the subarachnoid space of the brain or spinal cord of the deer; this location is the basis for a common nickname for *P. tenuis*: "the meningeal worm".[2] Eggs produced by female worms may be carried by the venous circulation to the lungs where they hatch or the eggs may hatch on the meninges and then the first-stage larvae enter the venous circulation to be carried to the lungs.[3] Either way, first-stage larvae in the lungs migrate up the bronchial tree and are coughed up, swallowed, and eventually passed in the feces. A wide variety of gastropod species can serve as intermediate hosts in which the larvae mature to third-stage larvae. Deer become infected when they accidentally ingest gastropods containing third-stage larvae. These larvae are released from the gastropod tissues in the abomasum and they then migrate into the peritoneal cavity, eventually following spinal nerves to enter the dorsal horn of the spinal cord.[2,4] Worm maturation to the adult stage occurs within the spinal cord in 20-30 days and the parasites then follow the dorsal nerve roots to enter the subarachnoid space.[2]

White-tailed deer are well-adapted hosts for *P. tenuis* and rarely display any clinical signs associated with infections. However, serious to fatal neurologic disease due to *P. tenuis* infection has been reported in numerous other ruminant and ruminant-like species.[2,4,5] Among domestic species, cattle are highly resistant to infection whereas llamas are particularly susceptible.[2] Goats and sheep appear to be intermediate in susceptibility.

The neurologic disease associated with *P. tenuis* infections in non-adapted hosts is caused primarily by prolonged and aberrant migration by the parasites within the spinal cord and/or brain; CNS damage secondary to the associated inflammation also plays an important role.[2,4] Trauma caused by the migrating worms produces multifocal necrosis and cavitation, sometimes with acute hemorrhage, followed by inflammation and axonal degeneration adjacent and distal to these foci. The inflammatory cell infiltrates usually consist primarily of lymphocytes, macrophages, and plasma cells, but may also include eosinophils.[2,3,5]

Clinical signs typically present initially as paresis or paralysis of one or more limbs – usually beginning with the hindlimbs – and reflect spinal cord injury. These may progress slowly or rapidly to recumbency; however, some animals may remain static or even recover.[2-4]

In goats, infection of the brain has been reported to cause circling – as in this case.[3] Larval migration through dorsal nerve roots has caused pruritus leading to self-trauma in goats.[3] Although nerve root involvement was not documented in this case, the goat

was noted to have an area of apparent pruritus over the right shoulder with secondary alopecia and cutaneous ulceration from self-trauma.

A tentative diagnosis of cerebrospinal parelaphostrongylosis may be made based on clinical signs, possible exposure to infected gastropods, and/or typical CNS lesions. However, the definitive diagnosis currently requires finding larvae with the appropriate morphology within the CNS; this is often difficult due to the low numbers of parasites present in aberrant hosts. An antigen-capture ELISA for detecting *P. tenuis* antigens in the cerebrospinal fluid has been developed but it is not yet commercially available.[2]

According to the vendor from which this goat was purchased, this animal was housed indoors and fed a commercial pelleted diet for 9 months before its shipment to USAMRIID; these conditions mean it is highly unlikely that the goat was infected at the vendor's facility. In natural *P. tenuis* infections of goats, the latest reported onset of clinical signs is 9 weeks after the animals were removed from pasture; therefore the possibility that this goat was infected before its being housed indoors at the vendor's facility is remote.[3]

This animal initially presented with clinical signs of neurologic disease 31 days after its arrival and placement into the outdoor pasture. Experimental studies of white-tailed deer have shown that *P. tenuis* can reach the spinal cord in as little as 6 days after ingestion of third-stage larvae.[2] Experimental infection of goat kids produced neurologic signs 11 to 52 days after infective larvae were inoculated into the peritoneal cavity.[3] Therefore, the timing of the development of clinical disease in this case appears to be consistent with infection after its arrival.

Although white-tailed deer had not been seen in the pasture where this goat was housed at the time it developed initial clinical signs, a high deer population is present in the fields that adjoin this pasture and *P. tenuis* infection is highly prevalent in deer from this area of Maryland. The goat's pasture and the surrounding fields are low-lying and moist; these conditions favor gastropods and transmission of *P. tenuis* to the intermediate hosts.[3] This goat most likely became infected when it ingested a snail or slug that had been infected in the adjacent fields and then crossed over into the goat pasture.

Measures that can be taken to try to control problems with *P. tenuis* infections include reducing deer populations and deer access to pastures. Reducing habitats favorable to gastropods – especially along fence lines – can also be beneficial. Frequent de-worming of livestock during seasons when gastropods are active is also recommended in order to kill migrating third-stage larvae before they can reach the CNS.[2]

AFIP Diagnosis: Spinal cord, white matter: Axonal degeneration and loss, diffuse, marked, with digestion chambers, gitter cells, and mild lymphocytic meningomyelitis, Nubian goat (*Capra hircus*), caprine.

Conference Comment: The contributor provides an extensive and thorough review of *Parelaphostrongylus tenius*. Residents considered caprine arthritis encephalitis (CAE) virus and copper deficiency as differentials for this case.

Aberrant larval migration of nematode parasites through the CNS is referred to as cerebrospinal nematodiasis. Grossly, nematode larval migration often appears as necrohemorrhagic serpentine or linear tracts in the affected tissue. Included below is a chart of nematodes that cause central nervous system disease in animals.[6]

Parasite	Normal Host	Aberrant Host
NEMATODE MIGRATION IN ABERRANT HOST		
Angiostrongylus cantonensis	Rat	Dog
Baylisascaris procyonis	Raccoon	Dog
Elaphostrongylus rangiferi	Reindeer	Sheep, goat
Parelaphostrongylus tenius	Deer	Sheep, goat
Setaria digitata	Cattle	Sheep, goat, horse
ABERRANT NEMATODE MIGRATION IN NORMAL HOST		
Angiostrongylus vasorum	Dog (coyote)	
Dirofilaria immitis	Dog (cat)	
Stephanurus dentatus	Pig	
Strongylus spp.	Horse	

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology.

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References:

1. Gardiner CH, Poynton SL: An Atlas of Metazoan Parasites in Animal Tissues, pp. 22-29. Armed Forces Institute of Pathology, Washington, D.C., 1999
2. Nagy DW: *Parelaphostrongylus tenuis* and other parasitic diseases of the ruminant nervous system. Vet Clin Food Anim 20:393-412, 2004
3. Smith MC, Sherman DM: Goat Medicine, pp. 149-151. Lea & Febiger, Philadelphia, Pennsylvania, 1994
4. Jortner BS, Troutt HF, Collins T, Scarratt K: Lesions of spinal cord parelaphostrongylosis in sheep. Sequential changes following intramedullary larval migration. Vet Pathol 22:137-140, 1985
5. Nichols DK, Montali RJ, Phillips LG, Alvarado TP, Bush M, Collins L: *Parelaphostrongylus tenuis* in captive reindeer and sable antelope. J Am Vet Med Assoc 188:619-621, 1986

6. Zachary JF: Nervous system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 897. Mosby Elsevier, St. Louis, Missouri, 2007
**Opinions, interpretations, conclusions, and recommendations are those of the author(s) and are not necessarily endorsed by the U.S. Army.

SLIDE 97
CONFERENCE 25 / CASE I – V05-03814 (AFIP 3032063)

Signalment: Unknown age, female Alpaca, *Lama pacos*

History: The owners of this alpaca were 'out of town.' Another person was feeding, watering, and serving as caretaker of the herd of 12 alpacas. No reportable abnormalities had been noticed by the substitute caregiver, until going to feed the group and one of the alpacas was found dead. There was no evidence of a struggle where the alpaca was found. A local veterinarian performed a necropsy and submitted multiple unfixed tissues to the laboratory.

Gross Pathology: The liver had multiple caseous abscesses throughout, varying from 0.5 to 2.0 cm diameter. Lung tissue had multiple abscesses throughout all lobes ranging from 0.3 cm to 2.0 cm diameter. Lymph node and thymus exhibited multiple abscesses. The myocardium contained multiple abscesses in both the free walls and the interventricular septum, up to 0.7 cm diameter. Kidney cortices had multiple 0.1 to 0.2 cm foci. Multiple firm nodules were present in the spleen and the abomasal lymph node was 3.0 cm diameter.

Laboratory Results: *Coccidioides immitis* was isolated from lung and liver samples. Identification and/or isolation of acid fast bacteria were negative from lymph node and liver samples.

Histopathologic Description: Multifocal abscesses were present throughout the liver, lungs, multiple lymph nodes, heart, spleen, adrenal glands and kidneys. These abscesses were characterized by central necrosis rimmed by cellular debris and neutrophils, with an outer rim of histiocytes. Fungal organisms were observed within these lesions. The fungal structures varied in size from 15 to 45 microns diameter, presenting as spherules with a 2 micron thick, refractile/double contoured capsule. The fungal spherules contained numerous 3-4 micron diameter basophilic bodies, considered to represent endospores.

Contributor's Morphologic Diagnosis: Lung: Pneumonia, multifocal, necrotizing, neutrophilic and histiocytic (granulomatous/pyogranulomatous), with moderate to marked patchy coalescing hemorrhage, edema and intralesional fungal spherules (*Coccidioides immitis*).

Contributor's Comment: This case represented a widely disseminated presentation of *C. immitis* infection. Follow up history with this herd of alpaca revealed that a second alpaca died within a few days of this case death. Because of this and the apparent efficient dissemination of the organism, and further because of the reported high susceptibility of the llama (a close relative to alpacas) to Coccidioidomycosis, we are proposing that the alpaca very likely shares this same susceptibility to infection and probably widespread dissemination of *C. immitis*. This is certainly not a sufficient number of cases to make a definitive broad assumption, but will add to the data that may accumulate as the alpaca is introduced and flourishes in areas to which the species is naïve and in which *Coccidioides immitis* may exist and/or be endemic (discussed later below). The abundant organisms present, in even small sections of affected tissue, in this case provides an excellent example of the variable morphology of *C. immitis* that may not always be afforded in some of the other animal species in which Coccidioidomycosis may be diagnosed.

Coccidioidomycosis is a systemic mycosis caused by the dimorphic fungus *Coccidioides immitis*. In soil, the fungus exists in a mycelial (saprophytic) phase while in tissues or body fluids, the fungus exists as a spherule (parasitic) phase. Spherules are large structures with a capsule filled with endospores.[6] The mycelial phase of *C. immitis* is found only in a specific ecologic region, the Lower Sonoran life zone in North America, Mexico, and Central and South America. This region includes the arid or semi-arid areas of the southwestern United States (California, Arizona, Utah, Nevada, parts of southwest Colorado, New Mexico, and southwestern Texas), northwestern Mexico, Argentina, Colombia, Guatemala, Honduras, Paraguay, Venezuela, and probably Bolivia.[4] Natural infection is found in many mammalian species, at least 22 species of free ranging and captive wild animals, as well as humans.[1] Domestic animal species reported to be susceptible to *C. immitis* infection include horses, cattle, sheep, pigs, dogs, cats and non-human primates.[2,3,11] Disseminated coccidioidomycosis amongst animals has been reported to occur in dogs, cats and non-human primates.[2,3] However, as this report and others have demonstrated, New World (South American) Camelids, such as the llama, are extremely susceptible to *C. immitis* infection and commonly develop widely disseminated disease. [4,8] In this case we demonstrate the apparent high susceptibility and disseminated disease due to *C. immitis* in the alpaca. Alpacas are New World Camelidae in the same genus as llamas.[7] Alpacas are smaller than llamas and valued as a producer of finest quality wool.[10]

Infection by *C. immitis* is mainly by inhalation of arthroconidia present in wind borne dust, frequently resulting after dust storms.[4,6,7] Local traumatic inoculation and infection can occur, resulting in a fluctuating abscess, but is rare and dissemination from such a focus would be very unusual.[3,6] After inhalation, the arthroconidia transform into spherical endospores, which subsequently enlarge and undergo internal cleavage to form large spherules containing hundreds of endospores, a process taking several days.[6]

Spherules subsequently rupture, releasing endospores and the cycle becomes repetitive. Arthroconidia and endospores can be engulfed by macrophages, but are not killed, resulting in spread through the circulation within macrophages to other sites of localization. Dissemination commonly occurs to bones, skin, abdominal viscera, heart, genital tract and eyes. The bones and eyes are two of the most common sites of dissemination in animals.[1,3,6,7] Bone involvement accounts for one of the more commonly described clinical signs in dogs, that being a shifting leg lameness. Other signs commonly described in disseminated coccidioidomycosis of dogs include: persistent and/or fluctuating fever; anorexia and weight loss; weakness; localized peripheral lymphadenopathy; draining skin lesions, keratitis, uveitis and acute blindness.[7] Microscopic lesions are typically pyogranulomatous, more neutrophils are associated with arthroconidia and endospores, while more epithelioid macrophages and giant cells are observed in association with spherules.[3,6] In this case of disseminated coccidioidomycosis in an alpaca, histopathological lesions tended to be similar in location and character.

Diagnosis of coccidioidomycosis can be made with serological testing using agar gel immunodiffusion and complement fixation techniques. Culture from exudates, body fluids or tissue can be utilized, but requires specialized handling and equipment and can potentially result in human exposure, unless unscrupulous laboratory technique is used. Direct observation of the organism from body fluids or exudate is possible and histopathological identification of the organism from fixed tissue using H&E staining and enhancement of identification by special stains such as Gomori's methenamine silver (GMS) or periodic acid-Schiff (PAS). The size of the spherule, presence of endospores and presence of a double contoured wall characterize the spherules of *C. immitis* compared to other potential fungal pathogens.

Treatment in animals can be successful with long term antifungal systemic therapy (Ketaconazole, Itraconazole, Fluconazole or Amphotericin B).[2,6,7]

Coccidioidomycosis is not a true zoonosis, in that transmission from animals to man does not occur. This is because the infective form, arthroconidia, is not produced in tissue or body fluids. The relationship of animal infection to human infection is that exposure to the same environmental source of infection can be common. One exception could be the possibility of inhalation of endospores by humans performing necropsy procedures upon infected animals.[4]

AFIP Diagnosis: Lung: Pneumonia, granulomatous, multifocal, moderate, with diffuse edema and numerous fungal spherules, etiology consistent with *Coccidioides immitis*, alpaca (*Lama pacos*), camelid.

Conference Comment: The contributor provides a complete overview of *Coccidioides immitis*. The two most common organs affected by *C. immitis* with systemic dissemination are bone and eye. As pointed out by the contributor, the arthrospores are

the infective form. Endospores are not infective. *C. immitis* is a primary pathogen capable of infecting immunocompetent hosts and may also be an opportunistic pathogen in contrast to the opportunistic fungi (*Aspergillus* sp., *Candida* sp., *Zygomycetes*) in which immunodeficiency is necessary for infection. Cell-mediated immunity is more important than humoral immunity in containing/clearing the fungus and for resistance to reinfection.[1,2,5]

As pointed out by the contributor this case provides an excellent example of the variable morphology of *C. immitis*. Sporangia ranging from 30-200 um in diameter containing numerous 2-4 um endospores (mature spherules) and immature spherules ranging from 5-30 um in diameter containing flocculent material are present in the slides provided.[1,2,5] Splendore-Hoeppli material surrounds spherules in some sections. Many conference participants commented on the striking multinucleated giant cells present in this case. Conference participants reviewed the histomorphologic features of the systemic mycoses, the fungi/algae that endosporulate, and cell-mediated immunity.

Fungi/algae that reproduce by endosporulation include:

5. *Chlorella* sp.
6. *Prototheca* sp.
7. *Coccidioides immitis*
8. *Rhinosporidium seeberi*
9. *Batrachochytrium dendrobatidis*

Readers are encouraged to review WSC 1 / Case I, 2006-2007 – a case of *Blastomyces dermatitidis* in the eye of a cat and WSC 21 / Case I, 2006-2007 – a case of *Cryptococcus neoformans* in the lung of a dog.

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References:

1. Burek K: Mycotic diseases. In: Infectious Diseases of Wild Mammals, eds. Williams ES, Barker IK, 3rd ed., pp. 514-517. Iowa State University Press, Ames, Iowa, 2001
2. Kahn CM: The Merck Veterinary Manual, 9th ed., pp. 513-514. Merck & Co., Whitehouse Station, New Jersey, 2005
3. Coccidioidomycosis. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 3rd ed., vol. 2, pp 518-520. Academic Press, Inc., San Diego, California, 1985
4. Acha PN, Szyfres B: Zoonoses and Communicable Diseases Common to Man and Animals, 3rd ed., vol. 1, pp. 320-325. Pan American Health Organization, Washington, D.C., 2001
5. Greene RT: Coccidioidomycosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd ed., pp. 391-398. WB Saunders Company, Philadelphia, Pennsylvania, 1998

6. Hirsh DC, Biberstein EL: Agents of systemic mycoses. In: Veterinary Microbiology, eds. Hirsh DC, Maclachlan NJ, Walker RL, 2nd ed., pp. 285-292. Blackwell Publishing, 2004
7. Fowler ME: Camelidae. In: Zoo and Wild Animal Medicine, eds. Fowler ME, Miller RE, 5th ed., pp. 612-625. Saunders, St. Louis, Missouri, 2003
8. Fowler ME, Pappagianis D, Ingram I: Coccidioidomycosis in llamas in the United States: 19 cases (1981-1989). J Am Vet Med Assoc 201:1609-1614, 1992
9. Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC: Veterinary Microbiology and Microbial Disease, pp. 243-244. Blackwell Science Ltd., Cornwall, Great Britain, 2002
10. The South American Camelids: Llamas, Alpacas, Guanacos, and Vicunas 1943-2006. AWIC Resource Series No. 12. USDA-ARS-NAL-Animal Welfare Information Center. Updated February 2006
11. Ziemer EL, Pappagianis D, Madigan JE, Mansmann RA, Hoffman KD: Coccidioidomycosis in horses: 15 cases (1975-1984). J Am Vet Med Assoc 201:910-916, 1992

SLIDE 98
CONFERENCE 25 / CASE II – 05-25021 (AFIP 3026812)

Signalment: 10-year-old castrated male, Cocker Spaniel dog (*Canis familiaris*)

History: Dog presented for a 5.0 to 6.0 cm in diameter, ulcerated mass on the fifth digit of the left forepaw. The mass had grown rapidly in four months. Fine needle aspiration cytology was performed. The affected digit was amputated and submitted for histologic examination.

Gross Pathology: The amputated fifth digit of the left forepaw with an approximately 4.5 x 4.5 x 4.0 cm firm, partially haired, ulcerated mass was received. On cut section the mass was homogeneously white.
Laboratory Results: Fine needle aspiration cytology revealed a poorly cellular preparation comprised of clusters and individual round to polygonal cells with distinct to indistinct cell borders and a moderate nuclear to cytoplasmic ratio. The nucleus was eccentric, round with coarsely clumped to reticular chromatin. The cytoplasm was moderate and blue. Some cells possessed a perinuclear clear zone, interpreted to be a Golgi apparatus. Anisokaryosis and anisocytosis were moderate. There were occasional binucleate and rare multinucleated forms. The cells stained positively with methyl green pyronine. There were scattered macrophages and occasional multinucleated giant cells were seen. Clusters of sebocytes were also present. Free erythrocytes and platelet clumps were abundant. There were clumps of cellular debris and occasional nondegenerate neutrophils. The cytological diagnosis was a discrete/round cell tumor and a cutaneous plasmacytoma was considered most likely.

In formalin-fixed, paraffin-embedded sections of the digital mass tumor cells stained positively with methyl green pyronine. The tumor cells also exhibited variable levels of staining with anti-CD79a and anti-CD45RA and did not stain with CD18. Some of the tumors stained moderately with anti-IgG.

Histopathologic Description: Extending from the limits of the hair follicles is an expansile, pseudoencapsulated mass that comprises large coalescing deposits of an amorphous, homogeneously pink, slightly isotropic material; multinucleated giant cells; packets of individual round to polygonal cells; thick collagen fibers, spindled cells and scattered adipocytes. Cell borders for the round to polygonal cells are distinct. The nuclear to cytoplasmic ratio is moderate to high. The nucleus is centric to eccentric, round to oval with coarsely stippled chromatin and usually a single nucleolus. The cytoplasm is scant to moderate and blue. Most cells possess a paranuclear clear zone. Cellular features of malignancy include: bi-, tri- and multinucleation (up to 5 nuclei per cell) often with nuclei of unequal size; moderate anisocytosis and anisokaryosis; megalocytosis and karyomegaly. Mitoses are present (2 to 4 per 40X objective field). There are individual macrophages with bi-, tri- and multinucleated forms. The multinucleated giant cells are partially or totally engulfing the collections of amorphous, homogeneously pink, slightly isotropic material, interpreted to be amyloid. This material, when stained with Congo red, has apple green birefringence with polarized light and this birefringence was retained following treatment with potassium permanganate, consistent with immunoglobulin-derived or primary amyloid composed of lambda light chains.

Contributor's Morphologic Diagnosis: The histological diagnosis was a digital, cutaneous (extramedullary) plasmacytoma with intralesional, primary amyloid and granulomatous inflammation.

Contributor's Comment: Cutaneous plasmacytomas have been recognized in dogs and cats. The incidence of cutaneous plasmacytomas has been reported to be 1.5% of all canine skin tumors.[1] In most instances canine cutaneous plasmacytomas develop as solitary lesions, but multiple masses may occur and may be numerous in some cases. Cocker Spaniels appear to be predisposed to this tumor.[1,2] Tumors occur frequently in at or near areas of chronic immune stimulation. Common locations are the pinnae, lips, digits, chin and oral cavity.[1-4]

Approximately 10% of canine cutaneous plasmacytomas show varying degrees of amyloid production.[1] Primary or immunoglobulin-associated (AL) amyloid is derived from immunoglobulin light chains.[2,4] Plasmacytomas with amyloid are invariably composed of well-differentiated tumor cells.[1] Occasional tumors are overwhelmed by amyloid and contain only scattered islands of residual tumor cells.[1] Amyloid usually is accompanied by a granulomatous inflammatory reaction, which includes foreign body-type multinucleated giant cells.[1,3] Similar giant cells have been reported in association with amyloid in both respiratory plasmacytomas and solitary plasmacytomas of bone in human beings as well as cutaneous amyloidosis of horses.[1] No correlation has been

described for the presence of amyloid in plasmacytomas and the cell type, location, recurrence or biological behaviour.[3,4]
Dense cytoplasmic staining with methyl green pyronine stain (MGP) lends support to the diagnosis of a cutaneous plasmactyoma but it is not specific for plasma cells and must be interpreted with caution. Canine plasmacytomas consistently express the common leukocyte marker CD45 but are often negative for CD18.[1] About 80% of canine plasmacytomas express CD79a [MB-1].[1]

Most canine cutaneous plasmacytomas are benign and surgical excision appears curative.[1-4] However, some authors suspect that digital plasmacytomas as well as plasmacytomas of the oral cavity or subcutis may be more behaviorally aggressive.[1] Occasionally solitary and multiple cutaneous plasmacytomas exhibit malignant behavior and may metastasize to internal organs.[1]

AFIP Diagnosis: Haired skin and subcutis: Plasmacytoma with amyloid, Cocker Spaniel (*Canis familiaris*), canine.

Conference Comment: The contributor provides a concise summary of cutaneous plasmacytomas in the dog. Canine cutaneous plasmacytomas are usually circumscribed, unencapsulated, uni- or multilobular neoplasms primarily confined to the dermis. There is often a narrow uninvolved zone of the superficial dermis (Grenz zone). The neoplasms are composed of round to polygonal cells arranged in sheets, packets, and cords separated by a fine fibrovascular stroma. The plasmacytic character of the neoplasm is typically more apparent at the periphery. Marked variation in tumor cell differentiation is common, anisocytosis and anisokaryosis can be moderate to marked, and there is often variation in chromatin pattern. Previous studies have presented subclassifications of canine plasmacytomas based on the variable morphologic features, and while the features may be useful as a diagnostic tool, they are of no prognostic significance.[1,3,4]

The differential diagnosis for cutaneous plasmacytomas includes other cutaneous round cell tumors including histiocytoma, lymphoma, transmissible venereal tumor, and mast cell tumor. Conference participants reviewed common tumors of the canine digit including melanoma, squamous cell carcinoma, and subungual keratoacanthoma. Participants also reviewed the ultrastructural features of plasma cells and amyloid as well as types of amyloid (readers are encouraged to review Conference 8, Case 4 – a case of amyloidosis in a macaque - for a more in-depth discussion about amyloid).

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References:

1. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: Skin Diseases of the Dog and Cat, 2nd ed., pp. 866-872. Blackwell Science Ltd, Oxford, United Kingdom, 2005
2. Rowland PH, Valentine BA, Stebbins KE, Stebbins KE, Smith CA: Cutaneous plasmacytomas with amyloid in six dogs. Vet Pathol 28:125-140, 1991
3. Platz SJ, Breuer W, Pfleghaar S, Minkus G, Hermanns W: Prognostic value of histopathological grading in canine extramedullary plasmacytomas. Vet Pathol 36:23-27, 1999
4. Cangul IT, Wijnen M, van Garderen E, van den Ingh TSGAM: Clinico-pathological aspects of canine cutaneous and mucocutaneous plasmacytomas. J Am Vet Med Assoc 49:307-312, 2002

SLIDE 99
CASE III – 46349 9A (AFIP 3027711)

Signalment: 3-year-old, male, canine, Shih Tzu

History: The animal was presented to The Animal Medical Center on 11/05/05 with a history of 3 days of decreased appetite, vomiting and diarrhea with melena. On physical examination, the dog had hyphema and abdominal pain. While hospitalized, the dog collapsed, was resuscitated once, but died after 3 hours on a ventilator. The clinical suspicion for the cause of death was acute internal hemorrhage.

Gross Pathology: On necropsy, the gross findings were as follow:
1. Severe, multifocal gastric ulceration with moderate amount of dark brown hemorrhagic content in gastric lumen
2. Severe fibrinous pericarditis with multifocal myocardial hemorrhage
3. Mild hydrothorax and ascites
4. Moderate lymphadenomegaly with hemorrhages (retropharyngeal and mesenteric lymph nodes)
5. Severe, diffuse pulmonary congestion and edema
6. Severe, diffuse small intestinal mucosal hyperemia

Laboratory Results: There was significant increase of creatinine (4.2 mg/dl [normal: 0.4 - 1.8]), blood urea nitrogen (135 mg/dl [7 – 27]) and phosphorus (>25 mg/dl [2.1 – 6.3]) values. The serologic tick-borne disease panel was positive for exposure to *Borrelia burgdorferi* (1:256), and negative for *Ehrlichia canis* and *Rickettsia rickettsii*.

Histopathologic Description: Two sections of kidney reveal diffuse glomerular, interstitial and tubular changes. The glomerular lesion is characterized by increased overall glomerular size and severe thickening of the glomerular capillaries by a deep acidophilic and glassy homogeneous material, evenly deposited along the capillary walls, with occasional nodular areas. The glomerular tufts also have increased cellularity, with moderate amounts of pyknotic nuclei, and synechiae are multifocally seen. The Bowman's capsules reveal mild to moderate hypertrophy and hyperplasia of

the visceral epithelium, with thickening of the basement membrane, and lamellar peri-glomerular fibrosis. A deposition of an irregularly floccular and deep acidophilic fibrinous material is occasionally seen in the interstitium surrounding the glomerular capsules. The interstitium has multiple scattered aggregates of moderate numbers of plasma cells and fewer lymphocytes. Minimal amount of loose fibrous tissue is multifocally seen between tubules. Small numbers of tubules reveal intra-luminal sloughed epithelial cells with pyknotic nuclei. A few tubules exhibit cells with enhanced cytoplasmic acidophilia and loss of nuclear detail, associated or not with an attenuated epithelial lining. Moderate numbers of tubules are dilated and contain acidophilic (proteinaceous) casts. There is mild and multifocal mineralization of cortical tubular basement membranes, glomeruli and Henle loops. A finely granular dark brown pigment is rarely seen within the cytoplasm of the tubular epithelium.

Contributor's Morphologic Diagnoses: 1. Kidneys: Marked, chronic, diffuse, global membrano-proliferative glomerulonephritis
2. Kidneys: Moderate, multifocal lymphoplasmacytic interstitial nephritis with minimal interstitial fibrosis
3. Kidneys: Mild, acute, multifocal tubular necrosis with moderate tubular regeneration

Contributor's Comment: In this case, the most significant gross changes, such as gastric ulcerations with hemorrhage, fibrinous pericarditis, hydrothorax, hydroperitoneum and hyphema, were secondary to severe multi-systemic necrotizing fibrinoid vasculitis caused by uremia. Although not grossly visible, microscopic evaluation of the kidney revealed a severe nephropathy, which had two major components: a marked membranoproliferative glomerulonephritis, and an interstitial nephritis, in which plasma cells were the predominant cell type, without significant interstitial fibrosis. A third component was mild acute tubular necrosis with mild tubular regeneration. The histologic features of the nephropathy observed in this dog is compatible with the distinctive renal lesion that has been putatively associated with *Borrelia burgdorferi* infection in dogs, and that has been referred to as "Lyme nephropathy" in recent literature.[1,4,5] Exposure to *Borrelia burgdorferi* was documented in this dog by a positive serologic titer.

Lyme borreliosis is caused by *Borrelia burgdorferi*, of the order *Spirochetea*, which also contains other genera of pathogenic bacteria such as *Leptospira* spp. and *Treponema* spp. *Borrelia* spp. are spiral-shaped, 10-30 x 0.18-0.25 μm, Gram-negative and have an outer sheath encasing endofibrils.[2,4] They are not free-living organisms (quickly die outside the body) and have special culture requirements.[4] Unique features are a linear chromosome and life cycles that require arthropod vectors and mammalian hosts.[4] Mechanisms for survival in the hosts include change in surface protein expression, a lack of requirement for iron, and the fact that they can change their shape and hide in folds of cellular membranes, which might contribute to protection against the host's immune response and antibiotics.[4] Lyme disease occurs in humans and multiple nonhuman animal species, and is transmitted by ticks of the *Ixodes* genus.[2,3,4] *B. burgdorferi sensu lato* is divided into multiple strains (genospecies) that include *B.*

burgdorferi sensu stricto (the cause of Lyme disease in North America), and others such as *B. garinii* and *B. afzelii* which occur in Europe and Asia.[3,4]

The commonly recognized clinical signs and lesions associated with spontaneous canine Lyme disease are fever and polyarthritis with reactive lymphadenopathy. Neurologic and cardiac disease (common disorders in human infection) has been rarely reported in naturally infected dogs, and cutaneous rash does not occur. [2,3,4] Histological studies of experimental canine Lyme disease have also shown dermatitis at the site of tick bite, and a mild subclinical meningitis and/or encephalitis.[6] A protein loosing nephropathy has been described in a relatively small number of natural cases, but the exact prevalence of this manifestation has not been determined.[5] Lyme nephropathy has been described as morphologically and clinically unique, and as the only fatal form of Lyme disease in dogs.[1] It affects mostly dogs under 5 years of age, and has a rapid progression. Usually the clinical course is of 6 to 8 weeks, but sudden onsets of lethargy, anorexia and vomits can occur, and the clinical course can be as acute as 24 hours.[1] A predilection for Labrador and Golden Retrievers and Shetland sheepdogs has been suggested.[1,5,6] In canine spontaneous glomerulonephritis of other etiologies, the dogs are usually older, the disease is slowly progressive, and there is no breed predilection. The presence of tubular necrosis and regeneration is an unusual feature not seen in other canine glomerular diseases, in which tubular changes are usually restricted to dilation with luminal protein casts. In Lyme nephropathy, the glomerular lesion is membranoproliferative in most cases, and associated with C3, IgG and IgM deposits, which supports an immune-mediated pathogenesis.

Ultrastructurally, the lesion is characterized by the presence of electron-dense subendothelial deposits, multifocally along the glomerular basement membrane. Although these deposits are not seen within the mesangium, mesangial matrix is increased. Glomerular endothelial cells present with cytoplasmic vacuolation and separation from the basement membrane. Also parietal cells will present swollen and with cytoplasmic vacuolation.[1] Another microscopic feature described on Lyme nephritis is a lamellar periglomerular fibrosis of the Bowman's capsule, which was also seen in our case.[6] The exact cause for the tubular necrosis is unknown. Deposition of complement or immunoglobulins has not been detected along tubular basement membranes. The suggested mechanisms for tubular necrosis in these dogs include hypoxia (possibly related to a perfusion disorder caused by the glomerular lesions) or nephrotoxicity. In spite of the glomerular and tubular changes, and the interstitial infiltration by lymphocytes and plasma cells, interstitial fibrosis is not a significant feature of Lyme nephropathy. Other necropsy findings of dogs with Lyme nephropathy are usually related to uremia.[6]

In cases of Lyme nephropathy, the glomerular deposits are PAS positive and Masson's trichrome and Congo Red negative, although amyloidosis is an occasional component of the lesion, and then be positive for Congo Red. Silver stains can help for the visualization of spirochetes within the lesions, although spirochetes are rarely found and can also be seen in dogs with Lyme disease but without renal disease.[1] Experimental infection of dogs and other species have failed to reproduce the renal syndrome

putatively associated with *B. burgdorferi* in dogs, therefore Koch's postulates has not been satisfied.[1,5,6] It is important to note that no studies of experimental Lyme disease have been conducted in dogs of the breeds that are presumably susceptible to Lyme nephropathy.[5] Why the Lyme nephropathy occurs in some dogs and not in others, and why the disease cannot be reproduced experimentally is unknown at this point.[5] Factors that might be involved in the specificity of the development of canine Lyme nephropathy include age and breed of the dogs, genetic susceptibility, different strains of *B. burgdorferi*, likelihood of multiple exposures and the presence of coinfections.[1,5]

It has been suggested that *B. burgdorferi* may not be the causative agent of Lyme nephropathy, but just a marker for tick exposure. Since mixed infections with *Ehrlichia* spp., *Babesia* spp. and *Bartonella* spp. can occur in dogs exposed to ticks, and thrombocytopenia, glomerulonephritis and neurologic signs (also seen in Lyme disease) have been seen in dogs infected by *Ehrlichia* spp. and *Babesia* spp., it has been suggested that another agent, rather than *B. burgdorferi* may cause the nephropathy.[4] It is also unknown if vaccination for Lyme disease in dogs can have a role in the development of the lesion.[1,3] This specific renal lesion has been seen in vaccinated and non-vaccinated animals, and titers for *B. burgdorferi* are higher for dogs with Lyme nephropathy than for healthy dogs exposed to the agent.[1,5]

AFIP Diagnosis: Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, marked, with tubular degeneration, necrosis, regeneration, proteinosis, and moderate chronic lymphoplasmacytic interstitial nephritis, Shih Tzu (*Canis familiaris*), canine.

Conference Comment: The contributor provides an excellent overview of Lyme nephropathy in the dog caused by *Borrelia burgdorferi*.

Conference participants reviewed causes of immune complex glomerulonephritis in different species. Readers are encouraged to review WSC 17 / Case IV, 2006-2007 – membranoproliferative glomerulonephritis in a macaque – which includes a chart summarizing the cause of immune complex glomerulonephritis in various species.

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References:

1. Dambach DM, Smith CA, Lewis RM, Van Winkle, TJ: Morphologic, immunohistochemical and ultrastructural characterization of a distinctive renal lesion in dogs putatively associated with *Borrelia burgdorferi* infection: 49 cases (1987-1992). Vet Pathol 34:85-96, 1997
2. Fritz CL: Lyme borreliosis. JAVMA 223: 1261-1270, 2003
3. Greene CE, Straubinger RK: Borreliosis. In: Infectious diseases of the dog and cat-Revised reprint. ed. Greene CE, 3rd ed., pp. 417-434. Saunders, 2006
4. Littman MP: Canine borreliosis. Vet Clin Small Animals 33:827-862, 2003

5. Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE: ACVIM Small animal consensus statement on Lyme disease in dogs: diagnosis, treatment and prevention. J Vet Intern Med 20:422-434, 2006
6. Summers BA, Straunbinger AF, Jacobson RH, Chang YF, Appel MJ, Straunbinger RK: Histopathological studies of experimental Lyme disease in the dog. J Comp Path 133:1-13, 2005

ELECTRON MICROGRAPH
CONFERENCE 25 / CASE IV – EM (AFIP 3030458)

Signalment: 10-week-old Sprague-Dawley, *Rattus norvegicus*, rat

History: The rat was part of a high dose group in a one month oral toxicity study using a proprietary compound. Necropsy was conducted at the end of the treatment period, followed by routine ultrastructural evaluation of the kidneys.

Gross Pathology: Kidneys were unremarkable at necropsy.

Histopathologic Description: Kidney: The image on the right is that of a renal glomerulus, characterized by capillary loops, recognizable by its content of a tangential section of an erythrocyte in one loop. The capillaries are lined by a thin layer of fenestrated endothelial cytoplasm and an endothelial cell body can be seen bulging into one of the capillary lumens. At least three podocytes can be seen giving rise to several primary and many secondary foot processes that rest on the glomerular basement membrane. A mesangial cell, grazing sections of mesangial matrix and the tortuous course of the urinary space can also be recognized. The cytoplasm of podocytes, endothelial cells and mesangial cells contain four to eighteen predominantly unicentric, electron dense, round to oval bodies of varying size that are composed of multi lamellar membranous whorls (lamellar bodies).

The image at the left is that of a renal proximal tubular epithelial cell, characterized by simple cuboidal epithelium and a prominent brush border of tall microvilli. The epithelial cell contains at least twelve predominantly multicentric lamellar bodies of variable size, randomly distributed in the cytoplasm. A few lamellar bodies are also present in the lumen.

Contributor's Morphologic Diagnosis: Kidney: Lamellar bodies, intracytoplasmic, multiple, glomerulus, proximal tubular epithelium, consistent with renal phospholipidosis.

Contributor's Comment: Similar ultrastructural changes were also observed in the liver (hepatocytes, Kupffer cells, biliary epithelial cells and endothelial cells), lungs (alveolar macrophages, type I pneumocytes, type II pneumocytes, Clara cells, ciliated bronchial epithelial cells and endothelial cells, and circulating lymphocytes. The changes observed are consistent with phospholipidosis consequent to administration of

cationic amphophilic drugs (CADs). Many drugs belonging to this family of compounds are known to induce this lesion. Aminoglycosides (Gentamycin) are good examples, and cause renal toxicity in humans and animals.

There are three mechanisms by which this group of compounds causes phospholipidosis:
1. By direct interaction with cellular phospholipids. CADs bind to phospholipids and the complex resists degradation by lysosomal enzymes.
2. By inhibition of the enzymes (phospholipases) that degrade phospholipids.
3. By upregulating the synthesis of phospholipids in the cell.

Excessive intracellular accumulation of poorly degraded phospholipids occurs in the lysosomes. They often acquire a multilamellar morphology that is recognizable ultrastructurally. Although such lamellar bodies can potentially occur in any tissue, they are commonly present in lung, liver, kidney, eye, brain, and occasionally in circulating lymphocytes. There is wide variation in species, breed and strain susceptibility to phospholipidosis. Because the distribution of specific phospholipids varies by tissue, target tissues for specific compounds vary, and because composition of specific phospholipids within a tissue varies by age, susceptibility may also vary by age. In the context of most of the compounds studied, phospholipidosis is thought to be largely an adaptive response and its functional consequences are not completely known, although accumulation of lamellar bodies in large numbers in a cell is known to induce apoptosis.[1]

AFIP Diagnosis: Kidney, glomerulus and proximal convoluted tubular epithelium: Lamellar bodies, intracytoplasmic and extracellular, consistent with renal phospholipidosis, Sprague Dawley rat (*Rattus norvegicus*), rodent.

Conference Comment: The contributor provides a concise summary of phospholipidosis and the mechanisms by which cationic amphophilic drugs (CADs) can cause phospholipidosis.

Conference participants reviewed the ultrastructural components of a glomerulus to include capillary loops, fenestrated endothelial cells, podocytes and mesangial cells. The ultrastructure of renal proximal tubular epithelial cells was also reviewed to include cuboidal to columnar epithelium, a prominent brush border with tall microvilli, and mitochondria arranged perpendicularly to the basement membrane. Additionally, ultrastructural abnormalities in various organelles and reversible and irreversible ultrastructural changes were discussed.

Reversible cell injury manifests as morphologic and functional changes that are reversible if the damaging stimulus is removed. The hallmarks of reversible injury are reduced oxidative phosphorylation, depletion of ATP, and cellular swelling. If the damage continues, the injury becomes irreversible. Certain structural changes such as

amorphous densities in mitochondria, indicative of severe mitochondrial damage and loss of membrane permeability, indicate that cells have undergone irreversible injury.[3]

Included below are two charts summarizing basic ultrastructural abnormalities in various organelles as well as reversible and irreversible ultrastructural changes.[2,3]

Basic Ultrastructual Abnormalities in Various Organelles

Plasma membrane
- Loss of surface specialization
- Breakdown of attachments
- Cytoplasmic blebs

Cytosol
- Rarefaction (swelling)
- Depletion of glycogen
- Presence of myelin figures

ER
- Swelling/dilation
- Detachment of ribosomes

Lysosome
- Swelling
- Rupture

Mitochondria
- Cristolysis
- Condensation
- Swelling
- Rupture
- Matrix flocculent densities
- Vacuolation

Nucleus
- Clumped chromatin
- Pyknosis, karyorrhexis, karyolysis

Other
- Inclusions
- Parasites, bacteria, fungi, algae

Reversible and Irreversible Ultrastructural Changes

Reversible	Irreversible
Plasma membrane alterations: Blebbing, blunting, distortion, creation of myelin figures, loosening of intercellular attachments	Overt discontinuities in plasma membrane Disruption of lysosomes
Mitochondria: Swelling, rarefaction, small amorphous densities	Large amorphous densities in mitochondria
Dilation of ER with detachment of	Pronounced myelin figures

ribosomes	Amorphous osmophilic debris
Clumping of nuclear chromatin	Profound nuclear changes

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References:

1. MJ Reasor, Kacew S: Drug-induced phospholipidosis: are there functional consequences? Exp Biol Med 226(9):825-830, 2001
2. Williams BH: Ultrastructural pathology. Descriptive Veterinary Pathology Course, Washington, D.C., 2007
3. Kumar V, Abbas AK, Fausto N: Robbins and Cotran Pathologic Basis of Disease, 7th ed., pp. 11-12, 19-20. Elsevier Saunders, Philadelphia, Pennsylvania, 2005