The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2004-2005

SLIDE 1
CONFERENCE 1 / CASE I – N03-516 (AFIP 2933954)

Signalment: 12 year-old, female, Alpine Swiss, caprine, goat.

History: This 12 year-old goat presented with coughing, dyspnea and white mucous membranes. Radiographs and ultrasound revealed a 1.5-inch diameter mass on the left lung lobe. A CBC revealed evidence of anemia due to blood loss. A transtracheal wash indicated reactive respiratory epithelial hyperplasia and hemorrhage. The animal died.

Gross Pathology: The lungs were moist and poorly collapsed. Several white nodules up to 2 cm in diameter were present on the surface of all lobes. On cut section, the lung was gelatinous and bulged. Small numbers of *Haemonchus contortus* were present in the abomasum. Caseous material exuded from an abscess over the medial aspect of the right hock joint.

Laboratory Results: No parasite ova were present on fecal examination. Alpha-hemolytic streptococci were isolated from the hock joint abscess. On AGID the serum was positive for CAE virus antibody.

Contributor's Morphologic Diagnosis: Pneumonia, lymphocytic, bronchointerstitial, diffuse, severe, with alveolar proteinosis, lung.

Contributor's Comment: The histologic appearance of the lung is consistent with pneumonia caused by caprine arthritis-encephalitis (CEA) virus disease, with findings of type II pneumocyte proliferation, lymphoid infiltration and proteinaceous fluid within alveoli (alveolar proteinosis). Electronmicroscopy has shown that the proteinaceous fluid found in CAE is lung surfactant.

CEA syndrome is a viral disease of domestic goats that manifests as chronic proliferative synovitis and periarthritis and progressive pneumonia in adult goats and afebrile leukoencephalomyelitis in goat kids.[1] The causative agent, a Lentivirus, is transmitted from adult goats to kid goats via colostrum or by lateral transmission. The CAE virus has a worldwide distribution. All breeds and ages of goats are susceptible to infection and once established the infection persists throughout the animal's life. A diagnosis is established on clinical signs, demonstration of serum antibodies on ELISA and pathological changes such as diffuse pulmonary consolidation or hypertrophic, proliferative synovitis with intra-articular rice bodies. There is a microscopic component of interstitial mastitis leading to induration and agalactia.

Viral RNA can be identified in macrophages where viral transcription occurs. Infected macrophages may be detected with immunohistochemistry techniques in tissues such a lung, udder, and lymph nodes.[2,3]

Interestingly, the mass identified radiographically in the left lung lobe turned out to be a secondary granulomatous pleuritis with intralesional *Cryptococcus neoformans* yeast organisms.[4]

AFIP Diagnosis: Lung: Pneumonia, interstitial, proliferative, lymphocytic, diffuse, moderate, with alveolar proteinosis and secondary acute bronchopneumonia, Alpine Swiss, caprine.

Conference Comment: Conference attendees discussed slide differences with some sections containing distinct lymphoid nodules, while others contained variable numbers of interstitial and perivascular lymphoid infiltrates. However, all slides were characterized by prominent type II pneumocyte hyperplasia and abundant alveolar proteinosis, which are characteristic of CAE virus.[5] Most slides also had features consistent with bronchopneumonia; bronchioles and alveoli filled with an exudate composed of degenerate neutrophils, necrotic debris, and proteinaceous fluid. This is not surprising, as secondary bacterial infections are common in animals with CAE.[5] However, no organisms were seen with H&E, B&B, B&H, or PAS.

CAE virus is a lentivirus (subfamily Lentivirinae, family Retroviridae). Lentiviruses are non-oncogenic retroviruses with clinical disease characterized by long incubation periods, persistent infection, and a progressive course. Other lentiviruses include: maedi-visna virus in sheep (ovine progressive pneumonia); equine infectious anemia virus in horses and, human, simian, bovine and feline immunodeficiency viruses.

Other causes of pneumonia in sheep and goats include:
<u>Viral:</u>
Maedi-visna virus (ovine progressive pneumonia)
Caprine Morbillivirus (Peste des petits ruminants)
<u>Bacterial:</u>
Mannheimia (*Pasteurella*) *haemolytica* (ovine pneumonic pasteurellosis)
Mycoplasma ovipneumoniae (chronic enzootic pneumonia)
Mycoplasma mycoides spp. *mycoides* large colony (contagious caprine pneumonia)
Mycobacterium bovis (tuberculosis)
Mycobacterium avium (tuberculosis)
<u>Parasitic:</u>
Dictyocaulus filaria
Muellerius capillaris
Protostrongylus rufescens

Contributor: University of Florida College of Veterinary Medicine, Dept of Pathobiology, Box 110880, Gainesville, FL 32610

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SLIDE 2
CONFERENCE 1 / CASE II – 03N0138 (AFIP 2936140)

Signalment: 2-year-old male castrated Great Dane.

History: The dog presented with a several day history of weight loss, vomiting, diarrhea, and urinary incontinence. The dog had been treated for six months with immunosuppressive doses of prednisone (1 mg/kg PO q12h for 2 weeks) for chronic lymphocytic-plasmacytic enteritis (inflammatory bowel disease).
The dog continued to have weight loss and diarrhea but also developed profound lethargy and hematuria. Despite treatment, the clinical signs worsened and two weeks later, the dog presented to the hospital recumbent, comatose, and with intermittent seizures and rotary nystagmus. Due to the poor prognosis, the owners elected to euthanize the dog.

Gross Pathology: Necropsy revealed numerous small, randomly distributed granulomas restricted to the kidneys and brain. In both kidneys, the lesions spread from the cortico-medullary junction to the cortex. The kidney lesions consisted of 0.3 to 1.0 cm in diameter soft raised tan well-demarcated raised nodules that were distributed randomly throughout the parenchyma. In the brain, lesions were found in the right occipital lobe and right caudal cerebellar peduncle. The brain lesions were well-demarcated unencapsulated gelatinous tan foci.

Laboratory Results: Blood cultures were negative and the kidney culture revealed one colony of hemolytic *E. coli*.

Contributor's Morphologic Diagnosis: Kidney: Multifocal severe granulomatous and necrotizing nephritis with intralesional amoeba organisms.

Contributor's Comment: The histologic features observed throughout this kidney consisted of marked interstitial edema and tubular necrosis associated with marked perivascular inflammatory infiltrates mainly comprised of macrophages, lymphocytes, Langerhans type giant cells, plasma cells and small numbers of neutrophils. Admixed with these cells were large numbers of amebic organisms in two different life stages, trophozoites and cysts. The trophozoites ranged in size from 15-45 _m in diameter, and were round to oval with occasional short plump cytoplasmic processes (pseudopodia). Their cytoplasm was pale, eosinophilic, and partially vacuolated and contained a 4-6 _m in diameter round, pale-staining vesicular nucleus. In contrast, the cysts were smaller, more uniformly round, and surrounded by an undulating outer wall and an internal thick round basophilic inner wall. The cytoplasm was scarce and contained multiple, hyperchromatic basophilic granules. The nucleus was centrally located with 1-2 prominent eosinophilic karyosomes. The cystic forms were distributed throughout the renal interstitium, but were most prominent within collecting tubules. The trophozoites were more abundant near blood vessels.

In the brain, lesions associated with this case had histologic features of fibrinoid necrosis of the blood vessels and a florid perivascular infiltration of neutrophils and macrophages admixed with lakes of fibrin. There were also extensive areas of malacia associated with astrogliosis, surrounding the affected vessels. Large numbers of trophozoites were concentrated primarily in the perivascular regions of the gray matter, the meninges and the choroid plexus. The cystic forms were located further away from the blood vessels and were associated with less inflammation and necrosis.

The amoeba in this case were diagnosed as *Balamuthia mandrillaris* based on positive immunohistochemical results (performed at San Bernardino) and PCR, which used specific primers designed for this case at Dr. Sykes' laboratory (UC Davis, VMTH).

Balamuthia mandrillaris is a free-living ameba of the order Leptomyxida capable of causing fatal granulomatous amebic meningoencephalitis (GAE) in humans and animals[1]. It was first isolated from a mandrill[1,6] (*Papio sphinx*) at San Diego Zoo Wild Animal Park, then subsequently in gorillas[2] (*Gorilla gorilla gorilla*), an orangutan[3] (*Pongo pygmaeus*) and Old World primates, including a colobus monkey[4] (*Colobus guereza kikuyuensis*) and a gibbon (*Hylobates concolor leucogenys*). In recent years, *B. mandrillaris* has also been increasingly identified as a cause of GAE in humans.[1,5,6] So far, the only reports in a non-primate species have involved a horse[7] and a sheep.[8] *B. mandrillaris* has been recently isolated from environmental samples suggesting it occupies similar habitats as other opportunistic ameba such as *Naegleria fowleri* and *Acanthamoeba* spp..[5,6] The route of invasion is still unknown, however penetration of skin or respiratory tract and subsequently hematogenous spread has been postulated.[6] Infections with *B. mandrillaris* are reported to be more common in immunosuppressed hosts such as patients with AIDS, although immunocompetent individuals also have been affected.[6] Reports of canine disease caused by free -living amebas are rare.

There is a single report of kidney lesions associated with dissemination of *Acanthamoeba castellani.*[9]

There are two forms of *B. mandrillaris*, a trophozoite form and a cyst form. The trophozoite amoebic form ranges in size from 15-60 _m in diameter and has a round nucleus and a dense nucleolus. More than one nucleolus can be observed, which aids as a distinguishing feature between *B. mandrillaris* and *Acanthamoeba*.[6] The trophozoite has pale, eosinophilic, and partially vacuolated cytoplasm that can occasionally be observed branching into short plump cytoplasmic processes (pseudopodia). In contrast, the cyst form, which is the dormant form, is smaller, more spherical, uninucleated, measuring 15-60 _m, and has a prominent bilayer wall with granules positioned beneath the inner cell wall.[6]

The precise pathogenesis of *B. mandrillaris* is unknown. However, a recent review discusses the life cycle and pathogenesis of *Entamoeba histolytica.* Initial infection is through ingestion of contaminated food or water with *E. histolytica* cysts. Cysts are ingested and excysted in bowel lumen. The invasive trophozoite form invades intestinal epithelium and spreads to other sites.[10] In the case of *B. mandrillaris*, infection may occur through ulceration in skin or lower respiratory tract infection. Spread to other sites, especially the brain, is thought to be hematogenous.

AFIP Diagnosis: Kidney: Nephritis, interstitial, necrotizing, pyogranulomatous, multifocal to coalescing, moderate, with amebic trophozoites and cysts, Great Dane, canine.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. The contributor provides a thorough description of the gross and histologic lesions associated with *B. mandrillaris*. Other important amebic organisms to consider include: *Acanthamoeba* spp.; *Entamoeba histolytica*; *Naegleria fowleri*; and, *Hartmannella* spp..

Acanthamoeba spp. is a free-living organism found in fresh water, soil, or sewage. Transmission is via inhalation with the lung being the primary target organ. However, the central nervous system and other organs may be affected through hematogenous spread. This organism is also known to cause granulomatous amebic encephalitis (GAE) in humans and other species. Trophozoites are 10-30 _m in diameter, contain an eccentric nucleus, a single nucleolus, and eosinophilic cytoplasm with glycogen vacuoles. Cysts are generally rare.[11]

Entamoeba histolytica is often a non-pathogenic inhabitant of the large intestine, only occasionally causes amebic dysentery in humans and nonhuman primates, and rarely affects other species. Transmission is via ingestion resulting in characteristic flask-shaped intestinal ulcers. Hematogenous and lymphatic dissemination to the brain, liver,

or other organs may occur. Trophozoites are 6-50 _m in diameter, often surrounded by a clear halo, and contain an eccentric nucleus with a distinct karyosome.[12]

Naegleria fowleri is a free-living ameba that causes acute and fulminating primary amebic meningoencephalitis (PAM) primarily in young healthy humans, with rare reports in animals. *Naegleria* is commonly found in fresh water, soil, and sewage. Transmission is via inhalation with invasion of the olfactory neuroepithelium. Trophozoites are 6-12 _m in diameter with a centrally located nucleus and a large single nucleolus. Unlike *Acanthamoeba* and *Balamuthia*, cysts are generally not found in neural tissue.[13]

Hartmannella sp. is a non-pathogenic, free-living amoeba. Older isolates of *Hartmannella* suspected of being opportunistic pathogens (like *Acanthamoeba* and *Naegleria*) have now been reclassified as *Acanthamoeba*. True *Hartmannella* sp. are not known to cause CNS infections in humans or other species.[5]

Contributor: University of California, Davis, Dept. of Veterinary Pathology, Vet. Med. Teaching Hospital,1 Garrod Drive, Davis, CA 95616

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SLIDE 3
CONFERENCE 1 / CASE III – L02-3969-1A or -1B or L04-3969-1C (AFIP 2936455)

Signalment: Adult, unknown sex, *Boa constrictor*.

History: History of lethargy, regurgitation, head tremors and death.

Gross Pathology: Uric acid deposits in multiple visceral organs (visceral gout).

Contributor's Morphologic Diagnoses:
Liver: There are multifocal, large, circular areas of acute hepatocellular necrosis surrounded by minimal inflammation. Within the center of most foci are shards of clear spaces surrounded by cellular debris, histiocytes and acidophilic fibrillar material (dissolved uric acid tophi). Approximately 80% of hepatocytes contain single to multiple, prominent, eosinophilic, circular 5-10 micron diameter, intracytoplasmic inclusion bodies. Randomly scattered individual to small clusters of hepatocytes are shrunken, hypereosinophilic and have fragmented nuclei (necrosis).

Kidney: There is severe architectural distortion by scattered large aggregates of necrotic cellular debris, mineralized basophilic material and dilated tubules containing shards of clear material and mineralized debris. Dilated tubules are lined by fragmented, shrunken, hypereosinophilic epithelial cells, many containing large eosinophilic prominent intracytoplasmic inclusion bodies (as previously described). Tubule lumens are filled with mineralized basophilic granular debris and shards of clear material surrounded by histiocytes and lymphocytes. The interstitium is widened by bands of edema fluid mixed with scattered lymphocytes and histiocytes.

Stomach: Lining epithelial cells contain large numbers of 5-15 micron diameter, deeply eosinophilic intracytoplasmic inclusion bodies.

Lung: Epithelial cells lining bronchi and alveoli contain single to multiple, brightly eosinophilic 5-7 micron diameter, intracytoplasmic inclusion bodies.

1. Hepatitis, granulomatous with uric acid deposition and multifocal hepatocellular necrosis.
2. Urate nephrosis, severe, chronic with tubular dilatation and mineralization.
3. Inclusion bodies, multifocal/multiorgan, epithelial cells and hepatocytes, diffuse.

Contributor's Comment: Histologic lesions indicate the cause of death is visceral gout secondary to Boid inclusion body disease (IBD). Gout is a common sequela to chronic infection or debilitating conditions in reptiles with dehydration often being a primary compounding issue. Underlying viral infections (IBD) also lead to secondary bacterial infections due to immunosuppression and debilitation.

Boid inclusion body disease (IBD) is a fatal disorder of boid snakes caused by a retrovirus that was described in 1994.[1] Clinical signs of IBD include chronic regurgitation and central nervous system disorders manifested in head tremors, disorientation and paresis and/or paralysis. Histologic examination reveals numerous eosinophilic intracytoplasmic inclusion bodies in epithelial cells of all major organs, in hepatocytes and within neurons of the CNS. Inclusion bodies contain an antigenically distinct 68 kDa protein.[2] In all snakes with CNS disease, nonsuppurative meningoencephalitis with neuronal degeneration and perivascular cuffing was present and viral particles resembling type C retrovirus were detected in the brain, pancreas, and kidney as well as in cultured kidney cells. The disease was shown to be transmissible by cell free primary kidney culture supernatants from infected *Boa constrictor* snakes to young Burmese pythons (*Python molurus bivittatus*) or by liver homogenates from *Boa constrictor.*[2] These data imply that a C type retrovirus may indeed be the causative agent of IBD.

AFIP Diagnoses: 1. Liver: Mineral deposition (gouty tophi), multifocal, with minimal granulomatous inflammation, boa constrictor (*boa constrictor*), reptile.
2. Liver, hepatocytes: Inclusion bodies, eosinophilic, intracytoplasmic, multifocal.
3. Liver, hepatocytes: Degeneration, multifocal, moderate, with random single cell necrosis.

Conference Comment: The contributor provides a thorough overview of boid inclusion body disease (IBD), a fatal disorder of boid snakes, thought to be caused by a retrovirus. It is well established that snakes with IBD have increased susceptibility to secondary infections and a variety of neurological signs. It is not surprising this snake likely developed gout secondary to IBD.

Gout is a common clinical finding in reptiles and may also affect birds, humans, non-human primates, and the Dalmatian dog. There are two forms of gout in birds and reptiles: articular and visceral. Articular gout is less common and presents as white deposits on tendon sheaths. Visceral gout is more common and presents as chalky white patches on visceral surfaces. Gout results from hyperuricemia (elevated plasma uric acid concentration), which may be caused by impaired renal function and clearance of urates, nephrotoxic drugs, dietary excesses (protein and calcium) and deficiencies (vitamin A), and dehydration.[3] Nucleic acids ingested in foods undergo enzymatic hydrolysis to yield free purine (adenine, guanine) and pyrimidine bases. In humans, the

formation of uric acid from purine degradation has been extensively studied and requires xanthine oxidase:

Adenine>hypoxanthine>xanthine>uric acid>allantoin>allantoic acid>urea

Guanine>xanthine>uric acid>allantoin>allantoic acid>urea

In order to conserve water, birds and reptiles excrete uric acid rather than urea.[4,5] The majority of uric acid excretion is via renal tubular secretion and is largely independent of urine flow rate. When hyperuricemia occurs, uric acid crystals may be deposited in joints, viscera, or extra-visceral sites.[6] The crystals that are deposited in tissues (urate tophi) are often dissolved during processing. Histologically, all that remains are large aggregations of clear acicular clefts, frequently surrounded by granulomatous inflammation.

In humans, gout may be the result of a deficiency in hypoxanthine guanine phosphoribosyl transferase (HGPRT), leading to increased production of uric acid due to synthesis of purine nucleotides from non-purine precursors. A complete lack of HGPRT occurs in the uncommon X-linked Lesch-Nyhan syndrome seen in males that is characterized by hyperuricemia, severe neurologic deficits, and occasionally gouty arthritis.[7]

Contributor: Utah Veterinary Diagnostic Laboratory, 950 E 1400 N, Logan, UT, 84322 (www.usu.edu)

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SLIDE 4
CONFERENCE 1 / CASE IV – 0404975 (AFIP 2937765)

Signalment: Approximately 16 month-old female B6C3F1 mouse (*Mus musculus*)

History: The mouse was a sentinel animal and did not receive any treatment. It was found dead at approximately 16 months of age.

Gross Pathology: Macroscopic examination revealed about 5 cc of a red fluid in the abdominal cavity. The spleen was enlarged (32x6x3 mm) and the mediastinal lymph node was enlarged (3x) and dark. A red mass was found on the right ovary (47x31x19 mm).

Contributor's Morphologic Diagnosis: Yolk Sac (Carcinoma) Tumor

Contributor's Comment: The tumor is composed of nests, clusters or ribbons of discrete cells embedded within an abundant eosinophilic matrix. The cells vary in size from small to large and have distinct borders and hyperchromatic nuclei.[1,2,3]

AFIP Diagnosis: Ovary: Yolk Sac Carcinoma, B6C3F1 mouse, rodent.

Conference Comment: Conference attendees had difficulty with tissue identification. Some sections contain rare follicles at various stages of development. Tumors arising from ovarian tissue are divided into three broad categories based on embryological origin: tumors of surface epithelium, tumors of gonadal stroma, and tumors of germ cells. Tumors may also arise from more than one of the three embryological lineages (mixed tumors) or from nongonadal support tissues, including smooth muscle, vascular, or fibroblastic tissue.[4]

Epithelial tumors may arise from ovarian surface epithelium, subsurface epithelial structures (SES) in canines, and the rete ovarii. These tumors commonly appear as unilateral or bilateral multinodular, cystic outgrowths extending from the ovarian surface. Examples of epithelial tumors include papillary or cystic adenoma, or less frequently papillary or cystic adenocarcinoma. These tumors are common only in the dog as SES are unique to this species.[5]

Gonadal stromal tumors, also known as sex cord-stromal tumors, arise from granulosa, theca, or interstitial cells. Granulosa cell tumors (granulosa-theca cell tumor) are the most common ovarian tumor in the cow and mare. Granulosa cell tumors in the mare frequently produce inhibin, which is thought to cause atrophy of the contralateral ovary. Theca cells may be present in these tumors and either cell population may be luteinized. Thecomas (theca cell tumor) are rare tumors composed of lipid-containing cells of stromal origin, resembling theca interna cells. Interstitial cell tumors (luteoma,

lipid cell tumor, steroid cell tumor) are composed of large rounded cells with round central nuclei, resembling Leydig, luteal, or interstitial gland cells. However, the origin of these cells has not been clearly identified.[6]

Germ cell tumors may fail to differentiate (dysgerminoma), differentiate into somatic tissue (teratoma), or differentiate into extraembryonic structures (yolk sac carcinoma, choriocarcinoma). The dysgerminoma is an uncommon tumor, which is comparable to the more common seminoma of the testicle. This malignant tumor is composed of cells that resemble primordial germ cells. Teratomas are rare tumors thought to arise from totipotential germ cells that have differentiated into two or more embryonic layers (endoderm, mesoderm, ectoderm). Most of these tumors are composed of a variety of tissues and are generally benign.[6] Yolk sac carcinomas can be distinguished histologically by nests and cords of neoplastic polygonal to cuboidal cells embedded in abundant amounts of a characteristic eosinophilic PAS-positive matrix.[2]

Contributor: Toxicology Battelle Columbus, Pathology Dept, Room 7-0-37A, Battelle Columbus Labs, 505 King Ave, Columbus, OH, 43201-2693 (www.battelle.org)

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SLIDE 5
CONFERENCE 2 / CASE I – N04-188 (AFIP 2937770)

Signalment: 27 year-old, female, mongoose lemur (*Eulemur mongoz)*, non-human primate.

History: The lemur presented with a history of rapidly progressive neurological symptoms, marked polyuria and polyphagia. A neurological examination revealed a wide-based stance, circling, ataxia and falling to the right, as well as a weak grip in the right hand and right foot. Cranial nerve reflexes and anal tone were within normal limits. Additional findings included patchy truncal alopecia and a cataract in the left eye. The very thin and debilitated lemur was euthanized due to poor prognosis and advanced age.

Gross Pathology: The submitting veterinarian reported multifocal to coalescing atheromatous plaques on the intima of the proximal abdominal aorta. On gross examination of the brain after formalin fixation, there were multifocal random (approx. 10), pinpoint to 1 mm, red-brown foci in the gray and white matter of the cerebrum, cerebellum and brain stem.

Laboratory Results: Serial serum chemistry profiles revealed marked persistent hyperglycemia and on the day of euthanasia, the blood glucose was >700 mg/dL and the urine glucose was >500 mg/dL.

Contributor's Morphologic Diagnosis: Brain: 1) Severe, regionally extensive to diffuse, granulomatous meningoencephalitis with intralesional myriad fungal yeast forms (*Cryptococcus* sp.).
2) Mild to moderate, multifocal atherosclerosis with vascular thrombosis and acute and chronic infarcts.

Contributor's Comment: The brain contains a combination of lesions that are associated with persistent diabetes mellitus, namely exacerbation of atherosclerosis with vascular thrombosis and opportunistic fungal infection.[1] The fungal meningoencephalitis dominates the tissue changes. The yeast forms within these lesions are highlighted with Gomori's methenamine silver (GMS) stain and have morphology consistent with *Cryptococcus*. (Although cultures were not performed, infection with *C. neoformans* is suspected.) The precise mechanisms associated with susceptibility to infection and immune compromise in diabetic patients is unclear, but appear to involve both humoral and cellular mechanisms.[2] In human patients with diabetes mellitus, the common opportunistic fungal infections include sino-orbital aspergillosis, rhinocerebral mucormycosis, and cryptococcal meningitis.[3]

Vascular thromboses and/or infarcts are present in the majority of brain sections; some lesions are prominent and acute, while others are subtle and may be evidenced by focal accumulations of hemosiderin. The cause of vascular disease in diabetes mellitus is due in part to accelerated atherosclerosis, hypertension, and thickening of small vessels (microangiopathy).[1] Vascular thromboses and infarcts in the brain are recognized as a complication of diabetes mellitus; although, there is evidence that cerebral infarction may occur in cases of chronic meningitis.[4,5]

AFIP Diagnoses: 1. Brain, cerebrum: Meningitis, granulomatous, multifocal, moderate, with numerous yeast, etiology consistent with *Cryptococcus neoformans*, mongoose lemur (*Eulemur mongoz)*, primate.
2. Brain, cerebrum: Infarcts, multifocal, acute and chronic.

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. Infected animals are often immunosuppressed. Lesions can occur in any organ, but are most common in the central nervous and respiratory systems, followed by the integumentary system and eyes. *Cryptococcus neoformans* is a cause of mastitis in cattle. Cryptococcosis is the most frequent systemic mycoses in cats, and often occurs in the nasal cavity.

Gross lesions of *Cryptococcus neoformans*, are usually gelatinous due to the organism's mucopolysaccharide capsule. The capsule hinders phagocytosis and is a major diagnostic feature of the organism. However, acapsular forms 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 and Alcian blue.[6,7] The immune response varies from sparse to granulomatous depending on the presence of a capsule and the host's immune status.
Chronic diabetes mellitus (DM) results in immunosuppression and damage to multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels. In humans, diabetic macrovascular disease is a common complication of DM and is characterized by accelerated atherosclerosis that often leads to infarction. Atherosclerotic vessels were not evident in the sections of brain examined during the conference, which is attributed to variation among slides noted by the contributor.

Contributor: North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606

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SLIDE 6
CONFERENCE 2 / CASE II – ND-1 (AFIP 2935562)

Signalment: 1-year-old female domestic shorthair cat (*Felis domesticus*).

History: This animal developed a sinus infection following surgery (procedure not specified). Following antibiotic therapy the cat improved for a week then regressed to prior condition. The owners requested euthanasia.

Gross Pathology: There was marked, bilateral, pulmonary stiffness with mottling of the tissue. Fibrin strands were present on the pleural surface. Mucopurulent exudate was present at the opening of both nares.

Laboratory Results: Aerobic culture of lung resulted in no growth. Fluorescent antibody (FA) examination of frozen sections of lung was positive for feline herpesvirus-1.

Contributor's Morphologic Diagnosis: Pneumonia, necrotizing and fibrinous, diffuse, acute, severe with intranuclear eosinophilic inclusion bodies.

Contributor's Comment: Feline herpesvirus-1 (FHV-1) infections in cats typically result in keratoconjunctivitis, upper respiratory tract disease and abortion. Along with *Chlamydophila psittaci* and feline calicivirus, FHV-1 is part of an upper respiratory disease complex referred to as "feline rhinopneumonitis". Young animals are more severely infected. Transmission is accomplished through direct contact with infectious secretions or excretions. Vaccination does not prevent illness, but will limit clinical signs. The disease tends to be more common where crowding, poor management and carrier animals exist.

Acutely infected animals shed large amounts of virus for several weeks. The virus is typically transmitted horizontally, but can be passed from mother to fetus as well. Most infections are restricted to the upper respiratory tract; however, less common involvement of the trachea and lung can occur. Systemic infections can lead to

reproductive tract disease and reproductive failure. Kittens infected *in utero* may be aborted or delivered normally and develop "fading kitten" syndrome. More typical presentations of the disease result in sneezing, oculonasal discharge, rhinitis, conjunctivitis, fever, anorexia, ulceration of the tongue and hard palate and herpetic keratitis. Healthy cats recover in one to two weeks.

Multifocal necrosis typical of herpesvirus infections may be seen microscopically, often leading to secondary bacterial infections. Acidophilic intranuclear inclusion bodies can be detected up to a week after infection. This case is an example of the less common necrotizing pneumonia seen in fulminant viral infections.

AFIP Diagnosis: Lung: Bronchopneumonia, necrotizing, acute, diffuse, severe, with syncytia and epithelial eosinophilic intranuclear inclusion bodies, domestic shorthair, feline.

Conference Comment: The contributor gives a thorough overview of feline herpesvirus-1. Although upper respiratory tract infections are common in cats, pneumonia is uncommon except when there is immunosuppression.[4] Some differentials that conference attendees considered for pneumonia in a cat included feline calicivirus, *Toxoplasma gondii*, aspiration pneumonia, and toxins.

Feline caliciviral disease in cats has clinical and pathological similarities to feline herpesviral infection. Clinical signs include oculonasal discharge, rhinitis, conjunctivitis, and ulcerative stomatitis. Feline calicivirus has an affinity for epithelium, and additional lesions include interstitial pneumonia and necrotizing bronchiolitis. The primary viral infection is transient, but secondary bacterial infections are common.[4]

Toxoplasmosis is a worldwide disease that is often triggered by immunosuppression and affects humans, dogs, cats, and many wild mammals. Pulmonary lesions include severe necrotizing interstitial pneumonia, with prominent proliferation of type II pneumocytes and infiltrates of histiocytes and neutrophils.[4]

Aspiration pneumonia, resulting from vomiting, regurgitation, dysphagia, or anesthetic complication, is a common condition in cats. Lesions may be unilateral or bilateral and most often affect the right cranial lobe. Histologically, the severity of the lesion often depends on the chemical and microbial composition of the aspirated material.[4]

Toxins, such as paraquat, a broad-spectrum herbicide, can cause severe and often fatal interstitial pneumonia in dogs, cats, humans and other species. Gross lesions include interstitial emphysema, bullous emphysema and pneumomediastinum. Histologically, there is extensive necrosis of alveolar epithelial and endothelial cells, interstitial and alveolar edema, intraalveolar hemorrhage and proliferation of type II pneumocytes.[4]

Contributor: North Dakota State University, Veterinary Diagnostic Laboratory, Fargo, ND 58105 (www.vdl.ndsu.edu)

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SLIDE 7
CONFERENCE 2 / CASE III – 04-0825 (AFIP 2936447)

Signalment: 14-month-old female Angus, *Bos taurus*, bovine

History: This cow was from a herd of 32 cattle maintained on a partially forested pasture in west-central Georgia. The cattle had been purchased in October 2003 and had shown no clinical signs of disease until April 2004, when two adult cows were found dead without precipitating clinical signs. This animal displayed depression, ataxia, anorexia, and ptyalism. Clinical examination revealed central blindness, tremors, and reduced rumen motility. Therapy included thiamine, vitamin B_{12}, oral electrolytes, and antibiotics. The cow was euthanized for diagnostic purposes. Differential diagnoses included polioencephalomalacia (PEM), thromboembolic meningoencephalomyelitis (TEME), plant toxicosis, infectious bovine rhinotracheitis (IBR), rabies, and lead toxicosis.

Gross Pathology: The body of a 14-month-old female Angus bovine in good body condition weighing 635 kg was presented for necropsy. Excessive saliva was present in the oral cavity. A thick cloudy discharge exuded from the nares. The reticulum and rumen contained approximately 10-15 liters of white to light-green fibrous contents, which incorporated many angular irregularly shaped fragments of metal measuring 5-15 mm. Upon further examination, metal fragments were found not to be attracted to a magnet. Scattered pieces of black plastic were scattered throughout the rumen contents.

Laboratory Results:
Clinical Pathology:

WBC: 14,990 cells/_l
Neutrophils: 5,097 cells/_l (some toxic neutrophils present)
Lymphocytes: 8,095 cells/_l
Basophils: 300 cells/_l
Fibrinogen: 700 mg/dl
Aspartate aminotransferase (AST): 317 U/L
Creatine kinase (CK): 3,631 U/L
Calcium: 8.2 mg/dl
Phosphorus: 11.3 mg/dl
Magnesium: 1.5 mg/dl
Glucose: 80 mg/dl
Iron: 175 mg/dl

Fluorescent antibody testing of brain for rabies virus: Negative
Lead analysis of kidney: 85ppm (wet weight)
Arsenic analysis of kidney: <1ppm (wet weight)
Lead analysis of metallic rumen contents: 17%

Contributor's Morphologic Diagnosis: Neuronal necrosis, acute, locally extensive (laminar), severe, with capillary endothelial hypertrophy and astrocytosis, cerebral cortex.

Contributor's Comment: Lead levels in the kidney of this case (85 ppm) were within the reported toxic range for cattle (5-700 ppm).[1] A diagnosis of acute lead toxicosis was confirmed by the presence of lead-containing metal fragments within the rumen and a laminar pattern of neuronal necrosis and capillary endothelial swelling within the cerebral cortex. Further examination of the pasture revealed the recently destroyed remnants of an automotive battery. Acid-fast intranuclear inclusion bodies were demonstrated within the renal tubular epithelial cells.

Cattle are usually exposed to lead through the ingestion of automotive batteries, petroleum products, roofing felt, or lead-based agricultural products. Clinical signs are indicative of central nervous system dysfunction, and include depression, tremors, ataxia, blindness, seizures, and dementia.[2] Gross lesions are uncommon after acute lead toxicosis. Histologically, lesions include laminar cortical necrosis of the cerebrum with swelling of capillary endothelium, cerebral edema, congestion, and astrogliosis. These lesions are thought to reflect ischemic-anoxic injury, and are therefore not specific for lead toxicity. Ischemia may be induced by capillary endothelial swelling, the pathogenesis of which is not understood. Lead does not accumulate appreciably in CNS tissue. Renal tubular epithelial necrosis is evident in some cases; tubular epithelial cells often contain acid-fast intranuclear inclusion bodies.[3]

Lead is present in the environment in three forms: metallic lead, lead salt, and organic lead.[2] Many man-made products incorporate metallic lead or lead salts; these include automotive batteries, lead weights, lead-based paints, lead shot, various plumbing waste products, computer equipment, and lead arsenate pesticides.[1,2] The industrial

process of lead smelting has resulted in livestock exposure through the airborne contamination of pasture. Organic lead (tetraethyl- and tetramethyl-lead) is found primarily in leaded petroleum products.

The toxicity of lead is attributable to multiple mechanisms, including binding and inactivation of enzymatic sulfhydryl groups, competition with calcium ions, and alteration of vitamin D metabolism. Sulfhydryl binding is most evident in enzymes involved in heme synthesis, such as gamma-aminolevulinic acid dehydratase (ALAD) and ferrochelatase; hence, toxicity often results in red blood cell abnormalities. Inhibition of heme synthesis may contribute indirectly to neurological abnormalities through the increased production of serotonin, resulting in aberrant neurotransmission in the brain. Lead competes with calcium ions in bone resulting in the formation of lead precipitates ("lead line") in the long bones of young animals. Lead also competes with calcium at the neuromuscular junction, resulting in tremors and paresis.[2]

The absorption of lead is dependent on its form and the route of exposure. In general, organic lead is better absorbed than lead salts and metallic lead. Ingested lead is most readily absorbed from the acidic environment of the stomach, yet the majority of ingested lead is passed in the feces. Once absorbed, more than 90% of absorbed lead is bound to erythrocytes, making whole blood the tissue of choice for the clinical diagnosis of lead toxicosis. Blood lead levels in excess of 0.6 ppm (60 _g/dl) are diagnostic for lead toxicosis. Other tests include the detection of reduced blood ALAD activity (<50 nmol porphobilinogen/ml erythrocytes/hr in adult cattle) or excessive urinary ALA levels (>500 _g/dl).[2] Upon post-mortem examination, kidney, liver, and bone harbor the highest concentrations of lead. As in this case, liver and kidney levels in excess of 10 ppm (wet weight) are considered diagnostic for lead toxicosis.[1,2]

AFIP Diagnosis: Brain, cerebrum: Neuronal necrosis, laminar, with gliosis and hypertrophic endothelial cells, Angus, bovine.

Conference Comment: The majority of the sections exhibit laminar neuronal necrosis in the middle to deep layers of the cerebral grey matter. Additionally, scattered throughout all layers, there is neuronal necrosis evidenced by shrunken, angulated, hypereosinophilic and often karyorrhectic or pyknotic neurons.

The contributor gives a thorough and complete overview of lead toxicity in cattle. Lead toxicosis, or plumbism, has been reported in many different mammals, birds, and reptiles.[2] Unlike cattle, equine lead poisoning is usually chronic and affected individuals characteristically present with laryngeal and pharyngeal paralysis, and ingestion of large amounts can produce generalized paralysis.[3] Lead toxicity is not uncommon in humans. Humans are exposed to lead by two main routes, occupational and non-occupational. Occupational exposures include inhalation during spray painting, foundry work, mining and extracting lead, and battery burning. Non-occupational exposures, which may be more difficult to track in relation to individual exposure, include ingestion of

contaminated water due to lead plumbing, lead solder in food and soft drink cans, paint dust and flakes in homes with interior lead paint, soil contaminated with exterior lead paint, newsprint, automotive exhaust, and illegally produced alcoholic beverages (moonshine).[4]

Similar to cattle, intestinal absorption in humans is enhanced by calcium, iron, or zinc deficiency. Children have a greater absorption capacity than adults and thus are more vulnerable to lead toxicity. Absorbed lead clears rapidly from the blood, but is often deposited in bones where it has a half-life of 30 years. Therefore, high blood lead levels indicate a recent exposure but are not indicative of total body burden.[4]

As with cattle, lead toxicosis is a multisystemic disease in humans, affecting the central and peripheral nervous systems, hematopoiesis, gastrointestinal system, kidneys, and bones. Symptoms include headache, dizziness, memory deficits, decreased nerve conduction velocity, a microcytic hypochromic anemia with characteristic basophilic stippling of erythrocytes, colic, anorexia, and damage to the proximal renal tubules with classic intranuclear lead inclusions. Chronically, lead may cause diffuse interstitial fibrosis, gout and renal failure, in addition to infertility and hypertension.[4]

Contributor: Auburn University, College of Vet Med, Auburn AL 36849-5519 (www.vetmed.auburn.edu)

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SLIDE 8
CONFERENCE 2 / CASE IV – 04F36 (AFIP 2935878)

Signalment: Adult 4-6 kg Chinook salmon (*Oncorhynchus tshawytscha*), mixed gender.

History: During June, 2004, mortalities at a salt water net pen facility along the coast of British Columbia increased from 0.5 to 10% over a week span. These fish had previously had episodes of low-grade bacterial kidney disease and sporadic losses due to vibriosis (*Listonella anguillarum*).

Fish initially presented with inappetence and lethargy, which progressed over 1-2 days to listlessness, opercular flaring, swimming high in the water column and increased mortality.

Gross Pathology: The most significant lesions involved the gills. Involving multiple gill arches, randomly throughout the primary, and to a much lesser extent secondary lamellae, there were 2-3 mm diameter, firm, glistening white to grey spherical cysts with scattered foci of acute hemorrhage. Within a small proportion of fish, there were miliary granulomas within the anterior and posterior kidneys as well as scattered foci of acute hemorrhage within the mesentery.

Laboratory Results: Aerobic culture yielded variably mixed isolates of *Vibrio* spp, *Pseudomonas* spp, and *Escherichia spp*. Immunofluorescence of pooled liver, kidney and spleen was positive for *Renibacterium salmoninarum* and polymerase chain reaction of pooled gill tissue was positive for *Loma salmonae*.

Contributor's Morphologic Diagnosis: Gills: Branchitis, moderate, multifocal to coalescing, fibrinohemorrhagic and necrotizing, acute, with telangiectasia, respiratory epithelial hyperplasia and intralesional protozoal xenomas morphologically consistent with *Loma salmonae*.

Contributor's Comment: In British Columbia, because of the emergence of infectious hematopoietic virus (IHNV) and *Kudoa* spp in farmed Atlantic salmon (*Salmo salar*), producers are opting to further domesticate and raise Pacific salmon species (*Oncorhynchus* spp). With expansion and intensification of Pacific salmon aquaculture, there has been an emerging concern about a branchial microsporidian parasitic infection, *Loma salmonae*. This parasite has a narrow host range and is a recognized pathogen of Chinook (*O. tshawytscha*) and to a much lesser extent, Coho salmon (*O. kisutch*). Atlantic salmon, brook trout (*Salvelinus fontinalis*) and Arctic char (*Salvelinus alpinus*) appear refractory to experimental infection.

Parasites are acquired through ingestion of infective stages. The sporoplasm either invades between or through enterocytes, localizing within the lamina propria by 24 hours post infection. In experimental models of rainbow trout (*O. mykiss*) maintained at 15C, the merogonic stages are consistently detected within the heart as early as 2 days post infection and remain for 2 weeks.[1] After 2 weeks, and up to 5 weeks post infection, parasitic xenomas (which have undergone sporogony) are detected within the gills and spleen.

Electron microscopy has revealed uni- and binucleate meronts in sub-intimal host cells of the capillary channels of secondary lamellae and lamellar arteries. Involvement of phagocytic pillar cells has also been reported. Localization to these cells may be due to embolism, receptor mediated internalization, or evasion (by directly transferring from monocytes or lymphocytes). Respiratory distress is attributed to dissolution of the

xenoma with inflammation directed against the chitin-rich wall of spores, and necrosis. Horizontal transmission is believed to result from rupture of the xenomas.

Temperature has a profound influence on the kinetics of infection; at 5C or 20C xenoma formation is interrupted, but branchial infections can still occur. At 5C, parasites localize to the heart in 7 days with no detectable xenomas within the gills to 4 weeks post exposure. At 20C, parasites are detected within the spleen, heart, and gills 3 days post exposure with no apparent xenoma formation. Maintaining fish in ambient water temperatures less than 5 C and greater than 20C appears to interrupt the life cycle of this parasite and stock that recover are resistant to re-infection.[2] Ectoparasitic copepods, such as sea lice (*Lepeophtheirus salmonis*) appear to enhance susceptibility of stock to *Loma salmonae* infection.

At present there is no recognized treatment for *Loma salmonae* infection. Efforts are currently underway for vaccine development and possible management schemes to limit development of clinical disease.

AFIP Diagnosis: Gill: Branchitis, necrotizing and proliferative, multifocal, moderate, with mucus cell metaplasia, and numerous protozoal cysts, Chinook salmon (*Oncorhynchus tshawytscha*), piscine.

Conference Comment: Microsporidians are generally taxonomically specific intracellular parasites with a direct life cycle. After spore ingestion by the host, the sporoplasm is discharged and migrates to the target organ, begins the proliferative phase (merogony), producing large numbers of cells (meronts) by binary and multiple fission. Meronts then give rise to sporonts that undergo sporogony, producing mature spores. These spores are then released from lesions on the body surfaces or after death of the host.[3]

Nodular microsporidial lesions may grossly resemble those of other pathogens including myxozoans, ich, lymphocystis, and dermal metacercariae, bacterial granulomas, or neoplasia. However, these can easily be differentiated histologically. Microsporidial spores are typically 7 µm or less, egg-shaped to elliptical, and contain a posterior vacuole. Myxozoans, except during autogamy (sexual reproduction), all have multinucleated forms that have enveloping (primary) cells that contain enveloped (secondary) cells.[3] Their spores contain two polar capsules which stain intensely blue with Geimsa.[6] Ich, or white spot disease, is caused by *Ichthyophthirius multifilis*. The trophonts are large and characterized by a uniform layer of external cilia and a unique horseshoe-shaped macronucleus.[4] Lymphocystis is caused by a piscine iridovirus that preferentially infects dermal fibroblasts and inhibits mitosis, producing tremendous cellular hypertrophy.[5] Dermal metacercariae of digenean trematodes, result in white to yellow or black raised nodules that contain the parasite.[3]

Contributor: Animal Health Center, Abbotsford, British Columbia, Canada V3G 2M3

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SLIDE 9
CONFERENCE 3 / CASE I – CRL2 (AFIP 2936451)

Signalment: Female, 4-month-old, RAG1 mouse (*Mus musculus*).

History: Formalin-fixed tissues from a 4-month-old female RAG1 mouse were submitted for evaluation because of increased mortality in the colony.

Gross Pathology: None given

Contributor's Morphologic Diagnoses: Lung: 1. Acidophilic macrophage pneumonia, multifocal, moderate.
2. Intraalveolar *Pneumocystis carinii*, multifocal, mild.
3. Bronchopneumonia, subacute, suppurative, multifocal, marked.

Contributor's Comment: In the sections of lung, many alveoli are filled with macrophages, some multinucleated, with prominent intracytoplasmic eosinophilic acicular crystals. Although in this case the crystals are easily visualized on the H&E-stained section, their appearance can be enhanced with Grocott's methenamine silver stain or Gram stain. There is some alveolar septal thickening, type II pneumocyte hyperplasia, and a mild to moderate neutrophilic, histiocytic, and eosinophilic infiltrate in the areas with the acidophilic macrophage pneumonia. Scattered among the foci of acidophilic macrophage pneumonia, as well as in unaffected regions of the lung, is intraalveolar granular eosinophilic extracellular material typical of *Pneumocystis carinii*, with minimal associated inflammation. Bronchi are distended with an exudate of neutrophils and fewer macrophages with multifocal necrosis of epithelium, and extension into the adjacent pulmonary interstitium. On some of the sections, there is a

well-developed abscess with mineralization where an airway has been destroyed. A tissue Gram stain demonstrates intralesional gram-negative bacilli.

RAG1 [V(D)J recombination activating protein 1]-deficient mice do not produce mature B or T lymphocytes, and are therefore deficient in both humoral and cell-mediated immunity.[1] Loss of the RAG1 gene prevents rearrangement of both immunoglobulin and T cell receptor genes early in lymphocyte differentiation. RAG1-deficient mice have a greatly increased susceptibility to murine pathogens of all types.

Pneumocystis carinii is an opportunistic fungal pathogen of many species, which can cause fatal pneumonia in immunocompromised individuals. There are two tissue forms of *Pneumocystis carinii* – trophozoites, and cysts containing sporozoites, which can be demonstrated in tissues sections by immunohistochemistry or special stains, such as GMS.[2] This case is typical of the minimal inflammation associated with *Pneumocystis carinii* pneumonia in an immunodeficient mouse.

Acidophilic macrophage pneumonia is characterized by alveolar accumulations of macrophages containing eosinophilic crystals. The crystals may also be found free in alveoli and in the cytoplasm of airway epithelial cells. The macrophage infiltrates are accompanied by variable numbers of eosinophils, neutrophils and lymphocytes. Acidophilic macrophage pneumonia is often found in association with other pulmonary lesions, such as *Pneumocystis carinii* pneumonia or lung tumors. The development of this lesion is strain and age dependent, with lesions commonly found in nude mice, C57BL/6 or Sv/129, or genetically engineered mice, particularly those generated on a C57BL/6 or Sv/129 background. Ultrastructurally the crystals are indistinguishable from Charcot-Leyden crystals. However, biochemical analysis indicates that they are composed of Ym-1 protein, a member of the chitinase family. This protein is also known as T lymphocyte-derived Eosinophilic Chemotactic Factor (ECF-L).[3,4]

Bacterial culture could not be performed, as the tissues were fixed in formalin. *Pasteurella pneumotropica* has been associated with bronchopneumonia in association with *Pneumocystis carinii* in immunocompromised mice,[2] but other gram negative bacilli are possible etiologies as well.

AFIP Diagnoses: 1. Lung: Pneumonia, acidophilic macrophage, diffuse, mild to marked, RAG1 mouse, rodent.
2. Lung: Bronchopneumonia, suppurative, lobar, severe.
3. Lung: Intra-alveolar fungal organisms, multifocal, etiology consistent with *Pneumocystis murina*.

Conference Comment: Studies have recently show that the *Pneumocystis* found in laboratory mice is phylogenetically distinct from *P. carinii*; it was named *Pneumocystis murina* sp. nov., and was formerly known as *Pneumocystis carinii* f. sp. *muris*.[5]

The first transgenic (introduction of ectopic DNA) mouse was created in 1980 by random insertion, a process that remains common today. However, as technology has evolved, transgenes are now often targeted to specific sites on the genome for either gain of function (knock in) or loss of function (knockout or null mice). Gene alteration, both random and targeted, can lead to unexpected phenotypes, severe immunodeficiency, and embryonic or fetal death.[2]

Primary and opportunistic pathogens often significantly affect transgenic mice, not only because they cause overt disease, but they may also alter the biological responses of the mice to experimental pathogens. The pathologist must be aware of strain-related patterns of pathology in relation to spontaneous and infectious disease, developmental and comparative pathology, and the predicted and unexpected outcomes of gene alteration.[2]

Contributor: Charles River Laboratories, 251 Ballardvale Street, Wilmington, MA 01887 (www.criver.com)

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SLIDE 10
CONFERENCE 3 / CASE II – RT04-1470 (AFIP 2937327)

Signalment: 14 week-old, male, Norwegian Rat, (*Rattus norvegicus*), Strain: Sprague-Dawley

History: This 14 week-old rat underwent surgery using an anesthetic combination of pentobarbital, magnesium sulfate and chloral hydrate of an unknown concentration. The anesthetic, "Equithesin", was injected intraperitoneally. The procedure implanted a microdialysis guide cannula in the ventral tegmental area, a region of the mesencephalon. Over the next five days, the rat's weight dropped from 273 grams to 247 grams. Four days after the operation, the animal looked depressed with "ruffled

fur". Palpation revealed dilated gut loops, especially in the left ventral abdominal quadrant. Five days following the operation, an eight-hour period passed with no fecal pellets. The animal was euthanized. The attending veterinarian performed the necropsy and submitted tissue in formalin.

Gross Pathology: Gross pathology findings reported by the clinician were as follows: The animal had very little fat stores and the stomach and duodenum had very little content. The ileum, cecum and colon were dilated and filled with ingesta. Contents of the colon were dry and firm. The dilated portion of digestive tract ended abruptly at an area of adhesion between body wall and colon. The terminal colon and rectum were only moderately dilated. The anterior surface of the liver had round, blunted edges and multiple adhesions to the stomach and diaphragm. The lungs were moderately congested.

Contributor's Morphologic Diagnosis: Colon: Peritonitis, fibrosing, diffuse, with moderate leiomyositis.

Etiologic Diagnosis: Chloral hydrate induced peritonitis.

Contributor's Comment: Chloral hydrate administered intraperitoneally to rodents can injure abdominal organs. Documented effects include gastric mucosal injuries, adynamic ileus, and peritonitis,[1,2] fibrosis of the serous membranes, and steatitis.[3] A study in 1999 documented a marked decrease in intestinal peristalsis and acetylcholine-induced contractions in rats following intraperitoneal injections of chloral hydrate.[4]

In this case, histopathological changes include fibroplasia of the colonic serosa and muscularis, giving it a pleated form. Individual myofibers of the muscularis externa are necrotic. There is inflammation of the serosa of the colon with neutrophils, lymphocytes, macrophages and plasma cells with small numbers of inflammatory cells extending into the muscularis externa. Hemorrhage is present in muscular layers. Some sections of colon have ulcers. Gastric ulcers often accompany intraperitoneal (IP) injections of chloral hydrate.[2] However, no gastric sections were submitted in this case.

Differential diagnoses included peritonitis secondary to perforating ulcer of the colon, or a needle perforation of the bowel with leakage of contents. A perforating ulcer or other cause of leakage of intestinal contents would be expected to cause more suppurative inflammation with peritoneal effusion and large numbers of intraperitoneal bacteria. Although not used in this case, the anesthetics tribromoethanol (Avertin) and pentobarbital have been reported to produce peritonitis and ileus in rats.[5, 6]

In 1961, The Walter Reed Army Institute of Research reported losing some rats and guinea pigs to ileus and intestinal blockage. They attributed it to a side effect of intraperitoneal injections of chloral hydrate.[1]

A 1977 study reporting chloral hydrate toxicity is commonly cited in literature. The study involved the implantation of brain electrodes into rats. Several rats became ill and died. Symptoms were lethargy, anorexia, abdominal distension, and constipation. After an infectious etiology was ruled out, chloral hydrate was administered in the second leg of the experiment. Of 27 rats, 20 developed typical signs of gastric distress seen in the initial study. Fourteen of the 20 rats died.[7]

Chloral hydrate usage is controversial but it remains widely used in surgical procedures. The drug is often chosen for intra-cranial surgeries because inhalation anesthetics present access problems. Barbiturates often depress respiratory and cardiovascular systems and require long recovery times.[8] Regardless of the drug, intraperitoneal injections in rats are often utilized because of their small muscular mass and relatively inaccessible vasculature.[6]

The pathogenesis of colonic ileus is unclear but it may involve a decrease in parasympathetic activity coupled with an increase in sympathetic inhibition of the colon. The most common cause of paralytic ileus is abdominal surgery. Abdominal trauma, serum electrolyte imbalance, septicemia, and intrathoracic conditions such as pneumonia, myocardial infarction and lower rib fractures have contributed to paralytic ileus. Narcotics, calcium channel blockers, and anticholinergic medications can also cause this condition. Treatment usually involves discontinuing use of narcotic and anticholinergic drugs and correction of electrolyte imbalance.[9]

Chloral hydrate is thought to be a chemical irritant. In a study that tested chloral hydrates' anesthetic effect, 83% of rats that were injected intraperitoneally had mild to moderate inflammation of the abdominal area involving the splenic capsule and peritoneal surface of the body wall.[6] Intestinal inflammation was not reported in this study.

Some studies attribute disease to the concentration of the drug. However, ileus has been reported to occur sporadically even at lower concentrations.[4] Preceding its use with a low dose of barbiturates, opioids, alpha-2 agonists, or phenothiazine tranquilizers has been reported to reduce negative side effects of chloral hydrate.[2] A study in 1995 concluded that "Equithesin" without chloral hydrate is an effective anesthetic that can be maintained for several hours by supplemental doses.[8]

AFIP Diagnosis: Colon, serosa and tunica muscularis: Serosal fibrosis, diffuse, mild, with leiomyocyte degeneration and necrosis, and neutrophilic inflammation, Sprague-Dawley rat, rodent.

Conference Comment: As many pathology residents realize, it is often more difficult to recognize the absence of cells or a structure than the addition of cells or a change in morphology. This is especially true in this case. Many conference participants did not recognize the focally extensive loss of smooth muscle that normally comprises the

external longitudinal layer of the tunica muscularis and replacement by fibrous connective tissue. This histologic feature is highlighted with a Masson's trichrome stain. Of interest to many of the conference participants, is the relative paucity of inflammation. This is somewhat surprising given the extent of myocyte degeneration and necrosis present in most tissue sections and the short clinical history of only five days post injection. Whether these changes are due to species, strain, individual, anesthetic, or inflammatory mediator differences is uncertain. Nonetheless, these findings are consistent with those in published reports.[4,5,6,7]

Contributor: National Institute of Health, Diagnostic and Research Services Branch, Division of Veterinary Resources, 28 Service Road West, Building 28A--Room 115, Bethesda, MD

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SLIDE 11
CONFERENCE 3 / CASE III – CF13 (AFIP 2935883)

Signalment: 4.33 year-old male rhesus macaque (*Macaca mulatta*).

History: This animal was one of a group used to evaluate viral dynamics in acute simian immunodeficiency virus (SIV) and recombinant simian-human immunodeficiency virus (SHIV) infection and the role of $CD8^+$ T cells. It was inoculated with SHIV162p3

six months prior to necropsy. Multiple biopsies of peripheral lymph nodes were performed at determined intervals prior to euthanasia. Due to the development of severe emaciation, weight loss, and dehydration this animal was euthanized.

Gross Pathology: Approximately 10 ml of pale yellowish fluid was present in the peritoneal cavity. Fibrous adhesions of the colon to the abdominal wall were observed in moderate severity. Yellow fluid feces were observed in the colon. The walls of the gallbladder and bile ducts were extremely thickened, and folds of mucosa protruded into the lumina, which contained cloudy bile. The pancreas was slightly firmer than normal. Mesenteric, colonic and iliac lymph nodes were enlarged four times normal size, and the palatine tonsils were prominent.

Contributor's Morphologic Diagnoses: 1. Gallbladder and liver: Marked diffuse chronic lymphoplasmacytic and suppurative cholecystitis with adenomatous hyperplasia and intralesional protozoal parasites (*Cryptosporidium sp.*).
2. Moderate widespread chronic lymphoplasmacytic and neutrophilic cholangiohepatitis with bile duct hyperplasia.
3. Marked focal intimal myocyte hyperplasia with minimal lymphocytic and neutrophilic phlebitis.

Contributor's Comment: Major bile ducts are markedly expanded by a proliferation of epithelium that occludes the lumen, by cellular inflammatory infiltrates of the lamina propria, and by variable degrees of periductal fibrosis. The epithelium is thrown into deep folds and crypts are lengthened, giving the tissue a multilocular appearance. The tall columnar ductular epithelium has more variability in its height, more prominent nucleoli, and markedly increased mucus production when compared to normal macaque bile duct epithelium. Segmentally, the apical epithelial surface has numerous 2-6 _m diameter lightly basophilic spherical bodies interpreted as *Cryptosporidium sp.* The lumina of the ducts and crypts contain increased mucus and scattered foci of neutrophils and necrotic cellular debris. The lamina propria is markedly expanded by infiltrates of plasma cells, neutrophils, lymphocytes, and eosinophils. Scattered lymphoid nodules are present. The tunica muscularis is incorporated into hyperplastic folds and is discontinuous. There is variable fibrosis surrounding the muscularis. Within the hepatic parenchyma there are portal infiltrates of lymphocytes, histiocytes, eosinophils, and plasma cells that rarely cross the limiting plate and there is multifocal marked hyperplasia of bile ducts. Cryptosporidia were not detected in small bile ducts. Rare random foci of lymphocytic inflammation are present in sinusoids. A prominent hepatic vein has a well-demarcated plaque-like thickening of the intima due to myocyte hyperplasia and deposition of matrix with a high content of ground substance. A few scattered lymphocytes and neutrophils are present within the lesion. In tissues not represented on the distributed slide, there was minimal cryptosporidiosis of the pancreatic duct, mild chronic myelitis, and lymphoid depletion. There was also mild chronic colitis, characterized by lymphoplasmacytic infiltration of the lamina propria, crypt dilatation, and the accumulation of necrotic debris in crypt lumina, but no cryptosporidia were detected. Colitis is a frequent finding in SIV-infected macaques.

The proliferative cholangitis and cholecystitis observed in this SHIV-infected macaque resembles the cholangiopathy previously reported in SIV-infected monkeys[1,2] and described in human AIDS patients.[3] AIDS-cholangiopathy, characterized by sclerosing cholangitis and acalculous cholecystitis is presumptively due to *Cryptosporidium* infection. Unlike primary cryptosporidial infections in immune competent human hosts, which are usually limited to the small intestine, cryptosporidial infections in AIDS result in persistent infections and extension beyond the intestine to include the stomach, and biliary and pancreatic ducts, lung, and inner ear.[3] Among human immunodeficiency virus (HIV)-positive persons with diarrhea, *Cryptosporidium* infection was present in 14-24%.[4] *Cryptosporidium* infection of nonhuman primates immunosuppressed by either SIV or simian type D retrovirus occurs in the small intestine, biliary and pancreatic tracts, conjunctiva, and lung.[5,6,7] Intestinal infection is associated with a profuse persistent watery diarrhea leading to dehydration and mortality.[8,9,10]

Primary intestinal cryptosporidial infections occur in neonatal macaques[11], as in many other mammalian species, and are associated with diarrhea that resolves within 16 days. Neonatal cryptosporidiosis is an important cause of diarrhea in cattle and humans worldwide and is usually due to sporocyst-containing fecal contamination of water supplies. Reportedly, 20% of US adolescents have been infected while over 90% of children living in an urban shantytown in Northeast Brazil were seropositive.[4] The resistance of sporocysts to chlorination, their small size (4-6_m), and their low infective dose (as low as 10 sporocysts) challenge the ability of water treatment plants to remove this agent from drinking water supplies.

Cryptosporidium spp. cause primary infections in immunocompetent hosts across many, if not all, vertebrates.[12] The separation of *Cryptosporidium* into species remains a controversial task and is based on morphology, host specificity, virulence, and genomic characterization. Currently, at least two mammalian (*C. parvum*, *C. muris*), two avian (*C. meleagridis*, *C. baileyi*), one reptilian (*C. serpentis*), and one fish (*C. nasorum*) species are established, although mammalian infections with avian cryptosporidia are documented.[4,13] The gastric cryptosporidia, *C. muris*, *C. serpentis*, and *C. baileyi* have larger cysts and are distinct from other cryptosporidia. *C. parvum* is divided into two genotypes where type 1 is restricted in host range to humans and nonhuman primates and type 2 is more commonly associated with bovine infections.[13]

Resolution of cryptosporidial infection depends upon interferon gamma (IFN-gamma), interleukin 12 (IL-12) and CD4+ T cells in mouse models.[14] However, differences in immune responses to *Cryptosporidium* infection between species limit further generalizations regarding the mechanisms of immune response. Secretory immunoglobulins can provide protection at the sporozoite attachment and entry stages.[14] The lack of effective chemotherapeutics has led to investigation of maternal vaccines for protection through maternal antibody transfer in cattle and humans and immunoglobulin therapeutics for immunocompromised patients.

The recent sequencing of the genome for *Cryptosporidium* parvum type 2 revealed a paucity of metabolic and mitochondrial enzymes, indicating that the organism depends

heavily on the host cell for nutrients. However, the identification of metabolic enzymes that resemble enzymes of plant and bacterial origin has provided new targets for drug development.[15]

Cryptosporidial infection causes epithelial hyperplasia in a variety of species, but the mechanism remains unknown. Cryptosporidial infection also results in villus blunting, which may be due in part to induction of apoptosis in epithelial cells.[16] Recently, oligonucleotide microarrays identified host cell genes which are differentially regulated during infection of epithelial cells in culture. Several genes involved in control of cell proliferation and apoptosis were significantly modulated[17], which may lead to a better understanding of this characteristic pathological effect of *Cryptosporidium sp.* infection.

The intimal myocyte hyperplasia and minimal phlebitis are interpreted as a SIV-related change, similar to the arteriopathy that has been described.[18]

AFIP Diagnoses: 1. Liver: Choledochitis, proliferative, chronic-active, diffuse, severe, with mucus cell metaplasia, apical protozoa, and multifocal mild cholangiohepatitis with bile duct hyperplasia, etiology consistent with *Cryptosporidium* sp., rhesus macaque, primate.
2. Large muscular vein, intima: Fibromyxomatous proliferation, focally extensive, moderate.

Conference Comment: The contributor provides a thorough overview of cryptosporidiosis in several species. Transmission is direct with ingestion or inhalation as the main routes of infection. Upon ingestion of oocysts, sporozoites excyst, invade mucosal epithelial cells, and undergo both asexual (merogony) and sexual (gametogony) replication resulting in meronts or macro- and microgametocytes respectively. Oocytes sporulate internally to contain four sporozoites that then either contribute to autoinfection or exit the body.[19]

The ultrastructure of *Cryptosporidium* sp. in tissue is unique. In the intestine, the organism attaches to enterocytes via a specialized feeder organelle, displaces microvilli, and is surrounded by a host cell derived membrane (parasitophorous vacuole). This results in the organism being intracellular but extracytoplasmic and is characteristic for *Cryptosporidium* sp.[20]

Contributor: Tulane National Primate Research Center, Division of Comparative Pathology, 18703 Three Rivers Road, Covington, LA (http://www.tpc.tulane.edu)

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SLIDE 12
CONFERENCE 3 / CASE IV – PA-4039-1 or PA-4039-4 (AFIP 2888624)

Signalment: 8 week-old New Zealand White (NZW) rabbit (*Oryctolagus cuniculus*).

History: Because of recent unexplained deaths, 6-week-old sentinel animals were placed in this rabbit room. They were exposed to dirty bedding from other cages and euthanized after two weeks. At the time of their sacrifice the animals were clinically healthy and normal in appearance.

Gross Pathology: The terminal ileum was enlarged and prominent in appearance. The mucosal surface was markedly thickened and had a corrugated appearance.

Contributor's Morphologic Diagnoses: 1. Enteritis, proliferative, diffuse, moderate-marked, with severe crypt epithelial hyperplasia and focal crypt abscesses.
2. Villous blunting, focal, mild (some sections).
3. Central lacteal dilatation, patchy, mild.
4. Intraepithelial protozoa consistent with *Eimeria*, villous epithelium, multifocal, minimal-mild (some sections).

Contributor's Comment: Multiple sections of bowel were cut to provide slides for the conference. Lesions vary slightly from block to block. Histological changes were limited to the ileum and consisted of a significant thickening of the mucosa associated with hyperplasia of both villous and crypt epithelium. The crypt epithelium was severely crowded with a marked increase in mitotic rate and crypts occasionally demonstrated branching. Focal areas of crypt luminal dilatation with attenuated epithelium and central necrotic debris were observed (crypt abscesses). A moderate mixed inflammatory infiltrate was observed in the deep mucosa that in areas included a significant histiocytic component. Areas of villous shortening & blunting (some sections) and dilatation of the central lacteal structure were occasionally observed.

In some of the H&E stained sections, numerous small punctate structures consistent with coccoid and small bacilliform bacteria were observed adjacent to villous and crypt epithelium, extending in some sections into the submucosal regions.

A Warthin-Starry silver stain revealed the presence of myriads of short, curved rod-shaped argyrophilic bacterial organisms primarily within the apical cytoplasm of hyperplastic crypt epithelium. These organisms were strongly positive on immunohistochemical staining with a porcine *Lawsonia intracellularis* -specific monoclonal antibody. Additionally, the presence of this organism was further confirmed by amplification of 319-base-pair-(bp) and 182-bp products specific for porcine *L. intracellularis* chromosomal DNA and 16S rRNA genes, respectively.

The gross and microscopic findings, in conjunction with the immunohistochemical and molecular verification are consistent with a diagnosis of *Lawsonia intracellularis* induced subclinical proliferative enteritis.

Lawsonia intracellularis is a recently identified intracellular pathogen, phylogenetically unrelated to other pathogens.[1] It is an obligate intracellular organism and has been associated with hyperplastic intestinal processes in a growing number of mammalian and avian species. Although the disease can manifest sub-clinically in many animals, moderate to severe clinical disease is often seen in a wide variety of hosts, most notably in pigs and hamsters.

Infection in rabbits with *Lawsonia* has been well documented,[2,3,4,5] including dual involvement with enteropathogenic *E. coli*. Some of these reported outbreaks have been associated with high mortality and the presence of lesions including severe necrosis, ulceration and suppurative inflammation in association with the proliferative changes. In some reports, histiocytic enteritis is described as a result of *Lawsonia* infection.[2,6] In other sentinel animals from this cohort sacrificed at later time points, a distinctly granulomatous pattern of inflammation was noted - suggesting that the histiocytic response may be associated with a later stage/resolution of infection.

The *Eimeria* organisms noted in this case were thought to be incidental. Concurrent infection of this animal with other enteric organisms and agents was not excluded.

AFIP Diagnoses: 1. Small intestine: Enteritis, proliferative, histiocytic and heterophilic, diffuse, moderate, New Zealand White rabbit, lagomorph.
2. Small intestine: Intraepithelial protozoa, multifocal, few, etiology consistent with *Eimeria* sp.

Conference Comment: As the contributor mentioned, *Lawsonia intracellularis* has been identified as the causative agent of a proliferative enteropathy in a number of species, including rabbits, hamsters, guinea pigs, rats, ferrets, non-human primates, swine, sheep, horses, white-tailed deer, dogs, Arctic and blue fox, emus, and ostriches.[1,7] Regardless of the species affected, two hallmarks are constant, namely proliferation and intracellular bacteria.[1]

The gross lesions in pigs, hamsters, foals, white-tailed deer, and guinea pigs are similar. They predominately occur in the distal ileum, beginning as small raised opaque islands that progress to a confluent, irregularly nodular, surface which may have areas of hemorrhage and/or necrosis. Histologically, the thickened epithelium results from expansion and elongation of the crypts, with actively dividing epithelial cells, and an absence of goblet cells.[1]

Lesions in other species differ in location and histologic changes. In ferrets, rabbits, and blue foxes lesions are most commonly found in the cecum and proximal colon. In ferrets, histologic changes may include organisms that penetrate the muscular tunics and appear in the serosa or draining lymph nodes. In rabbits, infiltrating histiocytes may expand the lamina propria, and the organism often has a single polar flagellum and is located in a membrane-bound vacuole, a feature not found in pigs. [1]

Contributor: University of Pittsburgh, Div. of Lab Animal Resources, S 1040 BioMedical Science Tower, 3500 Terrace Street, Pittsburgh, PA (http://www.pitt.edu)

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SLIDE 13
CONFERENCE 4 / CASE I – 04-19232-7 (AFIP 2936303)

Signalment: Four and a half year-old, castrated, male Vizsla, canine.

History: This dog had a history of chronic back pain (localized to the thoracolumbar junction) and generalized skin lesions (consistent with pyogranulomatous dermatitis /panniculitis on biopsy) for 9 months and was treated with cyclosporine, antibiotics and corticosteroid treatment with no clinical improvement. One week prior to death, the dog presented clinically with a head tilt and mild ataxia, but otherwise normal behavior and mentation. Medications were continued (prednisone, tetracycline, niacinamide, safflower oil for back pain and skin condition). About six days later, the dog became

acutely anorexic and depressed in the morning, progressing to lateral recumbency by evening, and died.

Gross Pathology: The auricles, pericardium, epicardium and endocardium have numerous, multifocal to coalescing, pinpoint to approximately 0.5 cm in diameter, white to tan, firm, slightly raised foci (Fig.1). The foci are diffusely tan on cut section and extend approximately 2-3 mm into the myocardium.

Laboratory Results: *Aspergillus* sp. was cultured in small numbers mixed with heavy *Cladosporium* sp. growth (likely environmental contaminant).

Contributor's Morphologic Diagnosis: Severe, multifocal, pyogranulomatous myocarditis with intralesional septate fungal hyphae (*Aspergillus* spp.)

Contributor's Comment: Histologic evidence of fungal hyphal structures were found in the heart (Figs. 2, 3), brain, lungs, liver, adrenal glands, lymph nodes, kidney, adipose tissue, subcutaneous tissues and vasculature. The gross and histologic lesions in this dog are consistent with a severe, disseminated fungal infection. The histologic appearance of the fungal hyphae with hematoxylin & eosin and special fungal stains (GMS, PAS) is compatible with *Aspergillus* sp. (Fig. 4). The central nervous system involvement and multiple organ failure resulted in clinical neurologic disease and ultimately led to this animal's demise. It was possible that the fungal infection occurred secondarily to chronic steroid-induced immune suppression and antibiotic therapy, and may not be associated with the inciting cause for the initial presenting complaint 9 months prior. The cause of the back pain was undetermined. There were no histologic indications of spinal cord lesions or fungal infiltration in sections from the thoracolumbar junction.

Aspergillus sp. is a saprophytic, ubiquitous mold that ordinarily occurs as an opportunist rather than a pathogen.[1] Two forms of aspergillosis have been described in dogs – localized infection of the nasal cavity and paranasal sinuses (primarily caused by *Aspergillus fumigatus* or *A. flavus*) and disseminated mycotic infection.[2] In dogs, disseminated aspergillosis is relatively rare. Most cases of aspergillosis have been reported in the German Shepherd Dog suggesting a possible inherent inability to mount an effective immune response against the organism.[2,3] In people, disseminated aspergillosis has been seen exclusively in immune suppressed or immune-deficient individuals; however, the same association has not been documented in infected dogs.[2] The use of corticosteroids and other immunosuppressive drugs may increase the susceptibility and incidence of systemic aspergillosis in treated dogs.[4]

AFIP Diagnosis: Heart: Epicarditis and myocarditis, necrotizing and granulomatous, multifocal to coalescing, marked, with vasculitis, and pigmented and non-pigmented fungal hyphae, Vizsla, canine.

Conference Comment: Mycotic diseases may be broken down into two groups: those caused by opportunistic fungi and those caused by primary pathogens associated with systemic "deep" mycoses.[5] Examples of both primary and opportunistic fungi are listed below:

Opportunistic fungi infections
1. Aspergillosis (*Aspergillus* sp.)
2. Zygomycosis
   Mucorales: *Rhizopus* sp.
   Absidia sp.
   Mucor sp.
   Entomophthorales: *Basidiobolus* sp.
   Conidiobolus sp.
3. Pythiosis (*Pythium insidiosum*)
4. Phaeohyphomycosis (*Cladosporium* sp.)

Primary pathogenic fungi infections
1. Blastomycosis (*B. dermatitidis*)
2. Histoplasmosis (*H. capsulatum*)
3. Cryptococcosis (*C. neoformans*)
4. Coccidioidomycosis (*C. immitis*)

In the slides examined by conference attendees there are high numbers of non-pigmented fungal hyphae (Aspergillus sp.) and low numbers of pigmented fungal hyphae (Cladosporium sp.) with some sections containing a prominent vasculitis. As mentioned by the contributor, *Aspergillus* spp. are ubiquitous fungi. They are an important cause of disease in birds but are opportunistic pathogens in immunosuppressed domestic animals and humans. Typical gross findings include multifocal to coalescing pale nodules. Histologically, aspergillosis is characterized by fungal granulomas or pyogranulomas composed of a central area of necrosis containing hyphae that are 3-5 _m wide, with regularly septate parallel walls, and dichotomous acute angle branching, surrounded by variable numbers of neutrophils, lymphocytes, epithelioid macrophages, and fibroblasts. Many cases also demonstrate vasculitis.[6]

The pigmented fungal hyphae are consistent with *Cladosporium* sp. with 2-6 _m wide, long, closely septate hyphae with non-parallel walls, non-dichotomous branching, and occasional thick-walled vesicular swellings.[6] Phaeohyphomycoses are uncommon opportunistic infections caused by a number of dematiaceous (pigmented) fungi. *Cladosporium* spp. primarily affect dogs and cats with a predilection for the central nervous system, only occasionally causing systemic disease. Grossly, the granulomas may appear pigmented. Histologically, the fungal elements may be very pale to very dark depending on the amount of pigment. The pigment is melanin and generally stains with Fontana-Masson. As with other opportunistic mycoses, conformation of the etiology is based on concomitant demonstration of hyphae in tissue and culture of a morphologically compatible organism from properly obtained tissue specimens.[7]

Contributor: University of Illinois, College of Veterinary Medicine, Department of Veterinary Pathobiology, 2001 S. Lincoln Avenue, Urbana IL (http://www.cvm.uiuc.edu/vp)

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SLIDE 14
CONFERENCE 4 / CASE II – CP04-1187-02 (AFIP 2938293)

Signalment: Six month-old, male, p53 -/- Background strain: C56BL/6;129SJ mouse.

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

Gross Pathology: The thymus was white, soft and markedly enlarged. There was a small, slightly dark, raised nodule in the heart.

Contributor's Morphologic Diagnoses: Thymic lymphoma; Cardiac hemangiosarcoma

Contributor's Comment: Multiple neoplasms are present. The thymus is effaced by a monomorphic population of densely packed sheets of lymphocytes. The cells have round to oval nuclei with finely dispersed chromatin and one prominent nucleolus. Individual cells have a small rim of cytoplasm and discernable cell borders. Anisokaryosis and anisocytosis are moderate. The mitotic rate is high, 8-12 per 400x field of view. Apoptotic cells are common, giving the mass a starry sky appearance. Focally in the intraventricular septum there is a multinodular poorly circumscribed proliferation of plump polygonal cells that line blood-filled spaces. Neoplastic lymphocytes can also be seen along the epicardium in some sections.

Mutation of the *p53* tumor suppressor gene occurs in a high percentage of human tumors including, colon, breast, lung and brain. In most cases the *p53* mutation is acquired in somatic cells. However, some individuals inherit a mutant *p53* allele and are predisposed to develop a malignancy when a "second hit" or loss of the normal allele occurs. This is referred to as Li-Fraumeni syndrome.

The *p53* protein (P53) is localized to the nucleus and functions to prevent the proliferation of cells with DNA damage due to irradiation, UV light, or mutagenic chemicals by way of repair or apoptosis. When damage occurs, P53 levels rapidly increase and arrest the cell cycle by transcription of *p21*, an inhibitor of cyclin dependent kinases that in turn prevent the phosphorylation of Rb, preventing entry into the S phase of the cell cycle. Transcription of *GADD45*, (Growth Arrest and DNA Damage) by *p53*, assists in DNA repair. If DNA damage is repaired, P53 activates *mdm2* whose product binds to and down-regulates *p53* releasing the cell cycle arrest. If DNA repair is unsuccessful, *p53* initiates apoptosis through *bax* and IGF-BP3. IGF-BP3 binds insulin-like growth factor receptor and *bax* antagonizes *bcl-2*.[3]

Mice deficient in *p53* are prone to the development of tumors. The tumor spectrum in *p53*-mutant mice includes thymic lymphoma (T-cell type), rhabdomyosarcoma, fibrosarcoma, hemangiosarcoma, teratoma, anaplastic sarcoma, osteosarcoma, lung adenocarcinoma, hair matrix tumor, leiomyosarcoma, and rarely brain tumors. Mice with a homozygous null mutation of *p53* have a higher incidence of lymphomas usually in the thymus. In mice with a heterozygous mutation, sarcomas predominate. Loss or decline of the wild type P53 activity has been demonstrated in tumors of *p53* heterozygotes. Mice homozygous for *p53* mutation have an accelerated rate of malignancy with the majority of the animals dying by six months of age. Occurrence of two distinct tumor types, like this case, is not uncommon. Lymphoma with a sarcoma or a teratoma is the most common observation.[1,2]

AFIP Diagnoses: 1. Thymus: Lymphoma, *p53* -/- C56BL/6;129SJ mouse, murine.
2. Heart, myocardium: Hemangiosarcoma.

Conference Comment: Not all sections contained the myocardial hemangiosarcoma and only some sections contained neoplastic lymphocytes within the epicardium. The contributor provides a thorough overview of *p53* and its critical role in cell cycle modulation. Carcinogenesis is a multistep process at the phenotypic and genetic level. The fundamental changes in cell physiology that determine malignancy include: self-sufficiency in growth signals (proliferation without external stimuli, often due to oncogene activation); insensitivity to growth-inhibitory signals (especially transforming growth factor beta (TGFß) and direct inhibitors of cyclin-dependent kinases); evasion of apoptosis (often through inactivation of *p53*); defects in DNA repair; limitless replicative potential (associated with maintenance of telomere length); sustained angiogenesis (induced primarily by vascular endothelial growth factor (VEGF)); escape from immunity and rejection; and the ability to invade and metastasize.[4]

Simply put, a malignant neoplasm is the result of acquired (environmental) DNA damage that is not repaired or an inherited mutation in genes affecting DNA repair, cell growth or apoptosis. Regardless of the initiating events, the end result is a mutation in the genome of somatic cells leading to activation of growth-promoting oncogenes, inactivation of tumor suppressor genes, and/or alterations in genes that regulate apoptosis. The mutated cells then undergo unregulated cell proliferation and sometimes decreased apoptosis. Within this clonal expansion, there may be cells that undergo additional mutations, eventually resulting in tumor progression, invasion and metastasis.[4]

Contributor: St. Jude Children's Research Hospital, Dept. of Pathology, Room 5017C, 332 N. Lauderdale, Memphis TN

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SLIDE 15
CONFERENCE 4 / CASE III – 423102 (AFIP 2938284)

Signalment: Eleven-month-old, spayed female, Cocker Spaniel, *Canis familiaris,* dog.

History: After an elective ovariohysterectomy in January 2004, the patient developed waxing and waning fevers with persistent neutrophilic leukocytosis. The dog was re-examined by the referring veterinarian in June 2004. A mass was identified in the patient's lower left abdominal quadrant, and a fine needle aspirate of the mass revealed suppurative inflammation. Exploratory laparotomy revealed an omentum-encased surgical sponge.

Gross Pathology: The mass was submitted as a surgical biopsy specimen. It was 6.5 cm x 5.5 cm x 3.5 cm, lobulated, firm, tan to pale brown and was covered by flecks of clotted blood. In the center of the mass there was entrapped surgical sponge material.

Laboratory Results: Abnormal hematology and serum biochemistry test results were as follows:

1. Leukocytosis (25.5 x 10^3/µl; Reference range - 6.0 to 14.3) with neutrophilia (20.4 x 10^3/µl; Reference range - 3.3 to 10.1) and monocytosis (1.4 x 10^3/µl; Reference range - 0.1 to 0.9)
2. Normocytic normochromic anemia
    - Low erythrocyte count - 5.50 x10^6/µl (Reference range - 5.8 to 8.9)
    - Low hemoglobin – 12.7 g/dl (Reference range - 14.3 to 21.1)
    - Low hematocrit - 37.8% (Reference range - 41.7 58.1)
    - Normal Mean Corpuscular Volume - 68.7 fl (Reference range - 63.2 to 76.8)
    - Normal Mean Corpuscular Hemoglobin Concentration - 33.6 g/dl (Reference range - 32.4 to 38.4)
3. Hypoalbuminemia (3.0 g/dl; Reference range - 3.1 to 4.2)
4. High serum alkaline phosphatase (202 Units/liter; Reference range - 7 to 128)
5. Low serum alanine aminotransferase (14 Units/liter; Reference range – 21 to 97)

There was scant growth of beta hemolytic *Streptococcus* serotype group G on aerobic culture.

Contributor's Morphologic Diagnosis: Adipose tissue, omentum**:** Marked chronic regionally extensive pyogranulomatous omentitis with intralesional surgical sponge material (gossypiboma).

Contributor's Comment: The technical term for a surgical sponge accidentally left inside a patient's body is "gossypiboma." The word is derived from the Latin word *Gossypium* for cotton and the Swahili word *boma* for *"place of concealment"*.[3] Retained surgical sponges seem to be fairly common in human surgical practice and a higher incidence of retained laparotomy sponges has been reported in association with gynecological procedures.[1,3] Intraperitoneal "forgotten" foreign bodies tend to create adhesions and become encapsulated, with or without an accompanying bacterial infection.[1] Clinical presentations for patients with a retained surgical sponge may be acute or chronic. Acute presentations generally follow a typical septic course with abscess or granuloma formation.[3] Delayed presentations may occur months or even years after the original surgical procedure and be heralded by adhesion formation and encapsulation.[3] Occasionally there may be intestinal obstruction and rarely fistulation, perforation or even extrusion.[3] The animal of this report was clinically normal within 36 hours of its exploratory laparotomy, and the dog's post surgical recovery continues to be uneventful.

AFIP Diagnosis: Omentum (per contributor): Omentitis, pyogranulomatous, sclerosing, marked, focally extensive, centered on abundant fibrillar anisotropic foreign material (surgical sponge), Cocker Spaniel, canine.

Conference Comment: Conference attendees discussed the clinical pathology findings and how these relate to the molecular basis of acute and chronic inflammation.

The normocytic normochromic anemia is likely due to the chronic inflammatory condition and was probably non-regenerative (anemia of inflammatory disease, AID). AID occurs in chronic infectious, inflammatory, or neoplastic disorders and is mediated by a variety of cytokines, including tumor necrosis factor (TNF), IL-1, and interferon gamma. The resultant anemia is due to decreased bone marrow responsiveness to erythropoietin, decreased release of erythropoietin, and impaired availability of iron to the erythron. When the primary cause of AID is removed, the anemia will resolve.[4]

Other systemic affects of inflammation include fever, release of acute phase proteins, and leukocytosis. Fever is produced in response to pyrogens that act by inducing prostaglandin synthesis in the hypothalamus. Exogenous pyrogens (bacterial products) stimulate leukocytes to release IL-1 and TNF (endogenous pyrogens) that result in increases in cyclooxygenases, which then convert arachidonic acid (AA) to prostaglandins. Prostaglandins, especially PGE_2, stimulate the production of neurotransmitters that reset the temperature set-point at a higher level. Acute phase proteins are plasma proteins that are primarily synthesized in the liver and often function as opsonins and fix complement. Three of the best-known examples include C-reactive protein (CRP), fibrinogen, and serum amyloid A protein (SAA). Cytokines such as IL-6 (for CRP and fibrinogen) and IL-1 and TNF (for SAA) induce hepatocytes to upregulate production of these acute phase proteins. A common feature of inflammatory reactions, especially those induced by bacterial infection, is a leukocytosis. Initially the increase in WBCs is due to accelerated release of cells from the bone marrow postmitotic pool, induced by IL-1 and TNF. Prolonged infection induces production of colony stimulating factors (CSFs) and results in increased bone marrow output.[5,6] The neutrophilia and monocytosis in this case is due to the inflammatory reaction and release of cytokines as a result of the foreign material in the abdomen.

Contributor: Angell Animal Medical Center, Pathology Service, 350 South Huntington Avenue, Boston, MA

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SLIDE 16
CONFERENCE 4 / CASE IV – NIAH-1 (AFIP 2937643)

Signalment: 3-week-old, male, white leghorn, specific-pathogen-free chicken.

History: This chicken was inoculated by eye drop with 0.1ml of inoculum containing 10^7 plaque-forming units (PFU) of viscerotropic velogenic Newcastle disease virus. The chicken died three days after inoculation, and was necropsied.

Gross Pathology: There was bilateral reddening and swelling of the lower conjunctiva. The lesions on the inoculated side were more severe than on the opposite side. There were multifocal white foci on the spleen, and occasionally hemorrhages in the mucosa of the proventriculus, duodenum, and cecal tonsil in the chicken.

Laboratory Results: Newcastle disease virus was isolated from the conjunctiva using a cell culture originating from chicken kidney cells.

Contributor's Morphologic Diagnosis: Conjunctiva: Conjunctivitis, acute, severe, with fibrinoid vascular necrosis, fibrinous thrombi, hemorrhage, and edema, white-leghorn, chicken.

Contributor's Comment: Newcastle disease (ND), a serious world-wide poultry disease, is caused by ND virus (NDV), a member of the genus *Rubulavirus*, family *Paramyxoviridae*. ND is divided into five pathotypes.[1] Viscerotropic velogenic ND (VVND) (Doyle's form), is a very acute and lethal form of ND with hemorrhagic lesions of the digestive tract. Neurotropic velogenic ND (NVND) (Beach's form) has neurological and respiratory lesions. Mesogenic ND (Beaudette's form) is an acute respiratory and sometimes lethal nervous infection of young chicks. Mortality is rare in older birds. Lentogenic ND (Hitchner's form) is a mild or inapparent respiratory infection of chickens. Asymptomatic-enteric form (Ulster type) manifests chiefly as gut infections with lentogenic viruses, causing no obvious disease.

Lymphoid, vascular, respiratory, neural, and reproductive lesions are seen in the chickens as pathological features of ND.[1] It is also well known that NDV causes conjunctivitis[1-4,6] and induces conjunctivitis in humans.[5] In these human cases, an epidemic of Newcastle disease occurred in turkeys in 1965 and 1966 in the United States, and several workers in close contact with the turkeys at the processing plant developed a follicular conjunctivitis with a rise of antibodies against NDV.[5] Therefore, ND is one of a few chicken zoonotic diseases. Clinically, conjunctivitis is a significant sign of ND.

Virulent avian influenza virus infection and VVNDV infection, acute fatal diseases with systemic hemorrhages in the chickens, are very important diseases of the poultry

industry. It is necessary to diagnose and differentiate them as rapidly and correctly as possible. There are few reports on conjunctivitis induced by virulent avian influenza except one report of avian-influenza-virus-induced conjunctivitis,[7] while there are many reports on macroscopical NDV-associated –conjunctivitis.[1-4,6] Detection of conjunctivitis with vascular necrosis can be important in the diagnosis of VVND infection in the chickens, although more pathological studies of chickens infected with NDV are necessary.

AFIP Diagnosis: Eye, conjunctiva: Conjunctivitis, acute, focal, moderate, with necrotizing vasculitis, white leghorn chicken, avian.

Conference Comment: The contributor provides a brief overview of the various pathotypes of Newcastle Disease (ND). Viscerotropic velogenic ND (VVND) is the most severe form of ND and is likely the most serious disease of poultry throughout the world. VVND affects numerous species of exotic pet and exposition birds, waterfowl, and domestic poultry. Transmission between birds is via aerosolization and transmission between premises is often via fomites. In affected birds, morbidity rates approach 100 percent and mortality often reaches as high as 90 percent. Neurologic and gastrointestinal signs are most common. However, non-vaccinated birds may be found dead without prior signs of illness. Gross lesions include subcutaneous and periocular edema, hemorrhagic and catarrhal tracheitis, airsacculitis, necrohemorrhagic enteritis (often with Peyer's patch and cecal tonsil ulceration), petechial hemorrhagic proventriculitis, and yolk peritonitis.[1,8] Histologic findings include necrotizing vasculitis, thrombosis, diffuse lymphoid depletion, and nonsuppurative encephalomyelitis.[1]

ND belongs to the Paramyxoviridae virus family, and like other members of this group, has two important surface proteins, hemagglutinin/neuraminidase (HN) and fusion (F) protein. HN is important in initial attachment of the virus to the host cell receptor. F protein has a critical role in virus and cell fusion and penetration of the host cell membrane. Other viruses in the family Paramyxoviridae include the following:[9]

<u>Subfamily Paramyxovirinae</u>
Genus *Paramyxovirus*
- Bovine parainfluenza virus-3
- Sendai virus (mouse parainfluenza-1)
- Human parainfluenza virus-1 and –3

Genus *Rubulavirus*
- Avian paramyxovirus-1 (NCDV)
- Simian virus-5
- Mumps virus
- Human parainfluenza virus-2

Genus *Morbillivirus*
- Canine distemper virus
- Rinderpest virus

<u>Subfamily Pneumovirinae</u>
Genus *Pneumovirus*
- Bovine respiratory syncytial virus
- Pneumonia virus of mice
- Turkey rhinotracheitis virus
- Human respiratory syncytial virus

Peste-des-petits-ruminants virus
Dolphin Morbillivirus
Phocine distemper virus
Measles virus

Contributor: National Institute of Animal Health, Laboratory of Chronic Diseases Pathology, 3-1-5, Kannondai, Tsukuba, Ibaraki 305-0856, Japan (http://www.niah.affrc.go.jp/index-j.html)

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SLIDE 17
CONFERENCE 5 / CASE I – 04-113 (AFIP 2942979)

Signalment: 9-year-old, neutered male, Rottweiler/Shepherd dog mix.

History: The dog had a one-month history of "not doing right". On presentation, lethargy and vomiting had developed. The clinician noted anemia (PCV – 27%) and increased liver enzymes. On physical exam a palpable abdominal mass was noted and by imaging was determined to be an enlarged spleen. Peripheral lymph nodes were not enlarged. On exploratory laparotomy, the spleen was confirmed to be extremely large with irregular borders and a mottled surface. A splenectomy was performed and the

spleen submitted for histopathology exam. The left lateral and medial lobes of the liver appeared mildly enlarged with rounded edges. No biopsy was taken of the liver. No other gross abnormalities were found within abdomen.

Gross Pathology: The spleen was markedly, diffusely enlarged (approximately 2-3 times normal size) and firm.

Laboratory Results: CBC day of surgery:

CBC	Patient Value	Reference Range	Units	Percentage
WBC	66	5.5 – 16.9	X 10^3/µl	
Neutrophils	7.06	2.0 – 12.0	X 10^3/µl	10.5
Monocytes	19.28	0.1 – 1.4	X 10^3/µl	28.8
Lymphocytes	39.48	0.7 – 4.9	X 10^3/µl	58.9
Basophils	0.36	0.0 – 0.1	X 10^3/µl	0.5
Eosinophils	0.81	0.1 – 1.49	X 10^3/µl	1.2
RBC	2.72	5.5 – 8.5	X 10^6/µl	
Hb	7.2	12.0 – 18.0	g/dl	
Hct	21.6	37.0 – 55.0	%	
Reticulocytes	147.3		X 10^3/µl	5.4
MCV	79.5	60.0 – 77.0	Fl	
MCHC	34.1	31.0 – 37.0	g/dl	
MCH	27.07	19.5 – 24.5	Pg	
RDW	16.3	14.7 – 17.9	%	
PLT	154		X 10^3/µl	
MPV	20.38		Fl	
PCT	0.3		%	
PDW	20.9		%	

Contributor's Morphologic Diagnosis: Spleen: T-cell LGL (large granular lymphocyte) lymphoma/leukemia, Rottweiler/Shepherd mix, canine.

Contributor's Comment: The splenic red pulp is diffusely expanded by numerous medium to large neoplastic round cells with scant amounts of eosinophilic cytoplasm and round to indented nuclei. Scattered germinal centers in the white pulp are still evident but are mildly atrophic. Minimal extramedullary hematopoiesis is present. Immunohistochemical staining demonstrates neoplastic cells to be strongly positive for CD3 and negative for CD79a and lysozyme.

Venous blood smears reveal a leukemic blood profile with a moderate regenerative anemia. Neoplastic lymphocytes are characterized by medium to occasionally large cells with moderate amounts of basophilic cytoplasm, round to irregularly indented nuclei with moderately clumped chromatin and variably visible nucleoli. Many cells have low numbers of variably sized, azurophilic granules that are often perinuclear. Occasional larger blast cells are noted. A rare mitotic figure is present. A manual WBC differential count on the blood smear results in 97% of cells being neoplastic lymphocytes, with 2% segmented neutrophils and 1% eosinophils. When compared to the automated differential count, it is apparent many of the neoplastic lymphocytes were erroneously classified as monocytes by the hematology analyzer.

Large granular lymphocyte (LGL) leukemias/lymphomas are either of cytotoxic T-cell (CD8+CD3+) or natural killer (NK) (CD3-) cell origin.[1] LGLs have characteristic azurophilic granules in their cytoplasm often in a perinuclear location; ultrastructurally, these granules appear as membrane-bound structures with an electron-dense core. In normal dogs up to 10% of peripheral lymphocytes in the blood can be LGLs.[2] Unlike

acute lymphocytic leukemias, which originate in the bone marrow, canine LGL lymphoma/leukemias are thought to originate from $\alpha_d\beta_2^+$ lymphocytes in the red pulp of the spleen.[3]

In addition to surface antigens, cytotoxic granule proteins such as TIA-1, granzyme, and perforin are used immunologically and in molecular studies to identify LGL leukemic cells in people.[4] Perforin-like immunoreactivity has been demonstrated in feline LGL lymphomas[5] as well as rats, mice, and guinea pigs.[2]

LGL lymphoma/leukemia in some dogs can be an aggressive disease involving spleen, liver, and bone marrow. Leukemic blood profiles with lymphocyte counts of up to 138 x 10^6/ml have been reported, with the majority (80-97%) being LGLs. In other dogs, the disease progress is slower, behaving like chronic lymphocytic leukemias.[2] LGL lymphoma in cats is usually associated with the gastrointestinal tract and tends to be an aggressive disease that spreads rapidly to the spleen, liver, and lymph nodes.[5, 6] Leukemia is variably seen in cats. LGL lymphoproliferative disorders have also been reported in horses[7], ferrets, birds,[2] and is common in Fischer 344 rats. Non-neoplastic proliferations of LGL in dogs have been reported with chronic ehrlichiosis.[3]

In humans, LGL leukemia is thought to arise from apoptosis dysregulation due to abnormalities in the Fas/Fas ligand pathway.[8] LGL leukemia cells are resistant to Fas-mediated apoptosis and express high levels of both Fas and Fas ligand.[8] Multiple genes involved in cytotoxic functions are upregulated in LGL leukemia including granzymes, cathepsin, calpain, perforin, and capase-8, similar to that seen in activated cytotoxic T-cells.[8] LGL lymphoma/leukemia is often associated with autoimmune disorders, particularly rheumatoid arthritis, and other lymphoproliferative disorders. Clonal proliferations of LGLs in humans have been associated with Epstein-Barr virus, human immunodeficiency virus, and human T cell lymphotropic virus.[2] Type C oncovirus has been reported associated with a cell line derived from peripheral lymphocytes from a dog with LGL leukemia.[9]

AFIP Diagnoses: 1. Spleen, sinusoids: Large granular lymphocyte leukemia, mixed breed, canine.
2. Spleen, white pulp: Lymphoid atrophy, diffuse, moderate.

Conference Comment: Some sections contain discrete nodules of white pulp that are unaffected by the neoplastic cells suggesting this neoplasm originates from the red pulp rather than the white pulp. It can be difficult (if not impossible) to differentiate lymphoma from leukemia when there is advanced disease with infiltration of neoplastic cells into widespread tissues. In these instances, special methods such as immunophenotyping of the cells are necessary to help differentiate the conditions.

Lymphoma is a neoplasm of lymphocytes arising as a solid tissue mass in organs other than bone marrow.[10] Lymphoma can be classified into subtypes according to anatomic

distribution, histiologic pattern, cellular morphology, cytochemistry, and expression of cluster designation (CD) markers. Subtypes of lymphoma according to distribution include: multicentric, alimentary, mediastinal, cutaneous, and miscellaneous. Multicentric lymphoma is the most common type of lymphoid neoplasia in dogs, cattle, and horses, while alimentary lymphoma is the most common form in cats. The histologic pattern in most cases is diffuse and characterized by sheets of neoplastic lymphocytes that efface and replace normal tissue architecture.[11]

Leukemia is defined as hematopoietic neoplasia with neoplastic cells in the blood and/or bone marrow. Lymphocytic leukemia arises from the bone marrow and may be either acute or chronic. Acute lymphocytic leukemia (ALL) is most common in younger animals, arises from undifferentiated lymphocytes in the bone marrow that are often of B-cell origin, and usually presents as large blastic cells. Chronic lymphocytic leukemia (CLL), is more common in older animals, arises from relatively differentiated lymphocytes that "home" to secondary lymphoid organs such as the spleen. These cells resemble small lymphocytes and are often of T-cell origin, specifically the CD8+ subset.[11]

There are several accepted classification schemes for hematopoietic tumors, including the REAL (Revised European and American Lymphoma) system and WHO (World Health Organization) system. The REAL system categorizes tumors based on their histogenetic derivation and biological behavior, while the WHO system also includes acute and chronic myeloproliferative diseases as well as myelodysplastic syndromes. Although the classification schemes may appear complex, the differentiation of specific hematopoietic tumors is important for veterinary oncologists to provide optimal tumor management.[1]

The contributor provides a thorough overview of LGL lymphoma/leukemia in domestic animals and humans. Although the azurophilic granules are difficult to appreciate on the H&E slides, granules can be seen in lymphocytes particularly around the fibromuscular trabeculae. When LGL lymphoma/leukemia is suspected, it is often easier to diagnose using cytologic touch imprints of affected organs or peripheral blood smears.

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

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SLIDE 18
CONFERENCE 5 / CASE II – N04-95 (AFIP 2937501)

Signalment: 3.5-year-old, female, French Alpine goat, *Capra hircus*, caprine.
History: This doe was presented with a 2-month period of dyspnea and progressive emaciation after parturition. The animal was euthanized and necropsied.

Gross Pathology: The doe was in poor body condition and exhibited minimal fat stores. There were multifocal fibrous adhesions between the parietal and visceral layers of the pleura (Fig. 1). Both lungs exhibited multifocal, green yellow nodules (abscesses) ranging from 0.5-2 cm in diameter. These nodules were primarily located in the cranial lobes. The abdominal cavity contained approximately 1 litre of clear, light yellow watery fluid (ascites). The liver showed multifocal confluent nodules ranging from 0.5-5 cm in diameter. Abundant pale green viscous material (pus) oozed freely from the nodules on cut surface (Fig.2). The rumen had a focal, approximately 1.5 cm in diameter, area of hemorrhage located in the serosal surface near the cardia. At this site, the mucosa exhibited blunting and erosion of the ruminal papillae. The mesenteric lymph nodes were enlarged twice their normal size. Both kidneys displayed numerous, miliary white foci scattered throughout the cortex. The rest of the internal viscera were unremarkable.

Laboratory Results: An hour previous to euthanasia, a complete blood count revealed a leukocytosis, neutrophilia, and mild monocytosis associated with chronic active inflammation. A biochemical profile revealed azotemia, hyperproteinemia, hyperglobulinemia, hypoalbuminemia, hypocalcemia, hyperkalemia, hyponatremia, and hypochloremia and elevated GGT and CK.

CBC/Chem	Patient Value	Reference Range	Units
WBC	30.2	4.0 – 13.0	X 10^9/L
Neutrophils	22.7	1.2 – 2.7	X 10^9/L
Monocytes	0.6	0.0 – 0.55	X 10^9/L
BUN	19.9	3.5 – 6.66	mmol/L
Creatinine	643	97 – 159	mmol/L
Protein	87	59 – 76	g/L
Globulin	78	20 – 60	g/L
Albumin	9	27 – 39	g/L
Calcium	1.08	2.22 – 2.91	mmol/L
Potassium	7.82	3.5 – 6.7	mmol/L
Sodium	127	142 – 155	mmol/L
Chloride	94	99 – 110	mmol/L
GGT	60	< 56	U/L
CK	3351	< 8.9	U/L

Bacteriology: A heavy growth of *Arcanobacterium pyogenes* was isolated in pure culture from the lung and liver lesions.

Parasitology (fecal flotation): *Eimeria* spp.

Contributor's Morphologic Diagnosis: 1. Liver: Multifocal confluent abscesses.
2. Liver: Hepatitis, necrosuppurative, plasmacytic and histiocytic, multifocal, severe, chronic, with myriads of intralesional bacteria, and abundant, diffuse, intercellular, intrahistiocytic, radiating to homogeneous, eosinophilic material consistent with Splendore-Hoeppli material.
3. Kidney: Glomerulonephritis, proliferative, neutrophilic, fibrinous, global, diffuse, severe, with glomerular hemorrhage, tubular dilation, proteinosis and degeneration, and scant, multifocal, extracellular, radiating to homogeneous, eosinophilic material consistent with Splendore-Hoeppli material.
4. Kidney: Nephritis, tubulointerstitial, neutrophilic and lymphoplasmacytic, multifocal, severe, subacute.
5. Lung: Multifocal abscesses and fibrous pleural adhesions (slides not submitted).
6. Rumen: Rumenitis, necrosuppurative and hemorrhagic, focal, acute, moderate (slides not submitted).

Contributor's Comment: The section of liver shows large, multifocal areas of liquefactive necrosis containing myriad bacteria intermixed with numerous degenerate neutrophils and cellular debris. These areas are surrounded by large numbers of plasma cells, with lesser numbers of histiocytes, neutrophils and rare lymphocytes. Multifocal aggregates of neutrophils, plasma cells and lymphocytes are scattered

throughout the parenchyma. The presence of abundant, homogeneous, eosinophilic, hyaline material often with radiating projections (spiculated), diffusely distributed among the parenchymal cells and sometimes within Kupffer cells is remarkable. This material ranges approximately from 20 to 30 microns in diameter, is usually surrounded by moderate numbers of neutrophils and is considered as consistent with Splendore-Hoeppli substance. Same material was ruled out as amyloid through Congo red staining (not submitted).

The section of kidney exhibits increased cellularity of the glomerular tufts caused by proliferation of epithelial and mesangial cells, with hypertrophy of the epithelium and focal adhesions between the glomerular tuft and Bowman's capsule (synechiae). Most of the glomeruli have small clusters of neutrophils and small deposits of fibrin involving both the glomerular tufts and the urinary space. In some urinary spaces the inflammatory cells are mixed with abundant red blood cells. Most of the tubules exhibit marked dilation and contain abundant, intraluminal, pale eosinophilic, proteinaceous material. The tubular epithelium has multifocal vacuolar degeneration with occasional intracytoplasmic hyaline droplets. Multifocal small aggregates of neutrophils, plasma cells and lymphocytes are scattered throughout the interstitium. There are also numerous intraluminal clusters of neutrophils mainly located in the medullary region. Occasional deposits of Splendore-Hoeppli material are present in some glomerular tufts.

The order Actinomycetales includes several families of pathogenic organisms. These organisms, referred to as higher bacteria, often generate lesions that resemble those produced by fungi. The genera *Actinomyces, Nocardia, Rhodococcus, Corynebacterium, Dermatophilus, Streptomyces,* and *Mycobacterium* are included in this order.[1] *Arcanobacterium pyogenes*, formerly known as *Corynebacterium pyogenes* and *Actinomyces pyogenes*, is widespread throughout the word as a common cause of pyogenic processes in cattle, sheep, swine, goats, and wild ungulates.[1,2] It is a facultatively anaerobic, small, pleomorphic, gram-positive bacterium, which is considered a normal inhabitant of the mucous membranes of several domestic species. *Arcanobacterium pyogenes* can induce purulent inflammation almost anywhere in the body. Usually this takes the form of localized abscesses, but the lesions may be more diffuse in internal viscera, joints, or tendon sheaths.[2] In goats, purulent pneumonias and abscesses in the upper respiratory tract,[1] as well as mandibular osteomyelitis[3] have been described as lesions associated with *A. pyogenes* infection.

A distinctive morphologic feature of certain fungal and bacterial diseases is the presence of a homogeneous, brightly eosinophilic substance, known as Splendore-Hoeppli material, surrounding individual organisms or colonies of organisms. It exists as a surrounding collar of radially arranged clubs, or as a variably-sized rim often with a serrated edge. The exact nature of this material is unknown, but it appears to be a product of the host, most likely antigen-antibody complexes. Splendore-Hoeppli material is a relatively consistent feature of coccidioidomycosis and sporotrichosis, as well as certain diseases caused by higher bacteria, such as actinomycosis.[4] Other bacterial

infections associated to this phenomenon are those produced by *Actinobacillus lignieressi*[5] and *Staphylococcus aureus* (Botryomycosis).

To our knowledge, the Splendore–Hoeppli phenomenon is not a common histomorphologic feature in infections caused by *Arcanobacterium pyogenes*. However, it was a remarkable finding in the liver section of this goat. It is also interesting that the material described in this case was not surrounding bacterial colonies, as it has been described in the literature.

AFIP Diagnoses: 1. Liver: Abscesses, multifocal and coalescing with myriad bacilli and, moderate, random, portal, neutrophilic and plasmacytic hepatitis, with abundant eosinophilic spiculated material, French Alpine goat, caprine.
2. Kidney: Glomerulonephritis, necrotizing, hemorrhagic and neutrophilic, global, diffuse, severe, with moderate multifocal, neutrophilic and plasmacytic, tubulointerstitial nephritis, numerous fibrin thrombi, and multifocal eosinophilic spiculated material.

Conference Comment: Within the liver sections, two prominent features are present, the focal areas of necrosis with large colonies of bacteria and the abundant eosinophilic spiculated material.

There are several gram-positive and gram-negative bacteria that form large colonies in tissue. They are known by the pneumonic YAAACCSS[5,6,7,8]:

Yersinia sp.	Gram-negative
Actinomyces sp.	Gram-positive
Actinobacillus sp.	Gram-negative
Arcanobacterium sp.	Gram-positive
Clostridium sp.	Gram-positive
Corynebacterium sp.	Gram-positive
Staphylococcus sp.	Gram-positive
Streptococcus sp.	Gram-positive

There is abundant eosinophilic, often spiculated material surrounding areas of necrosis and within sinusoids in unaffected areas. Conference attendees discussed whether this could be fibrin thrombi, Splendore-Hoeppli material or amyloid. Fibrin thrombi often create a long cast of the vascular lumina and tend to contain more enmeshed erythrocytes. PTAH (Phosphotungstic Acid Hematoxylin) stains fibrin dark blue, but did not stain the material. The spiculated morphology is most consistent with Splendore-Hoeppli material. Although the exact nature of Splendore-Hoeppli material is not known, it is thought to be antigen-antibody complexes. These complexes are often found closely associated with the bacteria. In this case, a significant amount of the material was not associated with bacteria and often not associated with inflammation. Tissue Gram stains did not reveal bacteria within the eosinophilic material. Amyloid is a pathologic proteinaceous substance that histologically appears as an amorphous, eosinophilic, hyaline, extracellular material. Within the liver, amyloid first appears in the

space of Disse, and with progressive accumulation, produces pressure atrophy of adjacent cells. It is congophilic, and exhibits green birefringence when polarized. After staining with Congo Red locally, the eosinophilic material is multifocally congophilic with green birefringence. There are several forms of amyloid, but the most common are AL (amyloid light chain) and AA (amyloid associated). AL protein is composed of partial or complete immunoglobulin light chains and is most commonly associated with B cell/plasma cell dyscrasias. AA protein is produced in the liver from SAA (serum amyloid-associated), which is an acute phase protein, and is most commonly seen in association with chronic inflammatory disorders.[9] Without further testing, it is not possible to definitively determine the composition of the eosinophilic material in this case.

Contributor: Departamento de Patología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán, MEXICO, D.F. 04510.
http://www.veterin.unam.mx

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SLIDE 19
CONFERENCE 5 / CASE III – 04-437 (AFIP 2933948)

Signalment: 3-year-old, intact female, Duncan Hartley (strain HsdPoc:DH) guinea pig *(Cavia porcellus)*.

History: This colony guinea pig was a short-haired albino with a recent history of muscle mass loss and a palpable intra-abdominal mass. There had been no recent experimental manipulations or breeding; this group of guinea pigs was kept mainly for environmental enrichment. The guinea pig was found dead and presented for necropsy on the same day.

Gross Pathology: At necropsy, there was marked muscle thinning on the cervical, thoracic and abdominal regions. The lungs were diffusely firmer than normal and the sternal lymph nodes were wet and red. The liver was diffusely red/brown, friable and granular and the spleen contained a 1 cm diameter soft, red nodule. Both kidneys had a granular appearance. Both ovaries were markedly enlarged by fluid-filled, thin walled cysts, probably accounting for the abdominal mass palpated prior to death.

Contributor's Morphologic Diagnoses: 1. Liver, lung: Cavian leukemia.
2. Liver: Mild centrilobular hepatocellular fatty change.

Contributor's Comment: Microscopically, the hepatic sinusoids were expanded by a fairly monomorphous population of round, lymphoid cells with scant to small amounts of pale eosinophilic cytoplasm and centrally placed euchromatic to hypochromatic, faintly stippled nuclei. Nuclei ranged from oval to round or indented; nucleoli were inconspicuous. Mitotic figures averaged 6 per 10 hpf (40X) and scattered tingible body macrophages created a "starry sky" effect, particularly in portal areas, where the lymphoid cells were more numerous and solidly packed. The capsular surface was a little irregular due to the subcapsular accumulation of lymphoid cells. Larger veins throughout the liver contained similar round cells. Mild, centrilobular hepatocellular fatty change was noted. In the lung, there was diffuse interstitial thickening due to the presence of lymphoid cells similar to those described above. These also occupied larger, congested veins. Incidental foci of heterotopic bone were also present.

Other infiltrated organs included lymph nodes (although peripheral lymphadenopathy was not appreciated at gross necropsy), the spleen, kidneys, intestinal tract, adrenal and thyroid glands. Most blood vessels contained similar cells. Bone marrow cellularity was 95-100% but the cells were still mixed, although there was a predominance of larger, non-segmented cells. Due to decalcification, it was difficult to be certain if these were the same population as was present throughout the rest of the body. However, reportedly there is lesser involvement of the bone marrow.[1]

Cavian leukemia is a spontaneous form of hematopoietic neoplasia that can occur in inbred (strains 2/N and 13/N) and non-inbred lines. Disease tends to arise in young adults under three years of age. The incidence varies in different reports, ranging from under 0.01% in a 16-year period[2] to 0.2% over a three-year period.[3] Early experimental work[4] demonstrated transmission of a "leukemogenic agent" via cell-free inoculation or cell transplantation to hybrid guinea pigs. Electron microscopy revealed Type C virus particles in neoplastic lymphoid cells, suggesting a role for retrovirus infection.

Experimentally, this is also a 100% transmissible (transplantable) form of neoplasia in inbred strains and some hybrids (e.g. strain 2/Hartley), usually by intraperitoneal or subcutaneous inoculation of fresh or thawed frozen whole blood or tissue. This has allowed maintenance of leukemic lines and further study of the pathogenesis, although it is not entirely clear if more recent spontaneous cases reflect exactly the same disease process.[2] The degree of transmissibility decreases quickly with age in non-inbred strains but can be facilitated by prior corticosteroid treatment.[1] While this disease is typically thought of as retrovirus-associated, guinea pig herpes-like virus may also contribute to the development of the leukemia. Herpes-like virus particles have been isolated from lymphoid cells derived from leukemic guinea pigs but only *in vitro*[5] and the cells were also derived from guinea pigs infected with retrovirus. Cavian leukemia has been postulated as a model for acute lymphoblastic leukemia in humans.[1]

AFIP Diagnosis: Lung; liver: Leukemia, lymphoblastic, Duncan Hartley guinea pig, cavian.

Conference Comment: Cavian leukemia is a rare condition that occurs spontaneously in various inbred and non-inbred strains of guinea pigs. Affected animals are usually young adults and may present with either a generalized lymphadenomegaly and/or a leukocytosis (up to 180,000 mm^3) with a significant number of circulating lymphoblastic cells. At necropsy, in addition to enlarged peripheral lymph nodes, there is marked splenomegaly and hepatomegaly. Histologically, as was noted in this case, there is diffuse infiltration of lymphoblastic cells in the liver, interstitium of the lung, as well as, spleen, bone marrow, thymus, alimentary tract lymphoid tissue, heart, eyes, and adrenal glands. As the contributor mentions, an endogenous retrovirus, is associated with, but not proven to cause Cavian leukemia. Although a retrovirus appears to play an important role in this disease, C-type virus particles have been noted in lymph node germinal centers from normal guinea pigs.[6]

Many viruses have been associated with tumor induction in both animals and humans. Most of these are DNA viruses and include the following[7]:

Family/Genus	*Virus*	*Associated Tumor*
DNA Viruses		
Poxviridae		
Leporipoxvirus	Rabbit fibroma virus	Myxoma
	Squirrel fibroma virus	Fibroma
Yatapoxvirus	Yaba monkey tumor virus	Histiocytoma in monkeys

Family/Genus	*Virus*	*Associated Tumor*
Herpesviridae		
Alphaherpesvirinae	Marek's disease virus	Lymphoma in fowl

Gammaherpesvirinae	Ateline herpesvirus-2	Lymphoma
	Saimirine herpesvirus-2	Lymphoma
	Epstein-Barr virus	Lymphoma in monkeys
	Baboon herpesvirus	Lymphoma in baboons
	Cottontail rabbit herpesvirus	Lymphoma in rabbits
Ungrouped	Lucké frog herpesvirus	Renal adenocarcinoma
Adenoviridae		
Mastadenovirus	Many adenoviruses	Solid tumors in rodents
Papovaviridae		
Papillomavirus	Cottontail rabbit papillomavirus	Papillomas in rabbits
	Bovine papillomavirus-4	Papilloma, carcinoma of intestine/urinary bladder
	Bovine papillomavirus-7	Papilloma, carcinoma of the eye
	Human papillomavirus-5, 8	SCC
	Human papillomavirus-16, 18	Genital carcinoma
Polyomavirus	Murine polyomavirus	Solid tumors in rodents

Reverse Transcribing Viruses

Hepadnaviridae (DNA virus)		
Orthohepadnavirus	Woodchuck hepatitis virus	Hepatocellular carcinoma
	Human hepatitis virus	Hepatocellular carcinoma
Avihepadnavirus	Duck hepatitis virus	Hepatocellular carcinoma
Retroviridae (RNA virus)		
Alpharetrovirus	Avian leukosis virus	Lymphoma/leukemia
	Rous sarcoma virus	Sarcoma in fowl
	Avian erythroblastosis virus	Erythroblastosis in fowl
	Avian myeloblastosis virus	Myeloblastosis in fowl
Betaretrovirus	Mouse mammary carcinoma virus	Mammary carcinoma
	Mason-Pfizer monkey virus	Sarcoma and Immunodeficiency
Gammaretrovirus	Feline leukemia virus	Leukemia
	Feline sarcoma virus	Sarcoma
	Murine leukemia virus	Lymphoma/leukemia
	Murine sarcoma virus	Sarcoma
	Avian reticuloendotheliosis virus	Reticuloendotheliosis
Family/Genus	*Virus*	*Associated Tumor*
Deltaretrovirus	Bovine leukemia virus	Leukemia
	Jaagsiekte virus	Adenocarcinoma of the lung in sheep
	HTLV-1 and –2	Human adult T cell

		leukemia and hairy cell leukemia
	Simian HTLV virus	Leukemia in monkeys

RNA Viruses

Flaviviridae		
Hepacivirus	Hepatitis C virus	Hepatocellular carcinoma in humans

Contributor: University of Tennessee, Dept. of Pathobiology, College of Veterinary Medicine, P.O. Box 1071, Rm. A201, Knoxville, TN
http://www.vet.utk.edu/departments/path/

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SLIDE 20
CONFERENCE 5 / CASE IV – R 18 (AFIP 2937814)

Signalment: 13-month-old, male, Cavalier King Charles Spaniel.

History: A porto-azygos shunt was diagnosed by the veterinarian and 2 surgical interventions were undertaken within an 11 week interval.
Before the second intervention, the basal portal pressure was 9 mm Hg. After the ligature, it was 24 mm Hg and euthanasia was elected.

Jan 29th 2004, convulsive seizure:
Abdominal echography: very small liver, porto-azygos shunt, cholelithiasis, increased kidney size.

March 18th 2004, first surgery

June 2nd 2004, echography

Early signs of portal hypertension and persistent shunt.
Anorexia, polyuria polydipsia, convulsive seizure.

June 10th 2004, second surgery

Ascites
Euthanasia

Gross Pathology: Porto-azygos shunt, small-sized liver

Laboratory Results: Jan 29th 2004, convulsive seizure:

Alkaline phosphatases: 495 UI
ALAT: 540 UI
Pre-prandial bile acids: 110 mmol/L
Post-prandial bile acids: 261 mmol/L

Contributor's Morphologic Diagnosis: Liver: Arteriolar hyperplasia, portal, diffuse, moderate, with terminal hepatic vein hypoplasia, lobular atrophy, periportal and bridging fibrosis, consistent with congenital portosystemic vascular shunt.

Contributor's Comment: The liver architecture is modified, as the hepatic lobules are difficult to identify due to loss of hepatocytic plates and terminal hepatic veins. The lobules seem small as evidenced by a subjective decrease in distance between portal triads, and there is slight periportal and bridging fibrosis. Serpentine arrangements of arteriolar smooth muscle cells that are disposed in a perilobular pattern represent portal arteriolar hyperplasia. Strikingly, portal veins are still discernible. Hepatocytes are small, dissociated, and a proportion of them show some medium-sized vacuoles. Ito cells are abundant and show a large and unique vacuole. Brown pigment is found in scattered Kupffer cells and bile canaliculi (cholestasis).

Portosystemic shunts are congenital or acquired communications between the portal and systemic vasculature.[4] Congenital vascular shunts are described more often in dogs, less frequently in cats, and sporadically in other domestic animals.[1] Rarely, young dogs may have arteriovenous (arterioportal) fistulae and develop portal hypertension, ascites, and acquired shunts.[3]

Clinically, the animals may be small for their age and show neurological signs due to hepatic encephalosis resulting from inadequate clearance of enterically derived toxins in portal blood.[2] In addition to neurological signs, animals with shunts may suffer from renal, cystic, or urethral calculi due to increased urinary excretion of ammonia and uric acid. The formation of ammonium biurate crystals in urine is frequent.

Biologically, increased post-prandial bile acids are typical of this condition. Dogs with portosystemic shunts often have hypoalbuminemia in the absence of proteinuria, low blood urea nitrogen, hypoglycemia, and hypocholesterolemia due to decreased hepatic function. Erythrocytic microcytosis is a common finding in animals with portosystemic shunts although iron metabolism is normal.[1]

AFIP Diagnosis: Liver: Portal arteriolar hyperplasia and venule hypoplasia, diffuse, moderate, with lymphangiectasia, hepatocellular atrophy and fatty change, periportal and bridging fibrosis, and bile stasis, Cavalier King Charles Spaniel, canine.

Conference Comment: The blood flow to the liver is unique with the hepatic artery providing oxygenated blood, the portal vein providing blood flow from the intestinal tract and spleen, and the hepatic vein returning blood from the liver to the systemic circulation. In health, portal blood contains constituents absorbed from the intestinal tract, including bile acids, amino acids, glucose, ammonia, medium-length fatty acids, and intestinal antigens that are largely removed by the liver before they reach systemic circulation. In acquired or congenital shunts, the portal blood largely bypasses the liver, and directly enters the systemic circulation. Therefore, systemic blood concentrations of the substances normally removed by hepatic processing are increased (i.e. bile acids, ammonia). Hyperammonemia may lead to CNS signs (hepatic encephalopathy).[5]

Portosystemic shunts often result in hepatic atrophy, often with concomitant loss of functional mass, due to decreased concentrations of intestinal and pancreatic hepatotrophic factors that normally reach the liver through the portal circulation. Approximately 70% or more of the functional hepatocytes must be lost before alterations of hepatic function are detectable by serum chemistry. When the functional mass is significantly reduced, it may result in hypoproteinemia, hypoalbuminemia, hypoglycemia, hypocholesterolemia, decreased BUN, and hyperbilirubinemia.[5]

The clinical pathology findings in this case are classic for animals with portosystemic shunts. The fasting and postprandial bile acids are elevated due to the portal vein largely bypassing the liver and delivering the blood to the systemic circulation. The increased ALAT (ALT, alanine aminotransferase) indicates hepatocellular injury with enzyme leakage from the cytosol of the hepatocytes into the blood. ALP (alkaline phosphatase) is an inducible hepatic enzyme with several isoenzymes, including liver, corticosteroid, bone, intestinal, and placental. These isoenzymes can be differentiated by their electrophoretic mobility. The liver ALP isoenzyme is a sensitive indicator of intrahepatic or extrahepatic cholestasis, whereas the bone ALP isoenzyme is frequently observed in young, rapidly growing animals. In this case, both isoenzymes may be contributing to the elevated ALP enzymatic activity.[5]

Contributor: Pfizer PGRD Amboise, Pathology Department, BP 109, 37401 Amboise, Cedex, France

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SLIDE 21
CONFERENCE 6 / CASE I – 04-3248 (AFIP 2937763)

Signalment: Two-year-old, male, Golden Retriever.

History: The dog presented with weakness, fever, weight loss, and muscle atrophy. He was treated with multiple antibiotics for an undiagnosed condition. The patient was euthanized due to severe emaciation and failure to respond to treatment.

Gross Pathology: A necropsy performed by the referring clinician revealed generalized muscle atrophy and slight bilateral enlargement of the kidneys.

Contributor's Morphologic Diagnoses:
1. Multifocal pyogranulomatous myocarditis with intralesional "zoites" and random meronts and merozoites.
2. Diffuse lymphoplasmacytic enteritis with neutrophils, intralesional sporozoites, blunting and fusion of villi, crypt abscesses, and mild lymphangiectasia.

Contributor's Comment: American canine hepatozoonosis (ACH) is distinctly different from "Old World" canine hepatozoonosis and the two entities have recently been compared in excellent reviews.[1,2] ACH is an emerging tick-borne disease caused by *Hepatozoon americanum* and transmitted by *Amblyomma maculatum* (Gulf Coast tick). The life cycle, route of infection, and pathogenesis are unique and require that the dog ingest an infected tick rather than being bitten by the tick. Sporozoites released from tick oocysts penetrate the intestine, enter the circulation and are carried to muscle and other tissues where they undergo asexual reproduction. A Gulf Coast tick feeding on a dog with circulating gamonts becomes infected and they undergo sexual reproduction. The necropsy and histological findings in a series of naturally-occurring and

experimental cases have been reported.[3-5] Antemortem diagnosis is best made from muscle biopsies from the biceps femoris or semitendinosus muscles because detection of gamonts in neutrophils on peripheral blood smears is rare. Pelvic radiographs reveal periosteal proliferation at muscle attachments to bone. An ELISA test has been developed. Marked leukocytosis is a consistent finding.

The tissues in this case were selected because they contain rarely seen sporozoites in the tips of villi (Image 2), a developing meront (Image 1) and merozoites "zoites"/gamonts (Image 3) in pyogranulomas in heart muscle in addition to the commonly seen cysts. The persistence of schizonts in the intestinal tract in the terminal stages of the infection raises the question of whether this represents re-infection from a tick, delayed/retarded migration of the schizonts, or possible reproduction in the intestine. Re-infection by asexual stages has been shown to persist for >9 months.[5] Severe cachexia has been attributed primarily to muscle pain and weakness. Intestinal, as well as splenic (Image 5 – granuloma with zoites also present), amyloidosis has been reported and was also present in this case. Intestinal amyloidosis can lead to malabsorption and protein loss. In the intestinal section of this case, the lacteals were severely dilated, even though the amyloid deposits were minimal and stained poorly. Mesangioproliferative glomerulonephritis has been reported in cases of ACH. Proteinuria is common in chronic cases and nephrotic syndrome can develop. In this case, glomerular lesions were dramatic with focal accumulations of large foam cells. Many glomeruli were partially effaced and adhesions were noted (Image 4). A major veterinary pathology text states that glomerular lipidosis has no functional significance.

This case originated in rural Georgia and ACH is reported primarily from the Southeastern United States. A seasonal occurrence corresponding with the prevalence of ticks (Apr-Oct) has been seen. Most cases occur in young adults and this may be related to their increased physical activity. Heavily infected dogs have a guarded prognosis and the mortality rate is high. Treatment with a combination of trimethoprim sulfa, pyrimethamine, and clindamycin has relieved clinical signs and prolonged treatment with decoquinate has reduced relapses and prolonged survival time.

AFIP Diagnoses: 1. Heart: Myocarditis and epicarditis, pyogranulomatous, multifocal, mild, with numerous protozoal cysts and merozoites, etiology consistent with *Hepatozoon americanum*, Golden Retriever, canine.
2. Small intestine: Enteritis, subacute, diffuse, mild, with villar blunting and fusion, and few sporozoites.

Conference Comment: The contributor provides a thorough overview of *Hepatozoon americanum*. Besides the differences in regional distribution, between *H. americanum* (United States, and possibly Central and South America) and *H. canis* (India, Africa, Asia, South America, the Middle East, and southern Europe), there are a few key distinctions between these apicomplexan protozoa. First, *H. canis* appears to be adapted to dogs and therefore often results in subclinical

disease or mild clinical signs. Moderate to severe clinical signs are only seen in immunosuppressed dogs. In contrast, *H. americanum* is poorly adapted to dogs and results in a more severe illness even in immunocompetent dogs. Secondly, the vectors differ, with the brown dog tick, *Rhipicephalus sanguineus* serving as the vector for *H. canis* and the Gulf Coast tick, *Amblyomma maculatum* serving as the vector for *H. americanum*. Lastly, the clinical presentation, pathology, and prognosis differ. *H. canis* causes anemia, and rarely an extreme leukocytosis. Radiographic bone lesions and significant histologic lesions within the muscle are absent. Meronts are found primarily in the spleen, bone marrow, and lymph nodes; they are rarely found in muscle and exhibit a wheel-spoke arrangement of merorozoites. The prognosis for dogs infected with *H. canis* is good. In contrast, *H. americanum* causes anemia, an extreme leukocytosis, and periosteal proliferation visible with radiography. Histologically, there are typical "onion-skin" cysts within muscle, meronts, and pyogranulomatous myositis. The prognosis for dogs infected with *H. americanum* is guarded.[1,7]

The sporozoite stage of *H. americanum* has not been well described in the literature. The contributor noted rare sporozoites within the villar tips. Although it seems logical that the organisms identified in the small intestine are sporozoites of *H. americanum*, they are very likely *Sarcocystis* oocysts containing multiple sporozoites. This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology, and Dr. J.P. Dubey. Dr. Dubey further described these organisms as having an oocyst wall, with two oval to elongate sporocysts, each containing two sporozoites. These characteristics are consistent with *Sarcocystis* sp. in the carnivore definitive host. In contrast, dogs are the intermediate host of *H. americanum*. Dogs become infected with *H. americanum* through ingestion of an adult tick containing oocysts. These oocysts are broken down in the intestinal lumen and the individual sporozoites then penetrate the intestinal wall.[6,7]

Contributor: University of Georgia-Tifton, Tifton Veterinary Diagnostic & Investigational Lab, P.O. Box 1389, 43 Brighton Road, Tifton, GA

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SLIDE 22
CONFERENCE 6 / CASE II – MK04-1836 (AFIP 2937351)

Signalment: 47-month-old male rhesus macaque, (*Macaca mulatta*).

History: This macaque was on a clinical trial to test the effectiveness of monoclonal antibody therapy to prolong renal graft survival after renal transplantation. Pre-transplant treatment consisted of daily injections of an anti-CD154 antibody, IDEC131, for three days preceding surgery. Post-transplant therapy was limited to weekly treatments of IDEC131 for eight weeks, after which, the animal did not receive additional immunosuppressive therapy. Renal biopsies were performed periodically to assess the character of renal graft inflammation. Monitoring also consisted of regular evaluation for increases in serum creatinine and blood urea nitrogen, and for the presence of anti-donor antibodies. There was no intervention after the graft started to fail. The animal lived 533 days post-transplant, the longest post-transplant period of any animal on this particular clinical trial.

Gross Pathology: The animal was in thin body condition with mild subcutaneous edema and pericardial effusion. The lungs were moderately pale. There were multifocal mild hemorrhages on the capsular surface of the transplanted kidney and the kidney was pale. No lesions were noted in the heart, lungs, liver, spleen, or gastrointestinal tract.

Laboratory Results:
Day 29 post-transplant: renal biopsy, moderate tubulointerstitial nephritis.
Immunohistochemistry of the interstitial infiltrate revealed $CD3^+$ cells.
Day 469: anti-donor antibodies detected in the serum
Day 533: BUN: 217 mg/dl Creatinine: 3.7 mg/dl

Contributor's Morphologic Diagnosis: Kidney: Mesangioproliferative glomerulonephritis, generalized, diffuse, moderate to marked, with periglomerular fibrosis, synechia formation and glomerular obsolescence; tubular degeneration, atrophy and loss and tubular proteinosis; interstitial nephritis; vascular smooth muscle hyperplasia and fibrosis with multifocal vasculitis and fibrinoid degeneration and medullary edema.

Contributor's Comment: The microscopic appearance of rejection in renal allografts generally is divided into three categories: hyperacute, acute and chronic. Hyperacute rejection occurs shortly after transplantation of non-HLA cross-matched organs or transplantation into patients who have been sensitized by previous allografts or

pregnancy. Histologically, the reaction is characterized by collections of neutrophils in arterioles, glomeruli, and peritubular capillaries as well as thrombi. This reaction is antibody mediated and the endothelium of the donor graft, along which antigen-antibody complexes are deposited, is the target of the immune response. Acute rejection may occur months to years later after transplantation and immunosuppressive therapy and it is mediated by cellular and humoral processes. Histologic changes suggestive of cellular rejection are: interstitial accumulation of mononuclear cells, edema, and interstitial hemorrhage.[1] The primary lesion associated with humoral rejection is vasculitis. Chronic rejection is characterized by disruption of vascular elastic lamina, interstitial fibrosis, and tubule loss.[2]

The Banff Working Classification of Renal Allograft Pathology has been developed by an international consortium of pathologists, clinicians, and investigators to serve as a definitive resource by which inflammatory changes in renal allografts are scored. The scoring system quantifies tubular, vascular, glomerular, and interstitial changes seen in acute rejection and chronic allograft nephropathy. The histological changes are then correlated with the severity of renal function deterioration seen clinically. This classification is revised periodically and is used by pathologists to standardize the diagnosis of renal allograft rejection, by clinicians to guide therapy for patients, and by investigators to evaluate clinical trial results. Acute or active rejection is characterized by tubulitis, tubulointerstitial inflammation, arteritis, and vasculitis. Mild, acute changes are thought to be primarily T-cell mediated but more severe acute changes are likely to involve an antibody mediated component.[2] Changes suggestive of an antibody mediated component include vasculitis with fibrinoid change, glomerular and small vessel thrombosis, infarction, glomerulitis and margination of polymorphonuclear leukocytes across peritubular capillaries and the presence of C4d, a component of the complement cascade, in peritubular capillaries and circulating anti-donor antibody.[3] Chronic changes due to immune reaction against renal allografts include interstitial fibrosis and tubular atrophy and loss. Deposition of basement membrane-like material to form “double contours” within capillary loops of glomeruli is a specific change associated with chronic transplant glomerulopathy. Additional chronic changes seen in allografts may be due to renal ischemia, hypertension, drug effects, infection, increased ureteral pressure, and nonimmune inflammation.[2] The changes seen in this case had many lesions similar to those seen in human acute and chronic allograft rejection, although interstitial fibrosis was not a significant finding.

One of the major obstacles facing successful organ transplantation is overcoming the recipient's immune response against alloantigens. Most post-transplant therapies focus on regulating the recipient's immune system by lifelong suppression, usually with a combination of a calcineurin inhibitor (cyclosporin or tacrolimus), steroids, and an antiproliferative agent, (mycophenolate, mofetil or azathioprine).[4] Calcineurin inhibitors specifically suppress T-cell activity. Calcineurin is a key signaling enzyme in T-lymphocyte activation.[5] Calcineurin inhibitors have improved short-term outcomes and reduced rates of acute rejection in renal transplant recipients; however, these drugs are nephrotoxic, and their use over a prolonged period of time contributes to chronic allograft nephropathy.[6] Antiproliferative agents non-selectively inhibit cell proliferation

and can cause bone marrow suppression and hepatoxicity. Steroids inhibit T-lymphocytes but also cause systemic immunosuppression.[7] Additional undesirable side effects of these immunosuppressive drugs include hypertension, hyperlipidemia, osteoporosis, and chronic allograft nephropathy.[4] In addition to chronic kidney damage by therapeutic agents and the undesirable effects of these agents, an allograft recipient receiving immunosuppressive therapy is predisposed to infection by adventitial microorganisms and to developing neoplastic disease.[4]

In order to decrease the use of immunosuppressive drugs with their attendant detrimental side effects, monoclonal antibodies are being developed to target cell surface molecules on the cells associated with rejection. By targeting a specific pathway, monoclonal antibodies may provide an adjunct to traditional therapy by rendering the recipient tolerant to donor tissue while incurring minimal systemic immunosuppression and, ultimately, leading to prolonged graft survival.[4]

The animal in this case was treated with 'costimulation blockade' monoclonal antibody therapy, which inhibits one of the two main signals necessary for T-cell activation. The first signal in T-cell activation is the presentation of antigen to T-cells in association with MHC-I or MHC-II molecules. Costimulatory molecules are the second immunological signal required for the activation of T-lymphocytes.[4,8,9] These molecules may activate or suppress T-cell response to an antigen. Monoclonal antibodies against costimulatory molecules are used at the time of transplantation when the recipient encounters foreign antigens from the graft for the first time. In theory, T-cells that are presented with novel antigens, such as those found on an allograft, without costimulation are rendered anergic or undergo apoptosis, removing the cells that potentially could mount an immune response against the graft.[4]

Monoclonal antibodies to CD-154 have been used to induce long term (years) renal allograft survival in nonhuman primates. CD-154 is found on the surface of T-cells and its expression is increased in activated T-cells. Interaction of CD-154 with CD40 found on antigen presenting cells (APCs) increases APC expression of B7 and MHC molecules and increases production of cytokines. The relationship between inhibition of CD-154:CD-40 binding and altered T-cell function has not been characterized completely. Use of anti-CD-154 agents in humans has not been as successful as predicted by studies in nonhuman primates. Some of these agents have had the undesirable side affect of predisposing the recipient to thrombi formation.[4]

There are several centers in the United States that now offer kidney transplantation for the treatment of end-stage renal disease in companion animals. While immunosuppressive regimes are similar between feline and human graft recipients, histological patterns of renal rejection differ between the two species. In a recent issue of *Veterinary Pathology*, the authors attempted to classify histologic changes in feline renal transplants using the Banff 1997 Guidelines. However, the scoring system did not accurately reflect the severity of lesions based on serum creatinine and BUN levels. Criteria for acute rejection in humans, tubulitis, lymphocytic glomerulitis, and vasculitis

were seen rarely in cats. Additionally, subcapsular and interlobular phlebitis were seen in this series of cats but have been reported rarely in man.[10]

The classification and treatment of allograft rejection continues to be an ongoing challenge. New therapies, such as monoclonal antibodies targeted against effectors of the immune response, may alleviate the need for multiple transplants by inducing life-long tolerance in organ recipients. However, at the present time, there is still no effective and definitive treatment or group of treatments to prevent the main cause of late graft loss, chronic rejection. An even more urgent problem than chronic allograft rejection is the tremendous demand for donated organs. In the United States, 52,000 people wait for a kidney each year.[6] The waiting period for a kidney is in excess of 800 days, and people often die waiting for a donor.[8] It may come to pass that, in refining our knowledge of the mechanisms of allograft rejection, we may be able to understand the causes of, and to develop effective treatments for, renal disease before transplantation becomes the only treatment option.

AFIP Diagnosis: Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, chronic, moderate, with lymphoplasmacytic interstitial nephritis, and arteritis with intimal fibromuscular proliferation, rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: As the contributor discussed, one of the most important goals of immunologic research is successful transplantation of tissues in humans without rejection. Although the surgical techniques for transplantation of many tissues, including kidneys, skin, heart, lungs, liver, spleen, bone marrow, and endocrine organs are well refined, the ability to confer permanent acceptance of foreign grafts is still out of reach. The basis of graft rejection involves differences in HLA proteins that are expressed on cells. HLA genes are highly polymorphic and any two individuals, other than identical twins, will express some HLA proteins that are different. Therefore all individuals will recognize some difference in HLA molecules as foreign and mount an immune response to them. Conference attendees discussed, in detail, the two types of hypersensitivity, cell-mediated (Type IV hypersensitivity) and antibody-mediated (Type II hypersensitivity) that are fundamental to transplant rejection.[11]

Type II hypersensitivity is mediated by antibodies directed toward antigens present on cell surfaces. In the case of transplant rejection, these antigens are the HLA proteins of the donor organ. This process is called humoral rejection and can take two forms: hyperacute and acute. Hyperacute rejection occurs when the recipient has preformed antidonor antibodies in circulation. These can develop in multiparous women who receive grafts from their husband, children, or unrelated individuals who share HLA alleles with the husband; people who have received prior blood transfusions; and, people who have already rejected a kidney transplant. The rejection is immediate, with circulating antibodies reacting to graft endothelium, inducing complement fixation, and resulting in thrombosis of graft vasculature. The acute humoral rejection occurs in recipients who are not previously sensitized. The antibodies formed cause injury via

complement-dependent cytotoxicity, inflammation, and antibody-dependent cell-mediated cytotoxicity, targeting the graft vasculature. [11]

Type IV hypersensitivity is T-cell mediated, is called cellular rejection, and is induced by two mechanisms: CD8+ cytotoxic T-lymphocyte (CTL) mediated destruction of graft cells, and CD4+ helper T-cell mediated delayed hypersensitivity. The direct pathway, mediated by the CD8+ CTLs occurs when the T-cells of the recipient recognize donor HLA (MHC) molecules on the surface of antigen presenting cells (APCs) in the graft. However, both CD8+ and CD4+ T-cells are activated and differentiate into mature CTLs and Th1 cells, respectively. Mature CTLs mediate cell destruction by either perforin-granzyme-dependent killing or Fas-Fas ligand-dependent killing. Th1 cells secrete cytokines that increase vascular permeability, are chemotactic for lymphocytes and macrophages, and activate macrophages, resulting in graft injury.[11]

Contributor: National Institutes of Health, Diagnostic & Research Services Branch, Division of Veterinary Resources, 28 Service Road West, Bldg 28A--Room 115, Bethesda, MD

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SLIDE 23
CONFERENCE 6 / CASE III – 04-14567 (AFIP 2938299)

Signalment: 4-week-old, female, Golden Retriever-cross (*Canis familiaris*).

History: This individual was one of two puppies from a litter of seven that died suddenly. The puppy had been nursing and then became cyanotic, dyspneic and died.

Gross Pathology: The carcass was mildly autolyzed and was in good nutritional condition. The thoracic cavity contained a few milliliters of watery yellow fluid and the lungs were diffusely edematous and had a rubbery consistency. There was marked pallor of the left ventricle. Gastrointestinal contents were sparse and the urinary bladder was contracted.

Laboratory Results: Abundant canine parvovirus antigen was detected in the myocardium by immunohistochemistry.

Contributor's Morphologic Diagnosis: Heart: Myocarditis, lymphohistiocytic with cardiomyocyte degeneration, necrosis and loss and intranuclear inclusion bodies.

Contributor's Comment: Multifocally, there are foci of cardiomyocyte degeneration and necrosis characterized by hypereosinophilia, loss of cross striations and fragmentation. The interstitium is mildly expanded by lymphocytes, macrophages and rare plasma cells. Within the cardiomyocyte nuclei, there are moderate numbers of 5-8 um, amphophilic to basophilic intranuclear inclusion bodies that often cause margination of the chromatin. Based on the lesion, this was suspected to be a case of parvoviral myocarditis and immunohistochemistry confirmed the presence of parvovirus antigen within cardiomyocytes.

The *Parvoviridae* are non-enveloped viral particles about 18-26 nm in diameter with a single-stranded DNA genome.[1] In the late 1970's, a new parvovirus emerged worldwide as the cause of severe enteritis and myocarditis in dogs. The agent was named canine parvovirus type 2 (CPV-2) to distinguish it from the less pathogenic canine parvovirus type 1 (Minute Virus of Canines) which is associated with mild diarrhea, fetal losses, myocarditis and fading puppy syndrome.[2] Historically, it was believed that CPV-2 evolved from the closely related feline panleukopenia virus; however, recent work suggests that a wild carnivore may have harbored the immediate ancestor of CPV-2.[3] Since its emergence in the 1970's, CPV-2 has been replaced by two antigenic variants CPV-2a and CPV-2b. Interestingly, CPV-2a and CPV-2b regained the ability to infect both domestic and large cats. It is believed that approximately 5% of parvovirus infections in cats are caused by either CPV-2a or CPV-2b.[4]

Parvoviruses may infect cells at any phase of the cell cycle but replication is dependent on cellular mechanisms functional only in the S phase prior to mitosis. For this reason, the effects of parvovirus infection are greatest in tissues with a high mitotic rate, including hematopoietic tissue, intestinal crypt epithelium and neonatal cardiomyocytes (<2 weeks of age).[1] In the original outbreaks of CPV-2 in naïve populations, myocarditis was a common finding. CPV-2 myocarditis is most often seen in puppies under 4 weeks of age and usually all pups in the litter are affected. Pups are often found dead or die after a brief period of dyspnea, crying and retching. Signs of cardiac dysfunction may be preceded by the enteric form of the disease or may occur suddenly without apparent previous illness.[2] The myocardial form of the disease has virtually disappeared as a result of population immunity. Virtually all bitches are immune and pass immunity to their
pups via colostrum resulting in protection during the critical period when infection of the myocardium is possible.[5] Myocarditis is still occasionally found in pups born to isolated, unvaccinated bitches or in cases where adequate colostrum is not received.[2]

AFIP Diagnosis: Heart: Myocarditis, lymphohistiocytic, multifocal, minimal, with myocyte degeneration and necrosis, and basophilic intranuclear inclusion bodies, Golden Retriever-cross, canine.

Conference Comment: The contributor provides a thorough overview of canine parvovirus type 2 (CPV-2) and compares it to the less pathogenic canine parvovirus type one (CPV-1). Members of the genus *Parvovirus* infect many other species of laboratory and domestic animals, including cats, mink, calves, and swine.[1]

Canine parvovirus enteritis was first recognized in dogs because the gross and microscopic lesions were identical to feline parvoviral enteritis, caused by feline panleukopenia virus (FPV). FPV affects all members of the Felidae, as well as mink, raccoons, and some other members of the Procyonidae.[1] It is the cause of panleukopenia, also known as cat distemper, feline enteritis, and mink enteritis,[6] and is

very similar to CPV-2. Panleukopenia virus is ubiquitous in environments frequented by cats; infection is common, but generally subclinical. Transmission is primarily through oronasal exposure and results in uptake of the virus by epithelium over the tonsils and Peyer's patches. The virus infects lymphoblasts and disseminates to other lymphoid organs (spleen, bone marrow, thymus, lymph nodes). Viral infection of these organs results in lymphocytolysis and viremia. Infection of the gastrointestinal epithelium is a secondary event and leads to destruction of the cells in the crypts of Lieberkuhn. If severe enough, this will result in focal or widespread villus atrophy, mucosal erosion or ulceration. Proliferating cells in the bone marrow are also affected during viremia, resulting in cytolysis and bone marrow depletion of both erythroid and myeloid elements. Infection of the fetus during late prenatal life by FPV results in cerebellar hypoplasia.

A summary of parvoviruses that affect domestic and laboratory animals are listed below:[7,8]

Virus	**Disease**
Feline parvovirus	Panleukopenia, cerebellar hypoplasia, enteritis
Canine parvovirus-1	Mild diarrhea
Canine parvovirus-2	Enteritis, myocarditis, leukopenia
Porcine parvovirus	Stillbirth, mummification, abortion, embryonic death, infertility (SMAEDI)
Mink enteritis virus	Panleukopenia, enteritis
Aleutian disease virus of mink	Chronic immune complex disease, encephalopathy
Minute virus of mice	Lymphotrophic, erythrocyte-associated anemia
Mouse parvovirus	Lymphotrophic, immunomodulation
Kilham's rat virus	Multifocal hemorrhage (brain, liver, testes)
Toolan's H-1 virus of rats	Nonpathogenic
Rat parvovirus	Nonpathogenic
Hamster parvovirus	Domed calvaria, malformation of incisor teeth
Lapine parvovirus	Mild to moderate enteritis in rabbits
Goose parvovirus	Hepatitis, myocarditis
Duck parvovirus	Hepatitis, myocarditis

Contributor: Department of Veterinary Pathology/ Prairie Diagnostic Services Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK Website: www.usask.ca/wcvm/vetpath

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SLIDE 24
CONFERENCE 6 / CASE IV – D-04-0229 (AFIP 2936454)

Signalment: Three-month-old, female, albino rat (*Rattus norvegicus*).

History: This was one of a group of 12 rats and mice imported from Los Angeles to Hong Kong four days previously. When examined subsequently they were showing upper respiratory tract disease signs and ruffled coats. Four younger rats appeared weak. They were all medicated with Baytril, but one rat died the next day (this case) and a second died 2 days later. The animals had been held in a warehouse for an extended period before departure and the flight was delayed in arriving at Hong Kong.

Gross Pathology: The submitted carcass was a 3-month-old white female rat in normal body condition, but dehydrated. The right cranial lung lobe was completely consolidated, dark red, and had a large amount of pale mucoid exudate in the bronchioles. The rest of the lungs were congested and edematous. The stomach contained no ingesta. The small intestine was distended with gas and the colon contained several fecal pellets.

Laboratory Results: A pure growth of *Streptococcus pneumoniae* was isolated from the rat's lung and liver.
Contributor's Morphologic Diagnosis: 1. Brain: Leptomeningitis, suppurative, diffuse, subacute, severe, and severe focal paraventricular encephalitis with numerous intralesional diplococcoid bacteria present.
2. Lung: Bronchopneumonia, suppurative, multifocal, severe, subacute with some alveolar septal destruction.

Contributor's Comment: This is a case of severe subacute suppurative meningoencephalitis and bronchopneumonia caused by *Streptococcus pneumoniae*. In addition to the lesions in lung and brain, the left ventricle of the heart had a focal area of severe myocarditis involving the endocardium and deeper myocardium with degenerate myocardiocytes and an infiltration of fibroblasts, macrophages and fewer lymphocytes. There was also a mild tracheitis with an infiltration of moderate numbers of degenerate

neutrophils and macrophages in the lamina propria with exudation into the lumen. The tracheal mucosal epithelium showed squamous metaplasia and loss of mucous glands.

Streptococcus pneumoniae is a gram-positive, capsulated, non-motile, facultatively aerobic coccus that occurs singly, in pairs, or in short chains in its natural habitat, the upper respiratory tract of humans or other mammals.[1] In infected tissues and pus this bacterium commonly occurs as pairs of cocci which gave cause for its previous generic name of *Diplococcus*. It is an important pathogen in humans causing primarily lobar pneumonia (giving rise to the name pneumococcus), but also causing septicemia and meningitis.[2] In the past, this infection was recognized as a common problem in rodent colonies with clinically normal carrier animals maintaining infection in the nasoturbinates and tympanic bullae, but human carriers have also been implicated as a source of infection to laboratory rodents.[3] There are also reports of *S. pneumoniae* causing bovine mastitis, calf septicemia and septicemia and septic arthritis in cats.[4]

S. pneumoniae is alpha-hemolytic in culture on blood agar but can show some variation in colony type. Compared to other alpha-hemolytic streptococci, *S. pneumoniae* can be identified by rapid lysis in bile salts and optochin (ethylhydrocupreine hydrochloride) sensitivity.[2] There are over 84 serotypes of this bacterium, but most infections are caused by less than 10 serotypes.[4]

In lesions, pus, or blood smears *S. pneumoniae* has a distinctive polysaccharide capsule which enables it to resist phagocytosis by host cells.[2,3] *S. pneumoniae* is not known to produce soluble toxins but several serotypes produce tissue damage by activation of the alternate complement pathway.[3]

Other lesions that have occurred in outbreaks of this infection in rodent colonies have included fibrinopurulent polyserositis, suppurative rhinitis, otitis media and embolic suppurative lesions in organs such as liver, spleen and kidney.[3]

AFIP Diagnosis: 1. Lung: Bronchopneumonia, necrotizing, suppurative, subacute, moderate, rat, rodent.
2. Midbrain: Meningoencephalitis, suppurative, diffuse, moderate, with myriad bacterial diplococci.

Conference Comment: *Streptococcus pneumoniae* was previously a common problem in laboratory rats. Today, outbreaks of clinical disease are rare in barrier-maintained facilities. Clinical signs may include serosanguinous nasal discharge, rhinitis, sinusitis, conjunctivitis, and vestibular signs consistent with middle ear involvement. Asymptomatic animals may develop clinical disease when there is a concurrent infection or a change in environment. Gross lesions are varied depending on the organ system(s) involved and include: serous to mucopurulent exudates in the nasal passages and/or tympanic bullae, fibrinopurulent pleuritis, peritonitis, pericarditis, periorchitis, meninginitis, or polyserositis. Histologically, in the acute form of the

disease, fibrinopurulent pleuritis and pericarditis are typical findings. Pulmonary lesions vary from localized suppurative bronchopneumonia to acute fibrinopurulent bronchopneumonia, with obliteration of normal architecture in the affected lobes. Fibrinopurulent lesions may be identified in any organ affected by *S. pneumoniae*. Embolic suppurative lesions have been identified in the liver, spleen, and kidney. Differential diagnoses for *S. pneumoniae* in a rat include corynebacteriosis, salmonellosis, pseudomoniasis, and pasteurellosis.[3]

Streptococcus pneumoniae is known to cause similar lesions in other animals, including guinea pigs, hamsters, and non-human primates.[3,5] Mice are resistant to *S. pneumoniae*[6] but can develop suppurative lesions when infected with beta-hemolytic streptococcal organisms. However, SCID mice may develop systemic disease with alpha-hemolytic streptococci.[3,6] Rabbits have developed acute diplococcal infections on rare occasions and streptococcal septemia has been reported in young rabbits.[3]

Contributor: Tai Lung Veterinary Laboratory, Agriculture, Fisheries and Conservation Department, Government of Hong Kong SAR, Lin Tong Mei, Sheung Shui, New Territories, Hong Kong SAR, China

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SLIDE 25
CONFERENCE 7 / CASE I –D-04-0312 (AFIP 2936453)

Signalment: Two month old, female, large white-cross, pig.

History: A pig farm has had chronic problems for two years involving respiratory signs in young pigs. Symptoms exhibited by this weaner were typical of affected animals, and included labored breathing, mucoid nasal discharge and poor growth.

Gross Pathology: There was a diffuse lesion throughout the lungs, with all lobes discolored and consolidated. The cut surface was effusive and showed multiple white spots that appeared to be material in airways, but there was no pus. The thoracic and mesenteric lymph nodes were enlarged.

Laboratory Results: Microbiology results showed no significant bacterial growth on routine aerobic culture from the lungs, liver, or large intestine.

PCR test results were negative for porcine reproductive and respiratory syndrome virus (PRRSV) from lung tissue and a clotted blood sample. Virus culture on MARC cells showed cytopathic effect, and the culture medium was PCR-positive for PRRSV (US strain). The lung tissue was also PCR-positive for porcine circovirus 1 (PCV1), but the lymph nodes were negative. Lungs and lymph nodes were negative for PCV2.

Contributor's Morphologic Diagnosis: 1. Lungs: Pneumonia, interstitial and proliferative, severe, non-suppurative, subacute to chronic, coalescing lesions, with marked proliferation of type II epithelial cells.
2. Bronchioles: Bronchiolitis, severe, necro-suppurative, subacute, coalescing lesions, with caseating material present in airways.

Contributor's Comment: Porcine reproductive and respiratory syndrome (PRRS) has become a world-wide problem for pig producers since slightly differing syndromes (North America in 1987 and Europe in the 1990s) were united into one syndrome caused by genetic relatives of the Lelystad virus, now referred to as PRRSV.[1] The American and European isolates have been found to have marked genotypic and phenotypic differences, and variants of each have arisen even within their respective continents. The widely varying syndromes involve late-term abortion, stillborn and weak pigs, lowered farrowing rates, high death rates of neonates and weaned pigs, and delayed returns to estrus.[2]

Infection and disease associated with PRRSV are common in Hong Kong pig farms, and many cases are complicated by co-infection with porcine circovirus 2 (PCV2). This case was negative for PCV2, so the interstitial pneumonia was demonstrative of uncomplicated PRRSV infection. The pulmonary and bronchiolar lesions were given distinct morphological diagnoses because of the likelihood that secondary bacteria were involved in the bronchial lesion. The bacterial and mycoplasma culture results from this laboratory were negative, but the bronchial lesion is more consistent with secondary bacterial involvement than with pure PRRS, and many pigs in Hong Kong receive prophylactic antibiotics in the feed. The experimental disease in the lungs associated with uncomplicated PRRSV is limited to an interstitial pneumonia, is non-suppurative and dominated by mononuclear reaction.[2] Additional airway disease with neutrophils is usually attributed to bacteria or *Mycoplasma* spp..

The lymph nodes of this pig also showed severe diffuse changes, including a general loss of follicular architecture and replacement by histiocytes, lymphocytes and plasma cells. The overall appearance of the lymph nodes was of diffuse granulomatous

lymphadenitis. The periphery of the lymph nodes showed areas of necrosis. The brain and meninges showed very mild perivascular cuffing, but this was not considered significant enough to have contributed to the pig's symptoms. The heart was normal but there were a few foci of monocytic infiltration in the liver.

Unfortunately it was not possible to obtain a more detailed history from the farm as to whether there were additional reproductive problems or other manifestations of PRRS disease. Records are not kept at many traditional farms and there are often multiple problems interacting at once.

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, subacute, diffuse, moderate, with multifocal type II pneumocyte hyperplasia, cross-bred pig, porcine.

Conference Comment: Porcine pneumonias are a major problem faced by the contemporary swine industry. The incidence, prevalence, and mortality rates of pneumonias in pigs are linked to many interdependent factors, including: the host (age, genetic makeup, immune status), infectious agents (viruses, bacteria, mycoplasmas), environmental conditions (humidity, temperature, ammonia concentrations), and management practices (crowding, mixing of animals, air quality, nutrition, stress).[3]

Porcine reproductive and respiratory syndrome (PRRS) is endemic in many swine producing countries and is a major cause of late-term abortions, stillbirths and respiratory disease in young pigs. PRRS is caused by PRRS virus (PRRSV), an enveloped single stranded RNA virus in the family *Arteriviridae*, genus *Arterivirus*. Other arteriviruses include equine arterivirus (EAV) and simian hemorrhagic fever virus (SHFV).[4]

PRRSV is transmitted by direct contact between infected and naïve pigs, although the exact route of transmission (aerosol, body fluids, fecal) has not been proven experimentally. The virus replicates in alveolar macrophages and glial cells. However, the virus antigen or RNA has been identified in macrophages of multiple tissues, monocytes, endothelial cells, smooth muscle cells, and fibroblasts. PRRSV infection has been limited to domestic swine, with a single report of PRRSV infection in Mallard ducks.[4]

The clinical presentation of PRRSV infection depends on the age, pregnancy status, and trimester of gestation of the infected pig. Clinical presentation on a farm varies from sporadic abortions to abortion storms. Individual animals may present with late-term abortion, premature farrowing with stillborn fetuses, partially autolyzed fetuses, or mummified fetuses. Clinical signs in infected sows or gilts vary from none to anorexia, fever, lethargy, pneumonia, agalactia, cyanosis of the ears and vulva, edema, delayed return to estrus, and less commonly, death.[4]

Gross lesions associated with PRRSV infection vary widely from none to diffuse tan consolidation of the lungs, and are commonly complicated by lesions resulting from concurrent bacterial infection. Lymph nodes may be markedly enlarged and vary from solid to polycystic. Fetuses from PRRSV abortions are late term and the body condition ranges from fresh to autolyzed.[4]

Light microscopic lesions most commonly involve the lung and lymphoid tissue. However, other lesions include vasculitis, myocarditis, and encephalitis. Lung lesions are characterized by septal thickening by macrophages, type II pneumocyte hyperplasia, necrotic debris, macrophages and syncytial cells within alveoli, peribronchial lymphoid hyperplasia, and lymphoplasmacytic perivascular cuffing. Lymphoid tissue exhibits lymphoid hyperplasia and necrosis.[4] Other infectious causes of pneumonia in swine are listed below[3]

Viral
Swine influenza virus
Porcine circovirus (Postweaning multisystemic wasting syndrome, PMWS)
Porcine respiratory coronavirus (PRCV)

Bacterial
Mycoplasma hyopneumoniae (Porcine enzootic pneumonia)
Actinobacillus pleuropneumoniae (Porcine pleuropneumonia)
Haemophilus parasuis (Glasser's disease)
Pasteurella multocida
Streptococcus suis type II
Mycobacterium spp. (*M. avium*, *M. bovis*, *M. tuberculosis*)
Salmonella spp. (*S. choleraesuis*, *S. typhisuis*)

Parasitic
Metastrongylus spp. (*M. apri*, *M. salmi*, *M. pudendotectus*)
Ascaris suum

Contributor: Tai Lung Veterinary Laboratory, Agriculture, Fisheries and Conservation Department, Lin Tong Mei, Sheung Shui, NT, Hong Kong SAR of China
http://www.afcd.gov.hk

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SLIDE 26
CONFERENCE 7 / CASE II – 15303 6a or 15303 6e (AFIP 2946723)

Signalment: Adult, female, intact, rabbit (*Oryctolagus cuniculus*).

History: This rabbit received intrahepatic inoculation of VX2 carcinoma cells in the development of a tumor model to assess the response to treatment by various gene therapy agents. One week post inoculation, the rabbit developed anorexia and was noted to be losing weight and condition. After 2 days of supportive therapy with no clinical improvement, the animal was euthanized.

Gross Pathology: The rabbit was thin with scant body fat. Abdominal organs, including the liver and kidney, contained multiple tumor masses composed of pale, yellow-white, soft tissue. There was palpable thickening of the pyloric region of the stomach, with pale transmural tumor-like masses noted on cut section.

Contributor's Morphologic Diagnosis: Stomach: Carcinoma, invasive, transmural, intravascular, compatible with VX2 carcinoma, rabbit, *Oryctolagus cuniculus.*

Contributor's Comment: The wall of the stomach is thickened by multiple unencapsulated, poorly circumscribed and highly infiltrative, multilobular masses that expand the submucosa and tunica muscularis, and extend into the serosa and mucosa. Lobules of various sizes and shapes separate muscle bundles, connective tissue and structural elements, and are supported on a fine to coarse fibrovascular stroma. Coalescing areas of coagulative and liquefactive necrosis occupy 10-40% of tumor area, depending on the section, and sometimes are associated with intravascular thrombi and hemorrhage (infarction). Within lobules, neoplastic cells form cords and packets supported on a fine fibrovascular stroma. The cells are polygonal with variably distinct borders, and moderate amounts of pale eosinophilic and vacuolated cytoplasm, a round vesiculate nucleus with dispersed chromatin and 1 to 3 small nucleoli. There are 3 to 8 mitoses per high power field (HPF) with numerous bizarre mitotic figures. There are scattered large karyomegalic and multinucleated atypical cells. Vascular invasion seems to be primarily within thin-walled lymphatic vessels, but also sometimes in blood vessels. There is mild to marked submucosal edema, depending on the section. There is mild to moderate lymphoplasmacytic infiltration of the mucosa and submucosa.

VX2 carcinoma was established from a carcinoma induced in a rabbit by the Shope cottontail rabbit papillomavirus (CRPV) in 1940.[1] Papillomaviruses induce benign and malignant tumors in humans and animals. VX2 tumor cells contain multiple copies of CRPV genome integrated into their cellular DNA as tandem repeats. VX2 tumor is considered to be an anaplastic carcinoma, composed of poorly differentiated keratinocytes that do not keratinize (cornify). VX2 cells grow rapidly in adult allogenic recipients, frequently metastasizing to the lungs. They are known for having extremely aggressive behavior in vivo, and are used to model various types of aggressive epithelial cancers including liver tumors and lung tumors.[1] Auricular VX2 carcinoma of the New Zealand White rabbit is an animal model for human squamous cell carcinomas of the head and neck region (HNSCC), since both tumors tend to metastasize lymphatically, leading to early lymph node and subsequent distant metastasis.[3] It has also been used as a model of tumor-induced hypercalcemia.[4]

AFIP Diagnosis: Stomach: Carcinoma, with intravascular tumor emboli, rabbit, lagomorph.

Conference Comment: Without the above history, an obvious differential is metastatic uterine adenocarcinoma, as it is the most common spontaneous neoplasm of rabbits. The incidence is relatively low, approximately 4%, in younger does (2-3 years old) and much higher, approximately 80%, in older does (5-6 years old). Grossly the tumors are nodular, often multicentric, and usually involve both horns. On cut surface, they are firm, often with a cauliflower-like surface and central ulcerations. Carcinomatosis and/or metastasis to the lung and liver may occur. Histologically, cells invade the underlying muscular tunics and form acinar and tubular structures.[6]

Conference attendees also discussed the multistep model of carcinogenesis, which involves the sequential stages of initiation and promotion. Initiation causes permanent DNA damage (mutations), is rapid and irreversible. However, initiation alone is not sufficient for tumor formation. On the other hand, promoters can induce tumors in initiated cells, but are nontumorigenic by themselves. In contrast to the effects of initiators, the cellular changes resulting from promoters do not affect DNA directly and are reversible. Promoters enhance the proliferation of initiated cells, which may lead to additional mutations.[7]

Initiation involves nonlethal genetic damage that may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line. There are four classes of normal regulatory genes that are the principal targets of genetic damage: the growth-promoting protooncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repair.[7]

Carcinogenesis is a multistep process at both the phenotypic and genetic levels. Characteristics such as excessive growth, local invasiveness, and the ability to form

distant metastases are phenotypic attributes that are acquired sequentially, a process known as tumor progression. At the molecular level, progression is a result of cumulative genetic damage that may be favored by defects in DNA repair.[7]

There are eight fundamental changes in cell physiology that together determine malignancy. They include the following: self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, defects in DNA repair, limitless replicative potential, sustained angiogenesis, ability to invade and metastasize, and the ability to evade the immune system. Mutations in genes that regulate these cellular traits are seen in every cancer.[7]

Contributor: Baylor College of Medicine, Center for Comparative Medicine, 600D, One Baylor Plaza, MS BCM 145, Houston, TX
www.bcm.tmc.edu

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SLIDE 27
CONFERENCE 7 / CASE III – 04-11575 (AFIP 2937341)

Signalment: 1 month old, female, Spotted Saddle Horse (*Equus caballus*).
History: Apparently healthy filly became acutely ill and died overnight.

Gross Pathology: The liver was slightly swollen and icteric. The kidney was 20% swollen. The spleen was 3X normal size with petechial hemorrhages.

Laboratory Results: Replicate sections of liver stained with Steiner silver stain reveal clusters of long bacterial rods within adjacent hepatocytes. Low numbers of *Listeria monocytogenes* as well as *Salmonella typhimurium* were isolated from the liver of this foal. *Listeria monocytogenes* was also isolated from the kidney in slightly higher numbers. Replicate sections of liver stained with Brown and Brenn (tissue gram stain) reveal rare extracellular short bacterial rods, most of which are gram positive.

Contributor's Morphologic Diagnosis: Liver: Severe, acute, multifocal random, necrotizing hepatitis with intracellular bacteria.

Etiology: Tyzzer's disease **(***Clostridium piliformis***)** with concurrent *Listeria monocytogenes* and *Salmonella typhimurium* infection**.**

Contributor's Comment: Tyzzer's disease is a fatal necrotizing hepatitis caused by *Clostridium piliforme* (previously named *Bacillus pilliformis*). This disease is most commonly encountered in young foals[1,2] and laboratory rodents[3], but occasional cases in cats and dogs are observed.[2] The disease is characterized by multifocal to miliary hepatic necrosis with the adjacent hepatocytes containing intracellular 10 to 40 micron long, spore forming, gram-negative clostridial organisms[4] arranged into "match stick" bundles. The organism is difficult to culture and diagnosis is dependent upon demonstration of the organism within the lesions.[3,4,5] It is principally a disease of foals 1-5 weeks of age[1] and is characterized by sudden onset of fever, shock, terminal coma and death within a few hours to two days. Clinical symptoms include tachycardia, tachypnea, jaundice and severe diarrhea. Affected foals are usually leukopenic and have highly elevated liver enzymes.

Although clinical disease is rare, the prevalence of antibody in horses suggests that *Clostridium piliforme* infection is common. Why only certain foals are susceptible to fatal infections is not known. Adult inapparent carriers can infect newborn foals, which are normally coprophagous. Spores survive moderate heating, freezing and thawing. Contaminated litter remains infective for months.[6]

Salmonellosis and listeriosis are uncommon forms of septicemia in the horse.[7]

AFIP Diagnosis: Hepatitis, necrotizing, acute, random, severe, with intrahepatocytic bacilli, etiology consistent with *Clostridium piliforme*, Spotted Saddle Horse, equine.

Conference Comment: Tyzzer's disease was first reported in Japanese waltzing mice by Ernest Tyzzer in 1917. The organism is now recognized to produce disease in a wide variety of other species including rats, gerbils, hamsters, guinea pigs, rabbits, and horses, and rarely in cats, birds and humans with AIDS. The etiologic agent, *Clostridium piliforme*, is a gram-negative, spore-forming, filamentous, obligate

intracellular bacterium that is difficult to isolate using standard bacteriologic techniques.[8,9]

The organism is shed in the feces and spores may persist in the environment for up to one year. Transmission is primarily through ingestion, although intrauterine infection has been produced experimentally in mice. Outbreaks of Tyzzer's disease are characterized by low morbidity and high mortality in affected animals. Animal strain, age, and immune status are important factors in susceptibility to the disease. Typically, the organism invades the intestinal mucosal epithelium and disseminates to other organs, particularly the liver and heart.[9]

Gross lesions include multifocal coagulative to suppurative hepatic necrosis, segmental necrotizing enteritis, primarily in the terminal ileum and cecum (except for the rabbit, in which the cecum and colon are the target organs),[10] and multifocal necrotizing myocarditis. Foci of necrosis may also be found in the mesenteric lymph nodes. Histologically, there are intracytoplasmic bundles of bacilli within enterocytes and hepatocytes adjacent to necrotic foci. The organisms are easily identified in Warthin-Starry 4.0 stained tissue sections.[9]

Contributor: C.E. Kord Animal Disease Diagnostic Laboratory, P.O. Box 40627, Melrose Station, Nashville, TN
http://www.state.tn.us/agriculture/regulate/labs/kordlab.html

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SLIDE 28
CONFERENCE 7 / CASE IV – N03-912 (AFIP 2946701)

Signalment: 11 year old adult female cynomolgus monkey (*Macaca fascicularis*).

History: This female cynomolgus presented to the clinician for marked lethargy, and with a previous history of seizures of unknown etiology. Examination and laboratory work-up revealed an inflammatory leukogram and low protein. The animal's condition did not respond over time to treatment and she was subsequently euthanized.

Gross Pathology: Gross postmortem examination revealed clear fluid in the thorax (hydrothorax).

Contributor's Morphologic Diagnosis: Pancreas and adjacent fibrovascular connective tissue: Arteritis, necrotizing, subacute to chronic, multifocal.

Contributor's Comment: The full range of the severity and duration of the polyarteritis in this animal was not evident in all sections. Mildly affected arteries were characterized by mixed populations of inflammatory cells located primarily in the tunica adventitia. More severely affected vessels displayed transmural inflammation, intimal proliferation and fibrinoid necrosis within the tunica media. The disease in this animal involved multiple tissues including pancreas, heart, kidney and mesentery.

Polyarteritis nodosa (PAN) -like diseases have been described in several species including humans, dogs, cats, pigs and rodents, but only rarely in nonhuman primates. The contributor is aware of four cases of idiopathic PAN reported in cynomolgus macaques. The disease in humans is multisystemic and characterized by necrotizing lesions in small and medium-sized arteries. Other shared features of the disease as it is observed in both cynomolgus monkeys and people include segmental distribution in affected vessels, predilection for areas of arterial branching, and coexistence of acute and chronic lesions. Although the pathogenesis of PAN is not well understood, it is generally thought to involve an immune-mediated disease process. Immune complex deposition with complement activation may play a role in initiating the disease, while cell-mediated immune interactions are likely to contribute to the progression of the lesion.

In rats, polyarteritis nodosa (polyangiitis, panarteritis) is a well-described entity and the incidence varies among different strains. Grossly, the disease typically presents as nodular lesions affecting the pancreatic, mesenteric and/or spermatic arteries. Depending on the chronicity of the lesion, microscopic features include fibrinoid necrosis of muscular arteries, mixed inflammatory cell infiltrate of vessels and surrounding tissue, and fibrosis. Spontaneous arterial diseases are also described in dogs including idiopathic necrotizing polyarteritis in Beagle dogs (Beagle pain syndrome) and idiopathic extramural coronary arteritis. Drug administration can be

associated with both non-necrotizing (often hypersensitivity induced) and necrotizing inflammatory vascular lesions. Some examples of drug induced vascular injury include the mesenteric vasculitis in rats treated with phosphodiesterase III inhibitors and the coronary and systemic arteritis associated with endothelin A receptor antagonists in dogs and monkeys.

AFIP Diagnosis: Pancreas: Arteritis, histiocytic and lymphocytic, necrotizing, multifocal, with intimal fibromuscular proliferation, cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: As the contributor mentions, there is slide variability, with the full range of severity and duration of the polyarteritis not evident in all slides. Within all slides the pancreatic arteries are affected to varying degrees. Within some sections, islet capillaries are also affected and occasionally contain thrombi. The arteries in the peripancreatic adipose tissue of some slides are also affected.

In rats, the disease most frequently occurs in the Sprague-Dawley and spontaneous hypertensive rat (SHR) strains, and in rats with late-stage chronic nephropathy. Classically, at necropsy, vessels are enlarged and thickened in a segmental pattern, with marked tortuosity. The lesions occur most frequently in the pancreaticoduodenal artery and medium-sized arteries of the mesentery, pancreas, and testis. Histologically, there typically is fibrinoid degeneration and thickening of the tunica media, and infiltration by monocytes and fewer neutrophils. There is marked variation in the luminal size of affected vessels, which are often thrombosed, and occasionally recanalized.[5] These features and the intimal proliferation are best appreciated with appropriate special stains and immunohistochemistry (IHC). The modified Movat's pentachrome method is very helpful as it stains elastic laminae black, collagen and reticular fibers yellow, ground substance and mucin blue, fibrin intense red, and muscle fibers red.[6] With the Movat's pentachrome method, the quantity of intimal proliferation is readily apparent as are disruptions of the elastic laminae. Other stains and IHC, such as Masson's trichrome and smooth muscle actin aid in differentiating increased amounts of intimal connective tissue from smooth muscle hyperplasia. Immunohistochemistry for CD68 confirms that most of the infiltrating leukocytes are macrophages.

Contributor: Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245

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SLIDE 29
CONFERENCE 8 / CASE I – XN2922 (AFIP 2940469)

Signalment: Adult male, ferret (*Mustela putorius furo*).

History: An adult male ferret of unknown age was one of several housed in a council animal park open to the public. It developed progressively worsening dyspnea, with lethargy and mild abdominal distension, in early June 2004 and was euthanized.

Gross Pathology: The adult male ferret weighed 1120 g and had a length from the nose to the tail tip of 60 cm. The pleural cavity contained 50 ml of light brown, opaque fluid. The heart was enlarged and globular, with a cross-section of 25 mm by 20 mm at mid-ventricular level. At this level, the thickness of the left ventricular free wall was 4 to 5 mm, the thickness of the right ventricular free wall was 2 mm and the interventricular septum was 3 to 4 mm thick. The heart had patchy areas of pallor on the epicardium and there were irregular, locally extensive, white to pale yellow areas of myocardium in the inner third of the left ventricle. The right ventricle was affected to a lesser degree. The subendocardial myocardium and the bases of the papillary muscles were most severely affected. The lungs had patchy, red to purple atelectasis affecting 60% of the total lung volume. The abdomen was mildly distended and contained 5 ml of slightly yellow, clear, transparent fluid. The liver was enlarged, with rounded borders, an irregular, granular capsule and exudation of fibrin on the surface. The lobular pattern of the liver was accentuated, with red-purple mottling. The kidneys and spleen were congested.

Contributor's Morphologic Diagnosis: Heart: Myocardium, degeneration, necrosis, fibrosis, nonsuppurative inflammation, subendocardial and subepicardial, severe, extensive, consistent with dilatative cardiomyopathy, ferret (*Mustela putorius furo*).

Contributor's Comment: Histologically, the subendocardial myocardium of this ferret exhibits degeneration and necrosis of cardiomyocytes, with extensive replacement by fibrous connective tissue. The bases of papillary muscles and the inner third of the myocardium of the left ventricle, including the left ventricular free wall and the interventricular septum, are most severely affected. Subepicardial degeneration and fibrosis tend to have a perivascular distribution. Mild cardiomyocyte degeneration and fibrosis are also evident in the subendocardial myocardium of the right ventricle,

especially in the interventricular septum. There are mild, diffuse and locally extensive infiltrates of macrophages, lymphocytes and plasma cells, as well as occasional neutrophils, in areas of degeneration and fibrosis. Other tissues had evidence of congestive heart failure. Congestion, atelectasis and edema were evident in the lungs. There was congestion, periacinar fibrosis, mild hemosiderosis and atrophy of subcapsular hepatocyte cords in the liver, reflecting chronic hepatic venous congestion. The kidney was congested and hemosiderosis was evident in renal tubular epithelial cells. The adrenal and thyroid glands were unremarkable. The gross and histological findings are consistent with dilatative (dilated or congestive) cardiomyopathy of ferrets.[1-3]

Cardiomyopathy is the most common cause of heart failure in the ferret.[2-5] The usual form of this condition in ferrets is dilatative (dilated or congestive) cardiomyopathy, although hypertrophic and restrictive cardiomyopathies have also been described in this species.[2,5] Ferrets are most frequently affected from 5 to 7 years of age, although some severe cases can develop clinical signs as early as 1 year of age.[4,5]

Clinical signs of dilatative cardiomyopathy in ferrets include weight loss, weakness, lethargy, dyspnea, tachypnea, tachycardia, coughing and abdominal distension.[4,5] Heart and lung sounds are muffled on auscultation of the thorax and moist rales may be evident. A holosystolic murmur is usually most intense on the left side of the thorax at the level of the seventh to eighth intercostal space. Ascites and pleural effusions may be present. Hepatomegaly and splenomegaly may be evident on palpation of the abdomen. Diagnosis of cardiomyopathy in ferrets is usually based on clinical signs, radiography and echocardiography.[2-4] Ancillary diagnostic tests may include electrocardiography[2,6] and cytological examination of fluids obtained by thoracocentesis or abdominocentesis. Hematology and biochemical testing including endocrinology are useful for evaluation of concurrent disease.

The cause of cardiomyopathy in the ferret is unknown. A genetic basis has been suspected in some lines of ferrets in North America.[5] Cardiomyopathy has been associated with hyperadrenocorticism in ferrets.[7,8] In one study, 10% of ferrets with hyperadrenocorticism had concurrent cardiomyopathy.[8] A ferret with meningitis due to *Cryptococcus neoformans* had concurrent congestive cardiomyopathy.[9]

Treatment of cardiomyopathy in ferrets usually includes diuretics such as furosemide, vasodilators such as enalapril, positive inotropes such as digoxin and a low sodium diet.[2-4,10] The long term prognosis for ferrets with cardiomyopathy is poor. There is insufficient evidence to determine if supplementation with taurine or carnitine is beneficial in preventing or treating the disease.[2,11]

AFIP Diagnosis: Heart, myocardium: Degeneration, necrosis, and loss, with replacement fibrosis, multifocal, marked, ferret (*Mustela putorius furo*), mustelid.

Conference Comment: Cardiac disease is common in older ferrets and is usually due to dilated cardiomyopathy, hypertrophic cardiomyopathy or valvular disease. The contributor provides a thorough overview of dilated cardiomyopathy. Hypertrophic cardiomyopathy has not been extensively studied in ferrets. Unlike cats, there is no known association with hyperthyroidism or hypertension. Grossly the interventricular septum and left-ventricular free walls are abnormally thickened with decreased internal dimensions, and often an enlarged left atrium. Histologically, fibrous connective tissue is present throughout the myocardium. Valvular heart disease is reported with increasing frequency, with gross lesions consisting of abnormally thickened valves and dilated atria. Histologically, there is myxomatous degeneration of the valve as with dogs with endocardiosis. Other, less common causes of heart disease in ferrets include myocarditis, which may be due to Toxoplasma-like organisms, parvovirus (Aleutian mink disease), septicemia, and *Dirofilaria immitis* (heartworm disease).[2]

Cardiomyopathies have been reported in a number of other species including the cat, dog, pig, cow, hamster, turkey, mouse, and man and are classified as primary or secondary. Primary cardiomyopathies, those without a known etiology, are further subdivided into dilated, hypertrophic, and restrictive. Secondary cardiomyopathies are associated with known etiologies, such as viral myocarditis.[1]

Dilated cardiomyopathy is an important cause of congestive heart failure in dogs and cats. Often cats will have low tissue concentrations of taurine and supplementation with taurine has reversed the clinical signs of cardiac failure. Affected dogs are often males of large breeds, such as Doberman Pinschers, Irish Wolfhounds, and Newfoundlands. Grossly the heart is rounded due to biventricular dilatation, often with a diffusely white, thickened endocardium. Histological changes are non-specific, may be mild or absent, and include interstitial fibrosis and myocyte degeneration. Hypertrophic cardiomyopathy occurs frequently in middle-aged male cats. Animals often present with congestive heart failure and approximately 10-20% will have posterior paresis from a concurrent thromboembolism of the caudal abdominal aorta (“saddle thrombus”). Grossly the heart is enlarged with prominent hypertrophy of the left ventricle and interventricular septum, the left ventricular cavity is small, and the left atrium is dilated. Histologically there is prominent disarray of cardiac myocytes, with interweaving rather than parallel fibers, interstitial fibrosis, and myocyte degeneration. Restrictive cardiomyopathy occurs infrequently and includes such conditions as endocardial fibrosis in certain strains of rats and congenital endocardial fibroelastosis in Burmese cats.[12]

Causes of secondary cardiomyopathy include infectious, hereditable, nutritional, toxic, physical injuries, endocrine disorders, neoplasia, and systemic hypertension in cats. Some infectious agents include:[12]

<u>Viral</u>:

Canine parvovirus (canine parvovirus type 2)
Encephalomyocarditis (cardiovirus)
Foot-and-mouth disease (picornavirus)
Pseudorabies (porcine herpesvirus)

Canine distemper (canine morbillivirus)
Cytomegalovirus (porcine betaherpesvirus)
Newcastle disease (avian paramyxovirus)
Eastern and western equine encephalomyelitis (alphavirus)
West Nile Virus (flavivirus)

Bacterial:

Clostridium chauvoei (Blackleg), *C. piliforme* (Tyzzer's disease)
Listeria monocytogenes (Listeriosis)
Fusobacterium necrophorum (Necrobacillosis)
Mycobacterium spp. (Tuberculosis)
Corynebacterium pseudotuberculosis (Caseous lymphadenitis)
Actinobacillus equuli
Staphylococcus sp.
Streptococcus pneumonia

Parasitic:

Toxoplasma gondii
Sarcocystis sp.
Encephalitozoon cuniculi
Trypanosoma cruzi
Cysticercus cellulosae
Trichinella sp.

Contributor: University of Glasgow Veterinary School, Institute of Comparative Medicine, Division of Pathological Sciences, Glasgow G61 1QH, Scotland, United Kingdom
http://www.gla.ac.uk/faculties/vet/.

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SLIDE 30
CONFERENCE 8 / CASE II – 39080 (AFIP 2936322)

Signalment: 1.5 year old, male-neutered, Domestic Shorthair cat.

History: Cat exhibited lethargy and anorexia, and presented with a temperature of 105 degrees Fahrenheit and respiratory harshness. Feline Leukemia Virus and Feline Immunodeficiency Virus tests in clinic were negative. All in-house lab work was normal. Cat declined despite antibiotic treatment and IV fluids. Within the last 12 hours preceding death, it became very agitated and aggressive. Clinical diagnosis was unknown.

Gross Pathology: The cat presented in fair flesh and poor postmortem preservation. There was moderate dilatation of the colon, with a dark red serosa. The descending colon's content was dark red.

Laboratory Results: No clinical pathology data was available. Bacteriologic examination: Lung, intestine: Nonpathogenic bacteria were isolated. Liver: No growth was observed.

Contributor's Morphologic Diagnosis: Protozoal schizonts, intravascular, multisystemic, feline.

Contributor's Comment: Multifocal intravascular protozoal schizonts were in the brain, heart, lung, intestine, spleen and kidney. Abundant acidophilic material admixed with RBC was in the colon. Tapeworms were present in the duodenum.

Cytauxzoonosis is a rare disease. In the U.S. it is most common in free-roaming felines in the south and is usually, but not always, fatal. Diagnosis is made by finding the piroplasms of *Cytauxzoon felis* in blood smears (although parasitized erythrocytes are seen in low numbers, or are absent in 50% of cases) or by identification of schizonts in tissues. Cytauxzoonosis should be considered in animals with anemia, icterus and fever. Cytauxzoonosis is probably spread by arthropod vectors (ticks). Clinical signs include anorexia, conjunctival/scleral injection, constant or increased vocalization, dehydration, dullness, dyspnea, fever, generalized weakness, hepatosplenomegaly,

hypothermia, icterus, increased respiratory rate, lymphadenopathy, mucoid nasal discharge, pale mucous membranes, palpably enlarged kidneys, petechiae or ecchymoses, prolapsed third eyelid, red or brown urine, reluctance to move and tachycardia.

AFIP Diagnosis: Brain, cerebrum; lung: Intramonocytic protozoal schizonts, many, etiology consistent with *Cytauxzoon felis*, domestic shorthair, feline.

Conference Comment: *Cytauxzoon felis* is an apicomplexan intracellular organism in the family *Theileriidae* that usually causes fatal disease in domestic and exotic cats in the south central and southeastern United States. The natural reservoir is the North American bobcat (*Lynx rufus*), which typically has only a subclinical infection. Domestic cats are considered dead-end hosts because the disease is rapidly fatal. In experimental infections, transmission has been through ingestion or through inoculation of infected blood or tissue or via ticks which have previously fed on infected bobcats.[8]

Cytauxzoon felis has both a leukocytic phase and an erythrocytic phase. The leukocytic (tissue) phase begins when *C. felis* infects mononuclear cells or macrophages and undergoes asexual reproduction, producing schizonts. As the schizonts accumulate and mature, leukocytes enlarge up to 75 µm, often resulting in blood flow obstruction in the liver, lung, lymph nodes, spleen, and bone marrow, leading to severe circulatory impairment. These schizonts then bud, forming merozoites, which lead to further host cell engorgement and cell rupture. Once the merozoites rupture from the host cells, they infect erythrocytes, leading to the erythrocytic phase, which often results in hemolytic anemia. During the erythrocytic phase, the piroplasms are approximately 1-2 µm in diameter, and are ring shaped (signet-ring pattern), and can be identified on peripheral blood smears usually 1-3 days prior to death. Normally only 1-2% of erythrocytes will be affected; however, in moribund cats up to 25% of erythrocytes may be affected.[8]

Gross necropsy findings may include pallor, icterus, petechial and ecchymotic hemorrhages over the surface of the lungs and heart, excessive clear yellow fluid in the pericardial sac, enlarged dark spleen, prominent distended intra-abdominal veins, and swollen, edematous, hyperemic, and sometimes petechiated lymph nodes. The characteristic histologic lesion of cytauxzoonosis, as is present in this case, is the occurrence of numerous intravascular large monocytes with intracytoplasmic schizonts containing multiple small, basophilic, granular bodies (cytomeres) that represent merozoites in various stages of development. The infected monocytes may be found in association with the endothelial lining of venous channels and sinusoids in most major organs, but are usually more abundant in the lung, spleen, lymph nodes, and bone marrow.[5]

Contributor: Livestock Disease Diagnostic Center, University of Kentucky, 1429 Newtown Pike, P.O. Box 14125 Lexington, KY

http://fp1.ca.uky.edu/lddc/

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SLIDE 31
CONFERENCE 8 / CASE III – CB04-13 (AFIP 2947494)

Signalment: An approximately 37 year-old, sexually intact female rhesus macaque, (*Macaca mulatta*), non-human primate.

History: This macaque was singly housed indoors and tested for 19 years in a neurobiology study involving interactions between catecholamine receptors in the prefrontal cortex and cognitive function. Seven months prior to presentation, a diagnosis of severe spinal osteoarthritis was made. After a 5 month period of biweekly intramuscular injections of a polysulfated glycosaminoglycan (Adequan®) treatment was changed to one month trials of oral nonsteroidal anti-inflammatories, carprofen and deracoxib. Seroconversion to Simian retrovirus was detected 10 weeks prior to presentation. Clinical signs at presentation included a sudden onset of profound lethargy, weakness, hypoglycemia and hypothermia. Treatment with intravenous dextrose, as well as fluid and thermal support resulted in a dramatic rapid improvement. Due to a poor prognosis for extended use, the investigator elected euthanasia and perfusion 4 days later.

Gross Pathology: A 2 cm round, slightly red mass is present within the body of the pancreas adjacent to the duodenum. Within the dorsal aspect of the uterine body a 4 mm round, white, firm raised myometrial mass extended to the perimetrium (serosa). A

0.5 cm paraovarian cyst was present. The liver was rounded with three large foci of hepatic nodules. Scattered, 0.1-0.2 mm, translucent pleural foci were present throughout all lung lobes.

Laboratory Results: Serum biochemistry: results from a blood sample taken at presentation revealed marked hypoglycemia (9mg/dl; reference range 84-131mg/dl) and relative hyperinsulinemia (51.1 _IU/ml).
Electronmicroscopy: neoplastic cells contain numerous polymorphic granules containing a dense, rectangular, crystalline core separated from the limiting membrane by a distinct, wide halo. Granules are consistent with those found in beta cells.
Immunohistochemistry: neoplastic cells stained negatively for glucagon and somatostatin; were diffusely moderately positive for chromogranin A and rarely weakly positive for insulin.

Contributor's Morphologic Diagnosis: Pancreas: Islet cell (beta) tumor; insulinoma.

Contributor's Comment: Within the pancreas there is a 2 cm round, discrete, expansile mass compressing the adjacent normal pancreatic tissue. Arranged in variably sized (20 - 500 _m) disorganized nests and clusters, neoplastic cells are suspended in a highly vascular, fatty, loose connective tissue stroma. These cells are 10-20 _m in diameter, round to oval with indistinct cell boundaries. They contain predominantly one and occasionally two, 5 _m diameter, basally aligned, basophilic nuclei with one or more small, indistinct nucleoli. The cytoplasm is amphophilic, abundant and vacuolated. Less than one mitotic figure is seen per 40 power field.

The most frequently described spontaneous neoplasms in nonhuman primates involve those of the digestive system.[1] Although reports of pancreatic tumors are limited, the most commonly described neoplasms involve islet cell adenomas; few of which present with gross lesions.[2] Clinical signs indicative of pancreatic disease have not been seen in any instance of nonhuman primate pancreatic tumors. Neoplasms of the pancreatic islets have been observed in several species including cattle[3], ferrets[4], cats[5], and mice[6]. In dogs, beta cell tumors have been observed in many different breeds and are more frequently carcinomas exhibiting clinicopathological evidence of hyperinsulinism rather than adenomas.[3] In humans, insulinomas, are exceedingly rare (1-4 reported cases per million yearly), but constitute 70-80% of the clinically symptomatic endocrine pancreatic tumors. Greater than 80% of these are solitary masses and, unlike other endocrine neoplasms of the pancreas where malignancy predominates, insulinomas are benign in more than 85% of cases.

The usual histological criteria for malignancy (nuclear pleomorphism, mitotic activity, infiltration of surrounding tissues) are considered unreliable markers in endocrine pancreatic tumors. The accepted parameter for classification of malignancy is local or capsular extension to adjacent organs or a demonstration of vascular invasion and metastases.

Insulinoma cells contain less insulin than do normal beta cells, yet neoplastic tissue has a higher concentration of insulin and proinsulin than the surrounding normal pancreatic cells. The presence of higher serum proinsulin levels in human patients with insulinoma has been attributed to a decreased storage capacity leading to inappropriate insulin release. Such release at times of low to normal blood glucose levels leads to hypoglycemia and associated neuroglycopenic symptoms (e.g. mental dullness, weakness, abnormal behavior and seizures).

Immunohistochemistry for Chromogranin A, a well established marker for tissues of neuroendocrine origin, is useful in classifying pancreatic neoplasms. Islet cell tumors can stain positive for several immunohistochemical stains and are named according to the hormone responsible for producing clinical signs (insulin for insulinoma).

This rhesus macaque exhibited clinical signs of acute hypoglycemia and a response to appropriate therapy. Concomitant hypoglycemia and elevated blood levels of insulin confirmed hyperinsulinism. In addition, a distinct solitary nodule was present with histological, immunohistochemical, and ultrastructural characteristics consistent with an insulinoma.

AFIP Diagnosis: Pancreas: Islet cell tumor, Rhesus macaque, primate.

Conference Comment: The contributor provides a thorough overview of insulinomas in various animal species and humans. Pancreatic islets are composed of six distinct cell types, each with specific secretory products that are best distinguished by immunohistochemical techniques. The alpha cells, which produce glucagon, compose approximately 15% of cells in the islets and are usually located at the periphery, but may not be found in all islets. The beta cells, which produce insulin, compose approximately 70% of cells in the islets, and are distributed throughout all islets. The delta cells are present in all islets and have two subtypes; one produces somatostatin, the other produces vasoactive intestinal peptide. Both the gamma cells, which produce pancreatic polypeptide, and the enterochromaffin cells, which produce serotonin, are sparsely and variably distributed.

Most islet tumors comprise a variety of peptide-producing cells and not all islet tumors are associated with clinical manifestations of hormone excess. There is poor correlation between the immunohistochemical profile of islet tumors and clinical disease,[7] with the exception of the adenomas/carcinomas of the insulin secreting beta cells, which are frequently endocrinologically active and are associated with functional disturbances related to hypoglycemia.[8] Another noteworthy exception is the gastrinoma. Although islet cell tumors are rarely responsible for the production of polypeptides with gastrin activity, they have been described in dogs and are associated with the Zollinger-Ellison syndrome. Gastrin is normally produced in the gastric and duodenal mucosa, where it stimulates glandular secretion. However, pancreatic gastrinomas producing gastrin

result in hypergastrinemia, which leads to gastric hyperacidity, mucosal hyperplasia of the antral region, and gastric and duodenal ulceration (Zollinger-Ellison syndrome).[7]

Contributor: Yale University, Section of Comparative Medicine, P.O. Box 208016, New Haven, CT
http://info.med.yale.edu/compmed/compmed/index.htm

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SLIDE 32
CONFERENCE 8 / CASE IV – S 3019/03 (AFIP 2936461)

Signalment: 3 week-old, male, Merino sheep (*ovis aries*), ovine.

History: This newborn lamb was nourished with colostrum only several hours after birth. The lamb suffered from omphalitis and shortening of the flexor tendons of the forelimbs. The animal was referred to the Clinic of Pigs and Small Ruminants, School of Veterinary Medicine, at one day after birth. It developed severe respiratory disease and was euthanatized at 2.5 weeks of age because of poor prognosis.

Gross Pathology: The lungs failed to collapse and exhibited a generalized reddish color and a firm consistency. The cranial lobes and ventral portions of the caudal lobes showed lobular atelectatic areas. A focal fibrinous exudate was present on the pulmonary pleura. On the cut surface, moderate amounts of a grey viscous fluid were present extruding from the large airways. The bronchial and mediastinal lymph nodes were moderately enlarged. The forelimbs could not be extended completely. One omphalic artery showed subacute, purulent inflammation.

Laboratory Results: Bacteriologic culture of the lung yielded a low degree of non-hemolytic streptococci, *Bacteroides fragilis*, *Pseudomonas aeruginosa*, *enterococci* and *Escherichia coli*.

Contributor's Morphologic Diagnosis: Lung: 1. Bronchitis and bronchiolitis, proliferative and necrotizing, diffuse, subacute, marked, with large basophilic intranuclear inclusion bodies and cytomegalic cells, Merino sheep, ovine, etiology consistent with ovine adenovirus infection.
2. Bronchopneumonia, suppurative, multifocal, subacute, moderate.
3. Pneumonia, interstitial, lymphohistiocytic, multifocal, subacute, moderate.

Contributor's Comment: A large number of bronchiolar and alveolar epithelial cells in this lung section contained large basophilic intranuclear inclusion bodies measuring up to 50 µm in diameter often resulting in cytomegaly with marked enlargement of the cell. In some areas, bronchioles and alveolar spaces are filled with moderate amounts of neutrophils, cellular debris, and numerous sloughed epithelial cells, with few containing inclusion bodies. Hyperplasia of type II-pneumocytes and bi- or multinucleated syncytial cells were found in the alveolar spaces. A severe infiltration of lymphocytes and macrophages was observed in the interstitium. In addition, interstitial hemorrhage and epithelial necrosis appeared in areas, where inflammatory infiltration and sloughing of epithelium was most severe.

Electron microscopical examination revealed intranuclear virus particles arranged in a paracrystalline array consistent with adenovirus infection.

Adenovirions are naked icosahedral particles measuring 60-90 nm in diameter. The genome is a single linear molecule of double-stranded DNA of a molecular weight between 20 and 25 x 10^6.[1] A typical paracrystalline array of virus particles in the nucleus can be found by transmission electron microscopy.[2]

Ovine adenoviruses have been classified into 7 serotypes, six of them are included in the genus *Mastadenovirus* and one in the new genus *Atadenovirus*, both are members of the family Adenoviridae.[3]

After natural infection of adenoviruses via the oronasal route, primary virus replication occurs in the respiratory and intestinal epithelium. Lesions in other organs, like nasal mucosa, lymph nodes, spleen, kidney and liver are due to a viremia appearing as early as 4 days post infection (p.i.). The virus is shed in the feces, urine and nasal discharge, starting on day 2-3 p.i.. Direct contact between animals is the most important factor for virus spread. Permanent shedding of the virus by recovered animals may occur and contributes to endemics in large farms.[4]

The serotypes differ in virulence and tissue tropisms. In naturally occurring infections, mild respiratory and enteric disease or a subclinical course can be observed.[4,5] Infection of other organs rarely leads to clinic or gross pathologic changes. Histopathologically,

hyperplasia of lymphoid follicles in lymph nodes, lung and intestine, tubular degeneration in the kidney, and activation of the reticuloendothelial system in liver and spleen has been described. [4]

The respiratory effects are more severe in experimental intranasal or intratracheal infections. Lesions are characterized by cytomegaly, large intranuclear inclusions and necrosis of epithelial cells in the upper and lower respiratory tract. This is accompanied by early infiltration of neutrophils followed by accumulation of mononuclear cells.[6] Secondary infection with *Mannheimia haemolytica* may complicate signs and lesions.

In natural infections, severe lesions are most likely due to immunodeficiency, and are described in young animals which are deprived of colostral antibodies or which are exposed to environmental stress or other concurrent diseases.[5]

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, proliferative, subacute, diffuse, moderate, with multifocal airway epithelial cell and pneumocyte cytomegaly, syncytia, and large basophilic intranuclear inclusion bodies, Merino sheep, ovine.

Conference Comment: Conference attendees discussed differences among slides, with some slides containing plant material in the bronchioles and alveoli. All slides have moderate numbers of both syncytia and multinucleated giant cells, which are not typical of adenovirus pneumonia. The multinucleated giant cells, primarily of the Langhans' type, may be due to a secondary bacterial infection, which is not uncommon with adenoviral pneumonia. However, no organisms were identified with tissue Gram stains.

Adenoviruses have been isolated from most animal species and humans. They have differing virulences and tissue tropisms, but frequently cause respiratory and enteric disease. Severe naturally occurring disease is usually seen only in immunodeficient animals. The most prominent lesion of the pneumotropic strains is a necrotizing and proliferative bronchiolitis.[5]

The family *Adenoviridae* now contains four recognized genera. The genus *Mastadenovirus* contains most of the mammalian adenoviruses. The genus *Aviadenovirus* contains the group I avian adenoviruses. The genus *Siadenovirus* contains the group II avian adenoviruses and frog adenovirus. The genus *Atadenovirus* contains the group III avian adenovirus and several mammalian viruses.[7] Some recognized adenoviruses include the following:[3,7]

Virus	Disease
Mastadenovirus	
Canine adenovirus-1	Infectious canine hepatitis
Canine adenovirus-2	Infectious canine tracheobronchitis
Equine adenoviruses-1,2	Mild respiratory disease (except CID foals)
Bovine adenoviruses-1,2,3,9,10	Enzootic pneumonia (one of many agents)

Ovine adenoviruses-1-6	Mild respiratory and enteric disease
Goat adenoviruses-1,2	Mild respiratory and enteric disease
Porcine adenoviruses-1-5	Enteritis and encephalitis
Guinea pig adenovirus-1	Adenoviral pneumonitis
Mouse adenovirus-1,2	Enteritis and encephalitis
Simian adenoviruses-1-25	Mild respiratory and enteric disease
Human adenoviruses-1-51	

Aviadenovirus

Fowl adenoviruses-1-11	
Fowl adenovirus-1	Inclusion body hepatitis (chickens)
Fowl adenovirus-4	Hydropericardium syndrome (chickens)
Goose adenoviruses-1-3	

Siadenovirus

Frog adenovirus-1	
Turkey adenovirus-3	Hemorrhagic enteritis (turkeys)
Pheasant adenovirus-1	Marble spleen disease (pheasants)

Atadenovirus

Ovine adenovirus-7	Mild respiratory and enteric disease
Bovine adenoviruses-4-8	Enzootic pneumonia (one of many agents)
Black tail deer adenovirus-1	Pulmonary edema, hemorrhage, vasculitis
Duck adenovirus-1	Egg drop syndrome (chickens)

Contributor: Hannover School of Veterinary Medicine, Department of Pathology, Bünteweg 17, D-30559 Hannover, Germany

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SLIDE 33
CONFERENCE 9 / CASE I – 03B5515 (AFIP 2941535)

Signalment: An adult American alligator (*Alligator mississippiensis*).

History: Between September and December of 2003, American alligators from several alligator farms in Louisiana died suddenly or showed various neurological signs including circling, ataxia, head and muscle tremor, and head tilt.

Gross Pathology: With the exception of gas-filled, empty gastrointestinal tracts and a diffuse thin pseudo-membrane on the colonic mucosa, no significant gross findings were noted.

Laboratory Results: All brain specimens taken from 13 alligators that showed the clinical signs were positive for West Nile virus (WNV) by RT-PCR. Viral isolation was successful from 10 out of the 13 brain specimens. Real-time PCR revealed a high viral load in a fecal sample. Immunohistochemistry for WNV showed strong immunoreactivity on the sections of brain, liver, pancreas, and small and large intestines.

Contributor's Morphologic Diagnosis: Colon: Colitis, heterophilic and granulomatous, multifocal, acute, moderate.

Contributor's Comment: The colonic mucosa is multifocally attenuated and fused with irregular paucity of colonic glands. The lamina propria is expanded with infiltration of moderate numbers of lymphocytes, plasma cells, and macrophages as well as heterophils. The inflammation often extends to the submucosa. The individual glandular epithelial cells are occasionally necrotic with pyknosis. Heterophilic exocytosis is evident. Some colonic glands are dilated with mucus and exfoliated degenerate epithelial cells. The submucosal lymphoid follicles are hyperplastic with heterophilic infiltration. There is a luminal core composed of a large amount of mucus admixed with exfoliated degenerate epithelial cells, heterophils, various bacteria, and a small amount of fibrin. Some tissue sections contain severe heterophilic infiltration surrounding bacterial colonies in the submucosal lymphoid follicle.

Other major microscopic findings were heterophilic meningoencephalitis, necrotizing and heterophilic hepatitis, heterophilic and histiocytic splenitis, and necrotizing, heterophilic pancreatitis, and generalized heterophilic lymphoid folliculitis.

Since it first emerged in 1999, West Nile virus (WNV) infection has been established as a seasonal epidemic in North America. WNV generally circulates between mosquitoes and birds. The infected birds commonly have a high level viremia and serve as reservoir hosts. WNV infection has been reported in various species but primarily in

warm-blooded animals. Recently, epizootic WNV infections in American alligators have been described from the states of Florida (2002) and Georgia (2003). Although the role of alligators in the transmission cycle of WNV infection is still largely unknown, there are concerns that these reptiles may be important regionally because they can develop high viremia and shed the virus in their feces. Immunohistochemistry for WNV confirmed that the viral antigens were located within the macrophages, intravascular monocytes, and intestinal epithelial cells in the colon. It is believed to be that alligators are initially infected by mosquito bites on the oral mucosa and then epizootics occur rapidly by orofecal transmission. In this case, the virus was detected from a fecal sample. In the report of the cases from Florida, the authors speculated that WNV in the horsemeat fed to the alligators might be the direct source of the infection among the alligators. However, it is considered unlikely because horses are the dead-end hosts of WNV infection with low numbers of viruses in the body after a short period of viremia. There have been 3 human cases of WNV infection, among workers on an alligator farm in Louisiana.

AFIP Diagnosis: Colon: Colitis, histiocytic and heterophilic, subacute, diffuse, moderate, American alligator (*Alligator mississippiensis*), reptile.

Conference Comment: West Nile virus (WNV) was first isolated from a woman in 1937 in the West Nile district of Uganda. Since then it has been reported in western Asia, the Middle East, Europe, southern Russia, and in 1999 in the United States. WNV is a member of the genus *Flavivirus*, family Flaviviridae, and is known to cause encephalitis in a wide variety of species, including humans, birds, horses, other mammals and reptiles.[3] Natural infections have been reported in bats, a chipmunk, a skunk, a domestic rabbit, reindeer[5] and several species of squirrels.[4] Mice and rhesus monkeys have been infected experimentally. Interestingly, dogs, rabbits, guinea pigs, hedgehogs, and sheep do not develop encephalitis after experimental inoculation with WNV.[5]

As mentioned by the contributor, mosquito vectors transmit the virus among reservoir bird populations, and susceptible mammalian species are infected incidentally. A wide variety of native and exotic birds are susceptible, although infection typically does not cause clinical signs in most birds. However, it does result in unusually high mortality in crows. Gross lesions in birds include meningeal hemorrhage, multifocal pale myocardial foci, splenomegaly, mucosal hemorrhage in the small intestine, and white foci in the kidneys. In birds, the most severe histological lesion is hemorrhage in the cerebellar folia, with degeneration and necrosis of the cerebellar molecular layer and Purkinje cells.[4] Other lesions include lymphoplasmacytic meningoencephalitis, necrotizing myocarditis, and lymphoplasmacytic enterocolitis. Subacute inflammation may be seen in the spleen, kidneys, liver, adrenal glands, or pancreas.[6] In horses, WNV exhibits a pronounced, if not exclusive, CNS tropism. As with WNV infection in humans and squirrels, the brainstem is the most severely affected area in horses.[4] Horses typically have polioencephalomyelitis, with prevalent involvement of the lower

brain stem and ventral horns of the thoracolumbar spinal cord.[7] WNV infections in humans are usually mild, with affected individuals exhibiting non-specific flu-like symptoms. Less than 15% of infected humans develop more severe forms of the disease, such as meningoencephalitis, hepatitis, pancreatitis, or myocarditis. Fatalities are more common in humans over the age of 50 and often a result of severe central nervous system disease.[8]

Contributor: Louisiana State University, School of Veterinary Medicine, Department of Pathobiological Sciences, Baton Rouge, LA.

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SLIDE 34
CONFERENCE 9 / CASE II – SA787-04 (AFIP 2937490)

Signalment: Twenty-day-old Ross broiler chicken (*Gallus domesticus*).

History: A large number (exact number unknown) of chickens died with symptoms of paralysis and incoordination. Parent flock young (less than 30 months old).

Gross Pathology: Negative, except for dehydration and nephrosis.

Laboratory Results: Serum from the affected chicks as well as from the parent flock tested negative for the presence of antibodies against Avian Encephalomyelitis virus (AEV) using the Enzyme Linked Immunosorbent Assay (ELISA) test.

Contributor's Morphologic Diagnoses: Multiple tissues presented for examination, including cerebrum, cerebellum, medulla oblongata (some sections), proventriculus, ventriculus (some sections) and pancreas.
1. Brain: Encephalitis, lymphocytic, subacute, multifocal to coalescing, severe with neuronal degeneration and necrosis, gliosis and perivascular lymphocytic infiltration. Neuronal degeneration and necrosis are characterized by chromatolysis and eosinophilic discoloration of some nuclei, which was particularly severe in the Purkinje cells of the cerebellum.
2. Proventriculus and ventriculus (some sections): Lymphocytic infiltration, multifocal, mucosal, submucosal and intermuscular, mild to moderate.
3. Pancreas: Lymphocytic infiltration, interstitial, multifocal, mild to moderate.

Contributor's Comment: The history of a high morbidity and a high mortality in young 20-day-old chickens, with neurological signs of paralysis and incoordination as well as severe neural lesions characterized by lymphocytic encephalitis with marked neuronal degeneration and necrosis, are consistent with the lesions described in the central nervous system of birds due to avian encephalomyelitis virus (AEV), a Picornavirus. The lymphocytic infiltrations in the proventriculus, ventriculus and pancreas provide further support for the diagnosis.[1]

The high mortality and severity of the lesions was probably the result of a high susceptibility in the affected chicks and their parents as supported by the negative ELISA test results.[1]

The most sensitive method, and the method of choice, for the detection of AEV appears to be the inoculation of serologically negative chickens at 2 weeks of age with infected brain tissue culture cells. These chickens should be observed for typical symptoms and positive serological reactions with the ELISA or immunodiffusion (ID) tests. In addition, brain, proventriculus and pancreas can be examined by indirect immunofluorescence and for the presence of typical histopathological changes.[1]

Picornavirus particles in crystalline array of 22-25 nm in diameter have been identified within the cytoplasm of Purkinje cells from infected chickens.[2,3] The AEV, a single-stranded RNA virus, has the capacity to induce nuclear DNA fragmentation with apoptosis in embryonal nerve cells through its structural VP3 –and non-structural 2C proteins.[4,5]

Avian encephalomyelitis (AE) should be differentiated from a number of diseases with similar clinical signs in young chicks such as Newcastle disease, equine encephalomyelitis virus infection, as well as from certain nutritional imbalances such as rickets, encephalomalacia and riboflavin deficiency. Newcastle disease in 1-3 week-old chicks can be distinguished from AE by peripheral chromatolysis in neurons; in contrast, AE results in central chromatolysis within neurons and multifocal infiltrations of lymphocytes within the proventriculus and the pancreas.[1] Rickets, encephalomalacia

and riboflavin deficiency cause very dissimilar lesions which cannot be confused with AE.[1]

Although AE occurs almost worldwide, the virus has a limited host range, and the incidence is very low due to vaccination and relatively high levels of immunity in commercial chicken and turkey production systems. Fatal natural infections have been reported in pheasants and quail[1] and also recently in pigeons in Turkey with typical symptoms and lesions.[6] Experimental infections of guinea fowl and ducklings have also been reported.[1]

Avian encephalomyelitis virus is most closely related to the human hepatitis A virus.[7] All isolates of AEV are serologically similar, but two distinct pathotypes of the virus exist. The natural field strains are enterotropic, and infection occurs via the oral route with fecal excretion of the virus. These strains have a low pathogenicity and only cause neurological signs in vertical or horizontal infection of susceptible chicks at a young age. Embryo-adapted strains represent the second group of pathotypes which are highly neurotropic by intracerebral inoculation and parenteral infection. These require high doses for oral infection and do not spread horizontally.[1]

AFIP Diagnoses: 1. Brain: Encephalitis, lymphoplasmacytic, multifocal, mild, with gliosis, and neuronal degeneration and necrosis, domestic chicken, avian.
2. Pancreas: Lymphoid infiltrates, multifocal, moderate.

Conference Comment: Avian encephalomyelitis virus (AEV) is a non-enveloped icosahedral single-stranded RNA virus of the Picornaviridae family and is pathogenic to young chickens, pheasants, quails, and turkeys, resulting in reduced hatching, ataxia, and tremors in 1-7 day old chicks.[5] Transmission may be vertical, fecal-oral, or via fomites. The only gross lesions associated with avian encephalomyelitis (AE) are areas of pallor in the tunica muscularis of the ventriculus. Histological lesions occur in the central nervous system (not the peripheral nervous system) and some viscera. Lesions in the CNS include diffuse nonsuppurative encephalomyelitis, Purkinje cell degeneration and gliosis in the molecular layer, and central chromatolysis, especially of large neurons in the midbrain. Dense aggregates of lymphocytes in the muscular wall of the proventriculus are pathognomic.[1] Similar lesions occur in the ventriculus muscle, myocardium, and pancreas. Although lymphoid nodules are normally found in the pancreas, in animals with AE, these nodules will be increased in number two to three fold.[1] Some common Picornaviruses include the following:[8]

Virus	Primary species affected	Disease
Aphthovirus		
FMD viruses	Ruminants, swine	Foot-and-mouth disease
Enterovirus		

Swine vesicular disease virus	Swine	Swine vesicular disease
Porcine enterovirus-1	Swine	Polioencephalomyelitis
Avian enteroviruses	Chickens	Avian encephalomyelitis
	Ducks , turkeys	Hepatitis
Coxsackieviruses	Humans	Aseptic meningitis, myocarditis, poliomyelitis

Cardiovirus

Encephalomyocarditis virus	Swine, elephants	Encephalomyocarditis
Theiler's murine encephalomyelitis virus	Mice	Murine polioencephalomyelitis

Rhinovirus

Bovine rhinovirus	Cattle	Mild rhinitis
Human rhinovirus	Humans	Common cold

Hepatovirus

Simian hepatitis A virus	Monkeys	Hepatitis
Human hepatitis A virus	Humans	Hepatitis

Contributor: Pathology Section, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Priv. Bag X4, Onderstepoort 0110, Republic of South Africa.
http://www.up.ac.za/academic/veterinary

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SLIDE 35
CONFERENCE 9 / CASE III – PA 4121 (AFIP 2943312)

Signalment: 8 year old, male castrate Pug dog.

History: This dog presented with a progressive history of weight loss and poor doing. Diarrhea developed later in the clinical course. Despite aggressive clinical supportive measures, the animal continued to deteriorate and was euthanized.

Gross Pathology: The kidneys were bilaterally enlarged, pale, firm and somewhat gritty on cut surface. No additional gross findings were specified.

Laboratory Results: Proteinuria was reported without additional specification/quantification.

Contributor's Morphologic Diagnoses: 1. Glomerular amyloidosis, global and diffuse, marked, with additional mild, patchy, interstitial (peri-tubular) amyloid deposits.
2. Tubular proteinosis, patchy to diffuse, moderate.
3. Interstitial nephritis, lymphoplasmacytic, patchy, mild.
4. Interstitial fibrosis, patchy, mild-moderate.
5. Tubular mineralization, multifocal, mild-moderate.
6. Vascular thrombosis, multifocal (some sections).

Contributor's Comment: The pale, amorphous, eosinophilic material effacing the architecture of most glomeruli was confirmed as amyloid via strong positivity under fluorescence with Thioflavin T staining.

Amyloidosis is well described in the canine. Although familial renal amyloidosis is reported in several breeds (Shar Pei, Beagle), concurrent neoplasia or chronic inflammatory disease is detected in over half of affected animals. Dogs often present with progressive protein-losing nephropathy and frequently demonstrate the nephrotic syndrome, including ascites, peripheral edema and hypercholesterolemia. A thromboembolic phenomenon is seen in up to 40% of affected dogs, and this lesion is seen in some of the submitted sections. Amyloid was not noted in a limited number of other organs submitted with this case, nor was an underlying chronic inflammatory or neoplastic disorder recognized.

The primary clinical differentials in dogs that present with renal associated protein loss include primary congenital glomerulopathy, described in the Samoyed, Bull Terrier and English Cocker Spaniel, immune-mediated glomerulonephritis, also known to have breed predilection, including Bernese Mountain Dogs and Soft-coated Wheaten Terriers and occasional functional renal tubular transport disorders.

The prognosis for dogs with renal amyloidosis is quite guarded, and most dogs die or are euthanized shortly after diagnosis.

AFIP Diagnoses: 1. Kidney: Amyloidosis, glomerular, diffuse, global, severe and interstitial, multifocal, mild, with tubular proteinosis, Pug, canine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, moderate.
3. Kidney, large pelvic vein: Thrombus, organizing, with mineralization.

Conference Comment: Amyloid, an insoluble fibrillar protein with a beta-pleated sheet conformation, is deposited between cells in various tissues. With progressive accumulation it encroaches on and produces pressure atrophy of adjacent cells. On standard tissue sections stained with H&E, amyloid appears as an amorphous, eosinophilic, hyaline, extracellular substance. It is congophilic, with apple green birefringence when polarized.[5]

There are three main types of amyloid protein: amyloid light chain (AL); amyloid-associated (AA); and beta-amyloid (Aß). AL protein is derived from plasma cells and contains immunoglobulin light chains, which may be complete immunoglobulin light chains, the NH_2-terminal fragments, or both. It is commonly seen in association with immunocyte dyscrasias, particularly multiple myeloma. AA protein is derived from serum amyloid-associated (SAA) protein that is synthesized in the liver, and is associated with chronic inflammatory conditions. Aß amyloid is found in the cerebral plaques of human patients with Alzheimer disease. Amyloidosis, a heterogeneous group of disease processes that result in the deposition of amyloid, may be classified as localized or systemic (generalized). Examples of localized amyloidosis include cerebral plaques in Alzheimer disease, islet associated amyloid polypeptide (IAPP) with type II diabetes, and some prion diseases. Systemic or generalized amyloidosis can be further classified as primary or secondary. Primary amyloidosis is associated with immunocyte dyscrasias, while secondary amyloidosis occurs as a complication of an underlying chronic inflammatory disease. [5]

Regardless of the inciting cause or type of amyloid, amyloidosis results from the abnormal folding of proteins, which are deposited as fibrils in extracellular tissue and disrupt normal tissue function. In this case, glomerular filtration was almost certainly severely compromised and resulted in proteinuria. Glomerular amyloid deposition initially causes selective loss of albumin, but as the disease progresses, large protein molecules (globulins) also may be lost. If protein loss is severe, then hypoproteinemia and edema develop.[6] As a result of the protein-losing nephropathy, animals can become deficient in antithrombin III, resulting in an increased tendency for thrombosis,[7] which may have been the cause of the thrombus formation in this case.

Contributor: University of Pittsburgh, Division of Laboratory Animal Resources, S-1040 BioMedical Science Tower, 3500 Terrace Street, Pittsburgh, PA
http://www.pitt.edu/

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SLIDE 36
CONFERENCE 9 / CASE IV – KL-8 M3 (AFIP 2936433)

Signalment: 2-year-old, male North American opossum (*Didelphis virginiana*).

History: Wild opossum rescued by a rehabilitation program. The opossum presented with dyspnea and pleural and abdominal effusions. He died post-thoracocentesis.

Gross Pathology: Heart, lungs, adrenal gland and portions of kidney and liver were submitted in 10% neutral buffered formalin. Gross lesions were restricted to the lungs and liver. All lung lobes were collapsed, firm and rubbery. On cut section, there were multifocal to coalescing, pale tan to reddish-brown, 3 mm diameter, perivascular and peribronchial nodules. On cut sections of the liver, an enhanced reticular pattern was apparent, somewhat resembling a nutmeg liver.

Contributor's Morphologic Diagnosis: Lung, granulomatous pneumonia and catarrhal bronchitis, chronic, multifocal, marked with intralesional *Didelphostrongylus hayesi*, pulmonary smooth muscle hyperplasia and widespread atelectasis.

Contributor's Comment: The majority of the pulmonary parenchyma is atelectatic. Within alveoli, bronchioles and bronchi are numerous transverse and longitudinal sections of nematodes characterized by a cuticle, coelom, gastrointestinal tract with intraepithelial black pigment, and reproductive tract with ova, sperm and developing larvae. Associated with scattered free larvae in alveolar spaces are variable numbers of multinucleated giant cells, lymphocytes, plasma cells and fewer eosinophils. Numerous

bronchial lumina contain large amounts of mucus admixed with free larvae. There are increased goblet cells distended with mucus lining most bronchial lumina. These bronchi are also associated with increased peribronchial mucous glands and prominent lymphoid nodules. Marked smooth muscle hyperplasia involving terminal bronchioles, alveolar ducts and less frequently, arterioles is apparent. Interstitial spaces around blood vessels are distended by proteinaceous fluid (edema). There are multiple foci of heterotopic bone in the pulmonary parenchyma, and mesothelial hyperplasia is present on all pleural surfaces. Some sections contain subpleural aggregates of lipid-laden macrophages with acicular (cholesterol) clefts. Additional histologic findings included chronic, multifocal, marked centrilobular hepatocellular degeneration and coagulation necrosis with fibrosis.

Didelphostrongylus hayesi, named in honor of Professor Frank A. Hayes who made great contributions to the study of North American wildlife diseases, is a common metastrongyloid lungworm in opossums.[1] In one retrospective study, pulmonary parasites were documented in 18/27 (67%) opossum necropsies with 13/18 (72%) being *D. hayesi*.[2] In another, *D. hayesi* larvae were found in 13/20 (65%) resident and 10/13 (77%) newly arrived opossums in an opossum care program.[3] Finally,

D. hayesi was documented in 11/20 (55%) opossum necropsies, and contributed to the cause of death in 8/11 (73%) of these cases.[4] The parasite has an indirect lifecycle similar to other metastrongyloid nematodes, with the intermediate hosts being the terrestrial snails *Triodopsis albolabris* and *Mesodon perigraptus*.[1]

Histopathologic findings reported with *D. hayesi* infection are as seen in this case.[2-6] Pulmonary inflammation is associated with free larvae rather than intact adult nematodes. A feature of *D. hayesi* infected lungs is bronchiolar and alveolar ductular smooth muscle hyperplasia, which is reminiscent of the marked pulmonary arteriolar smooth muscle hyperplasia seen in *Aelurostrongylus abstrusus* infected cats. The hepatic lesions in this opossum and reported in others were consistent with right-sided heart failure secondary to verminous pneumonia-induced pulmonary hypertension.[4] Foci of heterotopic bone have also been reported in the lungs of unparasitized opossums and are considered to be incidental findings.[5]

Although a complete blood cell count and differential were not performed in this case, opossums with *D. hayesi* display a regenerative erythrogram characterized by nucleated red blood cells and polychromasia.[4,6] Similar findings are reported in cats with *A. abstrusus*. However, it is unknown whether this is due to hypoxia or a direct stimulus from the lungworms.[6] In spite of the heavy parasitic burden in these opossums, a peripheral eosinophilia has not been reported.[4]

Evidence of endogenous lipid pneumonia was present in some slides. This lesion was present in 19/27 (70%) opossums from Louisiana, with 13/19 (68%) of these animals having pulmonary parasites.[2] In addition to *D. hayesi*, the pulmonary parasites included *Capillaria* sp. and *Besnoitia darlingi*. It is hypothesized that these parasites induce

pulmonary irritation which results in type II pneumocyte hyperplasia and surfactant overproduction with subsequent accumulation in alveolar macrophages.

AFIP Diagnosis: Lung: Bronchopneumonia, histiocytic, multifocal, mild, with many adult and larval metastrongyles, bronchiolar mucus cell hyperplasia with abundant catarrhal exudate, atelectasis, and bronchiolar and arteriolar smooth muscle hypertrophy, North American opossum (*Didelphis virginiana*), marsupial.

Conference Comment: The contributor provides a thorough overview of *Didelphostrongylus hayesi* infection in opossum. Conference attendees discussed lung parasites in other species as listed below:[7]

Species/Parasite	Location	Lesion/Comment
Canine		
Filaroides osleri	Tracheal bifurcation	Luminal nodules
Filaroides hirthi	Bronchioles, alveoli	Subpleural nodules
Paragonimus kellicotti	Bronchioles	Subpleural nodules
Angiostrongylus vasorum	Vasculature	Chronic arteritis
Dirofilaria immitis	Vasculature	Chronic arteritis
Crenosoma vulpis	Sm. bronchi/bronchioles	Catarrhal bronchitis
Feline		
Aelurostrongylus abstrusus	Bronchioles, alveoli	Subpleural nodules
Paragonimus kellicotti	Bronchioles	Subpleural nodules
Dirofilaria immitis	Vasculature	Chronic arteritis
Syngamus laryngeus	Larynx	"gapeworm"
Equine		
Dictyocaulus arnfieldi	Bronchi	Eosinophilic bronchitis
Parascaris equorum	Interstitium	Migrating larval stages
Bovine		
Syngamus laryngeus	Larynx	Asia and S. America
Dictyocaulus viviparous	Intrapulmonary bronchi	Bronchitis, BALT hyperplasia, edema
Ascaris suum	Bronchioles, alveoli	Interstitial pneumonia
Ovine/caprine		
Dictyocaulus filaria	Small bronchi	Catarrhal bronchitis; BALT hyperplasia

Muellerius capillaries	Subpleural alveoli	Pulmonary nodules
Protostrongylus rufescens	Bronchioles	Pulmonary nodules

Porcine

Metastrongylus apri	Bronchioles	Catarrhal bronchitis
Ascaris suum	Subpleural alveoli	Subpleural hemorrhage

Contributor: Weill Medical College of Cornell University / Memorial Sloan-Kettering Cancer Center / Rockefeller University, 1300 York Avenue, Box 40, RARC, Room C-701, New York, NY
http://www.mskcc.org; http://www.med.cornell.edu/; http://www.rockefeller.edu

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SLIDE 37
CONFERENCE 10 / CASE I – 03-1893 (AFIP 2948659)

Signalment: 6-7 month gestation, female, Holstein, bovine fetus.

History: Normal pregnancy. Cow was euthanatized because of luxated proximal left femur.

Gross Pathology: None in the fetus.

Contributor's Morphologic Diagnosis: Normal plexiform cortical bone, radius and ulna.

Contributor's Comment: Normal appositional growth (widening) of cortical bone can be circumferential lamellar, simple primary osteonal, plexiform, and saltatory.[1] Laminar bone is composed of lamellae deposited circumferentially in layers parallel to the convex surface of the cortex. Primary osteonal bone consists of anastomosing vascular Haversian canals surrounded by concentric lamellae forming Haversian systems. Plexiform bone is formed by multiple relatively widely spaced lamina of periosteal woven. The spaces between these woven laminae subsequently fill in (compact) with lamellar bone. In the horse, the compaction can form osteons (saltatory formation) with the orientation of the osteon and its vessels being perpendicular to the long axis of the bone.[1] In calves, the compaction (and blood vessels) are oriented parallel with the convex surface of the bone[2] without formation of osteons. Many species exhibit multiple patterns of primary periosteal cortical bone, dependent upon regional rate of growth, pattern of vascularization, and functional requirements.[3,4]

AFIP Diagnosis: Bone, radius and ulna (per contributor): Normal fetal bone, Holstein, bovine.

Conference Comment: There are structural variations in the microscopic organization of bone tissue in different animal species depending on the growth and remodeling processes for that species. Bones undergo change in size and shape as well as remodeling of the internal architecture during normal growth and due to changes in functional stresses throughout life. These processes result in bone deposition, resorption, and remodeling, which ultimately affect the amount of bone, the amount of mineralization, and type of bone tissue present within a given bone.[1]

Fetal bone development occurs in two ways, both of which involve the replacement of primitive collagenous supporting tissue by bone. The long bones, vertebrae, pelvis, and bones of the base of the skull are formed through a continuously growing cartilage model that is progressively replaced by bone (endochondral ossification). The bones of the vault of the skull, the maxilla and most of the mandible are formed by the deposition of bone within primitive mesenchymal tissue (intramembranous ossification). Regardless of the method of ossification, initially the bone that is formed is immature, or woven bone. The developing bone is then extensively remodeled by resorption and appositional growth to form mature, or lamellar bone.[5]

Contributor: The Ohio State University, College of Veterinary Medicine, Department of Veterinary Biosciences, 1925 Coffey Rd., Columbus, Ohio
http://www.vet.ohio-state.edu/docs/biosci/index.html

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SLIDE 38
CONFERENCE 10 / CASE II – X7764-6 or X7764-7 (AFIP 2946673)

Signalment: A juvenile female Black-crowned night heron (*Nycticorax nycticorax*).

History: Found dead. This was an individual among a wild flock that nests around the bird house at the National Zoo.

Gross Pathology: Poor nutritional condition; hepatomegaly with miliary hepatitis; splenomegaly; parasitism, roundworm, ventriculus and small intestine; arthritis, tibiotarsal-metatarsal joint, chronic.

Laboratory Results:
West Nile virus PCR – negative
Liver culture – *Salmonella typhimurium*
Liver cytology – Marked, subacute inflammation with intracellular, gram-negative coccobacilli
Heart blood culture – coagulase-negative *Staphylococcus* sp.

Contributor's Morphologic Diagnosis: Bone, tibiotarsus: Osteomyelitis, granulomatous, with trabecular resorption, periosteal new bone formation, marrow cavity bone and cartilage sequestrum, and intralesional coccobacilli, Black-crowned night heron (*Nycticorax nycticorax*), Ciconiiformes.

Contributor's Comment: Salmonellosis is a worldwide disease affecting a wide range of species from humans to birds to reptiles to insects. This bacterium used to be employed in the control of rodents, but the practice was banned in the US when the public health risk was realized. Clinical presentations most commonly linked to *Salmonella* include enteritis, colitis, abortion, and septicemia. Additionally, carrier states can be established with *Salmonella* sp. in a non-clinical or subclinical host. In our experience, infection in these hosts cannot be eliminated through antibiotic administration, and may only serve to create resistant strains of the bacterium.

In birds, salmonellosis is usually fatal in the animal's first 2-3 weeks of life. Older birds may survive the infection to become carriers of the pathogen. The two most common

Salmonella diseases in domestic fowl are Pullorum Disease and Fowl Typhoid, caused by *S. pullorum* and *S. gallinarum* respectively. In either disease, young birds may have splenomegaly, hepatomegaly, and caseous material within the yolk sac. Joints, especially the hock, may become enlarged and filled with viscous, yellow fluid. A common gross lesion in older, female birds is misshapen, firm, and discolored ovarian follicles. Pericarditis and hydropericardium may also be seen. Microscopically, inflammation is initially heterophilic and lymphocytic, and progresses to necrotizing and granulomatous lesions in target organs.
Wild waterfowl are less susceptible to *Salmonella*-induced disease. Pullorum Disease and Fowl Typhoid are only rarely reported in waterfowl. The most common *Salmonella* strain isolated from wild waterfowl is *S. typhimurium*, the strain isolated from this heron. In this case, infection was of utmost concern, as potential shedders of the organism live in and around the wetlands exhibit and defecate in close proximity to collection animals and the public.

AFIP Diagnoses: 1. Bone, tibiotarsus (per contributor): Osteomyelitis, granulomatous and heterophilic, multifocal, marked, with medullary bone and cartilage sequestrum, trabecular resorption, periosteal new bone formation, and colonies of coccobacilli, black-crowned night heron (*Nycticorax nycticorax*), avian.
2. Tendons, leg: Tenosynovitis, chronic-active, proliferative, multifocal, minimal to moderate.

Conference Comment: There are two species of *Salmonella*: *S. enterica*, which is very common and comprised of over 2000 serotypes; and *S. bongori*, comprised of 10 serotypes, all of which are rare. The Kauffmann-White classification system for serotypes is based on differences among somatic (O), capsular (Vi), and flagellar (H) antigens. Each serotype is named by where it was first isolated or by the clinical syndrome it produces in a particular host. In conventional terminology, the serotypes are treated as species. Based on host specificity, serotypes can be divided into two groups: those that are highly adapted to a specific host species, and those that affect a wide range of species. Most serotypes fall into the latter category, although there can be marked differences in virulence of a serotype in various hosts.[3]

Some common and important animal diseases caused by *Salmonella* sp. include the following:[4,5]

Species	***Salmonella* sp.**	**Disease/Lesions**
Porcine	*S. choleraesuis*	Septemia (piglets) (cyanosis of the skin, turkey-egg kidney); necroulcerative

		enterocolitis (button ulcers); hepatic paratyphoid nodules
	S. typhimurium	Enterocolitis; rectal stricture
	S. typhisuis	Ulcerative enterocolitis; caseous tonsillitis and lymphadenitis
Equine	*S. typhimurium*	Septemia (foals); enterocolitis (older horses)
	S. abortus-equi	Abortions (6-9 months of gestation); orchitis
Bovine	*S. dublin*	Fibrinous cholecystitis
	S. typhimurium	Septemia (calves); fibrinonecrotic enteritis and necrosis of the Peyer's patches
Ovine	*S. abortus-ovis*	Abortion
	S. typhimurium	Septemia (lambs); fibrinonecrotic enteritis
	S. dublin	Fibrinous cholecystitis
	S. enteritidis	Enterocolitis
Avian	*S. typhimurium*	Septemia
	S. pullorum	Pullorum disease: necrotizing typhilitis and necrotic foci in the liver, lung, myocardium, gizzard (chicks); oophoritis (adults)
	S. gallinarum	Fowl-typhoid: catarrhal enteritis and necrotic foci in the liver, myocardium, intestine, pancreas
Carnivores	*S. dublin*	Rare; associated with septemia in puppies
	S. typhimurium	Rare; gastroenteritis in immunosuppressed kittens
Lab animals	*S. typhimurium*	Uncommon; mice, gerbils, hamsters, rabbits, guinea pigs; non-human primates
	S. enteritidis	Uncommon; rats, hamsters, rabbits, guinea pigs; non-human primates

Contributor: Smithsonian National Zoological Park, Department of Pathology, 3001 Connecticut Ave, NW, Washington, DC
http://nationalzoo.si.edu/default.cfm

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SLIDE 39
CONFERENCE 10 / CASE III – N2004-0345 (AFIP 2942032)

Signalment: 10 day-old, male, boat-billed heron (*Cochlearius cochlearius*).

History: This animal was the first offspring produced from a group of boat-billed herons, and presented after falling from the nest. On physical exam, all long bones were very flexible. Radiographs revealed multiple fractures of the long bones with very poor bone density. Due to a poor prognosis, humane euthanasia was elected.

Gross Pathology: Gross findings confirmed moderate to severe flexibility of all bones, with multiple folding fractures. Additionally, there was a 0.5 cm diameter focus of subdural hemorrhage within the right frontal region of the cerebrum (consistent with trauma associated with the recent fall).

Laboratory Results:
Serum calcium: 6.0 MG/DL
Serum phosphorus: 10.5 MG/DL
Serum 25-hydroxy Vitamin D: 36 nmol/L*
*Laboratory comment: Reference values for this test are not defined for this species in our laboratory. However, this concentration is similar to values seen in chickens, turkeys, and conures.
Serum Vitamin A: Retinol = 610 ng/ml**
**Laboratory comment: We do not have serum vitamin A reference ranges for boat-billed herons. For comparison, in adult mammals, adequate serum vitamin A (retinol) concentrations range between 175 to 500 ng/ml.

Contributor's Morphologic Diagnoses: Long bone: Elongation and thickening of the zone of hypertrophic cartilage, with excess osteoid (unmineralized matrix), fibroplasia, and folding fracture (rickets).
Parathyroid (not submitted): Hyperplasia, diffuse, moderate.

Contributor's Comment: The gross and histologic findings within the long bones of this bird are consistent with rickets. Rickets is caused by the failure of mineralization of newly deposited osteoid and may be caused by deficiencies of vitamin D, calcium, or phosphorus, as well as excess calcium or phosphorus. Grossly, affected birds have

swollen joints, soft bones, and flared metaphyses. Curved deformities and folding fractures may occur.[1]

Much research has been done on the different histologic lesions associated with rickets depending on the specific nutrient imbalances present. With calcium deficiency, there is disorganization and thickening of cartilage within the zone of proliferation, with poor physeal vascularization. Only a small zone of hypertrophic cartilage is present in this type of rickets. Calcification of the hypertrophic cartilage is reduced, and there is little vascular invasion of this cartilage. At the base of the cartilage, few calcified bone trabeculae are present, with a loss of the normal longitudinal arrangement.[1,2] The defect of matrix calcification appears to be due to lack of continuation of mineral deposition, rather than failure of its initiation. In one study, electron micrographic studies revealed that failure of progression of matrix deposition is likely due to the presence of hypertrophic cells (responsible for the initiation of matrix deposition).[3] The bone marrow is often replaced by fibrous tissue, and osteoclasts are abundant. The defect in calcium-deficiency rickets is thought to be impaired hypertrophy of chondrocytes, rather than increased cell replication. In calcium-deficiency rickets, the parathyroid glands are often hypertrophied.

Conversely, with phosphorous deficiency, the zone of proliferation is unchanged, but the hypertrophic zone is elongated and thickened, and there is defective mineralization of the hypertrophic cartilage cells resulting in long columns of cartilage, surrounded by wide unmineralized osteoid seams extending into the primary spongiosa. There is normal invasion by metaphyseal blood vessels. Osteoclasts are reduced, osteoblasts are increased, and amounts of osteoid are limited.[1,2] This condition is thought to be due to decreased resorption of hypertrophic cartilage by chondroclasts, rather than an increased proliferation of chondrocytes.[4] Birds with phosphorus-deficiency rickets often have atrophy of the parathyroids.[2]

Vitamin D-deficiency rickets results in lengthening and disorganization of the proliferating zone and variable lengthening and dysplasia of the mineralizing zone. The primary spongiosa is short, thick cartilage columns.[1] As in calcium-deficiency rickets, there is often parathyroid gland hyperplasia.[2]

It is interesting to note that the morphologic appearance of rickets in this case is most consistent with decreased phosphorous. However, the presence of parathyroid hyperplasia and relatively low serum calcium with high serum phosphorous is suggestive of disease due to hypocalcemia. It should be stressed that the previously-described histologic patterns are general guidelines only, and may vary according to deficiency. Other causes of rickets, such as magnesium toxicity, excess vitamin A or fat in the diet, mycotoxins, and enteritis have also been reported, and may cause modifications in the typical morphology.[2]

AFIP Diagnoses: 1. Long bone: Failure of endochondral ossification and retained cartilaginous cores, with increased osteoid seams (rickets), boat-billed heron (*Cochlearius cochlearius*), avian.
2. Long bone: Fracture with callus formation.

Conference Comment: As mentioned by the contributor, rickets in avian species may be the result of a deficiency in vitamin D, calcium, or phosphorus as well as an excess of calcium or phosphorus. Although there may be histological differences in rachitic bones depending on the cause, the common denominator is the failure of mineralization of newly deposited osteoid (failure of endochondral ossification), which results in bone deformities and fractures.[1]

In domestic animals, rickets is most commonly caused by a deficiency in vitamin D or phosphorus. However, it may also result from chronic renal disease or chronic fluorosis. Histologically, the lesions in domestic animals are similar to those seen in birds. There is abnormal endochondral ossification characterized by failure of mineralization of the growth plate cartilage and osteoid. However, there is also disorganization of chondrocytes within the zone of hypertrophy, which may or may not be present in birds, depending on the etiology. It is unclear if the disorganization of chondrocytes in vitamin D-deficiency rickets is due to a primary affect of vitamin D metabolites or a mechanical consequence of the failure of endochondral ossification.[5]

Contributor: Wildlife Conservation Society, Department of Pathology, 2300 Southern Blvd., Bronx, NY
http://wcs.org/

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SLIDE 40
CONFERENCE 10 / CASE IV – AFIP 04 Case 1 (AFIP 2936425)

Signalment: Approximately 8-10 weeks of age, intact males, DBA/1LacJ mice (*Mus musculus*) from Jackson Laboratories.

History: Mouse with collagen-induced arthritis as a model for rheumatoid arthritis was used to investigate the therapeutic effect of a drug under development.

Gross Pathology: Swelling and redness of the fore and hindpaw joints with visible phalangeal distortion.

Contributor's Morphologic Diagnosis: Front and rear paw joints (with associated soft tissues): Polyarthritis, suppurative, severe, chronic with pannus formation, articular cartilage degeneration, periosteal bone remodeling and surrounding tissue involvement (bursitis, tendinitis and myositis), DBA/1LacJ mice (*Mus musculus*), rodent.

Contributor's Comment: The tissue section on the slide can be either the front or rear paw of the mouse. Microscopically, all joints (not present in all submitted slides) of the paws are severely disrupted with replacement of the synovium and capsule by severe fibrosis and marked infiltrations of neutrophils and a small number of macrophages and lymphocytes. Across the synovial cavity bridging the synovial capsule, a pannus was usually noted and consisted of a fibrous structure usually poorly vascularized, containing basophilic debris, and infiltrates of neutrophils and a few macrophages. The pannus formation is present with a partial to complete erosion and/or degeneration of the articular cartilage extending in the subchondral bone and occasionally in the underlying bone marrow containing mixed inflammatory cells and slight fibrosis. Along the metaphyseal and diaphyseal bone, a marked periosteal reaction is noted and consists of a marked thickening of the periosteum by a mild infiltration of mostly neutrophils, fibrous connective tissue, bone remodeling (cartilage metaplasia, osteoid matrix and/or woven bone formation with intense osteoclastic activities), and/or sometimes marked osteoblastic hyperplasia (i.e., cells with a large vesicular nucleus and abundant pale cytoplasm). Furthermore, in the surrounding tissues, a marked chronic multifocal suppurative bursitis, tendinitis and myositis are noted.

This submission is a severe case (i.e., grade 4) of arthritis induced by collagen [collagen-induced arthritis (CIA)] in mice as a model for rheumatoid arthritis (RA). The severity of the arthritis was scored on a scale of 0 to 4 by histological assessment as noted in Wooley and Wooley et al.[1,2] CIA is induced in MHC-susceptible mice immunized with bovine type II collagen emulsified in complete Freund's adjuvant. Typically the mice are boosted 3 weeks after immunization with bovine type II collagen in incomplete Freund's adjuvant. The resultant response is dependent on T cells, B cells, and cytokines. About 4 weeks after immunization, clinical signs of disease develop in the paws. Active inflammation of the paws remains for about 2-3 weeks and usually results in joint destruction and deformity.

In response to joint injury, hypertrophy and hyperplasia of synoviocytes of the synovial membrane, and villous and/or pannus formation are observed. Pannus is an intraarticular fibrovascular structure with inflammatory cell infiltrates that arises from the

synovial membrane and spreads over neighboring cartilage subsequent to chronic infectious nonsuppurative synovitis and/or immune-mediated diseases such as RA. With time, as observed in this case, opposing cartilaginous surfaces are united by fibrous tissue resulting in fibrous ankylosis of the joint. The pannus can act as a physical barrier between the synovial fluid and the cartilage preventing chondrocyte nutrition. The pannus macrophages with the proteolytic enzyme-producing neutrophils and the collagenase-producing fibroblasts enhance the cartilage degeneration that commonly extends into adjacent subchondral bone.[3]

CIA in the mouse and adjuvant-induced arthritis (AIA) in the rat are two models used to mimic the clinical manifestations of RA due to similarities in the development of synovial and cartilage lesions. RA is a chronic, erosive polyarthritic disease observed in both humans and dogs, but rarely observed in the latter. The cause of RA is not yet fully understood, but may involve both immune mediated processes i.e., humoral and cell-mediated immunity. IgG or IgM classes known as rheumatoid factors are produced in response to an unknown stimulus. The factors that might be involved are alterations in the stearic configuration of IgG, persistent bacterial cell wall components that cross-react with normal proteoglycans, anticollagen antibodies, and/or defective suppressor T cell activity. Immune complexes formed are ingested by neutrophils that release lysosomal enzymes (e.g., IL-1 promotes secretion of prostaglandins, nitric oxide, and neutral proteases inhibiting proteoglycan synthesis), which are responsible for the inflammatory reaction and destroy intraarticular structures.[3,4] The loss of proteoglycans from cartilage results in alterations in the hydraulic permeability of the cartilage, thus interfering with joint lubrication and leading to further mechanically-induced injury to the cartilage. TNF-_ has similar effects to IL-1: increasing concentrations of agents that will decrease matrix synthesis and increase matrix destruction.[3,4] Furthermore, matrix metalloproteinases (gelatinases, collagenases) activated by products of degenerating or reactive chondrocytes and inflammatory cells result in digestion of the matrix.[3]

AFIP Diagnosis: Paw, bones and joints: Polyarthritis and osteomyelitis, chronic-active, diffuse, severe, with articular cartilage erosion, subchondral pannus, cortical resorption, periosteal fibroplasia, reactive bone formation, and extensive soft tissue inflammation, DBA/1LacJ mouse, murine.

Conference Comment: The contributor provides a thorough overview of collagen-induced arthritis (CIA) and its use as an animal model for human rheumatoid arthritis (RA). There is significant variation in slides due to the necessary use of multiple animals and multiple paws. Therefore, as mentioned by the contributor, not all aspects of the described lesions are present on all slides.

Arthritis in the dog is often classified as erosive or nonerosive. Rheumatoid arthritis in the dog is a chronic, erosive polyarthritis that resembles RA in humans. The cause is unknown, but the process is immune-mediated, and as described by the contributor, involves both humoral and cell-mediated immunity, as well as inflammatory mediators

and fibroblasts. In dogs, RA is characterized by progressive lameness due to involvement of the peripheral joints of the limbs. Grossly, the lesions consist of marked villous hypertrophy of the synovial membrane, erosion of the articular cartilage, pannus formation, periarticular osteophytes, and occasionally fibrous ankylosis of affected joints.[3]

Nonerosive arthritis occurs in dogs with systemic lupus erythematosus (SLE), or chronic disease processes such as pyometra or otitis externa. In dogs with SLE, in addition to arthritis, these animals often present with anemia, thrombocytopenia, polymyositis, or glomerulonephritis. Immune complexes (type III hypersensitivity) can localize in the synovium and lead to synovitis. In contrast to erosive arthritis, in joints with nonerosive arthritis there is usually minimal villous hypertrophy, no pannus formation, no articular cartilage destruction, and the exudate in the synovial fluid is neutrophilic.[3]

Contributor: Wyeth Research, Department of Pathology, Chazy, New York

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SLIDE 41
CONFERENCE 11 / CASE I – 02-1123 (AFIP 2933943)

Signalment: 1-year-old, male castrated, Singapura, cat *(Felis domesticus)*.

History: Presented with lethargy, persistent fever, enlarged mesenteric lymph nodes, enlarged kidneys, abdominal and thoracic effusions. No history of diarrhea except when fed pumpkin/goat milk diet recommended by breeder.

Gross Pathology: Very thin, icteric cat with 20 ml of gelatinous serosanguinous thoracic fluid and 80 ml of similar abdominal fluid; enlarged lymph nodes (sternal, cranial mediastinal, mesenteric, colonic); multifocal to coalescing yellow-white nodules in liver, spleen, lung, lymph nodes, and kidneys; multiple tan-green 3 to 6 mm irregularly shaped raised ulcerated foci on mucosa of cecum.

Laboratory Results: Anemia, thrombocytopenia, hyperbilirubinemia, elevated alkaline phosphatase, negative feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) titers; Feline coronavirus titer 1:1600

Contributor's Morphologic Diagnoses: 1. Severe multifocal ulcerative typhlitis with innumerable intralesional trichomonads *(Tritrichomonas foetus)*.
2. Cecum: Severe multifocal necrotizing and lymphoplasmacytic vasculitis (FIP).

Contributor's Comment: This is a section of cecum with areas of mucosal necrosis and ulceration. The ulcers are filled with innumerable, irregularly pyriform to elliptical, 5 x 10 _m, pale-staining basophilic protozoal organisms (trichomonads). These organisms extend into the underlying submucosa and muscularis in some sections. There is granulation tissue and neutrophilic inflammation associated with the ulcers, but not always with the organisms. Many of the adjacent mucosal crypts contain trichomonads as well. Several blood vessels in the submucosa, muscularis, and serosa are surrounded or obscured by neutrophilic and lymphoplasmacytic inflammation. There are also areas of fibrinoid necrosis characterized by karyorrhectic debris (nuclear dust) and fibrin replacing and expanding the walls of blood vessels. There are mixed populations of bacteria (predominantly filamentous) on the ulcerated surface and extending into the areas of mucosal necrosis.

Tritrichomonas foetus is a recently recognized primary enteric pathogen of cats.[1] Natural infections have been associated with large bowel diarrhea and ulceration of the colonic mucosa.[2] Although the organism was previously identified as *Pentatrichomonas hominis*[3], rRNA gene sequence analysis, restriction enzyme digest mapping, and light, transmission, and scanning electron microscopy have identified the agent as *Tritrichomonas foetus*, the same organism that causes reproductive disease in cattle.[1] Experimental infection of domestic shorthair cats resulted in diarrhea which resolved after 7 weeks followed by persistent infection.[4] In previously reported natural and experimental cases, invasion through the mucosa has not occurred. In this case, the combination of feline infectious peritonitis and trichomonosis may have synergistically resulted in more severe enteric lesions.

AFIP Diagnosis: Cecum (per contributor): Typhlitis, necrotizing, histiocytic, neutrophilic and lymphoplasmacytic, transmural, multifocal, severe, with ulceration, granulation tissue and myriad protozoa, Singapura, feline.

Conference Comment: There is considerable variation among slides. In some sections, there is a perivascular inflammatory infiltrate suggestive of feline infectious peritonitis while in others the inflammation is more diffuse. Precise identification of protozoa in histologic sections is often impossible without utilization of special techniques. Methods of identification of intestinal flagellates include direct cytologic examination of feces suspended in saline solution, protozoal cultures, immunohistochemistry, electron microscopy and PCR. Characteristic cytologic features

of *Tritrichomonas foetus* include three anterior flagella, one posterior free flagellum, an undulating membrane, a single nucleus, a stout axostyle, and a stout costa.[1] In this case, the identity of the organism was confirmed by PCR (personal communication, Dr. Jody L. Gookin, North Carolina State University).

Genital trichomoniasis is a contagious venereal disease of cattle. Bulls can be carriers with early infections resulting in balanoposthitis with a purulent discharge. As the infection becomes chronic, there is no discharge, organisms are present in low numbers and often concentrated in the glans penis. Cows are infected during coitus, and a vaginitis with mucoid floccular discharge develops within a few days. Following the vaginitis, the organism localizes to the uterus and cervix, causing endometritis and cervicitis, and results in repeat breedings, abortion, or pyometra. There are no specific lesions in the aborted fetus. However, large numbers of organisms can be isolated from the fetal fluids and stomach. The placental lesions are not characteristic, and include a white to yellow flocculent exudate, placental thickening, and hemorrhagic cotyledons.[5]

T. foetus has been recently demonstrated to be a feline pathogen that causes large bowel diarrhea in young cats. The factors that result in the rare instances of invasive infections such as this one have yet to be determined.

Contributor: University of Tennessee, College of Veterinary Medicine, Department of Pathobiology, 2407 River Drive, Knoxville, Tennessee
http://www.vet.utk.edu/departments/path/

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SLIDE 42
CONFERENCE 11 / CASE II – HB3973 (AFIP 2943308)

Signalment: 5-year-old, female (spayed), Shiba-dog, canine.

History: The dog was presented for hematuria. By ultrasonography, a polypoid round mass, approximately 2 cm in diameter, protruding into the lumen of bladder was noted on the mucosal surface at the cranio-ventral wall of the bladder. Partial cystectomy was performed to remove the affected bladder wall.
Gross Pathology: The firm, 2 x 2 x 1.5 cm mass protruded on the mucosal surface. The polypoid mass had a smooth surface but was extensively ulcerated. The cut surface was solid, white and mildly edematous. The border on the cystic wall was well defined.

Laboratory Results: No atypical cells were detected by fine needle aspiration.

Contributor's Morphologic Diagnosis: Urinary bladder: Cystitis, eosinophilic, chronic, Shiba-dog, canine.

Contributor's Comment: Sections were obtained from either of two paraffin-embedded blocks.

The mass was located in the submucosa and was composed of a diffuse proliferation of fibrous tissue that contained fibrocytes, fibroblasts, lymphocytes, plasma cells, abundant blood vessels, and numerous eosinophils. Most of the mucosal epithelium on the mass was ulcerated and numerous neutrophils infiltrated the superficial layer of the mass. There was mild epidermal hyperplasia with Brunn's nests, mild proliferation of blood vessels, mild focal edema and hemorrhage with hemosiderin-laden macrophages in the mucosa adjacent to the mass. These histological findings are consistent with those of eosinophilic cystitis previously described in dogs.[2]

The lesion is considered a variant of polypoid cystitis, one in which eosinophils are the predominant component.[1] The alternative name of inflammatory fibrous polyps has been suggested.[3] A differential diagnosis includes fibroma, which lacks eosinophilic infiltration. There appears to be some overlap between the diagnosis of fibrous polyps and fibroma and the interpretations of such lesions are not unanimous.[3]

The etiology and pathogenesis of eosinophilic cystitis are still unknown.[2] The lesions occur in a variety of breeds of dogs ranging in age from 8 months to 15 years, with an average of 8 years.[3] Hematuria is the most common clinical sign in dogs. The lesions usually have a benign clinical course and surgical excision is curative.

AFIP Diagnosis: Urinary bladder: Polypoid eosinophilic cystitis, Shiba, canine.

Conference Comment: As the contributor noted, a variety of names have been utilized for similar lesions including fibroma, fibrous polyp, eosinophilic cystitis, polypoid eosinophilic cystitis, cystitis with fibroplasia, and mesenchymal tumor with inflammation.

Although pathologists may not agree on nomenclature, the histological features include: hyperplastic, often ulcerated transitional epithelium; a nodule of fibrous connective tissue confined to the propria/submucosa; abundant vascular supply; inflammatory cells with a predominance of eosinophils; and occasionally foci of granulopoiesis, eosinophilopoiesis, cystitis glandularis and Brunn's nests. The mesenchymal cells are surrounded by a material that stains as collagen with Masson's trichrome. Immunohistochemically, the mesenchymal cells are not immunoreactive for desmin and muscle specific actin.[3,4] Definitive differentiation of inflamed fibrous neoplasms from proliferative fibrous inflammatory lesions is often problematic.

Contributor: Hokkaido University, Graduate School of Veterinary Medicine, Laboratory of Comparative Pathology, Sapporo, Japan
http://www.hokudai.ac.jp/veteri/index-e.html

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SLIDE 43
CONFERENCE 11 / CASE III – 2004903384 (AFIP 2937492)

Signalment: Five-year-old, female, ferret (*Mustelae putorius furo*).

History: An intact female domestic ferret was evaluated for bloody stool. Clinical signs included emaciation, loss of body weight and abdominal swelling due to ascites. Generalized alopecia and anemia were also observed. Blood biochemical examinations revealed increased enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactose dehydrogenase (LDH) and alkaline phosphatase (ALP), and increased level of total bilirubin. Mild hypoglycemia was also detected. Despite various supportive treatments, the ferret died three months after first presentation to the animal hospital.

Gross Pathology: The liver was yellow to light tan and slightly enlarged, with a coarse multi-nodular appearance (Fig.1).

Laboratory Results:

At first presentation:

WBC 10400/_l	ALT over 1000 IU/l	T-Cho 252.2 mg/dl
Ht 26.3%	AST 540 IU/l	TG 87.6 mg/dl
Hb 7.8g/dl	ALP 200 IU/l	BUN 33 mg/dl
	T-Bil 0.1 mg/dl	Glu 118.5 mg/dl

About 1 week prior to death:

WBC 8800/_l	ALT 267 IU/l	T-Bil 4.3 mg/dl
Ht 29.7%	AST 154 IU/l	BUN 51.1 mg/dl
Hb 10.1g/dl	ALP 125 IU/l	Glu 136.3 mg/dl

Contributor's Morphologic Diagnosis: Massive hepatocellular necrosis and various degenerative changes in hepatocytes (ie; eosinophilic granules, lipid and bile pigment accumulations, severe vacuolation). Regenerative nodules of hepatocytes and cholangiocellular (bile ducts) proliferation (so-called toxic hepatopathy or chronic progressive hepatitis, consistent with Copper toxicosis).

Contributor's Comment: Copper storage hepatopathies in Bedlington terriers and West Highland White terriers are well documented in the literature. The disorder in Bedlington terriers is an inherited autosomal recessive defect. In West Highland White terriers, although the disorder is also thought to be an inheritable liver disease, not all of the affected dogs show signs of liver disease. In domestic ferrets, an identical disease condition with similar clinical features and histopathological changes is reported,[1] and thought to have an inheritable component.

The main histopathological changes in this specimen are massive to focal necrosis of hepatocytes, with mild fatty change and severe vacuolation. In addition to these changes, there are eosinophilic granules in the cytoplasm of degenerative hepatocytes. These granules stain positive for rhodanine, a copper-specific stain. Accumulation of an intracytoplasmic eosinophilic material is also present in Kupffer cells and macrophages.

Other lesions include mild fibrosis with mononuclear cell infiltration, reactive proliferation of cholangiolar epithelial cells, bile duct obstruction (bile thrombus or cholestasis), and regenerative hepatocellular nodules. This ferret also had bilateral adrenocortical adenomas and an islet cell tumor that are thought to be the cause of the generalized alopecia and hypoglycemia.
The genetic defect in the Bedlington terriers causes expression of an abnormal hepatic metallothionein. Defective metallothionein results in reduced biliary excretion of copper and excessive copper becomes sequestered in hepatocellular lysosomes. The association between an increased hepatic copper concentration and its relationship to tissue injury is controversial in dog breeds other than the Bedlington terrier.

Occlusion of the major bile duct has been shown to cause elevation of hepatic copper concentration in cats, but not in dogs. Chronic hepatitis, chronic cholestasis and cirrhosis have been shown to lead to increased tissue copper concentrations in dogs,

but these findings are not known in ferrets. The cause of copper toxicosis in this ferret is unknown, and a genetic predisposition is also unknown.

AFIP Diagnosis: Liver: Cirrhosis, characterized by multifocal necrosis, regenerative nodular hepatocellular hyperplasia, fibrosis, biliary hyperplasia, canalicular cholestasis, hepatocellular lipidosis, extramedullary hematopoiesis and eosinophilic refractile hepatocellular cytoplasmic granules, ferret, mustelid.

Conference Comment: Copper-associated liver diseases are well recognized in animals and humans. Copper toxicosis results from disruption of normal copper homeostasis or accumulation of copper in excess of metabolic requirements and may be either primary or secondary. Primary copper toxicosis results from an inherited metabolic defect. Secondary copper toxicosis results from an underlying pathologic process that leads to abnormally high intake, increased absorption, or reduced excretion of copper. To better understand copper-associated diseases, one must first understand copper homeostasis in mammals.[2]

Dietary copper is absorbed primarily in the proximal small intestine where transport from the lumen into the intestinal mucosa is a carrier-mediated process, with a saturable transport component, which may be influenced by other dietary factors. Most of the copper within the intestinal epithelium is found within the cytosol bound to metallothioneins. From here, copper enters the portal circulation bound to albumin or other carrier proteins, and is primarily transported to the liver, with small amounts entering the kidney. Once in the liver, copper undergoes three processes: hepatocellular uptake, intracellular distribution and utilization, and copper export, with each of these steps tightly regulated by transporters, chaperones, and other proteins. ATP7B protein is required for copper incorporation into ceruloplasmin in the liver, for biliary excretion, and possibly for transport of copper into a vesicular compartment, where it may be delivered to lysosomes and interacts with metallothionein. The main route of excretion of copper is in the bile. While some copper is excreted into plasma as a complex with ceruloplasmin, very little copper crosses the glomerular capillaries.[2]

Familial copper storage disorders occur in Wilson's disease in humans, Long Evans Cinnamon (LEC) rats, toxic milk mice, Bedlington terriers, and West Highland White terriers. Wilson's disease is an autosomal recessive inherited disorder of copper metabolism and results in copper accumulation in the liver, cornea, and brain. The gene defect has been localized on human chromosome 13 and codes for ATP7B, a copper transporting P-type ATPase. The mutations occur throughout the entire gene, leading to variable clinical presentations. The resulting liver disease may mimic a wide variety of common liver conditions, including fulminant hepatic failure, chronic hepatitis, and cirrhosis. The LEC rats and toxic milk mice are the only known valid animal models of Wilson's disease. The canine copper toxicosis locus in Bedlington terriers has been mapped to canine chromosome region CFA 10q26, and recently a mutated MURR1 gene was discovered in animals with the disease.[2]

Secondary copper toxicosis, characterized by copper retention secondary to an underlying disease has been documented in primary biliary cirrhosis in humans, chronic active hepatitis in Doberman pinschers, and Skye Terrier hepatitis. Primary biliary cirrhosis is a chronic, progressive, often fatal liver disease that results in cirrhosis and liver failure. Although the pathogenesis is unknown, an immune-medicated mechanism is suspected, and copper accumulation is a secondary event. Similarly, the cause of chronic active hepatitis in Doberman pinschers is unknown, but is also thought to be immune-mediated, with copper accumulation within the centroacinar (portal) areas occurring secondarily. Skye Terrier hepatitis is characterized by intracanalicular cholestasis, with copper accumulation, hepatocellular degeneration, and ultimately cirrhosis. In this disease, copper accumulates primarily in the periacinar (centrolobular) areas.[2]

Regardless of the cause, cirrhosis and hepatic failure are often the end result. Initially, as hepatocytes degenerate, become necrotic, and are lost, the liver will become hyperplastic, leading to both micro- and macro-nodular regeneration. These nodules form at varying times and therefore, as in this case, have varying histological appearances. Some regenerative nodules are composed of hepatocytes with intracytoplasmic lipid vacuoles while others are not. As this process continues, normal hepatic architecture is lost and, as is seen in this case, it may be difficult to identify a "normal" hepatic lobule.

Contributor: Setsunan University, Faculty of Pharmaceutical Sciences, Department of Pathology, 45-1 Nagaotouge-cho, Hirakata, Osaka, Japan

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SLIDE 44
CONFERENCE 11 / CASE IV – D04-23121 (AFIP 2938283)

Signalment: 12-year-old, spayed female, Labrador Retriever mixed breed dog (*Canis familiaris*).

History: The dog presented to a local practitioner for a soft tissue swelling, of unknown duration, that extended from the left metacarpal pad distally over the left forepaw. Systemic antibiotics in combination with epsom salt soaks failed to resolve the swelling. Incisional biopsies from the left metacarpal pad and adjacent interdigital skin were submitted for histopathological examination.

Gross Pathology: In a single biopsy, nests, islands, acinar and tubular arrangements of tumor cells extend from the dermal-epidermal junction to all surgical borders. Tumor cells have indistinct cell borders, are cuboidal to columnar to polygonal with a high nuclear to cytoplasmic ratio. One to two layers of cells line the tubular structures. The nucleus is centric, oblong to oval to round with finely stippled chromatin and a single nucleolus. The cytoplasm is scant and pale pink. Anisokaryosis and anisocytosis are moderate. Mitoses are present (0 to 1 per 40X objective field). Binucleate and trinucleate cells and karyomegaly are rare. Occasional angular nucleolar forms are noted. The arrangements of tumor cells are surrounded by an abundant fibrous connective tissue stroma (scirrhous reaction). A moderate infiltrate of plasma cells, small lymphocytes and eosinophils is present in the supporting stroma. Several normal eccrine glands are surrounded by the arrangements of tumor cells.

Contributor's Morphologic Diagnosis: Eccrine carcinoma

Contributor's Comment: Eccrine carcinoma is a malignant tumor showing differentiation to eccrine secretory epithelium.[1] The tumor is rare but has been reported in the footpads of the dog and cat where these glands are normally located.[1,2,3] Lesions may affect multiple toes in cats.[2] Affected areas are swollen and often the overlying epidermis is ulcerated.[1,2] There may be invasion of adjacent bones of the digit. Eccrine carcinomas are highly aggressive tumors that exhibit rapid metastasis to lymph nodes and subcutaneous tissues of the affected limb.[2,3] Visceral metastasis has not been reported for eccrine carcinomas.[1,3] Most cases are treated by excision of the tumor with wide margins.[1] If the spread of the tumor is confined to the leg then amputation of the leg is a reasonable option.[3]

AFIP Diagnosis: Foot pad: Adenocarcinoma, Labrador Retriever cross, canine.

Conference Comment: Exocrine glands are composed of highly specialized epithelial cells and discharge their secretory product via a duct onto an epithelial surface. Exocrine glands can be classified according to two major characteristics: the morphology of the gland, and the means of discharge of the secretory product. Exocrine glands are either simple, with a single, unbranched duct, or compound, with a branched duct system. These glands secrete their product in one of three ways: merocrine (eccrine) secretion involves the process of exocytosis; apocrine secretion involves the release of membrane-bound vesicles; and holocrine secretion involves the discharge of the whole secretory cell.[4]

The eccrine glands develop independently of the hair follicle, with the duct opening directly onto the surface of the epithelium.[5] They are found only on the glabrous skin, such as the footpad of dogs and cats, the frog region of ungulates, the carpus of pigs, and the nasolabial region of ruminants and pigs.[5,6] These glands have been designated eccrine based on their mode of secretion. However, it is now known that these glands

excrete their substances by a variety of secretory modes, including microapocrine blebbing. Some authors now prefer the term atrichial glands.[5]

The apocrine glands develop embryologically as part of the hair follicle complex and are found in all haired skin areas, although only associated with the primary hair follicles. The ducts of the apocrine glands open in the superficial portion of the hair follicle.[5] Apocrine gland activity is rarely visible in domestic animals, except in the horse. Other apocrine glands include the interdigital glands of small ruminants, glands of the external ear canal and eyelids of domestic animals, anal sac glands of dogs and cats, and the mental organ of pigs.[6] Previously, the mode of secretion was thought to involve the pinching off of apical blebs of cytoplasm. However, more recent studies have shown this to be largely artifact with sweat production resulting from a combination of processes including holocrine secretion, vesicle exocytosis, active ion and water transport, and a minor contribution from microapocrine blebbing. Due to this fact, some authors now prefer the term epitrichial or paratrichial glands.[5]

Differentiation of eccrine carcinoma from apocrine carcinoma is exceedingly difficult and requires knowledge of the site of origin of the tumor (footpad in the dog or cat). In dogs, the normal eccrine gland is composed of a secretory coil, a ductular segment that courses through the dermis and an intraepidermal segment. The secretory coil consists of a single layer of cuboidal to columnar epithelial cells and a single layer of fusiform myoepithelial cells. The ductular segment is composed of two layers of non-secretory cuboidal epithelial cells. The duct opens on the footpad surface.[7] Carcinoembryonic antigen (CEA) is present in both the ductular and secretory portions of the gland[8] and has been reported to be useful in identification of eccrine carcinomas in animals.[2,7] In this case, neoplastic cells are immunohistochemically negative for CEA and no unique features of eccrine differentiation were found. This neoplasm may be an eccrine carcinoma but the possibility of metastasis from another site should be excluded. Particularly in cats, occult pulmonary adenocarcinomas sometimes metastasize to one or more footpads and may be misdiagnosed as eccrine carcinomas.

Contributor: University of Saskatchewan, Western College of Veterinary Medicine and Prairie Diagnostic Services, Department of Veterinary Pathology, 52 Campus Drive, Saskatoon, Saskatchewan, Canada
www.usask.ca/wcvm/vetpath
www.usask.ca/pds

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SLIDE 45
CONFERENCE 12 / CASE I – 04-514 (AFIP 2941213)

Signalment: 6 year-old Rhodesian Ridgeback, neutered male, dog (*Canis familiaris*).

History: This dog initially presented with a chief complaint of vomiting. Over a period of several days its condition declined, and it developed lingual ulcers. Six days after initial presentation the dog developed tachypnea, a cough and excessive salivation. It fatigued easily and developed cyanosis after exercise. Therapy included fluids, antibiotics and oxygen via a nasal line. The dog's condition worsened over the next day. Due to its declining condition the dog was euthanatized. A postmortem examination was carried out at the veterinary hospital (on the day following the death of the animal) and tissues were submitted to the Veterinary Diagnostic Laboratory at Oregon State University for examination.

Laboratory Results: Chemistry panels showed elevated lipase, at > 6000. BUN and creatinine were not elevated. Multiple blood gases were performed over the last 3 days before euthanasia. Despite therapeutic oxygen insufflation pO2 fell from initial values of 170 mm Hg to 69, while pCO2 rose from initial values of 42.4 to 64 mm Hg.

Contributor's Morphologic Diagnosis: Diffuse pulmonary fibrosis with hemorrhage and edema and multifocal hyperplasia of type II pneumocytes.

Contributor's Comment: No gross description of lesions was available. A full set of tissues was submitted for histopathologic examination. Lesions were confined to the lung and kidney, which are presented in this submission.
In the lung there is diffuse hemorrhage and pulmonary edema, with alveoli containing eosinophilic proteinaceous fluid. Macrophages are present in many alveoli. There is diffuse thickening of alveolar walls and many alveoli are lined discontinuously by large, hypertrophied cells, presumably hyperplastic type II pneumocytes. There is increased collagenous tissue on the pleural surface. Pleural mesothelial cells are hypertrophied

and most appear to be ciliated. There is vascular congestion and some pulmonary vessels are thrombosed (in some sections). Trichrome stains reveal moderate, diffuse interstitial fibrosis. In the kidney there is diffuse congestion and scattered acutely necrotic tubules within the cortex.

This is one of seven cases presented to the Veterinary Diagnostic Laboratory within a period of about one week with similar clinical histories. All affected dogs had visited a particular park in Portland, Oregon. All had been seen to either ingest some material or to have vomited shortly after the visit. All developed gastrointestinal signs and progressive dyspnea. Some had elevated BUN and creatinine levels early in the clinical course, although that was not true for this case. Based on clinical signs and histopathology a presumptive diagnosis of paraquat intoxication was made. This was confirmed by the detection of paraquat in the urine of some of the cases.

Paraquat (1,1'-dimethyl-4,4'-bipyridillium dichloride; CAS # 1910-42-5) is a contact herbicide and its use is restricted in the US. Despite its restricted use, poisoning of pet animals remains a problem and in this series of cases is presumed to have been malicious.

The lung is the primary tissue affected in paraquat toxicity.[1] However, other tissues, including the kidney, liver and thymus also may be affected. In this series of cases lesions were consistently present in both lung and kidney. Experimentally paraquat produces pulmonary edema and hemorrhage with necrosis and loss of both type I and type II pneumocytes. Alveolar capillary endothelium is spared. These lesions may be followed by hyperplasia of type II cells. The extent of loss of pneumocytes and, thus, the capacity for regenerative hyperplasia of type II cells are dose dependent. In time diffuse interstitial fibrosis develops. The pulmonary lesions of paraquat toxicity are not unique and are similar to those induced by other agents that cause diffuse alveolar injury.

The targeting of the lung in paraquat toxicity is explained by its selective uptake in that tissue. Uptake is an energy dependent process that can occur against a concentration gradient. It utilizes the same biological uptake mechanisms that transport other polyamines, such as putrescine and spermidine. Toxicity is considered to result from cyclic oxidation-reduction reactions of the paraquat molecule, which result in the generation of large quantities of reactive oxygen species, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$). Toxicity is dependent on molecular oxygen and tissue damage is probably mediated, at least in part, by lipid peroxidation. Depletion of cellular NADPH may also play a direct role in toxicity.

Lesions in this case are consistent with paraquat toxicity. The discontinuous appearance of the hyperplastic type II pneumocytes is most likely explained by the dose received by this dog. Severe depletion of type II cells by the toxin would be expected to result in "patchy" hyperplasia. Renal lesions are relatively acute compared to those in the lung and the relative contributions of direct paraquat renal toxicity and hypoxia are

not clear. The presence of cilia, or rudimentary cilia, on pleural mesothelial cells has been reported in hyperplastic, metaplastic or neoplastic conditions affecting these cells.

AFIP Diagnoses: 1. Lung: Pneumonia, interstitial, hemorrhagic, diffuse, moderate, with type II pneumocyte hyperplasia, multifocal interstitial fibrosis, and emphysematous change, Rhodesian Ridgeback, canine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, minimal, with glomerular sclerosis.

Conference Comment: The contributor provides a thorough overview of paraquat toxicity in dogs. Conference attendees also considered other differential diagnoses for pulmonary edema and hemorrhage including warfarin toxicity, oxygen toxicity, nitrogen toxicity, ionizing radiation, and a neoplastic induced clotting deficiency.

Both paraquat and diquat are bipyridyl herbicides that have caused numerous deaths in humans and various animal species. Intoxication involves either carelessness or malicious poisoning and has been reported in cattle, sheep, horses, pigs, poultry, dogs, rabbits, and fish. Clinical signs and lesions vary with the species, dose received and route of administration.[2]

The most common route of administration for most species is ingestion. Cattle often present with neurological signs including dullness, incoordination, and staggering that progress to prostration, convulsions, coma and death. Oral lesions have also been reported in horses after grazing on freshly sprayed pasture. The lesions described in poultry and swine are similar to those seen in dogs, with dyspnea and pulmonary edema noted in both species. In sheep, intravenous administration of paraquat resulted in a dose-dependent decrease in glomerular and tubular function. The lesions in fish include massive desquamation of gill epithelial cells.[2]

In humans, accidental or intentional ingestion is the most common route of exposure; however, inhalation and dermal exposure have also been reported. Acute toxicity results in severe damage to all organ systems and death within 24 hours. Chronic toxicity results in progressive pulmonary fibrosis and pulmonary edema resulting in dyspnea and asphyxia.[3]

Contributor: Oregon State University, Veterinary Diagnostic Laboratory, 105 Magruder Hall, Corvallis, Oregon
http://www.vet.orst.edu/

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SLIDE 46
CONFERENCE 12 / CASE II – 98-1713 (AFIP 2694678)

Signalment: Tissue from a bovine.

History: Four of 21 cattle died suddenly during July 1998, while one recovered on a small farm close to Bloemfontein in the Free State Province of South Africa. When the farm was visited two days after the mortalities occurred, *Cestrum* spp. plants were found in an adjacent camp to which the cattle had gained access through a break in the fence. The tops of the plants had died off due to the effects of frost but at the bottom of the plants there were tufts of luscious green foliage which showed evidence of having been browsed.

Necropsies were performed on 2 of the 4 animals by the provincial state veterinarian and a range of organ specimens from the second case were submitted in unbuffered formalin to the Onderstepoort Veterinary Institute for histopathological examination. The plant involved was identified as a *Cestrum* sp. at the local herbarium.

Gross Pathology: Both of the animals necropsied presented with multiple hemorrhages throughout the serosal surfaces as well as on the endo- and epicardium, swollen and friable livers with marked hepatic lobular accentuation, hemorrhage and edema of the wall of the gall bladders, and colonic and cecal hemorrhages.

Contributor's Morphologic Diagnoses: 1. Necrosis and hemorrhage, coagulative, severe, acute, liver with hepatocyte vacuolation and presence of intracellular and loose intrasinusoidal segrosomes, midzonal, severe, acute.
2. Bile ductular proliferation, subacute, portal, moderate.

Contributor's Comment: As the tissues were fixed in unbuffered formalin, they became very fragile and several moderate to severe artifactual lesions are present in many sections:

- Acid hematin pigment accumulation
- Tears along edges
- Folds
- Presence of pink fluid in lumens of larger vessels
- Tearing and unraveling of walls of larger vessels

The liver revealed extensive periacinar coagulative necrosis and hemorrhage. Midzonal hepatocytes were extremely swollen and vacuolated, and many contained intracytoplasmic, single or multiple bright eosinophilic droplets of varying size, presumably cytosegrosomes. A few segrosomes were also present loose within the sinusoids in this region.

The history of ingestion of *Cestrum* spp. plants and the macroscopical- and histopathological changes of multiple hemorrhages and an acute hemorrhagic hepatosis correspond to the lesions described for acute to subacute cases of *Cestrum* poisoning.[1,2] The most likely differential diagnoses for this lesion in South Africa include acute seneciosis and acute algal toxicity.[1] No *Senecio* spp. plants could be found on the property and algal toxicity was unlikely in this incident as no algal blooms were noted in the stream from which the cattle drank, nor were mortalities reported in other groups of cattle drinking from the stream, either up- or downstream from the affected farm.

Species of the genus *Cestrum* are evergreen, ornamental shrubs which have been imported into South Africa from South America where they occur indigenously. In Africa suspected cases of poisoning of livestock due to *C. laevigatum, C. parqui* and *C. aurantiacum* have been reported from South Africa, Zimbabwe, Kenya and the island of St. Helena. In South Africa, outbreaks of poisoning by *C. laevigatum* have traditionally occurred in the Kwazulu-Natal Province.[1] The outbreak described above is one of 3 outbreaks attributable to *Cestrum* spp. toxicity, all of which occurred in the Free State Province of South Africa, an area not traditionally associated with *Cestrum* toxicity in South Africa. The *Cestrum* spp. are known for their ability for rapid establishment and spread, particularly along river banks and in recent years it appears that in South Africa, these plants have spread beyond the Kwazulu-Natal Province northwards into the Free State and Gauteng Provinces despite all 3 species having been proclaimed as weeds in South Africa.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, centrilobular and midzonal, diffuse, acute, severe, bovine.

Conference Comment: Hepatoxicity may be acute or chronic, but often is somewhere in between, and the agent's effect is usually a function of dose-rate. Nonetheless, agents that most often cause acute hepatotoxicity in cattle include: blue-green algae, *Cestrum* sp., cocklebur, poison peach, and sawfly larvae. Agents that most often cause chronic hepatotoxicity in cattle include: aflatoxin, pyrrolizidine alkaloids, sporidesmin, lantana, and nitrosamines.

There are several species of the genus *Cestrum*. In the United States, pathologists are most familiar with *Cestrum diurnum* as a cause of enzootic calcinosis in cattle. The species known to be hepatotoxic include: *C. parqui*, *C. laevigatum*, and *C. aurantiacum*, all of which cause similar hepatic disease. However, in the field, speciation within the genus is often uncertain due to hybridization.

Cestrum spp., the ink-berry plants, cause acute hepatotoxicity in South America, southern and central Africa, and Australia. Cattle are most frequently affected; however, sheep, goats, and fowl are susceptible. The young leaves and unripened

berries are the most toxic parts of the plant. The toxin, an atractyloside, has been recently identified. The histological lesions are consistently centrilobular (periacinar) and are identical to lesions caused by poison peach (*Trema aspera*) and cocklebur (*Xanthium pungens*).

Contributor: Onderstepoort Veterinary Institute, Pathology Section, P.O. Box 12502, Onderstepoort, South Africa
http://www.arc.agric.za/institutes/ovi/main/divisions/path2.htm

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SLIDE 47
CONFERENCE 12 / CASE III – S04-66 (AFIP 2948647)

Signalment: One-year-old, male, mixed-breed dog, canine.

History: The dog had clinical signs of gradual weight loss, vomiting, anorexia, and was fed with commercial dog food for several months.

Gross Pathology: The submitted samples were formalin fixed kidney, stomach and spleen. Prominent, white, powder-like deposits were observed on the kidney sample. The other samples had no significant gross lesions.

Laboratory Results: Moderate increases in WBC ($15.2 \times 10^3/\mu l$), neutrophils (95% of WBC), markedly increased BUN (407 mg/dl) and creatine (18.3 mg/dl).

Contributor's Morphologic Diagnosis: Kidney: Interstitial nephritis, fibrosing, lymphocytic, diffuse, severe, subacute, with severe tubular necrosis, mineralization, intratubular and pelvic oxalate crystals, canine.

Contributor's Etiologic Diagnosis: Oxalosis associated nephrotoxicosis.

Contributor's Comment: The lesions in the kidney were characterized by prominent interstitial fibrosis, crystal accumulation, atrophy, calcification, and mild lymphocytic

infiltration. The outline of the kidney was rough. Multifocally renal tubular lumina had accumulation of light yellow crystals. These crystals were round with many lines radiating from their center and had a birefringent appearance with polarized light. Affected tubular epithelium was degenerate to necrotic with mineral deposition on some tubular basement membranes and in the tubular epithelium. Some glomerular atrophy was also noticed. The interstitium had prominent fibrous connective tissue proliferation. The Alizarin red S stain was positive in the mineralized areas. Histopathologically, this case has marked renal tubular injury, prominent connective stroma proliferation and combined with the results of clinical pathology, renal failure is suspected clinically. The finding of renal tubular lumina with radially arranged crystal deposition could be induced by many causes; the most probable is calcium oxalate deposition. In a recent outbreak (March 2004) estimated at 3000 cases of canine renal failure in Taiwan, there was no sex, age or breed predilection. The ages of affected dogs ranged from several months to adult. The owners of the dogs told clinicians that their pets only ate a certain brand and type of commercial dry dog food. After eating this dog food for several days, dogs were presented with various degrees of renal failure. Finally, most of the affected dogs had gradual weight loss.

Oxalate is a simple organic carboxylic acid, which is excreted as calcium oxalate by many fungi. Oxalate also occurs as byproducts in various plant tissues, in urine, and in mantles of certain bivalves. In dogs, the most common cause of nephrotoxicosis is exposure to ethylene glycol. Ethylene glycol is rapidly absorbed from the gut, with peak plasma concentration achieved 2 to 3 hours after ingestion. It is predominantly metabolized in the liver in the following sequence: ethylene glycol to glycoaldehyde to glycolate to glyoxylate. Glyoxylate is converted to several final metabolites, including oxalate, glycine, and formate. Oxalate then binds with calcium and forms a soluble complex which is filtered by glomeruli. As water is reabsorbed by the tubules and the pH of the filtrate decreases, calcium oxalate precipitates and forms crystals. This results in nephrosis, and hypocalcemia if enough calcium is complexed. Other sources of oxalate may come from plant origin foods. Plants which may contain toxic amounts of oxalate are *Halogeton glomeratus* (halogeton), *Sarcobatus vermiculatus* (greasewood), *Rheum rhaponticum* (the common garden rhubarb), *Oxalis cernua* (soursob), *Rumex* spp. (sorrel and dock). In tropical and subtropical areas, certain grasses that are cultivated widely (genera of *Cenchrus*, *Panicum* and *Setaria*), accumulate large amounts of oxalate, and have been associated with renal oxalosis in cattle and sheep and with skeletal disease in the horse, the latter due to conditioned calcium deficiency.

The fungi *Aspergillus niger* and *A. flavus* can produce large quantities of oxalates on feedstuffs. Large doses of ascorbic acid have caused oxalate nephrotoxicosis in humans and in a goat; ascorbic acid is a metabolic precursor of oxalate. Primary hyperoxaluria, a rare inherited metabolic condition, occurs in humans, cats and perhaps dogs (Tibetan spaniels). Pyridoxine (vitamin B_6) deficiency and methoxyflurane anesthesia can also cause renal oxalosis.

Calcium oxalate is precipitated in the renal tubules during the process of elimination. A fatal outcome may occur from renal insufficiency and uremia after the earlier symptoms have abated. Conversely, recovery is possible, with blood urea levels slowly subsiding after about one month. Cystitis and urethritis may be a part of this syndrome.

AFIP Diagnosis: Kidney: Tubular degeneration, necrosis, and loss, diffuse, moderate, with interstitial fibrosis, and multifocal tubular mineralization and oxylate crystal deposition, mixed-breed, canine.

Conference Comment: The contributor provides a thorough overview of the most common causes of oxalosis, as well as the pathophysiology of calcium oxalate deposition in the kidney. As mentioned by the contributor, ethylene glycol is the most common cause of oxalosis in dogs and cats.

Intoxication with ethylene glycol is usually seasonal and coincides with the changing of antifreeze solutions in the spring and fall. Many antifreeze solutions are composed of up to 95% ethylene glycol. Toxicosis is common due to the sweet taste and very low minimal lethal dose (1.5 ml/kg for cats and 6.6 ml/kg for dogs). Ethylene glycol itself is of low toxicity. It is rapidly absorbed from the gastrointestinal tract and most is excreted unchanged in the urine. Only a small percentage is metabolized in the liver to the primary toxic metabolite glycolic acid (glycolate).[1,5]

The clinical signs of depression, ataxia, and osmotic diuresis develop within a few hours of ingestion of ethylene glycol. The nervous signs are due to the effects of aldehydes and severe metabolic acidosis, which develops due to accumulation of lactic acid, glycolate, and glyoxylate. Pulmonary edema, tachypnea, and tachycardia develop sequentially over the next 12 hours and are likely due to the systemic effects of the osmotic diuresis. If the animal survives for 1-3 days after ingestion, acute renal failure develops as a result of renal tubular damage caused by glycoaldehyde, glycolic acid, glyoxylic acid, and oxalate. The oxalate precipitates with calcium in the renal tubular lumina resulting in intrarenal obstruction, and tubular epithelial degeneration and necrosis. Using polarized light, the microscopic identification of large numbers of birefringent crystals in renal tubules is virtually pathognomonic for ethylene glycol toxicity in dogs and cats.[1,5] Alizarin red S stain will stain calcium oxalate crystals red at a pH of 7.0, but not at a pH of 4.2. In contrast, calcium phosphate and calcium carbonate stain red at a pH of both 7.0 and 4.2. In addition, calcium oxalate can be confirmed by its insolubility in 2M acetic acid, since both calcium phosphate and calcium carbonate are soluble.[6]

Contributor: Animal Technology Institute Taiwan, Division of Animal Medicine, P.O. Box 23, Chunan, Miaoli, Taiwan

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SLIDE 48
CONFERENCE 12 / CASE IV – UFSM-2 (AFIP 2940460)

Signalment: 7 year-old, castrated male, crossbred, bovine (*Bos taurus*).

History: The owner reported that for the last 60 days this draft ox had chronic bloat, regurgitation of food and loss of weight. The rumen had been punctured several times during this period to alleviate the bloat. At clinical examination the mucous membranes were pale, there was marked bloat, and subcutaneous emphysema extended from the ruminal area to the shoulder, lumbar and cervical areas on the left side. This ox was from an area where squamous cell carcinoma (SCC) of the upper digestive tract is endemic in cattle. There was heavy infestation of bracken fern (*Pteridium aquilinum*) in the pasture where this ox was kept. A clinical diagnosis of SCC of the upper digestive tract was made and the ox was euthanatized due to a poor prognosis.

Gross Pathology: Depletion of body fat deposits and pale mucous membranes were observed at necropsy. The extensive subcutaneous emphysema of the left side of the body was interpreted as being secondary to the ruminal punctures. Small papillomas (2-5 mm in diameter) were present in the surface of epiglottis, soft palate and pharynx. There was 1 liter of serous, clear yellow, partially coagulated fluid in the peritoneal cavity. The rumen was markedly distended by gas. The distal portion of the esophagus was distended (approximately 3 times its normal diameter), firm and contained a dark green plug of packed ingesta (grass) in its lumen. The ruminal contents were markedly dry and a mass, formed by multiple nodules (some edematous), protruded from the ruminal mucosa. The ruminal wall, just at and immediately distal (8 cm) to the esophageal entrance, was thickened (approximately 5 cm) and its cut surface was firm, gray-white, and speckled with yellow (keratinization) and dark brown (necrosis) foci.

This mass caused stenosis of the ruminal entrance. There was hypertrophy of the muscular layer of the walls of rumen and reticulum. Clear translucent, gelatinous edema was noted in the abomasal folds and multiple nodules due to *Oesophagostomum* sp. were present in the wall of the small intestine. There was atrophy of the left liver lobe and a dry, friable (sand-like) gallstone was located within the gallbladder.

Contributor's Morphologic Diagnoses: 1. Rumen, squamous cell carcinoma, well differentiated, with dystrophic mineralization, bovine, 7-year-old, castrated male, crossbred, bovine.
2. Rumen, squamous epithelial papillomas, bovine, 7-year-old, castrated male, crossbred, bovine (slides not included).
3. Pharynx and esophagus, squamous epithelial papillomas, bovine, 7-year-old, castrated male, crossbred, bovine (slides not included).

Contributor's Comment: *Pteridium aqulinum* (bracken fern) is a plant of worldwide distribution that causes intoxication in livestock in several regions of the world. Bracken fern is one of the more important toxic plants for cattle in Brazil, causing severe losses in the southern and southeastern regions. The plant also grows in other Brazilian regions including the states of Bahia, Amazonas, Acre, Mato Grosso and Pernambuco.[8]

There are three clinical manifestations of the poisoning by bracken fern and they were recently reviewed.[8] When cattle graze large amounts of the plant (between 10 and 30 g/kg/bw/day or more) for relatively short periods of time (weeks to few months, generally until the weight of ingested plant equals the weight of the animal) bone marrow aplasia develops, which results in an acute, usually fatal, clinical disease characterized by fever, hemorrhagic diathesis, thrombocytopenia and neutropenia. When cattle ingest less than10 g/kg/bw/day for longer periods (one year or more), a chronic disease characterized by intermittent hematuria is observed. This form is known as enzootic hematuria and is related to the development of tumors in the urinary bladder. Hemangioma is frequently found in these cases but several other types of benign and malignant neoplasms may occur.[7] Enzootic hematuria eventually leads to chronic anemia and death. As in this case, the development of SCC in the upper digestive tract of cattle is the third clinical manifestation related to the ingestion of bracken fern.[8] The occurrence of SCC in the digestive system of cattle is rare[6] and is virtually not observed in cattle grazing pastures where bracken fern is absent.[8] The clinical course associated with the SCC of the upper digestive tract in cattle is rather chronic (months to years) and the deleterious effects of the tumor are mainly mechanical and related to the interference with feeding and rumination. This leads to extreme malnutrition. Affected cattle are usually 5 years-old or older. It is presumed that the development of the SCC occurs when cattle ingest small amounts of bracken fern for extended periods (years) of time. The neoplasm occurs in one or more of the following anatomical sites in the bovine digestive tract:[5,8] base of the tongue, esophagus, cardia and rumen. Clinical signs include coughing, regurgitation of food, bloat, diarrhea and progressive loss of weight which eventually culminate in death. The clinical signs vary depending on the

location of the tumor, for example, coughing is related to tumors located in the base of the tongue and as in this case, bloat is related to tumors located in the esophagus or cardia. Metastasis occurs in some cases, mainly to the regional lymph nodes and lungs. However, SCC of the rumen may metastasize to the liver through the portal circulation.

A recent survey carried out by our lab that included necropsies of 14 cattle with SCC of the upper digestive tract (yet unpublished data) revealed multiple neoplasms in 57% of the cases and a single mass in 43%. The main affected anatomical sites were base of tongue (5/14), pharynx (3/14), epiglottis (6/14), proximal esophagus (3/14), distal esophagus (2/14), middle esophagus (3/14), entrance of the rumen ("cardia") (3/14) and rumen (2/14). Metastasis were observed in 50% of the cases and were identified in retropharyngeal (4/14), mediastinal (2/14), gastric/ruminal (3/14), hepatic (1/14), paravertebral (1/14) and mesenteric (1/14) lymph nodes; in the lungs (1/14) and in the liver (1/14). Usually a few or several papillomas were observed in the proximities of the malignant masses (SCC). Histological evidence of transition between benign (papilloma) and malignant (SCC) growths were occasionally found. The consistent finding of papillomas in the sites where SCC develop led to the suspicion that bovine *Papillomavirus* (BPV) has a role in the pathogenesis of bracken fern associated SCC in the upper digestive tract of cattle and this was later confirmed.[3,4]

There are six subtypes of BPV (BPV-1-6) that induce lesions with specific characteristics and distributions. SCC of the upper digestive tract in cattle is associated with prolonged ingestion of bracken fern and concomitant infection with BPV-4; whereas BPV-2 is associated with bladder tumors and enzootic hematuria in cattle feeding on bracken fern invaded pastures.[3,4] The BPV induced papillomas are benign growths that occasionally undergo malignant transformation due to genetic or environmental factors. In cattle, infection of the upper digestive tract with BPV-4 leads to the formation of papillomas which eventually regress (within approximately one year) due to a host-derived cell mediated immune response.[2] However, in cattle grazing bracken fern, which contains immunosuppressants, the papillomas persist for longer periods and may be transformed to carcinomas.[4] *In vitro* studies indicate that bracken fern is the co-carcinogen of BPV-4 in the pathogenesis of the SSC of the digestive tract in cattle. In addition, the flavonoid quercetin, a well known mutagenic compound of bracken fern, synergizes with BPV-4 in malignant transformation of papilloma cells.[3] The combination of increased viral BPV-4 transcriptional activity, the failure of cell arrest at G1 and the malfunction of the tumor suppressor protein p53 are thought to be the events contributing to transformation of the cell.[1,3] In fact, a strong epidemiological correlation between bracken fern consumption, high incidence of persistent papillomas, and SCC of the upper digestive tract has been noted. The progression from papilloma to SCC was experimentally reproduced in cattle fed bracken fern.[4]

In sheep, bracken fern causes progressive retinal degeneration referred to as bright blindness and in horses and pigs it has been described as causing a nervous disease due to thiamin deficiency.[5] However despite the high incidence of bracken fern poisoning in cattle, none of these conditions in sheep, pigs or horses were documented

in Brazil. The feeding of horses with high amounts of bracken fern failed to induce the neurological disease (Gava, personal communication).

Tumors of the digestive tract are reported in humans consuming the crosiers and rhizomes of bracken fern. In southeastern Brazil, a case-control study showed a 3.4 and 3.45-fold increased relative risk respectively for developing esophageal and gastric cancer in people who ingested bracken fern. Ptaquiloside, another carcinogenic substance isolated from bracken is present in the milk of cows fed this plant and it has been demonstrated that milk from these cows causes tumors in mice.[5]

AFIP Diagnosis: Rumen (per contributor): Squamous cell carcinoma, crossbred, bovine.

Conference Comment: The contributor provides a thorough overview of bracken fern toxicosis and the three clinical syndromes recognized in cattle, which are dependent on the dose and duration of ingestion. As mentioned by the contributor, bovine *Papillomavirus* (BPV) has a role in the pathogenesis of bracken fern associated SCC in the upper digestive tract of cattle.

Many viruses, both DNA and RNA, can cause neoplasia in humans and animals. The AFIP comments on conference 5, case III (October 2004) includes a list of common virally induced neoplastic diseases. At the cellular level, one of two consequences may follow infection with potentially oncogenic DNA viruses. If the infection is productive, the cells produce infective virus particles and the cells are lysed in the process. If the infection is non-productive, the cells survive and may be transformed, with introduction of specific gene sequences or gene products. Oncogenic DNA viruses contain specific viral oncogene products that are responsible for neoplastic transformation. The viral oncogene products are often virus specific with particular host cell targets. From the list below, it is apparent that many oncogenic DNA viruses share common mechanisms of cellular transformation mediated by their specific oncogene protein products. A prominent shared mechanism is the interaction with and functional inactivation of the tumor-suppressor gene products, Rb and p53, both of which play critical roles in processes responsible for cellular homeostasis, such as the cell cycle and apoptosis.[9]

Virus	Viral Oncogene Product	Cellular Target
Adenovirus	E1A (289aa)	Rb
	E1A (243aa)	Rb
	E1B (495aa)	p53
	E1B (175aa)	unknown
Polyomavirus	Large T-antigen	Rb
	Middle T-antigen	src
	Small T-antigen	unknown
SV40	Large T-antigen	Rb, p53

	Small T-antigen	unknown
Papillomaviruses		
BPV-1	E5	PDGF receptor
HPV-16	E6	p53
	E7	Rb

Contributor: Universidade Federal de Santa Maria, Departamento de Patologia, Santa Maria, RS, Brazil

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SLIDE 49
CONFERENCE 13 / CASE I – 1178/03 (AFIP 2948699)

Signalment: Four month-old, male, New Zealand White rabbit.

History: This rabbit was one of 20 rabbits in an experimental study inducing chronic cardiomyopathy. Doxorubicin (3 mg/kg) was given intravenously in the lateral ear vein

of three month-old male rabbits once a week for a six week period. The presented animal died after the fourth dose and was autopsied.

Gross Pathology: Mild alopecia was visible at the neck. There was 15 ml of watery fluid in the thorax. The myocardium was grayish-brown and the left ventricle of the heart was mildly dilated. The lungs showed moderate diffuse acute emphysema and edema. The testes were small and soft, and bone marrow was light-red but of normal consistency. The spleen and lymph nodes were light-brown, small and inactive. The gastrointestinal tract showed moderate diarrhea. The liver, kidneys and brain were without any specific morphological lesions.

Contributor's Morphologic Diagnoses: 1. Heart: Myocardial degeneration, eosinophilic and vacuolar, diffuse, with mild fibrosis, and multifocal, mild, mononuclear inflammation.
2. Bone marrow: Hypocellularity, diffuse, severe.
3. Testis: Degeneration and atrophy of the seminiferous tubular epithelium, diffuse, moderate.
4. Skin: Epidermal and adnexal atrophy, diffuse, moderate. (not submitted)
5. Intestine: Atrophy of the intestinal mucosa, moderate, with diffuse mild lymphoplasmacytic infiltration. (not submitted)
6. Lymph nodes, spleen: Hypocellularity, moderate. (not submitted)

Contributor's Comment: Anthracycline antibiotics, such as doxorubicin (adriamycin), epirubicin and daunorubicin, are effective anti-neoplastic agents which are widely used in cancer chemotherapy. However, administration of these agents is associated with a dose-related cardiomyopathy,[1] atrophy of hematopoietic tissues,[1] nephrotoxicosis,[2] and atrophy of skin and testes[1,3] as well as intestinal alterations.[4]

Cellular mechanisms of anthracyclines inducing heart failure are multifactorial and include local release of vasoactive substances,[5] cytotoxic effects of local free radicals,[6] inhibition of nucleic acid and protein synthesis,[7] and disturbed Ca^{2+} metabolism in cardiomyocytes.[8]

Acute doxorubicin toxicity is reflected by increased cytoplasmic eosinophilia and mild to moderate vacuolization of cardiomyocytes. Ultrastructurally, numerous vacuoles, swollen mitochondria with "onion ring" shaped cristae, and swollen sarcoplasmic reticulum occur. Myofibrillar loss as well as separation of intercalated discs and dilatation of the sarcotubular system is described.[3] In later stages small numbers of T-lymphocytes and histocytes surround the degenerating/necrotic myocytes[9] and interstitial fibrosis[1] is evident. Chronic effects may occur several weeks or months after repetitive exposure of cardiomyocytes to anthracycline. In humans, cardiovascular signs indicative of chronic cardiotoxicity include severe congestive heart failure.[10]

Further pathological systemic side effects due to anthracycline administration are described in organs with high turn over rate of cells leading to bone marrow depression, alopecia and atrophy of the skin, and testicular atrophy[1] as observed in the present

case. Additionally, in long term studies at about 17 weeks, necrosis and calcification of the liver, skeletal muscles and pancreas were observed.[1]

The lesions of the present case are typical findings induced by anthracyclines.[3,11] Since it is often used in experiments studying therapeutic models of cardiomyopathy, intensive investigations of this cardiac disease are available in literature. However, the effects on other organs are described sparsely[3,11] but may be responsible for the death of animals during experiments due to diarrhea, hemorrhagic diathesis and opportunistic infections.

AFIP Diagnoses: 1. Heart: Myocardial vacuolar degeneration, necrosis, and loss, diffuse, mild to marked, with multifocal mild fibrosis, New Zealand White, rabbit, lagomorph.
2. Bone marrow, hematopoietic cells: Hypocellularity, diffuse, marked.
3. Testis, spermatogenic epithelium: Degeneration and atrophy, diffuse, severe.

Conference Comment: The contributor provides a thorough overview of the dose-dependent cardiotoxicity of adriamycin. Both nutritional and toxic myopathies tend to result in degeneration and necrosis with little to no inflammation. However, if the animal survives long enough, inflammation may be present in response to the necrosis. Common causes are listed below:[12,13]

Disorder	Cause	Primary Species Affected
Ionophore toxicity	Monensin	Horses, pigs
	Lasalocid	Many
Plant toxicity	Cardiac glycosides (*Nerium oleander*)	Many
	Lantana camara	Small ruminants
	Gossypol	Young ruminants, pigs
	Cassia occidentalis	Many
	Hairy Vetch (*Vicia villosa*)	Many
	Calcinogenic plants (*Cestrum diurnum*) (*Trisetum flavescens)* (*Solanum malacoxylon*)	Many
Nutritional	Vitamin E/Selenium deficiency	Many
Other	Blister beetle (*Epicauta* sp.)	Horses

Many conference attendees considered nutritional causes of cardiomyopathy. Vitamin E and selenium deficiency is well recognized and has been described in many species, including rabbits. However, with rigid quality control standards for commercial feed, nutritional myopathy is relatively rare in laboratory colonies. Occasionally researchers

prepare specialized diets for specific research protocols, and deficiencies can result from errors in these formulations. Affected rabbits may present with stiffness and muscle weakness, infertility, or increased neonatal mortality. At necropsy, the musculature, especially of the diaphragm, paravertebral regions, and the hind limbs, may be pale with mineralized streaks. Microscopically, there is typically hyaline degeneration of affected myofibers and clumping and mineralization of the sarcoplasm. Interstitial fibrosis frequently occurs in lesions of longer duration.[14]

Contributor: Universität Leipzig, Institut für Veterinär-Pathologie, An den Tierkliniken 33, Leipzig, Germany

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SLIDE 50
CONFERENCE 13 / CASE II – 420230 (AFIP 2948690)

Signalment: Mixed breed dog, approximately 6 weeks of age.

History: Died acutely without any clinical signs.

Gross Pathology: The pup was in good to moderate body condition. The most prominent findings were ecchymoses in the mucosa of the digestive tract. The liver was congested and slightly enlarged. The gallbladder wall was edematous. The spleen and lymph nodes were edematous and congested.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, centrilobular, severe, with occasional intranuclear inclusion bodies consistent with Canine Adenovirus type 1 (CAV –1), mixed breed dog.

Contributor's Comment: Infectious canine hepatitis (ICH) caused by canine adenovirus -1 (CAV-1) was recognized as a specific viral disease of dogs in 1947.[1] The virus is a medium-sized DNA virus without a lipoprotein envelope. There is antigenic relationship between CAV-1 and CAV-2, and they provide cross-protective immunity.[2] Infection can cause severe disease in dogs, other canids, and also in bears (Family *Ursidae*).[3] The virus is ubiquitous, and is excreted in the urine of affected dogs for long periods of time. As with other adenoviruses, CAV-1 is resistant to environmental inactivation with chemicals such as chloroform, ether, acid and formalin. The virus survives for days at room temperature and remains viable for months at temperatures below 4° C. CAV-1 is inactivated after 5 minutes at 50 to 60° C. Chemical disinfection is successful using iodine, phenol and sodium hydroxide.[3]

Vaccination has greatly reduced the incidence of the disease and it is now rare in many countries. Infection with CAV-1 probably occurs in nature via the oral route.[4] The incubation period is from 4 to 7 days. Virus multiplication occurs first in the tonsils leading to tonsillitis and local lymphadenitis, and the infection reaches the blood via the thoracic duct. Viremia lasts between 4 to 8 days after infection and results in rapid dissemination of the virus to other tissues and body secretions, including saliva, urine and feces.[3]

The clinical signs caused by CAV-1 infections are due to cellular damage as a result of direct effects of viral replication. The virus of ICH has a special tropism for endothelium, mesothelium and hepatic parenchyma, and it is injury to these tissues that is responsible for the pathologic features of edema, hemorrhage and hepatic necrosis.

AFIP Diagnosis: Liver: Hepatocellular necrosis and loss, centrilobular and midzonal, diffuse, with marked congestion and hemorrhage, and basophilic hepatocellular intranuclear inclusion bodies, mixed-breed, dog.

Conference Comment: As mentioned by the contributor, due to vaccination infectious canine hepatitis is now rare in many countries in which it was once endemic.

Clinically, affected dogs may have inapparent infection, mild illness, or severe disease with vomiting, melena, high fever, abdominal pain, blanched mucus membranes with petechia, and occasionally icterus.[3] Virus-induced endothelial damage may lead to disseminated intravascular coagulation and hemorrhagic diathesis.[5] In the peracute form of the disease, the animal may be found dead without previously observed clinical signs.[3] Some recovering dogs will develop an immune complex uveitis (type III hypersensitivity) resulting in unilateral or bilateral corneal edema (blue eye).[5]

The virus has a special tropism for endothelium, mesothelium, and hepatic parenchyma, resulting in gross and microscopic lesions due to cellular injury. Grossly, the classic lesion is marked edema of the gallbladder wall. If the edema is mild, it may only be evident in the attachments of the gallbladder. The gallbladder may also be darkened by intramural hemorrhages.[3] Other lesions include edema and hemorrhage of the superficial lymph nodes, linear (paintbrush) hemorrhages on the serosa of the stomach,[3] widespread petechia and ecchymoses, fluid in serous cavities, fibrin strands on the surface of an enlarged, turgid and friable liver, or small foci of hepatocellular necrosis.[5] Gross lesions in other organs are inconsistent. Histologically, there may be hemorrhages in many tissues due to the endothelial tropism and the resultant destruction. At low magnification, the histologic changes in the liver appear similar to those caused by acute hepatotoxins producing a prominent centrilobular (periacinar) pattern. The virus is known to produce large, amphophilic to basophilic, solid, intranuclear inclusion bodies that often have a “smudgy” appearance and fill the nucleus. They may be found in hepatocytes, endothelial cells, Kupffer cells, renal tubular epithelium, bronchial epithelium, and primitive reticulum cells.[3] Ultrastructurally, adenoviruses are nonenveloped, 70-90 nm, icosahedral particles that form characteristic paracrystalline arrays within the nuclei of affected cells.[6]

Contributor: Kimron Veterinary Institute, Pathology Department, P.O. Box 12, Beit-Dagan, Israel

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SLIDE 51
CONFERENCE 13 / CASE III – 200404 (AFIP 2942014)

Signalment: Three-year-old, female, Holstein, bovine.

History: This animal was kept in a herd of cattle experimentally infected with bovine leukemia virus (BLV). Clinical examination did not reveal any abnormalities.

Laboratory Results: BLV serology: positive; nested PCR positive for BLV-proviral DNA

Hematology

Hct	24.0	%		
Hb	10.0	g/dl		
RBC	6.1	x 10^6/µl		
MCV	39.6	fl		
MCH	16.5	pg		
MCHC	41.7	%		
RBC morphology:	normal (echinocytes)			
Platelets:	347.0	x 10^3/µl		
MPV:	7.6	fl		
WBC:	16.9	x10^3/µl	H	(normal morphology)
Segm. Neu:	1.1	x10^3/µl (6.0 %)		
Band Neu:	0.1	x10^3/µl (1 %)		
Lymp:	14.5	x10^3/µl (86 %)	H	
Mono:	0.9	x10^3/µl (5.5 %)		
Eos:	0.3	x10^3/µl (1.5 %)		
Baso	0.0	x10^3/µl (0 %)		

Contributor's Morphologic Diagnosis: Pappenheim's stained peripheral blood smear: Parasitemia, numerous flagellated protozoa, etiology consistent with *Trypanosoma sp*.

Contributor's Comment: The blood smear contains on average in each 400x field two spindle-shaped curved protozoan organisms that are between erythrocytes and sometimes in proximity to aggregated platelets. The 30-35 µm long protozoa are characterized by tapered ends, an undulating membrane with a flagellum up to 30 µm long, a central nucleus and a large marginal kinetoplast. These morphological features, and the fact that they were isolated from a cow within Germany, are most likely consistent with *Trypanosoma (Megatrypanum) theileri.* These protozoan parasites occur with worldwide distribution and can be isolated from 50 to 70% of blood cultures from clinically healthy cattle. However, singular cases of higher parasitemia, serious clinical disease or even death are recorded in cattle severely stressed from concurrent disease or in newborn calves.[1] Parasitemia is common in cattle herds with concurrent BLV-infection.[2] *T. theileri* is transmitted mechanically by many biting fly species (*Tabanus*, *Haematopota*) and probably by ticks (*Rhipicephalus*, *Boophilus*, *Ixodes*). In general, the parasitemia is very low, and the trypanosomes are found incidentally in smears of blood or blood cell cultures. In many cases, detection is only possible after repeated blood culture (blood-agar-plates, Eagle's medium, BHJ-agar with 10% rabbit blood and incubation at 28 °C or 37 °C). Therapeutic approaches are usually not necessary.
The observed lymphocytosis in this case is related to the experimental infection of this animal with BLV and was shown by flow cytometry to be caused by an expansion of CD5+ sIgM+ B-lymphocytes. This is considered as characteristic for the persistent lymphocytotic (PL) stage of the disease.

AFIP Diagnosis: Peripheral blood smear: Trypomastigotes, numerous, and a relative lymphocytosis, Holstein, bovine.

Conference Comment: *Trypanosoma theileri* is classified within the phylum Sarcomastigophora, class Zoomastigophorea, order Kinetoplastida, family Trypanosomatidae, genus *Trypanosoma*, subgenus Megatrypanum. It is one of the largest mammalian trypanosomes and is commonly an incidental finding in clinically healthy cattle, but may cause serious disease in immunocompromised animals.[3]

Blood sucking arthropods, especially tabanid flies such as the common horsefly, serve as vectors. Following ingestion during a blood meal, trypomastigotes undergo cyclic development in the insect's gut. The infective stages are then excreted during subsequent feedings and enter the host through the bite wound or abrasions in the skin.[4] Other *Trypanosoma* spp. are listed below:[3,5]

Organism	Transmission	Species	Disease / Lesions
T. cruzi	Reduviid bugs	Dogs	"Chagas' disease"; myocarditis
T. brucei	Tsetse fly	Ruminants	"Nagana disease"; anemia
T. congolense	Tsetse fly	Ruminants	"Nagana disease"; anemia
T. vivax	Tsetse fly	Ruminants	Anemia
T. evansi	Biting fly	Many	"Surra"; edema, emaciation

T. equinum	Biting fly	Horses	“mal de Caderas”
T. equiperdum	Coitus	Horses	“Dourine”; genital plaques
T. gambiense	Tsetse fly	Humans	“African Sleeping Sickness”
T. rhodesiense	Tsetse fly	Humans	“African Sleeping Sickness”
T. cervi	Horsefly	Deer	Nonpathogenic
T. melophagium	Sheep ked	Sheep	Nonpathogenic

As mentioned by the contributor, *T. theileri*, may be pathogenic in animals that are stressed or otherwise immunocompromised. This animal was experimentally infected with bovine leukemia virus (BLV), the cause of enzootic bovine lymphoma. BLV is a retrovirus and has a high incidence in dairy cattle. Transmission is primarily horizontal via blood-sucking arthropods or by fomites. Clinical expression peaks at 6-8 years post-infection with animals often presenting with enlargement of one or more superficial lymph nodes. Clinical signs reflect the location of the lesions: unilateral or bilateral exophthalmus with involvement of retrobulbar lymphoid tissue; diarrhea with gastrointestinal involvement; congestive heart failure if the heart, most commonly the right atrium, is affected; or posterior paresis or paralysis with nervous system involvement. In addition, animals may have lesions in the liver and spleen with involvement of the bone marrow and leukemia during the terminal stages of the disease. A persistent lymphocytosis develops in approximately 30% of animals and may occur without, or prior to, clinical expression of the disease.[6]

Contributor: Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Boddenblick 5A, Greifswald – Insel Riems, Germany
www.bfav.de

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SLIDE 52
CONFERENCE 13 / CASE IV – N04-46 (AFIP 2937499)

Signalment: 8-year-old, female, mongrel dog, canine (*Canis familiaris*).

History: This animal was presented with a one-week history of hematuria and mild constipation. A mass was palpated in the caudal abdomen during physical examination. A pneumocystogram revealed a tumor within the urinary bladder. The growth was located on the ventral surface near the trigone area. It was removed surgically and submitted for examination.

Gross Pathology: A papillary mass measuring 5.5 x 5 x 2.5 cm was submitted for histological assessment. The papillary projections were gray white and intermixed with blood clots. The bladder wall underneath the tumor was thickened and sclerotic.

Laboratory Results: A complete blood count revealed lymphocytosis, granulocytopenia, and polycythemia.

Lymphocytes= 57% (10.0-40.0)
Granulocytes= 37.7% (50.0-80.0)
RBC= 9.24 m/mm^3 (5.5-8.5)
MCV= 75.1 fl (58.0-73.0)
Hct= 69.3% (35.0-55.0)
Hb= 19.6 g/dl (10.0-18.0)

Contributor's Morphologic Diagnosis: Urinary bladder: Transitional cell carcinoma, papillary, infiltrating.

Contributor's Comment: Sections of the submitted tissue reveal multiple, tall papillary growths covered by multiple layers of closely-packed, columnar to polygonal cells supported by thin stalks of fibrovascular connective tissue. Tumor cells have moderate amounts of pale eosinophilic, often vacuolated cytoplasm, with well-defined cell borders. Tumor cell nuclei are round to oval, euchromatic, with finely granular chromatin, usually single prominent nucleoli, and show moderate anisokaryosis. Mitotic figures are common (1-3 per random 40x field). Small clusters of neoplastic cells infiltrate into the stalks of the tumor, the lamina propria, submucosa, and muscle layers of the bladder wall. Multifocally, variably sized aggregates of lymphocytes mixed with lesser numbers of eosinophils are scattered throughout the stalks and lamina propria. Gross and histomorphologic features of this case are characteristic of the papillary and infiltrating variant of transitional cell carcinoma.

Neoplasia of the urinary bladder is common in dogs, relatively frequent in cats, and rare in all other species. Cattle rarely develop urinary bladder tumors spontaneously but have a high prevalence (as high as 25%) in endemic areas where bracken fern (*Pteridium* spp.) grows.[1] The etiology of bladder cancer in dogs is unknown. However, topical insecticides containing inert petroleum products commonly used for fleas and ticks, in addition to obesity, appear to increase the risk of bladder cancer in this species. Tryptophan, its metabolites, and the cytotoxic drug cyclophosphamide have also been

incriminated in the development of bladder cancer in dogs.[2] In humans, occupational exposure from employment as aniline dyes manufacturers, painters, farmers, rubber workers, electrical workers, pesticide applicators, hairdressers, truck drivers, petroleum and other chemical industry workers have a well-established higher risk of bladder cancer.[2] Cigarette smoking, chronic cystitis, schistosomiasis of the bladder, and certain drugs (cyclophosphamide) are also believed to induce a higher risk of bladder tumors.[3]

Transitional cell carcinoma (TCC) is the most commonly diagnosed tumor in the urinary bladder of domestic animals. Approximately, 75-90% of primary epithelial urinary bladder neoplasms in dogs are TCC.[1] This is a neoplasm of older dogs (average 9-11 years) and apparently, females are more susceptible (2:1 ratio of female to male).[4,5] Breeds that may have a greater risk include Airedales, beagles, and Scottish terriers. Nearly 90% of affected dogs present with clinical problems referable to the urinary system such as hematuria, pollakiuria, or dysuria.[4] Paraneoplastic diseases associated with bladder tumors include hypercalcemia, cachexia, hyperestrogenism, hypertrophic osteopathy, and, as in this case, polycythemia.[1]

The most common location of TCC in dogs is in the trigone area of the urinary bladder. Most tumors are solitary and only rarely are multiple on gross examination. These tumors are divided based on their patterns of growth as papillary (project into the lumen), or nonpapillary (sessile or flat) and infiltrating (90% in dogs) or noninfiltrating (10% in dogs).[1]

Transitional cell carcinomas are one of the most malignant neoplasms in domestic animals. Metastases are present in the majority (50-90% of cases) of dogs at necropsy;[4,5] lungs and lymph nodes are the two most common sites, but bones[6] are frequently involved. In dogs, reported rates to regional lymph nodes are 48% and for distant sites 51%.[4,5] Some features have been associated with survival such as sex and treatment selection. Some investigators found that spayed females survive significantly longer than castrated males (358 days versus 132 days) and dogs that received doxorubicin or mitoxantrone in addition to a platinum based chemotherapeutic (either cisplatin or carboplatin) lived significantly longer than those that received only a platinum compound (358 days versus 132 days).[7]

This bitch was clinically normal one month after surgical removal of the mass. Unfortunately, the owner rejected the option of chemotherapy and there was not further clinical follow up.

AFIP Diagnosis: Urinary bladder: Transitional cell carcinoma, papillary and infiltrating, mixed-breed, canine.

Conference Comment: The contributor provides a thorough overview of transitional cell carcinoma, including etiology, location, and histomorphologic categorization.

Conference attendees also discussed common causes of a positive urine occult blood test and how to differentiate hematuria from hemoglobinuria and myoglobinuria.

Hematuria is an increased erythrocyte concentration in the urine sediment and may be due to pathologic urinary system hemorrhage, iatrogenic hemorrhage, or genital tract hemorrhage. Causes of pathologic hemorrhage include the following: vascular damage due to trauma, inflammation, or renal infarcts; poor repair of small vessels due to thrombocytopenia, thrombocytopathia, or von Willebrand disease; or acquired or congenital coagulopathies. Iatrogenic hemorrhage may result from trauma during bladder palpation, cystocentesis, or catheterization. Genital tract hemorrhage is often associated with estrus in voided samples. Hemoglobinuria is most often due to intravascular hemolysis, while myoglobinuria is a result of muscle disease.[8]

With hematuria, the urine appears red and cloudy and will usually clear with centrifugation. Erythrocytes will be present in the urine sediment. There should not be clinical or laboratory evidence of hemolytic anemia or muscle disease. With hemoglobinuria, the urine is red to brown and does not clear with centrifugation and excessive numbers of erythrocytes will not be present in the sediment. There is a concomitant hemoglobinemia as free hemoglobin will discolor plasma before it saturates serum haptoglobin or causes hemoglobinuria. Clinically, there may be evidence of intravascular hemolytic anemia. With myoglobinuria, the urine is also red to brown, does not clear with centrifugation, and excessive numbers of erythrocytes will not be present in the sediment. Unlike hemoglobinemia, the plasma will be clear and of normal color. Clinical or laboratory evidence of muscle disease should be present rather than evidence of anemia. To differentiate urine hemoglobin from myoglobin in the laboratory, the addition of saturated ammonium sulfate solution will remove the color by precipitating the hemoglobin. Conversely, ammonium sulfate solution will not precipitate myoglobin and the urine will remain discolored. A better technique to differentiate hemoglobin from myoglobin is spectrophotometric analysis.[9]

Contributor: Departamento de Patología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán, Mexico
http://www.veterin.unam.mx

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SLIDE 53
CONFERENCE 14 / CASE I – 25078 (AFIP 2936460)

Signalment: One-year-old, male, Basset Hound, dog.

History: The dog was admitted at the Universidade Federal de Minas Gerais (UFMG) Veterinary Hospital with a history of lameness of the left hind limb for the past 4 months, and progressive weight loss. Radiological changes included a periosteal proliferative reaction in the metaphysis of the left femur suggestive of either an inflammatory or a neoplastic lesion (in spite of the age of the dog). Samples were obtained by fine needle aspiration for cytological exam, and a serum sample was processed for serological diagnosis of leishmaniasis.

Gross Pathology: Soon after euthanasia, fragments of the proximal metaphysis of the left femur were submitted for histopathology and a necropsy was not performed.

Laboratory Results: The serological tests for leishmaniasis yielded the following results: Indirect immunofluorescence: reactive 1/160; Complement fixation: reactive 1/160; ELISA: positive

Non-neoplastic mesenchymal cells and macrophages containing intra-cytoplasmic amastigotes were observed by microscopic examination of fine needle aspirates from the bone lesion. Immunohistochemical staining was strongly positive for *Leishmania* sp..

Contributor's Morphologic Diagnoses: 1. Bone, periosteum: Periostitis, histio-plasmacytic with periosteal proliferation and immature bone formation, osteoblastic hyperplasia and hypertrophy, intense osteoclasia, and innumerous intra-cytoplasmic amastigotes in macrophages, chronic, focal, moderate.
2. Bone marrow: Fibrosis, chronic, multifocal; and accumulation of macrophages containing intra-cytoplasmic amastigotes, diffuse, moderate.

Etiologic diagnosis: Protozoal periostitis

Etiology: *Leishmania chagasi*

Contributor's Comment: Visceral leishmaniasis (VL) is a disease caused by protozoa from the genus *Leishmania*, particularly *L. donovani*, *L. infantum*, and *L.* chagasi.[5] VL is a significant public health concern in several Brazilian States, including the State of Minas Gerais. The dog is considered the most important reservoir for human VL, particularly in urban areas.[1,2,3]

The clinical manifestation of canine VL is usually chronic, and associated with cachexia, cutaneous lesions, hepatomegaly, splenomegaly, and lymphadenopathy.[5] Osteo-articular involvement in cases of VL has been described in dogs,[4,6,8] and is usually due to either the inflammatory response to the parasite or accumulation of immune complexes in the joints.[6]

Although VL is widespread in Brazil, periosteal proliferation with the intensity and localization described here is extremely uncommon. As in several other areas in Brazil, VL is considered an emerging disease in the State of Minas Gerais. In spite of the large number of dogs with VL presented to the UFMG Veterinary Hospital, this was the first case in which the primary complaint was a skeletal condition due to periostitis associated with *Leishmania* sp.

Joint lesions occur in approximately 37.5% of VL cases. These changes are often associated with reluctance to walk, arthralgia, and periosteal proliferation in the periphery of the joint.[7] Interestingly, in this case the dog had no clinical signs of VL such as lymphadenopathy, which is one of the most frequently observed clinical signs.[5]

The diagnostic approach employed in this case allowed us to establish the etiology of the process to the level of classification as *Leishmania* sp.. However, considering the geographic distribution of the donovani complex Leishmania species, it can be assumed that the agent involved in this case was *Leishmania chagasi*, which is the agent of VL in the New World.

Considering the significance of the dog as a major reservoir for human VL, particularly in urban areas, it is important for clinicians to keep unusual clinical manifestations of VL including skeletal changes in their list of differentials.

AFIP Diagnosis: Bone, proximal metaphysis of left femur (per contributor): Osteomyelitis and periostitis, plasmacytic and histiocytic, multifocal, moderate, with reactive bone formation, periosteal fibroplasia, and myriad intrahistiocytic amastigotes, etiology consistent with *Leishmania* sp., Basset Hound, canine.

Conference Comment: Leishmaniasis is a zoonotic disease caused by many pathogenic species of the genus *Leishmania*. Histologically, the amastigotes are 2-4 _m, round to oval, with clear cytoplasm and a kinetoplast perpendicular to the nucleus. The kinetoplast is a specialized mitochondrion.[9] Organisms are usually located in the cytoplasm of macrophages, but have also been reported in neutrophils, eosinophils, endothelial cells and fibroblasts.[6] Clinical pathology findings in cases of leishmaniasis include hypergammaglobulinemia, hypoalbuminemia, nonregenerative anemia, thrombocytopenia, uremia and proteinuria.[7]

Differential diagnoses include *Histoplasma capsulatum, Sporothrix schenckii, Trypanosoma cruzi,* and *Toxoplasma gondii. H. capsulatum* and *S. schenckii* can be differentiated by special stains for fungal organisms, such as GMS or PAS. Unlike *Leishmania* sp., *T. cruzi* is located primarily within muscle and its kinetoplast is parallel to the nucleus.

As the contributor stated, periosteal proliferation to this extent is an unusual finding with leishmaniasis. More common causes of periosteal proliferation in dogs include hypertrophic osteopathy, *Hepatozoon americanum* infection, craniomandibular osteopathy, osteosarcoma and osteomyelitis.[11]

Although endemic throughout much of the world, there are only rare reports of leishmaniasis in dogs in the southern and midwestern United States. In 1999, *L. infantum* was diagnosed in an outbreak of foxhounds in the northeastern US. Beagles and Bassett hounds housed in the same kennel and with a similar travel history as the foxhounds were seronegative. The cause of the increased susceptibility of foxhounds to leishmaniasis is unknown.[10] The clinical disease depends largely on whether the animal mounts a predominantly Th1 or Th2 response to the parasite. The development of a Th1 immune response is important in the control of leishmanial infections. Th1 cells secrete interferon-gamma, which activates macrophages to kill the parasites. Whereas a predominantly Th2 response results in the release of IL-4, IL-10 and IL-13 which inhibit the activation of macrophages thereby preventing the killing of leishmanial organisms, and stimulate immunoglobulin production which may result in immune complex deposition.[7,9]

Contributor: Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
http://www.vet.ufmg.br

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SLIDE 54
CONFERENCE 14 / CASE II – 03-03451 (AFIP 2948752)

Signalment: 4 month-old Arabian foal.

History: This foal had a fever for 2 days and a 4 day history of dyspnea, coughing, and mild subcutaneous edema of the head and neck. Euthanasia was elected due to a poor prognosis. No clinical signs appeared in other foals or the adult horses of the same farm.

Gross Pathology: The cranioventral pulmonary areas (approximately 60-70% of total lung surface area) were bilaterally consolidated, failed to collapse, and had a corrugated surface. On cut section, there were random multiple and variably-sized (0.2 to 2 cm), multifocal to coalescing abscesses that contained casseated white necrotic material.

Laboratory Results: High numbers of *Rhodococcus equi* were grown on culture and seen on smears.

Contributor's Morphologic Diagnosis: Lung: Subacute Bronchopneumonia, multifocal to coalescing, suppurative and histiocytic with intralesional intrahistiocytic rods consistent with *Rhodococcus equi*.

Contributor's Comment: *Rhodococcus equi* (*R. equi*) is a Gram positive facultative intracellular pathogen that causes significant respiratory disease with an occasional enteric form in foals less than 6 months of age.[1] Histology is characterized by chronic suppurative/pyogranulomatous bronchopneumonia and ulcerative enteritis. Although rare, infections are also recorded in other mammals including goats. Transmission is primarily by inhalation and rarely by ingestion and may be facilitated by poor dusty conditions. *R. equi* is characterized by the presence of virulent and avirulent strains. Despite the fact that most environmental isolates are avirulent, the isolates from diseased foals are always virulent.[2] The virulent isolates are characterized by the presence of an 85 or 90 kb virulence-associated plasmid (Vap).[3] Virulent strains can survive within the macrophages likely due to the products of Vap genes.[4]

AFIP Diagnosis: Lung: Bronchopneumonia, pyogranulomatous, multifocal, severe, with myriad intrahistiocytic coccobacilli, Arabian foal, equine.

Conference Comment: *R. equi* causes pyogranulomatous pneumonia with abscessation, lymphadenitis, ulcerative enterocolitis, and less commonly, osteomyelitis in foals. *R. equi* has been reported to cause lymphadenitis in swine, sheep, cattle, llamas and cats. Disseminated infections are reported in goats, primarily causing hepatic and pulmonary abscesses. Unlike foals, avirulent strains of *R. equi* may cause disease in goats.[5] Although all foals are susceptible to *R. equi*, those with compromised immune systems from failure of passive transfer or combined immunodeficiency (CID), are particularly vulnerable to diseases such as those caused by *R. equi*, *Pneumocystis carinii* and equine adenovirus.

Contributor: Department of Veterinary Pathology-Alexandria University, Egyptian Society of Comparative and Clinical Pathology, Alexandria, Egypt

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SLIDE 55
CONFERENCE 14 / CASE III – 0303046 (AFIP 2937764)

Signalment: Approximately 6 month-old female laboratory Beagle (*Canis familiaris*) (9 kg).

History: Five day duration of sudden onset muscle rigidity especially noticeable in the neck and shoulder. Muscle mass appeared to be increased.

Gross Pathology: Most proximal muscles in each limb were pale and firmer than normal, especially in the thoracic limbs and neck. Fresh samples of affected muscles 20x10x5mm would stand out straight horizontally when held by one end (compared to samples from normal dogs, which hang down vertically).

Laboratory Results: Elevated CPK (762 U/l compared to lab normal of 242 U/l), normal liver enzymes, total protein, WBC normal (with no eosinophilia), RBC parameters normal.

Contributor's Morphologic Diagnosis: Myopathy, characterized by degeneration, necrosis, and regeneration, with minimal to mild histiocytic inflammation.

Contributor's Comment: The proximal muscles of the thoracic limbs were most severely affected, but pelvic limb muscles, diaphragm, temporalis and pharyngeal muscles were affected to a lesser extent. Section provided is from the rhomboideus or serratus ventralis muscle, both of which were markedly affected. Microscopically, most muscles examined, including several muscle groups that appeared grossly normal (e.g. diaphragm and esophagus), were characterized by differing degrees of myopathy (degeneration and regeneration). The myopathy was characterized by nuclei located centrally instead of peripherally (minimal in mildly affected muscles), wide variation in cross-sectional diameter, and in more extensively affected muscles by multifocal cytoplasmic hypereosinophilia (usually in central sarcoplasm), granularity, and loss of cross-striations. Granularity of sarcoplasm was due to abnormal organization of myofibrils (myofibril disarray). Occasional fibers were necrotic. Inflammation (predominantly histiocytic, with occasional neutrophils) was present in the most severely affected muscles, but was not a prominent feature (i.e. was considered a response to necrosis). Rarely, ring fibers were noted. Regeneration was present and characterized by rowing of nuclei, which were plump, euchromatic, and had prominent nucleoli; associated cytoplasm was usually slightly basophilic.

Based upon the intense rigidity of the muscles of this dog clinically and immediately post mortem, and on the microscopic findings, the myopathy in this dog is consistent with a diagnosis of "myotonic dystrophy". Many myotonic dystrophies are inherited (e.g. in Chow Chows, Miniature Schnauzers, Staffordshire terriers, myotonic goats, and humans), although myotonia can also occur following administration of cholesterol lowering agents, corticosteroids, and rarely in cases of hypothyroidism.

Myotonic Dystrophies:

Myotonic dystrophy is the most common form of muscular dystrophy in humans, with an estimated incidence of 1 in 8,000. The two types of heritable myotonic dystrophies in people are designated as type 1 (DM1, Steinert's disease) and type 2 (DM2, proximal myotonic myopathy or PROMM). Both are dominantly inherited, multiorgan diseases.

Muscle pathology in both DM1 and DM2 includes central nuclei (sometimes in chains), angular/atrophic fibers, hypertrophic fibers (hence wide variation in fiber diameter), necrotic fibers, fibrosis, and deposition of adipose tissue.[1] DM2 is known as proximal myotonic myopathy because muscle symptoms (pain, stiffness, myotonia, and weakness) characteristically involve proximal limb muscles.

Both DM1 and DM2 also cause a variety of extramuscular effects in a proportion of patients. These include cardiac conduction abnormalities, cataracts, diabetes, testicular failure, and hypogammaglobulinemia. DM1 also results in mental retardation and skeletal abnormalities in the congenital form.[1]

DM1 is due to DNA $(CTG)_n$ repeats that cause a "gain-of-function" at the RNA level, wherein $(CUG)_n$ RNA transcripts accumulate, resulting in aberrant splicing of chloride channel pre-mRNA, loss of CIC-1 (chloride channel) protein from the membrane surface, and therefore reduced membrane conductance to chloride.[3] DM2 results from $(CCTG)_n$ repeats having similar effects to DM1.[1] Reduced membrane conductance results in membrane hyperexcitability, with subsequent degeneration, necrosis, and attempts at regeneration.

Naturally-occurring animal models of the human disease include the myotonic goat and various dog breeds in which myotonia is inherited. An autosomal dominant mutation in the goat CIC-1 gene results in reduced channel conductance and hyperexcitability. Similarly, in miniature Schnauzer dogs, a missense mutation in CIC-1 has been identified causing recessive myotonia congenita by a similar mechanism.[4] These models have assisted in understanding the electrophysiology and function of the chloride channels, whereas transgenic mouse models of the human disease were used to elucidate the RNA splicing regulation abnormalities and "gain-of-function" mechanism of the $(CTG)_n$ and $(CCTG)_n$ repeats observed in humans.[2,5]

AFIP Diagnosis: Skeletal muscle: Myocyte degeneration and necrosis, multifocal, moderate, with regeneration, variation in fiber size, satellite cell proliferation, and endomysial fibrosis, Beagle, canine.

Conference Comment: Muscular dystrophies (MD) are a heterogeneous group of inherited disorders that cause progressive muscle weakness and wasting. In humans, MDs are divided into several groups, the most common of which are X-linked MD, autosomal MD, and myotonic dystrophy. The MDs all have similar histological lesions of muscle degeneration, necrosis and regeneration. Clinical correlation, genetic testing and electromyography are often used for definitive diagnosis of a specific MD.[6]

Myotonia refers to a sustained involuntary contraction of a group of muscles. Humans affected with myotonic dystrophy describe "stiffness" and an inability to release their grip after a handshake. The contributor has provided an excellent overview of the pathogenesis of myotonic dystrophy. Alterations in the CIC-1 (chloride channel) protein results in reduced chloride conductance, membrane hyperexcitability, and ultimately muscle degeneration. There are several animal models of myotonic dystrophy. Mutations in the CIC-1 (chloride channel) protein have been documented in the myotonic (or "fainting") goat and the miniature Schnauzer.[4] Myotonic dystrophy has also been reported in the Chow Chow and the Staffordshire Terrier. Gross lesions are variable, primarily depending on the stage of the disease, and range from muscular atrophy to hypertrophy. In this case, the clinical and gross findings of muscle rigidity correlate with the diagnosis of myotonic dystrophy.

The best known example of X-linked MD in humans is Duchenne MD. The cellular defect occurs in the gene encoding for the dystrophin protein. Dystrophin connects the intracellular contractile apparatus and the extracellular connective tissue matrix. Animal models include the *xmd* dog, *mdx* mouse and cats. In most species, X-linked MD causes muscular atrophy. However, affected cats develop muscular hypertrophy and the condition is known as "Hypertrophic feline muscular dystrophy". The muscles of the neck, tongue, diaphragm and pectoral girdle are most commonly affected.[7]

Contributor: Toxicology Battelle, Pathology Dept, Room 7-0-37A, Batelle Columbus Labs, 505 King Ave, Columbus, Ohio
www.battelle.org

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SLIDE 56
CONFERENCE 14 / CASE IV – 201602-1 (AFIP 2956376)

Signalment: 4 week-old female Simmental cow (*Bos bovis*).

History: This calf had watery and mucoid diarrhea over a long period of time. The calf was presented with dehydration and acidosis. It had lesions between the digits and on the muzzle.

Gross Pathology: At necropsy, especially in the esophagus, rumen and small intestine there were multiple linear erosions. In the small intestine, Peyer's patches were moderately depleted. Multiple erosions in the interdigital clefts and on the lower lip were seen.

Laboratory Results: Serology for Bovine Viral Diarrhea virus: antigen positive / antibody negative.

Contributor's Morphologic Diagnosis: Rumen: Rumenitis, erosive, multifocal, subacute, mild to moderate with single cell necrosis and hydropic degeneration of epithelial cells, bovine.

Contributor's Comment: Multifocally, mostly the basal layer of the epithelium and occasionally the upper layers show hydropic degeneration (swollen cells characterized by intracellular edema and clear pale eosinophilic vacuoles within the cytoplasm) and there are scattered apoptotic cells (characterized by hypereosiniphilia and condensed or karyorrhectic nucleus). There is some accumulation of lymphocytes surrounding these degenerated epithelial cells (satellitosis). In a focal extensive area, on the epithelium, there is a mild hyperkeratotic, orthokeratotic sometimes parakeratotic, layer with mild serocellular crusts and there are scattered small pustules (not on all slides). The epithelium is mildly eroded and mildly and irregularly hyperplastic.

Etiology: Bovine pestivirus

The Bovine Viral Diarrhea virus belongs to the family Flaviviridae, genus Pestivirus. It is a small, enveloped, positive strand RNA virus. The Bovine Viral Diarrhea virus (BVDV) is found in cattle; but can also infect sheep, goats and pigs and has been isolated in many wild and captive African species including the rhinoceros, giraffe and eland. There are two biotypes--cytopathic (CP) and noncytopathic (NCP), each with two serovars.

For the pathogenesis, the virus is shed in fluids (saliva, blood, oculonasal discharge, urine, feces, semen, uterine secretions, amniotic fluid, fetal tissue and blood). The primary replication takes places in the tonsils and oropharyngeal lymphoid tissues. The virus enters circulating monocytes. It is transported to lymphoid tissues and the subepithelial connective tissues of the dermis and the GI tract and spreads locally to the overlying epithelial cells. The outcome depends on viral strain and virulence, immune status of the host, whether or not the animal is pregnant, and the stage of pregnancy. Infection with some NCP-BVDV may produce a thrombocytopenia and hemorrhage. The virus can also inhibit macrophage chemotaxis and neutrophil function (animals may have concurrent pneumonia or mastitis).

<u>Acute Bovine Virus Diarrhea:</u>
Most BVDV infections in immunocompetent, nonpregnant cattle are subclinical and of the NCP type. Clinical disease is usually seen in seronegative, immunocompetent cattle from 6-months to 2-years old.

Transplacental infections can occur with CP strains:
Between 50-100 days: fetal death, abortion, mummification
Between 100-150 days: congenital defects (microencephaly, cerebellar hypoplasia, hydranencephaly, hydrocephalus, microphthalmia, thymic aplasia, hypotrichosis, alopecia, brachygnathism, growth retardation, pulmonary hypoplasia)

Transplacental infections can also occur with NCP strains:
Before 100-125 days: immunotolerance and persistent infection (bovine fetuses become immunocompetent at 150-200 days)
After 150-200 days: calves may be born with neutralizing antibodies and may be unthrifty, slow growers or show no clinical signs

<u>Persistent Infection:</u>
Several factors have influenced the persistence of BVDV in cattle. Non-lytic infections produced by non-cytopathic BVDV strains, and the ability to evade the host immune response, are the primary mechanisms of persistence. In addition, some man-made factors have provided opportunities for BVDV to persist in cattle populations. Others mechanisms unique to BVDV probably result from its adaptation to cattle as a primary host.

The ability to induce fetal persistent infections is a unique aspect of BVDV pathogenesis. An additional requirement of this mechanism is a non-lytic infection with BVDV, which does not adversely affect fetal development and maturation. This unique phenomenon is the primary mechanism whereby BVDV is maintained in cattle populations providing for direct and indirect transmission. Although persistently infected (PI) animals may represent approximately one percent of the cattle population, they shed virus and initiate further virus replication and genetic variation. Therefore, control and prevention programs must focus on prevention of persistent infections and identification and removal of PI animals. Breaking the cycle of exposure of pregnant animals in the first 125 to 150 days of gestation is the key to preventing persistent infections.

As an RNA virus, BVDV generates mutations that precipitate antigenic changes. Changes in the E2 glycoprotein are the primary sites of variation in neutralizing epitopes. Recently, the phylogenetic classification of BVDV isolates as type Ia, Ib, and II has emphasized the significance of genetic variation. Infection of immunocompetent animals with BVDV stimulates cross-reactive antibody and provides protection from disease due to infection with diverse strains. Due to the ease with which BVDV crosses the placenta, the fetus may remain susceptible to infection although the pregnant dam is protected by cross-reactive antibody. Variations in BVDV have led to vaccine failures against fetal infection due to differences between vaccine virus and field virus. However, continued genetic and antigenic variation is responsible for the circulation of BVDV in susceptible cattle and the development of persistent fetal infections in susceptible pregnant animals. The move toward multivalent vaccines is in response to the recognition of the importance of genetic variation of BVDV.

Recently, Voges et al. reported a chronic BVDV infection in the testicles of a bull that was previously acutely infected with the virus. The bull was not viremic and possessed high levels of anti-BVDV antibody while shedding approximately $10^3 CCID_{50}$ of virus/ml of semen. Currently, studies are being conducted to determine the prevalence and potential of chronic persistent infections that may follow acute BVDV infections. The establishment of chronic infections would provide an additional mechanism for BVDV to persist in cattle populations.

Recognition of the pathogenic mechanism of immunotolerant fetal persistent infections was an important step in the evolution of BVDV control and prevention. The identification and removal of PI animals is an important component of current prevention and control methods. Due to the prevalence of PI animals and their shedding of BVDV they represent a high risk and are justifiable targets of control methods. However, it is clear that BVDV has many mechanisms at its disposal to ensure that it can persist and be maintained in cattle populations. When this is considered, the slow progress in preventing and controlling BVDV infections is understandable. In addition, this aspect will be an important consideration as increased emphasis is placed on the eradication of BVDV from cattle.

Mucosal disease (MD) develops when immunotolerant cattle (infected with a NCP strain in utero) are infected with a CP strain, or it may occur with introduction of an exogenous CP virus or a mutation of the endogenous NCP virus that becomes CP. Cattle with MD can infect other animals in the herd. The greater the genetic homogeneity between the CP and NCP strains, the shorter the clinical course. Less similar viruses produce a disease with a more protracted clinical course. Case fatality rates approach 100%.

Typical gross findings are:

With BVD: Erosions or shallow ulcerations of the oral cavity

With early MD: Erosions in oral and nasal mucosa, esophagus (linear), rumenal papillae, abomasum, omasum, cecum, and colon; ulcers at the interdigital cleft, vulva and testis; blunting of the oral papillae; Peyer's patches swollen, necrohemorrhagic +/- diphtheritic membrane

With chronic MD: Alopecia and hyperkeratosis (especially on the neck), chronic erosive lesions in the mouth and skin at mucocutaneous junctions, and around hooves and horns

Typical histological findings are:

Severe, acute inflammation in intestinal mucosa, especially overlying Peyer's patches, destruction of underlying crypts, stromal collapse, lymphocytolysis

Hyaline degeneration or fibrinoid necrosis of blood vessels; vasculitis in multiple organs accompanied by a mild-to-moderate mononuclear cell infiltrate in the vessel walls and perivascular tissues

Mesenteric lymph nodes and spleen: lymphocytolysis and lymphoid depletion

Erosions in the skin similar to those in the mucosa

Differential diagnoses are:

Pestiviral thrombocytopenia: similar clinical signs, diarrhea less pronounced, with profound thrombocytopenia

Rinderpest (Paramyxoviridae-Morbillivirus): intranuclear/intracytoplasmic inclusion bodies, syncytia

Malignant catarrhal fever (Herpesviridae - gammaherpesvirus): similar gross findings plus conjunctivitis and corneal edema; lymphoblastic and lymphocytic necrotizing vasculitis

Infectious bovine rhinotracheitis (Herpesviridae - alphaherpesvirus): Similar gross findings; epithelial necrosis, intranuclear inclusions.

Diseases with oral lesions only: foot and mouth disease (Picornaviridae - Aphthovirus); vesicular stomatitis (Rhabdoviridae-Vesiculovirus); bluetongue (Reoviridae-Orbivirus); bovine papular stomatitis (Poxviridae-Parapoxvirus); and necrotic stomatitis or oral necrobacillosis (Fusobacterium necrophorum)

Diseases with diarrhea only: salmonellosis (*Salmonella dublin* and *S. typhimurium*), winter dysentery (“coronavirus”), paratuberculosis (*Mycobacterium avium paratuberculosis*), and intestinal parasitism

AFIP Diagnosis: Rumen: Rumenitis, erosive, subacute, multifocal, moderate, with epithelial degeneration and necrosis, Simmental, bovine.

Conference Comment: The contributor has provided an excellent overview of Bovine Viral Diarrhea virus (BVBV). BVDV is a common cause of erosive and ulcerative lesions on epithelial and mucosal surfaces. Histopathology and additional laboratory testing can be used to differentiate BVDV from the list of differential diagnoses that the contributor provided. Although there is some variation among slides, the majority of them have the erosions described by the contributor. Additional pestiviral diseases include Border Disease in sheep and Classical Swine Fever.

Contributor: Institute of Animal Pathology, University of Bern, Laenggassstrasse 122, Postfach, CH-3001 Bern, Switzerland
www.unibe.ch

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SLIDE 57
CONFERENCE 15 / CASE I – A040870057 (AFIP 2940514)

Signalment: Five year-old, female, mixed breed farm dog (*Canis domesticus*) weighing approximately 23 kg.

History: Over a period of several weeks, the dog developed progressive rear leg ataxia that worsened on exercise and then extended to the front legs. The dog also had a head tilt to the left and would stumble and fall to the left. During the course of the illness, the dog was alert, but unable to rise at times (astasia). The owner elected euthanasia, as the dog did not respond to treatment.

Gross Pathology: No overt gross findings were present on gross examination except for slight congestion of meningeal vessels.

Laboratory Results: Fluorescent antibody testing for rabies was negative. Immunohistochemistry using polyclonal antibody against both *Toxoplasma gondii* and *Neospora caninum* was positive.

Contributor's Morphologic Diagnosis: Brain, cerebrum at the level of hippocampus; meningoencephalitis, necrogranulomatous and eosinophilic, multifocally extensive, chronic, moderate to severe with intralesional protozoan cysts and tachyzoites.

Contributor's Comment: Several, smooth and thin-walled, nonseptate cysts (containing 1-2 _m bradyzoites) varying in size from 15-40 _m in diameter and individual tachyzoites are scattered in the vicinity of necrotic foci and inflamed leptomeningeal vessels. Meninges and Virchow-Robin spaces are moderately expanded by infiltration of moderate to large numbers of eosinophils, macrophages, lymphocytes and plasma cells. Glial cells including microglia and astrocytes surround the necrotic foci and vacuolated neuropil.

Naturally occurring or experimental disease caused by *Neospora caninum* or a Neospora-like coccidian has been recognized in a variety of animals including the dog, cat, cattle, sheep, and horse as well as laboratory rodents. The dog has been recently identified as the definitive host for the organism but other hosts may exist. The organism has some features similar to *Toxoplasma gondii*, including division of tachyzoites by endodyogeny and has both a proliferative (tachyzoite) and tissue cyst (bradyzoite) phase.[1] Although there are morphologic differences between the organisms (*N. caninum* has a thicker cyst wall), differentiation based on light microscopy is problematic and definitive diagnosis necessitates immunohistochemistry or electron microscopy. *N. caninum* does not develop within a parasitophorous vacuole as does *T. gondii*. Tachyzoite multiplication in both infections results in focal necrosis, followed by inflammation. Postnatal neosporosis is less common than toxoplasmosis. Felids (both domestic and wild) are the only definitive hosts for *T. gondii*. Toxoplasma can be transmitted to intermediate hosts via oocysts in feline feces, via cysts in host tissue (meat), and via tachyzoites transplacentally (vertical transmission).[2,3]

AFIP Diagnosis: Brainstem and cerebrum, at the level of the hippocampus: Meningoencephalitis, lymphoplasmacytic and eosinophilic, multifocal, moderate, with protozoal cysts and tachyzoites, mixed breed, canine.

Conference Comment: Dr. J.P. Dubey, USDA, Animal Parasitic Diseases Laboratory, performed immunohistochemistry using monoclonal antibody against both *Toxoplasma gondii* and *Neospora caninum*. The organisms in this case are positive for *N. caninum* and negative for *T. gondii*.

Neospora caninum is a recently recognized apicomplexan and until 1998, was misdiagnosed as *Toxoplasma gondii*.[2] *N. caninum* is a major pathogen for cattle and dogs, and occasionally causes clinical infections in goats, sheep, horses, and deer. Domestic dogs can be both the intermediate and the definitive host; canids are the only known definitive host.[4]

N. caninum has three infectious stages: tachyzoites, tissue cysts, and oocysts. The tachyzoites and tissue cysts are intracellular and found in the intermediate hosts. Tachyzoites are approximately 6 x 2 _m, while cysts are round to oval, up to 107 _m wide, and found primarily in the central nervous system. The tissue cyst wall is up to 4 _m thick and the enclosed bradyzoites are 8 x 2 _m.
N. caninum can be transmitted transplacentally in several hosts and transplacental is the main mode of transmission in cattle. Carnivores can acquire infection by ingestion of infected tissues. Domestic dogs will shed unsporulated oocysts in the feces, which sporulate and become infective outside of the host. Sporulated oocysts can be found in the soil, water, or food and are subsequently ingested by the intermediate host (cattle, sheep, goats, horses, and dogs). Upon ingestion, sporozoites excyst, multiply, spread to many tissues as tachyzoites, and eventually encyst as bradyzoites.[4]

N. caninum is a major pathogen of cattle, causing abortion and neonatal mortality. *T. gondii* is a major pathogen in sheep and humans, and not of cattle. In dogs, the most severe cases of neosporosis occur in young, congenitally infected pups. The disease may be localized or generalized and virtually all organs may be involved, including the skin. Neurologic signs depend on the site parasitized, but often the hind limbs are more severely affected and often in rigid extension. Subclinically infected bitches can transmit the parasite to their fetuses, and successive litters from the same bitch may be born infected.[4]

Another differential diagnosis considered by conference attendees was *Sarcocystis canis*, a related protozoan known to cause systemic illness in dogs. *S. canis* has been documented in fatal visceral and neural disease in dogs. Although there are subtle histomorphological differences between *N. caninum*, *T. gondii*, and *S. canis*, electron microscopy or immunohistochemistry should be employed to positively identify the organism.[5]

Contributor: Texas Veterinary Medical Diagnostic Laboratory, Texas A & M University, 6610 Amarillo Blvd. West, Amarillo, Texas

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SLIDE 58
CONFERENCE 15 / CASE II – CASE 1 (AFIP 2942328)

Signalment: 2.5 year old, white and brown, male Boer goat (*Capra hircus*)

History: A 2.5 year old buck presented with a 4-month history of multifocal proliferative, ulcerative and exudative epidermal lesions that were most severe on the right hind limb. Initial physical exam revealed a slightly thin, mildly dehydrated goat that was bright, alert and responsive. Orf, with a secondary bacterial infection, was considered the most likely diagnosis. Attempted treatments included systemic antibiotics, pain medications as well as topical antibiotics and antivirals applied to the skin lesions. No improvement was noted over several weeks and the owner elected humane euthanasia.

Gross Pathology: The main lesions were confined to the skin on the limbs and trunk. The skin on these areas displayed numerous and extensive proliferative epidermal lesions that consisted of firm, irregular, raised, gray, verrucous plaques or nodules with extensive superficial crusting, frequent ulceration and occasional purulent exudation. The lesions were limited to the epidermis and dermis and did not involve the subcutaneous tissues.

Other findings at postmortem examination include:
Focal spondylosis at the level of tenth thoracic vertebral body.
Mild diffuse pulmonary edema.
Multifocal moderate peripheral lymphadenopathy.

Laboratory Results: CBC and chemistry panel showed moderate anemia with severely decreased albumin levels. Aerobic bacterial culture resulted in isolation of *Proteus mirabilis* and *Pseudomonas aeruginosa*. Anaerobic bacterial culture resulted in

isolation of *Fusobacterium necrophorum* and *Bacteroides* spp.. Tissues were submitted to Dr. A. Dela Concha at Texas A&M and viral isolation and PCR identified parapoxvirus (orf).

Contributor's Morphologic Diagnosis: Skin: Locally extensive epidermal hyperplasia and intraepidermal vesiculo-pustular proliferative dermatitis with severe superficial perivascular to diffuse lymphoplasmacytic, histiocytic and suppurative dermatitis and dermal edema.

Contributor's Comment: The section consists of large, exophytic, papillary projections covered by thick serocellular crusts within which there are numerous degenerate neutrophils, red blood cells and colonies of coccoid bacteria. The epidermis is irregularly thickened with marked parakeratosis, occasional pustules and ballooning degeneration of numerous keratinocytes. Individual keratinocyte necrosis and occasional intracytoplasmic, eosinophilic inclusion bodies are present. Elongation of rete pegs is evident. The connective tissue core within papillary projections is loose, edematous, hemorrhagic and contains numerous small capillaries. It is heavily infiltrated by lymphocytes, plasma cells, macrophages and neutrophils.

Contagious ecthyma is also known as contagious pustular dermatitis, infectious labial dermatitis, scabby mouth, soremouth, lippengrind and orf (Old English for "rough").[2] It is a contagious viral skin disease of sheep and goats caused by a parapoxvirus related to those causing pseudocowpox and bovine papular stomatitis. It can be transmitted to humans.[4] The disease is usually more severe in goats than in sheep, where it affects primarily the lips of young animals.[1,2]

Typical contagious ecthyma lesions heal spontaneously over 3-4 weeks, and infection results in partial immunity to reinfection. Atypical contagious ecthyma infections have been described and the lesions are extremely severe and generalized and do not spontaneously regress.[1,2,3] These atypical cases have been described in Boer or Boer-crossed goats. The virus isolated from these cases was orf virus-San Angelo 2000 (OV-SA00). This is the same type of virus isolated from this case (Dr. Dela Concha, personal communication).

It has not been elucidated if Boer goats have a particular susceptibility to the virus or if they are immunosuppressed in some way.[2] However, the lesions described in the initial report include lymph node depletion.[5] In this case, lymph nodes were moderately enlarged with variable sized white to pale tan areas on cut section. Microscopically, the pale areas corresponded to large accumulations of amyloid, partially effacing normal lymphoid follicles (lymphoid depletion).

AFIP Diagnosis: Haired skin: Dermatitis, proliferative, lymphoplasmacytic and neutrophilic, chronic, diffuse, severe, with hyperkeratosis, intracorneal pustules,

epidermal intracellular edema, and epidermal intracytoplasmic eosinophilic inclusion bodies, Boer goat, caprine.

Conference Comment: Ovine parapoxvirus, the cause of contagious ecthyma, belongs to the family *Poxviridae*, and genus *Parapoxvirus*. Contagious ecthyma is an important disease of sheep and goats causing high morbidity and low mortality. Transmission occurs through direct contact or indirectly through fomites. It is zoonotic; however, lesions in humans are usually circumscribed, solitary, and confined to the hands. Parapoxviruses infect a wide range of species, generally causing only localized lesions. Infections of cattle, goats, sheep, and camels can be of economic importance. Parapoxviruses also infect several terrestrial and marine wildlife species (chamois, red deer, seals).[6]
In animals, gross lesions of typical ovine parapoxvirus consist of papules, pustules, and thick crusts that occur primarily on the muzzle and mouth. However, lesions may appear in the oral cavity, and on the eyelids, feet, or teats. Orf may prevent lambs from suckling and severely affected animals may loose weight and be predisposed to secondary infections.[6] Histologically, the pathognomic changes include marked proliferation of keratinocytes, extreme cell swelling resulting in ballooning degeneration, nuclear shrinkage, and eosinophilic cytoplasmic inclusions. The virus is highly keratinolytic, and inclusions appear to be floating in the fluid remains of the cytoplasm. Virions average 320 x 125 nm, but vary in size and shape. Small cytoplasmic inclusions must be differentiated from deeply basophilic keratohyaline granules and moderately eosinophilic, larger, intracellular keratin bodies.[7]

Other parapoxviruses of ruminants include bovine parapoxvirus (bovine papular stomatitis) and pseudocowpox virus. Bovine papular stomatitis (BPS) is a disease of calves characterized by proliferative lesions in the oral cavity and esophagus, with little to no systemic disease. BPS is transmissible to humans and results in lesions resembling those of orf. Pseudocowpox virus causes pox lesions on the teats of cattle and is the agent of milkers' nodules in humans.[7]

Contributor: Department of Biomedical Sciences, Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Ithaca, New York
www.vet.cornell.edu

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SLIDE 59
CONFERENCE 15 / CASE III – PM03-90 (AFIP 2937350)

Signalment: 4 year old, neutered female, English springer spaniel (*Canis familiaris*).

History: Variety of bizarre neurological and behavioral signs. These included stumbling and a periodic tremor. She was also reported to tire quickly. MRI showed changes suggestive of fucosidosis including swelling of the trigeminal nerve. Following laboratory investigations she was euthanized and submitted for full necropsy.

Gross Pathology: The cadaver weighed 19 kg. No gross abnormality of the brain was present but the trigeminal ganglia were twice the expected size. The vagosympathetic trunk and vagus nerves were also enlarged along with the cervical dorsal root ganglia. No other gross abnormalities were noted.

Laboratory Results: PCR screening on genomic DNA confirmed the animal was homozygous for the mutant gene producing alpha-L-fucosidase deficiency (Animal Health Trust, Newmarket, Suffolk, England).

Contributor's Morphologic Diagnosis: Trigeminal ganglion/Dorsal root ganglion. Neuronal degeneration with cytoplasmic vacuolation. Ganglionopathy, canine fucosidosis.

Contributor's Comment: Canine fucosidosis is a lysosomal storage disease which affects English springer spaniels. It occurs as a result of a frameshift mutation involving a 14 base pair deletion of exon 1 of the canine fucosidase gene.[1,2] The disease is inherited recessively. There is deficiency of the enzyme, which is present in plasma, leukocytes and other tissues including brain, which results in defective degradation of water-soluble glycoproteins containing fucose. Homozygotes have less than 5% of normal enzyme activity. Heterozygotes have intermediate activity.[3]

Clinical signs include behavioral changes as well as motor abnormalities such as wide-based stance and hypermetria. Male dogs may be infertile. Visual impairment has also been reported. The onset is usually around 6 months of age and is progressive. Affected individuals rarely survive beyond 4 years.

Microscopically there is neuronal swelling with cytoplasmic vacuolation. The vacuoles are large, single and displace the Nissl substance. Some are empty. Others contain fine floccular material. Similar changes were present throughout the CNS. In the springer spaniel, most symptoms are related to CNS pathology. It was first described in this breed in 1982.
Fucosidosis also occurs in humans. Mental retardation is a common sign. Twelve different mutations have been described in people and there is more widespread involvement of organs. The condition in dogs most resembles the intermediate form of the human disease and has been used as an experimental model for the CNS pathology. In addition to the neuronal changes in dogs, vacuolated macrophages are found in the meninges, perivascularly in the CNS, and in thickened peripheral nerves.[4]

Bone marrow transplantation has been shown to limit the severity and progression of the disease. Enzyme activity levels rise in a range of tissues including the CNS. If symptoms are already manifested, transplant is less effective.[5] Age at marrow transplantation has been shown to be important for survival, disease progression and the level of enzyme activity attained.[6] Gene therapy and recombinant enzymes have also been proposed as treatment modalities for humans.

AFIP Diagnosis: Ganglion: Vacuolar change, neuronal, multifocal, marked, with multifocal mild lymphoplasmacytic ganglionitis, English Springer Spaniel, canine.

Conference Comment: The contributor provides a thorough overview of fucosidosis, which is a lysosomal storage disease of complex carbohydrates, specifically a defect in the gene encoding the alpha-L-fucosidase enzyme, resulting in accumulation of fucose-containing sphingolipids and glycoprotein fragments.

Lysosomes are key components of the “intracellular digestive tract” and contain many hydrolytic enzymes that function as the acid milieu of the lysosomes. These lysosomal enzymes (acid hydrolases) are synthesized in the endoplasmic reticulum and then uniquely processed in the Golgi apparatus. Within the Golgi complex, these enzymes undergo post-translation modification, which involves the addition of terminal mannose-6-phosphate groups to some of the oligosaccharide side chains. This is an “address label” that is recognized by specific receptors found on the inner surface of the Golgi membrane. Lysosomal enzymes bind to these receptors, are segregated from other secretory proteins, and are delivered to lysosomes in transport vesicles.[7]

Lysosomal acid hydrolases catalyze the breakdown of a variety of complex macromolecules, from both metabolic turnover of intracellular organelles (autophagy) and from phagocytosis (heterophagy). With an inherited deficiency of a functional lysosomal enzyme, catabolism of its substrate remains incomplete, leading to accumulation of the partially degraded insoluble metabolite within the lysosomes. As this accumulation progresses, organelles increase in number and become enlarged, eventually interfering with normal cell functions.[7]

Lysosomal storage diseases may result from the lack of any protein essential for the normal function of lysosomes. Defects may include reduced synthesis of lysosomal enzymes, synthesis of a catalytically inactive protein that cross-reacts with the normal enzyme, defects in post-translational processing of the enzyme protein, lack of an enzyme activator, lack of a substrate activator protein, or lack of a transport protein.[7]

In general, the distribution of the organs affected is determined by two factors: the tissue where most of the material to be degraded is found; and, the cells or location where most of the degradation normally occurs. Lysosomal storage diseases can be divided into categories bases on the biochemical nature of the accumulated metabolite:[7]

Disease	Enzyme Deficiency	Accumulating Metabolites
Glycogenosis		
Type 2—Pompe disease	alpha-1,4-glucosidase	Glycogen
Sphingolipidoses		
GM1 gangliosidosis	GM1 ganglioside ß-galactosidase	GM1 ganglioside, Galactose-containing oligosaccharides

Disease	Enzyme Deficiency	Accumulating Metabolites
GM2 gangliosidosis		
Tay-Sachs disease	Hexosaminidase-alpha subunit	GM2 ganglioside
Sandhoff disease	Hexosaminidase-beta subunit	GM2 ganglioside, globoside
Variant AB	Ganglioside activator protein	BM2 ganglioside
Sulfatidoses		
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide
Krabbe disease (Globoid cell leukodystrophy)	Galactosylceramidase	Galactocerebroside
Gaucher disease	Glucocerebrosidase	Glucocerebroside
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin
Mucopolysaccharidoses (MPS)		
MPH I H (Hurler)	alpha-L-Iduronidase	Dermatan sulfate, heparan sulfate
MPH II (Hunter)	L-Iduronosulfate sulfatase	
Mucolipidoses (ML)		
I-cell disease (ML II)	Deficiency of phosphorylating enzymes essential for the formation of mannose-6-phosphate recognition marker	Mucopolysaccharide, glycolipid
Other Disease of Complex Carbohydrates		
Fucosidosis	alpha-fucosidase	Fucose-containing sphingolipids and glycoprotein fragments
Mannosidosis	alpha-mannosidase	Mannose-containing oligosaccharides
	beta-mannosidase	Mannose-containing oligosaccharides
Other Lysosomal Storage Diseases		
Wolman disease	Acid lipase	Cholesterol esters, triglycerides

Neuronal Ceroid-Lipofuscinosis	Unknown	Unknown

Contributor: University of Liverpool, Department of Veterinary Pathology, Crown Street, Liverpool, England
www.liv.ac.uk

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SLIDE 60
CONFERENCE 15 / CASE IV – P04-5855 (AFIP 2956372)

Signalment: 5 year-old, castrated male, European domestic shorthair cat (*Felis domesticus*).

History: A 5 year-old, European domestic shorthair, castrated, male cat was presented at a veterinary clinic in The Netherlands with progressive abnormal behavior, anorexia and loss of weight, ataxia, ocular mydriasis and nystagmus. The cat deteriorated over the next 2 months, was euthanized, and admitted to the Pathobiology Department of the Veterinary Faculty in Utrecht, The Netherlands.

Gross Pathology: At necropsy, gross pathologic findings were limited to the central nervous system (CNS). Between both cerebral hemispheres there was a large mass (3 x 1.5 cm diameter). This process was well demarcated and had a yellowish aspect with some empty spaces in it.

Laboratory Results: Earlier PCV, blood chemistry and FIV/FeLV tests showed no specific remarks.

Contributor's Histopathologic Description: Microscopically, between both hemispheres, there is a well demarcated, expansive growth that is partly encapsulated. This mass contains many cholesterol clefts and a large amount of foamy macrophages filled with lipophilic material. Many multinucleated giant cells with the same appearance are seen particularly surrounding the cholesterol clefts. Between these cells there are some fibrous strands and areas of mineralization. Multifocally, in the margins of the process, some lymphocytes and plasma cells are present. The nervous tissue around this mass is moderately compressed. Locally the process is continuous with the meninges.

Contributor's morphologic diagnosis: Brain: Xanthomatous meningioma, European domestic shorthair, (*Felis domesticus*).

Contributor's Comment: Meningioma is the most common primary CNS tumor in the cat. It rises within the meninges, has a mesodermal character and is composed of arachnoid cap cells and occasionally pial cells.[1] Usually it is in close association with the dura, and grows expansively, compressing but seldom invading the brain. Malignant meningiomas, which invade the surrounding brain tissue or metastasize, are rare in cats.[1,2] Meningiomas are seen more often in older cats (between 9 and 15 years old) and there is a slight predominance towards the male gender.[3] Meningiomas are quite common incidental findings at autopsy in the aged feline.[1] In cats, meningiomas have the tendency to be multiple and often arise from the tela choroidea of the third ventricle. They can be soft or firm and may be gritty on cut surface. They grow slowly (except for the malignant variant) and the clinical signs associated with the tumor reflect the neuroanatomical location of the tumor and the severity of any secondary pathology, such as edema, hemorrhage, brain herniation or hydrocephalus.[4]

Meningiomas show a remarkable diversity in histopathology probably due to the fact that both the mesoderm and neural crest contribute to the formation of the meninges. The range of patterns recognized is: meningothelial, fibrous, transitional, psammomatous, angiomatous, papillary, granular cell, myxoid and anaplastic (malignant) meningioma. Most meningiomas exhibit vimentin, and less often cytokeratin and S-100 protein immunoreactivity.[5]

In cats most are meningothelial or psammomatous and many have cholesterol deposits.[1] In humans xanthomatous infiltration in meningiomas has been described as metaplastic changes and rarely xanthomatous meningiomas are documented.[6,7,8] In 2004 a human case was presented with extensive xanthomatous change with focal lymphoplasmatoid infiltration and foci of necrosis with nuclear debris and cholesterol clefts. There were many epithelioid cells surrounding the areas of necrosis, forming granulomas. The xanthomatous change is often the result of lipid accumulation in meningeal cells, rather than infiltration by foam macrophages-lipid laden

"xanthomatous" cells. They have been shown to be meningeal in nature by immunohistochemistry and electron micrography but they also demonstrated the presence of the macrophage marker CD68. In between the xanthomatous zones there was presence of typical meningioma areas and there was a gradual transition from areas of typical meningioma to xanthomatous zones.[9] This suggests metaplastic changes are occurring in the meningothelial cells and that changes are not only from the entering of blood borne histiocytes from the bloodstream to ingest necrotic tumor cells.[6]

In this cat, next to the xanthomatous changes as could be found in a cholesterol granuloma, there are zones with evidence of neoplastic meningeal cells consistent with a meningioma. Those areas are positive in vimentin expression and negative for S100 protein, PAS and cytokeratin. Only three cases of a xanthomatous meningioma in a cat are documented: the case described here, in Veterinary Neuropathology and one case earlier described as a granular cell tumor, which in our opinion, is also a xanthomatous meningioma. It is remarkable that all three cases appear at the same site in the meninges.[1,10]

AFIP Diagnosis: Brain, cerebrum and meninges: Cholesterol granuloma, focally extensive, European domestic shorthair, feline.

Conference Comment: Although we gave careful consideration to the contributor's diagnosis of meningioma, we interpret the lesion in the provided sections as a cholesterol granuloma. Many feline meningiomas contain cholesterol deposits,[1] but to our knowledge a meningioma with diffuse xanthomatous metaplasia has not been reported in the veterinary literature. When present in feline meningiomas, xanthomatous/cholesterinic granulomatous inflammation is often located in areas of necrosis and hemorrhage. Although the lesion in this case is continuous with the meninges in some sections, transition of the xanthomatous /cholesterinic granulomatous inflammation to recognizable meningioma is not evident in the sections examined by the conference attendees. This case was reviewed by the Department of Neuropathology of the Armed Forces Institute of Pathology, which concurred with the diagnosis of cholesterol granuloma. Meningiomas usually affect cats significantly older than the one that had this lesion. Interestingly, meningiomas have been reported in young cats with mucopolysaccharidosis I.[11]

In veterinary medicine, cholesterol granulomas, also known as cholesteatomas or cholesteatosis, are most commonly seen as incidental findings in aged horses. In horses, they frequently occur in the fourth ventricle; those that occur in the lateral ventricle may be very large, leading to obstruction and hydrocephalus, which results in dilation and pressure atrophy of the walls of the ventricles. Possible causes of cholesterol granulomas include necrosis and hemorrhage or hyperlipidemia.[12]

Cholesterol granulomas are also occasionally documented in other animals, including a great plated lizard,[13] and meerkats,[14,15] as well as in humans.

Contributor: University of Utrecht, Faculty of Veterinary Medicine, Department of Pathobiology, Pathology Division, Yalelaan 1, 3508 TD Utrecht, The Netherlands

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SLIDE 61
CONFERENCE 16 / CASE I – 030928-41 (AFIP 2941564)

Signalment: 16-year-old, female, rhesus macaque (*Macaca mulatta*), nonhuman primate.

History: This adult female rhesus monkey was originally acquired from the Delta Primate Center, Louisiana, in 1990 by the United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, Maryland and transferred to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1992. She served as a subject on several Ebola virus protocols, and most recently as a blood donor for a whole-blood transfer study conducted in May 2003. The monkey was clinically normal during the study. The only anomaly noted was a slight change in eating habits that developed in November 2002, described as being "very picky" in her choice of foods. Although her weight was normal during the short course of the study, she became noticeably thinner throughout the month. A routine blood draw and clinical exam in early June demonstrated a significant weight loss of three kilograms. By late June, the monkey was still active, but began losing hair, did not eat regularly, and occasionally vomited. On July 7, 2003 the monkey had difficulty standing, demonstrated abdominal discomfort, and was shaking. She was euthanatized the following day.

Gross Pathology: The carcass was very thin, with significant loss of muscle mass and only a small amount of subcutaneous fat over the abdominal region. The fat had a white, chalky appearance (interpreted as steatitis). There was marked alopecia and moderate flaking of the skin, primarily over the back and the back of the head. Mild hair loss was present over the remainder of the body. Within the abdominal cavity there were multiple strictures in the distal colon and distal ileum, decreasing the lumen by 60% and 85%, respectively. Both the jejunum and the ileum proximal to the stricture were dilated up to five times normal and contained liquid fecal material (interpreted as reflux from the cecum and colon). The liver was yellow-brown and friable (hepatic lipidosis), and the gallbladder was distended by inspissated bile.

Gross Diagnoses: 1. Whole body: cachexia of chronic disease, moderate.
2. Subcutaneous fat: steatitis, moderate.
3. Colon and ileum: strictures, multiple, marked.
4. Liver: lipidosis, diffuse, severe.
5. Gallbladder: inspissated bile, moderate.

Contributor's Morphologic Diagnoses: 1. Colon, serosa: Endometriosis, multifocal, mild to moderate, with low numbers of hemosiderin-laden macrophages.
2. Uterus; ileum; and jejunum, serosal surfaces: Endometriosis, with few hemosiderin-laden macrophages (not submitted).

Contributor's Comment: The cause of this adult female monkey's weight loss, anorexia, and vomiting over the period before euthanasia was severe strictures of the

intestinal tract, especially the ileum, which significantly decreased the passage of ingesta. The pathogenesis of the intestinal strictures was attributed to chronic endometriosis. The intestinal strictures resulted from constant cyclical hemorrhage and menstruation from the ectopically located endometrial tissue within the abdominal cavity, followed by formation of abdominal adhesions. The endometrial tissue was present primarily on the serosa of the ileum, and to a lesser degree on the colon, uterus, and jejunum.

Endometriosis is the condition in which normal endometrial glands and stroma occur in abnormal locations outside the uterine cavity.[1] The ectopically located endometrial tissue physiologically responds to normal ovarian hormonal influences associated with the menstrual cycle. The aberrant endometrial tissue undergoes monthly desquamation and hemorrhage (menstruation), with the exception that the process occurs within the lower abdominal cavity rather than in the uterine lumen. The entrapped hemorrhagic menstrual fluid provokes an intense inflammatory reaction in the abdomen, often leading to fibrosis and adhesions among the pelvic organs. Infertility, abdominal pain, and occasionally bowel and/or urinary tract obstruction occur secondarily to the adhesions and strictures.[2]

Endometriosis occurs only in humans and animal species that menstruate.[2] In humans, endometriosis affects 10% of women and often causes dysmenorrhea, pelvic pain, infertility, and other problems; it is primarily a disorder of those in the active reproductive stage of life, especially during the third and fourth decades.[3]

Similarly, endometriosis is one of the most common reproductive disorders in Old World nonhuman primates, and has been proposed as a naturally occurring model of the disease in humans. While the disease occurs as a spontaneous condition, it is frequently a complication of repeated hysterotomies or caesarean sections.[2] As in humans, the most common clinical signs in affected monkeys and apes include abdominal discomfort and infertility; in some cases, the disease is asymptomatic. Other clinical signs include cyclical anorexia, depression, weight loss, and absence of feces for several days; there may be palpable masses within the abdominal and pelvic cavities.[1]

In nonhuman primates, endometriosis has been reported at various anatomical sites, although lesions most commonly occur in the pelvic cavity. Macroscopically, gross lesions often appear as soft, red-brown or white, masses of tissue adherent to the serosa of the pelvic organs, or masses of dense connective tissue containing fluid-filled cysts distended with brown menstrual blood ("chocolate cysts").[2] Common sites and organs involved include the ovaries, uterine tubes, urinary bladder, and, as in this case, the bowel. In some cases unilateral or bilateral hydroureter and hydronephrosis develop as a result of adhesions that develop among the lower abdominal organs which impinge upon the pelvic ureters. Infrequently, endometriosis may result in bowel infarction.[1]

Microscopically, endometriotic lesions consist of variably sized foci of normal appearing uterine glands surrounded by typical endometrial stroma and thick bands of fibrous connective tissue; scattered aggregates of hemosiderin-laden macrophages are often present throughout the bands of connective tissue.[2] In human pathology, the histological diagnosis of endometriosis is satisfied if two of the three following features are identified: endometrial glands; endometrial stroma; and hemosiderin pigment.[3] While present in a few locations, the number of hemosiderin-laden macrophages admittedly is less than overwhelming in the submitted histologic sections from this female monkey.

Although the pathogenesis of endometriosis is not understood, three potential but not mutually exclusive theories have been offered to explain both the origin and dispersion of the lesions:[3]

1. Regurgitation theory: retrograde menstruation or reflux of endometrial tissue through the fallopian tubes, with subsequent implantation and proliferation of viable endometrial fragments in the abdominal cavity.
2. Metaplastic theory: endometrial tissue arises directly from coelomic epithelium (itself the origin of the endometrium).
3. Vascular or lymphatic dissemination theory: this would explain the presence of lesions in the lungs and lymph nodes (described in both humans and nonhuman primates), which is not explained by the two previous hypotheses.

AFIP Diagnosis: Colon; mesentery: Endometriosis, multifocally extensive, Rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: The contributor provides a thorough overview of endometriosis in human and non-human primates. Although the gross lesions of endometriosis are often distinctive, other differentials to consider, especially in markedly fibrotic lesions, are adenocarcinoma and retroperitoneal fibromatosis.

In nonhuman primates, intestinal adenocarcinoma occurs most commonly in the cotton-top tamarin, which is the animal model for ulcerative colitis and associated carcinoma in humans. Approximately 50% of colony-maintained animals develop active colitis, with disease in 25-40% of those with active colitis progressing to colonic adenocarcinoma after 2-5 years of captivity. Grossly the lesions are nodular to annular, firm, gray-white, transmural, stenotic masses, often with proximal intestinal dilation or muscular hypertrophy. Histologically, there are four subtypes of adenocarcinoma based on the predominant cell type and growth pattern: papillary, tubular, mucinous, and signet ring. Although the pathogenesis is not completely understood, it is thought that chronic inflammation leads to hyperplasia and dysplasia, which may eventually progress to adenocarcinoma.[4]

Retroperitoneal fibromatosis is a disorder of macaques that primarily occurs in young animals (1-3 years of age), and is characterized by an aggressive proliferation of highly

vascular fibrous connective tissue, usually involving the ileocecal junction. This disorder is associated with a gammaherpesvirus - Retroperitoneal fibromatosis-associated herpesvirus (RFHV), and with an oncovirus, Simian type D retrovirus (SRV-2). SRV-2 is unique in its ability to induce both Simian Acquired Immunodeficiency Syndrome (SAIDS) and retroperitoneal fibromatosis. Lesions may be localized or progressive. Gross lesions in the localized syndrome include 1-4 cm single to multiple, firm, pale nodules beneath the peritoneum. In the progressive syndrome, lesions may encircle the intestines and adjacent lymph nodes leading to obstruction. Histologically, there are proliferating fibroblasts arranged in ill-defined bundles with occasional interweaving patterns within a disorganized matrix of collagen and reticulum fibers.[5]

Contributor: U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Ft. Detrick, MD
http://www.usamriid.army.mil/

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* 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.

SLIDE 62
CONFERENCE 16 / CASE II – CASE 2 (AFIP 2942335)

Signalment: Female,1.5-year-old miniature donkey (*Equus asinus*).

History: The animal had a chronic history of crusty skin lesions near her vulva and on her flanks. A skin biopsy was taken (tissue submitted). A month after the biopsy, the animal was noted to be lame, with a painful, swollen knee. The animal was humanely euthanized and submitted for a necropsy.

Gross Pathology: At the postmortem examination the animal was in poor body condition with minimal fat stores. The skin was thickened, irregular and crusty with variably-sized areas of alopecia and hypotrichosis. When sectioned, the skin, subcutis and superficial fascia had hundreds of small pinpoint white granular organisms. The scleral conjunctiva and vaginal mucosa contained dozens of similar foci. The distal phalanges of the left and right forelimb and right hind limb were ventrally rotated 32, 20 and 40 degrees, respectively, from the hoof wall.

Contributor's Morphologic Diagnosis: Skin Biopsy: Locally extensive, chronic lymphoplasmacytic and granulomatous dermatitis with intradermal protozoal cysts (*Besnoitia bennetti*, presumptive).

Contributor's Comment: Throughout the dermis there are dozens of randomly distributed, 0.5 to 1 mm diameter protozoal cysts within greatly distended and flattened fibroblasts (vimentin positive, smooth muscle actin negative cells). The cysts have a 25 µm thick hyaline internal capsule and are engorged by hundreds of fusiform bradyzoites, 8-9 µm long and 1-2 µm wide. There are variable numbers of macrophages around the intact cysts and larger numbers of macrophages mixed with lymphocytes and lesser numbers of plasma cells surround ruptured cysts. The epidermis is multifocally ulcerated, with granulation tissue and necrotic cell debris mixed with lymphocytes and plasma cells. The superficial dermis is moderately and diffusely expanded by dense fibrous connective tissue, with a mild to moderate perivascular to interstitial infiltrate of lymphocytes, macrophages, plasma cells and rare eosinophils.

Cutaneous besnoitiosis is a serious skin condition of cattle and horses characterized by painful swellings and thickening of the skin with loss of hair. Besnoitiosis is a rare disease in equines. It has been reported only in animals with a history of individual or herd travel outside the United States. *Besnoitia besnoiti* has been reported from southern Europe, Africa, Asia, and South America, but it has not been reported in cattle in the USA. *Besnoitia bennetti* has been reported from Africa, southern France, and in two imported burros in the USA.[5] However, this is the second case in a donkey from the northeastern USA that our service has diagnosed in the last two months.

Besnoitiosis is a contagious protozoal disease of various domestic and wild animals, including horses, burros, cattle, rodents, goats, antelope, reindeer, caribou and mule deer.[1] It is caused by members of the genus Besnoitia, which are characterized by having cysts containing bradyzoites within fibroblasts. The apicomplexan parasites under the subphylum Sporozoa, class Coccidian, and within the family Sarcocystidae include four genera: Toxoplasma, Sarcocystis, Besnoitia and Hammondia. The genus

Besnoitia has the following species: *B. bennetti* (horses and donkeys), *B. besnoiti* (cattle), *B. caprae* (goats), *B. oryctofelisi* (rabbits) *B. darlingi* (lizards), *B. jellisoni* (kangaroo and opossum), *B. tarandi* (reindeer and caribou), and *B. wallacei* (mouse).[1-2] The old name of the parasite was *Globidium* spp , which describes the characteristic large, thick-walled cysts filled with bradyzoites.[4] The life cycle involves a definitive host and an intermediate host. The cat has been identified as the definitive host for *B. besnoiti*, *B. wallacei*, and *B. darlingi*.[3] In the intermediate host, Besnoitia is found in the dermis, subcutaneous tissues and fasciae. The parasite produces characteristic thick-walled cysts containing bradyzoites within fibroblasts. It has been speculated that a biting insect vector spreads Besnoitia between intermediate hosts, but this is not proven. Besnoitia tissue cysts are characterized by hypertrophy of the infected host cell.[2]

AFIP Diagnosis: Haired skin: Dermatitis, chronic-active and eosinophilic, diffuse, moderate, with numerous intradermal protozoal cysts, etiology consistent with *Besnoitia* sp., miniature donkey, equine.

Conference Comment: As mentioned by the contributor, *Besnoitia* sp. are apicomplexan parasites within the class Sporozoasida, order Eucoccidiorida, family Sarcocystidae. Other genera of the family Sarcocystidae include *Toxoplasma*, *Sarcocystis*, *Neospora*, *Hammondia*, *Cystoisospora*, *Frenkelia*, and *Atoxoplasma*.[6]

All members of the family Sarcocystidae have a motile stage with apical complex, have a simple resistant spore, and undergo both sexual and asexual reproduction. Sexual reproduction results in the production of oocysts with two sporocysts in the intestine of the definitive host, while asexual reproduction results in spore formation within the intermediate host.[6]

Gross lesions caused by organisms of this family vary, but infection often results in acute necrosis from migration and multiplication of the tachyzoites and little tissue damage in organs with cysts containing bradyzoites. However, if the cysts rupture, the organisms often incite a granulomatous response. Histologically, these organisms appear very similar and immunohistochemistry or electron microscopy is needed for a definitive diagnosis.

Ultrastructurally, bradyzoites are found in the cytoplasm within a parasitophorous vacuole, which constitutes the innermost cyst wall layer. This is lined by a thin granular layer, which often contains one or more host cell nuclei. The outermost layer (secondary cyst wall) surrounds the host cell. The structures which help identify the organism as an apicomplexan parasite include a conoid, rhoptries, and micronemes.[2] The presence or absence, number, electron density, and/or location of each of these, as well as other organelles, assist in identifying the organism to the genus level.

Contributor:

Department of Biomedical Sciences, Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Ithaca NY
www.vet.cornell.edu

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SLIDE 63
CONFERENCE 16 / CASE III – 030926-44 (AFIP 2940303)

Signalment: Adult, female African green monkey (*Chlorocebus aethiops*), nonhuman primate.

History: This 4.3 kg female African green monkey (AGM) was procured from St. Kitts/Primate Products. The monkey was assigned to a protocol, but had not been exposed to any agent. Six weeks after arriving at the institute, blood was noted around the anogenital region by animal care technicians (day 1). Menses was suspected, but the condition was reported to Veterinary Medicine Division personnel after the animal had decreased appetite over the weekend.

Upon examination by a veterinarian, the suspected menses had persisted for 5 days (the normal menstrual period in AGMs is about 1-3 days, usually with scant discharge). Under ketamine sedation, the monkey had a body temperature of 100.7°F, pulse = 180, and respiratory rate = 28. The animal had lost 0.5 kg from the previous weight taken 7 days earlier. Formed stool with fresh blood and loose, stringy clots were noted in the catch pan beneath the cage. There was moderate periodontal disease with worn, stained teeth. Abdominal palpation was within normal limits. No vaginal bleeding was found; only rectal bleeding with decreased anal tone.

The differential diagnosis included stress-induced, dietary-related, or idiopathic inflammatory bowel disease; hemorrhoids, polyps, or neoplasia; and infection with intestinal pathogens such as *Campylobacter*, *Shigella*, *Salmonella*, *Yersinia*, or enteropathogenic *Escherichia coli*. Although this monkey was never positive for intestinal parasites, she was previously housed in a room where one monkey was diagnosed with whipworms (*Trichuris* sp.), so all monkeys in the room were treated at that time.

For initial work-up, a complete blood count (CBC), serum chemistry, clotting times, and fecal exam were ordered (results below). Gingival and anal swabs were submitted for bacterial culture. A B-vitamin injection was given to stimulate eating, and high-water content fruit treats were offered along with the regular diet. A fruit-flavored, sweetened water solution (Prang) replaced the monkey's normal water supply.

On day 6, the monkey showed no signs of improvement. She remained anorexic and continued to pass blood, but no stool. Based on the unremarkable laboratory results, flat film abdominal radiographs and a proctoscopic examination were ordered. A possible right side colonic stricture was noted on radiographs. Endoscopy revealed an advanced, severe hemorrhagic colitis. Difficulty was encountered in trying to pass the endoscope 30-33 cm (12-13 inches) proximal to the anus. The monkey was administered a broad spectrum antibiotic (enrofloxacin), subcutaneous fluids and given another B-vitamin injection. Exploratory surgery was planned for the following day.

Exploratory abdominal surgery revealed segmental areas of hemorrhage, restricted to the colon, that were visible through the serosa. Within the proximal colon, a 10-13 cm (4-5 inch) section contained markedly thickened mucosa, with hemorrhage and sloughed pseudomembrane formation that completely occluded the lumen. Additional colonic and gingival culture swabs were obtained. The decision to euthanize the monkey was based on the monkey's clinical history, the fact that she had lost 10-12% of her body weight and continued to deteriorate, and the severity and extent of the lesion found at surgery.

Gross Pathology: The body presented for necropsy was that of an adult, female African green monkey (*C. aethiops*). The carcass was in good body condition with adequate subcutaneous and cavitary fat. There was moderate periodontal disease with gingivitis, gingival hyperplasia, and worn canines. The stomach was empty and the small intestine contained a small amount of gas. Colon walls were edematous with swollen rugae and there were segmental areas of hemorrhage with blood clots in the lumen. Other areas in the colon were ulcerated or had a sloughed, pseudomembranous mucosal lining with dry, adherent fecal material.

Gross diagnoses: 1. Colon: Colitis, ulcerative and hemorrhagic, segmental, moderate, with pseudomembrane.
2. Gingiva: Gingivitis, multifocal, moderate, with mild gingival hyperplasia.

Laboratory Results: Initial work-up (Day 5)
Serum chemistry panel values were within normal limits, except for slightly elevated blood urea nitrogen, alkaline phosphatase, and triglycerides; albumin was slightly low, but the total protein was within normal limits. CBC values were all within normal limits (no anemia and no inflammatory leukogram). Fecal exam revealed no significant findings. Clotting times were within normal limits.
Bacterial culture results: Bacteria in the genus *Shigella* were isolated from the rectum, colon, and gingiva (growth was not speciated).

Contributor's Morphologic Diagnosis: Colon: Colitis, ulcerative and hemorrhagic, subacute, multifocal, moderate, with crypt abscesses and abundant luminal fibrinohemorrhagic and cellular debris (pseudomembrane), African green monkey (*Chlorocebus aethiops*), nonhuman primate.

Contributor's Comment: Histologically within the submitted section of colon, there are focally extensive areas of mucosal ulceration that are covered by a layer of sloughed mucosal epithelial cells, fibrin, hemorrhage, and necrotic debris. The lamina propria is expanded by many lymphocytes and plasma cells that widely separate crypts. Colonic crypts are often dilated and filled with many viable and degenerate neutrophils, mucus, and cellular debris (crypt abscesses). Subacute inflammation extends into the edematous submucosa where lymphatics are ectatic. Multifocally, similar inflammation and focal areas of hemorrhage are present within the tunica serosa.

By immunohistochemistry using a polyclonal anti-*Shigella* antibody, there is strong staining of necrotic mucosal epithelial cells and luminal necrohemorrhagic debris within affected sections of colon.

Shigella are gram-negative, non-motile, aerobic and facultatively anaerobic bacilli from the family Enterobacteriaciae.[1] *S. dysenteriae, flexneri, boydii,* and *sonnei* are highly infectious strains that can cause dysentery in humans with an ID_{50} of only 100-200 bacteria.[2] Nonhuman primates usually acquire the zoonotic infection from humans via a fecal-oral route and endemic infections can be maintained in monkey colonies via asymptomatic carriers.[1] Nonenteric *Shigella* infections in monkeys with gingivitis, air sacculitis, and abortion have also been reported.[1] The pathogenesis of diarrhea or dysentery among the strains is similar, with a typical incubation period of 1-4 days followed by watery and mucoid diarrhea mixed with blood. Although clinical disease usually requires a stressor in endemically infected monkeys, it is typically self-limiting in adults, requiring minimal supportive care.

Studies on the pathogenesis of *Shigella* have revealed unique methods of mucosal invasion that result in the lesions seen with infection. Because most lesions are often centered on gut-associated lymphoid tissue (GALT) and spread outward, it is suspected that the bacteria make their initial entry into the body through the normally phagocytic M cells overlying the lymphoid tissue.[2] Additional studies have revealed that through a complex process involving multiple genes found on both a large plasmid and on the *Shigella* chromosome, attachment of the bacteria to mucosal epithelial cells stimulates a

structural alteration of the normally non-phagocytic epithelial cell cytoskeleton and actin filaments to cause uptake of the organism in a manner similar to phagocytosis. Once within the intracellular vacuole of the invaded cell, a hemolysin produced by *Shigella* causes release of the organism into the cytoplasm. The *Shigella* then rapidly multiply and migrate along polymerized actin filaments to reach the plasma membrane so that adjacent cells can be invaded.[3] Early in the course of disease, low numbers of *Shigella* organisms can be found by electron microscopy within mucosal epithelial cell vacuoles. As the disease progresses, though, fibrinous exudate replaces the dead epithelial cells.[4] Death of epithelial cells and sloughing of mucosa creates the ulceration, pseudomembrane formation, hemorrhage, and inflammatory response that typifies shigellosis.

An additional aspect of virulence involves the production of an exotoxin, shiga toxin, by *S. dysenteriae*. Released during host cell lysis, shiga toxin stops host cell protein synthesis by inactivating the 60S ribosomal subunit (similar to the method of action of the plant toxin, ricin). Shiga toxin exerts effects similar to enterotoxins, neurotoxins, and cytotoxins, and can induce apoptosis in epithelial cells. Shiga toxin also enhances the lipopolysaccharide-mediated release of cytokines, such as interleukin-1 and tumor necrosis factor-alpha, which likely contributes to the vascular damage leading to renal failure seen in a complication of shigellosis, hemolytic uremic syndrome.[2]

AFIP Diagnosis: Colon: Colitis, necrotizing, subacute, diffuse, moderate, with a fibrinohemorrhagic pseudomembrane, African green monkey (*Chlorocebus aethiops*), primate.

Conference Comment: There is variation in slides with some slides exhibiting ulceration of the colonic mucosa, while others are only eroded. In this case, immunohistochemistry reveals that most of the *Shigella* organisms are located within the pseudomembrane and along the epithelial border, with few organisms found in the submucosa. However, in other cases, it is not uncommon to find moderate numbers of organisms within the submucosa.

Lesions of enteric shigellosis, as in this case, are primarily in the cecum and colon. The intestinal walls are thickened and edematous with luminal contents varying from fluid mucus with fibrin and cellular debris to frank hemorrhage, multifocal ulcerations and pseudomembrane (diphtheritic membrane) formation. Nonenteric *Shigella* infections have been reported, including gingivitis, abortion, and air sacculitis. With gingivitis, the gums are swollen, hyperemic, with scattered yellow-white foci of necrosis. Severely affected monkeys may have gingival recession and root exposure.[1,4]

The differential diagnosis should include yersiniosis, salmonellosis, and *Campylobacter*-associated enteritis, as well as *Clostridium piliforme* and *E. coli*. Definitive diagnosis requires culture of the organism from a rectal swab or fresh stool specimen.[1,4]

Contributor: U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD http://www.usamriid.army.mil/

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* 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 and are not necessarily endorsed by the U.S. Army.

SLIDE 64 & 65
CONFERENCE 16 / CASE IV – 04011035 (AFIP 2948689)

Signalment: 5-year-old, intact female, Shetland sheepdog.

History: (Per clinician) This canine patient was previously diagnosed with Ehrlichiosis and had been on a Imidocarb therapy regimen. Five months following the initial diagnosis of Ehrlichiosis, the dog presented with clinical signs of ataxia, vestibular signs, lethargy and pale mucus membranes. The patient exhibited anemia and thrombocytopenia. After a month, the neurological signs worsened and the dog started falling while walking. The PCV and platelet count decreased sharply. Horizontal, rotary and positional nystagmus appeared. Craniopropioceptive deficits were observed in the rear limbs and right front limb. Based upon a neurological examination, central vestibular disease, a cerebral cortical lesion, and a lesion between T2-T3 were suspected. Pulmonary radiographs revealed increased interstitial opacity. The dog continued to grow weaker with declining PCV and platelet count. The dog developed

hypoalbuminemia (1.5 g/L) and a markedly distended abdomen. The dog died in respiratory distress.

Gross Pathology: At necropsy, the dog was in poor nutritional condition characterized by prominent bony protuberances and absence of visceral and subcutaneous adipose tissue. The mucous membranes and subcutis were mildly icteric. The subcutaneous tissue in the ventral caudal cervical and cranial thoracic region was focally wet and jelly-like (subcutaneous edema). Large volumes of serofibrinous effusions were present within the peritoneum (2L) and pleural cavity (1L). The liver contained multiple, randomly scattered nodules with pale yellow margins and indented, soft, friable (necrotic) centers. Multiple, discrete, variably-sized, slightly raised, dark brown necrotic foci were present on the capsular surfaces and in the parenchyma of spleen, renal cortices and pancreatic lymph node. There was mild cerebellar coning and the leptomeninges over the frontal lobes were multifocally cloudy.

Laboratory Results:
Clinical pathology findings: January 10 – January 17; anemia (PCV-18% to low of 13%), thrombocytopenia (15,000 to low of 13,000/mm^3); hypoalbuminemia (1.5g/dL).
Ehrlichia canis serum titer: 1:40000
Fungus Testing Laboratory, The University of Texas Health Sciences Center at San Antonio identified the fungus as *Ochroconis gallopava.*

Contributor's Morphologic Diagnoses:
Submitted tissue:
1. Liver and kidney: Necrosis, multifocal, subacute, severe with vasculitis and intralesional fungus, Shetland sheepdog, canine.
2. Liver and kidney: Plasmacytic perivasculitis, multifocal, chronic, moderate.
3. Kidney: Membranous glomerulopathy, multifocal, chronic, marked with mild proteinuria.

Tissue not submitted:
4. Brain, spinal cord, gall bladder, pancreas: Perivasculitis, plasmacytic, multifocal, chronic, moderate to severe.
5. Lung: Pneumonia, interstitial, chronic, moderate.
6. Liver, spleen, lung: Extramedullary hematopoiesis.

Contributor's Comment: This patient has two overlapping disease processes. The perivascular lymphoplasmacytic infiltrate in multiple organs, interstitial pneumonia, glomerulopathy, multiorgan microthrombosis, anemia, thrombocytopenia and hypoalbuminemia, which are consistent with the clinical diagnosis of Ehrlichiosis. An unexpected finding in this case includes the necrotizing lesions in multiple organs secondary to a systemic fungal infection.

Phaeohyphomycosis is a collective term for cutaneous and systemic diseases caused by several genera of black molds that develop in tissue in the form of dark-walled, septate mycelium. Phaeohyphomycotic fungi belonging to the genera *Ochroconis*

(formerly *Dactylaria*) are known for their neurotropic potential and their predilection to cause severe necrotizing encephalitis in humans,[1] cats[2] and young birds. *Dactylaria gallopava* infection was first reported in 1962 in turkey poults in South Carolina.[3] Subsequent epidemics have been reported in young birds – chickens,[4] grey-winged trumpeters (*Psophia crepitans*),[5] Japanese quail (*Coturnix coturnix japonica*).[6]

The exact mechanism by which this fungus causes systemic disease is unknown. Respiratory exposure to spores has been shown to produce the disease experimentally in poultry.

Ochroconis is a thermophilic fungus and favors soil and decaying vegetation, which can undergo a composting phenomenon associated with the generation of heat and an acidic environment. It has been isolated from broiler house litter where similar environmental conditions prevail. It is also a contaminant of effluents of hot springs and nuclear reactors, thermal soils and self-heated coal waste piles.[7]

Though this fungus is more amenable to therapy, if not recognized and treated in time it can be a cause of significant mortality. Amphotericin B is considered the antimycotic agent of choice for systemic phaeohyphomycosis, including ochroconiosis. In a case report by Kralovic and Rhodes in 1995, a human liver transplant patient developed *Ochroconis* sp. infection despite receiving prophylactic fluconazole treatment.

AFIP Diagnoses: 1. Kidney: Necrosis, focally extensive, with moderate pyogranulomatous inflammation, vasculitis, and dematiaceous fungal hyphae, Shetland Sheepdog, canine.
2. Liver: Necrosis, multifocal, with neutrophilic inflammation, vasculitis, and dematiaceous fungal hyphae.
3. Kidney: Glomerulonephritis, membranous, global, diffuse, moderate, with multifocal mild plasmacytic interstitial nephritis.

Conference Comment: As mentioned by the contributor, this animal had a high titer to *Ehrlichia canis* as well as related clinical pathology abnormalities. Histologically, a characteristic change is generalized perivascular plasma cell infiltration. Infiltrates are evident in the section of kidney; although increased numbers of perivascular plasma cells might be expected in the liver, the numbers on the slides examined by conference attendees are deemed within normal limits.

Ehrlichiosis is a tick-transmitted rickettsial disease affecting several species of animals and humans. In dogs, disease is caused by *E. canis*, *E. chaffeensis*, *E. risticii*, *E. ewingii*, *E. equi*, *E. phagocytophilia*, and *E. platys* and in horses by *E. equi* and *E. risticii*. *E. risticii* is the causative agent of Potomac horse fever and *E. platys* is the cause of canine cyclic thrombocytopenia.[8]

The arthropod vector of *E. canis* is the brown dog tick, *Rhipicephalus sanguineous*. Following a short incubation period, *E. canis* induces acute disease in which organisms infect monocytes and spread throughout the mononuclear phagocyte system. During this stage, the morula of *Ehrlichia* species may be noted on cytological examination in neutrophils, lymphocytes, and monocytes. Endothelial invasion follows, resulting in vasculitis. There is then a subclinical phase from which the dog either recovers or develops pancytopenic bone marrow failure. The chronic phase is characterized by pancytopenia with depletion of erythrocytic, granulocytic, and megakaryocytic cells, with a persistence of plasma cells within the bone marrow. Gross findings include widespread petechiae and ecchymoses, splenomegaly, lymphadenomegaly, and either hyperplastic (acute disease) or hypoplastic (chronic disease) bone marrow. Histologically there is a perivascular plasma cell infiltration, nonsuppurative meningoencephalitis, interstitial pneumonia, and glomerulonephritis in most dogs. Ehrlichiosis in German Shepherd dogs causes a severe hemorrhagic disorder attributed to a depressed cell-mediated immune response to *E. canis* in this breed.[8]

Phaeohyphomycoses are uncommon opportunistic infections caused by a number of ubiquitous saprophytic and plant pathogenic molds with the characteristic of forming pigmented (dematiaceous) hyphal elements in tissue. The pigment is melanin and the fungus will generally stain with the Fontana Masson method. Other special histochemical stains commonly used to visualize the fungal morphology include Grocott's methenamine silver (GMS), or periodic acid-Schiff (PAS). With these stains, the fungal hyphae of *Ochroconis* (*Dactylaria*) *gallopavum* are characterized by thick, 2-4 µm wide, septate, non-parallel walls, with acute and right angle dichotomous branching, and yeastlike swellings.[9]

Contributor: Oklahoma State University, College of Veterinary Medicine, Department of Pathology, Rm. 250 McElroy Hall, Stillwater, OK
http://www.cvm.okstate.edu

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SLIDE 66
CONFERENCE 17 / CASE I – S03-2084 (AFIP 2936462)

Signalment: 3.5 year-old, male, Asian Elephant (*Elephas maximus*).
History: This juvenile elephant from the zoological garden, Zürich, refused to eat in the morning, showed signs of colic and depression and did not urinate. In the afternoon the tongue appeared cyanotic, the periorbital skin was swollen and the animal collapsed and died.

Gross Pathology: The main pathological changes were extensive hemorrhages particularly in the heart, the intestines and mesentery as well as in the subcutaneous tissues. The latter were also edematous in the head region. The myocardium, particularly the right atrium, was severely hemorrhagic and dark red. The liver was markedly swollen.

Contributor's Morphologic Diagnosis: Generalized severe petechial to extensive hemorrhage in nearly all organs and tissues. Intranuclear endothelial inclusion bodies (only in capillaries) in the heart, liver and tongue.

Etiology: Endotheliotropic Herpesvirus (Elephant herpesvirus-1)

Contributor's Comment: Histologically nearly every tissue was congested and most had areas of hemorrhage. There were early signs of hypoxic degeneration in the liver. Intranuclear inclusion bodies in the endothelium of the capillaries in the heart, liver and tongue were observed.

Death due to this disease typically occurs as a result of cardiac failure following herpesviral induced capillary injury and extensive myocardial hemorrhage. It is also typical that inclusion bodies may be found in the endothelium of capillaries of the heart, liver and tongue, but never in larger vessels.

The death of an Asian elephant due to Elephant Endotheliotropic Herpesvirus was first described in a juvenile Swiss circus animal in 1990.[1] The animal presented here is the third juvenile elephant which has died as a result of a herpesvirus infection in Switzerland.

The significance of this disease for the survival of elephant populations in captivity became apparent after a retrospective study published a decade later revealed that deaths due to herpesvirus, but unrecognized at necropsy, had occurred in both Asian and African elephants as early as 1983.[2,3] So far, the virus has not been isolated. However, molecular methods have provided evidence that several newly identified herpesviruses are involved. The current theory is that otherwise healthy African elephants with hyperplastic lymphatic tissue in the genital tract and nodules in the skin and in the lung, which harbor herpesvirus, may be the source for a fatal infection in Asian elephants.[3,4,5] This obviously has great significance for the risk of disease transmission in connection with the translocation of animals.[6]

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, centrilobular, diffuse, mild, with congestion, hemorrhage, and endothelial cell eosinophilic intranuclear inclusion bodies, Asian elephant (*Elephas maximus*), proboscidea.

Conference Comment: The Asian and African elephant endotheliotropic herpesviruses (EEHV) are two novel, distinct, yet related viruses that are an important cause of fatalities in young Asian elephants, and less commonly cause death in African elephant calves [2]

Clinical signs include sudden onset of lethargy, anorexia, edema of the head, neck and thoracic limbs, cyanosis of the tongue, lymphopenia, and thrombocytopenia. Gross lesions include pericardial effusion with widespread petechial to ecchymotic hemorrhages primarily involving the heart, liver, intestine and tongue. Oral, laryngeal and intestinal ulcerations often occur. Histologically there are extensive microhemorrhages, edema, and mild lymphohistiocytic infiltrates throughout the heart and tongue. Congestion and hemorrhage cause hepatic sinusoidal expansion with mild hepatocellular degeneration, and endothelial cells of capillaries in the myocardium, tongue, and liver contain amphophilic to basophilic intranuclear inclusion bodies. Ultrastructurally, the inclusion bodies are 80-92 nm and morphologically consistent with other herpesviruses.[2]

Currently it is thought that the African elephant may be the reservoir of the herpesviruses that can cause disease in the two elephant species. African elephants carrying EEHV have typical herpetic lesions on the skin and vulva. Transmission is thought to be through intimate contact. However, direct proof of transmission has not been established. Nonetheless, it is currently recommended that Asian and African elephants be housed separately.[2]

The differential diagnosis for widespread necrosis and hemorrhage in elephants includes encephalomyocarditis virus, orbivirus, salmonellosis or other bacterial septicemia, and vitamin-E deficiency.[7] The Smithsonian National Zoological Park is developing PCR and ELISA tests to diagnose elephant herpesvirus infections.

Additional endotheliotropic viruses of veterinary importance include equine viral arteritis virus, equine Hendra virus, equine orbivirus (African horse sickness), cervid orbivirus (epizootic hemorrhagic disease), ovine orbivirus (bluetongue), hamster parvovirus, rat parvovirus (Kilham rat virus), canine adenovirus type 1 (infectious canine hepatitis), porcine adenovirus, bovine adenovirus, and adenovirus of deer.[2]

Contributor: University of Zürich, Institute of Veterinary Pathology, Winterthurerstr. 268, CH-8057 Zürich, Switzerland
www.vetpathology.unizh.ch

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SLIDE 67
CONFERENCE 17 / CASE II – ND-2 (AFIP 2935568)

Signalment: 12-year-old American bison (*Bison bison*) cow.

History: The animal was found dead on pasture with no prior clinical signs of illness.

Gross Pathology: Pale, hemorrhagic nodules were present in multiple tissues: nearly all skeletal muscle groups (intercostals, epaxial and hypaxial groups, quadriceps,

gluteals, semimembranosus, semitendinosus, deltoids, etc.), heart, liver, kidney, spleen, walls of the forestomachs, small and large intestines, lung and brain.

Laboratory Results: Specials staining of replicate sections of tumor tissue yielded the following results: Desmin +, Factor VIII -, trichrome -, vimentin +, PTAH +, myoglobin +, and muscle specific actin +. Electron microscopy of tumor samples showed cross striations consistent with myocyte origin.

Contributor's Morphologic Diagnosis: Disseminated rhabdomyosarcoma.

Contributor's Comment: Rhabdomyosarcomas are described as embryonal, botryoid, alveolar and pleomorphic. The embryonal variety is classified into two types, large round cell and myotubular. It has been reported in a variety of species, including cows and sheep. Botryoid rhabdomyosarcomas are a distinct entity most commonly found in the canine bladder. It is considered a variant of the embryonal type. The alveolar subtype has been reported in large and small animals, and shows a distinct histologic pattern of tumor cells supported by a prominent fibrovascular stroma. Least common of the subtypes in animals is the pleomorphic variant. As the name implies, there is considerable cellular pleomorphism with minimal connective tissue involvement. Morphologic characteristics of the tumor in this bison cow would seem most consistent with the more rare pleomorphic variant.

Rhabdomyosarcomas of the limbs, trunk, and neck typically present as nodules within muscle. While usually pale on cut surface, hemorrhage and necrosis associated with continued growth can change the appearance. Necrosis occurs when tumor cells outgrow their blood supply. Metastatic disease occurs primarily in muscle tissue and less commonly in other organs. Microscopically, the cells are highly pleomorphic with unusual morphology characterized by racquet shapes, multinucleation, vacuolated cytoplasm, and strap cells. Striated myofibrils may or may not be visible by light microscopy. Immunohistochemical testing for specific proteins such as desmin, vimentin, myoglobin, and actin can help with diagnosis.

A search of the literature indicates that this is the first report of a rhabdomyosarcoma in a bison.

AFIP Diagnosis: Skeletal muscle; heart; kidney: Sarcoma, favor rhabdomyosarcoma, American bison (*Bison bison*), bovine.

Conference Comment: Based on the H&E histomorphology alone, conference attendees unanimously diagnosed a sarcoma with a differential list including rhabdosarcoma, fibrosarcoma, leiomyosarcoma, hemangiosarcoma, and neurofibrosarcoma.

Several histochemical and immunohistochemical stains are helpful in narrowing the differential diagnosis. With Masson's trichrome, moderately abundant blue-staining collagen is demonstrated separating neoplastic cells, which stain red. In the contributor's laboratory, neoplastic cells are negative for Factor VIII-related antigen, which does not support a diagnosis of hemangiosarcoma. Neoplastic cells exhibit diffuse immunoreactivity for vimentin, which supports mesenchymal origin, and exhibit multifocal immunoreactivity for desmin and muscle specific actin, suggesting it is a neoplasm of muscle origin. Neoplastic cells multifocally exhibit positive immunoreactivity for myoglobin, which is specific for striated muscle. In addition, phosphotungstic acid hematoxylin (PTAH) is used to highlight cross-striations, staining them blue. Depending on the laboratory results, immunohistochemistry can assist in determining the histogenesis of a tumor, but should never be used alone when making a diagnosis. In this case, the contributor also evaluated the tumor utilizing electron microscopy and results are consistent with myocyte origin. Conference attendees discussed the potential difficulty of evaluating an electron micrograph of a rhabdomyosarcoma within a skeletal muscle sample, especially if the sample were taken from the periphery, in areas where the neoplastic cells separate and surround pre-existing myocytes. In this case, ideally one would perform EM on the mass in the kidney.

Contributor: North Dakota State University, Veterinary Diagnostic Laboratory, 1523 Centennial Boulevard, Van Es Hall, Fargo, North Dakota
www.vdl.ndsu.edu

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SLIDE 68
CONFERENCE 17 / CASE III – 8053 (AFIP 2944793)

Signalment: Tissue from an adult female Vietnamese potbellied pig (*Sus scrofa*).

History: This animal came from a Vietnamese potbellied pig rescue establishment. The animal had been recently introduced into a herd of mixed sexes, most of which had been neutered. She had a history of hematuria for the previous 2 weeks. Ultrasound revealed pyelonephritis, along with pus in the uterus and urinary bladder. The owner attempted to give oral antibiotics without success and the animal was euthanized.

Gross Pathology: The animal had extensive subcutaneous and abdominal body fat. Thick, mucopurulent exudate was present in the vaginal lumen. The mucosa of the urethra and urinary bladder were streaked with hemorrhages, and the urine was cloudy.

The ureters were distended and thickened. Purulent exudate could be extruded into the pelvis of the kidneys if the ureters were compressed. The renal pelvis was bilaterally dilated by considerable tan flocculent debris. There was also edema of the adipose tissue of the mesentery, around the pancreas, and near the adrenal glands. The adipose tissue in this general area contained multiple chalky white areas. Two ascarids were present in the lumen of the duodenum; one of these protruded from the pancreatic duct and extended 12 cm into the duct itself.

Laboratory Results: A heavy growth of *Eubacterium suis* was isolated from the kidney.

Contributor's Morphologic Diagnosis: Acute fibrinosuppurative pancreatitis and fat necrosis, with pancreatic duct obstruction by an adult ascarid.

Contributor's Comment: *Ascaris suis* is the largest enteric nematode of pigs. Adults reside in the lumen of the small intestine, and the females may be up to 40 cm in length. Pigs are most often exposed in dirt lots where earthworms and dung beetles may ingest larvated eggs and serve as reservoirs. Piglets may ingest eggs attached to mammary nipples, but prenatal infection is not thought to occur. L2 larvae leave the eggs in the intestine, penetrate the hepatic portal system and molt to L3. These migrate via the blood from the liver to the lungs where they exit pulmonary capillaries, molt to L4, and migrate up airways. They are then swallowed and develop into enteric adults. Most pathology attributed to ascarids is a result of migration of large numbers of L3 larvae through the liver (white spot disease or milk-spotted liver) or lungs, where pneumonia results.

Ascaris suum is a close relative of *Ascaris lumbricoides*, the human ascarid, with which it shares a similar shape and size. Ascarids are sporadically responsible for blockage of the pancreatic duct in a variety of species, including human beings, especially in developing countries where 60% of the children and 30% of adults are parasitized. In people, the mechanism of pancreatitis is most often compression of the pancreatic duct by blockage of the bile duct or common duct, with obstruction of the pancreatic duct alone being less common. This pig had rather localized pancreatitis, and pigs have separate entrances for the pancreatic and biliary ducts at the duodenum. Living parasites slip in and out of ducts in people with relative ease, and occasionally bypass large obstructions such as gall stones in the ducts. Children are less commonly affected, perhaps because of the small diameter of the hepatic and pancreatic ducts, although they can develop severe pancreatitis. There is a 3:1 prevalence of women over men in adult patients. Chronic pancreatitis also has been described in horses as a result of migration of *Strongylus equinus*, occasionally with resulting diabetes, and there is a single report of an ascarid becoming lodged in the pancreatic duct of a horse. *Trichospiruria leptostoma* commonly inhabits the pancreatic ducts of wild-caught common marmosets. Occasional pancreatic fibrosis and exocrine insufficiency have been reported.

Characteristics of ascarid nematodes include coelomyarian musculature, and uninucleate intestinal epithelium with a low microvillous border. Numerous thick shelled eggs are present in the coelom, as characteristic of an adult female.

AFIP Diagnosis: Pancreas; peripancreatic fat; and duodenum: Pancreatitis, neutrophilic and eosinophilic, acute, multifocal, moderate, with vasculitis, fibrinous peritonitis, necrotizing steatitis, focal mural duodenitis, pancreatic duct ectasia, ulceration, and intraluminal adult ascarid, Vietnamese potbellied pig (*Sus scrofa*), porcine.

Conference Comment: The contributor provides a thorough overview of the lifecycle of *Ascaris suum* and the lesions associated with infestations by the nematode.

As pathologists, it can be tempting to play the "vet game" and diagnose *A. suum* infestation based on finding a large nematode in the pancreas of a pig. However, with a basic understanding of common histomorphological features of the various groups of metazoans, one can easily identify the parasite of this case as a nematode, and further classify it as an ascarid.

There are six groups of commonly seen metazoan parasites: nematodes, acanthocephalans, trematodes, cestodes, arthropods, and pentastomes. When evaluating a parasite histologically, it is important to note the following features: type of body covering and body wall, presence or absence of a body cavity, location and type of musculature, presence and type of digestive tract, presence and type of reproductive organs. Below is a simple table to identify the parasite to its group:[8]

GROUP	GENERAL SHAPE	BODY CAVITY	DIGESTIVE TRACT	STRIATED MUSCLE	SPECIAL DIAGNOSTIC FEATURES
Cestode	Flattened dorso-ventrally	--	--	--	1. calcareous corpuscles 2. scolex 3. tegument
Trematode	Flattened dorso-ventrally	--	+	--	1. suckers 2. tegument 3. blind ceca 4. yolk gland 5. hermaphroditic
Acanthocephalan	Spherical	+	--	--	1. hypodermis 2. lemniscus 3. two muscle layers 4. proboscis
Nematode	Spherical	+	+	--	1. cuticle 2. musculature
Arthropod	Tend to be	+	+	+	1. chitinized exoskeleton

	spherical				2. jointed appendages 3. tracheal tubes
Pentastomes	Spherical	+	+	+	1. chitinized exoskeleton 2. digestive glands 3. sclerotized openings

Once the organism has been identified as a nematode, it must be further classified into one of the following groups: Aphasmids or Phasmids. Aphasmids lack a tiny pair of sensory papillae (the phasmids) on the caudal end; however, these are not readily identifiable on histologic section. The morphological features that distinguish them from phasmid nematodes are hypodermal bands with associated nuclei, and prominent esophageal glands that form a stichosome. The Phasmids consist of the Rhabditoids, Oxyurids, Ascarids, Strongyles, Spirurids, and Filarids. Both the Rhabditoids and Oxyurids have a rhabditoid esophagus composed of a corpus, isthmus and bulb. The Strongyles have a cuticle, which occasionally is ridged, and all have an intestine composed of few multinucleated cells and a prominent brush border. Spirurids can be very diverse, but all adult females in this group produce embryonated eggs. Filarial nematodes are small and produce distinctive larvae called microfilariae.[2]

Ascarids are large worms that are found, as adults, in the intestines of their host. Larval ascarids may be found in other tissues in both the definitive and intermediate hosts. Adult ascarids have a cuticle, a pseudocoelom, coelomyarian musculature, large lateral chords, and less prominent dorsal and ventral chords, a simple esophagus, a large intestine lined by uninucleate cuboidal cells and a low brush border. Adult female ascarids produce eggs which contain a uninucleate zygote covered by a thick shell. Larval ascarids of mammals commonly have lateral alae.[2]

Contributor: University of Missouri, Veterinary Medical Diagnostic Laboratory, et P.O. Box 6023, Columbia, Missouri

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SLIDE 69
CONFERENCE 17 / CASE IV – 8-81-04 (AFIP 2956553)

Signalment: Male, yearling Mule Deer (*Odocoileus hemionus)*.

History: A male, yearling mule deer was observed lying down near a rural residence in southwest Montana. The animal was unable to stand, had tachypnea, and moist airway sounds were audible from a distance.

Gross Pathology: Numerous tan foci occurred throughout the lungs, kidney and liver. Tuberculosis was suspected and the carcass was buried. Two visiting veterinarians who performed a field necropsy submitted specimens.

Laboratory Results: *Yersinia pestis* was cultured from the lung and kidney.

Contributor's Morphologic Diagnoses: 1. Lung: Pneumonia, necrotizing, suppurative, multifocal to coalescing, severe, with intralesional bacterial rods.
2. Kidney: Nephritis, necrotizing, suppurative, acute, multifocal to coalescing, severe.

Contributor's Comment: Plague is endemic in many areas of the western United States. *Yersinia pestis* is maintained in the environment by a variety of rodent species and their associated fleas. Reports of plague are usually seasonal with the greatest incidence between March and October. Susceptibility differs both in domestic and wild species. In domestic animals, the cat and dog are most frequently infected. Besides rodents and rabbits, plague has been reported in mule deer, pronghorn antelope, mountain lions and bobcats in the western United States. There are three clinical manifestations: bubonic, septicemic, and pneumonic. Lymphadenitis of bubonic plague occurs after inoculation of the organism through the skin or mucous membrane by flea bites or direct penetration. Septicemia can occur subsequent to dissemination of the infection from the infected lymph nodes or by direct introduction into the blood vasculature. Primary pneumonic plague occurs after inhalation of infected material. This particular case represents the septicemic form. *Yersinia pestis* is occasionally isolated from domestic cats in this region in Montana.

AFIP Diagnoses: 1. Lung: Pneumonia, necrotizing, suppurative, subacute, multifocal and coalescing, severe, with vasculitis, and large colonies of bacilli, mule deer (*Odocoileus hemionus*), cervid.

2. Kidney: Nephritis, necrotizing, suppurative, subacute, multifocal and coalescing, severe, with vasculitis, and large colonies of bacilli.
3. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, with mineralization.

Conference Comment: Of the eleven species of *Yersinia*, family Enterobacteriaceae, only four are considered to be primary pathogens: *Y. pestis* (plague in mammals), *Y. enterocolitica* (yersiniosis in mammals and birds), *Y. pseudotuberculosis* (yersiniosis in mammals and birds), and *Y. ruckeri* (red mouth in fish).[3]

Y. pestis remains endemic in certain rodent populations on five continents due to an elaborate interaction of the agent, fleas, vertebrate hosts, and the environment. Enzootic hosts, such as voles, mice, and rock squirrels, serve as reservoirs and do not suffer 100% mortality. These animals may experience a transient bacteremia and thereby transmit the infection to fleas. The fleas may then transmit bacteria to other enzootic hosts, thereby maintaining the disease, or they may transmit it to epizootic rodent hosts. These hosts, such as prairie dogs, have a low resistance to plague morbidity and mortality. Subsequently there is a high death rate, which favors spread of plague, amplifies the intensity of the epizootic, and increases the risk of human infection as infected fleas disseminate from dead hosts.[3]

The primary mode of transmission of *Y. pestis* in mammals is via flea bite. Other modes of transmission include ingestion of, or exposure to, another mammal infected with *Y. pestis*. Another rare but effective mode of transmission is inhalation of aerosolized bacteria by a mammal in close proximity to an animal with pneumonic plague.[3]

As mentioned by the contributor, bubonic, pneumonic, and septemic plague are three clinical manifestations of *Y. pestis* infection. In many cases they represent a continuum as illness, if left untreated, will often progress from one form to the next. The bubonic form results from flea bite inoculation of bacteria and subsequent acute local inflammation of the lymph node draining the inoculation site. The Latin root “bubo”, meaning “swelling”, is descriptive of the lymphadenomegaly that occurs. Primary septemic plague is defined as bacteremia without the presence of palpable buboes. Secondary septemic plague occurs when bacteria from buboes enter the bloodstream. Primary pneumonic plague may result from inhalation of aerosolized droplets of *Y. pestis* from an animal with pneumonic plague.[3] This form has a very rapid incubation period (1-6 days) and is usually fatal if not treated within the first 24 hours of illness.[4] Secondary pneumonic plague may result from hematogenous spread of bacteria to the lungs in the septemic form. Clinically, the septicemic and pneumonic forms are much more severe and almost always result in death.[3]

Y. pestis is a potential bioterrorism agent, and if aerosolized, pneumonic plague could induce many human casualties. Accordingly, the Centers for Disease Control (CDC), lists it as a Category A agent. Category A agents are those agents that have the greatest potential for inflicting large numbers of human casualties, can be manufactured

and disseminated on a large scale, require significant efforts in public health preparedness, and are most familiar to the public.[4]

Contributor: Montana Veterinary Diagnostic Laboratory, P.O. Box 997, Bozeman, Montana
www.state.mt.us/liv/lab/index.asp

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SLIDE 70
CONFERENCE 18 / CASE I – 98-443 (AFIP 2681375)

Signalment: 10 year-old female spayed American Eskimo dog.

History: 3-4 month history of inflamed, ulcerated, and hyperkeratotic foot pads +/- oral mucocutaneous lesions. The dog was treated with glucocorticoids, which exacerbated lesions; then with antibiotics, dietary change, and antihistamines

- 3-4 week history of inappetance; treated with Baytril, Dicural, and Reglan
- One week history of polyuria/polydypsia, vomiting, icterus, and hyperglycemia with glucosuria and ketoacidosis; treated with insulin, Reglan, and centrine with no improvement
- Upon referral, a tentative diagnosis of hepatocutaneous syndrome was made and the owners elected euthanasia

Gross Pathology: The footpads, elbows, vulva, and lips were variably ulcerated and hyperkeratotic. The animal was severely icteric. The liver was firm, yellow-brown with multifocal to coalescing nodules which replaced the entire normal parenchyma. (See gross photo)

Laboratory Results:

Test Result	7-2-98	9-17-98	9-23-98	
ALP	1724	NA	>2400	NA=not available
ALT	158	142	45	
HCT	30	38.3	24.7	
WBC	16,500	22,900	28,500	
PMN	14,500	18,300	27,300	
GLU	NA	>700	221 (on insulin)	

ALB	NA	3.1	2.4

Contributor's Morphologic Diagnoses: 1. Skin, footpad: Superficial dermatitis with basal epidermal hyperplasia, epidermal pallor, and parakeratotic hyperkeratosis. 2. Liver: Severe lobular collapse with nodular regeneration, vacuolar hepatopathy, mild portal fibrosis and bile duct proliferation, and marked canicular bile stasis.

Contributor's Comment: Hepatocutaneous syndrome, also known as superficial necrolytic dermatitis, necrolytic migratory erythema, or diabetic dermatosis is the association of a specific skin lesion (hyperplastic basal epithelium, a zone of epidermal pallor, and parakeratotic hyperkeratosis) with severe liver disease. The liver lesion is typically a severe vacuolar hepatopathy with parenchymal collapse and nodular regeneration which mimics cirrhosis.

In humans the disease is most often associated with glucagon secreting tumors of the endocrine pancreas; however, some human cases have cirrhosis or chronic pancreatic disease with normal glucagon levels. In dogs, most cases are associated with liver disease with normal glucagon levels, but rare cases of glucagon secreting tumors have been reported. The pathogenesis is unknown.

AFIP Diagnoses: 1. Skin, footpad: Hyperkeratosis, parakeratotic, diffuse, severe, with marked basal epidermal hyperplasia, stratum spinosum edema and degeneration, mild subacute dermatitis, and focal ulcer with a serocellular crust, American Eskimo, canine. 2. Liver: Hepatocellular loss with stromal collapse, diffuse, severe, with nodular regeneration, multifocal vacuolar degeneration, mild bridging fibrosis, and biliary hyperplasia.

Conference Comment: Superficial necrolytic dermatitis (SND) is considered a paraneoplastic syndrome that more commonly occurs in association with hepatopathy than with glucagon-secreting neoplasia, giving rise to the familiar name of hepatocutaneous syndrome.[5]

Often the main presenting complaints of glucanoma-associated SND are progressive skin lesions of three weeks to many months, with concurrent lethargy and inappentance. The main dermatologic findings include erosions and ulcerations, with alopecia, exudation and adherent crusts on the feet, pressure points such as the elbows and hocks, flank, perineal area, muzzle, facial mucocutaneous junctions and/or oral cavity. Hyperkeratosis and fissuring of foot pads occur in all animals. Many dogs will also have hyperglucagonemia, hyperglycemia, and marked hypoanimoacidemia involving many amino acids.[5]

The histopathological findings of SND are distinctive and can be strongly suggestive of the disease. The epidermis has a "red, white, and blue" appearance on H&E. Parakeratotic hyperkeratosis and crusting create the upper eosinophilic layer. Edema

and necrosis of keratinocytes within the stratum spinosum make up the middle "white" layer. Hyperplasia of the stratum basale gives rise to the deep basophilic layer. In addition, there may be secondary clefting in the devitalized middle layer, leading to ulceration and secondary inflammation. A mixed inflammatory infiltrate may be present in the superficial dermis.[5]

Although the exact pathogenesis is unknown, there are several current theories. The first is that glucagon results in sustained gluconeogenesis and is involved in the catabolism of amino acids, and chronic elevation of the hormone, as seen in glucagonomas, may result in hypoaminoacidemia, leading to epidermal protein depletion and subsequent keratinocyte necrosis. This is supported by cases of glucagonoma-associated SND that rapidly resolve following surgical resection of the tumor. However, there are also documented cases of SND in which glucagon concentrations are within normal limits.[5] There may be other causes of hypoaminoacidemia. In one report, dogs with nonglucagonoma-associated SND, also had significantly lower amino acids concentrations than the control dogs or dogs with acute or chronic hepatitis.[6] Another theory involves deficiencies in zinc or fatty acids. However, there has been little response to replacement therapy. Finally, hepatic impairment has also been implicated as a possible mechanism. Many, although not all canine patients with nonglucagonoma-associated SND have elevated serum glucagon. It may be that impaired hepatic function leads to decreased metabolism of glucagon, resulting in increased serum levels.[5]

Although SND is an uncommon skin disorder, it is an important diagnosis because it is one of the relatively few skin conditions from which one can diagnose a life-threatening disease, with confidence, from a skin biopsy.

Contributor: University of Pennsylvania, School of Veterinary Medicine, Laboratory of Pathology, 3800 Spruce Street, Philadelphia, PA

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SLIDE 71
CONFERENCE 18 / CASE II – 03-56361 (AFIP 2937321)

Signalment: 1 year-old male Standardbred horse.

History: This colt was purchased at an auction sale 6 weeks prior to presentation. Nasal discharge, lethargy, and limb edema had been evident for the past 4 weeks and the colt had been treated unsuccessfully with 2 courses of antibiotics. Physical examination revealed peripheral lymphadenopathy, severe pneumonia with suspected microabscesses in lung, and ventral edema.

Gross Pathology: Severe generalized lymphadenopathy involved peripheral and many internal lymph nodes, including submandibular, internal iliac, and mesenteric nodes. The enlarged nodes were diffusely white and nodular on sectioning, with occasional areas of necrosis. Left lung contained 2 discrete, 2 cm white foci similar in texture and appearance to the affected lymph nodes. Scrotal skin contained numerous 3-4 mm pale, poorly pigmented, non-haired papules that were deemed an incidental finding.

Contributor's Morphologic Diagnoses: 1. Multicentric lymphosarcoma
2. Equine molluscum contagiosum

Contributor's Comment: Slides demonstrate lesions of equine molluscum contagiosum only. Although lymphosarcoma was the most significant disease process in relation to this horse's clinical demise, lesions of equine molluscum contagiosum presented an additional concurrent and interesting disease process.

Equine molluscum contagiosum (MC) is an uncommon, mildly contagious, cutaneous poxviral disease generally involving skin of the face, neck, chest, trunk, limbs, and genitalia.[1] The virus is transmitted by contact with either an infected individual or contaminated fomites. Multiple 2-8 mm papules develop in affected skin, and although these lesions are benign, they are refractory to therapy and persist for months to years. The lesions in this horse were typical of equine MC and consisted of focal, discrete lobulated areas of epidermal hyperplasia bulging into the underlying dermis. Keratinocytes of the stratum spinosum were swollen and contained large intracytoplasmic inclusions ('molluscum bodies'). In some sections, there is an impression of a central pore in the hyperplastic lobules, through which degenerate keratinocytes are exfoliated. Electron micrographs of the cutaneous lesions demonstrated numerous poxviral inclusions within epithelial cells. Although MC has been diagnosed in clinically normal horses, several literature reports describe MC in horses with other concurrent immunocompromised conditions, such as in this case.[1,2] In humans, lesions of MC are generally more severe and widespread in patients with compromised cell-mediated immune function.[3]

Equine MC bears clinical and histologic resemblance to the human skin disease of the same name, and recent *in situ* hybridization experiments have shown that the viruses causing lesions in these two species have significant nucleic acid homology, leading to the hypothesis that equine MC is an anthropozoonosis.[3] Equine MC has been identified in horses worldwide and clinical lesions are similar to those of Uasin Gishu disease, which has been identified in horses only in Kenya.[2,3] Although the Uasin Gishu virus can be cultivated, the virus associated with equine MC has yet to be isolated. The poxvirus of equine MC is currently classified in the subfamily Chordopoxvirinae, genus Molluscipoxvirus.

AFIP Diagnosis: Haired skin: Hyperplasia, epidermal, focal, marked, with large, eosinophilic intracytoplasmic inclusion bodies (molluscum bodies), Standardbred, equine.

Conference Comment: Equine molluscum contagiosum (MC) is caused by a molluscipoxvirus that is either identical with, or very closely related to, its human equivalent, molluscum contagiosum virus (MCV). MCV is a poorly characterized pox virus which causes a human skin disease characterized by benign but persistent papular lesions with a central opening or umbilicus. Lesions are commonly found on the face, trunk, lower limbs, and anogenital regions. Humans with immunodeficiency have a more severe, disseminated form of the disease.
Molluscum contagiosum has been reported in horses, chimpanzees, and kangaroos. In all of the cases, lesions clinically and histologically were very similar to those seen in humans. MC may represent an anthropozoonosis, wherein disease is transmitted from humans to animals.[3]

In horses, affected animals commonly have hundreds of lesions, especially on the chest, shoulders, neck, limbs, and external genitalia. Lesions on haired skin consist of papules that are initially tufted, but usually become alopecic and covered with a powdery crust or scale. Papules in glabrous skin may be smooth, shiny, hypopigmented, and umbilicated, or hyperkeratotic and hyperpigmented. Lesions are usually nonpruritic and nonpainful. Histologically, lesions are well-demarcated and characterized by epidermal hyperplasia and papillomatosis. Keratinocytes above the stratum basale become swollen and contain ovoid, eosinophilic, floccular intracytoplasmic inclusion bodies (molluscum bodies). These inclusions increase in size and density as the keratinocytes move toward the skin surface. Molluscum bodies exfoliate through a central pore that forms in the stratum corneum and enlarges into a central crater. Usually there is no dermal inflammatory reaction. Ultrastructural examination reveals mature virions that are brick-shaped and approximately 150 x 300 nm, with a biconcave nucleoid and two lateral bodies, typical of poxviral inclusions.[1]

Contributor: University of Guelph, Laboratory Services Division, Animal Health Laboratory, Guelph, Ontario, Canada
http://ahl.uoguelph.ca

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SLIDE 72
CONFERENCE 18 / CASE III – 04-2262 (AFIP 2941563)

Signalment: 10 year-old, female, mixed breed dog (*Canis familiaris*).

History: The dog was being treated for lymphoma and immune-mediated thrombocytopenia with a standard chemotherapy protocol (cyclophosphamide, doxorubicin, vincristine, prednisone). Small nodular dermal lesions developed after 11 treatments with progression to severe exudative dermatitis following the last treatment.

Gross Pathology: Variably sized irregular erythematous exudative skin lesions were randomly distributed on the trunk.

Contributor's Morphologic Diagnosis: Severe generalized necrotizing dermatitis and vasculitis with intralesional protozoal organisms.

Contributor's Comment: The slides vary somewhat in the degree of necrosis and ulceration. The lesion is characterized by extensive epidermal ulceration and necrosis that includes the follicular epithelium and adnexal structures. Necrosis extends from the epithelium to the superficial hypodermis with edema, hemorrhage and an inflammatory infiltrate of neutrophils, histiocytes, small lymphocytes and plasma cells that is diffuse to perivascular. The perivascular inflammatory infiltrates extend into the hypodermis. Small 1-2 µm oval organisms were found free in the necrotic tissue debris, within macrophages and, occasionally, within endothelial cells and epithelial cells of the epidermis, follicles and adnexal glands. Immunohistochemistry was performed and the organisms reacted strongly with a Polyclonal rabbit anti-*Toxoplasma gondii* antibody and did not react with a Polyclonal rabbit anti-*Neospora caninum* antibody.

Necrotizing dermatitis in the dog due to a *Toxoplasma gondii* – like apicomplexan organism and *Neospora caninum* has been previously described.[1,2] In addition to these two organisms, the differential diagnosis for protozoal dermatitis in the dog includes *Sarcocystis canis, Leishmania sp.,* and *Caryospora sp.*.[2,3] *Caryospora sp., Sarcocystis canis* and *Leishmania sp* have sufficiently distinctive morphologic characteristics to

assist in their diagnosis (i.e. caryocyts, schizont formation and the presence of a distinct kinetoplast, respectively).

Toxoplasma gondii and *Neospora caninum* are sufficiently similar in routine histological sections that immunohistochemistry and electron microscopy (EM) are usually employed to distinguish between these two apicomplexan organisms. The *Toxoplasma gondii*-like organism described by Dr. Dubey, et al[1] reacts with a polyclonal rabbit antibody to *T. gondii*, but its ultrastructural characteristics differ significantly. The *T. gondii*-like organism formed schizont-like organisms with a residual body that was best appreciated on EM but could be seen on close examination of the routine histological sections. In addition, the rhoptries in this organism were several in number and electron dense as compared to the few electron lucent rhoptries in *T. gondii.* Close examination of the sections in this case did not reveal any definitive residual bodies. However, electron microscopic examination would be necessary to determine if the protozoa in this case are *T. gondii* or the organisms described by Dr. Dubey.

Predisposing factors such as chronic ehrlichiosis, cardiomyopathy and immune-suppressive therapies for immune-mediated disease and neoplasia were reported in many cases of protozoal dermatitis in the dog.[1,2]

AFIP Diagnosis: Haired skin and subcutis: Dermatitis and vasculitis, necrotizing, acute, diffuse, severe, with multifocal erosion, and myriad intra- and extracellular protozoal tachyzoites, mixed-breed, canine.

Conference Comment: As mentioned by the contributor, organisms are noted both intra- and extracellularly. The intracellular organisms are present in many different cell types including fibroblasts, follicular and epidermal keratinocytes, sebocytes, apocrine ductular epithelium, endothelium, macrophages, adipocytes, and myocytes of the erector pili muscles.

This case was reviewed in consultation with Dr. J.P. Dubey, United States Department of Agriculture, Animal Parasitic Diseases Laboratory, who performed immunohistochemistry using polyclonal antibodies to *Neospora caninum* and *Toxoplasma gondii*. In his laboratory, the organisms exhibit weak positive immunoreactivity for *Neospora caninum* and strong positive immunoreactivity for *Toxoplasma gondii*. Electron microscopy is necessary to positively identify the organism.

Contributor: Arizona Veterinary Diagnostic Laboratory, 2831 N. Freeway, Tucson, AZ
http://www.microvet.arizona.edu/AzVDL/index.shtml

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SLIDE 73
CONFERENCE 18 / CASE IV – 433846B (AFIP 2948686)

Signalment: Three month-old lamb, belonging to a sheep flock of 380 lambs of which 40% died.

History: The animal presented with a fever, swollen eyelids, excessive lacrimation, and mucopurulent nasal discharge.

Gross Pathology: Skin lesions were found on the areas free from wool and were characterized by raised, circular plaques that occasionally had congested borders.

Laboratory Results: Sheep pox antigen was identified in cryostat sections by immunohistochemistry using monoclonal antibodies.

Contributor's Morphologic Diagnosis: Skin: Hydropic degeneration of stratum spinosum keratinocytes, epidermal hyperplasia, and intracytoplasmic eosinophilic inclusion bodies, predominantly in squamous epithelium of hair follicles, consistent with sheep pox.

Contributor's Comment: The epidermis is hyperplastic with hyperkeratosis, acanthosis and significant ballooning degeneration. Occasionally, within keratinocytes, there are pale eosinophilic intracytoplasmic inclusion bodies. In the dermis, there is multifocal infiltration of neutrophils, lymphocytes, and histiocytes.

Sheep pox is a malignant pox disease of sheep characterized by fever, multiple non-vesicular swellings on the skin and mucous membranes, rhinitis, conjunctivitis, respiratory distress (due to the pressure on the upper respiratory tract from the swollen retropharyngeal lymph nodes and developing lung lesions) and death. Sheep pox occurs in all ages of sheep but the disease is most severe in lambs, with mortality reaching 80-100%.

Skin lesions are often less obvious on post mortem examination of acutely infected animals than in live animals with disease. Gross findings include necrotic mucous

membranes, enlarged and edematous lymph nodes, and typical pox lesions characterized by papules, which may be ulcerated, on the abomasal mucosa, tongue, hard and soft palates, trachea and esophagus, and sometimes on the wall of the rumen and the large intestinal mucosa. Pale areas of approximately 2 cm in diameter may occasionally be seen on the surface of the kidney and liver, and have been reported on the testicles. Throughout the lung, but particularly in the diaphragmatic lobes, there are numerous white-gray firm lesions up to 5 cm in diameter. The pathological lesions and clinical signs mentioned above are pathognomonic for sheep pox.

Generalized contagious ecthyma (Orf) or parapox viral infection is rare. Confusion could occur between mild sheep pox and orf or even insect bites. Sheep pox causes extensive economic loss through high mortality, reduced meat, milk or wool yields, quarantine requirements, and the cost of disease prevention programs.

Capripox is endemic in Africa, north of the equator, the Middle East, including Israel, Turkey and Iran, and in Afghanistan, India, Nepal, parts of the Peoples Republic of China, and since 1984, Bangladesh. Recently, it has made frequent incursions into southern Europe.

Transmission of infection is by direct contact with diseased sheep or indirect contact via a contaminated environment. Transmission is usually via aerosolization, but virus can also be spread mechanically by insect bites or experimentally by intradermal or subcutaneous injection.

The incubation period is 4-7 days and is followed by a leukocyte-associated viremia. The virus localizes in many organs including the skin, where virus concentration peaks at 10-14 days post infection. The skin lesions develop 1-2 days later, especially in the sparsely wooled areas and typically involve the eyelids, cheeks, nostrils, vulva, udder, scrotum, prepuce, ventral surface of the tail, and the medial thigh.

Sheep pox lesions have a prominent vesicular stage. The vesicles are umbilicated, multiloculated, and yield only a small amount of fluid if punctured. Occasionally, a large vesicle forms as a result of separation of the necrotic epidermis from the underlying dermis. The pustule stage is characterized by the formation of a thin crust. There may be marked gelatinous dermal edema and in severely affected animals the lesions coalesce. Healing of the skin lesions is slow, taking up to 6 weeks and a scar may remain. Highly susceptible animals often develop hemorrhagic papules early in the course of the disease and later ulcerative lesions in the gastrointestinal and respiratory tracts. Approximately one third of animals develop multiple pulmonary lesions composed of foci of pulmonary consolidation.
The kidneys have multifocal, circular, fleshy nodules throughout the cortices.

Histologically, sheep pox lesions have the typical epithelial changes seen with poxviruses, including marked hydropic degeneration of keratinocytes in the stratum spinosum, microvesiculation, epidermal hyperplasia and eosinophilic intracytoplasmic inclusion bodies. The lesions affect both surface epithelium and follicular epithelium.

Marked dermal lesions reflect the systemic route of cutaneous involvement and may be due to immune mediated disease as well as direct viral damage. During the papular stage, large numbers of mononuclear cell accumulate in the increasingly edematous dermis. These cells, first described by Borrel, are called “cellules claveleuses” or sheep-pox cells, and are characteristic of the disease. The nuclei of sheep-pox cells are vacuolated and have marginated chromatin. The vacuolated cytoplasm contains single, occasionally multiple, eosinophilic intracytoplasmic inclusion bodies. Sheep-pox cells are virus-infected monocytes, macrophages and fibroblasts, but not endothelial cells.

Approximately 10 days post-infection, and corresponding with the peak of dermal infectivity, a severe necrotizing vasculitis develops. Virus particles have not been identified in endothelial cells and it is thought that the vasculitis may be due to immune complex deposition. Ischemic necrosis of the dermis and overlying epidermis follows. The pulmonary lesions are proliferative alveolitis and bronchiolitis with focal areas of caseous necrosis. Alveolar septal cells contain intracytoplasmic inclusion bodies. Histologic lesions, characterized by the accumulation of sheep pox cells, may involve the heart, kidney, liver, adrenal glands, thyroid gland, and the pancreas.

AFIP Diagnosis: Haired skin: Dermatitis, hyperplastic, subacute, multifocal, moderate, with epidermal and follicular keratinocyte ballooning degeneration, eosinophilic intracytoplasmic inclusion bodies, and sheep pox cells, breed not specified, ovine.

Conference Comment: As mentioned by the contributor, intracytoplasmic inclusion bodies may be present in several cell types. Conference attendees noted intracytoplasmic inclusion bodies in surface and follicular epithelial cells, and in dermal fibroblasts and macrophages.

Many strains of poxviruses are species specific and are given the name of the species that they infect (i.e turkeypox virus, canarypox virus, cowpox virus, etc.), while others may infect a wide range of hosts. Some pox diseases of vertebrates (family Poxviridae, subfamily Chordopoxvirinae) include the following:[5,6,7,8]

Genus	Virus	Major Hosts	Geographic Distribution
Orthopoxvirus	Variola virus (smallpox)	Humans	Eradicated globally
	Vaccina virus	Numerous: humans, cattle, buffalo, swine, rabbits	Worldwide
	Cowpox virus	Numerous: cattle, humans, rats, cats, gerbils, lg. felids, elephants, rhinoceros, okapi	Europe, Asia
	Camelpox virus	Camels	Asia, Africa
	Ectromelia virus (mousepox)	Mice, voles	Europe
	Monkeypox virus	Numerous: squirrels, monkey, anteaters, great apes, humans	Western and central Africa

	Uasin Gishu disease virus	Horses	Eastern Africa
	Tatera poxvirus	Gerbils (*Tatera kempi*)	Western Africa
	Raccoon poxvirus	Raccoons	North America
	Vole poxvirus	Voles (*Microtus californicus*)	California
	Seal poxvirus	Grey seals	North Sea
Capripoxvirus	Sheeppox virus	Sheep, goats	Africa, Asia
	Goatpox virus	Goats, sheep	Africa, Asia
	Lumpy skin disease virus	Cattle, Cape buffalo	Africa
Genus	**Virus**	**Major Hosts**	**Geographic Distribution**
Suipoxvirus	Swinepox virus	Swine	Worldwide
Leporipoxvirus	Myxoma virus	Rabbits (Oryctolagus and Sylvilagus spp.)	Americas, Europe, Australia
	Rabbit (Shope) fibroma virus	Rabbits (Oryctolagus and Sylvilagus spp.)	Americas, Europe, Australia
	Squirrel fibroma	Gray squirrels and woodchucks	Eastern United States
	Hare fibroma	European hares	Europe
Molluscipoxvirus	Molluscum contagiosum virus	Humans, horses, chimpanzees, kangaroos	Worldwide
Yatapoxvirus	Yabapox virus	Monkey, humans	West Africa
	Tanapox virus	Monkey, humans	West Africa
Avipoxvirus	Fowlpox virus	Chickens, turkeys, other birds	Worldwide
Parapoxvirus	Orf virus	Sheep, goats, humans	Worldwide
	Pseudocowpox virus	Cattle, humans	Worldwide
	Bovine papular stomatitis virus	Cattle, humans	Worldwide
	Auzdyk virus	Camels	Africa, Asia
	Seal parapoxvirus	Seals, humans	Worldwide

Contributor: Kimron Veterinary Institute, Pathology Department, P.O. Box 12, Beit-Dagan, 50250 Israel

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SLIDE 74
CONFERENCE 19 / CASE I – 04020321 (AFIP 2948688)

Signalment: Bovine, Angus, male, 5 months old.

History: Five-month-old Angus bullcalf found acutely dead. Lung tissues (only) submitted for microbiology and histopathology.

Laboratory Results: Aerobic culture of the lung retrieved trace numbers of contaminant bacteria: Alpha-*Streptococcus sp*., *Bacillus sp*., *Lactobacillus sp*. Moderate numbers of *Mycoplasma sp*. were obtained from *Mycoplasma* culture. Lung tissue was positive by PCR for bovine respiratory syncytial virus (BRSV).

Contributor's Morphologic Diagnosis: Severe acute bronchointerstitial pneumonia with syncytia.

Contributor's Comment: This case represents acute bronchointerstitial pneumonia secondary to BRSV infection in a young calf. The lung is diffusely hypercellular with widening of the interlobular septa by congestion, edema and inflammatory cells. The inflammation is intensified around bronchi and bronchioles with flooding of adjacent alveoli by edema. There is necrosis of bronchiolar epithelium and the bronchioles are partially occluded by large foamy macrophages, neutrophils, edema, fibrin and scattered multinucleated (syncytial) cells. Some bronchioles are surrounded by follicular type aggregates of lymphocytes implicating the role of *Mycoplasma* in the lesion.
The histological lesions and conspicuous syncytial cells suggest a viral pneumonia with the main differential diagnoses of BRSV or PI-3. The PCR test on DNA collected from lung tissue was positive for BRSV.

In the original sections obtained for diagnosis, the histological lesions were unique compared to normal field cases submitted to our laboratory because significant lesions from a secondary bacterial infection are not yet present. The features of the viral pneumonia were untainted. In the recuts of the tissue for WSC submission, there is regional variability in the purity of the viral lesion (vs. obscurity by the secondary bacterial component) and variability in the conspicuous numbers of syncytia.

Two excellent reviews on BRSV are provided.[1,2] BRSV was first isolated from an outbreak of respiratory disease in calves from Switzerland in 1970 and first reported in the United States in 1974. BRSV is a member of the pneumovirus genus, *Pneumovirinae* subfamily, *Paramyxoviridae* family within the virus order of *Mononegavirales*. The respiratory syncytial viruses are single stranded, negative-sense RNA viruses. The Pneumovirinae subfamily of paramyxoviruses is unique in that its members lack neuraminidase. The viruses attach to cells via membrane glycoprotein

G, and following infection, viral antigen is detected in bronchiolar and alveolar epithelium as well as alveolar macrophages. Although usually a prelude to bacterial infections as part of the bovine respiratory disease complex, BRSV can produce outbreaks of respiratory disease and occasional deaths on its own. Severe BRSV respiratory disease is usually restricted to calves less than 6 months old.

AFIP Diagnoses: 1. Lung: Pneumonia, bronchointerstitial, acute, multifocal, moderate, with necrotizing bronchitis and bronchiolitis, syncytia, and intracytoplasmic eosinophilic inclusion bodies, Angus, bovine.
2. Lung: Peribronchitis and peribronchiolitis, lymphoplasmacytic, multifocal, mild.

Conference Comment: Bovine Respiratory Syncytial Virus (BRSV) is a pneumovirus in the family Paramyxoviridae and is a causative agent of "enzootic pneumonia" or "calf pneumonia". BRSV occurs in a variety of breeds, although some reports state that certain breeds are more susceptible. During natural outbreaks, clinical disease is most severe in 1 to 5 month old calves, is seldom seen in calves less than 2 weeks of age, and is virtually absent in calves over 9 months of age. The high prevalence of antibodies against BRSV suggests that infection is endemic in most areas.[2]

Although the mode of transmission has not been determined, it is thought to occur through direct contact or aerosolization over short distances. Many factors influence the severity of disease, including: the animal's immune status, environmental conditions, animal management, and the presence of other infectious agents. The pathogenesis of BRSV is not clear; however, some research indicates that immune-mediated mechanisms play a dominant role. BRSV enhances bacterial colonization and adherence and alters the specific and non-specific defense mechanisms of the respiratory tract.[2]

Gross lesions are characterized as typical interstitial pneumonia involving the cranio-ventral region of the lungs. In affected areas, the lungs are consolidated and bronchi and bronchioles often are filled with mucopurulent exudate; hemorrhage and emphysema may also be present. The interlobular septa are expanded by pronounced edema. The cranio-dorsal and dorsal regions of the lungs often appear normal, but may also be markedly distended due to edema and severe alveolar, interstitial, and subpleural emphysema. Bronchial and mediastinal lymph nodes are often markedly enlarged, edematous, and occasionally emphysematous. Histologically, there is a bronchitis and peribronchitis accompanied by a large number of syncytial cells in the nasal and tracheal mucosa and alveolar and bronchiolar epithelium. Viral antigen is first detected in the bronchiolar epithelium, later in type I and type II pneumocytes, and may also be detected in alveolar macrophages. The virus is capable of cell-to-cell transmission, resulting in the generation of characteristic syncytial giant-cells. Degeneration, necrosis, and hyperplasia of bronchial epithelium and of lymphoid tissue around the bronchi are consistently present. The bronchial and bronchiolar exudates

consist primarily of epithelial cells, neutrophils, and occasionally eosinophils, and are often accompanied by edema and hyaline membrane formation.[2]

Contributor: Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Room 250 McElroy Hall, Stillwater, OK 74074 http://www.cvm.okstate.edu

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SLIDE 75
CONFERENCE 19 / CASE II – 03-21575 (AFIP 2936448)

Signalment: Unknown age, castrated male, mixed breed, *Bos taurus*, bovine.

History: Five out of 100 castrated male feedlot calves in the same pen died suddenly with no clinical signs prior to death.

Gross Pathology: The heart had numerous epicardial, myocardial and endocardial hemorrhages mixed with a few inconspicuous pale streaks.

Laboratory Results: *Haemophilus somnus* was isolated in pure culture from the heart, lung, and a pericardial swab. Immunohistochemical staining of an ear notch biopsy and the heart was negative for bovine viral diarrhea (BVD) virus.

Contributor's Morphologic Diagnosis: Heart: Multifocal and perivascular suppurative myocarditis and epicarditis with vasculitis, thrombosis, hemorrhage, myocardial degeneration and necrosis, and intralesional gram-negative coccobacilli.

Contributor's Comment: The submitted heart sections contain multiple perivascular and random infiltrates of numerous intact and degenerate neutrophils within the myocardium and epicardium. The tunica media of many small venules contains intact and degenerate neutrophils with necrosis of the vascular wall. Many of the affected venules contain fibrinous and fibrinocellular thrombi. The epicardium and myocardium contain multiple hemorrhages, which are often associated with the affected blood vessels. A few of the affected venules and inflammatory foci in the myocardium contain variable numbers of small gram-negative coccobacilli. There are a few foci where the cardiomyocytes are degenerate and necrotic, especially those entrapped within the inflammatory foci.

Haemophilus somnus is a small, gram-negative bacillus that can cause various and numerous clinical disease presentations in cattle.[1] The disease presentations in cattle include, but are not limited to, pneumonia, meningoencephalitis, myocarditis, myocardial abscesses, myositis, polyarthritis, abortion, endometritis, orchitis, epididymitis, placental vasculitis, intestinal thrombosis, laryngeal ulceration, and a single case report of a urachal abscess.[1-9] Although some of the disease presentations can be primary localized infections, such as pneumonia, many of the disease manifestations of *H. somnus* infections in cattle are due to septicemia, such as meningoencephalitis, polyarthritis, and myocarditis.[1]

Often, the septicemic form results in meningoencephalitis, but polyarthritis and myocarditis can be seen singly or in combination with other affected organs.[1] Although the lung can be involved with *H. somnus* septicemia, pneumonia is an uncommon feature of the septicemic disease. When the lung is involved, it more commonly results in a primary fibrinous pneumonia or suppurative bronchopneumonia. The pneumonia caused by *H. somnus* can be microscopically indistinguishable from that caused by *Mannheimia* (*Pasteurella*) *haemolytica*.[1,10] Most affected cattle develop a fibrinopurulent polyarthritis, particularly in the atlanto-occipital joint.[1] Myocarditis supposedly following an asymptomatic episode of septicemia is a major manifestation of *H. somnus* infection in some parts of North America.[1] In one study performed in Canadian feedlots, *H. somnus* infection was found in 70 out of 92 cases of myocarditis in calves in the feedlot.[11]

The most common macroscopic lesions seen with *H. somnus* septicemia in cattle are multiple foci of hemorrhage and necrosis in multiple organs.[1] Microscopically, the consistent feature of *H. somnus* septicemia is an intense vasculitis, usually of small venules and veins. The inflammation can extend into the surrounding parenchyma of the affected organ. The vasculitis often results in hemorrhage and can result in infarction of the organ. The affected venules often contain fibrin thrombi, which commonly contain colonies of bacteria. These colonies of bacteria are believed to proliferate at the site of thrombosis and are believed not to be bacterial emboli.[1] Although vasculitis is a common feature of *H. somnus* septicemia in cattle, the exact pathogenesis of the vasculitis is not known, but it is believed to be due to *H. somnus*–induced apoptosis of endothelial cells.[12]

AFIP Diagnosis: Heart: Myocarditis and epicarditis, suppurative, perivascular and random, moderate, with vasculitis, thrombi, myocardial degeneration and necrosis, and colonies of coccobacilli, mixed breed, bovine.

Conference Comment: Conference attendees discussed the histopathological changes seen in cardiomyocyte degeneration and necrosis. Cardiac muscle is structurally similar to skeletal muscle and is subject to the same anatomic changes associated with degeneration. Cardiac myocyte degeneration is characterized by a swollen vacuolated sarcoplasm, while necrosis is generally characterized by shrunken,

hypereosinophilic or fragmented sarcoplasm, loss of cross-striations, and karyorrhexis, karyolysis, or pyknosis. However, the gross and microscopic appearance of myocardial necrosis is dependent on the interval between the initial insult and death.

In this case, the inflammation, degeneration, and necrosis occurred around, and frequently obscured and disrupted vessels. Other causes of embolic myocarditis include *Salmonella* sp., *E. coli*, and rarely *Erysipelothrix rhusiopathiae*.[13] Clinically, *Clostridium chauvoei* is another common cause of acute death in cattle with no clinical signs prior to death. However, gross and histologic lesions of *C. chauvoei* differ from those of this case. *Clostridium chauvoei* affects skeletal muscle and cardiac muscle, with gross lesions characterized by focally extensive myonecrosis with edema and emphysema; histologically, the lesions are not vasocentric, but are focally extensive.

Histophilus somni is the proposed new name for *Haemophilus somnus,*[14] and infection causes a variety of disease syndromes, as previously mentioned by the contributor. However, *H. somni* is an opportunistic pathogen that is a relatively non-invasive commensal of the bovine respiratory and reproductive mucosal surfaces. Nonetheless, when factors that favor disease and compromise immunity, such as the stress of transportation, concurrent viral infection, overcrowding, pregnancy, lactation, and harsh weather significantly affect the animal, disease ensues.[15]

Vasculitis is the hallmark of systemic *H. somni* infections; however, the pathogenesis of vascular damage is poorly understood.[15] Identifying the mechanism by which *H. somni* induces endothelial cell damage is difficult because the virulence factors are not well characterized. In one report, the *H. somni* virulence factor LOS (lipo-oligosaccharide) induced endothelial cell apoptosis in a time- and dose-dependent manner in-vitro.[12] Another recent report noted virulence factors such as LOS phase variation, induction of endothelial apoptosis, intraphagocytic survival, and immunoglobulin Fc-binding proteins were important to survival and colonization of *H. somni*.[15]

Contributor: Kansas State University, Department of Diagnostic Medicine/Pathobiology, 1800 Denison, Manhattan, KS
http://www.vet.k-state.edu/depts/dmp/index.htm

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SLIDE 76
CONFERENCE 19 / CASE III – 03-9400 (AFIP 2933956)

Signalment: 3 month old female, Jersey calf, bovine (*Bos taurus*).

History: Three calves from a group of approximately 70 had bloody diarrhea and were recumbent. Animals were euthanized by the practitioner and tissues were submitted to the diagnostic laboratory.

Gross Pathology: The only gross lesions noted on the necropsy report by the practitioner were ulcers throughout the abomasums of the calves.

Laboratory Results: Rare oocysts of *Eimeria zuernii* and *Eimeria ellipsoidalis* were noted on routine fecal examination. A fluorescent antibody test of colon and an immunohistochemical stain of colon were positive for bovine coronavirus.

Contributor's Morphologic Diagnosis: Colon: Colitis, lymphoplasmacytic and eosinophilic, diffuse, moderate with numerous coccidial schizonts and gametocytes, Jersey, bovine.

Contributor's Comment: Diffusely throughout the colon, there is mild to moderate lymphoplasmacytic eosinophilic colitis with many second generation coccidial schizonts and gametocytes present within epithelial cells of colonic crypts. In addition, multifocally there is marked dilation of colonic crypts with occasional epithelial attenuation and crypt hyperplasia. Occasionally colonic crypts are collapsed. Within sections of small intestine examined (sections not submitted with this case), there is multifocal crypt dilation and hyperplasia with occasional crypts containing inflammatory debris.

Histologic lesions within the colon in this case are characteristic for coccidial enteritis in calves. The second generation schizonts of the more pathogenic coccidia enter the crypt epithelial cells of the colon and cecum approximately 14 – 18 days after infection.[2] Virtually all cells lining cecal and colonic glands can be infected.[2] As cells rupture and oocysts are released, the remaining intact glandular epithelium can become markedly attenuated and the glands may even collapse.[2] Crypt hyperplasia occurs in an attempt to regenerate mucosal epithelium in areas where it has been ulcerated and/or denuded.[2]

Eimeria zuernii and *Eimeria bovis* are most often implicated in cases of clinical coccidiosis in cattle up to 2 years of age[3]; however, other coccidia including *E. ellipsoidalis* and *E. auburnensis* are also known to cause less severe diarrhea.[2]

Additionally, laboratory testing indicated infection with bovine coronavirus in this case. It is likely the characteristic histologic lesions of this viral infection are masked by the coccidial colitis present; however, some of the lesions present may non-specifically support the presence of viral enteritis. For example, colonic lesions indicative of coronavirus infection include a mixed inflammatory reaction within the lamina propria, dilated colonic glands with attenuated epithelium and glandular hyperplasia.[1] Additionally, crypt epithelium in the small intestine may also be hyperplastic as a result of coronavirus infection.[1]

AFIP Diagnosis: Colon: Colitis, lymphoplasmacytic and eosinophilic, diffuse, moderate, with crypt loss, regenerative hyperplasia and ectasia, and myriad intracellular coccidia, etiology consistent with *Eimeria* spp., Jersey, bovine.

Conference Comment: Over a thousand species of Eimeria are known that primarily infect intestinal epithelial cells of domestic and wild mammals and birds. The life cycle of each species is host specific and direct. Unsporulated oocysts are shed in the feces and sporulate in the environment to become infectious. Following ingestion, sporozoites excyst, invade intestinal epithelial cells, form trophozoites and undergo asexual multiplication (schizogony, merogony) within a schizont or meront. Merozoites are released and eventually form sexual stages (micro- and macrogametes), which unite to form oocysts.[4] Some common coccidia species of domestic and wild mammals and birds include the following:[3,4,5]

Animal	Coccidia	Organ affected
Cattle	*E. bovis*	1st gen schizont – Jejunum 2nd gen schizont – Cecum and colon
Sheep	*E. ahsata* *E. bakuensis* *E. ovinoidalis*	Small intestine Small intestine Ileum/Large intestine
Goats	*E. christenseni* *E. arloingi* *E. ninakohlyakimovae*	Small intestine Small intestine Large intestine
Equine	*E. leuckarti*	Small intestine
Swine	*I. suis*	Small intestine
Canine	*I. canis*	Ileum, colon occasionally
Feline	*I. felis*	Small intestine, colon occasionally
Mice	*E. falciformis*	Colon
Rabbit	*E. stiedae* *E. intestinalis* *E. flavescens*	Bile ducts Ileum & cecum Ileum & cecum
Birds		
Chickens	*E. acervulina* *E. necatrix* *E. maxima* *E. tenella*	Duodenum Mid-intestine Mid-intestine Ceca
Turkey	*E. adenoeides* *E. meleagrimitis* *E. gallopavonis*	Ceca Mid-intestine Colon, rectum
Geese & ducks	*E. truncata* *E. anseris*	Kidney Mid-intestine

Contributor: South Dakota State University, Veterinary Science Department, P.O. Box 2175, North Campus Drive, Brookings, SD
http://vetsci.sdstate.edu

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SLIDE 77
CONFERENCE 19 / CASE IV – 04-K0227 (AFIP 2937322)

Signalment: 2 month old female polled Hereford calf.

History: This calf was found dead in the field where she was housed with her dam and 90 other cows and calves. The calf had recently (< 1 week ago) been moved to pasture from a calving pen, where management had been of good quality (adequate colostrum, good navel care, perinatal vitamin E / selenium injection). No previous illnesses had been identified in this calf.
Gross Pathology: Body condition was fair. The liver was pale and mottled, with capsular petechiation. Mesenteric lymph nodes were slightly enlarged. Lungs were red, wet, and heavy, with obvious interlobular edema in some areas. The rumen contained black, semi-fluid, odiferous debris.

Laboratory Results: Bacteriology: *Listeria monocytogenes* was isolated in large numbers from liver and spleen.

Contributor's Morphologic Diagnoses: 1. Acute multifocal necrotizing adrenalitis and hepatitis with intralesional bacilli.
2. Multifocal renal intravascular bacterial emboli.
3. Multifocal microvascular pulmonary thrombosis.

Contributor's Comment: Histologic lesions were consistent with septicemic listeriosis but were more severe than typically seen.[1] In the liver, multiple foci of acute coagulation necrosis involved over 75% of hepatic parenchyma, and large numbers of plump gram-positive bacilli were present within hepatocytes and extracellularly at the margin of necrotic and viable parenchyma. Lesions were similar in the adrenal gland, with multiple discrete foci of coagulation necrosis, involving approximately 50% of cortical parenchyma and large numbers of bacilli present among necrotic cellular debris within most foci. In the kidney, dense clusters of similar bacilli filled the lumens of several cortical and medullary interstitial blood vessels and glomerular capillaries. Pulmonary alveoli were flooded with proteinaceous edema fluid occasionally mixed with

fibrin or low numbers of macrophages, and a moderate number of alveolar capillaries contained luminal fibrin thrombi.

Small intestinal submucosal lymphoid aggregates were sparsely populated and rare individual mucosal crypts had widely dilated lumens filled with neutrophils and necrotic cellular debris. Immunohistochemistry (IHC) for bovine viral diarrhea virus (BVDV) identified abundant viral antigen in mononuclear cells in the intestinal lamina propria, submucosa, and Peyer's patches; in the tunica media of submucosa blood vessels; and in scattered mucosal epithelial cells. Abundant positive staining for BVDV was also evident in Kupffer cells and few intact hepatocytes in liver; mononuclear cells in lymph node; and tunica media of myocardial blood vessels. IHC was negative for bovine herpesvirus-1 antigen in liver and adrenal gland. Immunosuppression in cattle due to BVDV infection can promote susceptibility to other infectious agents, and BVDV may have predisposed this calf to severe septicemic listeriosis.[2]

AFIP Diagnosis: Liver: Hepatitis, necrotizing, acute, random, severe, with myriad bacilli, Hereford, bovine.

Conference Comment: *Listeria monocytogenes* is a small, rod-shaped, gram-positive intracellular bacterium that causes disease in most species of animals and humans. The organism is ubiquitous in nature and can be found in soil, vegetation, dairy products, animal feces, and sometimes the oropharynx and tissues of healthy animals.[1]

Listeriosis occurs as three distinct syndromes, which ordinarily do not occur together: systemic infection (septemia) in humans, cattle, sheep, swine, dogs, cats, and rodents; encephalitis in humans, cattle, sheep, goats, and swine; and abortion in humans, cattle, and sheep. Less commonly, *L. monocytogenes* is a cause of endocarditis and purulent lesions in other tissues.[1]

Systemic infection is the more common form of listeriosis in monogastric animals and in human infants. The most characteristic lesion in this form is focal necrosis of the liver. However, lesions may also occur in the spleen, lymph nodes, lungs, adrenal glands, gastrointestinal tract, and brain. Microscopically, there are areas of necrosis infiltrated by mononuclear cells and some neutrophils. The organisms may be seen in sections stained with H&E (Hematoxylin and Eosin) or can be easily demonstrated with B&B (Brown-Brenn) and B&H (Brown-Hopps).[1]

Encephalitis is the most characteristic form of the disease in ruminants. Clinical signs include abnormal posturing of the head and neck, walking aimlessly in a circle ("circling disease"), nystagmus, blindness, and paralysis. The organism is thought to reach the central nervous system by ascending peripheral nerves, particularly the trigeminal nerve, and localizing in the brain stem, particularly the medulla oblongata, and in the spinal cord. Gross lesions are usually absent; however, leptomeningeal opacity and foci of necrosis in the terminal brain stem have been noted.[3] Microscopically, there are

perivascular mononuclear cell infiltrates, with or without neutrophils. Diffuse cellular infiltration and microabscessation involving both the gray and white matter may occur, but there is usually relatively little tissue necrosis.[1] However, necrosis and accumulation of gitter cells can be prominent in some cases. Other changes include neuronal necrosis and leptomeningitis.[3]

Listeric abortion in animals is important in cattle and sheep. Abortion usually occurs in the last quarter of gestation without signs of infection in the dam. The fetus dies in utero and may be severely autolyzed when expelled.[1] Placental lesions include severe diffuse necrotizing and suppurative inflammation of both the cotyledons and the intercotyledonary areas. The fetal lesion is an enlarged liver with numerous 1 mm yellow foci. Microscopically, severe inflammation involves the mesenchyme of the villi and the upper intercotyledonary chorion. Chorionic epithelial cells, especially in areas between the villi, are filled with gram-positive bacilli. The cells in the areas of acute multifocal necrotizing hepatitis are also filled with organisms.[4]

Contributor: University of Guelph, Laboratory Services Division, Animal Health Laboratory, Guelph, Ontario
http://ahl.uoguelph.ca

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SLIDE 78
CONFERENCE 20 / CASE I – 99-10725 (AFIP 2683903)

Signalment: 2-month-old, male, Arabian horse (*Equus caballus*).

History: This colt had a spastic gait, ataxia and head tremors for about two weeks prior to elective euthanasia.

Gross Pathology: No significant gross lesions were noted in the thoracic or abdominal viscera. The cerebrum and cerebellum appeared normal in size and development,

externally. A sagittal section of the cerebellum revealed less prominent folia in the cranial and dorsal areas of the vermis.

Laboratory Results: A computerized tomography (CT) scan of the brain was inconclusive.

Contributor's Morphologic Diagnosis: Cerebellar cortical abiotrophy.

Contributor's Comment: The history and gross and microscopic lesions in this case are consistent with a diagnosis of cerebellar cortical abiotrophy. Affected animals are usually neurologically normal at birth with clinical signs developing at various times in postnatal development. Spasticity and ataxia are observed in the gait, especially in the forelimbs. Grossly, the cerebellar folia are best developed (or least degenerative) in the caudal and ventral areas of the cerebellum (see gross photograph). The cerebellum was not weighed in this case but in normal horses the cerebellum should be about 10-12% of total brain weight. Microscopically, there are degenerative and missing Purkinje cells with a reduction in all layers where Purkinje cells are absent. There is accentuation of radial astrocyte processes in the molecular layer. The cause of this condition is unknown, although a hereditary component (autosomal recessive trait) is suspected.

AFIP Diagnosis: Brain, cerebellum: Purkinje and granular cell degeneration and loss (cerebellar cortical abiotrophy), diffuse, moderate, with mild Wallerian degeneration in the folia white matter, and mild gliosis of cerebellar nuclei, Arabian, equine.

Conference Comment: Conference attendees discussed the relationship between the normal development and physiology of the cerebellum and the clinical and histopathological changes identified in this case.

The term abiotrophy literally means the lack of a life-sustaining nutritive factor. Implicit in its use in veterinary medicine is the presumption that the premature neuronal degeneration is not an acquired insult, but rather the consequence of an intrinsic metabolic disorder. However, the specific metabolic derangement may vary from syndrome to syndrome. The hallmark of cerebellar abiotrophic diseases is the premature demise of discrete and often functionally related populations of neurons, after the organ has developed its full cellular complement.[1]

In abiotrophic conditions encountered in veterinary medicine, neurological deficits usually begin in the first few weeks to months of life, are progressive, and are inherited in studied populations. The clinical hallmark of cerebellar cortical abiotrophies is neurological normality at birth, followed by the development of cerebellar deficits that progressively worsen in the post-natal period. In contrast, in-utero damage, such as with viral agents that may damage the developing cerebellum at a very precise stage of fetal life, results in cerebellar ataxia from the time of birth. Because the injury is not

ongoing, the neurological deficits are often static and may even slowly improve as the animal learns to compensate.[1]

Microscopically, cerebellar cortical abiotrophies are characterized by ongoing neuronal degeneration and loss, with reactive gliosis in the background of a normally developed cerebellum. Neither folial dysplasia nor neuronal heterotopia occurs in cerebellar cortical abiotrophies; they are features of in-utero viral infections that can disrupt normal cerebellar development. Purkinje cells are usually affected first in abiotrophies, and, in general, a reduction of the granule cell neurons follows. There is often proliferation of astroglia (Bergmann astrocytes) in the affected folia and a mild gliosis of the molecular layer. Due to Purkinje cell degeneration, Wallerian degeneration may be found in the white matter of the folia and low numbers of spheroids may be present in the granular layer, cerebellar white matter, or the nuclei of the cerebellar medulla. Cerebellar nuclei are often gliotic.[1]

Cerebellar abiotrophies have been reported in a number of domestic and laboratory species, including dogs, cattle, sheep, Yorkshire pigs and Arabian horses and Gotland ponies. The time of onset of clinical signs varies with the species and breed affected. Common dog breeds affected include Kerry Blue Terriers, Gordon Setters and Rough-Coated Collies. Veterinary Neuropathology provides a more thorough list of predisposed breeds.[1] The syndrome in Kerry Blue Terriers is unique since the caudate nucleus and the substantia nigra are also affected. Cerebellar abiotrophy of Gordon Setters is unusual since it has a delayed onset, with clinical signs not typically appearing until six to 24 months of age. Kerry Blue Terriers and Rough-Coated Collies may have extracerebellar involvement, with Wallerian degeneration in the brainstem and spinal cord.

Contributor: University of Minnesota, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, 1333 Gortner Avenue, St. Paul, MN

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SLIDE 79
CONFERENCE 20 / CASE II – 51304 (AFIP 2947490)

Signalment: 14-year-old, male-castrated, Golden retriever.

History: In early spring 2003, the dog began to become bilaterally paretic in the rear legs. This progressively got worse. In November 2003, a cervical laminectomy was performed. The dog did not get any better following surgery. After six months, the owner opted for euthanasia.

Gross Pathology: No gross abnormalities were noted.

Contributor's Morphologic Diagnosis: Meninges: Pachymeningitis, ossifying, diffuse, severe with fibrosis.

Contributor's Comment: The case resembles closely a case described in the Journal of the American Veterinary Medical Association,[1] except that it is far more extensive, with involvement of the entire spinal cord. A comment on their report was written by Dr. John McGrath,[2] who felt that they had over-interpreted the lesion. The case and lesion are unusual, but the effects on the animal were severe, as are the lesions. A differential diagnosis that might be considered is Lyme disease.

AFIP Diagnoses: 1. Leptomeninges, spinal: Fibroplasia, diffuse, with vascular hyalinization, and multifocal arachnoid cell proliferation, Golden Retriever, canine.
2. Dura, spinal: Osseous metaplasia, focal.
3. Spinal cord, ventral funiculi: Axonal degeneration and loss, mild, with dilated myelin sheaths, axonophagia, and rare spheroids.

Conference Comment: There is significant variation in slides and not all features may be present on all slides. In the sections reviewed by conference attendees, the major histological changes in the arachnoid layer included fibroplasia, vascular hyalinization, and multifocal arachnoid cell proliferation. The osseous metaplasia noted in some slides is within the dura mater.

The literal definition of pachymeningitis is inflammation of the pachymeninges, also known as dura mater. Metaplastic ossification of the spinal dura occurs most frequently in the lumbar region of large and giant breed dogs, and is sometimes referred to as ossifying pachymeningitis. These grayish islands of lamellar bone may contain adipocytes and myeloid elements and are an incidental, age-related change. An association with intervertebral disk prolapse has been suggested, but this metaplastic change can be found in the cranial dura also. In addition, thickening (sclerosis) as a result of fibrosis, with acellular collagen deposition or hyalinization, may be found in the leptomeninges and choroid plexus stroma of old animals.[3] Residents also noted small amounts of perivascular lipofuscin accumulation, particularly in the gray matter. This too is a common finding in older dogs.

Of additional interest in this case is the axonopathy in the ventral funiculi, which is likely due to focal or diffuse disease rostral to this segment and may be the cause of the clinical signs noted in this case. Nonetheless, the histopathological lesions in the spinal cord are likely not the result of the meningeal lesions. The causes of the leptomeningeal changes and the spinal cord axonopathy are not evident in the slides examined in conference.

Contributor: Johns Hopkins University School of Medicine, Department of Comparative Medicine, 733 North Broadway, Suite 811, Baltimore, MD

References:

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SLIDE 80
CONFERENCE 20 /CASE III – 03-1499 (AFIP2935569)

Signalment: 4-month-old, female mixed breed goat.

History: Recumbent for one week. The animal had some tremors and stiff hind limbs.

Gross Pathology: None.

Contributor's Morphologic Diagnoses: 1. Cerebellar abiotrophy.
2. Neuronal and axonal degeneration, spinal cord, mild.

Contributor's Comment: The cerebellar folia have depletion of the granular cell layer and loss of Purkinje cells. Chromatolysis is seen among the remaining Purkinje cells and in the large neurons within the brainstem. The spinal cord has mild Wallerian degeneration of the ventral white matter tracts and chromatolysis of neurons in the ventral horn. The cerebellar lesion is consistent with cerebellar abiotrophy, but when taken in consideration with the spinal cord lesions, this case is an example of a multisystem neuronal degeneration.

Multisystem neuronal degeneration is a degeneration or abiotrophy of neurons in multiple locations throughout the nervous system. In humans these include both familial and acquired conditions and may affect single or multiple neuronal systems, including motor, sensory and autonomic neurons. Neuronal system degenerations have been reported in a pig and several breeds of dogs, including the Swedish Lapland reindeer-herd dog, Cairn Terrier, Cocker Spaniel and Miniature Poodle. These primarily affect the

brain and spinal cord and are inherited in all but the Miniature Poodle. Cerebellar abiotrophy and multisystem neuronal degeneration have not been reported in the goat.

AFIP Diagnoses: 1. Brain, cerebellum: Purkinje and granule cell degeneration and loss, diffuse, moderate, with Purkinje cell ectopia, and molecular gliosis, mixed breed, caprine.
2. Brainstem, medulla oblongata; spinal cord, ventral column: Neuronal degeneration (chromatolysis), multifocal, mild to moderate, with gliosis.

Conference Comment: In contrast to the first case, not only is there Purkinje cell degeneration and loss, but there is also Purkinje cell ectopia with moderate numbers of Purkinje cells located in the molecular layer. The latter indicates this degenerative disease began in-utero when the Purkinje cells were still migrating from the internal germinal layer adjacent to the fourth ventricle.[2]

The histopathological changes present in the cerebellum, brain stem, and the spinal cord, suggest copper deficiency as a possible etiology. Copper deficiency can cause two clinical neurologic disease syndromes in sheep and goats: congential (swayback) and acquired (enzootic ataxia).

In the congenital form (swayback), the condition develops in-utero and clinical signs are present at birth with affected animals being totally recumbent or severely ataxic. Other signs include depression, head shaking, trembling, and most affected animals die soon after birth. Grossly, there may be small foci of gelatinous softening, or cavitation of the cerebral hemispheres. Microscopically there is absence or destruction of the white matter of the cerebral hemispheres with chromatolysis of large motor neurons of the red and vestibular nuclei. Demyelination of the motor tracts of the white matter of the spinal cord has also been reported.[3]

The delayed form (enzootic ataxia) develops after birth with animals appearing normal at birth and developing signs of the disease from one week to six months of age. Clinical signs include incoordination, ataxia, and posterior paresis. Lesions are limited to the large neurons of the brain stem and spinal cord. However, goats with enzootic ataxia may have well defined lesions in the cerebellum, including patchy cerebellar hypoplasia, necrosis, and loss of Purkinje cells and depletion of the granular cell layer.[3]

Swayback and enzootic ataxia may result from either primary or secondary copper deficiency. Primary copper deficiency is caused by a diet that is low in copper. Secondary copper deficiency results from dietary composition, which determines the proportion of dietary copper that is absorbed. It is well known that other minerals, such as molybdenum, sulfur, and iron can interfere with proper copper utilization. However, food type and the interaction between food type and mineral composition will also affect copper absorption and ultilization.[3]

Contributor: Virginia Tech, College of Veterinary Medicine, Department of Biomedical Sciences & Pathobiology, Blacksburg, VA
www.vetmed.vt.edu

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SLIDE 81
CONFERENCE 20 / CASE IV – TAMU 04-02 (AFIP 2941201)

Signalment: 10-day-old, male Brangus calf.

History: The calf was born in a pasture and found recumbent with a "hole in the back." The calf never walked, and was brought into the clinic as a donation. At presentation, the animal was moribund and considered septicemic. He had a withdrawal reflex in all limbs but could not stand. A skin defect over a deep hole was noted on the dorsum in the thoraco-lumbar area. No work-up was conducted, and a clinical diagnosis of spina bifida preferred. The animal was euthanized.

Gross Pathology: Decubital ulcers were over bony prominences. A 2X1.5 cm, open, skin defect was at the T13-L1 junction. Another depression in dorsal, axial tissues was at the L6-S1 junction, but was not associated with a skin defect. The dorsal arch of the T13-L1 junction was absent and a hyalinized membrane connected the spinal canal to the "hole" seen grossly. The spinal cord became attenuated and deviated dorsally into the membrane. No spinal cord was seen from L1 to approximately L3 (spinal dysraphism in its broadest definition; segmental spinal cord aplasia or necrosis). The spinal column began again and continued normally from L4 caudally. A fibrous band was in the area of the arch at L6-S1, and the dura and spinal cord appeared normal in this area (spina bifida occulta).

The cortex of the occipital pole of the brain extended caudally over the cranial aspect of the cerebellar cortex at the midline with a redundant or hamartomatous growth of the marginal gyrus (local, cerebral-cortical dysplasia/redundancy). Meninges were slightly opacified. The lateral ventricles were twice normal volume (hydrocephalus)

Macroscopic Diagnosis: Multiple spina bifida aperta and occulta; spinal dysraphism/focal spinal cord aplasia/necrosis; meningitis, hydrocephalus, cerebral cortical hamartomatous dysplasia.

Contributor's Morphologic Diagnosis: Severe, subacute/chronic suppurative meningomyelitis, with granulation tissue and numerous bacteria; spinal meningocele; spinal dysraphism with agenesis of the spinal cord, OR absence of spinal cord, OR hypoplasia of the spinal cord, OR duplication of the spinal canal AND/OR diastematomyelia

Contributor's Comment: Malformations offer special challenges to the diagnostician. The most important decision is determining if the malformations are hereditary. I do not believe this is a hereditary condition. The low incidence of such lesions and the inadequate histories in our necropsy population usually make that determination impossible. The variation in malformations between individuals makes comparison between cases of malformations difficult, and usually, the diagnosis is a descriptive exercise.[1,2,3,5,7,8,12,13] Often, one malformation is accompanied by or leads to more malformations. Sometimes, names given to cases are inaccurate. Depending on the level of the section received, the lesion you see on your slide will vary in this presented case. A unifying theme is subacute to chronic inflammation with granulation tissue, neutrophilic and histiocytic exudates, and bacteria associated with fibrin and necrosis. An occasional thrombus is noted (considered a consequence not a primary lesion). The animal could not move and history suggested the animal's back lesion (the myelocele) had been pecked at by birds. Thus, with the opening to the CNS, sepsis became rampant in this case (and other similar cases in our files). The meninges surround the cord as the cord 1) moves dorsally, 2) becomes smaller, 3) loses its central canal, 4) is progressively bisected to give diastematomyelia, 5) becomes a small core of nondescript neural tissue, and then, 6) disappears from the section.

A series of images is provided for all participants to follow. The entire affected area of cord was blocked and cut.

Figure 4. Cranial to the meningocele (2X).
Figure 5. Cord at start of the "coele" (2X).
Figure 6. Cord with ventral fibrous septum dissecting the cord (2X).
Figure 7. Fibrous septum dividing the cord (diastematomyelia) (2X).
Figure 8a. Fibrous tissue and remnant neural parenchyma (2X).
Figure 8b. Remnant neural tissue of 8a, note thrombus (10X)
Figure 9. Intact cord with two central canals distal to the meningocele (2X).

The sections where spinal cord is not present are not submitted. One could argue that the infection destroyed the cord in this area; however, the progressive diminution of tissue and persistence of remnant cord in caudal sections of the affected area as described above argues against this theory. Segmental loss of spinal cord with reappearance in both cervical and lumbo-sacral areas is described in calves.[5,7,10,11] Such segmental loss in areas where the development of a canal and mesenchymal structures is complete suggests to me that the cord was present at one time to allow induction of somite development, and only later, the cord underwent necrosis. Subsequently, the lumbar and sacral cord reappears as normal except for a brief caudal lumbar segment with duplication of the central canal and the continued inflammatory

change seen at all levels of the cord and brain. The loss of the dorsal bony arch in the lumbo-sacral junction was associated with no defect in the associated cord and is spina bifida occulta. The withdrawal reflex noted clinically is a spinal reflex and not demonstrative of perception of deep pain. It is tempting to say that the premature termination of the cord with its filamentous end not going into the meningocele may represent tethering of the cord.

Spina bifida is a form of rachischisis/cleft vertebral canal.[4,6,14] Dysraphism is failure of a fusion of a raphe. Interestingly, the term per se is not defined in or not even used by some current human neuropathology texts. However, some authors[3] have (incorrectly I believe) used the term more broadly to include any "myelodysplasia" including: aplasia, hydromyelia, syringomyelia, fusion failures of the neural tube, etc.. Unfortunately, I was taught and remember that Weimaraner dogs had a condition of spinal dysraphism, which is an incorrect diagnosis for the condition.[15] It is syringomyelia, hydromyelia, and central canal dysplasia. I think the term, dysraphism, should be used generically when there is a neural tube fusion disorder. Most cases of spina bifida have a closed neural tube. We will not discuss the process of closure of the neural tube (Chapter 8 in Greenfield's), but it occurs as a bidirectional process occurring multifocally in both the spinal cord and brain. The caudal spinal cord develops by secondary neurulation via a growth of neural cells caudally, NOT from a tube. The lumbosacral spina bifida occulta of this case is probably the result of a defect in this secondary neurulation process. Spina bifida's pathogenesis is thought to involve: 1) abnormal proliferation of neural tissue in this area, 2) focal ischemic injury, or 3) an idiopathic/undefined maldevelopment of the tail bud.

Spinal cord anomalies often are associated with brain malformations, especially Chiari II malformations; however, the cerebellar vermis in this animal is normal. The redundant cortex seen is not part of a described syndrome. Hydrocephalus is common in cases of spina bifida as well.

AFIP Diagnosis: Spinal cord: Myelodysplasia, severe, with duplication of spinal roots, chronic suppurative meningitis, granulation tissue, and numerous bacteria, Brangus, bovine.

Conference Comment: The contributor provides an excellent description, possible pathogenesis, and discussion of the lesions present in this case. There is considerable variation in slides and not all lesions described may be present on all slides.

Spina bifida in its customary usage refers to absence of the dorsal portions of the vertebrae. It is an imperfect name as the various forms of the defect largely represent differences in the degree of defective closure of the neural tube, its separation from the ectoderm, and its induction of a skeletal investment. Often, the defect is divided into several classes on the basis of severity. Myeloschisis, spina bifida occulta, spina bifida cystica with meningocele, and spina bifida with myelomeningocele apply to the vertebral

defect. Amyelia, diastematomyelia, hydromyelia, and dysraphism apply to the spinal cord defect. The most severe forms of myelodysplasia occur in association with spina bifida, as seen in this case.[15]

Contributor: Texas A&M University, College of Veterinary Medicine, Department of Veterinary Pathobiology, College Station TX
http://vtpb-www.cvm.tamu.edu/

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SLIDE 82
CONFERENCE 21 / CASE I – 166-04 or 1250-03 (AFIP 2941192)

Signalment: 4 year old, spayed female, Dachshund, dog (*Canis familiaris*).

History: The dog first presented in April 2003 for cutaneous petechiae, and was diagnosed with immune-mediated thrombocytopenia and anemia. She was treated with vincristine, cyclosporine and prednisone, and the anemia and thrombocytopenia resolved. She was then prescribed long term prednisone and cyclosporine. In May, the dog presented for an acute onset of reddening of the left eye. Ophthalmic examination revealed fibrinous anterior uveitis and increased intraocular pressure. At that time, she was also showing stranguria. *Candida* spp. was isolated from her urine, and treatment with itraconazole was initiated for fungal cystitis. Topical prednisone and glaucoma medications failed to control the inflammation and high intraocular pressure in the left eye, which became buphthalmic and was enucleated in June. The signs of urinary tract infection resolved, but the dog presented later in June, for evidence of uveitis in the right eye. The right eye partially responded to therapy with topical steroids, until January 2004 when the dog became blind. The right eye was then buphthalmic, and was enucleated. In the meantime, cystitis recurred several times in 2003, despite treatment with itraconazole and ketoconazole.

Gross Pathology: The right globe was 20 mm in diameter. The cornea was moderately thickened and opaque, and the anterior chamber was filled with friable tan material. The lens was irregular in shape, opaque, and partially surrounded by a thick layer of tan to brown tissue. The retina was detached, and the subretinal space was partially filled with clotted blood. The vitreous humor was cloudy. Focally at the limbus, the sclera was markedly thickened.

Contributor's Morphologic Diagnoses:
Right eye (166-04):
1. Pyogranulomatous endophthalmitis, severe, chronic, with lens rupture and fungal yeasts and pseudohyphae.
2. Keratitis, ulcerative, neutrophilic, moderate, chronic, with vascularization.
3. Retinal detachment with severe full thickness degeneration and severe retinitis.
4. Limbus: Focal suppurative scleritis and conjunctivitis, severe, acute.

Left eye (1250-03):
1. Pyogranulomatous endophthalmitis, severe, chronic, with lens rupture and fungal yeasts and pseudohyphae
2. Keratitis, ulcerative, neutrophilic, moderate, chronic, with focal abscess and vascularization
3. Retinal detachment with moderate degeneration of inner layers

Contributor's Comment: Both eyes had a similar endophthalmitis with lens rupture, and numerous fungal organisms consistent with *Candida* spp. in the anterior segment. The route of ocular infection was believed to be hematogenous, originating from a primary cystitis. *Candida* spp. are normal inhabitants of the gastrointestinal, upper respiratory, and genital mucosa of dogs, and alterations in local and systemic immunity can result in opportunistic infections of mucosae and mucocutaneous junctions.[1]

Urinary tract infection by *Candida* spp. in dogs and cats is often associated with concurrent diseases or drug therapy (e.g. immunosuppressive drugs, broad-spectrum antibiotics) that may interfere with host defense mechanisms and alter normal bacterial flora.[2] Systemic candidiasis is an uncommon sequela of candidal cystitis.[2]

While *Candida albicans* is the most common cause of fungal endophthalmitis in humans, ocular candidiasis is rare in domestic animals.[3] Fungal endophthalmitis in dogs is most commonly caused by dimorphic fungi such as *Blastomyces dermatitidis*.[4] Endophthalmitis caused by *Candida* spp. has been occasionally reported in dogs and cats, resulting from hematogenous spread,[3,6] or inoculation into the eye, secondary to candidal keratitis.[5] In our case, both eyes showed lens rupture, and organisms were most numerous within the lens capsule and degenerate lens fibers, suggesting a tropism for lenticular tissues. However, this particular distribution of organisms has not been reported in published cases.

Candida spp. have a distinct morphology in histologic sections: they consist of round to oval yeasts that are 3 to 6 microns in size, reproduce by narrow-based budding, and form chains of elongated yeasts separated by constrictions (pseudohyphae).[7] While *Candida albicans* is the most common isolate of this genus, at least five other species have been isolated from dogs and cats with urinary tract infections: *C. tropicalis*, *C. rugosa*, *C. krusei*, *C. parapsilosis*, and *C. glabrata* (previously called *Torulopsis glabrata*).[2]

AFIP Diagnosis: Eye: Endophthalmitis, pyogranulomatous, severe, diffuse, with retinal detachment, lens rupture, intracorneal abscess, and numerous yeast and pseudohyphae, Dachshund, canine.

Conference Comment: *Candida albicans* is a dimorphic, saprophytic, opportunistic fungus that is a normal inhabitant of the gastrointestinal, upper respiratory, and genital mucosae of dogs. Infections may develop as a result of breaks in the normal mucosal barrier, immunosuppression, or treatment with broad-spectrum antimicrobials. Antibiotic therapy reduces the number of anaerobic bacteria, allowing proliferation of *Candida* spp., and resulting in an overall change in the mucosal flora.[1,6]

Candidiasis is mainly a disease of keratinized epithelium in young animals, especially pigs, calves, and foals. In pigs, *Candida* spp. often invade the parakeratotic material that accumulates on the gastric squamous mucosa. Thrush, candidiasis of the oral cavity, occasionally occurs in young pigs. Lesions may be confined to the tongue, hard palate, or pharynx, but often involve the esophagus, and gastric squamous mucosa as well. In calves, lesions are most often in the ventral sac of the rumen but may also involve the omasum and reticulum, and occasionally the abomasum. Gastroesophageal candidiasis in foals involves the squamous epithelium and is associated with ulceration adjacent to the margo plicatus. Grossly, the lesions are yellow-white, smooth, or wrinkled plaques that cover the mucosa. Histologically, the

epithelium is spongiotic and contains yeast and abundant hyphae admixed with neutrophils and bacteria beneath the cornified layer.[8]

Localized candidal infections in dogs are reported in chronically immunosuppressed dogs and include infections of the skin and nailbed, urinary tract, ears, and gastrointestinal tract.[1] Additional factors that promote candidal urinary tract infections are thought to include an increased intestinal *Candida* spp. population (i.e. post-antibiotic treatment) and local alterations in the urinary tract environment (i.e. diabetes mellitus or aciduria).[2] Systemic dissemination is by embolization from primary sites of colonization and local invasion. Clinical signs of generalized infection include pyrexia and erythematous skin lesions, myositis, osteomyelitis, and ocular infections.[1]

Candida spp. are unique fungi in that they often form yeast (blastospores, blastoconidia), pseudohyphae, and hyphae in tissue. If only blastospores are seen, they can be confused with morphologically similar yeast forms in tissue, such as *Histoplasma capsulatum* var. *capsulatum*, *Blastomyces dermatitidis*, and poorly encapsulated *Cryptococcus neoformans*.[9] *Histoplasma capsulatum* var. *capsulatum* and *Blastomyces dermatitidis* only very rarely form pseudohyphae in tissue.[7] In some instances Candida hyphae and pseudohyphae may resemble dematiaceous fungi or other filamentous fungi. However, *Candida* spp. are not pigmented and usually there is more than one type of fungal element present. In mucocutaneous candidiasis, masses of branching, septate hyphae, pseudohyphae, and round to oval budding yeast forms measuring 3-5 _m in diameter are seen on the surface and within the epithelium. In systemic candidiasis, either all or any combination of these fungal elements may be seen. The presence of blastospores mixed with characteristic pseudohyphae or hyphae in tissue enables the pathologist to identify the fungus as a species of *Candida*.[9]

Contributor: The Caspary Institute for Veterinary Research, The Animal Medical Center, Department of Pathology, 510 East 62nd Street, New York, NY www.amcny.org

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SLIDE 83
CONFERENCE 21 / CASE II – 8004-476 (AFIP 2940131)

Signalment: 10 month old, neutered male Golden Retriever dog (*Canis familiaris*).

History: A 6 month old male Golden Retriever was presented for chronic vomiting. Clinical chemistry showed a BUN of 83 (7-27) and creatinine of 3.3 (0.5-1.8) mg/dl with a hematocrit of 34 (37-55)%. The animal was maintained with periodic medical treatments and diet (Science Diet® K/D) but clinical signs and azotemia (BUN > 130 mg/dl, creatinine 13.35), hyperphosphatemia (15.43, 2.5-6.8 mg/dl) and anemia (PCV 21%) slowly progressed until the dog was euthanized at 10 months of age. The referring veterinarian noted that both kidneys were small and nodular and these were submitted in fixative for histological examination.

Gross Pathology: Grossly, kidneys were irregularly multinodular, firm, pale and small.

Laboratory Results: Final BUN > 130 (7-27) mg/dl, creatinine 13.35 (0.5-1.8) mg/dl, hyperphosphatemia 15.43 (2.5-6.8) mg/dl and PCV = 21%

Contributor's Morphologic Diagnoses: 1. Renal dysplasia.
2. Chronic suppurative pyelonephritis.
3. Multifocal mild tubular necrosis and regeneration with intratubular and intraepithelial birefringent crystals, hyaline and granular casts.

Contributor's Comment: In both kidneys, the cortex is irregularly contoured and composed of radially arrayed zones of fibrosis interspersed with zones of dilated tubules. Throughout the kidney, renal corpuscles have cystically dilated Bowman's spaces containing eosinophilic fluid; as well, a few small glomeruli with external nuclei and inapparent capillary loops (immature glomeruli) are scattered throughout, predominantly in the outer cortex. Areas of fibrosis have many large ductular structures mainly in the medulla, with degeneration of tubules, coalescence of glomeruli and moderate lymphoplasmacytic inflammation in the cortex. Medullary ducts have flattened basophilic (immature) and columnar (mesonephric) epithelium. Dilated tubules contain intraluminal eosinophilic fluid, many hyaline and fewer granular casts and

scattered birefringent pale intratubular and intraepithelial crystals (calcium oxalate?). Tubules are separated by expanded proliferative pale to collagenous stroma. Areas of pale, poorly differentiated, stellate (immature) stroma are present primarily in the medulla. Cortical tubules have variable epithelium with occasional necrosis, flattening of epithelium and hyperplasia (regeneration). The pelvic and ureteral urothelium is underlain by moderate mixed lymphoplasmacytic and neutrophilic inflammation and fibrosis with varying degrees of epithelial degeneration, transmigration of neutrophils and intraepithelial micropustules (chronic pyelonephritis). Some sections show a focus of necrosis with exudation of fibrin and degenerating neutrophils in the renal papilla (fibrinosuppurative pyelitis, not in all sections).

Renal dysplasia is thought to arise as a consequence of perturbations in the complex chains of events involved in the embryological development of the kidneys resulting in arrest of full maturation with retention of immature structures. Histological features of renal dysplasia include fibrosis, immature glomeruli, large columnar-lined (mesonephric) ducts, immature ducts lined with flattened hyperchromatic cells and the persistence of pale poorly differentiated (immature) mesenchyme.[1,2,3] Clinical signs of renal failure are variable and are commonly recognized at several months age.[1,2] Animals with renal dysplasia show increased susceptibility to pyelonephritis; however, cortical fibrosis is found independent of inflammatory disease. Renal dysplasia has been reported to be caused by canine herpesvirus, feline panleukopenia virus, bovine virus diarrhea virus and porcine hypovitaminosis A.[3] The influence of inheritance is controversial,[3] but multiple occurrences in litters[4] and breeds, notably Golden retrievers[2], Lhasa apsos, Shih Tzus, Boxers, Finnish harriers, Dutch kooiker, and Cocker spaniels[1,4,5,6] have been reported demonstrating hereditary determination or predisposition. Dogs with renal dysplasia may suffer from renal dysfunction-related conditions such as anemia and fibrous osteodystrophy[7] (renal hyperparathyroidism). Nephrogenesis is largely undefined but transgenic manipulations in mice indicate cytokine and other apparently dual-purpose genes function in intercellular communication during organogenesis and that interruption or over-expression of certain genes can lead to increased incidence of renal dysplasia.[8,9] Renal dysplasia is an important cause of renal failure necessitating renal transplantation in children. Renal dysplasia has also been described in the adult horse.[10]

AFIP Diagnoses: 1. Kidney: Dysplasia, with severe interstitial fibrosis, persistent metanephric ducts and primitive mesenchyme, fetal glomeruli, cystic glomerular spaces, tubular ectasia, degeneration and loss, and chronic mild lymphoplasmacytic pyelonephritis, Golden Retriever, canine.
2. Kidney, tubules: Necrosis, multifocal, with intratubular crystals.

Conference Comment: The contributor provides a thorough overview of renal dysplasia in animals. By definition, renal dysplasia is disorganized development of renal parenchyma due to abnormal differentiation. Lesions associated with dysplasia include the presence of structures inappropriate to the stage of development of the host or the development of structures that are anomalous. Associated with and often

obscuring dysplastic lesions are a number of secondary compensatory, degenerative, and inflammatory changes.[1]

In humans, about 10% of all people are born with potentially significant malformations of the urinary tract. Renal dysplasias and hypoplasias account for 20% of chronic renal failure in children. Congenital renal disease can be hereditary, but is most often the result of an acquired developmental defect in utero. Dysplasia can be unilateral or bilateral and is almost always cystic. Grossly, the kidney is usually enlarged, extremely irregular, and multicystic. The cysts vary in size from microscopic to several centimeters in diameter. Microscopically, there is abnormal lobar organization and persistence of abnormal structures, including cartilage, undifferentiated mesenchyme, and immature collecting ductules. The characteristic histologic feature is the presence of islands of undifferentiated mesenchyme, often with cartilage, and immature collecting ducts.[11]

Although microscopic features of human renal dysplasia are present in dogs, a number of differences are apparent. The consistent segmental cortical pattern of asynchronous differentiation of nephrons is not a characteristic feature of human dysplasia. In man, ducts lined by tall columnar epithelium are interpreted as persistent metanephric ducts with no analogous structure in the normally developed kidneys. The pseudostratified columnar epithelium lining medullary ducts in canine cases may similarly represent persistent metanephric ducts.[1]

Contributor: Mississippi State University, College of Veterinary Medicine, Diagnostic & Field Services Program, Box 9825, Mississippi State, MS www.cvm.msstate.edu

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SLIDE 84
CONFERENCE 21 / CASE III – Kiupel (AFIP 2942010)

Signalment: A 60 pound, 2.5 year old, female spayed, mixed breed (Border collie) dog.

History: The dog presented to the Veterinary Teaching Hospital (VTH) at Michigan State University for a previously diagnosed retinal detachment of the right eye. The dog was bright, alert and responsive, with a good appetite. Physical examination revealed a small skin lesion on the lateral surface of the hock, and bilateral retinal detachment. CBC and serum chemistry were submitted to the clinical pathology laboratory. Fine needle aspirate of the ocular fluid of the right eye and right hock were obtained for cytologic examination. Fungal cultures were obtained from the ocular fluid.

Gross Pathology: This 60 pound female spayed dog presented with mild dehydration, adequate nutrition, and minimal autolysis. The eyes had been enucleated prior to necropsy for special processing. The primary lesions were widespread foci of suspected granulomatous inflammation in multiple parenchymal organs. There were hundreds of pinpoint white foci scattered across the capsular surface of the right and left kidneys. These lesions did not extend into the inner parenchyma. There were hundreds of multifocal to coalescing, white foci scattered across the epicardial surface of the heart. These white foci extended throughout the myocardium of the left ventricular free wall and the interventricular septum. There were multifocal areas of moderate mucosal hyperemia diffusely throughout the small and large intestine. The spleen was diffusely congested and oozed blood on cut surface. There were no other gross lesions of diagnostic significance in this animal.

Gross Morphologic Diagnoses: 1. Kidney: Multifocal, moderate, granulomatous, interstitial nephritis (suspected).
2. Heart: Multifocal to coalescing, granulomatous cardiomyopathy (suspected).

Histopathologic Findings: Only heart was submitted to the conference. Sections of heart examined had multifocal areas of myocardial necrosis surrounded by moderate to severe lymphoplasmacytic and histiocytic inflammation admixed with low numbers of neutrophils and eosinophils. The lesions caused destruction of approximately 40-50%

of the sections of heart examined. Within the centers of the necrotic areas were numerous Prototheca. These organisms ranged from 5-15 µm in diameter with a thin refractile wall. Some organisms had a granular basophilic cytoplasm with a small central basophilic nucleus. Others had undergone endosporulation and contained as many as 20 daughter cells. Most of the inflammation appeared centered around the spent theca cells/mother shells, which represented the remaining empty capsule wall following release of endospores. Many of the protothecal aggregates consisting of spent theca cells elicited only a moderate lymphohistiocytic inflammatory response. However, several of the larger granulomas had necrotic centers that consisted of central mineralization surrounded by cellular debris, caseous exudates, and fragmented Prototheca shells.

Other lesions: 1. Multifocal, mild, lymphoplasmacytic, and eosinophilic meningoencephalitis with necrosis and intralesional Prototheca.
2. Multifocal, moderate, plasmacytic, and lymphohistiocytic interstitial nephritis with intralesional Prototheca.
3. Diffuse, moderate to severe, plasmacytic, and eosinophilic colitis with intralesional Prototheca.
4. Multifocal, mild to moderate, lymphoplasmacytic, periportal hepatitis with intralesional Prototheca and moderate, diffuse, vacuolar hepatopathy.
5. Multifocal, moderate to severe, plasmacytic, and lymphohistiocytic pancreatitis with intralesional Prototheca.
6. Multifocal, moderate, plasmacytic, and lymphohistiocytic thyroiditis with intralesional Prototheca.
7. Bilateral, severe, pyogranulomatous panophthalmitis with complete retinal detachment, glaucoma, and intralesional Prototheca.

Laboratory Results: CBC and serum chemistry profiles had no significant abnormalities.
Right ocular fluid
Subretinal fluid from the right eye contained frequent spherical to oval-shaped basophilic organisms with a thin clear wall and central 'nucleus'. The organism measured from approximately 3-9 µm in diameter and approximately 3-10 µm in length with a clear cell wall of approximately 0.5-1 µm thick. It appeared consistent with *Prototheca* sp. A mild inflammatory response consisting of predominately moderately to severely degenerate neutrophils was observed. Culture was recommended.

Right hock skin lesion
Numerous extracellular oval to reniform-shaped organism with thin clear walls were seen. The organisms varied greater than two-fold in size. Frequent mildly degenerate neutrophils were present and frequently could be seen phagocytizing the organisms.

Urinalysis
No significant findings.

Cerebral spinal fluid

Cytospin preparations of CSF were made. They were moderately to highly cellular with a large preponderance of eosinophils and lesser numbers of lymphocytes and non-degenerate neutrophils and macrophages. There were occasional macrophages with phagocytized protothecal organisms and spent organisms. Microprotein was measured to be 50 mg/dl. A 300 cell count revealed 146 eosinophils, 65 mononuclear cells, 40 small lymphocytes, and 49 neutrophils.

Contributor's Morphologic Diagnosis: Heart: Multifocal, moderate to severe, granulomatous and eosinophilic, necrotizing myocarditis with intralesional *Prototheca* sp. and mineralization.

Contributor's Comment: Prototheca are saprophytic achlorophyllous algae that are closely related to the green algae of the genus Chlorella._ They reproduce by endosporulation and may have asymmetrical cytoplasmic and nuclear cleaving leading to anywhere from 2 to greater than 20 endospores. The mother cells rupture releasing the daughter spores, which are tiny replicas of their mothers. They mature and repeat the life cycle. Empty shell casings from ruptured mother cells are usually seen amongst the intact population of organisms._

Prototheca sp. are ubiquitous organisms and may be found in sewer treatment plants (Fetter et al., 1971), potato skin (Negroni and Blaisten, 1940), tree flux (Fetter, Klintworth and Nielson, 1971),_ and in freshly voided human and animal feces._ Prototheca rarely causes disease, but will adversely affect its host when the immune system is suppressed or challenged by a pre-existing or concurrent disease. Intact protothecal organisms normally elicit a minimal inflammatory response. Once the mother cells rupture and release the endospores, a strong lymphoplasmacytic and histiocytic inflammatory response is initiated against the spent theca shell. It has been speculated that a defect in the host's cell mediated immune system is a more important factor in protothecal infections than a defect or decrease in the humoral immune response._ A defect in neutrophils may allow for protothecal infections. In some hosts the neutrophils are able to phagocytose the organisms but are unable to destroy them. In these cases, there was no evidence of humoral or cell mediated immune deficiencies.

To date there are three recognized *Prototheca* species: *P. stagnora*, *P. zopfii*, and *P. wickerhamii*. Only *P. zopfii* and *P. wickerhamii* are known to cause disease in animals and humans. Both species appear morphologically similar to one another and can be differentiated based on sugar and alcohol assimilation or fluorescent antibody tests. In humans, both cutaneous and disseminated protothecosis has been reported. *P. wickerhamii* is most commonly associated with cutaneous lesions and *P. stagnora* usually results in disseminated disease in humans.

In cats and dogs, infection with *Prototheca* sp. is rare. There have been no reports of disseminated protothecosis in cats. Cutaneous infections in cats are caused almost exclusively by *P. wickerhamii*. Dogs, however, predominantly contract the disseminated form of protothecosis and invariably it is caused by *P. zopfii*._ Collies appear to be more susceptible (7 out of 20 cases) than other breeds._ Dogs with disseminated

protothecosis normally present with a history of bloody diarrhea that is unresponsive to treatment. The animal continues to eat and drink well and remains bright and responsive. As the organism disseminates, clinical signs usually develop depending on the organ system affected. Besides the gastrointestinal tract, the eyes, heart, brain, liver, kidney, and skin are most commonly affected. As the disease progresses, dogs become more depressed and develop CNS signs such as ataxia, incoordination, paresis, deafness, circling and depression. Two-thirds of the reported cases include bilateral or unilateral ocular involvement and the animals normally present with retinal detachment and blindness. CBC and serum chemistry are often within reference range, but occasionally hepatic enzymes may be increased with the involvement of the liver or other organ systems. The predominant inflammatory response invoked by protothecal organisms within a dog is lymphoplasmacytic and histiocytic regardless of the organ system affected.

In this case, culture performed at MSU did not further differentiate the Prototheca organism. It was unknown if the organism is *P. zopfii* or *P. wickerhamii*. Based on the wide dissemination of the organisms, it was speculated that *P. zopfii* was the pathogen.

AFIP Diagnosis: Heart: Myocarditis, granulomatous and necrotizing, multifocal, moderate, with numerous extracellular and intrahistiocytic algae, etiology consistent with *Prototheca* sp., mixed breed, canine.

Conference Comment: As mentioned by the contributor, *Prototheca* spp. rarely cause disease but occasionally infections result in severe gastrointestinal, ocular, cutaneous, or disseminated disease. The most commonly affected domestic animals are dogs, cats, and cows. In cows, manifestation of the disease is usually in the form of mastitis caused by *P. zopfii*. Cats are most commonly affected with the cutaneous form of protothecosis (*P. wickerhamii*) and present with large, firm nodules on the limbs and feet; however, the nose, pinnae, forehead, and tailbase may also be affected. The most common clinical presentation of protothecosis in the dog is protracted hemorrhagic enterocolitis, with the colon being most severely affected. Grossly, the colon is diffusely reddened with multiple raised white nodules and multifocal ulcerations with hemorrhage. Disseminated disease involving the eyes, ears, skin, skeletal muscles, kidneys, liver, heart, spinal cord, and brain has been reported.[8]

Prototheca spp. reproduce via asexual endosporulation and have a characteristic microscopic appearance. Histologically, there are intra- and extracellular organisms that may be either small single endospores with granular cytoplasm, or large sporangia that are round to oval, 8-20 µm in diameter, have a clear 2-4 µm thick wall, and contain multiple (2-20) wedge-shaped endospores arranged radially ("Mercedes Benz emblem-like"). The cells eventually rupture leaving empty theca (mother shells) in the lesions. Organisms may be readily identified using special stains such as PAS (Periodic Acid-Schiff) or GMS (Grocott's Methenamine Silver). Other organisms that reproduce via endosporulation include *Chlorella* sp., *Rhinosporidium seeberi*, and *Coccidioides*

immitis. Ultrastructurally, a paucity of chloroplasts differentiates *Prototheca* sp. from *Chlorella* sp.[10]

Contributor: Michigan State University, Diagnostic Center for Population and Animal Health, Department for Pathobiology and Diagnostic Investigation, PO Box 30076, Lansing MI
http://www.dcpah.msu.edu
http://cvm.msu.edu/vetpath/faculty.htm

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SLIDE 85
CONFERENCE 21 / CASE IV – 04/05 #1 (AFIP 2948745)

Signalment: A 13-year-old male castrated Irish Setter canine.

History: This dog had a history of anorexia, fever, and septic peritonitis.

Contributor's Morphologic Diagnosis: 1. Gallbladder: Malignant neuroendocrine neoplasia (malignant carcinoid).

2. Gallbladder: Cholecystitis, suppurative and lymphoplasmacytic, multifocal, moderate.

Contributor's Comment: The submitted specimen consists of a section of gallbladder, which contains a portion of a non-encapsulated, invasive, multilobulated mass that expands, effaces, and replaces up to 90 percent of the wall. Individual lobules, which are separated by a moderate fibrovascular stroma, consist of discrete and coalescing, variably cellular sheets and packets of mildly atypical and pleomorphic cells which are separated by a delicate fibrovascular stroma. Cells are large, round to polygonal with distinct cell borders and contain a moderate amount of pale eosinophilic, faintly granular and often vacuolated cytoplasm. Nuclei are round to oval, bland, and contain coarsely granular chromatin and single, prominent nucleoli. Anisokaryosis and anisocytosis are mild and 0-1 mitotic figures are identified per 400X field. Throughout the mass, there are multiple, scattered foci of hemorrhage and necrosis and rare, entrapped biliary, mucosal crypts. The gallbladder lumen contains a variable amount of sloughed, necrotic and degenerate tumor and epithelial cells admixed with fibrin, hemorrhage, and varying numbers of degenerate and viable neutrophils. Within the adjacent gallbladder tissue, the lamina propria-submucosa contains modest numbers of plasma cells and lymphocytes with scattered, hemosiderin-laden macrophages, with mild, multifocal cystic mucosal hyperplasia. Adherent to the serosa is a moderate cellular coagulum composed of hemorrhage, mixed leukocytes, and fibrin.

In this case, based upon the cytomorphologic and architectural characteristics, and the demonstration of neoplastic cell argyrophilia using the Grimelius method, a diagnosis of malignant neuroendocrine neoplasia (malignant carcinoid) was made. Further characterization (immunohistochemical or ultrastructural analysis) of the cells was not conducted. Additional sections, which were not included in the submitted slide series, demonstrated multiple nests of identical cells throughout a section of liver. Based upon their comparatively small size and multicentric nature, these were presumed to represent metastatic foci rather than the primary lesion.

Carcinoids are rare neuroendocrine neoplasms of both humans and domestic animals, which arise from dispersed neuroendocrine cells located in the gastrointestinal tract and other organ systems (liver, pancreas, urogenital, and tracheobronchial). Historically, the term carcinoid has been used as an umbrella term in the diagnosis of all gastroenteropancreatic neuroendocrine tumors (GEP-NET's) regardless of biologic or clinical behavior. Recently, in an attempt to reflect their diverse histogenesis and biologic behavior, carcinoids were reclassified with the more neutral and inclusive terms: neuroendocrine tumor and neuroendocrine carcinoma. However, the term carcinoid was retained for neuroendocrine gastrointestinal tumors of both benign (carcinoid) and malignant (malignant carcinoid) forms, thus removing pancreatic neuroendocrine neoplasms from this group.[3] Carcinoids may synthesize and secrete either a short chain polypeptide and/or biologically active amine, including serotonin (5-HT), somatostatin, gastrin, or histamine.[2,4,7] The classic carcinoid syndrome of flushing, hypotension, diarrhea, and wheezing is due to serotonin secretion.[3]

Definitive diagnosis is based upon histologic features, cytochemical (argyrophilia) and immunohistochemical (neuron specific enolase, chromogranin A and synaptophysin) techniques, and the ultrastructural identification of secretory granules. Hepatic and biliary carcinoids are typically negative for cytokeratin.[2,7]
In the dog, carcinoids are most common in aged animals and have been reported in the gallbladder, liver, lung, and throughout the gastrointestinal tract.[1,2,4,6,7] Hepatobiliary carcinoids have been described in the dog, cat, and one cow.[2,7] Additionally, there are reports of intestinal carcinoids in the horse and cow and three cases of maxillary sinus carcinoid tumors in the horse.[4] In humans, carcinoids are most commonly diagnosed in the gastrointestinal tract (73.7%) and bronchopulmonary (25.3%) system.[5]

Based upon their rarity, the usual biologic behavior of gastrointestinal carcinoids in dogs is uncertain, however in the previous reports, the vast majority of hepatic carcinoids demonstrated aggressive, metastatic behavior, with spread most common to the peritoneal cavity and peritoneal lymph nodes.[6] In humans, the overall 5-year survival rate of all types of carcinoid tumor was 50.4%, with a localized disease (79.7%) having a predictably better prognosis than if regional (50.6%) or distant (21.8%) metastatic lesions are present. The prognosis of gallbladder carcinoids (41.3% 5-year survival rate) is poor.[5]

AFIP Diagnosis: Gallbladder: Carcinoid, Irish Setter, canine.

Conference Comment: Neoplasms derived from neuroendocrine cells of the gastrointestinal mucosa are known as carcinoids because, histologically, they closely resemble some carcinomas of intestinal epithelial origin. As mentioned by the contributor, mucosal neuroendocrine cells can secrete several different hormones; however, individual cells synthesize and store a single hormone, with the active secretion being either short chain polypeptides and/or biologically active amines. It has been shown that not all neuroendocrine cells can decarboxylate an amine precursor, and the APUD (amine precursor uptake and decarboxylation) system concept has been modified and renamed the diffuse endocrine system.[2]
In animals, alimentary tract and hepatobiliary carcinoids are considered malignant.[8]

Theoretically, tumors of the diffuse neuroendocrine system should invoke a recognizable clinical syndrome related to their secretory products, but this is not consistently observed. However, carcinoids that secrete gastrin (G cell tumors) are responsible for the Zollinger-Ellison syndrome, characterized by severe gastric hypersecretion and peptic ulceration, with watery diarrhea. The syndrome has been reported in dogs and cats, usually associated with a non-beta cell pancreatic islet cell tumor rather than a gastrointestinal tumor.[2]

Grossly carcinoids are yellowish or tan on cut surface and range from annular stenosing thickenings to nodular masses and the overlying epithelium may be eroded or ulcerated.[2] Tumors often have characteristic neuroendocrine features histologically,

with large polygonal cells arranged in nests and packets, or more solidly cellular areas, separated by a fine fibrovascular stroma, with palisading of peripheral cells along the stroma. Cells have distinct cell borders with abundant granular cytoplasm and irregularly round, often centrally located nuclei. However, two other patterns have been recognized. One is characterized by groups of rosettes or acinar-like structures that contain eosinophilic secretions separated by similar stroma. The other is composed of anastomosing groups and rows of cells like ribbons, consisting of mostly ovoid or spindle cells with fibrovascular stroma. Silver stains such as modified Fontana-Masson and Churukian-Schenk are useful. However, immunohistochemical stains, such as neuron-specific enolase (NSE) and chromogranin, which stain almost all neuroendocrine tumors, are now more commonly used. A varying number of neoplastic cells may stain with antibodies such as serotonin, somatostatin, gastrin, glucagon, synaptophysin, and calcitonin. Ultrastructurally, neoplastic cells contain a variable number of intracytoplasmic neurosecretory granules that are typically round, composed of an electron dense core and surrounded by an electron dense membrane.[8]

Contributor: Colorado State University, College of Veterinary Medicine and Biomedical Science, Department of Microbiology, Immunology, and Pathology, Fort Collins, Colorado
www.cvmbs.colostate.edu/mip/

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SLIDE 86
CONFERENCE 22 / CASE I – 04-2871 (AFIP 2936449)

Signalment: Three near term fetuses, mixed sex, unknown breed, ovine (*Ovis aries*).

History: Six out of forty yearling ewes in the flock became mildly lethargic and would often stand alone in a corner away from the rest of the flock. Affected ewes had slight vaginal discharge followed by abortion. The older ewes had no clinical signs or abortions. All ewes were vaccinated, and the yearling ewes were vaccinated twice, including vaccinations for *Campylobacter* species.

Gross Pathology: The cotyledons in the placenta submitted with one of the fetuses were edematous and mottled with multiple 1-4 mm diameter, white to tan foci. The intercotyledonary placenta occasionally contained similar foci.

Laboratory Results: A pure culture of *Campylobacter jejuni* was isolated from the abomasal fluid of all three lamb fetuses.

There was no bacterial growth on aerobic or *Brucella* cultures. Fluorescent antibody testing of the lung, liver, kidney, placenta, and thyroid gland was negative for bovine viral diarrhea (BVD) virus (used to test for border disease virus). Fluorescent antibody testing of the placenta, lung, and liver was negative for *Chlamydophila abortus*. Fetal titers were negative for BVD virus at less than 1:2, negative for *Toxoplasma gondii* at less than 1:8, and negative for bluetongue virus.

Contributor's Morphologic Diagnoses: 1. Placenta: Multifocal, necrotizing and suppurative placentitis with mineralization, vasculitis, and thrombosis
2. Liver: Multifocal, suppurative and necrotizing hepatitis
3. Liver: Extramedullary hematopoiesis

Contributor's Comment: There is moderate postmortem decomposition in the submitted placenta, with loss of most of the trophoblastic epithelial cells. The chorionic villi contain multiple foci of intact and degenerate neutrophils with necrosis and mineralization. The chorioallantois is edematous and contains a few perivascular and multifocal infiltrates of macrophages, neutrophils and lymphocytes. The tunica media of a few small arterioles in the chorionic villi is infiltrated with intact and degenerate neutrophils and the vascular wall is necrotic. A few of these affected arterioles contain fibrin thrombi. In the submitted liver, there are multifocal areas of necrosis filled with variable numbers of neutrophils. There is extramedullary hematopoiesis in the liver. In the placenta of another lamb (not submitted), there is mild suppurative inflammation with many of the trophoblasts containing numerous gram-negative coccobacilli. In the lung of all three lambs (not submitted), there is suppurative bronchopneumonia.

Campylobacter species are small, curved, highly motile, noncapsulated, microaerophilic, gram-negative bacilli. In sheep, the most common manifestation of *Campylobacter* infections are late term abortions, stillbirths, premature births, the birth of weak lambs, and occasional ewe fatalities due to metritis.[1,2] The most common ovine *Campylobacter* is *Campylobacter fetus* subspecies *fetus*, but *Campylobacter jejuni* can

also infect sheep.[1-4] One study showed an increase in ovine abortions caused by *Campylobacter jejuni* versus *Campylobacter fetus* subspecies *fetus* in the later years (1983-1989) of the study.[5] In this same study, *Toxoplasma gondii*, *Campylobacter* species, and *Chlamydophila abortus* were the most common identifiable causes of ovine abortions. Placentitis was the most prominent lesion.[5]

The macroscopic and microscopic lesions in aborted fetuses and their placentas are similar in infections with *Campylobacter fetus* subspecies *fetus* and *Campylobacter jejuni.* The lesions include necrotizing edematous placentitis, fetal suppurative bronchopneumonia, and multifocal hepatic necrosis.[1-3] The most common lesion seen with *Campylobacter* abortions in sheep is placentitis.[4] In some cases, the placentitis is macroscopically apparent. The liver lesions can be large enough to be seen grossly and have a "target" appearance.[1,3]

The transmission of *C. jejuni* and *C. fetus* subsp. *fetus* is believed to be orally due to fecal contamination of water and feedstuffs.[1] The *Campylobacter* organisms then become transiently bacteremic with localization of the bacteria in the gut and bile.[1] In nonimmune ewes, *Campylobacter* can localize in the uterus during the bacteremic phase.[1] In nonimmune ewes that are pregnant, *Campylobacter* first localizes in the hilar zone of the placentomes causing vascular necrosis and thrombosis.[4] This results in separation of the chorion with invasion of the chorion and chorionic capillaries, with subsequent necrosis.[4] If the fetus survives the hypoxia secondary to the necrosis of the placenta, then the fetus can be invaded by the *Campylobacter*, and in some cases, undergoes fetal death.[4]

AFIP Diagnoses: 1. Chorioallantois (cotyledon): Placentitis, necrotizing, suppurative, diffuse, severe, with multifocal vasculitis, thrombi, and mineralization, breed not specified, ovine.
2. Liver: Hepatitis, necrotizing, neutrophilic, random, mild.

Conference Comment: The contributor provides a thorough overview of campylobacteriosis in sheep. The placentitis is characterized by an edematous intercotyledonary chorioallantois and friable, yellow cotyledons. Grossly, about 25% of the fetuses have multiple, yellow, "targetoid", areas of hepatic necrosis that are characteristic of the disease. *Flexispira rappini* causes similar lesions in the placenta and fetus, but infections are sporadic.[6]

Infectious causes of abortion in sheep include the following (C=cotyledonary; IC=intercotyledonary):[6,7,8]

Organism	Placental Lesions		Fetal Lesions
	Gross	Histological	
Campylobacter fetus fetus	C=friable, yellow IC=edema, exudate	Often vasculitis, inflammation severe in chorionic villi with gram-negative bacteria	Liver: large targetoid areas of necrosis

Toxoplasma gondii	C=pinpoint white foci of necrosis IC=edema	Chorionic epithelial hypertrophy and hyperplasia with rare intracellular zoites	Focal necrotic lesions in the brain, liver, kidney, lung
Neospora caninum	C=necrosis IC=normal	Zoites rarely seen within trophoblasts	Multifocal encephalitis with gliosis and necrosis
Chlamydophila abortus	C=necrosis IC=brown exudate	Necrotizing placentitis with neutrophilic vasculitis and organisms within trophoblasts	Inflammatory/necrotic foci in the liver, lungs, muscle, etc.
Coxiella burnetii	C=less affected IC=thick, yellow, with exudate	IC necrotizing placentitis with gram-negative rickettsial organisms within chorionic epithelium	Inconsistent; lymphocytic infiltrates in the lungs, kidneys, liver
Brucella ovis	C=necrosis IC=brown exudate	Vasculitis; gram-negative bacilli intra- and extracellularly	Nonspecific
Listeria monocytogenes	C=necrosuppurative IC=necrosuppurative	Severe diffuse necrosuppurative placentitis with gram-positive bacteria within chorionic epithelial cells	Hepatomegaly with numerous 1mm yellow necrotic foci

Other less common causes of ovine abortion include *Salmonella dublin, S. typhimurium*, *S. abortusovis*, Ovine orbivirus (Bluetongue virus), Ovine pestivirus (Border disease), and bunyaviruses (Akabane virus, Cache Valley virus, Rift Valley fever virus). [6,7,8]

Contributor: Kansas State University, Department of Diagnostic Medicine/Pathobiology, 1800 Denison, Manhattan, KS
http://www.vet.k-state.edu/depts/dmp/index.htm

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SLIDE 87
CONFERENCE 22 / CASE II – UCONN 2004#2 (AFIP 2942012)

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

History: The dog is a free-ranging outdoor dog from the U.S. Virgin Islands. A protruding vulvar mass of two weeks duration was surgically removed.

Gross Pathology: A section of the vulvar mass, measuring 3 cm x 1.5 cm x 1.5 cm is submitted for histopathologic examination. The tissue has a uniform pale tan color and is homogeneous on cut section.

Contributor's Morphologic Diagnosis: Vulva: Transmissible venereal tumor, canine.

Contributor's Comment: Vulva. The section is of non-haired, non-cornified, stratified squamous epithelium and associated submucosal connective tissue, within which there is a raised, sessile, superficially eroded mass. The mass is well-demarcated, nonencapsulated, highly cellular and expansile, extending into the submucosa. The mass is composed of loosely packed sheets and cords of round cells, separated by fine strands of fibrovascular connective tissue. Cells are uniform, with scant eosinophilic cytoplasm and distinct cytoplasmic margins. Nuclei are large, round with marginated chromatin and a single prominent nucleolus or occasionally two nucleoli. There are moderate numbers of mitotic figures (2 – 4/HPF). At the deep and lateral margins, the stroma is infiltrated by small numbers of lymphocytes, fewer plasma cells, and macrophages. Toluidine blue stain shows few mast cells in subepithelial tissues; there are no metachromatic granules detected in the cytoplasm of tumor cells.

Canine transmissible venereal tumor (CTVT) is a naturally occurring contagious round cell neoplasm with a primarily histiocytic immunophenotype, with immunopositivity to lysozyme and vimentin.[1] Tumors are primarily found in the mucus membranes of the external genitalia of dogs of both sexes. Commonly, single or multiple masses are found on the caudal part of the penis, from crura to bulbis glandis and glans penis, and, in females, in the posterior part of the vagina and at the junction of the vestibule and the vagina. The tumor occurs also extragenitally in the nasal and/or oral cavities. This tumor is most often seen in young, roaming, sexually active dogs. CTVT is transmitted only by the transplantation of viable tumor cells to mucus membranes at coitus or during other contact. CTVT have been reported in the lymph nodes and skin, and occasionally in the tonsils, liver, pancreas, spleen, lungs, and kidneys. These neoplasms typically regress without treatment via an IgG-mediated immune response; however, metastasis does occasionally occur.[2,3] Distinctively, karyotyping of the cells of this tumor reveals 58-59 chromosomes, with 13-17 metacentric, compared to the normal canine component of 78 with 2 metacentric.[2]

The differential diagnosis includes mast cell tumor, histiocytoma, plasmacytoma, cutaneous lymphoma, and, grossly, other neoplasms of the vagina and vulva including papilloma, squamous cell carcinoma, epidermoid carcinoma, fibroma, and leiomyoma.

There is patchy worldwide distribution of this tumor, with endemic areas in the Caribbean, where this dog lived.[2] Transmission to the fox, coyote and jackal is possible.[4]

AFIP Diagnosis: Vulva (per contributor): Transmissible venereal tumor, mixed-breed, canine.

Conference Comment: Canine transmissible venereal tumor (CTVT) is the only known naturally occurring tumor that can be transplanted as an allograft across major histocompatibility (MHC) barriers within the same species, and to other canids, such as foxes, coyotes, and wolves.[5]

The histogenesis of CTVTs is not yet certain, but immunohistochemical studies suggest the cells are of histiocytic origin. The cells are immunohistochemically positive for vimentin, lysozyme, ACM1 (an epitope on canine mononuclear phagocyte stem cells), and alpha-1-antitrypsin (a good marker for benign and malignant histiocytes). These antigens are not expressed by other mesenchymal round cells, except those of histiocytic origin. Nonetheless, CTVT cells are unique in that they contain only 59 chromosomes. The normal diploid number of chromosomes in the somatic cell of the dog is 78.[5]

Like histiocytomas, the growth pattern of CTVTs includes a progressive growth phase, a static phase, and a regression phase. The progressive growth phase occurs after sexual transmission and is characterized by rapid proliferation of neoplastic cells. The static phase follows and is characterized by indolent local tumor growth or progression with metastasis. Some tumors regress spontaneously. CTVTs evoke both humoral and cell mediated immune responses, and as seen in this case, infiltrating lymphocytes may be present within and around the neoplasm during the regression phase.[5]

Contributor: University of Connecticut, Department of Pathobiology and Veterinary Sciences, U-3089, 61 North Eagleville Road, Storrs, CT
http://www.canr.uconn.edu/patho/

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SLIDE 88
CONFERENCE 22 / CASE III – 2004B (AFIP 2937641)

Signalment: 2-year-old, CD-1, male mouse (*Mus musculus*).

History: This mouse was from a control group on a 2 year carcinogenicity study. There were no adverse antemortem findings.

Gross Pathology: At necropsy, the left lobe of the seminal vesicle was irregular, with red/brown discoloration.

Contributor's Morphologic Diagnosis: Seminal vesicle: Granular cell tumor, benign.

Contributor's Comment: Granular cell tumors are generally a single mass in the male and female genital tract of the mouse, but may occur in other organs. These benign tumors typically grow by expansion but can include a more infiltrative pattern. Individual cells have abundant pale cytoplasm filled with numerous eosinophilic, course granules, and small, round to oval nuclei. The cytoplasmic granules are consistent with secondary lysosomes (residual bodies) and are PAS-positive and diastase resistant. The histiogenesis of the granular cells is unknown, although Schwann cells or primitive mesenchymal cells have been proposed as the cell of origin. Synonyms include myoblastoma and benign Abrikossoff's tumor. [1]

AFIP Diagnosis: Seminal vesicle: Granular cell tumor, mouse, murine.

Conference Comment: Granular cell tumor (GCT), once called granular cell myoblastoma, is an uncommon neoplasm of uncertain origin. They have been most frequently reported in the dog and horse, but also occur in laboratory rodents, cats, and birds. In the dog, GCT occurs most commonly in the tongue, but they have been reported in the ear, lip, palate, cerebral cortex and meninges, heart, lymph node, orbit, and the skin. In the horse, GCT appears to be exclusively a tumor of the lung, and is frequently found in association with the bronchi, but may be disseminated throughout the lung.[2] Granular cell tumors have been described in the genital system, brain and

meninges in mice and rats.[1,3] In cats, GCTs have been reported in the tongue, palate, vulva, and digits.[2]

Grossly, the neoplasm is often nodular, whitish, and firm. Microscopically, the tumor very characteristically consists of nests of large, round to polygonal cells with prominent, coarsely granular, eosinophilic cytoplasm. The cytoplasmic granules are PAS (periodic acid-Schiff) positive and diastase resistant. The circumscribed nature of many of the tumors and a lack of mitotic activity suggests a benign course. However, some reported GCTs were invasive and/or mitotically active. Granular cell tumors are immunohistochemically variably positive for vimentin, S-100 protein, and neuron specific enolase (NSE), emphasizing the heterogeneous nature of these tumors. Ultrastructurally, the cells contain packed lysosomes and phagosomes (myelin bodies).[4]

Contributor: Merck Research Laboratories, Department of Safety Assessment, WP45-227, Sumneytown Pike, West Point, PA

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SLIDE 89
CONFERENCE 22 / CASE IV – CASE #2 (AFIP 2940162)

Signalment: Seven-week-old, male Sprague-Dawley rat.

History: Administered 200 mg/kg/day ethylene glycol monomethyl ether (EGME) for 4 days.

Gross Pathology: None

Contributor's Morphologic Diagnosis: Testis: Stage-specific spermatocyte necrosis, and spermatocyte and round spermatid loss.

Contributor's Comment: EGME is a well-characterized experimental testicular germ cell toxicant in rats.[1] One day following a single 200 mg/kg dose, degeneration of spermatocytes (both those undergoing meiotic division and early pachytene stage) in Stage XIV tubules is evident. Occasionally, spermatocyte degeneration is also evident among Stage I and XIII tubules.

In rats examined following dosing with EGME for 4 consecutive days (such as the submitted case), similar spermatocyte degeneration predominantly within Stage XIV tubules is evident, as is depletion or absence of round spermatids and spermatocytes among Stage I-V tubules. There are occasional Stage IV or V tubules having absence of pachytene spermatocytes but retention of round spermatids, consistent with loss of Stage XIV early pachytene spermatocytes (but not spermatocytes undergoing meiotic division) following exposure to EGME on day 1 of dosing. Most conspicuous are tubules having Sertoli cells, spermatogonia, and elongate spermatids present, but lacking spermatocytes and round spermatids (presumably Stage I to VI tubules). These represent tubules having lost all spermatocytes when at Stage XIII or XIV, some time in the previous 4 days. Some of the affected tubules contain multinucleate germ cells (syncytia of round spermatids). However, Stage VII through XII tubules are generally unaffected.

Staging of the seminiferous tubular epithelium is useful for identifying temporal changes in germ cell associations in the course of spermatogenesis.[1,2,3] Each stage identifies a morphologically distinct array of spermatogonia (proliferating diploid germ cells), spermatocytes (meiotic [tetraploid] germ cells), and round and elongate spermatids (differentiating haploid germ cells) at a particular phase of development, supported in layers by basilar Sertoli cells. Staging schemes are based on light microscopic morphologic characteristics (usually related to details of spermatid development) and vary among species and among investigators describing them. Stages are designated by a Roman numeral and are of variable temporal duration (ranging from 7 [e.g. Stage IX] to 58 hours [Stage VII] in the rat). The most widely accepted staging scheme for the rat has one 12.9-day cycle divided into Stages I through XIV. Four and a half cycles (56 days) are required for spermatogenesis - development of a mature rat (step 19) spermatid from a type A1 spermatogonium. Maturation of sperm (spermiogenesis) is described by morphologic changes of spermatids designated by Arabic numeral as steps 1 through 19 over the course of one and a half cycles. Familiarity with spermatogenic staging aids recognition and description of testicular injury in acute toxicologic studies.

In the rat, Stage I through VII tubules are characterized by a single layer of pachytene spermatocytes and two populations of spermatids (both round and elongate). At Stage VIII, step 19 spermatids are released into the lumen and the round (step 8) spermatids begin to elongate. Stage IX through XIII tubules have two layers of spermatocytes (the luminal layer being large pachytene spermatocytes, and the basilar layer smaller preleptotene, leptotene, or zygotene spermatocytes) and a single layer of elongating spermatids. Stage XIV tubules have the luminal spermatocytes undergoing meiotic

division to secondary (diploid) spermatocytes and then (haploid) round spermatids, and basilar spermatocytes progressing to the pachytene stage.

AFIP Diagnosis: Testis, seminiferous epithelium: Degeneration, necrosis and loss, segmental, with multinucleated germ cells, Sprague-Dawley rat, rodent.

Conference Comment: The contributor provides a thorough overview of the staging of seminiferous tubules and the importance of stage specific changes that may occur with testicular germ cell toxicants. For the toxicologic pathologist, the ability to identify the tubular stages of the spermatogenic cycle and a sound understanding of the spermatogenic process are essential in order to detect and characterize toxic effects to the male reproductive system. There are several excellent references listed below which cover this topic.

Contributor: Abbott Laboratories, Department of Pathology, AP13A/R 469, 100 Abbott Park Road, Abbott Park, IL

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SLIDE 90
CONFERENCE 23 / CASE I – 03-10192 (AFIP 2942336)

Signalment: 7-year-old Polypay ewe.

History: This ewe was a chronic poor doer and was positive serologically for ovine progressive pneumonia virus.

Gross Pathology: The ewe was in poor body condition. The lungs were heavy and did not collapse. The cranial ventral portions were consolidated and contained several abscesses. Hilar lymph nodes and gastrohepatic lymph nodes were enlarged and replaced by abscesses. The kidneys also contained abscesses.

Laboratory Results: Mycoplasma cultures and immunohistochemistry for PI3 virus were negative. *Streptococcus* sp. and *Staphylococcus aureus* were isolated from the lung.

Contributor's Morphologic Diagnoses: 1. Severe, diffuse, chronic lymphoid interstitial pneumonia
2. Multifocal, severe, chronic suppurative bronchopneumonia with abscessation

Contributor's Comment: Ovine progressive pneumonia (OPP) and maedi-visna (from the Icelandic words for 'shortness of breath' and 'wasting')[1] are virtually identical diseases caused by closely related ovine lentiviruses. OPPV is transmitted primarily by close contact and droplet transmission and infects cells of monocyte-macrophage lineage, including dendritic cells.[2] Recent studies suggest transmission via milk and transplacental transmission are of minimal significance. Certain breeds are more susceptible to infection than others.[3] Recently, researchers in Iceland have identified a gene (vif) in maedi-visna virus responsible for infectivity in vivo and in vitro.[4]

The gross pulmonary lesions in this case were typical of OPP complicated by secondary bronchopneumonia.[1] Heavy, grey, non-collapsing lungs often have cranioventral consolidation. Pulmonary and systemic abscessation in this ewe suggested a secondary infection with *Corynebacterium pseudotuberculosis*, although this organism was not isolated from the lungs.

The histologic lesions were diagnostic. The most characteristic feature was extensive lymphofollicular proliferations around airways and pulmonary vasculature, both arteries and veins; some had germinal center formation. Also present was the characteristic smooth muscle hyperplasia of terminal bronchioles and alveolar ducts and lymphohistiocytic interstitial pneumonia. Severe pulmonary fibrosis in the submitted sections was most likely secondary to chronic, suppurative bronchopneumonia, but 'microatelectasis' due to collapse of alveolar spaces has also been described in chronic cases.[1] Hyperplasia of type II pneumocytes and bronchiolar epithelium was not prominent, in contrast to cases of pulmonary adenomatosis, which has been associated with a type B/D retrovirus.[3] Other lesions attributed to OPPV infection seen in this ewe but not represented in these tissues were diffuse lymph node cortical hyperplasia and moderate lymphoplasmacytic synovitis in multiple joints. Also present were membranous glomerulopathy and splenic amyloidosis, which were attributed to chronic antigenic stimulation.

AFIP Diagnoses: 1. Lung: Pneumonia, interstitial, lymphohistiocytic, chronic, diffuse, severe, with perivascular and peribronchiolar lymphoid hyperplasia, and smooth muscle hyperplasia, Polypay, ovine.
2. Lung: Bronchopneumonia, suppurative, multifocal, moderate, with focally extensive abscess.

Conference Comment: Some sections contain a large abscess composed of a central area of necrosis with many small aggregates of gram-positive cocci around the periphery. This area is bounded by moderate numbers of lymphocytes, plasma cells, and fewer epithelioid macrophages. There are reactive fibroblasts and fewer inflammatory cells at the periphery.

The contributor provides a thorough overview of ovine progressive pneumonia virus disease, which is caused by a lentivirus in the *Retroviridae* family. In addition to the closely related ovine lentivirus known as maedi-visna virus, additional lentiviruses of veterinary significance include simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), and equine infectious anemia virus.

The gross pulmonary lesions of uncomplicated OPP include expanded, heavy, rubbery to firm lungs that fail to collapse, and have rib impressions on the pleura. The lungs are diffusely mottled gray to grayish-tan. The bronchial and mediastinal lymph nodes are enlarged with soft grayish-white, homogeneous thickening of the cortical regions. Microscopically, the most characteristic feature of OPP is lymphocytic interstitial pneumonia with perivascular and peribronchial lymphofollicular proliferations that often have germinal centers. Other features include smooth muscle hyperplasia in the walls of terminal bronchioles and alveolar ducts, interstitial fibrosis, and microatelectasis. Hyperplasia of bronchiolar epithelium and type II pneumocytes is not a prominent feature of OPP. Other lesions associated with OPP include lymphofollicular mastitis, chronic proliferative arthritis, nonsuppurative meningoencephalitis, and vasculitis.[1]

Ovine Progressive Pneumonia (OPP) shares many clinical and pathological features with caprine arthritis-encephalitis (CAE), which is caused by a closed related caprine lentivirus. CAE virus primarily causes nonsuppurative leukoencephalomyelitis in young goats, and chronic proliferative arthritis and synovitis in adults. Less commonly, mastitis and lymphocytic interstitial pneumonia occur in adult goats CAE virus often induces two prominent pneumonic features lacking in OPP. One is extensive alveolar filling with dense, acidophilic, proteinaceous to lipoproteinaceous material, and the other is type II pneumocyte hyperplasia.[1]

Similar to goats with CAE viral pneumonia, sheep with OPP seldom have a pure viral infection, and as in this case, often develop a secondary bacterial pneumonia. It is important in such cases to separate the two processes and understand which agent is likely causing which lesion(s). When OPP is complicated by bronchopneumonia, the gross appearance should include the typical cranioventral consolidation with pus-filled airways. Additionally, there can also be coexistent lungworm lesions.[1]

Contributor: Washington State University, College of Veterinary Medicine, Department of Veterinary Microbiology and Pathology, Pullman, WA www.vetmed.wsu.edu

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SLIDE 91
CONFERENCE 23 / CASE II – 1140-99 (AFIP 2689105)

Signalment: 2-year-old, Quarter Horse, gelding.

History: Six, 1-2-year-old horses, out of a total of 40, died after 2-3 days of blindness and going down. One horse and another horse head were submitted. These 40 horses ate 5 tons of corn and oat screening from January 26-February 7 (our submission was on February 16). Other horses on the premises were hand-fed 5 lbs of these screenings/day/horse and were not affected.

Gross Pathology: One brain had focal, mild, yellow softening in the posterior cerebral white matter on one side. The other brain had severe yellow softening throughout the cerebral white matter, but limited to one side. There were no gross lesions in the other organs, except that the stomach was filled with roughage and finely cracked corn and oats.

Laboratory Results: Screening analysis:
Fumonisin B_1: 8.3 ppm (1.0 considered "positive" and above 5.0 ppm associated with leukoencephalomalacia) (Texas Veterinary Diagnostic Laboratory)
Aflatoxin: 5 ppb ("negative"/normal)
Rabies F.A.: negative

Contributor's Morphologic Diagnosis: Multifocally severe cerebral leukoencephalomalacia with vasculitis, hemorrhage, and gitter cells (Fumonisin toxicity).

Contributor's Comment: Equine leukoencephalomalacia (ELEM) is a neurotoxic disease caused by fumonisin production in *Fusarium moniliforme*-infected corn, usually screenings. The disease is characterized by sudden onset of circling, anorexia, head pressing, paresis, ataxia, blindness and depression that may progress to hyperexcitability. The horses usually become comatose within 1 to 10 days but can recover with some neurologic defects.

Fumonisin toxicity is cumulative so the exact "safe" level is unclear. In one study, the Fumonisin B_1 (FB_1) level never exceeded 8 ppm in horse feed not associated with ELEM, whereas the FB_1 ranged from less than 1 to 126 ppm in feed associated with ELEM with most of these being above 10 ppm. Another study indicated that 5 ppm was considered toxic. The affected horses averaged about 19 lbs/day/horse. The areas of malacia and cavitation in the cerebral white matter may be unilateral or bilateral. Microscopically, the malacic areas have gitter cells with degenerating axons and myelin and adjacent multifocal hemorrhage and slight perivascular cuffing of lymphocytes and eosinophils. Lesions occur but are less common in the spinal cord, brainstem, and cerebellum.

Fumonisins are structurally similar to sphingosine and act by blocking enzymes in sphingolipid synthesis and thus disrupt endothelial cell membranes. This might explain the vasculitis and rare fibrin thrombi in dilated vessels in this case. There is species variability in toxicity to fumonisin: horses develop neurotoxicity and sometimes hepatotoxicity, swine develop pulmonary edema, several laboratory animals develop hepatotoxicity and fumonisin is carcinogenic in rats and possibly in humans (esophageal cancer).

AFIP Diagnosis: Brain, cerebrum: Necrosis, white matter (leukoencephalomalacia), multifocal to coalescing, with vasculitis, edema, and hemorrhage, Quarter Horse, equine.

Conference Comment: There is slide variability and not all sections contain good examples of vasculitis and fibrin thrombi. Often it can be difficult to determine if there is a true vasculitis, or if the vascular changes are induced by the changes in the surrounding tissue (inflammation and necrosis). However, if fibrin thrombi are present, the vascular changes are likely not simply a result of the surrounding necrosis.

Equine leukoencephalomalacia (ELEM), also known as moldy corn poisoning, is a mycotoxicosis caused by fumonisin B_1, and is usually associated with consumption of moldy corn or grain contaminated with *Fusarium moniliforme*.[5]

In the horse, fumonisin causes two syndromes: neurotoxic and hepatotoxic. The neurotoxic syndrome is most common, with clinical signs including depression, head pressing, or seizures. Gross lesions include degeneration and liquefactive necrosis of the subcortical white matter, especially in the frontal and parietal lobes. Lesions are often bilateral, but are not always symmetrical. The characteristic gross lesion is yellow gelatinous malacia and liquefaction of the affected white matter, with hemorrhage. Microscopically, areas of liquefaction are surrounded by diffuse or perivascular edema, hemorrhage, and small leukocytic cuffs. Blood vessels may be degenerate or necrotic with occasional thrombi. Less characteristically, there may be edema and perivascular cuffing in the leptomeninges and neuronal necrosis in the deeper layers of the gray matter. The hepatotoxic syndrome is characterized by a swollen, yellow-brown liver

with multifocal pale areas. Microscopically, there is centrilobular necrosis and fibrosis that are similar to those seen with aflatoxicosis.[5]

The primary toxin isolated from *F. moniliforme* is fumonisin B_1, although other fumonisins have been extracted. The exact mechanism of injury has not been fully defined; however, vascular damage has been inferred as the primary injury. Fumonisins inhibit the enzyme ceramide synthase, interfering with the synthesis of sphingolipids. Fumonisins disrupt cellular membranes, are associated with lipid peroxidation of cells and cellular membranes, inhibit synthesis of macromolecules and DNA, and may enhance production of tumor necrosis factor-alpha by macrophages.[5]

Other animals including pigs and avian species (chickens, ducks) are susceptible, but clinical disease and lesions generally include pulmonary, hepatic, or renal injury. In pigs, the disease is called porcine pulmonary edema and is characterized by severe pulmonary edema and hydrothorax.[5] Recently, meningoencephalitis secondary to *Fusarium solani* has been reported in a German Shepherd Dog.[6]

Contributor: Arkansas Livestock & Poultry Commission Lab, P.O. Box 8505, One Natural Resources Drive, Little Rock, AR 72205

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SLIDE 92
CONFERENCE 23 / CASE III – A03-286 (AFIP 2948654)

Signalment: Adult (age unknown) intact male cynomolgus macaque (*Macaca fascicularis*).

History: In April 2003, the animal underwent experimental kidney transplantation and was treated with thymoglobulin and rapamycin to inhibit transplant rejection. Three

weeks later the animal had labored breathing and appeared to be in pain. While sedated for a physical exam, the animal died spontaneously.

Gross Pathology: At post-mortem, the animal was thin with bilateral enlargement of the axillary and inguinal lymph nodes. There was bicavitary effusion (hydrothorax and hydroperitoneum) and the lungs had multifocal red, wet, heavy areas with intervening pink, crepitant areas. On the surface of the right kidney there was a focal, radiating white streak.

Laboratory Results: Serial Creatine values: 5/2/03 Creatine 1.9 mg/dl
5/4/03 Creatine 3.2 mg/dl
5/5/03 Creatine 3.0 mg/dl

Contributor's Morphologic Diagnoses: 1. Body as a Whole**:** Disseminated cytomegalovirus (CMV) infection.
2. Spleen: Severe acute multifocal necrosuppurative splenitis with intralesional CMV inclusions and CMV arteritis.

Contributor's Comment: Within the section of spleen, there is widespread loss of the white pulp denoted by severe lymphoid depletion with a conspicuous angiocentric distribution (most prominent around the central arteries). Discrete foci of intact and degenerate neutrophils admixed with bland cell detritus, karyomegalic cells and occasional small "plugs" of fibrin are predominately located within the white pulp. The karyomegalic cells are misshapen and pleomorphic with variable amounts of amphophilic cytoplasm and large oval to cigar-shaped eosinophilic glassy intranuclear inclusions surrounded by a distinct halo (Cowdry type-A inclusions). Karyomegalic inclusion-bearing cells are also scattered within the red pulp. Occasionally, endothelial cells and smooth muscle cells of large and small caliber blood vessels are karyomegalic and contain Cowdry type-A intranuclear inclusion bodies. In some sections, a focus of foreign body giant cells enveloping fragments of suture material is external to the splenic capsule.

Immunohistochemistry was performed using a polyclonal CMV antibody and cytomegalic cells show positive nuclear staining for CMV.

Cytomegalovirus (CMV) is a herpesvirus of the subfamily Betaherpesvirus and is a naturally occurring infection in many species of nonhuman primates. Following initial infection, which is often asymptomatic, the virus establishes latency in its natural host.[1] In immunocompromised animals the virus may reactivate and disseminate as the result of a variety of predisposing conditions, including immunosuppressive viral infections (SIV, type D retrovirus) and immunosuppressive drug therapy (cyclophosphamide, corticosteroids, and antithymocyte globulin).[1] Organs targeted by CMV include the eye, lung, meninges, skin, heart, intestines and testicle.[2] It is known that CMV has a predilection for mesenchymal cells, although infected cells are frequently misshapen and difficult to identify using conventional methods. The typical, almost pathognomonic cytopathological lesions produced by CMV infection are cytomegaly and large, Cowdry

type-A intranuclear inclusion bodies, although both intranuclear and intracytoplasmic inclusion bodies can be seen with CMV infection.[2] In this particular animal, a satisfactory pathoanatomical explanation for the bicavitary effusion is provided by the widespread endothelial injury caused by CMV infection, resulting in increased vascular permeability and effusion into the body cavities and lungs.

AFIP Diagnosis: Spleen: Splenitis, necrotizing, acute, diffuse, moderate, with marked lymphoid depletion, and myriad cytomegalic cells with eosinophilic intranuclear inclusions, etiology consistent with cytomegalovirus, cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: Cytomegalovirus (CMV) is a common asymptomatic infection of humans and many nonhuman primates. CMV resembles other herpesviruses ultrastructurally, but differs from alphaherpesviruses in several aspects. First, CMV is slowly cytolytic and tends to cause enlargement of the nucleus and the cytoplasm (cytomegaly). Cytomegaly is a result of the accumulation of enveloped virions in large cytoplasmic vacuoles during viral replication instead of the virions being released into intercellular spaces. Secondly, CMV tends to be restricted in its host range, unlike many of the cytolytic alphaherpesviruses. Lastly, latent infections tend to persist in glandular tissue, lymphoreticular cells, and kidneys rather than in neurons.[1]

Cytomegalovirus is transmitted horizontally in a variety of body secretions, including saliva, blood, urine, milk, and semen. Infection is usually not associated with disease. However, disease occurs in immunocompromised individuals or following intrauterine infection. Cytomegalovirus persists as a latent infection and may periodically be shed in body secretions. In immunosuppressed macaques, reactivation of the virus may be associated with disseminated lesions in the brain, lymph nodes, liver, spleen, kidney, small intestine, nervous system, and arteries. Disseminated CMV disease may be initiated by a variety of immunosuppressive events, including viral infection (simian immunodeficiency virus or type D retrovirus) and drug therapy (cyclophosphamide, cortisone, antithymocyte globulin).[1]

Immunosuppressive therapy is commonly given to animals and humans undergoing renal transplantation. Rapamycin is an immunosuppressive macrolide antibiotic that inhibits T and B cell proliferation, while thymoglobulin is a rabbit polyclonal antithymocyte antibody that is a potent T-cell depleting agent. Thymoglobulin is effective in depleting T-cells in both the blood and secondary lymphoid organs through the apoptotic pathway.[3]

It is important to understand the primary and secondary effects that immunosuppressive agents will have on animals when evaluating histopathological changes in specific organs. In this case, the splenic necrosis and lymphoid depletion are primary effects of the immunosuppressive agents given to this nonhuman primate. The disseminated CMV disease is likely a result of the immunosuppressive agents reducing the numbers

of B and T cells, effectively diminishing this animal's immune response, and therefore allowing reactivation of the latent virus.

Contributor: Harvard Medical School, New England Regional Primate Research Center, Division of Comparative Pathology, 1 Pine Hill Drive, PO Box 9102, Southborough, MA

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SLIDE 93
CONFERENCE 23 / CASE IV – N2004-96 (AFIP 2942031)

Signalment: 9-year-old, female, Goeldi's marmoset (*Callimico goeldii*).

History: This Goeldi's marmoset presented with an open fracture of the proximal right humerus. The tissue distal to the fracture was largely devitalized. On physical examination the animal was icteric and had clinical signs of shock. Elective euthanasia was performed.

Gross Pathology: The carcass was in moderately thin body condition. Most tissues were discolored yellow (icterus). Both kidneys had tan mottling of the capsular surfaces as well as mottling of the medulla and cortex on cut section. The spleen was mildly enlarged, and the liver was diffusely pale tan. The open fracture of the humerus and the associated extensive devitalization of the distal right arm were confirmed.

Laboratory Results: Fluorescent antibody assay demonstrated *Leptospira* spp. antigen in kidney and liver. Microagglutination testing (MAT) (tested for 18 Leptospira serotypes) of serum obtained at the time of euthanasia revealed an antibody titer of 1:12800 for serotype Ballum.

Contributor's Morphologic Diagnoses: 1. Kidney: Nephritis, tubulointerstitial, chronic-active, diffuse, severe, with multifocal tubular regeneration, and bilirubinuria. 2. Kidney: Mineralization, tubular epithelial, multifocal, mild.

Contributor's Comment: The clinical and gross findings together with the pathological changes in the kidneys, presence of rare intratubular argyrophilic spirochetal bacteria

(Steiner's silver stain), positive Leptospira fluorescent antibody (FA) result, and high antibody titer are consistent with leptospirosis in an "incidental host." In this case, infection with serotype Ballum was demonstrated.

Histologically there was, in addition to the extensive infiltration of the renal interstitium by lymphocytes, plasma cells, histiocytes, and scattered neutrophils, multifocal tubular epithelial degeneration, necrosis, regeneration, and occasionally intratubular proteinosis. Several tubules contained ongoing tubulonephritis in which infiltrating neutrophils predominated. Intratubular and intraepithelial cytoplasmic yellow-brown, granular pigment was present in several of the tubules (bilirubin pigment; Hall's bilirubin stain positive). Minimal, multifocal interstitial fibroplasia and deposition of collagen were demonstrated by trichrome staining. Few mitotic figures in cells of possible lymphocytic origin were seen in areas of inflammation. Mineralization of epithelial cells and associated epithelial degeneration/necrosis were seen in some tubules.

Leptospirosis is a zoonotic disease of global importance that affects a broad range of mammalian species. It is caused by the various serotypes of *Leptospira interrogans* (old classification scheme; see below). The bacteria are ubiquitous. Although not able to replicate in the environment, they can persist in the environment under optimal, warm and wet conditions for months.[1,2] *Leptospira* spp. bacteria are tightly coiled spirochetes, 0.1 _m in diameter and 6-20 _m long, with hooked ends.[1] They require special methods for visualization including silver stains, fluorescent antibody methods, immunohistochemical stains, darkfield microscopy, or electron microscopy. Leptospires have also been isolated from arthropods, amphibians, reptiles, and birds, including serovars pathogenic for mammals from amphibians.[1]

"Maintenance hosts" are distinguished from "incidental hosts."[3] In the maintenance host there is a stable host-parasite relationship characterized by high susceptibility of the host species, occurrence of frequent and direct infection between individuals (usually at an early age), little or no signs of clinical disease, relatively low antibody response, and persistence of the bacteria in the renal tubules with chronic excretion via the urine. Animal species can be maintenance hosts of some serovars but incidental hosts for other serovars. Throughout the world, there are distinct variations in the maintenance hosts and the serovars they carry.[3] In domestic animals cattle may be carriers for serovar Hardjo and Pomona; pigs may carry Pomona, Tarassovi, or Bratislava; and dogs may carry serovar Canicola.[3] Examples of maintenance hosts in wild animals include raccoons and skunks in North America harboring serovar Grippotyphosa, and various species of wildlife worldwide including ungulates, harboring serovar Pomona. The most important maintenance hosts are rodents. Rats are generally thought to be carriers of serogroup Icterohaemorrhagiae serovars and mice are generally carriers for the serogroup Ballum serovars.[3] Infection with the *L. interrogans* serotype Ballum was indicated in this case by the high antibody titer.

The "incidental host" is characterized by relatively low prevalence of infection in the host population but high pathogenicity for the host, development of acute and possibly fatal disease, a short renal phase of infection with comparably short period of leptospira

shedding, and a marked antibody response.[4] Possible routes of infection include contact with urine, bite wounds, and ingestion of infected tissues, as well as venereal or placental transfer.

In zoological collections, the majority of leptospirosis cases represent incidental host infections.[4] Heated buildings, that house temperature-sensitive species (i.e., tropical primates) provide an excellent, hard-to-control, year-round environment for both the Leptospira bacteria and the maintenance host; in this setting usually rodents. In this case, environmental testing to determine possible sources of infection, including water supply and the food preparation area were negative.

The mortality rate and the extent of damage to the internal organs vary with virulence of the serovar and host susceptibility; more animals encounter infections than will develop recognizable disease.[2,4] More than one serovar may occur in a given animal.[2] The bacteria penetrate either through mucous membranes or percutaneously through injured skin. After a variable incubation period (4-20 days)[4] there is a biphasic progression of the disease; a septicemic phase lasting for about a week is followed by the immune phase. In the septicemic phase, there is leptospiremia with rapid multiplication and spread via the bloodstream**.** During this period, the bacteria can enter and replicate in any tissue, e.g., liver, kidney, spleen, lung, eye, central nervous tissue and genital tract.[2] Hemorrhage, intravascular hemolysis, nephritis, pneumonia, and meningitis are thought to be due to the action of bacterial toxins and the inflammatory response to the bacteria that lead to damage of the vascular endothelial lining. In some Leptospira strains hemolysins were isolated [2], some of which have been shown to mediate hemolytic and cytotoxic activities by pore formation on the mammalian cell membranes. The leptospiral lipopolysaccharide has lower endotoxic activity compared to other gram-negative bacteria.[2] Icterus, as seen in this case, is often due to a combination of intravascular hemolysis and liver injury. The spirochetes are cleared from the blood and most tissues with increasing antibody titers, but can persist and multiply for some time in renal tubules, uvea, and brain. Within the kidney, leptospires invade the interstitium with the help of their two periplasmic flagella [2] and subsequently penetrate the tubular epithelium, which results in an acute inflammatory response dominated by neutrophils. In this case, tubulointerstitial nephritis is still ongoing in some of the renal tubules, demonstrating the chronic-active nature of the inflammatory process. The acute phase of leptospirosis is transient and replaced by the immune phase, in which antibody production and excretion of leptospires via the urine are key features. Lymphocytes, plasma cells, and macrophages infiltrating the interstitium are the predominant inflammatory cells during this phase. The organisms can persist within the renal tubules of the incidental host, protected from antibody and other host defenses, for a few days to several weeks. Renal insufficiency and failure are associated with the tubular damage, and possibly with decreased glomerular filtration and hypoxia due to the overall organ swelling that may impair renal blood perfusion.[2] If infection takes place during pregnancy, fetal infection may occur, resulting in abortion (usually last trimester), stillbirth, birth of weak neonates, or birth of healthy but infected neonates.[4] Detectable gross lesions are often absent in infected neonates; thus, failure to further test for leptospira may result in under-reporting of the disease.[5]

In the old phenotypic classification system, the *Leptospira* were serologically divided into two species. *Leptospira interrogans* included all the pathogenic serovars, and *Leptospira biflexa* included all the environmental, nonpathogenic serovars.[3] The more recent classification, based on genotypic properties (12 named and 5 unnamed genomospecies), is somewhat problematic in the clinical situation, since it is incompatible with the former system of serogroups (e.g., species in the new system do not correspond to the previous two species and their serovars, and include pathogenic and nonpathogenic within some species).[3] Therefore, the phenotypic classification is likely to remain in place until simpler molecular identification methods are developed.[3]

Fluorescent antibody (FA) testing is frequently used to confirm the presence of leptospiral antigen in fresh urine or fresh or frozen tissue collected at the time of necropsy.[5] If serum is available, microagglutination testing (MAT) may help to determine the infecting serovar. The highest titer is considered the infecting serovar, with lower positive titers considered to be cross-reactivity.[2] Leptospires can be isolated from blood and CSF during the first phase of illness, and from urine after the first week (data from humans);[3] overall, culture is problematic due to the fragile and fastidious nature of the organisms.[2,5] The use of PCR assays is also still limited due to the inability of most assays to identify the infecting serovar.[3] Killed leptospiral vaccines are frequently used in domestic species and occasionally in exotic species such as the black rhinoceros, in which leptospira infections have been associated with hemolytic crises and high mortalities.[4]

AFIP Diagnosis: Kidney: Nephritis, interstitial, lymphocytic, multifocal, moderate, with neutrophilic tubulitis, Goeldi's marmoset (*Callimico goeldii*), primate.

Conference Comment: The contributor provides a very thorough overview of leptospirosis in animals, including the epidemiology, transmission, histopathological changes, and diagnostic techniques.

Prior to being informed of the contributor's laboratory results, some conference attendees considered lymphoma as a primary differential diagnosis based on the relatively monomorphic population of interstitial lymphocytes that are associated with moderate numbers of mitotic figures, and in some slides the apparent blurring of vessel walls by high numbers of lymphocytes.

Although the angiotropic lymphomas were considered, there are several histopathologic changes for which one must account. First, although the infiltrating inflammatory cells are predominantly lymphocytes, there are low numbers of plasma cells and histiocytes within the interstitium. Therefore, there is not truly a monomorphic population. Secondly, lymphoma classically forms sheets of cells that obliterate normal tissue architecture and often induce a mass effect; neither is evident in this section. And, although the angiotropic lymphomas could be considered, the lymphocytes are not

predominantly associated with vessels, nor are they occluding vessels resulting in ischemic infarction. Lastly, one must account for the renal tubular inflammation, degeneration, and necrosis.

When considering all of the changes apparent with H&E alone: tubular degeneration and necrosis, neutrophilic tubulitis, and lymphoplasmacytic and histiocytic interstitial nephritis, the most logical differential is leptospiral nephritis.

Contributor: Wildlife Conservation Society, Department of Pathology, 2300 Southern Blvd, Bronx, NY
www.wcs.org

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SLIDE 94
CONFERENCE 24 / CASE I – 98-4356 (AFIP 2681370)

Signalment: Adult, male, timber rattlesnake (*Crotalus horridus*).

History: Five of 7 rattlesnakes within an exhibit have died and the remaining 2 are sick. The snakes are bloated, off feed and have seizures.

Gross Pathology: Little body fat is present. The coelom contains caseous material. There are numerous 1 mm white foci in the coelom and the lung.

Contributor's Morphologic Diagnoses: 1. Liver: Cytoplasmic inclusions, hepatocytes.
2. Kidney: Nephritis, interstitial, lymphocytic, multifocal, mild with cytoplasmic inclusions in tubular epithelial cells.
3. Lung: Pneumonia, necrotizing, purulent and granulomatous, with rare intraepithelial cytoplasmic inclusions and numerous bacteria.

Contributor's Comment: This is a case of paramyxovirus infection in rattlesnakes. A secondary bacterial pneumonia is the predominant lesion in this case and a bacterial coelomitis was also present. Ophidian paramyxovirus infection has been recently described in snakes and produces immunosuppression like the mammalian virus. Secondary bacterial infections are common and are the usual cause of death. The lung and nervous system are the usual organs affected but secondary bacterial infections can occur in a variety of organs. Intracytoplasmic viral inclusions are seen in a variety of epithelial cells but formation of syncytial cells, as occurs in mammalian paramyxovirus infections, is rare.

AFIP Diagnoses: 1. Lung: Pneumonia, bronchointerstitial, granulomatous and heterophilic, diffuse, severe, with multifocal necrosis, epithelial hyperplasia, and rare epithelial eosinophilic intracytoplasmic inclusion bodies, etiology consistent with ophidian paramyxovirus, timber rattlesnake (*Crotalus horridus*), reptile.
2. Kidney: Nephritis, interstitial, lymphocytic and histiocytic, subacute, multifocal, mild, with tubular epithelial degeneration and necrosis.
3. Esophagus, epithelium: Necrosis, multifocal, with rare eosinophilic intracytoplasmic inclusion bodies.

Conference Comment: The respiratory system of snakes is similar to that of mammals with a few distinct differences. The left lung is vestigial in all snakes except boas. The right lung has a posterior avascular portion, known as the air sac. The air sac regulates pressure inside the body cavity. The anterior portion of the lung is composed of faveoli, similar to mammalian alveoli, separated by relatively thin septae that are lined by capillaries and type I and type II pneumocytes. Since snakes do not have a diaphragm, air enters and leaves the lung due to action of the body muscles and movement of the ribs.[3]

Ophidian paramyxovirus (OPMV) is a single-stranded enveloped RNA virus and is an extremely important pathogen of viperid snakes. There have also been reports of OPMV in colubrid, boid, and elapid snakes. Clinical signs typically include loss of muscle tone, abnormal behavior and dilated pupils. However, in many of the outbreaks of OPMV, the snakes are found dead with minimal or no clinical signs noted.[4]

The most significant gross lesion of OPMV disease is hemorrhage of the lung and air sac with caseous necrotic debris. Additional gross lesions include pancreatic hyperplasia, and hepatic granulomas or caseous necrosis. Typical microscopic findings include cellular debris and exudate within airways, type II pneumocyte hyperplasia, thickening of faveolar septa, and few epithelial cells containing intracytoplasmic inclusion bodies.[4]

Like mammalian paramyxoviruses, OPMV also causes immunosuppression. Animals infected with OPMV, as in this case, commonly have secondary bacterial infections with gram-negative organisms. The most common gram-negative organisms isolated from

reptiles with pneumonia include *Pseudomonas* spp., *Providencia* spp., *Proteus* spp., *Salmonella* spp., *Aeromonas hydrophila*, and *Escherichia coli*. Thus, it is not surprising that snakes infected with OPMV often succumb to secondary bacterial pathogens.[4]

Contributor: Virginia Tech, College of Veterinary Medicine, Department of Biomedical Sciences & Pathobiology, Blacksburg, VA

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SLIDE 95
CONFERENCE 24 / CASE II – PP1481D (AFIP 2936457)

Signalment: 1.5 year old, female, harbor porpoise (*Phocoena phocoena*), cetacean.

History: A juvenile, female harbor porpoise, weighing 41 kg, was rescued in April 1999 from a pound net close to Baaring Vig and taken to the Fjord and Belt Centre, Kerteminde for rehabilitation. The animal was regularly treated with anthelmintics and clinically examined. A couple of weeks later, the porpoise developed pox-like lesions on the skin. The lesions persisted for 6 to 8 months but healed without scars. On 12th February 2000, the porpoise developed clinical signs of illness consisting of reduced appetite, floating on the surface and being unable to support itself in the water. The animal had two crises, with high respiratory rate and low body temperature. The porpoise was treated intensively, including antibiotics. In spite of intensive medical care, the animal died on February 22nd, 2000 after 10 days of illness.

Gross Pathology: The porpoise was in a good nutritional status. There was an acute fibrinous to suppurative pleuritis and pericarditis with approximately 0.4 to 1 liter of exudate. One myocardial abscess, approximately 5 cm in diameter, was found in the left ventricle with fistulous tracts to both, the endocardium and epicardium. Another myocardial abscess, 0.5 cm in diameter, was located at the base of the heart. There was a severe diffuse, fibrinous, suppurative peritonitis with 1 litre of exudate. In the esophagus few ulcerations were detected. On the skin of the left side of the body there were multiple round foci approximately 1 cm in diameter consisting of a white center surrounded by a black rim. In the bronchial tree and pulmonary blood vessels a mild to

moderate parasitic infection with *Pseudalius inflexus* and *Torynurus convolutus* as well as a moderate parasitic burden of *Stenurus minor* in both aural peribullar cavities were observed. In the first compartment of the stomach one nematode (*Anisakis simplex*) and a few fish eyes were found. In the second compartment of the stomach few submucosal hemorrhages were noted with a diameter of about 0.5 cm. In the right lower jaw, the third tooth was dislocated towards the buccal gingiva.

Laboratory Results: *Staphylococcus aureus* was cultured from a sample taken from the blowhole while the animal was alive. Postmortem samples from cardiac abscesses, liver, spleen, and kidneys yielded *Staphylococcus aureus*. In addition, a few colonies of streptococci and *Serratia liquefaciens* were found. Skin biopsies revealed moderate cytoplasmic swelling and vacuolization of keratinocytes; however, ultrastructurally pox virus particles could not be detected. Morbillivirus antigen was not detected by immunohistochemistry.

Contributor's Morphologic Diagnosis: 1. Heart: Myocarditis, pyogranulomatous, chronic, multifocal to coalescing, severe with necrosis, colonies of cocci, homogenous eosinophilic material (consistent with Splendore-Hoeppli material), fibrosis, harbor porpoise (*Phocoena phocoena*), cetacean.
2. Heart: Myocarditis, lymphocytic, chronic, multifocal, mild.
3. Heart: Epicarditis, lymphohistiocytic, chronic, diffuse, moderate.

Contributor's Comment: The submitted tissue section includes myocardium, epicardium and fibrous connective tissue of a harbor porpoise. The main lesion consists of a multifocal extensive myocardial necrosis with myriad cluster-forming cocci which are surrounded by homogenous eosinophilic substance in a club-shaped radiating corona (consistent with Splendore-Hoeppli material). The surrounding tissue is markedly infiltrated with inflammatory cells, predominantly neutrophils, macrophages and epithelioid macrophages admixed with lymphocytes and plasma cells. The periphery of the lesion is demarcated by a poorly vascularized fibrous tissue. Multifocal areas of the myocardium showed a mild, interfascicular infiltration of lymphocytes. The epicardium was irregularly thickened by an infiltration of lymphocytes and histiocytes.

Upon histological examination of other tissues, a multifocal acute hepatocellular, centrolobular necrosis without inflammatory reaction, and chronic suppurative pericholangitis, were detected. Follicular hyperplasia was found in the spleen. In the adrenal gland a mild focal lymphoplasmacytic infiltration with multifocal hemorrhages was observed.

Staphylococcus aureus was isolated from the cardiac abscess and other organs. Chronic infections caused by *Staphylococcus aureus* are termed botryomycosis. This is a poorly understood pyogranulomatous bacterial disease of the skin and subcutis or, more rarely, the viscera. In humans, cutaneous, pulmonary, and hepatic botryomycosis have been reported. In veterinary medicine botryomycosis is recognized as a staphylococcal wound infection in horses and pigs. In addition, pulmonary botryomycosis has been reported in horses and guinea pigs.[3] In cattle, swine and

elephants it is an unusual manifestation of mastitis.[1,2] One case of disseminated staphylococcal botryomycosis has been described in a cat with perforating gastric ulcer.[4] Intraabdominal botryomycosis has been diagnosed in a dog.[5] In two harbor porpoises (*Phocoena phocoena*), one case submitted here, a pyogranulomatous myocarditis due to *Staphylococcus aureus* septicemia has been reported.[6] In three harp seals (*Pagophilus groenlandicus*) the presence of subcutaneous and systemic botryomycosis was described.[7]

The pathogenesis of botryomycosis is still unclear. An imbalance between virulence of the organism and host resistance may result in incomplete removal of bacteria by the host which leads to formation of bacterial granulomas.[8] The exact composition of the infiltrating population of inflammatory cells depends of the stage of the infection. At initial examination, the lesion may have a purulent exudate with small white granules with a diameter of less than 1 mm. These granules are indistinguishable from those of actinomycosis, nocardiosis, and mycetoma unless special stains are used. In botryomycosis the capsules of the grains were homogenously eosinophilic (Splendore-Hoeppli material), periodic acid-Schiff (PAS) positive, slightly acid-fast, weakly positive with Gomori's methenamine silver stain, and were unstained with the MacCallum-Goodpasture modification of the Gram stain.[3]

Differential diagnoses include systemic mycosis, chronic bacterial abscesses, and foreign body reaction. Fungal hyphae are best demonstrated with silver stains (for example Grocott's stain). *Actinomyces* and *Nocardia* are Gram-positive filamentous organisms, and acid-fast in the case of *Nocardia* (for example Ziehl-Neelsen stain). Most reported cases of botryomycosis are caused by *Staphylococcus aureus*, but *E. coli*, *Pseudomonas aeruginosa*, *Actinobacillus lignieresii*, *Bacteroides*, *Streptococcus* and *Proteus species* have also been implicated.

Staphylococcus aureus is an uncommon finding in marine mammals. Some authors described *Staphylococcus aureus* as a commensal in the blowhole flora, whereas others classify it as a potential pathogen in clinically normal dolphins.[9] In the literature *Staphylococcus aureus* was described in association with septicemia in a killer whale,[10] a cerebral abscess in a dolphin,[11] and a septic embolic nephritis in the same species.[12] Furthermore it was described as a pathogen in pneumonia in dolphins,[13] cutaneous lesions, subcutaneous abscesses, and omphalitis in pinnipeds. In this case, the origin of the staphylococci remained unclear; the potential port of entry might be the pox-like lesions.

AFIP Diagnosis: 1. Heart: Myocarditis, pyogranulomatous, multifocal to coalescing, severe, with fibrosis, Splendore-Hoeppli material, and numerous colonies of cocci, harbor porpoise (*Phocoena phocoena*), cetacean.
2. Heart: Epicarditis, lymphoplasmacytic and histiocytic, chronic, diffuse, moderate.

Conference Comment: The contributor provides a thorough overview of staphylococcal infections and botryomycosis. As mentioned above, botryomycosis has been reported in many species and in a variety of organ systems, including the skin (humans, horses, pigs), lungs (horses, guinea pigs), mammary gland (cattle, pigs, elephants), stomach (cat), abdomen (dog), and most recently the heart (harbor porpoise).

Another large group of animals commonly affected by staphylococcal botryomycosis is laboratory animals, including mice, rats, gerbils, guinea pigs, hamsters, and rabbits. Staphylococci are common inhabitants of the skin and mucous membranes and are often carried asymptomatically. Many factors, such as immune status, lack of competing bacteria, nutritional deficiencies, trauma to the skin, and prevalence of staphylococci in the environment are recognized as contributing factors.[14]

Clinical signs in mice include abscessation of the cervical lymph nodes, inflammation and abscessation of the preputial and lacrimal glands, conjunctivitis, periorbital abscesses, superficial pyoderma, and severe ulcerative dermatitis. B6 mice are prone to ulcerative dermatitis, while nude mice tend to develop furunculosis around their muzzles, lacrimal gland abscesses, and preputial gland infections. In rats, staphylococcal ulcerative dermatitis characteristically is localized to the dorsal neck and interscapular regions. In young gerbils, *S. aureus* causes a diffuse moist dermatitis involving the face, nose, feet, legs, and ventral body surface. The nasal dermatitis in gerbils is associated with porphyrin-containing lacrimal gland secretions. When these secretions accumulate on the external nares, they act as a chemical irritant which leads to scratching, hair loss, and dermatitis. In guinea pigs, staphylococcal infections lead to ulcerative pododermatitis, also known as bumblefoot. Predisposing factors include trauma due to defective caging and poor sanitation. However, guinea pigs may also develop acute staphylococcal dermatitis (exfoliative dermatitis), which most frequently involves strain 13 guinea pigs. This disease is characterized by alopecia and erythema on the ventral abdomen with exfoliation of the epidermis. In hamsters, cutaneous and cervical abscesses are colonized by a variety of organisms including *S. aureus*, *Actinomyces bovis*, *Streptococcus* spp., and *Pasteurella pneumotropica*. Outbreaks of staphylococcosis occur sporadically in commercial rabbitries, with disease varying from localized abscessation to acute, and frequently fatal, septemia. In rabbits, lesions may occur in the skin, mammary glands, genital tract, conjunctiva, footpads, and respiratory tract. The acute septemic form typically occurs in suckling kits during the first week of life and leads to multifocal suppurative lesions in the subcutaneous tissue, lung, kidney, spleen, heart, and liver.[14]

Contributor: Department of Pathology, School of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany

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SLIDE 96
CONFERENCE 24 / CASE III – 05-0119 (AFIP 2964502)

Signalment: 1+ year old, male, Siamese fighting fish (*Betta splendens*).

History: Prior to euthanasia, Calvin had a two-month history of behavioral changes, hiding in his rock cave, and a three-week history of progressive coelomic enlargement.

Gross Pathology: Coelomic organs are displaced ventrally and caudally by a 1.2 cm diameter mass that occupies two-thirds of the centrodorsal portion of the coelom. The mass is composed of a cystic center filled with a viscous slightly opaque fluid and is surrounded by a 1-3 mm thick wall.

Contributor's Morphologic Diagnosis: Kidney: Nephroblastoma, Siamese fighting fish (*Betta splendens*).

Contributor's Comment: Nephroblastoma (Wilms' tumor, embryonal nephroma) occurs commonly in young pigs and chickens, less frequently in cattle and dogs, and is observed in various species of fish, amphibians and reptiles. It is the most common primary renal tumor in children (Marilyn J. Wolfe, personal communication).[1-3] Since these neoplasms arise from metanephric blastema, primitive pluripotential tissue, the triphasic histologic features resemble the developmental stages of embryonic kidneys with predominantly a myxomatous mesenchymal component interspersed with primitive tubules, embryonic glomeruli, occasional nests of cells resembling metanephric blastema, and rarely contain muscle, cartilage, bone and fat.[2,3]

The WT1 gene protein is a transcriptional activator of genes responsible for regulating cell growth and is essential in renal and gonadal differentiation and for the development of blastema into epithelium. Tissues that express WT1 are the uterus, spinal cord, spleen, abdominal wall musculature, mesothelium lining thoracic organs and the central nervous system. Mutations in the WT1 gene, a tumor suppressor gene, are associated with the development of Wilms' tumor in animals and humans. In addition, mutations in Beta-catenin, important in the *wnt* (*wingless*) signaling pathway, are demonstrated in 15% of humans with Wilms' tumor.[1,4] Thoracolumbar spinal cord tumors of young dogs that share similar histologic features and stain for the Wilms' tumor gene product are considered extrarenal nephroblastomas.[3]

This case was reviewed in consultation with Marilyn J. Wolfe, DVM, PhD, DACVP, Principal Investigator, Registry of Tumors in Lower Animals, Sterling, Virginia 20166-4311.

AFIP Diagnosis: Kidney: Nephroblastoma, Siamese fighting fish (*Betta splendens*), piscine.

Conference Comment: There is significant variation among slides and not all slides have all of the characteristic features of a nephroblastoma. As mentioned by the contributor, the diagnostic features of a nephroblastoma include the triphasic histologic features: myxomatous mesenchyme; interspersed primitive tubulesand/or glomerular-like buds; and, nests of cells resembling metanephric blastemain various amounts. Rarely, these tumors contain non-epithelial tissue such as muscle, cartilage, bone and fat.[3] In this case, the mesenchyme, primitive tubules, and blastema are present on all slides. However, there are very few glomerular-like buds and they are not present on all slides.

Adenocarcinoma of the swim bladder was considered by most residents. However, unlike many other fish, the swim bladder in Siamese fighting fish is located ventral to the kidney. Therefore, if the neoplasm originated from the swim bladder, one would expect

that the unaffected kidney would be dorsal to the swim bladder. In all slides examined by conference participants, the unaffected portions of the kidney are ventral to the tumor. In many slides, it was difficult to determine if the tumor was originating within the kidney; however, in a few slides, it is clearly arising from the kidney.

In humans, nephroblastomas (Wilm's tumor) often present as a large, solitary, well-circumscribed mass; however, in 10% of the cases, the tumor is either bilateral or multicentric at the time of diagnosis. On cut section, the tumor is soft, homogeneous, tan to gray, with occasional hemorrhage, cyst formation and necrosis. Microscopically, the classic triphasic combination of blastemal, stromal, and epithelial cell types is observed in most tumors, although the percentage of each component is variable. Stromal elements are usually fibrocytic or myxoid, and skeletal muscle differentiation is not uncommon. Rarely, other elements, such as squamous or mucinous epithelium, smooth muscle, adipose tissue, cartilage and osteoid and neurogenic tissue are identified.[1]

This case was reviewed in consultation with Dr. Isabell A. Sesterhenn, Chair, Department of Genitourinary Pathology, The Armed Forces Institute of Pathology.

Contributor: Walter Reed Army Institute of Research, Department of Pathology
503 Robert Grant Avenue, Silver Spring, MD
http: //wrair-www.army.mil/

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SLIDE 97
CONFERENCE 24 / CASE IV – G 6573/8 (AFIP 2956261)

Signalment: 1-year-old, female, Goeldi´s monkey (*Callimico goeldii*), non-human primate

History: For a period of several months the animal showed oral, perioral and nasal alterations which were characterized by swelling, edema, emphysema and incrustation. Treatment consisted of antibiotic therapy and regular removal of the overlying crusts to

ease breathing, but clinical recovery could not be achieved. The swollen and hyperemic lips were still remarkable when the animal was necropsied after its accidental death. Retrospectively, similar clinical signs were present in both parents.

Gross Pathology: At necropsy the young Goeldi´s monkey was in poor nutritional condition and showed marked abdominal edema. Main post mortem findings were located in the upper respiratory and gastrointestinal tracts. Both nasal and oral tissues were swollen and edematous and the nose was covered with crusts. The overgrowing crusts and hyperemic lesions were mainly present on the mucocutaneous membranes of the lips and the nasal region. Numerous whitish nematodes up to 1 cm in length could be detected macroscopically within the mucous membranes of the lips, tongue, pharynx and intestine.

Laboratory Results: Bacteriological examination of a swab that was taken from the altered tissue - *Staphylococcus* sp. was cultured. Routine flotation of a fecal sample was negative for parasites and parasite eggs.

Contributor's Morphologic Diagnosis: 1. Skin (mucocutaneous junction): Dermatitis, minimal to mild, chronic, diffuse, with irregular epidermal hyperplasia and parasitic structures consistent with *Spirurida*, Goeldi´s monkey, non-human primate.
2. Intestine (not present in all slides): Several parasitic structures consistent with *Spirurida* within the lumen of the duodenum, Goeldi´s monkey, non-human primate.

Contributor's Comment: Histologically, the dermatitis is characterized by an irregular epidermal hyperplasia forming prominent rete ridges and pseudocarcinomatous proliferations in several locations. Focal erosions are present on the skin surface. In consequence of the parasite infection there is a mild lymphocytic inflammatory reaction associated with spongiosis in the superficial parts of the dermis. The parasitic structures are located within the epidermal layer where they are arranged in cystic cavities. In sections the nematodes do not cause any inflammatory response in the adjacent tissue. No parasites are seen inside the hair follicles.

The parasites were identified as nematodes of the order *Spirurida.* Characteristic histologic features are the muscular and glandular esophagus, lateral cephalic alae, the morphology of the lateral chords and the presence of embryonated eggs.[1] In the present case, further classification was not possible on the basis of histological examination. Because of the gross pathologic findings and the site of infection it is presumed that the parasites belong to the genus *Gongylonema*.

Spirurids of the genus *Gongylonema* are parasites of the upper digestive and respiratory tract in a variety of birds and mammals including non-human primates. Coprophagous arthropods (dung beetles and cockroaches) serve as intermediate hosts in the indirect life cycle. Embryonated eggs are deposited by the female worms, and liberated after epithelial desquamation with the host's feces. The first-stage larvae hatch in the insect's intestines, migrate into the body cavity and develop into the second larval stage after encapsulation. The maturation of the infectious third-stage larvae is

completed after about four weeks. Final hosts acquire the parasite infection by ingestion of infected intermediate hosts or by drinking contaminated water. The migration pathway in the definitive host is still unknown for the most part, especially in primates. The larvae are released from their capsules under the influence of gastric acid and probably migrate within the wall of the upper intestinal tract, where they develop into adult worms.[2,3]

Gongylonema are considered to be non-pathogenic in most host species. Generally there is little tissue reaction with no extensive lesions. By contrast, reports about gongylonemiasis in non-human primates, especially in New World monkeys, indicate that these nematodes may cause profound oral inflammation and irritation, particularly in times of high parasite exposure, and predispose the affected animals to fatal secondary infections.[1,4]

AFIP Diagnosis: Oral mucosa, multiple sites: Intraepithelial adult spirurids, with multifocal minimal lymphocytic inflammation, etiology consistent with *Gongylonema* sp., Goeldi's monkey (*Callimico goeldii*), primate.

Conference Comment: There is significant slide variation with some slides having a section of pancreas and attached duodenum with intraluminal and submucosal nematode parasites.

As mentioned by the contributor, the key histologic features of spirurids include a characteristic small, usually thick-shelled, embryonated egg; cuticular ornamentations around the buccal cavity; coelomyarian musculature; uninucleate multicellular intestine often lined by microvilli that form a brush border; and, lateral chords that may be quite large. In some sections, the spirurids within the oral mucosa have cuticular bosses and lateral alae. The cuticular bosses are characteristic for *Gongylonema* sp. and are located on the anterior end of the parasite. Grossly, *Gongylonema* sp. are long thin worms that often form a zigzag pattern in the mucosa and submucosa and appear "stitched" into the tissue.[5]

Some sections contain pancreas and duodenum with intraluminal and submucosal spirurids. These spirurids are *Pterygodermatites* sp., which have characteristic lateral alae in a sublateral position on the anterior end of the parasite. Adult parasites may be found in the lumen of the small intestine with their anterior ends embedded in the mucosa. The larvae, when present, are deeper in the submucosa.[6]

Other intraepithelial parasites include: *Capillaria* sp., *Anatrichosoma* sp., and *Trichosomoides* sp.

Contributor: German Primate Center, Department of Primate Medicine and Husbandry, Kellnerweg 4, 37077 Gottingen, Germany

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SLIDE 98
CONFERENCE 25 / CASE I – 04-01930 (AFIP 2948759)

Signalment: 2-year-old Baladi (native Egyptian breed) mare.

History: Baladi horses are used as dancing and parade horses. Two days post-dancing parade; this mare had acute progressive muscle stiffness and considerable distress with transient pyrexia. Shortly before euthanasia she had very stiff thigh muscles and occasional lateral recumbence.

Gross Pathology: The paraspinal lumbosacral muscles and the thigh muscles of both legs, especially the gluteal muscles, were moist, mildly swollen, and had massive patchy to regionally extensive pale streaks.

Contributor's Morphologic Diagnosis: Skeletal muscles: Acute degenerative and necrotizing myopathy with intermyofibrillar edema, moderate, multifocal.

Contributor's Comment: The current gross and histologic lesions are typical of equine recurrent exertional rhabdomyolysis (ERER). Polysaccharide storage disease was ruled out by negative PAS (periodic acid-Schiff) stain. ERER, or myoglobinuria, belongs to a group of muscle diseases called exertional myopathies. Exertional myopathies also include capture myopathy and porcine stress syndrome (malignant hyperthermia), characterized by a common initiating factor of intensive or exhaustive muscle activity.[1] ERER is characterized clinically by sudden onset of stiff gait, reluctance to move, and

swelling of affected muscles groups especially gluteal muscles.[1] Diagnosis is based on clinical signs in association with an increase in serum creatinine kinase (CK), aspartate amino-transferase (AST), and in severe cases myoglobinuria. The exact etiology and pathogenesis of ERER are still unknown. Recently, a heritable defect in muscle cell calcium regulation of muscle excitation-contraction coupling was suggested as the primary factor for this disease.[2] Few reported cases of ERER have an underlying polysaccharide storage myopathy; however, the extent of PSM in the majority of ERER is unknown.[3]

AFIP Diagnosis: Skeletal muscle: Degeneration and necrosis, acute, multifocal, moderate, Baladi horse, equine.

Conference Comment: Conference attendees discussed the differential diagnosis of the gross lesion, pale streaks in striated muscle. Differentials include: exertional rhabdomyolysis, equine polysaccharide storage myopathy (EPSSM), nutritional myopathy (Vitamin E/Selenium deficiency), ischemic myopathy due to anesthesia, plant toxicity (*Cassia occidentalis*, coffee weed), ionophore toxicity (monensin), clostridial myositis (malignant edema, or botulism), malignant hyperthermia-like syndrome, protozoal myopathy (*Sarcocystis* spp.), and Streptococcus-associated myopathy.

In this case, the top three differentials are equine recurrent exertional rhabdomyolysis (ERER), EPSSM, and nutritional myopathy. EPSSM is an inherited polysaccharide storage disease of quarter horses, warmbloods, and draft-related breeds. Histologically this disease can be diagnosed by the accumulation of periodic acid-Schiff (PAS)-positive and amylase resistant material in affected muscles. ERER is a group of myopathies which include EPSSM and other often unidentified causes of rhabdomyolysis. ERER is also known as tying up, azoturia, or Monday morning disease. There is some evidence to suggest that ERER in Thoroughbreds is due to abnormal calcium homeostasis within skeletal muscle. Vitamin E and selenium deficiency most commonly occurs in foals and young adult horses. In foals, the most severely affected muscles are those that have the highest workload (cervical muscles, proximal limb muscles, tongue, and masticatory muscles). In young adult horses, the most severely affected muscles are often the temporal and masseter muscles. Histologically, the lesions in the affected muscles are those of a multifocal, multiphasic segmental necrosis.[3]

Comparatively, wildlife, especially deer, can exhibit capture myopathy, which is identical to exertional rhabdomyolysis. Cattle, sheep, racing greyhounds, and sled dogs can exhibit exertional rhabdomyolysis. And pigs, dogs, and humans can have malignant hyperthermia, which is a hereditary molecular defect in the ryanodine receptor which is involved with calcium regulation in muscle.[3]

Contributor: Egyptian Society of Comparative and Clinical Pathology (ESCCP), Alexandria University, Department of Veterinary Pathology, Alexandria, Egypt.

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SLIDE 99
CONFERENCE 25 / CASE II – S 2/04 (AFIP 2942015)

Signalment: 15-years-old male, Lady Amherst's Pheasant (*Chrysolophus amherstiae*).

History: This animal was kept in a zoological garden and was found dead in its aviary.

Gross Pathology: The pheasant was emaciated. The cecal wall was multifocally thickened with numerous, up to 3 mm large, round, subserosal and intramural white nodules. In the lumen there were several, up to 1.5 cm long, nematodes.

Laboratory Results: Bacteriology revealed *Salmonella enteritidis* in all parenchyma and intestine.

Contributor's Morphologic Diagnosis: Cecum, submucosa: Typhlitis, granulomatous and fibroplastic with intralesional larval and adult ascarid nematodes, etiology consistent with *Heterakis* spp.

Contributor's Comment: Infections with *Heterakis* spp. occur worldwide and affect ducks, chickens, quails, grouse and especially pheasants. Besides *H. dispar*, *H. gallinarum* and *H. isolonche* are the species most commonly found in pheasants.[1] These nematodes are morphologically characterized by prominent cuticular alae, large lateral chords, polymyarian-coelomyarian musculature, and triradiate intestine with uninucleate columnar intestinal cells. The exact identification of the species can be made by measuring the length and estimating the shape of the spicules and oesophagus.[2] *H. isolonche* has a direct life cycle, lives within the cecal submucosa and induces nodular granulomas with considerable mesenchymal proliferation or even leiomyomas.[2,3] Defecated non-embryonated eggs gain their infectivity outside the host. After hatching in the small intestine, the larvae reach the cecal submucosa and develop into adult worms.

AFIP Diagnosis: Cecum: Typhlitis, nodular and granulomatous, multifocal, moderate, with marked mesenchymal infiltration and intralesional adult and larval nematodes, etiology consistent with *Heterakis* spp., Lady Amherst's pheasant (*Chrysolophus amherstiae*), avian.

Conference Comment: Conference attendees discussed the histogenesis of the cecal nodules. Some reports classify them as granulomas, while others describe them as fibrous hyperplastic tissue or even leiomyomas.[1] The spindle cells forming the nodules in this case are not producing collagen as identified with Masson's trichrome stain, nor are they immunohistochemically positive for smooth muscle actin. Therefore, these cells may be of histiocytic origin; however, further immunohistochemistry would be necessary to determine the definitive histogenesis.

Heterakis spp. are known to cause nodular typhilitis in a number of avian species, including chickens, turkeys, ducks, geese, grouse, guinea fowl, partridges, pheasants, and quail. However, the chief economic importance of *Heterakis gallinarum*, the cecal worm, lies in its role as a carrier of *Histomonas meleagridis*.[5]

Histomoniasis is a parasitic disorder of the ceca and liver of many gallinaceous birds. Grossly, the disease is characterized by well-demarcated necrotic foci surrounded by a raised hyperemic ring in the liver and necroulcerative lesions in the ceca that often lead to the development of cecal cores composed of necrotic debris. Microscopically, histomonads are pale, lightly stained, 15-20 _m, oval bodies within lacunae in the lamina propria and muscularis mucosa and are admixed with lymphocytes, macrophages, and heterophils. Histomonad invasion with necrosis may extend well into the muscular tunics, nearly to the serosa. With time, large numbers of giant cells form nodules that may be seen grossly as granulomas bulging from the serosal aspect of the cecum.[5]

The life cycle of *H. meleagridis* is complex with histomonads being found in the intestinal epithelial cells of *H. gallinarium*. *Histomonas meleagridis* infected *Heterakis gallinarium* eggs are passed in the feces of susceptible avian species. The eggs then embryonate and may either be swallowed by a susceptible host (direct transmission) or they may be ingested by the earthworm (indirect transmission). In the earthworm, eggs hatch and larvae may live for months. The earthworm is then eaten by a susceptible host, resulting in infection with both *Heterakis gallinarium* and *Histomonas meleagridis*.[5]

Contributor: Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Boddenblick 5A, 17493 Greifswald – Insel Riems, Germany
www.bfav.de

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SLIDE 100
CONFERENCE 25 / CASE III – NADC MVP-1 2004 (AFIP 2936146)

Signalment: Full term fetus, white-tailed deer (*Odocoileus virginianus*).

History: Twin full-term fawns were born to a 2.5 year-old white-tailed deer. One fawn was stillborn while the other appeared healthy.

Gross Pathology: The stillborn fawn had marked abdominal distention due to marked bilateral nephromegaly. Kidneys maintained their reniform shape but were enlarged (8.8 x 6.0 cm), pale, tan and smooth. The capsule was thin, tightly adherent and translucent, through which could be seen numerous fluid-filled cysts. On cut section, there were numerous 1-5 mm, round to fusiform cysts that contained clear fluid. The ureters and bladder were grossly normal. On cut surface of the liver, the intrahepatic bile ducts were variably ectatic with irregular outlines and intraluminal bile. Gross lesions were not seen in other organs.

Contributor's Morphologic Diagnosis: Kidney, glomeruli, tubules and collecting ducts: Ectasia and cysts, diffuse, severe, white-tailed deer (*Odocoileus virginianus*).

Contributor's Comment: Microscopically, there is severe dilatation of all renal tubules. The corticomedullary junction is obscured. Dilated tubules are lined by low cuboidal to flattened squamous epithelium. Most dilated tubules are empty, but some contained a flocculent eosinophilic material. The number of glomeruli is greatly reduced; those present are small and located within a dilated Bowman's capsule. The liver (not submitted) is characterized by marked biliary hyperplasia. Bile ducts are dilated to 1 to 5 mm in diameter, irregular in contour and contain intraluminal inspissated bile.

In humans, polycystic kidney disease (PKD) is characterized by progressive enlargement of the kidneys due to numerous expansile cysts and ultimately leads to

renal failure. In humans, PKD is heritable and recognized in at least 2 genetically distinguishable forms; autosomal recessive PKD (ARPKD) or autosomal dominant PKD (ADPKD). The autosomal dominant form is often associated with a variety of extrarenal manifestations and usually leads to death from renal failure in late adulthood. The autosomal recessive form is rare, often diagnosed in early infancy by massive nephromegaly, and is rapidly progressive.[1] Syndromes resembling both the recessive and dominant forms of human PKD have been recognized in animals including cats,[2,3] with Persian cats appearing disproportionately affected,[4] dogs,[5,6] mice,[7] pigs, raccoons[8] and ruminants such as cattle,[9] goats,[10,11] sheep[12] and Springbok (*Antidorcas marsupialis*).[13] PKD has not been reported previously in any member of the family Cervidae.

In domestic animals, PKD is most often consistent with the human ARPKD in that disease manifests as stillbirths or death within the first few weeks of life, although manifestations consistent with the ADPKD have also been described. Reported extrarenal manifestations of PKD in animals include biliary and pancreatic cysts.[3,5,6,10-12]

Many humans with ARPKD have been found to have mutations in the gene, polycystic kidney and hepatic disease 1 (PKHD1). This gene is predicted to code for a protein that is known as fibrocystin or polyductin.[14,15] The protein is expressed on adult and fetal kidney, liver and pancreas and may be a receptor protein that plays a role in collecting duct and bile duct differentiation. The basic defect in ARPKD may, therefore, be a failure of terminal differentiation in collecting and bile ducts.[15] PKHD1 gene products are members of a novel class of proteins that share structural features with hepatocyte growth factor receptor and plexins, members of a class of proteins involved in the regulation of cell proliferation, cellular adhesion and repulsion.[14,15] Genetic factors may be involved in congenital PKD of Cairn Terriers, springbok and Persian cats as the condition has been described in groups of related animals.

AFIP Diagnosis: Kidneys, glomeruli and tubules: Cystic change, diffuse, severe, white-tailed deer (*Odocoileus virginianus*), cervid.

Conference Comment: The contributor provides a thorough overview of polycystic kidney disease in humans and animals. As mentioned by the contributor, animals with polycystic kidney disease (PKD) often have biliary cysts and pancreatic cysts in addition to the renal changes. Extrarenal cysts are not found in conditions leading to congenital or acquired renal cysts.

Congenital renal cysts can occur in cases of renal dysplasia or can occur as a primary entity. There may be only one cyst or there may be many cysts that often distort the contour of the kidney. Some cysts may cause no alteration in renal function, and are therefore considered incidental findings. Cysts may arise anywhere along the nephron and can be located in either the cortex or the medulla and may range in size from barely visible to several centimeters in diameter.[16]

Acquired renal cysts can occur as a result of renal interstitial fibrosis, as in chronic renal disease, or they may occur in renal diseases that cause intratubular obstruction. These cysts are usually small, only 1-2 mm in diameter, and occur primarily in the renal cortex. In all cases with renal cysts, the cysts must be differentiated from hydronephrosis.[16]

Contributor: National Animal Disease Center, ARS, USDA/ARS, 2300 Dayton Avenue, Ames, IA 50010
http://www.nadc.ars.usda.gov/

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ELECTRON MICROGRAPH
CONFERENCE 25 / CASE IV – Case #2 (AFIP 2946684)

Signalment: Young female striped skunk (*Mephitis mephitis*) of unknown age with an approximate weight of 4 pounds (1.8 Kg).

History: The skunk had a history of seizures and accumulation of ocular/periocular crusty debris. The skunk was found in a populated area where dogs and cats resided, and was transferred to a wildlife rehabilitation center where it subsequently died. The carcass was refrigerated until a necropsy was performed. Selected tissues were collected and preserved in a 10% buffered neutral formaldehyde solution for histopathologic examination. Small fragments of lung and gastric mucosa were collected and preserved in a 3% buffered glutaraldehyde solution for examination by transmission electron microscopy.

Gross Pathology: The stomach and duodenum had numerous white nematode parasites that measured approximately 2.5 mm long. The uterus contained six fetuses each measuring approximately 1.5 cm in length.

Laboratory Results:
1. Canine Distemper Virus (CDV) and Rabies Virus fluorescent antibody tests – positive CDV antigen immunoreactivity in the lungs, tongue, and brain. Negative for Rabies Virus antigen immunoreactivity. Viruses were not isolated from the lungs, brain, or tongue.
2. Histopathology (Tissues not submitted for review) –
    a. Lung: Interstitial pneumonia with bronchial epithelial inclusion bodies.
    b. Brain: Meningoencephalitis, lymphocytic.
    c. Bronchial lymph node: Histiocytosis and follicular lymphoid hyperplasia
    d. Liver: Congestion and hepatocellular vacuolar degeneration with biliary epithelial inclusion bodies.
    e. Spleen: Lymphoid depletion
    f. Epithelium (multiple sites): Inclusion bodies

Contributor's Morphologic Diagnosis: Ciliated columnar epithelium (bronchus): Intracytoplasmic and intranuclear viral inclusions consistent with CDV.

Contributor's Comment: The transmission electron photomicrograph of bronchial epithelium contained ciliated columnar epithelial cells with intracytoplasmic and

intranuclear viral inclusion bodies consistent with CDV. Intracytoplasmic inclusion bodies were consistent with accumulated viral nucleocapsids and were characterized most frequently by amorphous aggregates of moderately electron dense granular material, and less frequently by amorphous aggregates of tubular-like structures. The nuclei of most cells were of decreased electron density due to dispersion of the chromatin pattern. One cell had intranuclear inclusions characterized by parallel, stacked arrays of electron dense, tubular to filamentous material. Other findings in the bronchial epithelial cells consisted of vacuolar epithelial cell degeneration characterized by mild dilatation of the smooth endoplasmic reticulum and the perinuclear cisterna, loss of apical microvilli, low numbers of secondary lysosomes, and cellular debris in the luminal surface. Examination of fine mitochondrial detail was not possible due to mild autolysis.

CDV is grouped in the family *Paramyxoviridae*, genus *Morbillivirus,* and is related to Measles Virus, Peste de Petits Ruminants Virus, and Rinderpest Virus.[1] It is an RNA virus that displays spherical to filamentous morphology ultrastructurally. It is reported that there is a single serotype of CDV; however, more than one genotype is known to exist. CDV isolates vary in biologic activity and tissue tropism.

CDV is transmitted primarily by contact with respiratory, ocular, or oral fluids.[1] CDV may also be transmitted less frequently by contact with infected shed skin cells, feces, and urine, and by the transplacental route. Transmission is likely enhanced by increased density and contact between susceptible animal populations, animal behaviors conducive to transmission, increasing dose of virus, and immunosuppression.[1] Other factors that influence susceptibility to CDV are age, species of host, virus strain, and environmental conditions. A strong antibody response to infection by the virus is reportedly protective, while weak antibody responses are associated with illness.

CDV may infect and cause clinical disease in a wide variety of carnivores including canids, felids, mustelids, procyonids and others.[1] Striped skunks are reportedly less susceptible to disease caused by CDV, but may suffer from the disease nonetheless. CDV-infected wild and domestic carnivores present a significant risk to zoological collections with susceptible species. The incubation period reportedly varies from one week to > 1 month, and clinical disease may last approximately 1-6 weeks. Infection may be fatal, particularly in highly susceptible species such as domestic ferrets (*Mustela putorius furo*). Clinical signs associated with CDV infection include depression, mucopurulent oculonasal discharge, fever, cough, anorexia, vomition, diarrhea, and central nervous system (CNS) dysfunction.[1-4] Clinical signs consistent with CNS dysfunction in CDV-infected animals include seizures, convulsions, paresis, paralysis, and other clinical signs. CDV-infected mustelids, such as striped skunks, may have symptoms similar to those described above.[1]

Macroscopic lesions include oculonasal discharge, diarrhea, hyperkeratosis (in prolonged infections primarily), poor body condition, and pulmonary changes consistent with pneumonia.[1] Other less frequently seen macroscopic findings may occur and in some cases lesions may not be evident. Commonly seen microscopic findings include

interstitial and/or bronchopneumonia, and lymphoid depletion in the spleen, lymph nodes, and thymus.[1] Formation of intracytoplasmic and intranuclear eosinophilic inclusions are a characteristic microscopic feature and can be seen in epithelial and neural cells of infected animals. In the present case, inclusions were seen in multiple epithelial tissues by light microscopy. CDV-induced primary lesions may also be seen in other tissues including tissues of the CNS, stomach, and intestines.[1-4] Concurrent opportunistic infections may occur in CDV-infected animals secondary to immunosuppression, and may obscure CDV-induced lesions.[1,5] This skunk had microscopic lesions consistent with CDV.

Laboratory tests, other than light microscopic examination, utilized to diagnose CDV-infection include transmission electron microscopy, fluorescent antibody testing (FAT)/immunohistochemistry, PCR assays, nucleic acid hybridization, virus isolation, determination of antibody titers, and cytologic examination of tissue samples or blood smears.[1,6-15] In the present case the ultrastructural characteristics of the viral particles and the FAT results were consistent with CDV.

The most important differential diagnosis in a suspected CDV-infected carnivore, particularly those with CNS dysfunction, is Rabies Virus.[1] In the present case Rabies was considered an important differential diagnosis and specific testing indicated that the skunk was not infected with Rabies Virus. Infection with other microbiologic agents or exposure to toxins are differential diagnoses that must also be considered.[1]

AFIP Diagnosis: Ciliated respiratory epithelium: Degeneration and necrosis, with intracytoplasmic and intranuclear viral inclusions, striped skunk (*Mephitis mephitis*), mustelid.

Conference Comment: The contributor provides a thorough overview of Canine Distemper Virus (CDV) including species affected, clinical signs, transmission, microscopic and ultrastructural findings, diagnostic tests, and etiologic differentials.

For most pathologists, describing ultrastructural changes and interpreting electron micrographs can be challenging. However, when examining electron micrographs one must only consider three cellular alterations: degeneration, necrosis, and something added or taken away. Then one must systematically evaluate and describe the cells present and their organelles in order to identify the cells/tissue and the process. When describing electron micrographs it is important to start with a brief description of the normal features which allow one to identify the cells and or tissue. Such features may include: number and arrangement of cells, plasma membrane, surface decorations, cellular junctions, cytosol, endoplasmic reticulum (smooth and rough), lysosomes, mitochondria, nuclei, and other intra- and extracellular features. Below are two helpful charts to assist in evaluating electron micrographs.[16,17]

Organelle	Normal features and changes to note on EM
Plasma membrane	Cilia, villi; loss of surface specialization; cytoplasmic blebs; types and locations of intercellular junctions
Cytosol	Rarefaction (swelling); presence of myelin figures; inclusion bodies
ER (smooth and rough) SER / RER	Relative amounts of SER and RER; swelling/dilation; detachment of ribosomes; increased amounts of SER
Mitochondria	Relative number and location; low amplitude / high amplitude swelling; matrix flocculent densities; calcification; vacuolization; rupture
Lysosome	Relative number; swelling; rupture
Nucleus	Clumped, dispersed, or marginalized chromatin; heterochromatin, euchromatin; pyknosis, karyorrhexis, karyolysis; viral inclusions
Other	Intranuclear or intracytoplasmic inclusions; bacteria; parasites; fungi; algae

Organelle	Reversible Changes	Irreversible Changes
Plasma membrane	Blebbing, blunting, distortion; loosening of intercellular attachments	Disruption of cellular membranes;
Mitochondria	Swelling, rarefaction, small amorphous densities	Marked dilation; large amorphous densities
ER	Dilation, detachment of ribosomes	
Nucleus	Chromatin clumping	Pyknosis, karyorrhexis, karyolysis
Other	Cellular swelling; creation of myelin figures	Increased cellular swelling; swelling and disruption of lysosomes; increased numbers of myelin figures

Contributor: Eli Lilly and Company, Lilly Research Laboratories, 2001 West Main Street, Greenfield, IN

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