The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2003-2004

SLIDE 1
CONFERENCE 1 / CASE I – 02RD1062 (AFIP 2888047)

Signalment: Ten-year-old neutered male Australian Shepherd dog (*Canis domesticus*).

History: One month prior to presentation, the owners noticed a reddened cornea in the right eye which later spread to the surrounding portions of the eye. Yellow discharge was also noted from the right eye, and one morning a few days before presentation the owners found the dog with both eyes "crusted shut". The owner eased the eyes open after the use of a warm compress. The owners also reported that the dog would rub at the right eye, and appeared to have no vision in that eye. At presentation, no menace response was invoked in the right eye. Intraocular pressure in the right eye was recorded as 15 mmHg. The clinician noted buphthalmia, conjunctivitis, and an iris neoplasm filling the anterior chamber of the right eye. Enucleation of the right eye was then elected by the owner.

Gross Pathology: Dyscoria, wrinkled cornea, raised white tissue adherent to iris and obscuring pupil.

Laboratory Results: The iris morphology is distorted and thickened due to the presence of a spindle cell neoplasm extending from the iris base a short distance into the ciliary body and obliterating most of the iris stroma. There is broad posterior synechiae. The retina and optic nerves suggest chronic glaucoma. On one side of the globe the tissue within the tumor, the ciliary body, and the choroid are pigmented. On the other side of the globe the tumor, the ciliary body, and the choroid are nonpigmented.

Contributor's Morphologic Diagnoses:
1. Partially amelanotic globe
2. Spindle cell iridal tumor of blue-eyed dogs
3. Secondary glaucoma

Contributor's Comment: This dog has a pleomorphic spindle cell tumor effacing much of the iris and extending into the ciliary body. The tumor varies considerably in morphology with areas that have elongate spindle cells and other areas with more myxomatous features. Karyomegalic cells and multinucleate cells are easily found. Neoplastic cells infiltrate well into the ciliary body. This is one of the more malignant appearing of these iridal spindle cell tumors that we have evaluated.

Careful evaluation of the pigmentation of this eye reveals that there is fairly abundant melanin pigment in the portion of the iris with the least tumor invasion, and, likewise, there is a normally pigmented choroid on the same side as the pigmented iris. The tumor itself has almost no areas of trapped melanin pigment and the choroid on the side of the eye with more tumor is devoid of melanin. This tumor almost always occurs in blue or partly blue eyes. For that reason it is most commonly found in Husky dogs and other breeds which have heterochromia, such as the Australian Shepherd [1,2].

Melanomas and melanocytomas will occasionally present as spindle cell tumors. Other spindle cell tumors of the iris are extremely rare. These tumors stain positive for S100 about 50% of the time. They all stain positive for vimentin, and about 50% stain positive for GFAP. Occasional tumors have morphologic features which suggest a peripheral nerve origin such as Antoni A and Antoni B patterns, and one of two cases in which electron microscopy was performed had broadly deposited basal lamina at the cell membrane. The inconsistent S100 staining is difficult to interpret if this is, indeed, a tumor of peripheral nerve origin.

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AFIP Diagnosis: Eye, iris: Spindle cell neoplasm, favor peripheral nerve sheath origin, Australian Shepherd, canine.

Conference Comment: Spindle cell tumor in blue-eyed dogs is a recently described neoplasm that occurs in dogs with poorly pigmented uveal tissue. The cell of origin has not been determined, but based on immunohistochemical stains and cellular morphology, peripheral nerve sheath origin is suggested. Metastatic spread has not been described.[3,4]

This tumor is vimentin positive, GFAP positive, and S-100 negative. Conference attendees favored melanoma but did not have the benefit of the immunohistochemistry stains prior to the conference. The GFAP positivity eliminates melanoma as a possibility and supports the diagnosis of spindle cell tumor in blue-eyed dogs, as described by the contributor. Also, the nuclear regimentation and occasional herringbone pattern support a tumor of peripheral nerve sheath origin.

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SLIDE 2
CONFERENCE 1 / CASE II - Seal-1 (AFIP 2888451)

Signalment: Adult male Harbor seal, *Phoca vitulina*.

History: A male 18.9 kg Harbor seal was found stranded at Barnegat Lighthouse State Park in New Jersey. At time of recovery the animal had labored breathing and what appeared to be blood on the abdomen. The animal was treated at the local marine mammal stranding center with 500 cc electrolyte solution, vitamins, levamisole, and Baytril. The animal was inactive and shivering. It died approximately 7 hours later and was submitted for necropsy.

Gross Pathology: At necropsy, the urinary bladder was severely distended with a translucent wall. It contained red, watery fluid with a strong odor of ammonia. The mucosa was red with a cobblestone appearance. The ureters were distended measuring 4 mm in diameter bilaterally, but both kidneys were grossly normal. The periurethral soft tissue was swollen and multinodular and contained a central 1 cm cavitation with a firm, green-black wall. It oozed a yellow opaque fluid when cut. Numerous crystals were present around the preputial opening but the urethra remained unobstructed.

    A large nematode was found adjacent to the rectum in the retroperitoneal space. Other necropsy findings include numerous lice on the dorsal skin and gingival ulcers. On the surface of the lung, dilated lymphatic vessels are seen.

Laboratory Results: Aerobic bacterial culture of the fluid in the cavitation was negative.

Contributor's Morphologic Diagnoses:
1. Severe multifocal subacute to chronic granulomatous cellulitis with numerous intralesional nematode ova, periurethral soft tissue, Harbor seal.
2. Severe diffuse necrotizing cystitis with vascular fibrinoid necrosis and superficial bacterial colonization, urinary bladder, Harbor seal.

Contributor's Comment: The recovered nematode was female, brownish-red, and measured 22.3 cm long by 4.5 mm wide. The worm was alive when recovered

(approximately 1 day after the death of the seal). The anus was terminal and the mouth was surrounded by 2 circles of 6 papillae. This led to its identification as *Dioctophyma renale*. Eggs seen in histological sections had a characteristic thick mamillated shell and were consistent with this identification. Ten eggs recovered from formalin were measured with an ocular micrometer, with a mean size of 41.3 ± 2.3 um wide by 68.9 ± 3.7 um long. Parasite identification was made by Dr. Thomas J. Nolan from the laboratory of Parasitology, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania.

Dioctophyma renale, the "giant kidney worm", is one of the largest species of nematodes. The usual definitive host for *D. renale* is the mink. The adult worms are normally found in the right kidney. This is thought to occur when the larvae migrate through the stomach wall (or duodenum) and liver. They are blood-red, and females can measure up to one meter long and one centimeter in diameter. Males are somewhat smaller (less than 40 cm). Eggs are brownish and thick-walled, measuring 68 by 44 um. The eggs are passed out in the urine in the one- or two-cell stage and mature, in water, to the first larval stage in approximately 35 days. They are then infectious to *Lumbriculus variegates*, a fresh-water oligochaete annelid worm, and develop into the infective third larval stage. Fish or frogs that ingest the infected oligochaete may act as paratenic hosts. Life cycle of *D. renale* is completed when the infected oligochaete or paratenic host is ingested by a final host[1].

The giant kidney worms cause a widely distributed parasitic infection in mustelids, canids (mostly fish-eating carnivores), swine, and occasionally man. In human beings, *D. renale* infection has been reported to manifest as a hemorrhagic cyst at one pole of the right kidney, mimicking a retroperitoneal neoplasm[2]. Liesegang rings (LRs) are periodic precipitation zones from supersaturated solutions in colloidal systems. In histologic sections, LRs are eosinophilic, acellular, laminated, sharply outlined and sometimes have a central nidus of pyknotic debris. They occur in the kidney, synovium, conjunctiva and eyelid of men and can be sometimes mistaken for eggs, larvae or adults of *D. renale*[3].

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AFIP Diagnoses:
1. Urinary bladder: Cystitis, necrotizing, fibrinosuppurative, diffuse, severe, with fibrinoid necrosis, and mixed population of bacteria, harbor seal (*Phoca vitulina*), pinniped.
2. Fibromuscular tissue: Cellulitis, pyogranulomatous, diffuse, moderate, with numerous mamillated nematode eggs.

Conference Comment: According to the literature, this is the first case where *Dioctophyma renale* has been definitively identified in a Harbor seal. *D. renale* was previously described in a Caspian seal (*Phoca caspica*).[4] The case presented in the conference is especially interesting because the life cycle of *D. renale* is associated with fresh water fish as paratenic hosts. Although the exact history of this particular free-living seal is unknown, access to fresh water fish would have been necessary for this

animal to become infected, unless the infective third stage larvae may now use salt or brackish water fish as paratenic hosts.

D. renale has a wide host range, including mink, weasels, river otters, coyote, wolves, fox, domestic dogs, raccoons, and occasionally cattle, horses, and swine.[4]

Conference attendees had difficulty in identifying the two tissues. Identification of urinary bladder was made based on the appearance of the outer musculature in a thin-walled tubular organ. Although the exact morphology of the inflammatory cells was difficult to discern, it is likely they are neutrophils based on the presence of abundant fibrin and, therefore, an acute process. Within the section of fibromuscular tissue, the presence of more loosely arranged, immature granulation tissue towards the central cavitary lesion and progression to more mature granulation tissue at the periphery helped to elucidate the pathogenesis of this lesion.

This case was reviewed in consultation with Dr. C. H. Gardiner, Parasitologist.

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SLIDE 3
CONFERENCE 1 / CASE III – HN 1825 (AFIP 2892546)

Signalment: 10-year-old, male Miniature Schnauzer, canine.

History: This dog had neurological signs including torticollis, circling, mental confusion, convulsive seizures, and unconsciousness for about a month. The dog showed severe hypoglycemia and infusions of glucose were performed. The dog died 3 days after the onset of treatment.

Gross Pathology: Autopsy was performed seven hours post-mortem and a mass was found in the left lobe of pancreas. The mass was white and 2_1_1 cm in size. The surface of the mass was slightly granular and showed lobular patterns on cut surface. Small whitish nodules were also observed in liver and heart. The cortex of the cerebrum was mildly clouded on cut surface.

Laboratory Results: Serological exam revealed that this dog showed persistent hypoglycemia of 15-42 mg/dl. In contrast, insulin concentrations were at conspicuously high levels at 100 mIU/l (reference 10-25 mIU/l).

Contributor's Morphologic Diagnoses:
1. Pancreas: Islet cell carcinoma, Miniature Schnauzer, canine
2. Cerebrum: Cortical necrosis, severe, Miniature Schnauzer, canine

Contributor's Comment: The neoplastic cells observed in the pancreas showed invasive growth into the pancreatic parenchyma and through the fibrous capsule. The closely packed neoplastic cells were sometimes subdivided into small lobules by fine connective tissue. The individual neoplastic cells were well-differentiated and mitotic figures were seen infrequently. Histologically, the whitish nodules grossly observed in the liver and heart were metastases of the pancreatic neoplastic cells.

A number of intrinsic or acquired disorders in animals produce transitory or prolonged hypoglycemia[1], for example, hypoadrenocorticism (Addison's disease), severe hepatic or renal disease, and constitutional hypoglycemia of pups. Pancreatic beta-cell tumors are also one of the important causes of canine hypoglycemia. To show that primary and metastatic tumor cells are beta-cell origin, Gomori method, immunohistochemical reactions for insulin and ultrastructural evidence of secretory granules are useful. By immunohistochemical examination performed in our laboratory, the tumor cells stained positively for anti-insulin antibody. So, the neoplastic cells originated from insulin secreting beta-cells. The clinical evidence of: 1) severe neurological signs, 2) decline of blood glucose concentrations, and 3) reaction to administration of glucose also suggests that the tumor cells are functional[2]. Later in the disease, however, animals may become unresponsive to supplemental glucose therapy.

Neurological signs in this dog were induced by prolonged hypoglycemia, and hyperinsulinism. In the cerebrum, ischemic nerve cells with eosinophilic shrunken cytoplasm and pyknotic or lytic nucleus were found in the superficial layer of the cortex. Microglial cells with elongated nuclei (rod cells), enlarged astrocytic nuclei with dark nuclear membrane, and enlarged nuclei of vascular endothelium were also observed. Ischemic changes associated with hypoglycemia of dogs is previously reported[3], and the pathological findings in those dogs were consistent with the present case. Therefore, we diagnosed this dog with an insulin-secreting islet cell carcinoma accompanied by secondary cortical necrosis of the cerebrum.

The mechanism of neuronal damage induced by hypoglycemia is not fully understood. The distribution of hypoglycemic brain damage was discussed in insulin-induced hypoglycemic model of rats[4], and the existence of neurotoxic substance (excitotoxin) has been suspected[5]. Excitotoxic neuronal death with DNA fragmentation and activation of immediate early gene expression is thought to contribute to neuropathogenic conditions in hypoglycemia[6].

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AFIP Diagnoses:
1. Pancreas: Islet cell carcinoma, Miniature Schnauzer, canine.
2. Cerebral cortex: Neuronal necrosis, superficial, laminar, multifocal.

Conference Comment: Neuronal necrosis has been described in hypoglycemia associated with the biochemical disturbances related to other conditions, specifically pregnancy toxemia in ewes[7], and is similar to the neuronal necrosis described in a dog with an insulinoma[8] and in the case presented here. The pattern of neuronal necrosis related to hypoglycemia is confined to the superficial laminae of the cerebral cortex. Ischemia also causes similar lesions of neuronal necrosis but, in contrast, it affects the middle laminar cortex.[9]

The mechanisms of cell death in hypoglycemia and ischemia have many parallels. Both ischemic and hypoglycemic cell death are caused by the release of excitotoxins that bind to neuronal surface receptors and cause necrosis. One difference is that the predominant excitotoxin responsible for hypoglycemia-induced necrosis is aspartate and for ischemic-induced necrosis is glutamate. Another basic difference between these two causes of necrosis is that ischemia results in a pan-necrosis (necrosis of neurons, glial cells, and vessels) due to lack of blood supply, whereas hypoglycemia only causes selective death of neurons because blood supply is not compromised.[9, 10]

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SLIDE 4
CONFERENCE 1 / CASE IV - 4188 (AFIP 2852792)

Signalment: Juvenile female varying hare (*Lepus timidus*).

History: The hare was found dead.

Gross Pathology: Normal body condition. A few subcutaneous hemorrhages. Slightly enlarged spleen. Multifocal tiny grayish-white foci on the liver. Numerous intestinal *Cittotaenia sp*.

Laboratory Results: *Francisella tularensis* was isolated from the bone marrow.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal.

Contributor's Comment: This represents a typical case of tularemia in hares. The disease in this species is an acute fatal septicemia. Gross findings include moderately enlarged spleen and numerous pinpoint, pale foci in the liver, spleen, and bone marrow. Often there are also subcutaneous hemorrhages. Histologically, acute multifocal complete necrosis is seen in the liver, spleen, and bone marrow. Infrequently, a hemorrhagic necrotic enterocolitis has been detected.

   Francisella tularensis is a gram-negative, pleomorphic, nonmotile coccobacillus. It is classified into three biovars: *F. tularensis tularensis* (type A), *F. tularensis palearctica* (type B), and *F. tularensis mediaasiatica*. Biovar *palearctica* is divided into three biotypes: *I, II*, and *japonica*. Biovar *tularensis* is the most pathogenic and occurs in North America. It has recently been isolated also from Central Europe. Other biovars are less virulent. Biovar *palearctica* (holoarctica) occurs in Eurasia and, to a lesser extent, in North America. Biovar *mediaasiatica* occurs in Central Asia and biovar *japonica* in Japan. The other species, *F. philomiragia* and *F. novicida*, are of unknown pathogenicity.

Tularemia occurs all over the Northern hemisphere, in Scandinavia, Central Europe, Russia, Japan, China, the USA, and Canada. The natural hosts of the disease are wild rodents and lagomorphs. Ticks are considered to be important biological vectors of the disease and can also transmit the bacteria vertically to their ova. Other arthropods such as mosquitoes, flies, fleas, and lice can transmit the disease. The bacteria are quite resistant and can survive in cold environments, in water, soil, and cadavers for several months.

Over 250 animal species can be infected, including man. The severity of the disease varies from fatal septicemia to asymptomatic infection. Rodents and lagomorphs are the most susceptible and carnivores are usually resistant. Among domestic animals, severe infections have been described in sheep, foals, fur animals, and cats.

The disease in man can manifest in several different forms: ulceroglandular (the most common form), glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal. Also meningitis has been described. Infection is acquired through skin wounds or mucous membranes when handling sick animals, from dust or soil contaminated with rodent feces, from blood sucking insects or tick bites, or from contaminated drinking water or food.

In Finland epizootics of *F. tularensis* occur among wild hares (varying hares (*Lepus timidus*) and European brown hares (*Lepus europaeus*)). Only very few cases have been seen in muskrats (*Ondatra zibethicus*) or beavers (*Castor canadensis, Castor fiber*). The annual number of cases in humans as well as animals varies. The disease in man is most common in the late summer and autumn. The annual incidence in humans is 1-2/100,000 population and most of the cases are mild. Most human outbreaks have been associated with mosquito bites (ulceroglandular form) or with hay or soil dust (pneumonic form).

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AFIP Diagnosis: Liver: Necrosis, random, multifocal, varying hare (*Lepus timidus*), lagomorph.

Conference Comment: The contributor gives a thorough overview of this highly infectious, zoonotic disease. The organism enters via percutaneous inoculation by arthropods, penetration of skin or mucous membranes, ingestion, or inhalation. Organisms are phagocytized by macrophages and disseminate via the lymphatics, and can invade and damage vascular endothelium causing vasculitis and thrombosis. The bacteria cause inflammation and multifocal necrosis in the liver, spleen, kidneys, lungs, and lymph nodes.[4]

Along with *F. tularensis*, the differential diagnosis for hepatic necrosis includes *Clostridium piliforme* (Tyzzer's disease), *Salmonella* sp., *Yersinia* sp., *Toxoplasma gondii*, and *Listeria monocytogenes*. The following may be used to differentiate these from tularemia: *C. piliforme* is present within the cytoplasm of hepatocytes and will stain

positively with silver stains; Salmonellosis causes paratyphoid nodules in the liver, characterized by individualized necrotic hepatocytes surrounded by inflammatory cells; *Yersinia* sp. produce large, lobulated colonies of bacteria in the liver; intralesional tachyzoites are observed with toxoplasmosis; and *Listeria monocytogenes* is a gram positive coccobacillus.

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SLIDE 5
CONFERENCE 2 / CASE I – S55/00 (AFIP 2888672)

Signalment: 14-week-old, female, ZIKA-hybrid rabbit, *Oryctolagus cuniculus*.

History: This animal was inoculated experimentally with 10^2 hemagglutination units/ml of an infectious agent. 4 days p.i. the rabbit developed progressive jaundice, became anorectic and was euthanized on day 10 p.i. At this time a large, continuously bleeding hematoma had appeared on the ear after blood sampling the previous day.

Gross Pathology: At necropsy, the moderately emaciated rabbit was severely icteric. All lobes of the diffusely brownish-yellow liver showed multifocal to confluent, sharply demarcated, irregularly shaped, reticulated, greyish to red, gritty areas (Fig. 1). The kidneys were enlarged and bright yellow. The thymus was atrophic, the spleen was moderately enlarged, and the lungs showed diffuse, severe, alveolar emphysema.

Laboratory Results: RT-PCR and in situ hybridization revealed RHDV-positive-strand RNA in the liver, located mainly in macrophages of periportal areas and, rarely, in periportal hepatocytes. Macrophages and reticulocytes in the sinuses and red pulp of the spleen were also positive for RHDV-RNA (Fig. 2).

Contributor's Morphologic Diagnosis: Liver: Hepatitis, necrotizing and histiocytic, subacute, centrilobular to bridging, severe, with calcification and biliary hyperplasia. Etiology consistent with Rabbit hemorrhagic disease virus (RHDV).

Contributor's Comment: Periportal and midzonal hepatocytes are variably increased in size, some of them binucleate with basophilic cytoplasm, large, chromatin-poor nuclei and two, or sometimes more, prominent nucleoli (regeneration). Most midzonal hepatocytes contain large cytoplasmic lipid vacuoles, some of them bile droplets. Centrilobular hepatocytes are hypereosinophilic, pyknotic, or karyorrhectic (degeneration). Many centrilobular areas show dystrophic, granular calcification and are surrounded by large numbers of histiocytes and lymphocytes. Bridging of necrotic areas obliterates the sinusoidal and lobular architecture of the liver. Portal tracts show marked hyperplasia of bile ducts accompanied by moderate to severe lymphocytic and histiocytic infiltration and mild fibrosis[1].

This animal was inoculated with liver homogenate, obtained from a rabbit that had died from "Rabbit hemorrhagic disease" caused by RHDV strain "Eisenhüttenstadt"[2]. However, the clinically subacute course as well as the gross and histopathologic presentation of this case is rather uncommon for RHD. RHDV, a positive-stranded RNA virus, has been recently classified as type species within the genus *Lagovirus*, family *Caliciviridae*. Experimentally infected and susceptible domestic and wild rabbits *(Oryctolagus cuniculus)* usually exhibit hepatic necrosis and pulmonary hemorrhages 30 h p.i. with subsequent involvement of lymphatic tissues and kidneys. In adult rabbits the disease is peracute (usually not more than 3 days) with a high morbidity (100%) and mortality (80-90%), whereas rabbits 45 days and younger are susceptible to RHDV infection but do not develop clinical signs. Submassive necrosis of the liver is thought to be central to the pathogenesis leading to a primary or secondary depletion of coagulation factors and endothelial lesions causing the hemorrhagic syndrome after which the disease was named.
The initial necrosis of the liver is caused by viral replication within hepatocytes. The current hypothesis is that alteration of these cells is responsible for the activation of clotting factors. Hepatic diseases leading to severe tissue destruction stimulate fibrinogen synthesis and release massive amounts of tissue thromboplastins. The defective clearance of activated clotting factors by the liver, combined with decreased levels of coagulation inhibitors in the plasma trigger disseminated intravascular coagulation (DIC), which in turn further promotes hepatic necrosis. Fatty degeneration of hepatocytes and centrilobular bridging necrosis are interpreted as final stages of the temporary severe hypoxia to the liver, coinciding with the DIC during the acute phase of the disease[3]. Monocytes and macrophages are considered to represent further cellular targets and their infection may also be relevant to the development of DIC[4]. Using *in situ* hybridization to detect RHDV-RNA in formalin-fixed, paraffin-embedded tissues of infected rabbits, it was shown that 10 days p.i. hybridization signals in hepatocytes become sparse, and predominantly macrophages show strong signals in the liver (Fig. 2).

The protracted nature of the infection in this case possibly may have been caused by factors such as immaturity at time of inoculation, innate immunity, or breed, alone or in combination. Bile duct proliferation, hepatocellular necrosis and regeneration together with a moderate inflammatory reaction can be summarized as early stages of cirrhosis

of the liver. These chronic changes are uncommon findings with RHD, and even in endemic areas, end-stage livers are not a frequent condition in rabbits.

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AFIP Diagnoses:
1. Liver: Hepatitis, necrotizing, acute, centrilobular, moderate, with hepatocellular regeneration, ZIKA-hybrid rabbit (*Oryctolagus cuniculus*), lagomorph.
2. Liver: Fibrosis, portal and bridging, multifocal, with lymphoplasmacytic and histiocytic cholangiohepatitis, and biliary hyperplasia.

Conference Comment: The contributor provides an excellent overview of RHDV and its mechanisms of hepatic necrosis. Cytokine release by activated macrophages and monocytes is thought to be a key component in the pathogenesis of many of the hemorrhagic diseases, including RHDV. The release of tumor necrosis factor (TNF) and interleukin-1 (IL-1) by activated macrophages induces the expression of procoagulant proteins on the endothelial surface, leading to DIC. [4]

Conference attendees considered toxic hepatopathy as the primary differential diagnosis due to the centrilobular pattern, paucity of inflammation, and lack of hemorrhage. Portal fibrosis and inflammation are not typical features of RHDV. It is unclear whether they are associated with the prolonged course in this case or are unrelated to the virus. Calcification, as described by the contributor, was not present on all slides.

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SLIDE 6
CONFERENCE 2 / CASE II - 40752 (AFIP 2890228)

Signalment: 2 month-old, female, North American wood duck, *Aix sponsa*, avian.

History: This North American wood duck was hatched at the San Diego Zoo on 5 May 1999 and was housed in an outdoor exhibit with multiple species of birds. It was seen alive early on the morning of 28 July 1999, but was found dead later that morning.

Gross Pathology: The animal was in good body condition. There were numerous pinpoint tan foci throughout the liver. The spleen was speckled with dark red foci. At the esophageal-proventricular junction there was a 1mm wide, circumferential band of green discoloration of the mucosa. A similar wider circumferential band (3 to 4 mm) was present in a section of the distal small intestine. At this site the mucosal surface was covered with green-tan material and was rimmed by red margins.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Esophagus: Moderate diffuse epithelial hyperplasia and hypertrophy with multifocal acute erosive and ulcerative esophagitis and intranuclear and intracytoplasmic eosinophilic inclusion bodies.
2. Esophagus: Intraepithelial nematodes (not present in all sections).

Contributor's Comment: Histologic examination of the esophageal-proventricular junction revealed diffuse hyperplasia of the esophageal stratified squamous mucosal epithelium. The epithelial cells showed a mild degree of disorderly maturation toward the luminal surface. Nearly all cells were markedly swollen with partial clearing of the cytoplasm and prominent intercellular desmosomal junctions. Nuclei had peripheralized chromatin and contained a 5 to 7 um diameter round to oval eosinophilic inclusion body within the center. Similar smaller (2 to 3 um diameter) eosinophilic inclusions were also present within the cytoplasm of the squamous epithelial cells.

In most sections, at the esophageal-proventricular junction there was an abrupt transition between the hyperplastic epithelium and a large esophageal ulcer, which extended through the lamina propria and into the underlying muscular tissue. The ulcer bed was lined by necrotic cellular debris and fibrin with superficial colonies of bacteria. There were prominent aggregates of inflammatory cells along the margins of the ulcer, composed predominantly of heterophils and a few macrophages. The majority of the heterophils were necrotic. A mild infiltrate of heterophils with few macrophages and lymphocytes was present within the lamina propria of the proventriculus. Multifocal mild erosions were seen elsewhere in the esophageal epithelium in many sections. Varying degrees of necrosis and inflammation with intranuclear inclusions bodies were present in liver, intestines, cloaca, spleen, and thymus in this case.

As an incidental finding, there were cross-sections of adult nematodes within the esophageal mucosa. These nematodes were approximately 100 to 130 um in diameter with a 1um thick smooth cuticle, prominent platymyarian musculature, a digestive tract with uninucleate cells and a brush border, and a single oval reproductive tract. These most likely represent *Capillaria* sp. However, none of the examined sections contain hypodermal bands or esophageal gland cells (stichocytes). Nematodes were not present in all submitted slides.

The above findings of epithelial hyperplasia and ulcerative esophagitis with intranuclear and intracytoplasmic inclusion bodies are diagnostic for duck viral enteritis, an acute contagious disease of Anseriforme birds (ducks, swans, geese) caused by the alpha-herpesvirus Anatidae herpesvirus 1.[4] It affects both wild and domestic waterfowl, however it most commonly causes large outbreaks in duck or domestic waterfowl raising facilities. Transmission is via the oral route through direct contact with infected birds or contaminated soil or water.[1] At domestic facilities it is most often transferred by wild waterfowl flying over the farms.[4] Vertical transmission from a carrier bird to its young has also been reported.[2] There is a variable susceptibility between Anseriformes. Pintails (*Anas acuta*) and the European teal (*Nettion crecca*) are resistant to disease despite infection. Mallards (*Anas platyrinchos*) are infected and in some cases can be inapparent shedders. Muscovy ducks (*Cairina moschata domesticus*) seem to be the most highly susceptible.[3,4] Recovered animals may shed virus orally and through excretions for up to 4-5 years.[1,4] To date the natural reservoir has not been identified.[3]

Clinical signs develop within 3 to 14 days (typically 3 to 7) and may include diarrhea, dehydration, extreme thirst, weakness, bloodstained vent, cyanotic bill, photophobia, drooping plumage and in males, phallic prolapse.[1,4] When moved, animals may also demonstrate head, neck and whole body tremors.[2,4] In most cases, however, there are typically no clinical signs and dead birds are found floating on the surface of the water.

Common gross findings include pinpoint areas of necrosis throughout the liver, petechiae in the liver, pancreas and along the intestinal serosa, and a hemorrhagic enteritis. The enteritis is characterized by a dark red mucous membrane and band shaped areas of ulceration covered by a thick tan to yellow diphtheritic cast. The esophagus and cloaca may also show similar ulceration.[1-3] In our experience, intestinal lesions are uncommon; lesions are most often seen in the esophagus and cloaca.

The reported histologic findings are similar to those described above. The intranuclear inclusions are a consistent finding; intracytoplasmic inclusions have been described[5] and are not unusual in our experience. The intranuclear inclusions may also be found in hepatocytes and pancreatic acinar cells surrounding areas of necrosis, as well as renal tubular epithelial, interstitial cells of the kidney and bursa of Fabricius, and in mononuclear cells of the spleen.[1,4] Lymphoid organs may show reactive follicles, lymphoid depletion and necrosis.[4] Secondary opportunistic fungal and bacterial infections are also a common finding.[1]

On ultrastructural examination, viral particles are 105 to 115 nm in diameter, hexagonal and have an electron dense core. They are found both in cytoplasmic vesicles and budding through the nuclear envelope. When present, intracytoplasmic inclusion bodies may form clusters. Enveloped virions (200 to 250 nm in diameter) are occasionally seen free in the cytoplasm.

Prevention of disease in domestic ducks can be accomplished through vaccination.[1-4]

AFIP Diagnoses:
1. Esophageal-proventricular junction: Esophagitis, ulcerative, acute, focal, moderate, with diffuse lymphoid necrosis, epithelial hyperplasia, and eosinophilic intranuclear and intracytoplasmic inclusion bodies, North American wood duck (*Aix sponsa*), avian.
2. Esophagus: Intraepithelial nematodes with diffuse epithelial hyperplasia.

Conference Comment: The contributor provides a concise review of duck viral enteritis. This case was reviewed in consultation with Dr. C. H. Gardiner, Parasitologist. The intraepithelial *Capillaria* sp. are not present in all slides. Cross- sections of the parasite demonstrate coelomyarian polymyarian musculature, hypodermal bands and, occasionally, stichosomes, which are features of aphasmid nematodes.

Herpesviruses are 150nm diameter, enveloped, double-stranded DNA viruses with icosahedral nucleocapsids. Intranuclear and occasionally, intracytoplasmic inclusions are present in cases of Anatidae herpesvirus 1. Intracytoplasmic inclusion bodies are not typically characteristic of herpesviruses, except in cytomegalovirus and gallid herpesvirus 2 (Marek's disease).[5] Herpesvirus replicates in the nucleus where virion DNA becomes encapsidated, and these nucleocapsids then become enveloped by budding through the inner layer of the nuclear envelope[6]. Intracytoplasmic inclusions in cases of duck viral enteritis have been shown to contain enveloped herpesviruses.[4,5]

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SLIDE 7
CONFERENCE 2 / CASE III – 03N059 (AFIP 2890941)

Signalment: Three year-old female spayed Border collie, *Canis familiaris*.

History: Animal has two-month history of dermal and subcutaneous nodules and draining tracts. Lesions initially observed on the left front foot, but now involve all four distal extremities.

Gross Pathology: Physical examination revealed pyrexia, generalized lymphadenopathy, and pitting edema of all four limbs. There were multiple ulcerated cutaneous lesions, nodules, and draining tracts involving all four distal extremities. The draining exudate was serohemorrhagic to purulent. Some ulcerative lesions were covered by an eschar. A single 5x5 cm nodule was present in the left caudal mammary gland. The prescapular and popliteal lymph nodes were enlarged approximately 5x normal.

Laboratory Results: Hematologic and serum biochemical analyses revealed an inflammatory leukogram, hyperglobulinemia, and mild hypercalcemia. Cytologic examination of a fine needle aspirate of the left popliteal lymph node revealed pyogranulomatous inflammation with numerous broad, infrequently septate hyphae. Culture of a fine needle aspirate of the left popliteal lymph node yielded *Lagenidium* sp. Identification of the cultured isolate was made on the basis of morphology as well as PCR amplification.

Contributor's Morphologic Diagnoses:
1. Skin from rear leg: dermatitis, necrotizing and granulomatous, chronic, diffuse, severe with presence of broad, irregularly branching hyphae and multinucleated giant cells.
2. Popliteal lymph node: lymphadenitis, necrotizing and granulomatous, chronic, multifocal to diffuse, severe with presence of broad, irregularly branching hyphae and multinucleated giant cells.

Please note: Some sections do not show necrotic components in skin and lymph node. They show granulomatous inflammation with presence of hyphae and giant cells.

Contributor's Comment: Oomycetes are soil- or water-dwelling organisms that belong to the Kingdom Stramenopila (Chromista). Although they are not true fungi, many grow like fungi on mycological media and produce vegetative hyphae that are morphologically similar to those of fungi in the class Zygomycetes. *Pythium insidiosum*, an aquatic organism best known as a cause of cutaneous lesions in horses and of gastrointestinal or cutaneous disease in dogs, has long been considered to be the only mammalian pathogen in the class Oomycetes.[1] However in 1999, a second pathogenic oomycete was isolated from tissue taken from a dog with severe multifocal cutaneous lesions and regional lymphadenopathy.[2] The dog died acutely following rupture of a caudal vena caval aneurysm, and necropsy revealed severe sublumbar lymphadenitis and pyogranulomatous vasculitis. Sequencing of a portion of the ribosomal RNA gene of the isolate recovered from this dog identified it as member of the genus *Lagenidium*. Presently, more than 30 dogs with serologic, histologic, and/or culture evidence of *Lagenidium* sp. infection have been identified.

The clinical and epidemiologic features of lagenidiosis that have thus far been identified are similar in many respects to those associated with cutaneous pythiosis.[3] Affected animals are typically young to middle-aged dogs living in the southeastern U.S. Although most dogs have been from Florida or Louisiana, we have also identified cases in Texas, Tennessee, Virginia, and Indiana. A number of infected dogs have had frequent exposure to lakes or ponds. Infected dogs are typically presented for evaluation of progressive cutaneous or subcutaneous lesions (often multifocal) involving the extremities, mammary region, vulva, or trunk. Grossly, these lesions appear as firm dermal or subcutaneous nodules, or as ulcerated, thickened, edematous areas with regions of necrosis and numerous draining tracts. Regional lymphadenopathy is often noted, and may occur in the absence of cutaneous lesions. Animals with great vessel or sublumbar lymph node involvement often develop hindlimb edema. Similar to the clinical course associated with cutaneous pythiosis, skin lesions in dogs with lagenidiosis tend to be progressive, locally invasive, and poorly responsive to therapy. In contrast to pythiosis, however, the majority of dogs with lagenidiosis have been found to have lesions in distant sites, including great vessels, sublumbar and inguinal lymph nodes, lung, pulmonary hilus, and cranial mediastinum. *Lagenidium* sp. infection has not been identified in mammals other than dogs.

The histologic features of lagenidiosis are similar to those associated with pythiosis and zygomycosis, and are characterized by pyogranulomatous and eosinophilic inflammation associated with broad, irregularly branching, sparsely septate hyphae.[1] In contrast to *P. insidiosum*, *Lagenidium* sp. hyphae are usually visible on H&E-stained sections. On GMS-stained sections, numerous broad, thick-walled, irregularly septate hyphae are easily recognized. *Lagenidium* hyphae typically demonstrate a great deal of variability in size (even within the same tissue section), but in general are much larger than *P. insidiosum* hyphae, ranging from 7 to 25 μ in diameter, with an average of 12 μ. Immunoblot serology for the detection of anti-*Lagenidium* antibodies in canine serum can provide a presumptive diagnosis of lagenidiosis,[3] but must be interpreted in conjunction with results of serologic testing for *P. insidiosum* infection[4] because of the potential for cross reactivity. A definitive diagnosis of *Lagenidium* sp. infection is best

made by culture followed by identification of the pathogen via either ribosomal RNA gene sequencing[3] or genus-specific PCR.[5] This same PCR assay can also be used for the detection of *Lagenidium* DNA in infected tissue samples.[6]

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AFIP Diagnoses:
1. Haired skin: Dermatitis, pyogranulomatous, multifocal and coalescing, severe, with ulceration and fungal hyphae, Border Collie, canine.
2. Lymph node, popliteal (per contributor): Lymphadenitis, pyogranulomatous, diffuse, severe, with fungal hyphae.

Conference Comment: The contributor provides an excellent overview of this recently described cause of cutaneous pyogranulomatous inflammation, which is very similar to pythiosis. Although current literature does not address a specific association between hypercalcemia and lagenidiosis, a well-known association between hypercalcemia and granulomatous disease has been described.[7,8,9,10]

The primary mechanism by which granulomatous disease causes hypercalcemia involves alterations in vitamin D metabolism. In vitro, macrophages can convert 25-hydroxycholecalciferol to its active form, 1,25-dihydroxycholecalciferol (calcitriol) by 1-alpha hydroxylase found in macrophage mitochondria. Normally, 1-alpha hydroxylation takes place in the proximal renal tubular epithelium. The extrarenal production of calcitrol by macrophages is unregulated and causes excess absorption of dietary calcium, reabsorption of renal calcium, and osteoclast resorption of bone calcium, leading to hypercalcemia.[7,8,9,10]

Another proposed mechanism of macrophage-induced hypercalcemia is described in human sarcoidosis, a chronic granulomatous disorder.[12] Parathyroid hormone-related protein (PTHrP) is produced by many normal adult and fetal tissues, where it has autocrine and paracrine functions. PTHrP is a mediator of hypercalcemia of malignancy, and produced by neoplasms such as lymphoma and adenocarcinoma of the apocrine gland of the anal sac. PTHrP acts on PTH receptors in the bone and kidney to cause mobilization of calcium from bone by osteoclasts and calcium reabsorption in the kidney. Although PTHrP is normally secreted by macrophages, it was identified in the cytoplasm of macrophages and multinucleated giant cells in granulomas of human sarcoidosis, and was reported as the source of elevated PTHrP levels.[10,12]

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SLIDE 8
CONFERENCE 2 / CASE IV - NIAH-No.2 (AFIP 2888757)

Signalment: 7.5-year-old, female, Bernese mountain dog, *Canis familiaris*, canine.

History: According to the owner, the dog exhibited anorexia, weight loss, and finally anemic jaundice. Markedly swollen spleen and liver were palpable at the veterinary clinic. The disease developed rapidly over a couple of weeks before termination.

Gross Pathology: Systemic anemia and jaundice, splenomegaly, and hepatomegaly with congestion and cloudy swelling were observed. The bone marrow was yellowish.

Laboratory Results: Blood examination showed elevation of ALT (189 IU/L), ALP (740 IU/L), total bilirubin (3.2 mg/dl) and anemia.

Contributor's Morphologic Diagnosis: Spleen and lung: Malignant histiocytosis, hemophagocytic, Bernese mountain dog, canine.

Contributor's Comment: In the spleen, the proliferating and infiltrating cells are atypical pleomorphic round or histiocytic cells with abundant cytoplasm and anisokaryosis. Large binucleated and multinucleated cells are also seen. These cells frequently engulf erythrocytes, pigment, cell debris, and have vacuolated cytoplasm. Mitotic figures are not prominent. Megakaryocytes are scattered without associated erythroblastic lineage. Immunohistochemically, the proliferating cells are positive for lysozyme, CD68, and MAC 387 in paraffin-embedded sections. In the lung, the tumor cells were often larger than those in the spleen and other organs, and had a prominent nucleolus.

Well-defined proliferative histiocytic diseases in dogs include canine cutaneous histiocytoma, cutaneous histiocytosis, systemic histiocytosis, and histiocytic sarcoma (HS) / malignant histiocytosis (MH). The latter is known as a familial disease for Bernese mountain dogs. MH is indistinguishable from HS after dissemination.

In this case, the lung lesions had been confused with anaplastic lung carcinoma or giant cell variant of large cell anaplastic carcinoma. However, electron microscopy and immunohistochemistry revealed that the character of the tumor cells were identical.

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AFIP Diagnoses:
1. Lung: Malignant histiocytosis, Bernese Mountain Dog, canine.
2. Spleen: Malignant histiocytosis.
3. Spleen: Siderotic plaques.

Conference Comment: Malignant histiocytosis, also known as disseminated histiocytic sarcoma, is a rapidly progressive tumor of the mononuclear phagocyte system, and is an inherited disease in Bernese Mountain Dogs. It is also reported in Rottweilers, Golden Retrievers, Labrador Retrievers, and Flat-Coated Retrievers.[5]

Grossly, it causes solitary or multiple firm, white masses in the lung, liver, spleen, lymph nodes, bone marrow, or kidneys. Microscopically, multinucleated giant cells are a prominent feature and atypical histiocytes often show extensive erythrophagocytosis.[5]

The contributor mentions the differential diagnosis for proliferative histiocytic lesions in dogs. Systemic histiocytosis is a disease of non-neoplastic histiocytes that form dense perivascular cuffs, primarily in the skin and lymph nodes. Systemic histiocytosis, cutaneous histiocytosis, and cutaneous histiocytoma are considered reactive proliferative histiocytic diseases. Localized histiocytic sarcoma is a rapidly growing, solitary cutaneous or subcutaneous mass most frequently located on a distal limb

adjacent to a joint. Occasionally it is found in the spleen, liver, gastric wall, or tongue. Malignant fibrous histiocytoma is characterized by neoplastic cells with features of fibroblasts and histiocytes.[1,5,6]

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SLIDE 9
CONFERENCE 3 / CASE I – 02-3629 (AFIP 2885737)

Signalment: Three year-old, female, Barbados-Moreno X, ovine.

History: Three of forty sheep in a dry lot developed black, hard, encrusted skin of the ear tips, lips, and nose. The lesion involved only non-pigmented skin. The lesions developed three days after the sheep were fed a moldy, pelleted complete horse feed. The sheep were fed oat hay in addition to the pellets. Inspection of the baled hale revealed some mild discoloration of the edges of the bale but no obvious mold or extraneous weeds.

Gross Pathology: All three of the sheep had black, hard, encrusted skin of the lips, nose, eyelids and ear tips except where the skin was pigmented or covered by an ear tag (Fig. 1-5). Heavily wooled parts of the body and feet were not affected. The livers were diffusely pale.

Laboratory Results: Elevated GGT (201 IU/L) and bilirubin levels (Total bilirubin= 5.0 mg/dl) were present in serum of one ewe collected before euthanasia. Aerobic cultures of the lung, liver, and spleen yielded no significant isolates. The moldy, pelleted horse feed was negative for aflatoxin B1, aflatoxin G1, sterigmatocystin, zearlenone,

zearalonal, ochratoxin A, citrinin, diacetoxyscirpenol, neosolaniol, nivalenol, deoxynivalenol, fusarenone-X, T-2, and HT-2.

Contributor's Morphologic Diagnosis: Cholangiohepatitis, subacute, diffuse, severe, with intraluminal crystals, bile ducts, and occasional hepatocyte necrosis. Resulting in: Secondary photosensitization with multifocal cutaneous necrosis of skin of ear tips, lips, nose, and eyelids.

Contributor's Comment: In the liver there is diffuse periportal fibrosis and dilation of bile ducts. Basophilic, wispy, acicular crystals are present in bile duct lumina. Sometimes, these are surrounded by multinucleated giant cells. The portal connective tissue contains infiltrates of mononuclear cells, principally lymphocytes and plasma cells. There are increased bile duct profiles. Small numbers of necrotic hepatocytes, often surrounded by neutrophils, are present in lobules. A few hepatocytes contain crystalline material. There is mild, diffuse hepatocellular vacuolation.

The lesions are consistent with "crystal-associated cholangiohepatitis". This is a well-recognized syndrome of photosensitization secondary to liver injury associated with the formation of crystalline material in the bile ducts.[1,2] The disease is associated with the consumption of certain plants including Kleingrass (*Panicum coloratum)*, *Agave lecheguilla, Tribulus terrestris, Narthecium* sp*., Nolena texana* and *Brachiaria decumbens.* There is also a report of photosensitivity and liver disease with crystal formation in goats consuming young green oats (*Avena sativa*) infected with the fungus *Drechslera companulate.*[3]

The puncture vine (*Tribulus terrestris*), in combination with the mycotoxin, sporodesmin produced by *Pithomyces chartarum*, causes the disease known as geeldikop and is a major cause of secondary photosensitization in South Africa.[1] Mycotoxins have not been associated with the other plants listed. Steroidal saponins derived from the plants are suspected to be the source of the crystals but this has not been substantiated.[1]

The moldy, pelleted horse feed was temporally associated with the outbreak of disease in this flock but we were not able to identify a mycotoxin in the feed. None of the plants known to be associated with this syndrome were present in the environment except for the oat hay. The owner could not recall exactly when the bales were purchased relative to the first clinical signs. It is interesting to note the case report of goats developing hepatogenous photosensitization and crystal-associated hepatopathy following grazing of mold infected green oats. [3] The hay was not grossly moldy but fungal cultures were not performed.

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AFIP Diagnoses:

1. Liver: Fibrosis, portal, diffuse, moderate, with mild lymphoplasmacytic cholangiohepatitis, biliary hyperplasia, and intrabiliary crystals, Barbados-Moreno X, ovine.
2. Liver: Hepatitis, necrotizing, neutrophilic, random, multifocal, mild.

Conference Comment: Conference attendees agree that the portal distribution of the lesions is consistent with the pathogenesis of toxin concentration within the biliary system. The cause of the randomly distributed neutrophilic hepatitis is not evident. It is not thought to be associated with the intoxication.

There are three types of photosensitization, based on the source of the inducing agent, that produce gross lesions similar to those seen in this case. Gross lesions are comparable in all three types of photosensitization. Photosensitization results from the activation of photodynamic substances within the skin by ultraviolet light. This results in free radical formation, either directly or through activation of xanthine oxidase, which causes the tissue damage.[4]

Type I, or primary photosensitization, results from ingestion of a plant with photodynamic properties, such as *Hypericum perforatum* (St. John's wort), *Fagopyrum* sp. (buckwheat), *Ammi majus* (bishop's weed), or the anthelmintic, phenothiazine. Type II photosensitization is caused by a defect in porphyrin metabolism, such as congenital porphyria and congenital protoporphyria in cattle. In both cases, enzyme deficiencies lead to the accumulation of photodynamic pigments. In congenital porphyria, cattle are deficient in the enzyme uroporphyrinogen III cosynthetase. In addition to photodermatitis, affected cattle have discolored teeth and bones, anemia, and porphuria. Congenital protoporphyria occurs in Limousin cattle due to a deficiency of ferrochelatase, and causes photodermatitis. Finally, Type III is called hepatogenous photosensitization and is secondary to any hepatic injury that interferes with bile excretion. Phylloerythrin, a breakdown product of chlorophyll, is normally excreted in the bile. Accumulation of phylloerythrin causes photosensitization if the animal is exposed to the appropriate wavelength of solar radiation that activates the photodynamic agent.[4]   The contributor noted several plants associated with hepatogenous photosensitization.

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SLIDE 10
CONFERENCE 3 / CASE II - 03M0512 (AFIP 2888770)

Signalment: 12 month, male, mixed breed, bovine, *Bos taurus*.

History: This animal had a brief history of drainage from the right ear and swelling of the right side of the head. Loss of sight in the left eye was suspected. Euthanasia with captive bolt was performed.

Gross Pathology: In the calvaria, a 2.5 - 3 cm diameter tan, firm, multilobular mass was present extending from the right vestibulocochlear nerve medially into the brain on the right side. Lungs were adhered to the diaphragm and thoracic cavity.

Laboratory Results: Immunohistochemistry demonstrated immunoreactivity for *Mycoplasma bovis* in and around the caseous and purulent foci (Fig. 1).

Contributor's Morphologic Diagnoses: Ear/brain: chronic polypoid pyogranulomatous and plasmacytic otitis media, interna, and meningoencephalitis, associated with *Mycoplasma bovis* infection.

Contributor's Comment: Speculation in the present case is that an inflammatory polyp extended from the middle ear along the vestibulocochlear nerve with invasion of meninges and parenchyma of medulla oblongata and cerebellum. Not all sections contain cerebellum. The cause of the inflammation was likely chronic infection with *Mycoplasma bovis*. Immunohistochemistry, as in this case, has been used successfully to detect the organism in lung abscesses in calves with fatal pneumonias that were culture positive for the organism.[1] Otitis media caused by *Mycoplasma bovis* has been described in preweaned dairy calves. Clinical findings included ear droop, epiphora, head tilt, and recumbency.[4] Tympanic bullae often had fibrinosuppurative to caseous exudate with fibrous thickening of the tympanic mucosa and mononuclear cell infiltrates.[4] Other causes of otitis media in calves include *Haemophilus somnus*, *Pasteurella multocida*, *Streptococcus* spp., *Actinomyces* spp., and *Railletia auris* (an ear mite).[4]

Cases of otitis media in preweaned calves were likely associated with cases of subclinical *M. bovis* mastitis, given isolation of *M. bovis* from the bulk tank of the herd of origin.[4] *M. bovis* is an important bovine pathogen causing respiratory disease and arthritis in addition to mastitis, reproductive disease, and otitis media. In Europe it is reported to be responsible for 1/4 to 1/3 of calf pneumonias.[3] Its prevalence is likely underestimated with bacteria such as *Mannheimia haemolytica*, *Pasteurella multocida*,

and *Haemophilus somnus* being more commonly and easily isolated.[3] In one study, feedlot cattle with chronic respiratory disease and/or arthritis had high rates of *M. bovis* identification from lungs and joints by immunohistochemistry, often with bovine virus diarrhea virus (BVDV) infection also identified. *M. bovis* from lungs or joints and along with BVDV were the most common pathogens persisting in tissues of animals failing to respond to antibiotic therapy.[2]

Besides *M. bovis*, other mollicutes isolated from cattle include *Mycoplasma mycoides* subsp. *mycoides* (the cause of contagious bovine pleuropneumonia), *Mycoplasma dispar*, *Ureaplasma diversum*, *Mycoplasma bovirhinis*, and *Mycoplasma canis*.[3] Because of the lack of a cell wall, these organisms are inherently refractory to several groups of antibiotics and some strains are becoming resistant to other antibiotics, making control difficult. As of early 2003, no vaccine was available.[3]

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AFIP Diagnosis: Brain: Meningoencephalitis, pyogranulomatous, multifocal, moderate, with hemorrhage and neuronal and white matter necrosis, mixed breed, bovine.

Conference Comment: The lesions are most severe in the sections containing cerebellum; however, some slides contain only sections of less severely affected brainstem.

The contributor reviews *M. bovis* infection and its implication as a predisposing factor in infection with other bacterial and viral agents. This stresses the importance of considering mycoplasma as a primary pathogen, especially in respiratory disease, as it is frequently masked by other common and more easily cultured secondary bacterial agents.[3]

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SLIDE 11
CONFERENCE 3 / CASE III – 03-2702 (AFIP 2888614)

Signalment: 3 month old, intact female pit bull dog, *Canis familiaris*, canine.

History: This dog was euthanized by the County Animal Shelter due to neurologic signs that included incoordination, spasms, tremors, and abnormal use of its legs. The dog was also reported to have diarrhea and ocular mucous discharge.

Gross Pathology: Tan fecal material soiled the perirectal skin. Lung lobes were distended and had prominent rib impressions. Multifocal pinpoint white foci were present in all lobes. The right main-stem bronchus contained red, tenacious mucous. The thymus was markedly atrophied. Intestinal contents were watery and brown to clear.

Laboratory Results: Fecal parasite examination revealed high numbers of *Isospora* sp. oocysts. Rabies IFA of sections of brain were negative. Immunohistochemistry for canine distemper virus was positive on sections of spleen (performed by Washington Animal Disease Diagnostic Lab).

Contributor's Morphologic Diagnoses: Spleen: Marked, diffuse, lymphoid atrophy and multifocal follicular lympholysis, with intranuclear inclusions.

Contributor's Comment: There is severe depletion of lymphocytes in the splenic cords and periarterial lymphoid sheaths. The underlying reticular stroma, dendritic cells, and ellipsoids are exposed. Pale foci in the mantle and marginal zones of the follicles contain lymphocytes and mononuclear cells with nuclear pyknosis and karyorrhexis. In these regions, dendritic cells have swollen nuclei with marginated chromatin and central eosinophilic viral inclusions. Additional microscopic lesions (not submitted) include bronchointerstitial pneumonia with syncytial cells containing intracytoplasmic and intranuclear inclusions, and mild nephritis localized to the papillae with myriad eosinophilic intracytoplasmic inclusions within the transitional epithelium.

Canine distemper virus is a negative-strand RNA *Morbillivirus* of the family *Paramyxoviridae*. Morbilliviruses cause measles, rinderpest, peste-des-petits ruminants, equine morbillivirus infection, and phocine, dolphin, and porpoise distemper. Canine distemper virus is most commonly transmitted via respiratory aerosols; transplacental infection may also occur. Following respiratory exposure, the virus replicates within macrophages and disseminates to lymph nodes, the spleen, and to other organs[1].

Severe lymphopenia and immunosuppression are hallmarks of the disease and thymic atrophy is one of the most consistent gross lesions. Increased lymphocyte apoptosis has been demonstrated in lymph nodes and the thymus and may be the cause of immunosuppression.[2] CD4+ T cells have also been shown to be preferentially depleted.[3] This spleen nicely demonstrates the severity of lymphocyte depletion, along with the etiologic agent. Differential diagnosis includes canine herpesvirus and canine adenovirus 1 infection. The spectrum of lesions and positive immunohistochemistry results confirm canine distemper virus as the etiology.

Distemper virus infection of the CNS causes both grey and white matter lesions. It has been compared to toxic and metabolic myelinopathies and multiple sclerosis. The mechanism of demyelination has been the subject of investigation. Demyelination in the CNS is associated with down-regulation of myelin gene transcription and degeneration of oligodendrocytes. Interestingly, viral particles are much more abundant in microglia, astrocytes, ependyma, and neurons than oligodendrocytes.[4] In contrast to lymphoid depletion, demyelination does not appear to be result of oligodendroglial necrosis or apoptosis.[5] The presence of viral nucleic acid but not intact virions within oligodendrocytes suggests that defective infectious particles may be a cause of the demyelination. Other theories, including immune-mediated, antibody-dependent mechanisms, cytokine-mediated, and astrocyte-dependent processes have also been proposed.[4]

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AFIP Diagnosis: Spleen: Lymphoid necrosis and depletion, diffuse, marked, with reticuloendothelial eosinophilic intranuclear inclusion bodies, pit bull, canine.

Conference Comment: The contributor gives a concise review of this important infectious disease. Canine distemper virus (CDV) has a wide host range and affects multiple species in the families *Canidae* (dingo, fox, coyote, wolf, jackal), *Ailuridae* (pandas), *Mustelidae* (ferret, mink, skunk, badger, weasel), *Procyonidae* (raccoon, coati), *Ursidae* (bear), and *Felidae* (large cats - lions are especially susceptible). Similar disease syndromes occur in marine mammals caused by phocine distemper virus (pinnipeds), and viruses of the cetacean morbillivirus group (dolphins and porpoises).[6]

Secondary infections due to the immunosuppressive effects of CDV are especially important. Toxoplasmosis, bordetellosis, and canine adenovirus 2 are common sequelae to CDV infection.[6]

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SLIDE 12
CONFERENCE 3 / CASE IV - 02-4408 (AFIP 2888675)

Signalment: Twenty-day-old Holstein calf.

History: Calf submitted with a history of respiratory signs and diarrhea.

Gross Pathology: The calf had a small thymus and small erosions or ulcerations of the oesophagus and abomasum. Intestinal content was liquid and yellowish. Atelectatic lesions were present in cranioventral regions of the lungs.

Laboratory Results: BVD virus was detected in the colon with the immunofluorescence technique. Search for bovine coronavirus and rotavirus with the FA technique was negative. Bacterial cultures were negative.

Contributor's Morphologic Diagnosis:
Mucosal colitis with crypt necrosis

Contributor's Comment: In this colon, there is a mild and multifocal exfoliation of the superficial epithelium. Many crypts dilated and filled with mucus and necrotic cells are denuded or lined by flat, cuboidal or low columnar enterocytes. There is a multifocal infiltration of the lamina propria by lymphocytes and plasma cells. The extensive necrosis of the crypts of Lieberkühn noted in this colon is compatible with a BVD virus infection destroying their epithelial lining[1]. The bovine coronavirus causing a similar colitis was not demonstrated in the present case. This case emphasizes the fact that BVD virus should always be considered a potential cause of diarrhea in young calves.

AFIP Diagnosis: Colon: Colitis, neutrophilic, acute, diffuse, mild, with crypt abscesses, crypt epithelial cell necrosis, and epithelial regeneration.

Conference Comment: Bovine pestivirus consists of two biotypes, cytopathic (CP) and noncytopathic (NCP), based on their effects in cell culture. Infection with NCP biotype occurs primarily in immunocompetent, non-pregnant cattle and often produces subclinical infection. Affected animals may develop acute disease with fever, lethargy, diarrhea, and mild oral erosions. Whether the animals develop clinical signs or not, they are considered reservoirs of infection and are lifelong shedders. *[Clarification: Postnatal BVDV infection of immunocompetent, non-pregnant cattle is usually subclinical and the cattle clear the virus, usually in 2-3 weeks. They definitely do NOT become lifelong shedders. Even when they are shedding maximally, they are not very efficient transmitters, as several studies have shown. Only persistently infected (infected at 1-4 months of gestation) cattle are lifelong shedders.]* Some NCP BVDV strains have been associated with abortion, and some with thrombocytopenia and hemorrhagic disease in calves and, rarely, adult cattle; however, only NCP BVDV cause persistent infection.[1,2]

If a fetus is infected *in utero* with NCP biotype during the first four months of gestation (before the immune system fully develops), the calf may be aborted, born weak, or may exhibit congenital defects such as cerebellar hypoplasia. If the calf survives, it is considered immune tolerant because its immune system does not recognize the NCP virus as being foreign. This calf is now a persistently infected carrier.[1,2]

If the persistently infected animal is then infected with a CP strain (possibly by mutation from an NCP strain), the animal becomes superinfected and develops fatal mucosal disease. Both the CP and NCP biotypes are antigenically similar, so the immune system fails to recognize the CP strain as foreign and does not protect the animal. Mucosal disease is characterized by erosions in the oronasal, esophageal, and gastrointestinal mucosa, blunting of buccal mucosal papillae, and necrosis of Peyer's patches, often with a diphtheritic membrane. The animal often has severe diarrhea with dehydration, and may die quickly. If the animal survives the acute disease, chronic mucosal disease develops, characterized by healing ulcers in the oral cavity, erosive and ulcerative dermatitis of the pastern, and laminitis.[1,2]

Although not present in this case, additional classic histological lesions of BVD infection include Peyer's patch necrosis, lysis of gut-associated lymphoid tissue, and herniation of the crypts of Lieberkühn into the submucosal space previously occupied by Peyer's patches.[1,2]

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SLIDE 13
CONFERENCE 4 / CASE I – 10760 (AFIP 2886852)

Signalment: 190 lb, female, large white crossbred, commercial pig.

History: This pig is from a farrow to finishing operation that had experienced dermatitis with pruritus in all ages of pigs for the last 4-5 months.

Gross Pathology: The skin on both ears (pinna and external ear canal), face, and between the claws was thickened and covered with dry yellow-brown crusty exudate (Fig 1-3). Similar exudate was present on the dorsal midline, but the skin was not thickened.

Laboratory Results: Whole mites, dissected from the hyperkeratotic epithelium, were identified as adult *Sarcoptes scabiei* var. *suis* by the clinical parasitology laboratory at Purdue University School of Veterinary Medicine.

Contributor's Morphologic Diagnosis: Skin of ear (pinna); Dermatitis, eosinophilic with marked epidermal hyperplasia, hyperkeratosis, intracorneal abscesses, and numerous intracorneal mites morphologically consistent with *Sarcoptes scabiei*.

Contributor's Comment: Mange was apparently introduced to the farm with a shipment of grower pigs approximately 6 months prior to the submission of this pig. During that time, all areas of production (farrowing, nursery, grower, and finisher) became infested. Two clinical forms of mange are recognized.[1] A large percentage of pigs on this farm had either the hyperkeratotic form of mange as seen in these slides or the immediate hypersensitivity form with reddened skin and papules. Populations of mixed bacteria, mainly coccoid bacteria, within stratum corneum were considered either incidental or minimally contributing to the lesion.

AFIP Diagnosis: Haired skin, pinna: Dermatitis, eosinophilic and proliferative, subacute, diffuse, marked, with parakeratotic hyperkeratosis and intracorneal mites, large white cross, porcine.

Conference Comment: Other diseases in pigs that produce hyperkeratotic lesions include porcine juvenile pustular psoriasiform dermatitis, exudative epidermitis caused by *Staphylococcus hyicus*, and zinc-responsive parakeratosis.

Porcine juvenile pustular psoriasiform dermatitis is also known as pityriasis rosea or pseudoringworm and is a non-contagious, self-limiting disease of weanling pigs. The cause is unknown but a hereditary component has been proposed. Gross lesions are symmetrical, sharply defined, raised red plaques on the ventral abdomen or medial thighs. The lesions heal from the center outward so the skin in the center is normal and is surrounded by a zone of elevated erythematous skin and scales, forming targetoid lesions. These ring lesions often coalesce to form mosaic or serpiginous patterns. Microscopically, there is prominent parakeratotic hyperkeratosis, superficial eosinophilic and neutrophilic perivascular dermatitis, and psoriasiform epidermal hyperplasia.[2]

Exudative epidermitis, also known as greasy pig disease, is caused by *Staphylococcus hyicus* and is an acute, often fatal disease of suckling piglets. Grossly, there are focal cutaneous erosions around the head (eyes, ears, snout, and lips) that spread to the extremities, ventral thorax, and abdomen. A characteristic thick, yellow-brown greasy exudate covers affected erythematous areas and the piglets succumb to severe protein and electrolyte imbalance. Microscopically, there is a thick parakeratotic and orthokeratotic crust, suppurative folliculitis, subcorneal pustules in the epidermis and outer root sheath of hair follicles, and epidermal hyperplasia.[2]

Zinc deficiency causes marked parakeratotic hyperkeratosis, epidermal hyperplasia, and eosinophilic and lymphocytic perivasculitis. Grossly, symmetrical, erythematous macules on the ventral abdomen, medial thighs, face, scrotum, and tail progress to papules covered with a thick, dry crust that forms cracks and fissures.[2]

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SLIDE 14
CONFERENCE 4 / CASE II - 03-5830-02 (AFIP 2888625)

Signalment: Seven-week-old female crossbred German Shepherd Dog (*Canis familiaris*).

History: This dog was from an animal shelter; it was one of two that died with respiratory distress. A third dog in the same facility with similar signs and swollen cervical lymph nodes recovered after hospitalization.

Gross Pathology: The liver was yellow and swollen to approximately twice the normal size. Fine yellow strands of tissue (fibrin) were loosely adherent to the capsule. A finely mottled tan and red-brown pattern extended throughout the hepatic parenchyma. The colonic serosa was covered by blotchy red foci of hyperemia and congestion. The colonic lymph nodes were enlarged and swollen with a reddened, wet medullary region.

Laboratory Results: Immunohistochemical staining for canine adenovirus antigen performed on sections of formalin-fixed liver and kidney was positive (Prairie Diagnostic Services, Saskatoon, SK Canada)[1].

Contributor's Morphologic Diagnoses:
1. Liver: Necrosis, hepatocellular, multifocal and coalescent, acute, severe with intranuclear inclusion bodies, etiology consistent with canine adenovirus -1.
2. Kidney: Intranuclear inclusion bodies, glomerular endothelium, few.

Contributor's Comment: Liver sections contain random, multicentric and coalescing foci of coagulative necrosis of hepatocytes involving at least 50% of the parenchyma. Necrotic hepatocytes have pale eosinophilic cytoplasm and pyknotic or karyorrhectic nuclei. There are necrotic Kupffer cells scattered throughout these foci as well. Small to moderate numbers of hepatocytes contain eosinophilic intranuclear inclusions with a bluish tint that fill affected nuclei.

In sections of kidney, intranuclear inclusions are present in endothelial cells of small numbers of glomeruli. Chromatin is clumped against the inner aspects of nuclear membranes in nuclei with inclusions. Some sections of kidney may not have this change.

Infectious canine hepatitis is a ubiquitous disease of canids. Signs may include abdominal pain, high fever, vomiting, melena, ecchymotic hemorrhages and icterus[2]. The cause is canine adenovirus-1, a member of the genus Mastadenoviridae[3]. The virus's tropism for endothelium, mesothelium, and hepatic parenchyma is grossly evident as edematous and hemorrhagic tonsillar and cervical lymph nodes, blotchy serosal hemorrhages, edematous gallbladder, and enlarged yellow liver with capsular fibrin[2]. A later sequela routinely seen is corneal edema thought to be associated with hypersensitivity to viral antigen[4]. Gross lesions of other organs are inconsistent and linked to endothelial damage. After oral exposure, viral replication occurs in the tonsils and cervical lymph nodes followed by viremia with ensuing hepatic, renal, ocular, and endothelial lesions. Histologically, the yet unexplained periacinar necrosis resembles the zonal necrosis associated with acute hepatotoxicity. Severe lesions and death are

rare (it is thought that most dogs are exposed by two years of age with unapparent signs). Usually the viral cytotoxic effect on hepatocytes is self-limiting and limited to periacinar regions with rapid regeneration on the intact reticulin matrix.

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AFIP Diagnoses:
1. Liver: Necrosis, centrilobular and midzonal, diffuse, with intranuclear inclusion bodies, German Shepherd Dog, canine.
2. Kidney, glomeruli: Intranuclear inclusion bodies.

Conference Comment: There is wide variation among slides in the number of intranuclear inclusion bodies present within the glomeruli.

In addition to canine adenovirus type 1, important adenoviruses of animals include canine adenovirus type 2, equine adenovirus type 1, avian adenovirus type 1, and avian adenovirus type 2 in pheasants and in turkeys.[5]

Canine adenovirus type 2 is one of the etiologic agents of canine infectious tracheobronchitis. It causes necrotizing bronchiolitis with intranuclear inclusion bodies in bronchiolar epithelium. Most cases of canine adenovirus type 2 are secondary to immunosuppression, most often caused by canine distemper virus.[5]

Equine adenovirus type 1 causes disease in Arabian foals with severe combined immunodeficiency. Histologic lesions include a severe necrotizing (early) to proliferative (late) bronchiolitis with intranuclear inclusion bodies in bronchiolar epithelium and widespread atelectasis.[5]

Avian adenovirus type 1 causes inclusion body hepatitis in birds and is associated with hydropericardium syndrome of chickens. Co-infection with other viruses or immunosuppression may play a role in severity of adenovirus-induced lesions. Infection with avian adenovirus type 1 causes hepatocellular degeneration and necrosis and intranuclear inclusion bodies within hepatocytes.[6]

Avian adenovirus type 2 causes marble spleen disease in pheasants and hemorrhagic enteritis in turkeys. Lesions of marble spleen disease and hemorrhagic enteritis include splenic hyperplasia of white pulp, lymphoid necrosis, and intranuclear inclusion bodies within lymphoreticular cells. In addition, hemorrhagic enteritis is characterized by intestinal mucosal congestion, sloughing of mucosal epithelial cells, and hemorrhage in the villous tips.[7]

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SLIDE 15
<u>**CONFERENCE 4 / CASE III – 78105-N (AFIP 2889974)**</u>

Signalment: 4-month-old, male, Aberdeen-Angus, *Bos taurus*, bovine.

History: The calf presented to the Large Animal Clinic with neurologic signs. On physical exam, the calf demonstrated a staggering gait and nystagmus. The body temperature was 104°F, with normal respirations and pulse.

Possible diagnoses of polioencephalomalacia, trauma, infection with *Haemophilus somnus*, rabies virus, or *Listeria monocytogenes,* or lead intoxication were considered, and therapy with thiamine HCl was initiated. The body temperature returned to 101.1^{O}F. Neurologic signs progressed over the next day to include head pressing and depression, followed by sternal recumbency. The animal did not respond to the therapy (which had been expanded to include dexamethasone and florfenicol) and was euthanized.

Gross Pathology: The fourth ventricle is filled and the cerebellar vermis is compressed and partially replaced by an approximately 6 cm x 5 cm x 4 cm mass that appears to arise from the anterior aspect of the cerebellum (Fig 1). The mass also causes compression of the brainstem and occipital lobe of the cerebrum. The mass is very soft and gray-tan, with red mottling. The lateral ventricles are moderately dilated (internal hydrocephalus).

Laboratory Results: Cerebrospinal fluid obtained soon after presentation to the clinic was examined and the results include:

Red blood cell count	250/ml
White blood cell count	6/ml
Color/transparency	Colorless and clear
Protein	31.6 mg/dL

Cytology:
- 2% segs
- 40% lymphocytes
- 48% monocytes
- No evidence of abnormal cells

The serum lead level was within normal limits.

Contributor's Morphologic Diagnosis: Cerebellum and brainstem: Medulloblastoma, with cerebellar and brainstem compression.

Contributor's Comment: In the presented case, the cerebellum and brainstem are compressed by an expansile, soft to gelatinous, gray-tan mass, consistent with origination of the tumor in the cerebellum. Examination of a Diff-Qwik-stained touch impression of the cut surface of the mass is densely cellular and reveals a single population of pleomorphic cells with indistinct cell borders, scant, pale eosinophilic cytoplasm, and a large round to ovoid nucleus with an occasional nucleolus. Fibrillary processes occasionally extend from cells. Anisocytosis and anisokaryosis are present.

Histologically, sections of cerebellum or brainstem contain a non-encapsulated mass composed of sheets of a single, monomorphic population of variably densely packed, round to polygonal cells that infiltrate into and replace a portion of the cerebellum, and extend into the fourth ventricle, with extension to the leptomeninges of the brainstem. In areas of low cellularity, abundant fibrillar, eosinophilic cell processes separate cell groups, forming rhythmic palisades of neoplastic cells and fibrillar material. Cells often palisade around small blood vessels (pseudorosettes) and occasionally form vague, fibrillar (Homer Wright) rosettes. Individual cells have indistinct cell borders, a scant to moderately abundant amount of pale, eosinophilic, homogeneous cytoplasm, and an oval, darkly basophilic nucleus (abundant heterochromatin), sometimes with 1-2 basophilic nucleoli. Anisocytosis and anisokaryosis are moderate. Mitotic figures are 3-5 per 400X field. Areas of necrosis are present. Immunohistochemical staining reveals that the neoplastic cells are strongly positive for S-100 and faintly positive for synaptophysin, especially in the fibrillar areas of the tumor. Neoplastic cells are negative for neuron-specific enolase and glial acid fibrillary protein. Scattered endothelial cell (Factor VIII-positive) proliferation is present, resulting in multifocal thickening of capillary walls within the mass. The adjacent cerebellar folia and subjacent brainstem are compressed, with loss of Purkinje and granular layer cells in the cerebellum, and axonal and mild neuronal degeneration in the brainstem.

The clinical presentation and the gross, cytologic, and histologic lesions described here are typical of a medulloblastoma. Medulloblastomas are a type of malignant, primitive neuroectodermal tumor (PNET) that originate in the cerebellum, usually in young animals and, more commonly, in children.[1,2,3,4] Other PNETs are not histologically distinguishable from medulloblastomas, and are found in locations other than the cerebellum.

Embryonal nervous system tumors described as PNETs are believed to arise from progenitor cells that can differentiate along various cell lineages, including neuronal, glial, ependymal, and perhaps mesenchymal.[1,3] Medulloblastomas are thought to be derived from pluripotent cells of the external germinal cell layer or perhaps are derived from more than one cell type.[3,4] A number of genetic alterations have been identified in human medulloblastomas, including alterations in *hedgehog*, neurotrophin, ErbB receptor, and *Wnt* signal transduction pathways.

The macroscopic and microscopic features typical of medulloblastomas reflect features observed in this case. In addition to the pseudorosettes and Homer Wright rosettes observed in the presented case, Flexner-Wintersteiner-like rosettes have been reported in medulloblastomas of domestic animals.[1] Homer Wright rosettes are sometimes absent or poorly formed in medulloblastomas of humans.[5] The presence of carrot-shaped nuclei is a commonly described feature in medulloblastomas of humans, but poor tissue handling may cause compression of the nuclei of neoplastic lymphocytes, mimicking the nuclei of medulloblastomas.[5] The rhythmic palisading of nuclei and fibrils is observed not only in some human medulloblastomas, but also in several types of gliomas.[5]

Immunohistochemical staining of medulloblastomas in humans reveals consistent expression of synaptophysin.[5] Other reagents useful for the identification of medulloblastomas in humans include antibodies directed to GFAP, neurofilaments, protein gene product 9.5, and S-100.[5]

Ultrastructural examination of medulloblastomas should reveal features of embryonal neuronal cells: microtubules, scant intermediate filaments, and dense core granules.[1] Examination by electron microscopy of the presented case was not performed.

In human patients with cerebellar neoplasia, the differential diagnoses of small cell carcinoma and lymphoma are entertained. The presence of fibrillar cell processes and Homer Wright rosettes are important in the identification of medulloblastomas, and the lack of cytokeratin and CD3 expression aid in refuting carcinoma and lymphoma, respectively.

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AFIP Diagnosis: Cerebellum: Medulloblastoma, Aberdeen-Angus, bovine.

Conference Comment: The contributor gives a concise overview of the features of this neoplasm. Some sections contained only brainstem. Without a section of cerebellum, the most specific diagnosis would be primitive neuroectodermal tumor (PNET).

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SLIDE 16
CONFERENCE 4 / CASE IV - 1778 (AFIP 2887457)

Signalment: Seven-day-old Standardbred foal.

History: At four days of age, the foal experienced acute onset of progressive respiratory disease, unresponsive to antibiotic treatment. No other horses on the premises exhibited signs of respiratory disease prior to, during, or following the illness. The foal and mare were turned out daily with a mature Quarter Horse, which had recently returned from another farm with an unknown history.

Gross Pathology: The lungs failed to collapse, were edematous with stable tracheal froth and exhibited generalized reddish discolouration with scattered foci of tan mottling. All lung lobes were rubbery with small, firm, randomly situated nodules throughout. The bronchial lymph nodes were enlarged, wet, and mottled tan and red on cut surface. There was moderate mediastinal edema.

Laboratory Results: Serum immunoglobulin G levels measured 1100 mg/100 ml. Routine bacterial culture of the lung was negative. Cultures for equine herpes virus type 1 and adenovirus were negative. Influenza A was identified by egg inoculation and PCR analysis of lung tissue (pooled influenza A antigens). Immunohistochemistry

demonstrated positive staining for influenza A antigen in the epithelium of terminal bronchioles. The influenza virus isolated from the foal showed hemagglutination-inhibition with A2 (1:80) and A1 (1:20) equine flu subtypes using reference antisera for A2/Miami/63 and A1/Prague/56. Genetic analysis by nucleotide sequencing determined that the strain was most closely related phylogenetically to A2/Kentucky/97.

Contributor's Morphologic Diagnosis: Bronchointerstitial pneumonia, acute, necrotizing, with hemorrhage and hyaline membranes. Etiology - Equine Influenza A2.

Contributor's Comment: Influenza virus is a common cause of non-fatal respiratory disease in horses. The disease most often presents as outbreaks in susceptible horses following exposure to individuals that are shedding virus. Death can occur either as a result of secondary bacterial bronchopneumonia or severe viral infection[1].

Bronchointerstitial pneumonia in foals less than one week of age is considered uncommon. The peak occurrence of bronchointerstitial pneumonia at 1.5 to 2.5 months of age coincides with declining maternally-derived immunoglobulins, implying that passive immunity is protective in younger foals[2].

Equine bronchointerstitial pneumonia is considered to be primarily of viral etiology[2]. This condition has been reported as a sporadic cause of death in foals[2] but definitive etiological diagnosis of these cases has proven frustrating. Early clearance of virus with sloughed necrotic bronchiolar epithelium may render identification attempts futile by the time the lung lesion has progressed to regenerative epithelial hyperplasia and secondary bacterial pneumonia[3]. In the early stage of bronchiolar necrosis, influenza A antigen is more readily identified[3]. The presence of active bronchiolar epithelial necrosis in this case likely facilitated viral isolation and identification.

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AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing, acute, multifocal to coalescing, moderate, with hemorrhage and hyaline membranes, Standardbred, equine.

Conference Comment: Equine influenza is caused by infection with one of the virus subtypes, influenza A/equine-1 (H7N7) or influenza A/equine-2 (H3N8). The glycoproteins hemagglutinin and neuraminidase are surface antigens on the virus envelope. An antigenic drift occurs when there are point mutations in the gene coding for a surface antigen. Antigenic shift occurs when there is genetic reassortment between the two subtypes, resulting in a new subtype with completely different antigenicity. Antigenic drift of the H3N8 strain, such as that isolated in this case, has created subgroups of the virus that contribute to epizootics in equine populations.[4,5]

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SLIDE 17
CONFERENCE 5 / CASE I – Case 2 (AFIP 2889963)

Signalment: Juvenile female cynomolgus monkey, *Macaca fascicularis.*

History: Colony monkey presented with abdominal distention. Supportive care was given with no clinical improvement. As a result, the animal was electively euthanized.

Gross Pathology: At necropsy, there were severe diffuse intra-abdominal adhesions involving the gastrointestinal tract, liver, diaphragm, and uterus.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Colonic serosa, mesentery: Fibromatosis, severe, diffuse, with neovascularization, edema, and lymphoplasmacytic and histiocytic infiltrates.
2. Mesenteric lymph nodes: Atrophy, lymphoid, mild, diffuse.

Contributor's Comment: Microscopically, the colonic serosa and the mesentery were severely thickened by a disorganized fibroblastic proliferation infiltrating the fat and superficial region of the tunica muscularis longitudinal layer and surrounding the mesenteric lymph nodes. The microscopic changes are consistent with retroperitoneal fibromatosis characterized by two dominant morphologic patterns: proliferative and sclerotic. These can occur separately or, as in this case, the lesion can contain both variants. The proliferative pattern is characterized by randomly arranged plump fibroblasts with an interwoven network of collagen fibers and numerous blood vessels of variable size. In some areas, slit-like neovascular spaces are present with hypertrophic endothelium. There is a high nucleus-to-cytoplasm ratio. Mitotic figures are viewed occasionally (i.e., 0 to 2 at HPF). Edematous and myxomatous areas are observed as well as perivascular lymphocytes, plasma cells, and histiocyte infiltrates. The sclerotic pattern consisted of sparsely scattered elongated fibroblasts within a densely packed

bundle of collagen fibers with fewer blood vessels and inflammatory cells compared to the proliferative pattern[1].

Retroperitoneal fibromatosis was first recognized as a disease syndrome in 1976[2]. It is characterized by an aggressive proliferation of highly vascular fibrous tissue subjacent to the peritoneum[3]. Animals in the later stages of retroperitoneal fibromatosis disease often develop SAIDS, a simian acquired immunodeficiency syndrome, and present the following clinical signs: lymphoid depletion, weight loss, depressed immune functions, recurrent diarrhea, and chronic infections unresponsive to antibiotic therapy[1]. Affected monkeys often experience sudden death due to complications such as intestinal obstruction[1].

Simian retroperitoneal fibromatosis has many morphological and epidemiological similarities to human Kaposi's sarcoma (KS), which is highly associated with an immunodeficiency syndrome caused by viral infection (HIV, Simian retrovirus-2, SV40 and recently human herpes virus-8). While manifestations of KS are most severe in individuals with an immunodeficiency syndrome (epidemic KS), KS also can occur in immunosuppressed organ transplant recipients (iatrogenic KS). Furthermore, KS is often present in elderly Mediterranean men (classic KS) and is endemic in sub-Saharan Africa (endemic KS) where it is presently the most common malignancy[4].

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AFIP Diagnosis: Colon, mesentery: Atypical mesenchymal proliferation (retroperitoneal fibromatosis), diffuse, cynomolgus monkey (*Macaca fascicularis*), nonhuman primate.

Conference Comment: Although the retroviral status of this particular monkey is not provided, the contributor points out the well-known association between retroperitoneal fibromatosis and type D simian retrovirus, serotype-2 (SRV-2). The mechanism of cellular transformation by SRV-2 is not known but several hypotheses have been proposed, including transformation of multipotential mesenchymal stem cells toward endothelial cells, fibroblasts, and pericytes; promotion of cell growth by basic fibroblast growth factor (bFGF); and the presence of elevated levels of the growth factor IL-6 in SRV-2 infected monkeys.[1,5,6]

Simian retrovirus can cause simian acquired immunodeficiency syndrome (SAIDS), which is often seen in animals in the later stages of retroperitoneal fibromatosis. The association between retroperitoneal fibromatosis and SAIDS is very similar to the association between Kaposi's sarcoma (KS) and human immunodeficiency virus-1 (HIV-1). Another similarity that makes retroperitoneal fibromatosis a good model for studying the relationship of HIV-1 and Kaposi's sarcoma is the presence of herpesviruses in both entities. Human herpesvirus-8 (HHV-8) has been identified in KS tumors, and is thought to be a cofactor in the development of KS. Retroperitoneal fibromatosis-associated herpesvirus of macaques (RFHV) is a gammaherpesvirus closely related to HHV-8 that has been identified in macaques with retroperitoneal fibromatosis and is

activated by immunosuppression or SRV infection.[7] The association of these tumors with herpesviruses adds another dimension to this valuable animal model of KS.

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SLIDE 18
CONFERENCE 5 / CASE II - 02-427x (AFIP 2888842)

Signalment: 7 month old, female C57BL/6B2m-/-(B2-Microglobulin knockout) mouse, *Mus musculus*.

History: Mice from this group are part of a study that investigated the function of certain proteins that control cancerous proliferation of cells infected by oncogenes of a rodent virus called *Polyomavirus*. These mice were inoculated intraperitoneally with Polyomavirus at the neonatal stage (< than 18 hours old), then monitored for 28-33 weeks for the development of tumors. Most of them developed salivary gland masses, others became depressed, ill or paralyzed in the hindlegs. Salivary tumors are submitted for histopathological evaluation.

Gross Pathology: Salivary glands markedly enlarged, firm, pale tan.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Salivary gland, parotid, anaplastic / mixed type carcinoma.

Contributor's Comment: Polyoma virus of mice is a DNA *Papovavirus*, which has been extensively studied as an oncogenic virus that induces many (*poly*) types of tumors (*oma*). Polyoma-induced tumors are primarily a laboratory phenomenon and seldom occur under conditions of natural infection, except in nude mice. Nude mice also develop multifocal necrosis and inflammation, followed by tumor formation in multiple tissues reminiscent of experimentally inoculated neonatal mice.

Inoculation of neonatal mice with contaminated biologicals or cell cultures is a potential source of spread. Tumors appear 2-12 months after inoculation and, in most strains of mice, the parotid salivary gland is the prevalent site for tumor development. However, tumors can occur at other sites, especially skin, gastrointestinal tract, kidneys, and spinal cord. Paralysis is due to vertebral tumors as well as demyelination.

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AFIP Diagnosis: Salivary gland, parotid: Malignant spindle cell neoplasm, C57BL/6B2m-/-(B2-Microglobulin knockout) mouse, rodent.

Conference Comment: This case was reviewed in consultation with the AFIP Department of Soft Tissue Pathology. We cannot further classify this tumor with immunohistochemistry because our laboratory uses anti-mouse primary antibody. Attempts to use Mouse on Mouse (M.O.M.) immunodetection were unsuccessful.

Polyoma virus-induced neoplasms in the salivary glands of mice most commonly consist of a mixed population of mesenchymal-like cells and epithelioid cells with occasional acinar and ductular structures. Less frequently, there is a pure population of either epithelioid cells or mesenchymal-like cells.[2]

Polyomaviruses belong to the papovaviridae family (PAPOVA from the 3 originating viruses of the family - PApillomavirus, POlyomavirus, and VAcuolating agent). Other polyomaviruses in animals include Simian virus 40 (SV40) in macaques, budgerigar fledgling disease virus, K-virus of mice, hamster polyomavirus, and rabbit kidney vacuolating virus.[5] Important polyomaviruses in humans include JC virus, which causes the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML) in AIDS patients, and BK virus, which is associated with kidney infection in renal transplant patients.[8] Polyomavirus is also significant in humans because rhesus kidney cell cultures used in production of polio vaccines were contaminated with SV40. Many thousands of people were infected between 1954 and 1961 with no apparent harmful

effects, but recent studies using PCR have demonstrated the presence of SV40 in some human neoplasms. The causal relationship linking SV40 to human tumors is controversial and is an active area of research.[8,9]

Simian virus 40 causes inapparent infection in healthy macaques, but causes lesions in the brain (similar to PML in humans), kidney, and lung of immunocompromised macaques. It is oncogenic in suckling or young hamsters, causing undifferentiated sarcomas at the site of virus inoculation.[5] Budgerigar fledgling disease is an acute disease (unusual for a polyomavirus) that causes high mortality in budgerigars and other psittacines. This disease is characterized by hydropic degenerative changes in the epidermis, follicular epithelium, tubular and glomerular epithelium, splenic lymphoid depletion, hepatic necrosis, and amphophilic intranuclear inclusion bodies.[6] K-virus of mice is mostly of historical significance since it rarely occurs in laboratory mouse colonies today. It causes pulmonary vascular edema and hemorrhage in neonatal mice.[1] Hamster polyomavirus is the cause of transmissible lymphoma and keratinizing skin tumors of hair follicle origin. Like mouse polyomavirus, it causes multisystemic infection and is shed in the urine.[1] Rabbit kidney vacuolating virus is a common, nonpathogenic virus of cottontail rabbits that causes only latent infection.[7]

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SLIDE 19
CONFERENCE 5 / CASE III – MK0302215 (AFIP 2892676)

Signalment: Adult, 4.65 kg intact female rhesus monkey (*Macaca mulatta).*

History: The animal was inoculated with simian immunodeficiency virus (SIV) on 1/9/01. Several months prior to euthanasia gradual weight loss was observed. Three weeks prior to euthanasia the animal appeared to be completely blind. An MRI conducted on 12/16/02 revealed a large tumor in the left brain and possibly a small tumor infringing on the optic chiasm. The animal developed ataxia and had difficulty eating and was euthanized on 12/17/02.

Gross Pathology: The entire left caudal lung lobe is adhered by fibrous adhesions to the costal and diaphragmatic pleura. Tonsils are enlarged approximately 2x normal size. A cylindrical mass measuring approximately 1 cm in length by 4 mm in diameter is attached to the inner leaflet of the left atrioventricular valve. Diffuse reddening and hemorrhage of the dura is observed in the distal thoracic spinal column. The left cerebral hemisphere is swollen. No significant mass is observed in the brain. Petechiation is noted within the white matter of the left cerebral hemisphere. The spleen is slightly enlarged with prominent follicular structures. Bone marrow is diffusely reddened. Inguinal, axillary, mandibular, hilar, mesenteric and colonic lymph nodes are all enlarged approximately 2-3x normal, however there is a distinct cortico-medullary junction. Upon formalin fixation an approximately 1 cm in diameter light gray mass is observed in the left cerebral hemisphere white matter.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Brain, meningoencephalitis, granulomatous, with numerous multinucleated giant cells, multifocal to coalescing, marked.

Contributor's Comment: The lesions seen in this monkey are highly suggestive of simian immunodeficiency virus (SIV) infection. SIV is a retrovirus of the lentivirus family that is both immunosuppressive and neurovirulent. Lentiviruses often cause immunodeficiency (loss of CD4+ T-cells) in their hosts in addition to slow, progressive wasting disorders, opportunistic infections, neurodegeneration and death[1]. CD4+ T-cells coordinate a number of critical immunologic functions and the loss of these cells causes progressive impairment of the immune system and a deteriorating clinical course.

   Cellular entry of SIV is quite complex. The SIV envelope contains two glycoproteins, surface gp120 that is noncovalently attached to transmembrane gp41. CD4+ T-cells, Langerhans/dendritic cells and monocytes/macrophages are primary targets of SIV because of the affinity of the gp120 glycoprotein component of the viral envelope for the

CD4 molecule. The binding of gp120 to the CD4 molecule causes a conformational change in the gp120 glycoprotein that creates a new recognition site for a coreceptor. Coreceptors for the SIV virus include CCR5, a beta-chemokine receptor and CXCR4, an alpha-chemokine receptor. Macrophage-tropic strains of SIV can infect monocytes, macrophages and T-cells by binding to the coreceptor CCR5. T-cell-tropic strains infect T-cells utilizing the CXCR4 coreceptor. After the virus binds to the coreceptor conformational changes occur in gp41 that result in the insertion of a fusion peptide at the tip of gp41 into the cell membrane of the T-cell or macrophage. The binding of the virus results in the entry of the genome into the cell[1].

The most common means of retroviral infection is through sexual transmission at the genital mucosa. In this route of infection, Langerhans' cells are the first targets of the virus. After infection, these cells then infect CD4+ lymphocytes, spread to deeper tissues and within a few days reach regional lymph nodes[2]. Once monocytes are infected, they allow for transport of the virus throughout the bloodstream to the nervous system, a major target of SIV infection. SIV initially gains access to the CNS when infected monocytes/macrophages cross the blood-brain barrier[3]. The infection of the CNS occurs as early as one-week post infection[4].

Neurologic disease is common in SIV-infected macaques with simian AIDS, and 50% of rhesus macaques inoculated with SIV show a giant cell encephalitis. Histologically, this is characterized by multifocal, perivascular aggregates of macrophages and multinucleated giant cells in all levels of the CNS[5]. In this case, the monkey was injected with SIVsmE660, a macrophage-tropic strain of SIV known to cause granulomatous encephalitis with viral antigen-positive multinucleated giant cells. Multifocal infiltrates of multinucleated giant cells are present in the pia arachnoid and meninges, and multifocal, perivascular lymphocytic infiltrates are observed in the leptomeninges. The most severe infiltrates of macrophages and lymphocytes are in the cerebrum. Numerous individual, small grouped and large aggregates of multinucleated giant cells admixed with foamy macrophages and lymphocytes are present in the white matter as one large mass, a lesion atypical for SIV infection.

Various cytokines are involved in the formation of multinucleated giant cells and the spread of the virus. Multinucleated giant cells form when macrophages in the brain engulf viral particles and present them to T-cells. The T cells are then activated and secrete various cytokines such as IL-2 and IFN-gamma. The IL-2 activates other T-cells, perpetuating the response, while the IFN-gamma aids in the transformation of the macrophages into epithelioid cells and multinucleated giant cells[1].

In a study of rhesus macaques inoculated with SIV, viral antigen was found in the CNS regardless of the presence or absence of giant cell encephalitis. Viral antigen was found consistently within the CNS in infiltrates of macrophages and multinucleated giant cells with the cerebral white matter and cerebellum most commonly affected. Viral antigen was not limited to mononuclear cells and multinucleated giant cells and was also found commonly associated with scattered capillaries and small vessels in the brain, spinal cord, meninges and choroid plexus. Viral antigen was found less

commonly in the same tissues associated with cells or cell clusters but not in association with vessels. Antigen was found in the walls of vessels in the CNS in areas with and without macrophage/giant cell lesions. In SIV infected macaques, lentiviral transcripts were seen in vessels in the non-inflamed CNS. It is thought that the infection of vessels is key in the pathogenesis of SIV encephalitis. It is not clear whether both endothelial cells and mononuclear cells in the vessel wall and perivascular space are infected with the virus or if the endothelial infection is actually infected mononuclear cells in transit through the vessel wall. Many SIV-positive cells were found in the vessels in the parenchyma and choroid plexus suggesting these sites as the primary routes of infection of the CNS with parenchymal vessels being the major route. The meninges is less likely to be a major route of infection due to the fact that lesions are rarely seen in the adjacent cortical gray matter as compared with the cortical white matter[5].

AFIP Diagnosis: Cerebrum: Meningoencephalitis, histiocytic and lymphocytic, multifocal to coalescing, severe, with numerous multinucleated Langhans and foreign body-type giant cells, rhesus monkey (*Macaca mulatta)*, non-human primate.

Conference Comment: The contributor provides an excellent overview of simian immunodeficiency virus and its pathogenesis. It is interesting to note that this remarkable lesion was diagnosed antemortem as a possible tumor via MRI.

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SLIDE 20
<u>**CONFERENCE 5 / CASE IV - CP02-2337 (AFIP 2889949)**</u>

Signalment: 4 month old, male, WAS -/- Background strain: 129S7/SvEvBrd /C57BL/6J, *Mus musculus*.

History: Mice were irradiated and transplanted with stem cells. Several mice in the group developed rectal prolapse and were submitted for necropsy.

Gross Pathology: There was a 1.5mm prolapse of the rectum. The distal colon was moderately thickened.

Laboratory Results: PCR on fecal pellets was positive for *Helicobacter hepaticus*.

Contributor's Morphologic Diagnoses:
1. Large intestine: Severe inflammatory proliferative colitis and proctitis.
2. Rectum: Rectal prolapse.

Contributor's Comment: In the colon and rectum there is a focal area of mucosal ulceration and marked mucosal epithelial hyperplasia. Mucosal epithelial cells lining crypts are well differentiated. These cells are crowded and frequently pile up. Mitotic figures are numerous. The hyperplastic crypts extend upward toward the lumen and deep into the submucosa, and are often observed transversing the mucosa muscularis. Crypts are dilated and mucinous lakes are present in the submucosa. Inflammation is extensive in the lamina propria, submucosa, and tunica muscularis. The inflammatory infiltrate is composed of neutrophils, lymphocytes, plasma cells, macrophages, and eosinophils. The luminal surface is ulcerated, and coagulative necrosis of the superficial mucosa is observed. The invasion of the crypts deep into the submucosa and, in some slides, into the tunica muscularis suggests that over a period of time these lesions may progress to a neoplastic state. In this animal this process is considered benign because the mucosal epithelial cells were well differentiated, and there was no evidence of lateral migration of epithelial cells or metastasis to regional lymph nodes.

   Helicobacter species of mice have been associated with hepatitis and inflammatory bowel disease.[1,2,3,4] Members of this genus are microaerophilic, have curved to spiral rod morphology, and are propelled by flagella that vary in number and location. Transmission of these organisms is through the fecal-oral route. *Helicobacter hepaticus* and *H. bilis* have received the most attention because of their prevalence in rodent populations and their association with disease.

   These organisms cause a chronic active hepatitis characterized by portal, perivascular, or randomly distributed aggregates of lymphocytes, macrophages, and few polymorphonuclear cells. In some cases hepatocellular necrosis may be a predominant finding. Progression to hepatocellular carcinoma has been reported.[1,2,3,4] Histologically the lesion in the large intestine consists of mucosal hyperplasia (sometimes atypical) and inflammation. Inflammatory infiltrates vary from predominately neutrophils to a mixed population consisting of neutrophils, mononuclear cells, and macrophages, with the latter predominating in chronic lesions.[1,2,3,4]

Helicobacter hepaticus was first identified as a pathogen in a long-term carcinogenicity study when hepatitis developed in untreated A/JNCr mice. Later, the organism was identified in chronic proliferative typhlocolitis and proctitis in immunodeficient mice. *Helicobacter bilis* has been reported to cause similar lesions.[1,2]

Recently, *H. rodentium* and other novel urease-negative *Helicobacter* sp. have been associated with hepatic and inflammatory bowel lesions similar to those described for *H. hepaticus* and *H. bilis*.[3,4] Susceptibility to infection varies among mouse strains, with immunocompromised mice having severe infections.
Understanding *Helicobacter* infections is important because infection with this organism provides an important model for bacterial induced carcinogenesis, infections can significantly skew results for some research studies, and certain species may prove to have important zoonotic potential.[4]

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AFIP Diagnosis: Rectoanal junction: Proctitis, ulcerative, acute, focally extensive, severe, with crypt loss, herniation, abscesses, and regeneration, 129S7/SvEvBrd /C57BL/6J strain, mouse, rodent.

Conference Comment: As the contributor mentions, *Helicobacter* infection in laboratory mouse colonies not only causes disease in immunodeficient mice, but may also have a significant impact on research results.

In addition to the intestinal lesions described here, other mice may develop focal non-suppurative necrotizing hepatitis that progresses to chronic active hepatitis with minimal necrosis, often accompanied by erosion and ulceration of gallbladder mucosa. In these cases, *Helicobacter* organisms are best demonstrated in the bile canaliculi with silver stains, such as Warthin-Starry or Steiner.[5] In this case, a Warthin-Starry was performed but no organisms were identified.

In the A/JCr mouse strain, *H. hepaticus* has been associated with hepatocellular carcinoma. A more recent study was not able to replicate these findings, suggesting that bacterial strain and environmental conditions may be important factors in development of hepatic lesions.[6]

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SLIDE 21
CONFERENCE 6 / CASE I – 02-12465 (AFIP 2886652)

Signalment: Adult, pigeon (free-ranging), female.

History: Animal control wardens reported a "dramatic" increase in the number of dead pigeons in an Illinois city (population 81,860). In addition to collecting dead birds, the wardens also collected several birds that were mentally dull and unable to fly. Pigeons appeared to be the only avian species affected.

Two dead pigeons and 1 live pigeon were submitted to the Veterinary Diagnostic Laboratory for necropsy examination.

Gross Pathology: One bird was in poor body condition, with moderate skeletal muscle atrophy and no visible body fat reserves. The crop was markedly distended (10 x 10 cm), thin-walled, and filled with a large number of corn kernels. There was a focal perforation of the crop associated with necrotizing ingluvitis and surrounding cellulitis.

Laboratory Results:
Aerobic culture of heart blood - no growth

Gastric (ventriculus) contents, liver, and kidney - drugs, organic compounds, and pesticides were not detected by Toxilab

Kidney - 381 ppm lead

Liver - 14.4 ppm lead

Contributor's Morphologic Diagnosis: Kidney, proximal renal tubular epithelium: Myriad acid-fast intranuclear inclusion bodies consistent with lead toxicosis.

Contributor's Comment: Lead toxicosis is not uncommon in avian species, particularly in migratory waterfowl in the United States. In metropolitan areas, pigeons have been used to monitor environmental exposure to lead contamination. Both chronic and acute effects of lead ingestion have been evaluated in pigeons. In chronic, dose-controlled studies using lead acetate, it has been shown that a dose of 6.25 mg/kg resulted in only marginal behavioral effects and a dose of 12.5 mg/kg resulted in moderate to marked behavioral effects without producing either ataxia or gross lesions of lead toxicosis[1]. However, a dose of 25 mg/kg had pronounced effects on behavior and produced gross lesions of toxicity[1]. Acute lead poisoning has also been experimentally produced in pigeons by a single intra-peritoneal (IP) administration of lead acetate[3]. The primary signs of acute toxicosis following IP dosing included weight loss, anemia, and death. Interestingly, the per os (PO) administration of lead acetate has been shown to induce crop dysfunction (stasis, dilatation, feed impaction, and regurgitation of crop contents) and lead-induced ataxia[2]. The exact mechanism of the crop dysfunction is not fully known, although possible explanations include an indirect effect on crop activity following a primary action on the cerebellum or semicircular canals of the inner ears. Since the intramuscular injection (breast muscle) of lead also induces crop stasis, it does not appear to require direct, local contact with the crop.

In the present case, the pigeon presented in poor body condition with a markedly dilated crop. Since the bird died prior to presentation, it is unknown if neurologic deficits were also present. Aside from ingluvitis and cellulitis associated with crop rupture, the histologic changes in this case were restricted to the kidney and consisted of numerous acid-fast, intranuclear inclusion bodies in the proximal renal tubular epithelium. Based on studies in mourning doves, the presence of intranuclear inclusion bodies suggests that the bird was exposed to lead at least 4 or 5 days previously. In mourning doves which were experimentally dosed PO with lead shot, the renal epithelial inclusion bodies were primarily intracytoplasmic at day 4 post-exposure, while they were primarily intranuclear at day 9 post-exposure[4]. It was suggested that the presence of intracytoplasmic inclusions may represent the early cytoplasmic transport of lead, before final deposition in the nucleus, and may be an indicator of duration of exposure.

Lead toxicosis was confirmed by the toxicologic analysis of the kidney (381 ppm Pb) and liver (14.4 ppm Pb).

AFIP Diagnosis: Kidney, tubular epithelium: Degeneration, multifocal, minimal, with numerous intranuclear inclusion bodies, pigeon, avian.

Conference Comment: Anemia is a consistent clinicopathologic finding in animals with lead intoxication. Lead inhibits the enzymes delta-aminolevulinic acid synthetase and

ferrochelatase that are involved in hemoglobin synthesis. The inability to synthesize hemoglobin results in anemia. In addition, inhibition of nucleotidase causes increased fragility of red blood cells that also contributes to the anemia. Basophilic stippling is a morphologic change in erythrocytes characterized by aggregation of residual RNA. When basophilic stippling is accompanied by metarubricytosis with minimal polychromasia, indicating an inappropriate response, lead toxicosis is a likely cause.[5,6]

Grossly, a "lead line" may been seen as a blue discoloration in the gingiva adjacent to the teeth as a result of precipitation of lead sulfide. This change, however, is more frequently observed in humans and nonhuman primates. Radiographically, a linear metaphyseal density (lead line) may be present in young animals as a result of impaired osteoclast resorptive activity causing a band of mineralized cartilage.[6]

Ultrastructurally, lead inclusions have a discrete electron dense central core surrounded by an outer zone of fibrillar structures[6], giving its borders an indistinct, hazy appearance.

Contributor: University of Illinois at Urbana-Champaign, Veterinary Diagnostic Laboratory & Department of Veterinary Pathobiology

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SLIDE 22
CONFERENCE 6 / CASE II - 024-03-1 (AFIP 2888659)

Signalment: Pastured adult, female, Gelbvieh bovine.

History: On March 23, three Gelbvieh cows were discovered dead in their pasture at the morning feeding. Of the remaining 51 cattle, only a handful ran up to the feed truck in their usual manner. Clinical signs in affected cows included ataxia, "star-gazing", lassitude, recumbency and brief, agonal convulsions. Gross post-mortem examination by the referring veterinarian did not reveal any remarkable abnormalities beyond a few thistle-like seed heads in the rumen. Six animals were treated empirically with activated charcoal and mineral oil to no effect. By the following morning, another 6 cattle were dead. By the end of two weeks, a total of 42 died.

Gross Pathology: A second, recumbent, cow was admitted to the CSU College of Veterinary Medicine Teaching Hospital and died a few hours later. Lesions included subcutaneous edema and hemorrhage and a pronounced lobular pattern in the liver.

Laboratory Results: Tissues submitted to the Wyoming State Veterinary Laboratory (WSVL) were initially analyzed with normal results for cholinesterase, nitrate and toxic metals (As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Tl, V and Zn). Rumen pH was 7.0 and aqueous humor cations (Ca, Mg) were within normal limits. Brain sodium was elevated (2396 ppm, normal <1600) but aqueous humor was not. A serum chemistry panel revealed moderately elevated alanine aminotransferase and markedly elevated lactate dehydrogenase. A complete blood count revealed neutrophilia with a left shift and serum chemistry demonstrated severe hypoglycemia, hyperbilirubinemia, hypernatremia, hypokalemia, elevated creatine kinase, aspartate aminotransferase and gamma-glutamyl transferase, and decreased sorbitol dehydrogenase activity.

The kidney was analyzed for aflatoxin with negative results. Samples of the hay being fed were extracted with MeOH or water and bioassayed in mice, also with negative results. Post mortem samples and hay were submitted for mycotoxin analysis, herbicide screens, ethylene dibromide, dibromochloropropane, volatile and halogenated hydrocarbons, alkaloids, phenols and PCB's with negative results.

Liver and rumen from the index case were found to contain approximately 15 ppb microcystin by ELISA, a result later confirmed by mass spectrometry.

Contributor's Morphologic Diagnosis: Liver: necrosis, severe, diffuse acute periacinar to massive with vacuolar degeneration, Gelbvieh, bovine.

Etiologic Diagnosis: Toxic hepatic necrosis

Etiology: Microcystin

Contributor's Comment: Liver lesions from tissues examined at CSU and WVSL are similar. Section of liver with multifocal to massive hepatocyte loss with severe disruption of hepatic cords, primarily centrilobular and paracentral often with extension to the limiting plate of the portal areas. The hepatocyte necrosis is coagulative to lytic and individualization of hepatocytes is a notable feature. Nuclei are often swollen and there is margination of chromatin, with karyorrhexis and pyknosis found. Additionally,

there is rarefaction of hepatocyte cytoplasm with increased vacuolization and cell swelling. No mitotic figures are identified nor evidence of reactive change. There is flooding of the sinusoids with blood. There is a mild accumulation of lymphocytes and plasma cells in the portal areas, with an occasional neutrophil identified. Large rod-shaped bacteria, sometimes in chains, are encountered particularly within the portal areas and also within the expanded sinusoids (post-mortem putrefaction).

Cyanobacteria or blue-green algae constitute a large group of prokaryotic organisms characterized by the presence of chlorophyll a[14]. A variety of cyanobacterial species are capable of producing substances toxic to vertebrates. These substances are referred to as cyanotoxins and can be classified broadly into 3 structural groups: cyclic peptides (microcystins, nodularins) that target hepatic function; alkaloids (anatoxins, saxitoxins) that target the nervous system; and lipopolysaccharides that are potential irritants and are produced by all cyanobacterial species[14].

Microcystins are naturally occurring protein phosphatase inhibitors and potent hepatotoxins[6] with microcystin-LR being the most commonly occurring and at the same time most toxic congener[15], defined as having the highest capacity for protein phosphatase-1 and -2A inhibition[11]. Microcystin-LR is produced by the cyanobacteria *Microcystis aeruginosa*[7]. Globally it appears that blooms of microcystin-containing hepatotoxic species are a greater threat to health than the other groups and have been responsible for numerous incidents of acute animal poisoning episodes[10,12]. There are more than 60 different structural variants of microcystin identified with LD50's ranging from 50-800 ug/kg. Microcystins have also been associated with human toxicity resulting in acute sickness and death in dialysis patients in Brazil[10]. Moreover, it has also been suggested that microcystins play a role in the high incidence of hepatocellular carcinoma present in China[12]. Supporting this theory is laboratory data that incriminates microcystin-LR as an initiator in the promotion of liver tumor formation, hepatic neoplastic nodules and preneoplastic tumor growth[16].

The toxicity of microcystin-LR in mammals is characterized by fulminant intrahepatic hemorrhage, followed by hypovolemic shock[1], secondary to massive hepatocellular necrosis and collapse of hepatic parenchyma, and death[8,2,3,4]. Microcystin-LR is hydrophilic and does not readily cross lipid membranes[9]. Microcystins reabsorbed from the GI tract are taken up from the blood via a multispecific, rifampicin-sensitive, energy-dependent bile acid transport system of hepatocytes[7,12]. Rounding of hepatocytes occurs concurrently with the loss of normal hepatic architecture and is considered to result from the cytosolic interaction of microcystin-LR with serine/threonine phosphatases-1 and -2A[4,5], which are essential for maintaining the monomerization/polymerization equilibrium of cytoskeletal intermediate filaments. Through microcystin-mediated inactivation of these protein phosphatases, this equilibrium is shifted towards monomerization with resultant dissociation of the hepatocyte cytoskeleton[4]. Laboratory data have shown that within 10 minutes post-exposure to microcystin-LR a mild widening of centrilobular hepatocyte intercellular spaces can be identified[8]. Within minutes, plasma membrane invaginations occur with formation of intracytoplasmic vacuoles, loss of microvilli along the sinusoidal face, and

widespread pronounced hepatocyte separation. This is followed by a marked widening of the space of Disse and of the centrilobular areas containing necrotic cells and apparently intact, isolated organelles intermingled with erythrocytes and platelets[8].

In the past, microcystin diagnosis was sufficiently cumbersome that it was seldom attempted in any but the most typical cases. However, with the advent of sensitive methods capable of detecting the toxin in tissue, microcystin poisoning should be included in the differential diagnosis for any case of severe, acute massive necrosis in outdoor animals. This case is unusual in that blue green algae toxicoses are commonly held to be a warm-weather problem, however there are precedents in the scientific literature for cyanotoxin production in cold weather. In this case, the onset of signs was preceded by several years of drought, and several days of unusually warm, windy weather leading to a sudden snowmelt runoff that flooded the pasture, leaving puddles of warm stagnant water. We hypothesize that either a mat of a benthic cyanophyte such as *Oscillatoria* was dislodged and washed into the pasture via the irrigation system, or that the unseasonably warm weather permitted a bloom to occur in standing water in the pasture.

Differential diagnoses for acute centrilobular hepatic necrosis in ruminants includes numerous plant toxins including *Cestrum parqui*, *Helichrysum blandowskianum*, cocklebur (*Xanthium strumarium*)[17] and *Trema aspera* ("poison peach"). In cattle, Rift Valley fever and acute poisoning with aflatoxin should also be considered as differentials.

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AFIP Diagnosis: Liver: Necrosis, centrilobular and midzonal, diffuse, with hemorrhage and hepatocellular dissociation, Gelbvieh, bovine.

Conference Comment: The contributor gives a thorough overview of microcystin toxicosis.

Differential diagnoses for acute hepatic necrosis discussed during the conference and by the contributor may be differentiated from microcystin toxicity based on additional histopathologic features. The toxic plants *Cestrum parqui*, *Helichrysum blandowskianum*, *Xanthium strumarium*, and *Trema aspera* usually cause acute periacinar necrosis. Aflatoxicosis may be differentiated based on the presence of bile duct proliferation and megalocytosis. Rift Valley fever, caused by an arthropod-borne bunyavirus, is characterized by randomly distributed foci of hepatocellular necrosis that progresses to massive hepatic necrosis, and the occasional presence of elongated, eosinophilic intranuclear inclusion bodies within degenerate hepatocytes.[18]

Contributor: Colorado State University, Veterinary Diagnostic Laboratory, Ft. Collins, Colorado

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SLIDE 23
CONFERENCE 6 / CASE III – 2003-02 (AFIP 2888032)

Signalment: 12-week-old, male, Sprague-Dawley rats.

History: Rats were treated with 10 mg/kg cisplatin as a single dose administered via intraperitoneal injection then sacrificed on Day 4.

Gross Pathology: None.

Laboratory Results: Increases were observed in blood urea nitrogen (2.9x-3.1x controls) and creatinine (2.9x-5x controls) on Days 3 and 4 post treatment.

Contributor's Morphologic Diagnosis: Kidney, corticomedullary tubules (proximal and distal): Epithelial cell necrosis, acute, multifocal, moderate with vacuolar degeneration and tubular ectasia, multifocal, mild to moderate.

Contributor's Comment: Cisplatin represents a class of platinum coordination compounds with potent antitumor activity[1]. The adverse effects of cisplatin include renal impairment, intestinal toxicity, and myelosuppression, of which renal toxicity is the most serious dose-limiting factor clinically. The kidney is known to retain platinum at the highest tissue concentration among various organs examined and the renal accumulation of platinum most likely involves the excretory function of the kidney, since cisplatin and/or its metabolites are eliminated in the urine[1].

Tubular lesions associated with acute cisplatin nephropathy include cytoplasmic vacuolation and swelling of tubular epithelial cells with subsequent necrosis and sloughing of necrotic epithelial cells resulting in cast formation and tubular ectasia[1]. A single intraperitoneal dose of cisplatin (6 mg/kg) in the rat has been shown to induce marked acute tubular necrosis in the proximal and distal tubules with a maximum lesion on day 7[1]. One of the characteristic features of acute cisplatin nephropathy is that tubular lesions are primarily localized in the corticomedullary region, involving both the proximal and distal tubules. In contrast, nephrotoxicity of other heavy metal compounds usually causes epithelial damage in the proximal tubules in the cortex. The toxic injury of cisplatin in the corticomedullary region is likely a result of the large accumulation of platinum in that particular region[1].

Impairment of renal function is manifested as elevated serum levels of blood urea nitrogen and creatinine post exposure. Following a single injection of cisplatin (6 mg/kg

IP) to rats, serum levels of BUN and creatinine have been observed to rise to a peak of 12- and 11-fold, respectively, above controls on day 5[1].

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AFIP Diagnosis: Kidney, corticomedullary tubular epithelium: Degeneration and necrosis, acute, multifocal to coalescing, Sprague-Dawley, rodent.

Conference Comment: The proximal tubule is a common site of toxicant-induced injury. Three discrete segments of the proximal tubule are described: S_1 is the pars convoluta, S_2 is the transition between the pars convoluta and the pars recta, and S_3 is the pars recta. The S_3 segment, or the distal portion of the proximal segment, is the site-selective target of cisplatin in the rat.[2] As noted by the contributor, platinum accumulates in the corticomedullary region and induces injury in this area.

The exact mechanism of toxicity is not known, but it may involve the metabolites of cisplatin and not the platinum atom itself. *In vitro* studies have shown that inhibition of DNA synthesis is the mechanism of action by which cisplatin exerts its nephrotoxic effects.[2]

Contributor: Pfizer Global Research and Development, Groton, CT 06340
http://www.pfizer.com

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SLIDE 24
CONFERENCE 6 / CASE IV - 46822 (AFIP 2888627)

Signalment: Adult, male, C57BL6 mouse, *mus musculus*, rodent.

History: These animals had surgically-placed intraabdominal telemetric implants and were administered 300 mg/kg of acetaminophen PO pre- and post-operatively. There was a 10% mortality rate of the population within one week post-surgery.

Gross Pathology: The livers of the animals examined at necropsy were diffusely mottled red and tan.

Laboratory Results:
ALT 1638 U/L (10-35)

AST 1122 U/L (10-45)
BUN 504 mg/dL (9-30)
Creatinine 3.6 mg/dL (0.4-1)
Phosphorus 27.3 mg/dL (4.2-8.5)

Contributor's Morphologic Diagnosis: Liver, hepatocellular necrosis, centrilobular, diffuse, severe, acute with hemorrhage and mineralization.

Contributor's Comment: Acetaminophen is a widely used analgesic and antipyretic agent. Toxic levels of acetaminophen have been associated with massive liver cell necrosis, usually 3 to 5 days after the ingestion of the toxic doses.[1] Central lobular necrosis is the most frequent form of hepatocellular necrosis in animals exposed acutely to many hepatotoxic agents. Hepatocytes in this region of the lobule are highly susceptible to such agents because of their relatively high levels of cytochrome P450 and associated enzymes that metabolize and therefore activate xenobiotics. Many of these agents are not intrinsically toxic but become toxic once metabolized by the target organ.[2] Centrilobular hepatocytes also receive the blood with lower oxygen concentration further increasing their susceptibility to toxic injury.[3]

The toxic effects of acetaminophen are a result of increased production of a reactive intermediate, N-acetly-p-benzoquinone imine (NAPQI). The liver normally uses reduced glutathione to neutralize NAPQI, which can then be excreted in the urine. Further exposure to acetaminophen can eventually deplete glutathione stores and permit build-up of NAPQI. The excess NAPQI is then free to react with hepatic proteins, more specifically, mitochondrial proteins, causing morphologic changes and inhibition of mitochondrial respiration. Another hypothesis cites oxidative stress as the mechanism of hepatocellular damage. NAPQI depletes the cell of glutathione, which is normally protective against oxidative stress. This stress to erythrocytes causes methemoglobinemia and Heinz body formation, which is the most common manifestation of acetaminophen toxicity in cats.[4]

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AFIP Diagnosis: Liver, hepatocytes: Necrosis, centrilobular, diffuse, with hemorrhage and mineralization, C57BL6 mouse, rodent.

Conference Comment: The contributor gives a concise overview of the toxic principle of acetaminophen toxicity. Acetaminophen is metabolized via three pathways in the liver. The first is the pathway described above, whereby NAPQI is formed from oxidation of acetaminophen by the P-450 pathway. Acetaminophen may also conjugate to a sulfate compound or conjugate to a glucuronide compound by glucuronosyltransferase.[4]

In cats, the major pathway for acetaminophen conjugation is through the sulfate pathway because cats have very little glucuronosyltransferase activity. Cats, however,

have limited amounts of sulfate so this pathway becomes quickly overwhelmed, forcing metabolism via the P-450 pathway and increased formation of NAPQI.[4]

Contributor: The Johns Hopkins Hospital, Department of Comparative Medicine, 1-127 Jefferson Building, 600 North Wolfe Street, Baltimore, MD 21287

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SLIDE 25
CONFERENCE 7 / CASE I – 03-12068 (AFIP 2887460)

Signalment: 6.5 years, neutered male, American Staffordshire terrier, (*Canis familiaris)*, canine.

History: This 33 kg dog presented to the University of MN Veterinary Medical Center with a four-day history of vomiting, weakness, and ataxia after ingesting a two-pound (0.9 kg) bag of store-bought raisins [0.97 oz/kg or 27.3 g/kg]. During this time, the dog was anorexic and oliguric. The dog had no previous medical problems. On presentation, the dog was quiet, alert, and responsive. The dog was slightly ataxic, but the remainder of the neurologic examination was within normal limits. The dog remained oliguric and continued to vomit while in the ICU. Therapy included placing a urinary catheter, intravenous fluids, famotidine, furosemide, and dopamine. Due to the lack of response to diuretics and fluid therapy, the dog was euthanized.

Gross Pathology: At necropsy, the major findings were restricted to the stomach. The gastric mucosa was diffusely reddened and there was moderate submucosal edema at the level of the cardia, extending along the most proximal (orad) 1/3 of the body of the stomach. There were no significant macroscopic lesions noted in the other organs.

Laboratory Results:
Complete blood count (CBC): within normal limits.
Serum chemistry: blood urea nitrogen (BUN): 209 mg/dl; creatinine: 16.6 mg/dl; phosphorus: 18.1 mg/dl; calcium: 13.0 mg/dl.
Ocular fluid (aqueous): "BUN" ocular fluid equivalent: 204 mg/dl; creatinine: 7.3 mg/dl.

Contributor's Morphologic Diagnosis: Kidney, [renal cortex], bilateral, nephrosis with acute tubular necrosis, extensive, moderate to marked, and renal tubular epithelial regeneration, multifocal, moderate.

Contributor's Comment: There have been several publications in the past 3 years that discuss cases of acute renal failure in dogs, associated with ingestion of grapes and raisins. The exact mechanism and pathogenesis of this putative association is not currently known. To date, diagnostic screening for mycotoxins, heavy metals, pesticides, and other contaminants has been negative.

Since 1989, the Animal Poison Control Center (APCC) of the ASPCA (American Society for the Prevention of Cruelty to Animals) has documented a number of cases of renal failure in dogs that had eaten raisins or grapes[1]. In several recent publications, the amount of raisins or grapes ingested was estimated to be between 0.41 and 1.1 oz/kg [1,4,5]. There was a wide variety in the types of grapes ingested in these cases, including fresh, store-bought grapes, fresh grapes from private vineyards, and fermented grapes from wineries[1]. In the cases of raisin ingestion, the majority of the raisins were commercial, sun-dried raisins[1].

As more data were collected from these cases, it was noted that most of these dogs typically vomited within a few hours of ingesting the grapes or raisins. Signs of anorexia and diarrhea were also noted in many of these cases. Most of the cases had abnormalities in the serum chemistry profile, including hypercalcemia, hyperphosphatemia, increased Ca X PO_4 product, and elevated blood urea nitrogen (BUN) and serum creatinine concentrations [1,2,4].

Histopathologic findings from one necropsied dog were similar to those seen in this case, including mild renal tubular damage. The published case also had metastatic mineralization of numerous tissues. There has been some discussion as to whether the renal lesions were sufficient to cause the severity of the dog's clinical signs[1]. The history, clinical signs, and histologic lesions in this dog are at least suggestive of an association between the ingestion of raisins and acute renal failure. Additional screening for contaminants in several of the reported cases is currently being conducted at a number of institutions.

The exact cause of the renal damage in these cases is not known. Possible etiologies include nephrotoxins, fruit contaminants, mycotoxins, high levels of vitamin D, interference of sugar metabolism, or an idiosyncratic or anaphylactic reaction possibly leading to hypovolemic shock and subsequent renal ischemia[1,2,3,4].

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AFIP Diagnosis: Kidney, tubules: Necrosis, acute, multifocal, with regeneration, American Staffordshire terrier, canine.

Conference Comment: This is a classic example of acute tubular necrosis, although raisin toxicity is not widely reported in the literature. Although reference intervals were not given, conference attendees discussed clinical pathology data related to azotemia.

An increased serum blood urea nitrogen (BUN) and creatinine indicate azotemia and must be interpreted in light of other clinicopathologic parameters to determine if it is prerenal, renal, or postrenal. Prerenal azotemia is caused by a reduction in the glomerular filtration rate (GFR), and there is no decline in urine concentrating ability. This is an insensitive measure of renal disease since shock, dehydration, or cardiovascular disease may cause decreased renal perfusion, and thus decrease GFR. Renal azotemia occurs when 75% of the nephrons are nonfunctional and the kidneys lose the ability to concentrate urine. Postrenal azotemia is generally due to obstruction or postrenal leakage, and concentration abnormalities may or may not occur.[6]

The contributor notes that ocular fluid was obtained for evaluation. It is reported that postmortem urea nitrogen and creatinine concentrations in ocular fluid correlate closely with antemortem serum concentrations, and although it varies among species, are generally valid within 24 hours after death at a body temperature of 37 degrees Celsius.[7]

Contributor: Minnesota Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, 1333 Gortner Avenue, St. Paul, MN 55108 www.mvdl.umn.edu

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SLIDE 26
CONFERENCE 7 / CASE II - 03N156 (AFIP 2888669)

Signalment: Five year-old, female, spayed, Dalmatian, *Canis familiaris*, dog.

History: This five year-old Dalmatian had a history of intermittent vomiting which occurred within 10 minutes to 8 hours after eating, every 2-3 days for one week. Stool appeared normal. CBC and serum chemistries were submitted. All values were within normal limits. Metoclopramide and a bland diet were begun. Two days later, vomiting continued and plain and barium contrast films of GI tract were taken. No obstructions or masses were noted. Intermittent vomiting was still occurring ten days later, so endoscopy of the stomach was performed revealing mild "granularity" to the mucosa. A mucosal biopsy was taken with histopathological findings of lymphoplasmacytic gastritis. Carafate and prednisone were initiated. Intermittent vomiting was still occurring two weeks after the biopsy was performed. The dog has lost four pounds and now appeared depressed. Another CBC and serum chemistries were submitted; results showed an increase in liver enzymes and an elevated WBC. At this time the decision was made to wean the dog off of the prednisone. Three days later the dog's appetite improved. Another CBC and serum chemistries were submitted. Liver enzymes had improved, however the WBC was still elevated. Antibiotics (cephalexin) were initiated. Six days later, the dog was vomiting every 3-4 days. This time a mass was palpated in the left upper cranial abdominal quadrant. Another CBC and serum chemistries were submitted. The WBC count was within normal and the liver enzymes had improved. An exploratory was performed. A large firm lobulated mass was found cranial to the left kidney, adhered to the dorsal body wall and vena cava and wrapped around the renal vessels. The tumor was hemorrhagic, friable and unresectable due to its proximity to major vessels. The abdomen and skin were closed and the dog was removed from anesthesia and placed in ICU where it died shortly thereafter.

Gross Pathology: There are hemorrhagic sections of small intestine as well as colon. The liver has two yellow masses each two cm in one direction. There is a mottled, partially encapsulated left adrenal mass (9 x 6 x 2 cm) adhered to the left renal vessels and vena cava extending cranially to the liver and attached to the parietal peritoneum (Fig. 1).

Laboratory Results:
CBC/chemistries 4/9/03: All values within normal limits

After prednisone: 5/5/03: Bilirubin 4.2 mg/dl, Alk phos 1154 mg/dl, ALT 160 u/l, AST 79 u/l, GGTP 71 u/l. Sample was icteric. WBC 32.6 x 10^3/cmm, monocytes 2608 (8%).

Weaning off prednisone: 5/8/03: Bilirubin 1.6 mg/dl, Alk phos 2130 mg/dl, ALT 167 u/l, GGTP 97u/l. Sample was icteric. WBC 33.4 x 10^3/cmm, monocytes 3674 (11%).

Steroids discontinued post antibiotics: 5/14/03: Bilirubin 0.4 mg/dl, Alk phos 1282 mg/dl, ALT170 u/l, GGTP 51 u/l. WBC 15.8 x 10^3 /cmm

Contributor's Morphologic Diagnoses:
1. Pheochromocytoma, left adrenal gland.
2. Moderate diffuse hepatocellular swelling with mild lobar hyperplasia.

3. Minimal to mild acute hemorrhage, mucosa of the colon.
4. Mild acute congestion, mucosa of small intestine.

Contributor's Comment: The most common adrenocortical tumors in dogs are adenomas and carcinomas, and the most common tumors in the adrenal medulla of dogs are pheochromocytomas. All of these neoplasms may be functional.

Functional pheochromocytomas occur infrequently in animals. Norepinephrine is the catecholamine most commonly secreted from these tumors in dogs and may be secreted sporadically or continuously. Clinical signs reported are related to the release of catecholamines and include congestive heart failure, pulmonary edema, and ventricular fibrillation as a result of hypertension.[4] Pheochromocytomas may be large and invade the posterior vena cava, as seen in this case. Grossly, they are often light brown to yellow-red because of areas of hemorrhage and necrosis.[5]

Pheochromocytomas are tumors of chromaffin cells, which vary from small cuboidal or polyhedral cells to large pleomorphic cells often subdivided into small lobules by fine connective tissue septa and capillaries.[5] Immunohistochemically, neoplastic cells express chromogranin and synaptophysin. Neurosecretory granules are positive using Churukian-Schenk.

In bulls, pheochromocytomas may develop along with calcitonin-secreting C-cell tumors of the thyroid gland, which is part of a syndrome of multiple endocrine neoplasia (MEN) in which there is neoplastic transformation of numerous cells of neuroectodermal origin in the same animal.[1,5]

Functional tumors of the adrenal cortex secrete cortisol, however only about 50% of cases in one study had historical clinical signs, morphologic signs, or clinicopathological evidence of hyperadrenocorticism.[1]

Adrenal cortical carcinomas occur less frequently than adenomas. They have been reported most often in cattle, sporadically in old dogs and rarely in other species. Carcinomas develop in adult to older animals, and there is no particular breed or sex prevelance.[2]

Adrenal cortical carcinomas are generally larger than adenomas and may be more likely to develop in both glands. In dogs they are composed of a variegated, yellow to brownish red, friable tissue that incorporates most or all of the affected adrenal gland. They are often fixed in location because of extensive invasion of surrounding tissues and the posterior vena cava, forming a large tumor cell thrombus.[1]

Adrenal cortical carcinomas are composed of more pleomorphic cells when compared to adenomas, which are subdivided into groups by a fibrovascular stroma of varying thickness. The pattern of growth varies between individual tumors, and within the same carcinoma, resulting in the formation of trabeculae, lobules, or nests of tumors cells.[1]

Surgical resection of adrenal neoplasms can be achieved in dogs that do not have tumor invasion of the vena cava. Although long-term outcome in these patients is often good, adrenalectomy is technically difficult surgery, and perioperative complications are not uncommon. Perioperative complications include thromboembolism, pancreatitis, adrenal insufficiency, and cardiac arrest.[3]

AFIP Diagnosis: Adrenal gland: Pheochromocytoma, Dalmatian, canine.

Conference Comment: Conference attendees had some difficulty in tissue identification, but the packeted appearance of the tumor cells helped to classify this as a neuroendocrine tumor.

If an adrenal mass is present in a dog, an adrenocorticotropic hormone (ACTH) stimulation test, low-dose dexamethasone suppression test, and high-dose dexamethasone suppression test may be helpful in ruling out a functional adrenocortical tumor.

The ACTH stimulation test evaluates the ability of the adrenal gland to increase plasma cortisol in response to stimulation by exogenous ACTH. Normal dogs will have a two- to three-fold increase in plasma cortisol levels, compared to baseline values. This is the test of choice for diagnosis of iatrogenic hyperadrenocorticism because dogs will have little to no response to exogenously administered ACTH. Dogs with functional adrenocortical tumors may have a normal response, but at least 50% of dogs will have abnormal ACTH responses.[6]

The low-dose dexamethasone suppression test is used to screen animals for pituitary-dependent and adrenal-dependent hyperadrenocorticism. In healthy dogs, administration of dexamethasone inhibits cortisol secretion. Dogs with either pituitary-dependent or adrenal-dependent hyperadrenocorticism will have inadequate suppression of cortisol levels.[6]

The high-dose dexamethasone suppression test is used to differentiate pituitary-dependent hyperadrenocorticism from dogs with adrenal-dependent hyperadrenocorticism. Dexamethasone inhibits cortisol secretion in healthy dogs, identified at the lowest limit of detection by the cortisol assay when dexamethasone is administered at the high dose. Dogs with pituitary-dependent hyperadrenocorticism have similar suppression. Dogs with functional adrenal tumors will not adequately suppress plasma cortisol, nor will approximately 25% of dogs with pituitary dependent hyperadrenocorticism.[6]

The presence of an adrenal mass with normal dexamethasone suppression test results may suggest the presence of a pheochromocytoma. Hormonal tests for veterinary patients with pheochromocytomas are not specific because they have not

been adapted from their use in human patients.[1] The value in clinicopathologic testing and clinical findings remain important noninvasive methods of diagnosis in patients with a functional adrenal mass.

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SLIDE 27
CONFERENCE 7 / CASE III – 03-8778 (AFIP 2888891)

Signalment: Black lab X, neutered male; 5 years old, *Canis familiaris*.

History: Dog presented 5 days after hiking in wilderness area with elevated temperature (106F), lethargy, anorexia and pronounced malaise. These signs progressed to ataxia, focal seizures and then over a month to weakness, inability to rise and dysphagia with pronounced weight loss and muscle atrophy. Intermittent fevers were present throughout this time. The dog was treated with a variety of medications including anti-oxidants, anti-inflammatory drugs (meloxicam and prednisolone) and antibiotics including trimethoprim sulfa. Final treatment was with Clindamycin. The dog is recovering.

Gross Pathology: Not applicable.

Laboratory Results:
Initial laboratory results were:

Marginal leucopenia with degenerative left shift. ALT=3436 iu/l (reference range 0-113); ALP 312 iu/l (reference range 04-113) CK 841 iu/l(reference range 00-314) Bilirubinuria was present and urine SG was 1.032.

The PCV dropped from .051 to .036 l/l (reference range 0.390-0.560) over the first week of treatment. Azotemia developed, BUN 16.8 mmol/l (reference range 2.5-9.20) Creatinine 173 µmol/l (reference range 68-141) and urine SG was 1.020 with both bilirubinuria and hemoglobinuria. ALT dropped to 259 iu/l and ALP to 293 iu/l, GGT increased to 22 iu/l (reference range 2-20). CK dropped to 410 iu/l Antibody titer for ICH was positive at 1:24 (animal had been vaccinated).

One month later anemia had improved, PCV 0.416; the ALT was 764 iu/l but ALP and GGT were normal, the CK was 13206 iu/l. Mild azotemia persisted BUN 11.0 mmol/l. An ANA test was negative as was an IgG toxoplasmosis titer. IFA for *Neospora caninum* was positive at 1:800. Muscle biopsies submitted from biceps and semitendinosus muscle. Immunoperoxidase procedure on the muscle biopsy was positive for neospora and negative for toxoplasmosis.

Contributor's Morphologic Diagnosis: Pyogranulomatous myositis, severe, chronic, biceps and semitendiosus muscle; with numerous tissue cysts of *Neospora caninum.* Tachyzoites are also present in the areas of inflammation.

Contributor's Comment: The animal presented with a multisystemic disease that initially involved the liver, progressed to CNS signs and then to a profound muscle weakness with dysphagia. It is an unusual clinical presentation for this organism as the dog was 5 years old, and previously healthy with no evidence of immunocompromise. The most severe disease manifestations usually occur in animals <1 year of age. The initial manifestations were of hepatocellular injury, and although no biopsy was obtained, the subsequent findings of muscle tissue cysts and tachyzoites support this organism as the causative agent for the 8-week illness. The late involvement of the muscle tissue is also interesting but may reflect a resurgence of disease as a consequence of therapy that included both corticosteroids and nonsteroidal anti-inflammatory drugs.

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AFIP Diagnosis: Skeletal muscle: Myositis, necrotizing, subacute, diffuse, moderate, with regeneration and intrasarcoplasmic protozoal cysts, Labrador Retriever cross, canine.

Conference Comment: This case was reviewed in consultation with Dr. J. P. Dubey. By light microscopy, Dr. Dubey favors a diagnosis of *Sarcocystis* based on the presence of metrocytes within immature cysts and internal septa separating mature organisms into compartments within the cysts, neither of which are features of *Neospora caninum*. Immunohistochemistry performed by Dr. Dubey indicates this is *Sarcocystis*. Dogs are definitive hosts for *Sarcocystis*, where the organism sporulates then is excreted in the feces in an infective form. Since dogs are definitive hosts, it is unusual for tissue cysts of *Sarcocystis* to be present, and especially unusual for the organisms to cause

inflammation in tissue. In this case, the presence of tissue cysts and a substantial inflammatory reaction suggests either an aberrant life cycle of *Sarcocystis* or an unusual presentation of *Neospora*.[7,8] Dr. Dubey is working with the contributor to clarify the etiology and describe this case for publication.

Conference attendees discussed differential diagnoses for tissue cysts in skeletal muscle, including neosporosis, toxoplasmosis, and sarcocystosis.

Until recently, tissue cysts of *Neospora caninum* were reportedly only present in central or peripheral neural tissues, and have a characteristic thick (up to 4um) wall.[1] Recently, however, thin-walled (0.3-1um) tissue cysts have been reported in muscles of dogs and cattle that were naturally infected. This, however, has not been reproduced in experimentally infected animals.[6]

Dogs that develop generalized signs related to *Toxoplasma* infection are immunocompromised and young, most often less than one year of age. Older dogs develop specific clinical signs associated with lesions in neural and muscular systems, similar to clinical signs seen with *Neospora caninum* infection. Another similarity between *Neospora caninum* and *Toxoplasma gondii* is that the tissue cysts and tachyzoites of each have a comparable light microscopic appearance. In fact, it is believed that some previous reports of toxoplasmosis in dogs may have been neosporosis.[1,8]

Conference attendees discussed clinicopathologic alterations related to muscle disease. Creatine kinase (CK) and aspartate aminotransferase (AST) are important enzymes of muscle origin that may be altered in diseases when there is disruption of muscle cell membranes and subsequent enzyme leakage. Lactate dehydrogenase (LDH) may also be elevated with muscle damage and, when measured along with other enzymes, can support a diagnosis of muscle damage.

Creatine kinase is the enzyme of choice for detecting muscle disease because most serum CK activity is of muscle origin, although isoenzymes for cardiac muscle and brain also exist (the brain isoenzyme is only present in cerebrospinal fluid). Hemolysis affects CK activity because enzymes and intermediates released from erythrocytes may cause CK to become falsely elevated. Elevations in CK activity occur quickly, within 4-6 hours of muscle injury and return to reference intervals within 24-48 hours if muscle injury is not ongoing. Since CK is very sensitive to minor muscle injury, such as intramuscular injections, it is best evaluated in conjunction with other enzymes of muscle origin.[9]

Aspartate aminotransferase is not tissue specific, but muscle and liver are the major sources of this enzyme. Aspartate aminotransferase activity increases more slowly than CK and may persist for several days after cessation of muscle injury. Skeletal muscle, cardiac muscle, liver, and erythrocytes are sources of LDH activity. Erythrocytes contain very high activity, so even mild hemolysis may significantly alter LDH activity. The half-life of LDH is longer than that of CK or AST (approximately 5 days).[9]

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SLIDE 28
CONFERENCE 7 / CASE IV - 03 C 1607 (AFIP 2893688)

Signalment: Five-year old intact female Labrador retriever x German Shepherd cross.

History: A 5-year old Labrador retriever x German shepherd cross bitch was presented for left pelvic limb lameness. She was treated with a non-steroidal anti-inflammatory drug (carprofen; Rimadyl, Pfizer Animal Health)(87.5 mg BID). After 14 days of treatment the bitch was re-examined and a drawer sign detected in the left stifle. The animal was treated with another COX-2 inhibitor (deracoxib; Deramax, Novartis Animal Health); carprofen withheld for 4 days. The veterinarian performed surgery for a ruptured anterior cruciate ligament. The bitch was discharged and given a course of carprofen for 5 days BID. The dog was treated with carprofen for 19 days total.

At the end of the second course of carprofen, the bitch was presented by the owner. The bitch was unwell, constipated, icteric, and vomiting. A seroma developed at the

surgery site. The veterinarian hospitalized the animal and administered amoxycillin, prednisolone, and cimetidine. After four days the bitch appeared improved and the veterinarian planned to send her home. At that time she was vomiting but less icteric. The following day icterus was marked and petechial hemorrhages developed in mucous membranes. The veterinarian gave her half a unit of blood, the bitch arrested and was revived, but never regained consciousness.

Gross Pathology: The submitting veterinarian reported the presence of nutmeg liver, and blood in the peritoneal cavity, omental bursa, mesentery and bowel.   Internal hemorrhage was the presumed cause of death.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Hepatocellular necrosis, subacute, multifocal, periportal and centrilobular, with hemorrhage and lymphocytic-histiocytic periportal hepatitis.

Contributor's Comment: The presumed cause of subacute hepatopathy in this dog is idiosyncratic carprofen-associated hepatocellular toxicosis. The basis of the diagnosis is a temporal association following recent administration of carprofen and histological lesions consistent with toxic hepatopathy. The dog's breed (Labrador retriever cross) may be significant. Thirteen of 21 cases of carprofen hepatocellular toxicosis in one report occurred in the Labrador breed.[1]

   Histological changes of variable severity were reported in liver in association with carprofen administration.[1] They included multifocal to extensive hepatocellular necrosis, periportal neutrophilic and lymphocytic inflammation, bridging fibrosis, biliary hyperplasia, intracanalicular and hepatocellular bile pigment accumulation, and extramedullary hematopoiesis. Four of 21 dogs in that study died 3 - 5 days after presentation. In addition to hepatocellular necrosis, one of two dogs examined post-mortem had multifocal renal tubular necrosis with regeneration and a perforating jejunal ulcer. Only liver was available from this dog, so the presence or absence of lesions in other tissues could not be documented histologically.

   Carprofen (Rimadyl) is a non-steroidal anti-inflammatory drug (NSAID) in the propionic acid class. It is widely used of osteoarthritic and post-operative pain. The compound is a substituted carbazole, 6-chloro-a-methyl-9H-carbazole-2-acetic acid ($C_{15}H_{12}ClNO_2$) that inhibits cyclooxygenase activity, particularly the inducible cyclooxygenase COX-2. It is rapidly and nearly completely absorbed (>90% bioavailable) when administered orally, with peak blood plasma concentrations 1-3 hours after oral administration. It has a half-life of ~8 hours. It is eliminated primarily by biotransformation in liver followed by rapid excretion of metabolites in feces (70-80%) and urine (10-20%). Some enterohepatic circulation of the drug occurs. The manufacturer (Pfizer) has reported animal safety studies and adverse reactions.[2]   The most common adverse clinical reactions are vomiting (4%), diarrhea (4%), changes in

appetite (3%), lethargy (1.4%), behavioral changes (1%), and constipation (0.3%). Hepatopathy was not consistently reproduced in safety studies.

This dog was also treated with another NSAID, deracoxib. Hepatotoxicity is a rare adverse reaction associated with this drug.[3] The manufacturers recommend that deracoxib should not be given at the same time as other NSAIDs, including carprofen. Concurrent administration was not done in this instance.

The basis for hepatotoxicity in a small proportion of dogs given carprofen is unknown. The most likely explanation is an idiosyncratic toxic reaction. The drug was sold on the European continent for 10 years before an association was detected in North America between use of the drug and hepatic disease. A genetic feature unique to some Labrador bloodlines in North America might be an explanation, but that possibility was not pursued due to limited pedigree data.

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AFIP Diagnosis: Liver: Necrosis, Rappaport zone 3, diffuse, with mild subacute hepatitis and intrahistiocytic hemosiderin, German shepherd x Labrador retriever cross, canine.

Conference Comment: This case was reviewed in consultation with the Armed Forces Institute of Pathology's Hepatic Pathology department. We recognize that most of the necrosis is in the centrilobular area, but prefer to categorize it as zone 3 because the pathologic processes in this case correspond better to acinar landmarks based on the three-dimensional physiologic unit rather than the landmarks of the classic lobule, which are an artifact of two-dimensional microscopic sections. Based on the acinus described by Rappaport, zone 3 is mostly centrilobular but in some planes of the two-dimensional section it can also be periportal.[4]

Conference attendees noted the prominent cholestasis, as verified by a Hall's stain. Perl's iron stain also revealed abundant intracytoplasmic hemosiderin.

Although serum chemistries were not reported in this case, conference attendees discussed possible laboratory abnormalities with liver disease. The hepatocellular leakage enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and sorbitol dehydrogenase (SDH) are released with damage to the hepatocyte. The serum activity of these enzymes is dependent upon the number of hepatocytes injured, the severity of the injury, and the half-life of the enzyme. The inducible enzymes alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) are membrane-bound and increased activity is seen with cholestasis, or when induced by certain drugs or hormones.[5]

The cytosolic enzyme ALT may be elevated with liver or muscle damage, or may be induced by drugs. This enzyme is not useful in horses, ruminants, pigs, or birds due to low activity in these species. Aspartate aminotransferase is both a cytosolic and

mitochondrial enzyme, although more severe cellular injury is necessary for release of the mitochondrial isoenzyme. Like ALT, AST is not liver specific. It may be elevated in muscle disease or with in vivo or in vitro hemolysis since it is present in erythrocytes. Sorbitol dehydrogenase is liver specific in all animals studied, and is generally the enzyme of choice in horses, sheep, goats, and cattle.[5]

Alkaline phosphatase is bound to the plasma membrane of hepatocytes and biliary epithelium and is present in several isoenzyme forms, the most clinically important of which are hepatic, bone, intestinal, placental, and corticosteroid. The intestinal and placental isoenzymes do not contribute significantly to serum ALP activity because of their short half-life (less than 6 minutes in the dog). The bone isoenzyme has increased activity in young growing animals, animals with lytic or proliferative bone lesions, or those with active bone resorption. The liver isoenzyme is specific for liver disease and is a sensitive indicator of cholestasis. Cats have a lower hepatic ALP activity and shorter half-life than dogs. Increases in ALP activity with cholestasis in cats are less dramatic than increases in GGT, except with hepatic lipidosis where ALP increases more dramatically than GGT. The corticosteroid isoenzyme is only present in the dog and has increased activity with exogenous and endogenous corticosteroids. Initially, the hepatic isoenzyme increases with corticosteroid treatment but eventually the corticosteroid isoenzyme, which increases more gradually, becomes the predominant form of ALP. ALP is not a sensitive indicator of liver disease in large animals because it has a wide reference interval in these species.[5]

Gamma glutamyl transferase activity is induced in cholestasis, and is the preferred indicator of cholestasis in large animals and birds. It is generally a more specific indicator of cholestasis than ALP, but can also be induced by corticosteroids in dogs. Its activity is also high in the colostrum of dogs, sheep, and cattle so neonates have a very high serum GGT, which may be a useful indicator of passive transfer.[5]

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SLIDE 29
CONFERENCE 8 / CASE I – 03-1198 (AFIP 2887461)

Signalment: 5 months, female, Large White X Landrace, *Sus scrofa,* porcine.

History: Between December 31, 2002 and January 2, 2003, thirty-one gilts, approximately 5 months of age died and 300 were lethargic and/or febrile in a group of 988. This group of gilts entered this barn as 2 _ month old pigs in mid-October, 2002. There had been no new animal introductions since that time. The barn was one of three on a site with each barn containing approximately 1000 pigs. The pigs were considered to be negative for PRRSV and *Mycoplasma hyopneumoniae* as determined by repeated serological testing of the pigs themselves and the source herd. Prior to this severe death loss, there was only minimal death loss (less than 1.4% total from all three barns) due to swine influenza virus (H1N1) and co-infections with type 2 circovirus, *Streptococcus suis,* and *Pasteurella multocida*. A total of 182 pigs died (18.4%) in this one barn with 159 deaths (87% of total death loss) occurring between January 2, 2003 and January 10, 2003.

Gross Pathology: The entire remains of six gilts were submitted. All pigs had cloudy meninges and heavy, red, and wet lungs. Abundant fibrin covered the pleura and pericardium of one pig.

Laboratory Results: Aerobic culture of the meninges resulted in a pure or predominant growth of *Haemophilus parasuis* from all 6 pigs. The *Haemophilus parasuis* isolate was typed by enterobacterial repetitive intergeneic consensus based-polymerase chain reaction (ERIC-PCR) technique as serovar 2. *Haemophilus parasuis* was also isolated from the pleural and pericardial swab of one pig, the lungs of all 6 pigs, and in mixed growth with *Pasteurella multocida* from the lungs of 3 pigs. A pooled tissue homogenate was positive for type 2 circovirus by polymerase chain reaction (PCR). Reverse-transcriptase PCR tests of the pooled tissues were negative for influenza A virus, PRRSV (North American and European strains), and pestivirus. A PCR test of a pooled bronchial swab was negative for *Mycoplasma hyopneumoniae*. Fluorescent antibody examinations of the tonsils were negative for pseudorabies virus. Virus isolation attempts for porcine enterovirus, PRRSV and pseudorabies virus were negative after passages on PK-15, MARC-145, PAM, and BT cells. Liver mineral analyses revealed no evidence of excessive or deficient levels of arsenic, cadmium, cobalt, copper, iron, magnesium, manganese, molybdenum, lead, selenium or zinc.

Contributor's Morphologic Diagnosis: Meningitis, subacute, severe, diffuse, fibrinopurulent, with encephalitis, subacute, moderate, multifocal, perivascular, lymphoplasmacytic and histiocytic, *Haemophilus parasuis*.

Contributor's Comment: *Haemophilus parasuis* is a common bacterial infection in swine and causes polyserositis (Glasser's disease) primarily in pigs 4 to 8 weeks of age. The severity of disease in this older age pig is remarkable but has been reported in high-health status herds populated with naive pigs[1]. *Haemophilus parasuis* is a gram-negative, non-spore-forming, nonmotile, microaerophilic, rod-shaped bacteria requiring heme and/or nicotinamide adenine dinucleotide (NAD) factors for growth[2]. There are 15 serovars recognized[2,3] with much heterogeneity within and between serovars. Virulence is associated with serovar type but also with not yet clearly classified capsule and external membrane proteins. There is evidence that certain *Haemophilus parasuis* isolates of similar genotype as determined by ERIC-PCR have tropism for the brain[1]. *Haemophilus parasuis* is a frequent co-infection or opportunistic pathogen with other bacterial agents and viruses and the presence of multiple pathogens increases the severity of the disease[3]. In this case, at the time of severe death loss and illness, both *Pasteurella multocida* and type 2 circovirus were identified along with the *Haemophilus parasuis. Pasteurella multocida* is normal flora of the swine respiratory tract and its presence in the lungs is not surprising. The co-infection with type 2 circovirus is intriguing. However, it is important to note that there was no evidence of pathology associated with type 2 circovirus (histiocytic/ granulomatous lymphadenitis, lymphoid depletion, or interstitial pneumonia) noted in any pig from this case. Control and prevention of Glasser's disease is through the use of commercial vaccines, autogenous vaccines derived from homologous strains from the affected herd found to cause systemic disease, and through water and feed medication.

AFIP Diagnosis: Brain, leptomeninges: Meningitis, lymphocytic and neutrophilic, subacute, diffuse, moderate, with abundant fibrin, Large White cross Landrace, porcine.

Conference Comment: Glasser's disease causes severe meningitis, polyserositis, and/or polyarthritis in young pigs following a stressful episode. The classical gross finding with *Haemophilus parasuis* infection is fibrinous polyserositis, arthritis, and meningitis. The primary differential diagnoses for fibrinous serositis in pigs are *Mycoplasma hyorhinis*, *Streptococcus suis* type II, and septicemic salmonellosis. Like *Haemophilus parasuis*, *Mycoplasma hyorhinis* causes polyarthritis, but meningitis is usually not a feature of mycoplasmal infection. If meningitis is present, it is mild with lymphocytic inflammation. In addition to purulent meningitis and polyarthritis, *S. suis* type II can also cause endocarditis. *Streptococcus suis* type II is zoonotic and can cause meningitis with residual deafness or septic shock and death in humans.[4,5]

In this case, lymphocytes and neutrophils within the subarachnoid space multifocally extend into and expand Virchow-Robin space. The significance of the isolation of type 2 circovirus is unclear.

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SLIDES 30 and 31
CONFERENCE 8 / CASE II - C03-0733 (AFIP 2887161)

Signalment: 1-year-old Male, C.B-17-SCID-Beige mouse (*Mus musculus)*.

History: Streptozotocin was administered to induce diabetes mellitus at 6 months of age. Two months later, a single cell suspension of human islet cells was xenografted beneath the renal capsule. Four months later, the mouse developed an enlarged abdomen, dyspnea, ruffled haircoat, and a bluish tinge to the ventral abdominal skin.

Gross Pathology: The abdomen was enlarged due to approximately 2 ml of serosanguineous peritoneal fluid (Figures 1 and 2). The liver was also mottled tan and granular (Figure 3).

Laboratory Results:
WBC: 12.0 K/μL
Neutrophils: 72.2%
Lymphocytes: 6.2%
Monocytes: 16.8%
Eosinophils: 0.3%
Basophils: 4.5%
RBC: 7.66 M/μL
Hemoglobin: 9.56 g/dL
Hematocrit: 27.5%
Platelets: 2538 K/μL

Serum protein: <1 gm/dL
Serum glucose: 217 mg/dL

Negative by ELISA for: Mouse Hepatitis Virus, Sendai Virus, Mouse Encephalomyelitis
Virus (strain GDVII), Pneumonia Virus of Mice, Minute Virus of Mice, Epizootic Diarrhea
of Infant Mice Rotavirus, and *Mycoplasma pulmonis*.

PCR on formalin-fixed liver was performed by the Animal Diagnostic Laboratory,
College of Veterinary Medicine, Cornell University for *Mycobacterium avium* complex,
M. avium subsp. *avium*, and *M. bovis* or *tuberculosis*, all of which were negative. PCR
for *Mycobacterium tuberculosis* complex on formalin-fixed liver was also performed by
Focus Technologies (Cypress, CA). *M. tuberculosis* complex DNA was not detected;
however, the efficacy of the Amplicor MTB test has not been determined for non-
respiratory (sputum) samples.

Contributor's Morphologic Diagnosis: Liver, hepatitis, granulomatous, multifocal to
widespread, marked with intrahistiocytic bacilli, hepatocellular loss and micronodular
regenerative hyperplasia.

Contributor's Comment: Multifocal to coalescing aggregates of epithelioid
macrophages replace normal hepatic cords throughout the sections. These
macrophages contain myriad bacilli, which are gram-positive (Brown and Brenn stain,
not included) and acid-fast-positive (Ziehl-Neelsen, included). Macrophages containing
bacilli were also found in the lungs, submandibular lymph node, adrenal gland, kidneys,
and spleen. Immunohistochemistry (IHC) was performed on paraffin sections of liver
using a commercially available (DAKO), rabbit anti-human *Mycobacterium* sp. antibody,
by Dr. Fabio del Piero in the Department of Pathobiology at the University of
Pennsylvania, School of Veterinary Medicine. Macrophages throughout the liver were
strongly immunopositive for *Mycobacterium* sp. (Figure 4). Additional lesions in the liver
include an irregular capsular surface due to hepatocellular loss and micronodular
regenerative hyperplasia, as well as extramedullary hematopoiesis characterized by
aggregates of myeloid cells (neutrophils) at various stages of maturity around blood
vessels and within sinusoids.

Mycobacteria belong to the order Actinomycetales, which also includes the genera
Actinomyces, *Nocardia*, *Rhodococcus*, *Corynebacterium*, *Dermatophilus* and
Streptomyces. There are numerous species of *Mycobacterium,* which are pathogenic
for humans and animals. Classically, tuberculosis is caused by a group of mycobacteria
referred to as the *Mycobacterium tuberculosis* complex (MTC), which includes *M.
tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*[1]. The characteristic lesion caused
by these organisms is the tubercle, a classic granuloma composed of epithelioid
macrophages and multinucleated giant cells surrounded by a rim of fibroblasts.
Depending on the host's immune response, necrosis, calcification and ancillary
inflammatory cells such as lymphocytes might also be present[1]. *M. avium* and *M.
intracellulare* are closely related and comprise the *M. avium-intracellulare* complex
(MAIC)[1]. *M. avium* includes *M. avium* subsp. *avium* and *M. avium* subsp. *silvaticum*,

originally bird pathogens, as well as *M. avium* subsp. *paratuberculosis*, the cause of Johne's disease in ruminants and the purported cause of Crohn's disease in humans[1]. MAIC has also recently emerged as a significant cause of morbidity and mortality in immunocompromised humans and animals. The MAIC lesion differs from that of MTC in that sheets of epithelioid macrophages contain myriad organisms[1]. Leprosy in humans is caused by *M. leprae*, while feline and murine leprosy is caused by *M. lepraemurium,* both of which are characterized as either lepromatous with large numbers of lipid-laden macrophages containing large numbers of bacilli or tuberculoid[1]. Finally, other saprophytic and opportunistic mycobacteria such as *M. kansasii*, *M. fortuitum*, *M. smegmatis*, *M. xenopi*, *M. chelonae*, *M. thermoresistable* and *M. phlei* are considered atypical mycobacteria, generally causing cutaneous or subcutaneous to disseminated infections in which filamentous or beaded organisms are found within lipid vacuoles[1].

Paramount to the pathogenesis of mycobacterial infections is the ability of the organism to survive and replicate within the host's macrophage. This is made possible by numerous virulence factors residing in the cell wall, which result in biological activities such as inhibition of phagosome-lysosome fusion, scavenging of oxygen radicals and altered cytokine secretion among others[1]. CD4+ T cells and their secretion of interferon-γ (IFN-γ) are also important for macrophage activation and subsequent killing of the mycobacteria[1].

Although mice are used as experimental models of mycobacterial infections, naturally occurring infections are rare[2]. Sixty-three percent of C57BL/6N mice developed lesions due to MAIC while C3H/HeN and B6C3F1 mice as well as F344 rats housed in the same room did not. The animals appeared healthy; however lesions were found in the lungs, livers, spleens and mesenteric lymph nodes. Experiments were conducted to fulfill Koch's postulates and elucidate the pathogenesis of this naturally occurring outbreak[3]. It was determined that the most likely source of MAIC in the outbreak was the water since the water source for affected animals differed from unaffected animals, and direct transmission could not be demonstrated.

In this case, the histology of the multisystemic lesions was compatible with mycobacteriosis and confirmed by IHC. The gram-positive acid-fast organisms most likely represent MAIC but this could not be confirmed by PCR. SCID-beige mice, which were used in this experimental protocol, are immunocompromised. SCID mice are homozygous for the *Prkdc^scid* (protein kinase, DNA activated, catalytic polypeptide) mutation, resulting in a defect in V(D)J recombination and therefore a lack of T and B lymphocytes and low to undetectable immunoglobulin levels[4]. Beige mice, homologous to humans with Chediak-Higashi syndrome, have defective cytotoxic T cells and macrophages, as well as impaired natural killer cell function due to abnormally large lysosomal granules in all granule-containing cells[5]. The double mutation makes SCID-beige mice susceptible to a variety of opportunistic pathogens; however no other animals from this experimental group have been affected to date. The source of the organisms in this mouse is not apparent but is likely from the environment since MAIC

organisms have been isolated from soil, water and sawdust[1], as well as sporadic canaries and finches housed in our facilities.

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AFIP Diagnosis:
1. Liver: Hepatitis, granulomatous, multifocal to coalescing, moderate, with intrahistiocytic acid-fast bacilli, C.B-17-SCID-Beige mouse, rodent.
2. Liver: Nodular hyperplasia, multifocal, moderate, with biliary hyperplasia and hepatocyte atypia.
3. Liver: Granulocytic extramedullary hematopoiesis.

Conference Comment: The contributor provides an excellent review of mycobacteriosis. Conference attendees discussed the prominent nodular hyperplasia and atypical hepatocytes characterized by cytomegaly, karyomegaly, and multinucleated hepatocytes. Phone consultation with the contributor verified that this was the only animal to demonstrate these lesions, therefore these changes are not thought to be related to the experimental regimen but may be a response to the inflammation. Conference attendees also noted numerous intravascular bacilli that, upon further investigation, are gram-positive. Post-mortem overgrowth was considered, but ruled out based on the fact that the animal was euthanized via carbon dioxide and immediately necropsied. This may represent a terminal septicemia.

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SLIDE 32
CONFERENCE 8 / CASE III - 03060007 (AFIP 2890741)

Signalment: Adult (exact age unknown) male black-tailed prairie dog (*Cynomys ludovicianus*).

History: An entire group of 15 prairie dogs had become ill. Ten were dead. The prairie dogs exhibit ocular discharge, swollen eyelids, coughing, sneezing, wasting away, ulcers on tongue and death.

Gross Pathology: The patient has yellow mucoid material along the eyelids. There is a small, 0.3cm in diameter ulcer in the center of the tongue. The lungs exhibit bilateral, randomly distributed foci of pneumonia. These foci were deep red, firm and wet on cut surface. All total, 40-50% of the lung was affected.

Laboratory Results: CDC reports that tissues are positive by PCR for monkeypox virus. Also, immunohistochemistry is positive on formalin fixed lung, heart, lymph node and conjunctiva for monkeypox virus.

Contributor's Morphologic Diagnosis: Severe necrotizing bronchointerstitial pneumonia with syncytia.

Contributor's Comment: Submission of this patient preceded the reports of the monkeypox outbreak in humans and prairie dogs in the United States Midwest in early June. The prairie dog originated in Illinois from a pet distributor that also kept Gambian rats (*Cricetomys gambianus*) that were also reportedly ill. In addition, the pet distributor and his wife exhibited flu-like symptoms, but did not have any skin lesions. The referring veterinarian suspected tularemia (*Francisella tularensis*).
Histological examination (esp. the syncytia) suggested a viral infection. While attempts were being made to define the etiology, reports of the monkeypox outbreak surfaced and tissues were sent to the CDC where monkeypox was confirmed by PCR and immunohistochemistry.

As of this writing, most of the literature is focused on the virology and epidemiology of the disease with scant information on gross pathology or histologic lesions in prairie dogs. Human infections have been linked to prairie dogs that in turn appear to have been exposed from sick Gambian rats.[1,2] Apologies in advance for some of the "smudge" artifact present in the current submission. This is attributed to intrathoracic administration of euthanasia solution. However, the histological features of the pneumonia and numerous syncytial cells are still clearly present.

AFIP Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing, subacute, diffuse, severe, with vasculitis, syncytial cells, and eosinophilic intracytoplasmic inclusion bodies, black-tailed prairie dog (*Cynomys ludovicianus*), rodent.

Conference Comment: This case was sent to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland for immunohistochemical staining. Researchers there used a polyclonal anti-vaccinia antibody that cross-reacts with monkeypox, cowpox, camelpox, and smallpox viral antigen. The submitted section of lung is immunohistochemically strongly positive for orthopoxviral antigen within pulmonary macrophages and respiratory epithelial cells.

Monkeypox is a member of the Orthopoxvirus genus, and, until the spring of 2003 when it appeared in Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, had primarily caused disease in parts of Central and Western Africa. Wild squirrels have been implicated as reservoirs in Africa, whereas pet prairie dogs and a giant Gambian rat were identified as the source of the U.S. Midwest outbreak.[1] There is very little information in the published literature on the disease in prairie dogs, but it is well described in cynomolgus macaques causing, among many lesions, fibrinonecrotic bronchopneumonia with necrotizing vasculitis[3].

Other orthopoxviruses of veterinary importance include cowpox, camelpox, and ectromelia (mousepox). Cowpox causes disease in cows, domestic cats, humans, and zoo animals, including large felids, elephants, and rhinoceroses. Despite its name, cowpox is not endemic in cattle. It is usually self-limiting, causing lesions on the teats and udder. Cats may have more severe disease than humans or cattle, with systemic disease accompanying maculopapular eruptions in immunocompromised individuals. Camelpox causes typical pox lesions primarily concentrated around mucocutaneous junctions, but may cause more generalized skin lesions in young camelids. Ectromelia virus is of significant economic importance in mouse laboratory colonies. It causes either a rapidly fatal form of the disease, or a chronic form with ulceration, necrosis, and loss of the extremities and tail.[4,5]

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    Also refer to updates through CDC Morbidity and Mortality Weekly Report (MMWR) at: http://www.cdc.gov/mmwr/

SLIDE 33
CONFERENCE 8 / CASE IV - 02N427 (AFIP 2888046)

Signalment: 5 1/2-year-old Arab gelding.

History: Boomer presented to the VMTH on Thursday (10/10/02) evening after a 36-hour history of lateral recumbency. He had been seen by his referring DVM on Wednesday (10/09/02), and was prescribed prednisone and trimethoprim sulfa (TMS). Upon presentation, he was found to be comatose, dehydrated, with absent pupillary light reflexes and icteric oral mucous membranes. His lung sounds were normal. He was given 15 liters of Plasmalyte and 1 liter of hypertonic saline via a jugular catheter, and then transported to the recovery stall. After stabilizing him in a sling, he was treated overnight with IV fluids, thiamin, DMSO, gentacin and potassium penicillin. At 8:00 am on 10/11/02, he was found to be tachypneic, icteric, and tachycardic. Lung sounds were absent in his left lung fields. His fluids were stopped and he was administered two boluses of furosemide. His respiratory rate decreased slightly over 30 minutes, but his breathing became agonal shortly thereafter, and he expired quietly.

Gross Pathology:
1. Lungs:
    a. Moderate to severe pulmonary edema
    b. Moderate pulmonary emphysema
    c. Multifocal atelectasis
2. Urinary bladder: Multifocal mucosal petechiation
3. Integument: Multifocal abrasions
4. Brain: Multifocal petechiation (cut surface following fixation)

Laboratory Results:
Clinical Pathology Findings:
CBC: Lymphopenia (420/μl) with a slight left shift (140/μl)
Chemistry Panel:
        Hyperglycemia (223 mg/dl)
        Elevated BUN (49 mg/dl), creatinine (2.3 mg/dl)
        Elevated AST (776 μl/ml), LDH (1630 μl/ml), total bilirubin (4.5 mg/dl), ALT (47 μl/ml), Alk phos (502 μl/ml), total protein (8.1 gm/dl), CK (6990 μl/ml)
        Slightly low Na (134 mmol/l) and K (mmol/l), Total CO2 20 mmol/l, pH 7.245,

pCO2 = 66.1 mm Hg., pO2 = 39.3 mm Hg

CSF: Clear, colorless; no RBC seen; TP = 143.2 mg/dl
       TNCC = 48/μl (2% PMN, 47% lymphocytes, 51 % macrophages)
       Nondegenerate neutrophils, macrophages frequently vacuolated and
          occasionally contain cytoplasmic leukocytes
       Interpretation: mononuclear pleocytosis

Virology:
Rabies (-) IFA
West Nile Virus (-) PCR
EEE Viral RNA RT-PCR (+) & Virus Isolation (+)

Contributor's Morphologic Diagnosis: Moderate-to-severe multifocal
necrosuppurative and hemorrhagic vasculitis and meningoencephalitis with thrombosis.

Contributor's Comment: Lesions present are widespread, and disseminated
throughout most if not all sections of brain. The lesions consist of meningeal infiltrates
of variable intensity made up of plasma cells, lymphocytes and localized areas that
have neutrophils. Meningeal vessels are moderately congested and/or blood-filled.
Additionally, all blood vessels throughout all sections of the whole brain are congested
and blood-filled with focal areas of perivascular hemorrhage. Surrounding some blood
vessels and oftentimes extending into the adjacent parenchyma there are variable, and
oftentimes large numbers of lymphocytes, plasma cells, macrophages and neutrophils.
Numerous blood vessels contain this mixed inflammatory cell infiltrate within their walls.
Additionally, several exhibit fibrinoid degeneration and thrombosis. Throughout the
parenchyma in most sections, both white and gray matter, there are focal areas of
hemorrhage with necrosis and gliosis. Many of these foci also have degenerative
neutrophils. All major anatomical regions of the brain are affected to some degree.

Eastern Equine Encephalitis Virus
Equine Arboviral encephalomyelitis - member of the antigenic alpha viruses in the family
Togaviridae, a single-stranded, enveloped RNA virus.[1,2.]

TRANSMISSION - Primarily enzootic cycle with a wild bird reservoir, and mosquito
vector (*Culiseta melanura*) that feeds on small birds that serve as maintenance and
amplifying hosts; with environmental and biological changes, spills over into other
species of mosquitoes that feed on mammals at which time equine and human cases
can be seen. Viremia level in people and most horses are sufficiently small such that
they are considered dead end hosts.[1]

ZOONOTIC POTENTIAL - Major public health threat, although incidence is lower than
other viral encephalitides; severe, often fatal encephalitis in people during summer and
fall months with high mosquito densities. Severity of disease greatest in youngest (<10
years) often progressing to death; least in people 10-50 years of age, and the oldest
group (>50 years) either had a good outcome or died. Deaths in horses or wild birds
usually precede disease in people.[3]

OCCURRENCE - [young more susceptible] Mainly Atlantic & Gulf coasts & Caribbean. Following infection, horses may 1) develop inapparent infection, 2) develop viremia and high fever that resolves without CNS disease, or 3) develop clinical neurologic disease.[2]

EQUINE MORTALITY - lethal in up to 90% of the cases[4]

EQUINE CLINICAL SIGNS: Inapparent or systemic illness or CNS [reflects cerebro cortical damage] - Sensory changes, depression, cortical blindness, increased movements progressing to ataxia, circling, head pressing, hyperexcitability, recumbency, death

GROSS LESIONS: Usually none

MICROSCOPIC FINDINGS: [Grey matter, most marked cerebral cortices, thalamus, hypothalamus, milder caudally] Largely neutrophilic inflammation; microgliosis;+/- malacia w/gitter; vasculitis, thrombosis.

DIAGNOSIS: Clinical findings, epidemiological data, histopathology, serology, viral isolation, PCR.

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AFIP Diagnosis: Brain: Encephalitis, necrotizing, neutrophilic, diffuse, moderate to severe, with gliosis, vasculitis, hemorrhage, and thrombosis, Arab, equine.

Conference Comment: There are numerous viral causes of equine encephalomyelitis, among which are the Alphaviruses [Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), and Venezuelan equine encephalomyelitis (VEE)]; the Flaviviruses (West Nile virus and Japanese encephalitis virus); Rhabdovirus (rabies virus); and equine herpesvirus-1 (EHV-1).

Histologically, EEE, WEE, and VEE cause lesions predominantly in the gray matter and most severely in the cerebral cortex, thalamus, and hypothalamus, consisting of neuronal degeneration, neuronophagia, microgliosis, and perivascular and parenchymal inflammation. In EEE, the inflammatory response is mostly neutrophilic; in VEE it is a mixture of neutrophils and lymphocytes; and in WEE, it is mostly nonsuppurative. Vasculitis and thrombosis may also be present.[4,7]

West Nile virus primarily involves the ventral and lateral horns of the thoracic and lumbar spinal cord and lower brain stem, with perivascular lymphocytic infiltrates, neuronal degeneration, neuronophagia, and hemorrhage.[5,7]

Japanese encephalitis virus initially causes neutrophilic leptomeningitis and encephalitis that becomes nonsuppurative. It targets neurons and causes neuronal degeneration and necrosis.[6] Rabies virus causes nonsuppurative meningoencephalitis

with intraneuronal Negri bodies, but neuronal necrosis is rare. Equine herpesvirus-1 is not neurotropic but causes vasculitis, with secondary lesions in the neuroparenchyma resulting from infarcts.[4,7]

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SLIDE 34
CONFERENCE 9 / CASE I – 02-1657 (AFIP 2888700)

Signalment: Equine, Standardbred filly, 8 days of age.

History: The filly was presented with a 5-6 day history of patent urachus, bloody urine, and a bloody vaginal discharge. Left fetlock lameness was observed on the day of euthanasia.

Gross Pathology: Suppurative osteomyelitis and periostitis with osteolysis and necrosis of bone and cartilage of the left rear cannon bone, with extension into the fetlock joint (suppurative arthritis), were observed. A patent urachus with chronic fibrinopurulent and necrotizing omphalitis with fibrosis was also present. The right rear cannon bone had a well-demarcated focus of thickened distal physeal cartilage along the dorsal and medial aspect, which resulted in a cartilaginous lesion 3mm in width and 2mm in depth, as compared to 1mm depth in the remaining physis.

Laboratory Results: None provided.

Contributor's Morphologic Diagnosis: Metatarsus: Focal retention of physeal cartilage with metaphyseal infraction.

Contributor's Comment: Evaluation of the distal right metatarsal bone was done for comparison with the severely affected left. Lesions in the right metatarsal were considered clinically incidental. The focal retention of growth cartilage in the right metatarsal bone (delayed endochondral ossification) is consistent with the gross lesions seen in the clinical entity of physitis/epiphysitis of horses[1,2]. Such lesions of focal retention of growth cartilage can be secondary to any lesion that would interrupt the vascular penetration of the growth plate such as trauma or inflammation, or represent primary lesions of cartilage maturation (dysplasia of osteochondrosis). In this case, the focal retention of growth cartilage is interpreted to be secondary to trauma with infraction of primary trabecular bone. This interpretation is supported by the absence of cellular inflammation and the presence of irregularly arranged and fragmented cartilage cores (infractions) on the metaphyseal side of the lesion without presence of normal primary trabeculae. The presence of the cartilage cores indicates that cartilage mineralization and chondrocyte death had proceeded normally prior to lesion development. While trabecular infractions could be secondary to primary disorders of endochondral ossification, work in pigs has suggested that infractions are common causes of focal failure of endochondral ossification[3].

AFIP Diagnosis: Metatarsus: Retained physeal cartilage, focal, with infraction of primary trabeculae, Standardbred, equine.

Conference Comment: Conference attendees discussed two possible pathogeneses for this lesion. The first is failure to resorb physeal cartilage, resulting in retention of the physis and a microenvironment prone to fractures. The second is an acquired problem, most likely trauma. Regardless of the cause, the end result is disruption of the vascular supply to the metaphyseal-diaphyseal junction. Invasion of capillary loops through the transverse septa and into the tubes of mineralization surrounding the hypertrophied chondrocytes of the growth plate is essential for modeling of the primary spongiosa. The absence of these vessels (secondary to fractures and fibrosis) results in failure to model and mineralize the growth plate and culminates with retention of cartilage within the growth plate (i.e. formation of "cartilage cores"). Like the contributor, conference attendees speculate that the latter pathogenesis is more likely.

Discussion focused on the cut back zone at the proximal metaphysis, where primary and secondary trabeculae are remodeled to form cortical bone. Cortical bone is normally decreased or absent in this area due to physiological remodeling, so the thin cortical bone should not be misinterpreted as pathologic. Another physiologically normal change is the chondrification of blood vessels in the growth plate. After crossing

the growth plate, blood vessels normally undergo thrombosis and chondrification, so this also should not be misinterpreted as a pathologic change.

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SLIDE 35
CONFERENCE 9 / CASE II - 02-13490 (AFIP 2889976)

Signalment: 4-month-old, male, Suffolk, *Ovis aries*, ovine.

History: The lamb was born with kyphosis, and as the animal grew, he developed a "Roman" nose that became progressively more pronounced and angular limb deformities that worsened over time. At the time of presentation and euthanasia, the lamb had extreme difficulty walking and required special care and feeding. The lamb's twin was born with no musculoskeletal abnormalities and remained healthy.

Gross Pathology: The lamb was in good nutritional condition. The dorsal aspect of the skull and nose was rounded (Fig. 1). The distal aspect of the pelvic limbs had moderate to severe valgus deformity (Fig. 2). The thoracic limbs, especially the left thoracic limb, had mild varus deformity (Fig. 3). There was mild scoliosis and kyphosis of the vertebral column. The ventral aspect of the ribs, from the costochondral junctions to the sternum, and the sternum were flattened and bowl-shaped. On longitudinal section of the sternum, the sternum was undulating (Fig. 4) and the fourth and sixth sternebrae were replaced by multiple small pieces of bone surrounded by thick cartilage. The articular cartilage of the occipital condyles and atlas was roughened, dull, gray, and pitted. The occipital condyles were elongated (Fig. 5), and there was increased angulation of the occipital condyles. The width of the intercondyloid notch was decreased. On saggital section of the vertebrae, sternebrae, the occipital condyles, and the long bones of the left rear leg, the physes were irregular with multiple ossification centers and islands of cartilage in the primary and secondary cancellous bone (Fig. 6), cyst-like structures, and occasionally hemorrhage. There were no significant gross lesions in the thoracic viscera, abdominal viscera, or brain.

Laboratory Results: Postmortem radiographic examination of the sternum, skull and limbs was performed. The sternebrae were misshapen, misaligned and of irregular sizes (Fig. 7). The skull exhibited pronounced rounding of the dorsal silhouette (Fig. 8). The hind limbs had valgus deviation centered at the metatarsophalangeal joint (Fig. 9). There appeared to be widening of the physis of the distal metatarsal bone with areas of bony proliferation. Areas of radiolucency were consistent with multiple centers of ossification and the diagnosis of spider lamb syndrome.

Contributor's Morphologic Diagnosis: Vertebrae and long bones: Chondrodysplasia.

Contributor's Comment: Your section may have two vertebrae with an intervertebral joint or a section of a long bone. The physes of the vertebrae and long bones are multifocally widened by multiple islands of cartilage resulting in multiple ossification centers (Fig. 10). These islands of cartilage extend into the primary and secondary cancellous bone (Fig. 11) and occasionally the cortex. The islands of cartilage consist of disorganized clusters and rows of chondrocytes suspended in a chondroid matrix (Figs. 12, 13). The chondroid matrix is irregularly replaced by osteoid. There is occasional degeneration and necrosis of the islands of cartilage.

Spider lamb syndrome (ovine hereditary chondrodysplasia, spider lamb chondrodysplasia) is an autosomal recessive inherited trait in Suffolk and Hampshire sheep and their crossbreds[1,2]. A spectrum of gross lesions have been identified and include the following: tall lambs that are finely boned and poorly muscled with abnormally long legs and small heads, scoliosis, kyphosis, sternal deformities, a "Roman" nose, deviation of the nose, angular limb deformities, and, in all affected lambs, elongation of the occipital condyles leading to a decrease in the width of the intercondylar notch. Microscopically, there is nodular thickening of the cartilage in the centers of endochondral ossification in affected bones. This leads to multiple small ossification centers that may still be present in the metaphysis and impinge on the cortex. Occasionally, the affected cartilage can undergo degeneration. Irregularities of the cartilage columns in the ossification centers can occur.

The exact defect leading to spider lamb syndrome (SLS) is not known at this time. The abnormality has been localized to the distal end of ovine chromosome 6, and a defect in the gene encoding fibroblast growth factor receptor 3 (FGFR3) is believed to be the cause of SLS[3]. Fibroblast growth factor receptor 3 is included in a family of polypeptide receptors that contain a pair of cytoplasmic tyrosine kinase domains, three immunoglobulin-like domains, and a transmembrane domain.

Three types of human dwarfism (achondroplasia, hypochondroplasia, and thanatophoric dwarfism) are caused by mutations in FGFR3 [4,5], and mice lacking FGFR3 develop skeletal overgrowth and deafness[6]. Fibroblast growth factor receptor 3 is believed to be involved in the down regulation of chondrocyte growth in bone development[3,4,6,7,8]. The FGFR3 mutation in human dwarfism is believed to result in the continual activation of FGFR3 [4,8], while mutations disrupting the function of FGFR3 in

mice result in excessive proliferation of chondrocytes in the physis and skeletal overgrowth[6,7].

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AFIP Diagnosis: Vertebra (per contributor), bone and growth cartilage: Chondro-osseous dysplasia, diffuse, with osteopenia, Suffolk, ovine.

Conference Comment: The contributor provided a thorough discussion of spider lamb syndrome. Other congenital skeletal malformations in lambs may be caused by maternal ingestion of plant teratogens or administration of drugs during certain stages of gestation.

Ingestion of locoweed (*Astragalus* sp. or *Oxytropis* sp.) by pregnant ewes may cause arthrogryposis and limb rotation in lambs. Overdosing pregnant ewes with the anthelmintic parbendazole may cause compression or fusion of vertebral bodies and proximal ribs, curvature of long bones, and hypoplasia of articular surfaces in the lamb.[1] Ingestion of *Veratrum californicum* (skunk cabbage) by the dam around day 14 of gestation causes congenital cyclopean deformities, but other defects may occur depending on stage of gestation at plant ingestion. Ingestion around day 29 may cause shortening of metatarsal, metacarpal, and tarsal bones, medial bowing of forelimbs at the fetlock, and arthrogryposis.[1,9]

A syndrome known as bent-leg, or bowie, has been associated with ingestion of *Trachymene glaucifolia* (wild parsnip) by pregnant ewes in Australia and New Zealand. This disease affects the long bones of the forelimbs of lambs, causing flexion and lateral rotation of the knee joints and medial or lateral rotation of the fetlock joints. This has been reproduced experimentally by feeding a diet low in both calcium and phosphorus. Supplementation of the diet with phosphorus seems to reduce the incidence of disease.[1,9]

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SLIDE 36
CONFERENCE 9 / CASE III – 10629/93 (AFIP 2890204)

Signalment: 6-week-old Norwegian elkhound, female, canine.

History: Two out of four puppies in a litter were unable to move normally. These clinical signs appeared at an age of 3 to 4 weeks and deteriorated rapidly within the next couple of weeks. They were sacrificed at an age of 6 and 8 weeks, respectively. Autopsy of one of them revealed a skeletal disorder as the cause of the problems. The same parents produced a new litter where two out of four puppies developed the same clinical signs as in the two first cases. One normal and the two diseased puppies in this litter were sacrificed. The sections submitted are from one of the affected animals.

Gross Pathology: The chest cavity showed a conspicuous flat appearance with a marked reduction of the distance between the ventral part of the spine and the chest bone. Costochondral junctions were prominent. The costochondral reduction on the ribs was very prominent and consisted of a soft chondroid tissue. The limbs were moderately shorter than normal. The epiphyseal part of the tubular bones was enlarged with a club-like appearance. Cut sections through these areas revealed a soft chondroid tissue containing a bony core markedly reduced in size. The skull had a normal size. The length of the vertebral column was not reduced although a moderate increase of chondroid tissue was observed around the endplates.

Laboratory Results: The clinical chemistry revealed no abnormalities consistent with the skeletal deformities. All the values recorded in the puppies of the second litter were otherwise within normal limits.

Contributor's Morphologic Diagnosis: Chondrodysplasia.

Contributor's Comment: Chondrodysplasia in the Norwegian elkhound was originally described by Bingel and Sand in 1982.[1] These dogs had disproportionately short limbs where the front limbs might appear relatively shorter than the hind limbs. The body trunk was also shorter than normal. Histological examination revealed a disorganized endochondral ossification and a zone of chondrocyte proliferation, which was decreased in width. Chondrocytes in all zones contained inclusions. This type of chondrodysplasia appeared to be inherited as an autosomal recessive trait.[2] It may be added that the ability of these dwarfs to walk and stand was only slightly affected.

This description discloses both clinical and pathological differences from those demonstrated in the puppy of the present material. The age level of affected animals, the severity of the clinical signs and the type and distribution of macroscopical and microscopical lesions reveal that the present case represents a new type of chondrodysplasia in the elkhound.

Louw described in 1983 [3] chondrodysplasia in a litter of Bulldogs with some of the clinical and macroscopical features described in this case. More details regarding the puppies were, however, not reported.

A review of the morphological lesions in human skeletal dysplasia by Sillence et al. 1979 [4] reveals four disorders with significant lesions in the resting (reserve) zone. Hypercellularity in this zone as well as in other zones of the growth plate seem to be a conspicuous finding in two of them called achondrogenesis type 1 and 2. Enlarged and vacuolated chondrocytes with a reduced amount of intercellular matrix together with a disorganization of the endochondral ossification were also observed in these cases. The type 2 of achondrogenesis seems to be the most interesting disorder from a comparative point of view as it also has macroscopical and radiological findings which in many respects are similar to those observed in the elkhound puppy.

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AFIP Diagnosis: Long bone (site not specified), growth and articular cartilage: Chondrodysplasia, diffuse, with osteopenia, Norwegian Elkhound, canine.

Conference Comment: A differential diagnosis in dogs and cats with skeletal lesions and dwarfism should include lysosomal storage diseases. English Springer Spaniels with GM_1 gangliosidosis, a deficiency of beta-galactosidase, develop dwarfism, irregular intervertebral spaces, and degenerative articular changes in the femur and tibia. Mucopolysaccharidosis I (MPS I) is reported in Plott hounds and domestic shorthaired cats, causing facial and skeletal dysmorphia, corneal clouding, and cardiac valve insufficiency. Animals with MPS I have a deficiency in alpha L-iduronidase resulting in excess urinary excretion of heparin sulfate and dermatan sulfate. Mucopolysaccharidosis VI occurs in Siamese cats and manifests as skeletal abnormalities, including short stature, large paws, and facial dysmorphism. Affected cats have deficient activity of N-acetylgalactosamine 4-sulfatase and excrete dermatan

sulfate in their urine. In animals with MPS VI, stored mucopolysaccharides stain metachromatically with toluidine blue stain. There is no metachromatic staining of cells in animals affected by MPS I.[1,5,6]

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SLIDE 37
CONFERENCE 9 / CASE IV - P128-03 (AFIP 2892973)

Signalment: 11 months, neutered male, domestic shorthaired cat, "Tacos", feline.

History: Bilateral fractures of caput femoris, epiphyseal/metaphyseal border. No known trauma.
Radiology: Both columns of femur have inhomogenous decreased radiopacity, indicating lytic areas. Malalignment and a radiolucent line through the physis of both femoral heads indicate bilateral physeal fracture in the region. Both femoral heads have homogenous bone opacity. The mineralization in the skeleton appears normal with no sign of osteopenia. In conclusion, bilateral "Salter Harris-type" fractures with lytic changes in the column and no signs of osteopenia. Resection of the femoral heads was done and the specimens were sent for PAD.

Gross Pathology: The specimen comprised two femoral heads, both with a thick synovial membrane attached. Areas of dull frayed articular cartilage covering the periphery close to the synovial membrane attachments and part of the central femoral head could be seen.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Femoral head, bone necrosis and fibrous reactive tissue at the epiphyseal/metaphyseal border, remnants of growth plate cartilage, focal areas of fraying of articular cartilage, synovitis, chronic, proliferative, pannus-like, slipped capital femoral epiphysis, developing pseudoarthrosis, "spontaneous femoral capital epiphyseal fractures in the cat."

Contributor's Comment: Microscopically the femoral head is characterized by reactive fibrous tissue together with bone necrosis and remnants of the growth plate (the growth plate in the cat should close between 8-10 months of age). A synovitis with pannus-like properties was also found.

The lesions have similarities with slipped femoral epiphysis, Legg-Calve-Perthes disease, canine metaphyseal osteopathy, traumatic fracture of the femoral neck and osteomyelitis. However, the description of femoral neck metaphyseal osteopathy in the cat[2] is most compatible with the radiographic and microscopic lesions described above and these authors suggested a new idiopathic disease in cats.

Recently, similarities between "spontaneous femoral capital epiphyseal fracture"[1] and feline metaphyseal osteopathy have been discussed. It has been suggested that the metaphyseal osteopathy is a pseudoarthrosis formation following a spontaneous femoral capital epiphyseal fracture. The predominance of neutered male cats has led to the suggestions that a delay in closure of the growth plate and an immature vascular supply could predispose to the fractures[2].

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AFIP Diagnosis: Femoral head, physis/subphysis: Fracture, with fibrosis, woven bone formation, and synovial hyperplasia (callus), domestic shorthair, feline.

Conference Comment: Conference attendees discussed the paucity of cells relative to the thickness of the growth plate and that it may indicate delayed closure. The irregularity of the growth cartilage at the deep margin was determined to be a true change, based on separation and necrosis of the physeal cartilage and the presence of fibrous tissue growing perpendicular to the growth plate. Changes in the synovium include synoviocyte hyperplasia, and synovial hyperplasia and hypertrophy. Some slides demonstrate articular cartilage erosion.

It has been suggested that femoral capital physeal fractures may be a result of physeal dysplasia or delayed growth plate closure. The high proportion of gonadectomized cats noted in the literature supports the association with delayed growth plate closure and resulting increased susceptibility to spontaneous fracture. High body weight has been identified as an additional risk factor in these cats.[1]

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SLIDE 38
CONFERENCE 10 / CASE I – MVP1-03 (AFIP 2888424)

Signalment: 7 month old, male reindeer (*Rangifer tarandus*).

History: One of a group of 20 males (7-8 months of age) moved from Michigan to Ames, IA. Diarrhea was noted in 5 of 20 reindeer. Diarrhea persisted for 5-7 days. This animal, in which diarrhea was not observed, was found dead.

Gross Pathology: Variably sized ulcers (3-10 mm) were present on the nares, tongue, larynx, and rumen. Through the cecum and proximal colon were segmental areas of necrosis and hemorrhage with ulcerated areas covered by a fibrinonecrotic membrane. Ileocolic and cecocolic lymph nodes were markedly enlarged, edematous and hemorrhagic. Numerous petechiae were noted in both renal cortices. Although diarrhea had not been reported prior to death, the perineal area was stained with liquid fecal material.

Laboratory Results: Bacteriologic culture of cecum and cecocolic lymph node: *E. coli* (non-toxigenic), *Clostridium perfringens* type A.
PCR for Ovine Herpesvirus-2 (kidney): positive
PCR for BVD virus (cecocolic lymph node): negative

Contributor's Morphologic Diagnosis: Kidney: Vasculitis and perivasculitis, lymphocytic, multifocal, moderate with periglomerular interstitial nephritis and multifocal tubular mineralization, reindeer (*Rangifer tarandus*).

Contributor's Comment: The histologic lesions are consistent with malignant catarrhal fever (MCF). There are multifocal infiltrates of lymphocytes within the renal interstitium centered on vessels and glomeruli. Fibrinoid degeneration is present around vessels where lymphocytic vasculitis and perivasculitis are present. Thickening and hyalinization of Bowman's capsule is seen in some affected glomeruli. There are multiple mineralized tubules scattered throughout the cortex and medulla. Other organs with ulcerative lesions (not included) had vasculitis, perivasculitis and thrombosis in vessels of the submucosa subjacent to ulcerative lesions.

Malignant catarrhal fever is the clinical manifestation of the infection of certain ruminant species with one of a group of pathogenic gammaherpesviruses known as MCF viruses. Most domestic cattle and numerous exotic species of ruminants are susceptible to clinical disease that may be sporadic or occasionally epidemic in nature. Clinical disease can range from peracute to chronic and has been reported in various species of Cervidae including, white-tailed deer, black-tailed deer, mule deer, reindeer, muntjac deer, sika deer, Shira's moose, Pere David's deer, swamp deer, rusa deer, and red deer[1-8]. The disease is characterized primarily by lymphoproliferation, mucosal inflammation and vasculitis. Historically, 2 MCF viruses have been associated with clinical disease, one endemic in wildebeest, known as alcephaline herpesvirus-1 (AIHV-1), and the other endemic in sheep, ovine herpesvirus-2 (OvHV-2) known as sheep-associated MCF (SA-MCF). Only AIHV-1 has been propagated in vitro and partially characterized. OvHV-2 is the major MCF virus worldwide. Recently, however, 2 additional members of the MCF virus group have been associated with clinical disease. An MCF virus of unknown origin that causes clinical disease in white-tailed deer [9], and an MCF virus endemic in goats, provisionally known as caprine herpesvirus-2 (CpHV-2) that has been associated with chronic alopecia in sika and white-tailed deer [1,10]. The literature on MCF contains descriptions of various manifestations of disease with diverse lesion types and organ involvement. The variable nature of disease expression is thought to result from the possession of multiple regulatory genes in gammaherpesviruses acquired during evolution. Cell type as well as host species may alter the expression of these genes [1].

In the present case lesions were consistent with MCF and OvHV-2 was identified by PCR from formalin fixed sections of kidney submitted for analysis. There was no known association with sheep at the farm of origin; however, upon receipt in Ames, IA the reindeer were housed within 50 yards of several pens of adult sheep. PCR analysis of whole blood buffy coat samples from the remaining 19 reindeer did not demonstrate the presence of OvHV-2 DNA.

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AFIP Diagnoses:
1. Kidney: Nephritis, lymphocytic and lymphofollicular, perivascular and periglomerular, multifocal, moderate, with periglomerular and interstitial fibrosis, reindeer (*Rangifer tarandus*), cervid.
2. Kidney, cortex: Necrosis, tubular, acute, multifocal.

Conference Comment: This case was reviewed in consultation with the Department of Nephropathology at the Armed Forces Institute of Pathology. There is hyaline change within multiple blood vessels, and perivascular inflammation. However, conference attendees and the nephropathologists did not identify vasculitis in the slides evaluated. There is acute tubular necrosis (ATN) with retention of the basement membrane, distinct from the autolysis present. Conference attendees discussed differential diagnosis for acute tubular necrosis, including oak bud and aminoglycoside toxicity, but the cause of ATN is not evident in this case.

Conference attendees discussed the presence of small lymphocytes around vessels, rather than lymphoblasts typically present in malignant catarrhal fever. The classic lesions reported in malignant catarrhal fever are lymphoproliferation, mucosal disease, and vasculitis.[1] Typical gross lesions include oral and gastrointestinal mucosal erosions, lymphadenopathy, corneal opacity, mucopurulent nasal discharge, crusted muzzle, and cutaneous ulceration and necrosis. Compared to cattle, MCF in cervids is usually an acute disease with animals showing few clinical signs before death. Lesions are often hemorrhagic, and involve the viscera of the gastrointestinal tract.[11,12]

Differential diagnosis for diseases that cause ulceration and necrosis of the oral and gastrointestinal mucosa in ruminants include bluetongue (Orbivirus), epizootic hemorrhagic disease in deer (Orbivirus), bovine virus diarrhea-mucosal disease (Pestivirus), rinderpest (Morbillivirus), and vesicular diseases. Important vesicular diseases to consider are foot and mouth disease (Aphthovirus) and vesicular stomatitis (Vesiculovirus), which are grossly indistinguishable from one another.[11]

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SLIDE 39
CONFERENCE 10 / CASE II - MK03-4768 (AFIP 2892675)

Signalment: Owl monkey (*Aotus nancymae)*, adult female, wild caught, weighing 0.63 kg.

History: The animal had a 2-month history of weight loss and had received nutritional supplements. The animal presented with labored breathing and edema of the lower body. Based on poor prognosis, euthanasia was elected.

Gross Pathology: The animal is well hydrated with scant body fat stores and is thinly muscled. There is diffuse subcutaneous edema. There is serous effusion in the thoracic cavity and a small amount of serous effusion in the pericardial sac. The heart is rounded. Weights are as follows: total heart = 6.715 gm; right ventricle = 1.327 gm; left ventricle and septum = 4.27 gm. The right ventricle free wall measures 1.5 mm in width, the left ventricle 8 mm and the septum 5 mm. Kidneys are mildly pale and slightly enlarged. Lungs are edematous and tan.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Heart: Hypertrophic cardiomyopathy characterized by myofiber loss and fibrosis, multifocal, moderate.
2. Arterioles, heart, intestines and multiple other organs: Degeneration and fibrinoid change, multifocal, moderate to severe.

Contributor's Comment: The animal's poor condition was due to cardiomyopathy and congestive heart failure. The arteriolar changes, present in multiple organs, are consistent with underlying hypertension. The cardiomyopathy is likely secondary to the hypertension. Hypertension is classically subdivided into primary and secondary. Primary hypertension is attributed to either renal retention of excess sodium with vasoconstriction or increased peripheral resistance. In the former, genetic factors result in decreased sodium excretion by the kidneys, which cause an increase in fluid volume and cardiac output. To prevent overperfusion of tissues from an elevated cardiac output, autoregulation leads to increased vasoconstriction and along with it elevated blood pressure. In non-renal associated hypertension, peripheral resistance is the primary cause. This results from vasoconstrictive influences such as catecholamines or

structural changes in the vessel wall leading to a thickened wall and narrowed lumen[1]. Secondary hypertension results from renal, endocrine, vascular or neurogenic disturbances. In these cases, hypertension is secondary to a specific disorder of a particular organ or blood vessel, such as the kidney, adrenal gland, or aortic artery.

The gross and histopathological lesions seen in this case are consistent with those seen in previously studied captive owl monkeys and resemble those in humans with hypertrophic cardiac disease and hypertension[2]. Whether primary or secondary, hypertension frequently results in generalized vascular disease that can lead to end-organ damage. In arterioles, the chronic hemodynamic stresses result in hyaline arteriolosclerosis in which there is hyaline thickening of the wall due to accumulation of serum proteins in the subendothelial space and media[3]. The change is accompanied by a reduction in the lumen diameter. These changes were prominent in the intestine in this case and also evident in the heart, kidney, spleen, lymph nodes and stomach. Hypertension increases the workload of the heart and leads to left ventricular hypertrophy. When the heart can no longer compensate for the pressure increase, congestive heart failure will occur. Grossly, this monkey's heart was enlarged with its total weight exceeding 1% of body weight. The left ventricle was hypertrophied and measured 8 mm in width while the right ventricle measured 1.5 mm. Microscopically, there are areas of myofiber loss and fibrosis. The diffuse subcutaneous edema and serous effusion in the thoracic cavity and pericardial sac are due to congestive heart failure. Hypertension can cause renal disease, nephrosclerosis, characterized by sclerosed glomeruli and atrophied tubules. However, these changes may also be explained by infectious disease, parasites, environment and dietary factors[2]. Idiopathic renal disease is commonly encountered in *Aotus* monkeys, but its association with hypertension is not clear.

The vascular changes seen in this case suggest that the monkey suffered from hypertension. However, based on practical difficulties in handling these small nocturnal New World primates, hypertension is difficult to document clinically, and there are no established normative values for these species[2]. Capture of an unsedated monkey will, in itself, alter blood pressure, as will any method of sedation. Implanted telemetry devices are required to record blood pressure accurately.

While we cannot exclude the possibility that the cardiomyopathy in this case is due to familial hypertrophic cardiomyopathy, or cardiomyopathic diseases unrelated to hypertension, we believe a more likely explanation is that the stress of captivity induced hypertension, through a mechanism involving catecholamine release. In an unpublished study performed at the Oregon Primate Center, owl monkeys were implanted with telemetry devices and the blood pressures were continuously monitored. Even the simple entry of a caretaker into the room caused the owl monkeys' blood pressure to elevate and remain elevated, to a far greater degree than observed in rhesus monkeys exposed to similar stimuli (O. Smith, personal communication, 1994). Rozanski et al., in a study of cynomolgus monkeys, showed that some monkeys exhibited exaggerated heart rate and blood pressure responses when presented with engaging, challenging, or aversive stimuli[4]. Gozalo found no direct correlation between

severity of heart lesions in *aotus* monkeys and time in captivity. However, those animals in quarantine for 4 months or less showed a lower incidence of hypertrophic cardiac disease than those animals that died after more than six months in captivity, suggesting either an age or captivity related effect[2]. Stress, secondary to the threat of capture or the entry of a caretaker into the room, causes release of catecholamines and cortisol, and stimulates the sympathetic nervous system. This results in vasoconstriction, increased heart rate, elevated blood pressure, and subsequent endothelial cell injury and accompanying mural changes[4].

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AFIP Diagnoses:
1. Heart, myocardium: Myofiber degeneration and loss, multifocal, mild to moderate, with fibrosis, owl monkey (*Aotus nancymae*), nonhuman primate.
2. Heart and colon, arterioles: Hyaline degeneration, multifocal, moderate.

Conference Comment: The contributor gives a comprehensive overview of hypertrophic cardiomyopathy (HCM) and hypertension in owl monkeys.

Among the domestic species, hypertrophic cardiomyopathy occurs frequently in cats. The cause of feline HCM is unknown, but heritability has been suggested in Persian and Maine coon cats. Grossly there is symmetric hypertrophy of the ventricles, especially of the left ventricle. This must be differentiated from the concentric cardiac hypertrophy that commonly occurs with hyperthyroidism. Microscopically, HCM is characterized by myofiber hypertrophy, myofiber disarray, myofiber loss, and replacement by fibrosis. In hyperthyroidism, the myofibers are enlarged, but not in disarray in most cases. A common sequelae to HCM in cats is atrial thrombosis with thromboembolic hind limb ischemia (saddle thrombus).[5,6]

Although dilated cardiomyopathy is more common in dogs, HCM does occur and may be associated with carnitine deficiency. Hypertrophic cardiomyopathy is reported to have a familial tendency in the Doberman Pinscher and Cocker Spaniel.[5]

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SLIDE 40
CONFERENCE 10 / CASE III – 98-305 (AFIP 2784507)

Signalment: 128 days old, male, C57Bl/6 mouse, *Mus musculus*.

History: This mouse was from a group of mice that were experimentally immune suppressed. This mouse showed signs that preceded the death of similarly treated mice. Since the immune suppression had not been lethal in previous experiments the mouse was euthanized and selected tissues were submitted for histopathology.

Gross Pathology: None reported.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses: Moderate multifocal splenic necrosis - syncytial giant cell formation.

Contributor's Comment: The lesions are consistent with mouse hepatitis virus infection as seen in immunodeficient mice. Mice that are immune suppressed may not produce antibodies adequate for serologic identification of MHV infection, and the syncytial cells must be distinguished from megakaryocytes commonly found within the spleen of mice.

   There was also mild chronic capsulitis of the spleen and hepatic necrosis with syncytial cells visible within the liver (not present on the slides submitted). The diagnostic aspects of MHV infection have been clearly illustrated and succinctly reviewed[1]. The slide was submitted to illustrate the differences between the characteristic syncytial cells associated with MHV infection and megakaryocytes within spleen.

   MHV infection may still complicate research using mice because of the variation of sources of mice (many genetically manipulated stocks are not commercially available), the limitations of mouse containment and diagnostic funds, the need to balance the

access of the investigators to the mice, the effects of experimental reagents (many are derived from mice) and other interactions of experimental systems and the use of mice.

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AFIP Diagnoses:
1. Spleen: Syncytial cells, numerous, viable and necrotic, C57Bl/6 mouse, rodent.
2. Spleen: Plasmacytosis, diffuse, marked.

Conference Comment: Conference attendees discussed the presence of high numbers of plasma cells that efface the T cells of the periarteriolar lymphoid sheath (PALS). Attendees considered whether the plasmacytosis could be associated with the MHV infection, but decided a separate, unknown etiology was more likely.

The typical microscopic findings in the spleen of mice infected with MHV are necrotizing splenitis and syncytial cell formation. This case demonstrates both viable and necrotic syncytial cells. Other lesions of MHV include necrotizing hepatitis, necrotizing encephalitis and meningitis, necrosis of lymphoid tissues, and virus-induced syncytia in target organs. Mouse hepatitis virus infection of neonatal mice results in necrotizing enterocolitis with high mortality. Before MHV was identified as the cause of this syndrome, it was known as lethal intestinal virus of infant mice (LIVIM). [1]

Infection and clinical disease vary with age and immune status of the mouse, virus strain, and the associated tissue tropism. Mice of any age are susceptible, but clinical disease generally occurs in immunocompromised mice, or those less than two weeks of age.[1] It is reported that co-infection with *Helicobacter hepaticus* decreases the severity of lesions during the acute phase of the disease, but increases the severity of hepatitis and meningitis in chronic disease.[2]

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SLIDE 41
CONFERENCE 10 / CASE IV - N98-21 (AFIP 2893183)

Signalment: Sixteen years of age, male, Chimpanzee, *Pan troglodytes*.

History: This adult male *Pan troglodytes*, a first-generation captive-born, was born in March 1982 in Texas. He had not been part of any infectious disease studies, however he was part of a maxillary sinus augmentation study in 1996. His clinical history was uneventful until the age of 15. Chronic low-grade anemia was noted eight months prior to death, and a liver biopsy at that time revealed severe, diffuse amyloidosis (Congo-red stain positive, Masson's trichrome stain negative). One week prior to death, an abscess in the area of the right knee was lanced, debrided, and antibiotic therapy was initiated.

Gross Pathology: A complete necropsy was performed and on gross postmortem examination, a 20 x 20 x 10 cm necrosuppurative lesion was present in the subcutaneous tissues around the right knee, extending into the underlying skeletal muscle. The heart and kidneys were pale tan. The pericardial sac contained an excess of pericardial fluid with fibrin. The liver was diffusely pale and enlarged. On the left side of the liver, away from the gallbladder, was a round, white, lobulated 10 cm-in-diameter mass.

 Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5-um sections were mounted and stained with hematoxylin and eosin. Selected liver sections were stained with Congo red. Immunohistochemical staining of liver from chimpanzee 4X0392 for carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), cytokeratins 8/18, and cytokeratin 7 were performed by the Armed Forces Institute of Pathology.

 Histologically, the hepatic mass was unencapsulated and highly infiltrative, composed of anaplastic hepatocytes arranged into branching cords one to three cell layers thick. Large areas of necrosis and hemorrhage were present. Sinusoids contained necrotic cellular debris and small numbers of lymphocytes, and occasional sinusoids were greatly dilated. The hepatocytes within the mass formed rare glandular structures. The cells were polygonal and exhibited marked anisocytosis, anisokaryosis, and pleomorphism. They typically contained large amounts of eosinophilic granular cytoplasm and many had multiple small, round, clear, intracytoplasmic vacuoles (lipid). The nuclei were irregularly ovoid, with coarsely clumped chromatin and one to three nucleoli. Numerous multinucleated cells were present. The mitotic rate was high, with approximately eight mitotic figures per 40x field and frequent abnormal mitoses. Immunohistochemical staining revealed strong diffuse staining of neoplastic cells for cytokeratins 8/18, scattered positive staining for cytokeratin 7, strong canalicular staining with CEA, and negative staining for AFP.

Sinusoids in the surrounding hepatic parenchyma were diffusely and markedly expanded by eosinophilic, amorphous, Congo red-positive material (amyloid). Multifocal small to moderately-sized accumulations of amyloid were also identified in the spleen and smaller amounts of amyloid were present in the heart, lung, and kidney. Within the kidney, moderate, diffuse, global, membranoproliferative glomerulonephritis and mild, multifocal, tubular necrosis were present. The lumen of the large intestine contained small numbers of nematodes, morphologically consistent with *Enterobius vermicularis,* and small numbers of *Balantidium coli*. The cause of death was determined to be systemic amyloidosis.

Laboratory Results: Blood work revealed a marked leukocytosis, mild anemia, mild hypoalbuminemia, mild hyponatremia, and mild increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase.

Contributor's Morphologic Diagnosis: Hepatocellular carcinoma, liver, chimpanzee.

Contributor's Comment: Hepatobiliary tumors have been experimentally induced in nonhuman primates by a variety of chemical compounds. Spontaneous hepatobiliary neoplasms, however, have been only sporadically reported. Hepatic neoplasia is rare in chimpanzees. Only four hepatic neoplasms have been reported in chimpanzees, three of which were associated with viral etiologies[1,2,3].

Forty-four percent (30 of 68) of the tumors reported in the literature have been described as benign, with the majority of those being hepatocellular adenomas. Of the malignant tumors described in nonhuman primates, 58% (22 of 38) have been HCCs, previously termed hepatomas. HCC has been reported in prosimians, New World monkeys, Old World monkeys, and apes. Of the reported HCCs with histologic descriptions, the majority had a trabecular pattern, and tumors were evenly divided between low grade and high grade forms. Metastasis was reported in 8 of 22 tumors, with the lung being the most common metastatic site. HCCs appear to be much less common in nonhuman primates than in man, in which the majority of cases are associated with HBV infection, HCV infection, and/or alcoholism.

In humans, HCC is the most common primary malignant hepatic neoplasm and one of the most common of all malignancies. Four grades (I to IV) and several histologic growth patterns are recognized, including trabecular, pseudoglandular, compact, cirrhosis, fibrolamellar, and mixed. The HCC found in chimpanzee 4X0392 had a predominantly trabecular pattern with only rare formation of pseudoglandular structures. The high degree of nuclear atypia, high mitotic rate, multinucleated cells, and areas of necrosis are indicative of a high grade (grade IV) neoplasm. Other features described in human tumors that were not evident in this case include Mallory bodies, non-Mallory cytoplasmic globules, fibrinogen inclusions, and large amounts of intracytoplasmic glycogen.

In addition to having some typical microscopic characteristics of HCC, the carcinoembryonic antigen (CEA) immunohistochemistry staining pattern of this hepatic mass indicated HCC. Antibodies to CEA cross-react with biliary glycoprotein I in the bile canaliculi. A canalicular staining pattern with anti-CEA is considered useful in differentiating HCC from other malignancies. This tumor also stained positive for cytokeratins 7, 8 and 18. Cytokeratins 8 and 18 are commonly demonstrated in both normal and neoplastic hepatocytes, while cytokeratin 7 is a biliary-type keratin found in a subset of HCCs [2]. The neoplastic cells, however, did not stain for the tumor marker AFP, which is normally produced by liver cells during development of the embryo and is the dominant serum protein in early embryonic life. This protein can reappear in the adult serum during certain pathologic states, including neoplasms such as hepatoblastomas, hemangioendotheliomas, and HCC. The level produced by hepatomas, teratocarcinoma, and embryonal cell carcinomas, when present, can be used for monitoring of responses to treatment of these tumors. Human HCC patients often have elevated serum AFP, but the neoplastic cells are usually negative for this protein. However, the absence of AFP is not diagnostic[2].

The cause of death of chimpanzee 4X0392 was determined to be systemic amyloidosis, which is a recognized disease in adult chimpanzees. Systemic amyloidosis in chimpanzees is a chronic, progressive, and fatal disease. The amyloid is of the secondary or reactive type and the liver is the predominant organ affected, with amyloid accumulation leading to elevated liver enzymes and eventual liver failure.

Proliferative liver lesions are of special significance in the chimpanzee, a species that is currently an important model of human viral hepatitis. Our understanding of the role of viruses in hepatic carcinogenesis is far from complete, and the chimpanzee model may allow better understanding of these processes. Only a few hepatic tumors have been described in the chimpanzee, and more cases must be studied before meaningful correlations with human tumors can be made.

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AFIP Diagnosis: Liver: Hepatocellular carcinoma, chimpanzee, nonhuman primate.

Conference Comment: The contributor gives an excellent review of hepatic neoplasia in nonhuman primates and hepatocellular carcinomas in humans. As the contributor notes, the study of viral hepatitis is currently an important area of research. Woodchucks (*Marmota marmax*) and a variety of ground squirrels have been identified as developing hepatic disease in association with hepadnavirus infection and have been suggested as animal models.[4]

Woodchuck hepatitis virus (WHV) causes hepatitis and is associated with the development of hepatocellular carcinoma in this species. The woodchuck is a useful animal model for studying viral hepatitis and hepatocarcinogenicity, and for the development of antiviral drugs for treatment of chronic hepatitis B virus infection in humans. California ground squirrels (*Spermophilus beecheyi*) persistently infected with

ground squirrel hepatitis virus (GSHV) develop hepatitis and hepatocellular carcinoma, but at a lower frequency than that associated with WHV. Arctic ground squirrels (*Spermophilus parryi*) infected with arctic ground squirrel hepatitis virus (AGSHV) have a high incidence of hepatocellular carcinoma, as well. Pekin ducks (*Anas domesticus*) infected with duck hepatitis B virus (DHBV) also develop hepatitis; however, the role of DHBV in producing hepatocellular carcinoma in ducks remains to be elucidated.[4]

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SLIDE 42
CONFERENCE 11 / CASE I – 2768804 (AFIP 2887158)

Signalment: 2 week old, male and female, mixed breed, porcine, *Sus scrofa domesticus*.

History: Sudden outbreak in nursery of piglets with inappetence, liquid yellow feces, dehydration, weight loss, mortality approaching 50% in young piglets. Gilts exhibited decreased appetites and occasional vomiting.

Gross Pathology: Stomachs filled with curdled milk. Lacteals within the mesentery were empty. The intestines were thin-walled, with yellow foamy fluid contents. Close examination of jejunum and ileum revealed atrophied mucosal lining.

Laboratory Results: Direct fluorescent antibody examination of intestines: positive for coronavirus, negative for rotavirus. PCR on feces strongly positive for coronavirus. Bacterial isolation of >100 cfu of *Escherichia coli* from intestines. PCR testing of *E. coli* isolates negative for the following pilus and toxin genes: F41, K88, 987p, F18, K99, Stx2, STa, STb, and LT.

Contributor's Morphologic Diagnosis: Jejunum and ileum: acute, severe, atrophic enteritis with villous blunting and fusion, and superficial epithelial attenuation.

Contributor's Comment: This is a fairly classic acute outbreak of Transmissible Gastroenteritis (TGE) due to coronavirus. Rapid spread, high mortality in young piglets, and illness in gilts indicates a herd with limited immunity and a case of epizootic TGE. Diarrhea and mortality typically affect piglets less than 3-5 weeks of age; with milder signs in older piglets, and only transient inappetence and vomiting in adults. The duodenum is typically spared by the TGE coronavirus, which replicates in and destroys the crypt enterocytes. The jejunum and to a lesser degree the ileum exhibit the most severe villous atrophy; in this case the reduction in villous to crypt ratio approaches 1:1. Both FA and PCR are rapid means of confirming the diagnosis. More recently, a non-enteropathogenic variant of TGE known as Porcine Respiratory Coronavirus (PRCV) has been reported in Europe and North America. While TGE may be complicated by other enteric pathogens (rotavirus, *E. coli*, *Salmonella* sp.), this appears to be a relatively pure infection with TGE. *Escherichia coli* was isolated; however, no pilus or toxin genes were detected, nor was there microscopic evidence of bacterial colonization of the gut. In the face of an outbreak, supportive therapy and antibiotics are used to treat affected piglets, while intentional exposure of pregnant sows or vaccination can be utilized to limit further spread.[1]

AFIP Diagnosis: Small intestine: Villus blunting and fusion, segmental, with apical epithelial necrosis and multifocal regeneration, mixed breed, porcine.

Conference Comment: Conference attendees discussed the possible mechanisms for the age-dependent susceptibility to TGE virus. Neonates normally have tall villi (villus height to crypt depth is normally 7:1 to 9:1) with mature differentiated enterocytes and short inactive crypts of undifferentiated epithelium, resulting in a large population of susceptible villus cells and crypts that are slow to repair. A second mechanism may be associated with gastric secretions. Milk buffers gastric acid in neonates, so this acid-labile virus is better protected in the less acidic environment of the neonate's stomach. In addition to the above, neonates are inherently more susceptible to dehydration, electrolyte imbalances, and hypoglycemia, making them more susceptible to the effects of this virus.[1,2,3]

   Although all ages of pigs may be affected in a susceptible herd, TGE is generally a disease of high morbidity and mortality in pigs younger than 10 days of age, causing vomiting and profuse diarrhea. Differential diagnosis for diarrhea in young pigs includes *E. coli*, rotavirus, *Clostridium perfringens* type C, hemagglutinating encephalomyelitis virus, and coccidiosis. Enteric colibacillosis is a common cause of profuse diarrhea, without vomiting, in piglets less than 10 days of age with peak incidence at 3 days of age. Rotavirus causes disease in suckling and weaned pigs (1-5 weeks of age) with less severe villus atrophy than seen in TGE. Clostridial enterotoxemia is a rapidly fatal disease of newborn piglets less than one week of age, causing bloody diarrhea.

Hemagglutinating encephalomyelitis virus, another coronavirus, is the cause of vomiting and wasting disease. Vomiting and wasting disease affects pigs less than 10 days old and is characterized by vomiting and weight loss. Additionally, a number of affected pigs develop acute encephalomyelitis. Diarrhea may occur but, in contrast to TGE, is not severe. Coccidiosis causes diarrhea without blood in piglets 5-15 days of age, with peak incidence at 7-10 days of age.[4]

Grossly, TGE causes distension of the small intestine with gas and yellow frothy fluid and flaccid, thin, transparent intestinal walls. Of the differentials listed above, the gross lesions that most closely resemble TGE are those of *E. coli* and coccidiosis.[1,2,3]

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SLIDE 43
CONFERENCE 11 / CASE II - Experimental Path Labs (AFIP 2890229)

Signalment: 2-year-old, female, Chinese Shar Pei, domestic dog (*Canis familiaris*).

History: A two year old, female, Chinese Shar Pei presented with vomiting and diarrhea. Blood work and urinalysis revealed an elevated BUN and creatinine and isosthenuria. The dog responded poorly to symptomatic treatment for renal failure and euthanasia was elected.

Gross Pathology: The kidneys had an irregular to finely granular surface and were diffusely pale (necropsy was limited to the kidneys).

Laboratory Results:
Blood Work:
BUN: 82.8
Creatinine: 8.35

Total Protein: 8.5
Cholesterol: 310

Urinalysis:
Specific Gravity: 1.011
Protein: Trace

Contributor's Morphologic Diagnosis: Kidneys: Renal amyloidosis, medullary and glomerular, severe.

Contributor's Comment: Familial renal amyloidosis has been described in the Chinese Shar Pei[1]. These dogs usually present at a relatively young age (average 4.1 years) and sometimes have a clinical history of intermittent fever and swelling of the tibiotarsal joints[1]. Unlike most forms of canine renal amyloidosis, in which the amyloid deposition is primarily glomerular, Shar Peis with this familial form typically develop medullary amyloid deposits sometimes leading to renal papillary necrosis[1]. However, over half of these dogs also have some degree of glomerular amyloid deposition as well as deposition in other organs including the spleen, liver, heart, prostate, pancreas, lymph nodes and intestine[1]. The amyloid in these familial cases is Congo Red positive and sensitive to potassium permanganate treatment indicating that it is of the AA (reactive) type[1].

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AFIP Diagnoses:
1. Kidney: Amyloidosis, interstitial and glomerular, multifocal, moderate, Chinese Shar-Pei, canine.
2. Kidney: Nephritis, interstitial, lymphoplasmacytic, chronic, multifocal, moderate.

Conference Comment: The most common forms of amyloidosis are primary (immunocytic) and secondary (reactive). Primary amyloidosis is often seen in patients with plasma cell dyscrasias because AL (amyloid light chain) is composed of immunoglobulin light chains. In secondary amyloidosis, AA (amyloid-associated) is derived from serum amyloid-associated (SAA) protein, which is an acute phase protein produced in the liver as a result of chronic antigenic stimulation. Hereditary reactive amyloidosis is found in Chinese Shar-Peis, as demonstrated by this case, and is also reported in Abyssinian cats.[2,3,4]

Pulmonary arterial and renal vein thrombosis have been reported as sequelae in animals with glomerular amyloidosis. Glomerular amyloidosis results in loss of serum proteins leading to proteinuria and hypoalbuminemia. Renal loss of antithrombin III produces a hypercoagulable state that predisposes to thrombosis. Development of thrombi may also be exacerbated by stimulation of acute phase proteins, such as fibrinogen.[2]

In most animals Congo red is used to stain amyloid deposits, which shows green birefringence under polarized light. Congo red may not stain amyloid in cats. In these cases, thioflavin T may be used to demonstrate amyloid which fluoresces bright yellow when polarized.[2]

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SLIDE 44
CONFERENCE 11 / CASE III – L02-7504 (AFIP 2889975)

Signalment: These tissues are from an adult, female, three-spined stickleback, *Gasterosteus aculeatus*.

History: A group of three-spined sticklebacks were caught in Alaska and introduced to a laboratory colony for genetic studies. These fish have subsequently developed white lumps on body surfaces. Affected fish have been culled, but new infected individuals continue to appear.

Gross Pathology: Ten spherical, creamy white, smooth nodules approximately 2 mm in diameter were distributed on the body surface from the opercula and extending caudally along the lateral and ventral body walls. These nodules oozed white fluid on section. An additional 6 nodules were identified in the oral cavity after euthanasia. A 16x7x1.5 mm, dorsoventrally flattened, segmented larval cestode filled the coelomic cavity.

Laboratory Results: Microscopic examination of a wet preparation from one surface nodule revealed myriad 2 x 5 micron, slightly elongated microsporidian spores each with a terminal vacuole (Fig. 3).

Contributor's Morphologic Diagnoses:
1. Body: Multiple xenomas, body wall and oral cavity, consistent with *Glugea* sp. (Microsporiasis).

2. Intestine and kidney: Mild to moderate protozoiasis, minimal tissue changes, suspect *Eimeria* sp.
3. Liver: Moderate, diffuse glycogen depletion.
4. Liver: Mild, diffuse hepatic lipidosis.
5. Ocular lens: Encysted trematode metacercaria, suspect *Diplostomum* sp. (only present in a small number of slides).
6. Coelomic larval cestode (plerocercoid) consistent with *Schistocephalus* sp. (gross diagnosis).

Contributor's Comment: In multiple coronal sections of whole fish, the most significant changes are the multiple (11 profiles in the slide examined) roughly spherical masses (xenomas) measuring 1 to 1.5 mm diameter and distributed in the superficial body wall and oral cavity (Fig. 1). The xenomas are delineated by a prominent hyaline membrane and contain myriad approximately 2 x 5 micron bodies. Amphophilic to basophilic cell debris is distributed within these xenomas, especially near the periphery. One xenoma has ruptured into the peritoneal cavity and another has ruptured into the subcutaneous tissue of the operculum. Skeletal muscle degeneration is associated with some body wall xenomas.

All microsporidians infect host cells, but some, such as *Glugea*, induce severe cellular hypertrophy that, in conjunction with the parasite, forms a "xenoparasitic complex" or xenoma (Fig. 2 - xenoma in abdominal cavity of a stickleback)[1]. *Glugea anomala* was first described in sticklebacks in 1887 [2]. This organism causes a chronic infection characterized by the production of tumor-like masses that may have little effect on the host unless vital organs are affected. No intermediate host is required; spores released from xenomas can directly infect new tissues. Infection occurs via a polar filament that anchors the spore to the host while the sporoplasm is extruded through the everted filament.

Microsporidians are common in the environment, including water supplies, and infect all the major animal groups. These organisms are opportunistic pathogens of humans; eight genera of microsporidians have been identified in human infections[3]. Recent phylogenetic analysis suggests microsporidians are closely related to fungi. Diagnosis of microsporidian infection can be made based on identification within target tissues of spores that are 2 to 10 microns in length, egg-shaped to elliptical and have a prominent posterior vacuole (Fig 3)[1]. Ribosomal RNA sequences are useful as more specific diagnostic tools. Diagnosis to the species level in the present case was not pursued.

All wild fish are likely to have a significant parasite load. The encysted trematode and the suspect protozoal organisms in the intestine and kidneys are considered incidental findings. Hepatic glycogen depletion and lipidosis likely reflect poor body condition secondary to debilitation and/or inadequate food intake.

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AFIP Diagnoses:

1. Retroperitoneal and subepithelial tissues: Multiple xenomas, three-spined stickleback (*Gasterosteus aculeatus*), piscine.
2. Intestine, mucosa: Small number of protozoal zoites.
3. Kidney, tubules: Small number of protozoal zoites.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Microsporidia are obligate intracellular parasites with a direct life cycle. A characteristic feature of all microsporidia is the extrusion apparatus. This is composed of a polar tube attached to the anterior end of the spore by an anchoring disc, which contains 4-30 coils. The polar tube everts and "injects" sporoplasm into the host cell cytoplasm. All stages of this protozoa are gram positive, a unique feature of *Microspora*. Mature spores are acid-fast, have a PAS-positive polar tube, and are anisotropic.[3,4,5]

Another microsporidium of veterinary importance is *Encephalitozoon cuniculi*. This protozoa infects a wide variety of mammals and causes lesions predominantly in the brain, kidney, and vascular endothelium.[6] Several microsporidia are common enteric pathogens in immunocompromised, HIV-infected patients. Such pathogens include *Enterocytozoon bienueusi* (also reported in SIV-infected macaques), *Vittaforma corneae* (*Nosema corneum*), and *Trachipleistophra hominis*.[4,5]

There are coccidia present in the sections examined by conference attendees. Dr. Gardiner identified sporulated oocysts in the tissue. By definition, *Eimeria* does not sporulate in tissue; however, we were unable to further classify these organisms.

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SLIDE 45
CONFERENCE 11 / CASE IV - 00-0369 (AFIP 2741057)

Signalment: 13-year-old male owl monkey (*Aotus trivirgatus*).

History: This 13-year-old male owl monkey was previously on several malaria protocols, but has been in the issue pool for the last three years. Early radiographic signs of cardiac enlargement were noted in May 1999; however, the animal remained asymptomatic. Significant weight loss was recorded in February 2000. In the evening on 27 Mar 00 he was found sitting in the bottom of his cage (owl monkeys typically stay high in the cage on limbs or perches). At this time he was weak, but bright, alert and responsive, had a good appetite and normal respiratory rate and heart rate. He was found dead the next morning.

Gross Pathology:
Brain: A red friable mass approximately 1.5 x 1.5 x 0.7 cm was present in the white matter of the temporal and occipital lobes of the right cerebral hemisphere. The mass compressed and distorted adjacent structures including the corpus callosum, lateral ventricles, thalamus, hippocampus and midbrain. The overlying gray matter was markedly thinned (Fig A - gross photo).
Kidneys: The kidneys were bilaterally small and diffusely pale. On cut section white, radiating streaks extended from the renal pelvis to the capsular surface and the corticomedullary junction was mildly obscured.
Heart: Thin white streaks were scattered diffusely throughout the myocardium.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Brain, cerebrum: Hemorrhage, acute, focally extensive, severe, with cerebral cavitation, owl monkey (*Aotus trivirgatus)*, primate.
2. Brain, cerebral arteries: Atherosclerosis, chronic, multifocal, moderate to severe, with thrombosis, aneurysmal dilatation and perivascular hemosiderin.

Other histologic findings [tissues not submitted for conference]:
Kidney: Nephritis, interstitial, eosinophilic and lymphoplasmacytic, chronic, multifocal, mild to moderate, with membranous glomerulonephritis and hyperplastic arteriolosclerosis.
Heart: 1. Fibrosis, multifocal, moderate with arteriosclerosis.
        2. Perivasculitis, eosinophilic, multifocal, mild.

Contributor's Comment: The pathogenesis of atherosclerosis is complex and not fully understood. This case of naturally occurring atherosclerosis in an owl monkey is of interest in that it provides a framework in which to discuss this complex entity and the use of nonhuman primates as an animal model.

In the case submitted, meningeal arteries contain atheromatous plaques (Fig. 1), which are typically focal (not affecting the entire circumference of the artery) intimal lesions comprised of a fibrous cap and a central necrotic lipid rich core. In several areas this lesion is associated with aneurysm, thrombosis (Fig. 2) or both. In people, atherosclerosis most commonly leads to ischemic injury such as myocardial or cerebral infarction; it is also associated with aneurysms, typically in the aorta. In this case atherosclerosis is associated with cerebral hemorrhage, likely secondary to a ruptured aneurysm of a meningeal artery.

Current research suggests that atherosclerosis is a chronic inflammatory process initiated by endothelial injury (response to injury hypothesis). Factors thought to contribute to endothelial damage include hyperlipidemia (specifically elevated cholesterol-rich low-density lipoproteins (LDL)), hypertension, stress, cigarette smoking, diabetes mellitus, genetic alterations, elevated plasma homocysteine, and infectious agents. Ross[5], Woolf[7] and Schoen and Cotran[6] have recently reviewed the postulated pathogeneses of these risk factors. In this case it is interesting to note that although systemic hypertension was not documented clinically, the presence of renal hyperplastic arteriolosclerosis or "onion skinning" (Figs. 3, 4, 5) is highly suggestive of hypertension. Additionally, multifocal myocardial fibrosis suggests previous ischemic episodes. Naturally occurring and experimentally induced atherosclerosis is well documented in a variety of nonhuman primates. According to April and Keith[1] the most commonly affected nonhuman primates are baboons, rhesus monkeys, squirrel monkeys, cynomolgus monkeys and African green monkeys. There is at least one report in the literature[4] of naturally occurring atherosclerosis in the owl monkey. The cynomolgus monkey is currently the most commonly used nonhuman primate model. Kaplan and Manuck[3] recently published an interesting study on the association of environment and behavior to development of atherosclerosis in cynomolgus monkeys.

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AFIP Diagnoses:
1. Brain, cerebrum: Hemorrhage, acute, focally extensive, severe, with cerebral cavitation, owl monkey (*Aotus trivirgatus)*, primate.
2. Brain, cerebral arteries: Atherosclerosis, chronic, multifocal, moderate to severe, with thrombosis.

Conference Comment: Atherosclerosis is rare in animals. It has been reported as a sequela of hypothyroidism and diabetes mellitus in dogs. Miniature schnauzers have a breed predisposition for atherosclerosis that has been associated with idiopathic hyperlipoproteinemia.

Several animal models have been suggested for the study of atherosclerosis. Pigs are the only domestic animal that commonly develop atherosclerosis and may represent a natural animal model for this disease. Additionally, rabbits, chickens, pigs, and nonhuman primates fed high cholesterol diets also develop atherosclerosis.[8]

Two strains of rabbits commonly used as models of atherosclerosis are the Watanabe heritable hyperlipidemic rabbit and the St. Thomas's Hospital rabbit. The Watanabe rabbit was the first animal model of natural endogenous hypercholesterolemia, and is a well-established model with a deficiency of LDL receptors in the liver and other tissues. The St. Thomas's Hospital strain is named for the hospital in London where it was developed; these animals maintain hypercholesterolemia despite normal LDL receptors.[9,10]

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SLIDE 46
CONFERENCE 12 / CASE I - M03-1654 (AFIP 2886856)

Signalment: Four-year-old male corn snake (*Elaphe guttata guttata*).

History: This animal belonged to a collection of seventy-five snakes. Thirty-eight snakes were experiencing weight loss and regurgitation and some had died. This snake was euthanized due to chronic weight loss, regurgitation and diarrhea.

Gross Pathology: The referring veterinarian noted a thickened gastric mucosa at necropsy (Fig. 1). Tissues from this snake were fixed in formalin and mailed to the pathology department.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Diffuse gastric mucosal hyperplasia with intra-lesion *Cryptosporidium* and bacteria.
2. Mild multifocal lymphocytic plasmacytic gastritis.

Contributor's Comment: The gastric mucosa is markedly hyperplastic with prominent mucosal folds lined by multiple layers of columnar epithelium with basal nuclei. The chief cells are completely replaced by mucous neck and surface epithelial cells (Fig. 2). There are nests of hyperplastic branching glands extending deep into the lamina propria. Focal infiltrates of lymphocytes, plasma cells, and a few granulocytes are scattered throughout the lamina propria. Small 4-5um oocysts are present in a few gastric glands and are attached to the luminal surface of epithelial cells. Surface epithelium and gastric pits are overgrown with mixed populations of gram-negative and gram-positive bacterial cocci and rods (Figs. 3 and 4).

Coccidia of the genus *Cryptosporidium* infect mammals, birds, fish, and reptiles. Transmission is direct by sporulated or unsporulated oocysts shed in the feces. Cryptosporidiosis is a well-known cause of gastric hyperplasia (hypertrophic gastritis) in captive snakes. In the present case, there was overgrowth of bacteria on the surface and in gastric pits, possibly secondary to gastric hyperplasia. Chronic postprandial regurgitation and loss of body mass are the most common presentations in infected snakes. The disease is often protracted and fatal[1]. As in this case, outbreaks of cryptosporidiosis can devastate ophidian collections. Snakes shed large numbers of oocysts in the feces and the disease can be extremely difficult to eradicate from a collection[1,2]. Stringent sanitation and quarantine procedures are required to contain the disease. Hydrogen peroxide and formalin are effective disinfectants[1]. The classification and speciation of *Cryptosporidium* is still uncertain[3]. *Cryptosporidium serpentis* is the pathogenic species for snakes and other reptiles. The reptilian pathogen appears not to infect mammalian and avian species and mammalian and avian species are non-infectious for snakes. However, snakes can be infected with *C. serpentis* from other species of reptiles[2].

AFIP Diagnosis: Stomach: Mucosal neck cell hyperplasia, diffuse, moderate, with granular cell loss, submucosal edema, and apical protozoal organisms, etiology consistent with *Cryptosporidium* sp., corn snake (*Elaphe guttata guttata*), reptile.

Conference Comment: This case was reviewed in consultation with the Department of Pathology, National Zoological Park. Conference attendees noted the high number of bacteria within the gastric pits and on the surface epithelium and concluded that it is most likely overgrowth due to retention of ingesta and slowed digestion secondary to the *Cryptosporidium* sp.

Cryptosporidium sp. are located at the apical surface of epithelial cells. Ultrastructurally, the organism resides in an intracellular but extracytoplasmic environment. The organism is surrounded by a host cell-derived membrane (parasitophorous vacuole) and attaches to the epithelial cell by a specialized feeder organelle, displacing microvilli. While *Cryptosporidium serpentis* is the most common species in reptiles, *Cryptosporidium parvum* is most common in ruminants, and *Cryptosporidium baileyi* in poultry. Heavy infections are reported in immunocompromised animals, such as chickens with infectious bursal disease (birnavirus) or cats with feline leukemia virus infection (retrovirus).[4,6]

Proliferative gastritis is a characteristic finding in snakes infected with *Cryptosporidium* sp. Causes of proliferative gastritis/abomasitis in other species include *Ostertagia ostertagi* in cattle, *Ostertagia circumcincta* in sheep and goats, *Nochtia nochti* in nonhuman primates, *Trichostrongylus axei* in horses, *Hyostrongylus rubidus* in pigs, and *Ollulanus tricuspis* in cats.[4,5]

Histologically, the gastric mucosa of reptiles lacks the parietal cells present within the mammalian stomach. In reptiles, the gastric glands are composed solely of chief and clear cells.[7]

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SLIDE 47
CONFERENCE 12 / CASE II - 2 (AFIP 2891621)

Signalment: Captive-bred, adult of unknown age, male yellow-naped amazon parrot (*Amazona auropalliata*).

History: Sudden death.

Gross Pathology: Pale, mottled, hemorrhagic, enlarged liver. Hemorrhages in multiple tissues, particularly the mesenteric fat and liver.

Laboratory Results: Positive for psittacid herpesvirus (Pacheco's Disease Virus) by in-situ PCR.

Contributor's Morphologic Diagnosis: Massive, diffuse, acute hepatocellular necrosis with syncytial cell formation, intranuclear inclusion bodies, and multifocal hemorrhage (Pacheco's disease).

Contributor's Comment: Pacheco's disease (PD) is caused by a heterogeneous group of psittacid herpesviruses (PsHVs) closely related to Gallid herpesvirus-1.[1-3] Since its initial description in Brazil in 1929, Pacheco's disease has attained worldwide distribution with many reported outbreaks in the United States.[1] Recent investigations in Europe and the United States indicate that there are at least 5 serologic serotypes and 10 genetic variants, and that infection by one serotype/variant may not be protective against infection by other variants.[1-2] In spite of the heterogeneity, some variants occur more commonly than others. For example, in one study the majority (63%) of reported cases were caused by a single genetic variant.[1]

   High morbidity and mortality are typically seen in affected flocks, particularly those with poorly managed, densely populated aviaries; however, individual pet birds may also be affected.[4] Sudden death or minimal, brief, non-specific clinical signs characterize PD, and infected birds may die 3-14 days after exposure.[4-5] Latently infected birds are thought to be a source of infection and environmental contamination.[4] The bird in this case was from a single-bird household with no recent contact with other birds. We speculate that the bird may have been latently infected and suffered a

relapse of the disease or the owner may have brought the infection to the household from a contaminated source.

Macroscopic lesions that may be associated with PD consist of hepatomegaly, splenomegaly, pale discoloration of the liver and spleen (necrosis), and hemorrhage in multiple tissues.[3-6] Less frequently, macroscopic lesions may be identified in other organs. Microscopically there is multifocal to massive, peracute to acute hepatocellular necrosis without significant inflammatory infiltrates. Necrosis may be so severe and extensive that the hepatic lobular architecture is completely distorted as in the present case (Fig. 1).[3] Intranuclear inclusion bodies are commonly seen in hepatocytes and biliary epithelium. Syncytial cells may be identified in hepatic tissue but are reportedly not common.[3] Splenic necrosis with intranuclear inclusion bodies are a common finding.[3] Other tissues are affected less frequently and may contain intranuclear inclusion bodies (gastrointestinal tract, pancreas, lymphoid tissues, bronchi, kidneys, ovary, thyroids/parathyroids).[3,5] The present case was characterized by hepatic and splenic necrosis with hemorrhage in multiple tissues (Figs. 1 and 2). Hepatic syncytial cells were infrequently seen (Fig. 3), inflammatory infiltrates (heterophils) were minimal (Fig. 1), and intranuclear inclusion bodies were present in hepatocytes (Fig. 4), biliary epithelial cells (Fig. 3), and mononuclear cells in the spleen (Fig 2).

The macroscopic and microscopic lesions are not specific for PD/PsHVs since similar lesions may be seen with other viruses (avian polyomavirus, avian adenovirus).[3] Other causes of hepatic necrosis include bacterial infections (*Chlamydophila psittaci*, *Salmonella* sp., *Clostridium piliforme*),[3,7-9] viruses (avian reovirus, psittacine circovirus),[10,11] trematodes,[12] and toxins (aflatoxins).[3] A specific diagnosis is possible with in-situ PCR and whole tissue PCR assays, fluorescent antibody testing, and immunohistochemistry.[1-3] In the present case the histologic findings were strongly suggestive of PD and in-situ PCR was diagnostic for PsHVs. The primer probes utilized in this assay are based on a conserved nucleotide sequence present in multiple variants of PsHV (K. Latimer, personal communication).

Captions for figures:
Fig.1. Massive and diffuse hepatocellular necrosis with minimal heterophilic infiltrates. (H&E, 60X)
Fig. 2. Splenic necrosis with intranuclear inclusion bodies (arrows). (H&E, 60X)
Fig. 3. Numerous biliary epithelial cells with intranuclear inclusion bodies and syncytial cell formation (arrows). (H&E, 60X)
Fig. 4. Hepatocellular (arrows) and biliary (arrowhead) intranuclear inclusion bodies (arrows). (H&E, 60X)

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AFIP Diagnosis: Liver: Hepatocellular necrosis and degeneration, diffuse, severe, with rare syncytia and many eosinophilic intranuclear inclusion bodies, yellow-naped amazon parrot (*Amazona auropalliata*), avian.

Conference Comment: The contributor gives a concise review of Pacheco's disease and important differential diagnoses. In addition to Pacheco's disease virus (psittacid herpesvirus-1), other alphaherpesviruses of birds include gallid herpesvirus-1 (avian infectious laryngotracheitis), gallid herpesvirus-2 (Marek's disease), and anatid herpesvirus-1 (duck plague). Significant gross lesions associated with infectious laryngotracheitis include thickened and hemorrhagic tracheal mucos**a** with necrotic debris in the tracheal lumen. Marek's disease virus causes atypical lymphocyte proliferation in a variety of tissues, including peripheral nerves, bursa, thymus, iris, and visceral organs. Duck plague, or duck viral enteritis, causes hemorrhage and necrosis in the gastrointestinal tract, liver, and lymphoid organs, to include the gastrointestinal associated lymphoid tissue (GALT), which produces the characteristic dark annular bands in the intestinal mucosa.[4]

The contributor provided a list of differential diagnosis for hepatocellular necrosis in psittacines, most notably *Chlamydophila psittaci*, adenovirus, and polyomavirus. Clusters of *Chlamydophila psittaci* organisms within hepatocytes and macrophages are diagnostic for psittacosis but may require special stains such as Gimenez. Polyomavirus and adenovirus cause large intranuclear amphophilic to basophilic inclusions that expand the nucleus.[3]

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SLIDE 48
CONFERENCE 12 / CASE III - 44678 (AFIP 2890230)

Signalment: 2 year 8 month-old, female, frilled lizard, *Chlamydosaurus kingii*, reptile.

History: This lizard was hatched at the San Diego Zoo on 7 November 1999. It was presented on 18 July 2002 for weight loss and swelling of the right carpus. On physical examination the animal was emaciated and had a poor righting reflex. Euthanasia was elected.

Gross Pathology: At necropsy the right forelimb was mildly swollen from the elbow to the phalanges. The limb measured 1.0 cm diameter at its widest point. All other organs examined were grossly normal.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Severe regionally extensive articular and periarticular deposition of amphophilic material with granulomatous inflammation and bone remodeling (Severe articular and periarticular gout).
2. Moderate focal subcutaneous deposition of amphophilic material with granulomatous inflammation (Moderate subcutaneous gout).

Contributor's Comment: The section examined is through the radius and carpus. It includes the articular surfaces of the carpal bones, adjacent skeletal muscle and a section of the overlying skin. Lesions vary in severity and distribution from slide to slide. Surrounding the carpal joint and dissecting between the muscle fascicles are large aggregates of amphophilic, finely granular material. These aggregates are surrounded by a thin fibrous connective tissue capsule and mildly compress the adjacent skeletal muscle fibers. Thin fibrous connective tissue trabeculae often extend from the capsule, further subdividing the amphophilic material. Small deposits of amphophilic material within the skeletal muscle demonstrate radiating thin crystals, classic for gout tophi. A similar aggregate of material is present between the skin and the underlying skeletal muscle. Amphophilic granular material and scanty inflammatory cell aggregates are present within the carpal joint spaces. In some areas near the capsule the material is birefringent.

The fibrous connective tissue capsule is lined by an inner rim of inflammatory cells. The inflammatory cells are predominantly epithelioid macrophages with frequent multinucleated giant cells, few lymphocytes and plasma cells. Similar inflammatory cells are intermingled with the amphophilic granular material in the joint spaces. The articular cartilage surfaces are irregular and mildly fibrillated. Along one margin of the radius there is proliferation of woven bone beneath the periosteum associated with a small aggregate of amphophilic material.

Gout is a relatively common disease of certain captive and wild reptiles. It is also a disease of humans, non-human primates, birds and canids, particularly the Dalmatian dog. The disease is caused by either increased production of uric acid (humans, Dalmatian dog) or a disruption in the balance between uric acid production and excretion (reptiles, birds). The most common causes of disruption in reptiles are a high protein diet, (eg., herbivorous reptiles fed a carnivorous diet), and chronic disease, especially renal tubular disease and dehydration. Administration of specific drugs (eg., furosemide, aminoglycosides, sulfonamides) can also result in decreased excretion of uric acid either through retention of urates (furosemide) or renal tubular damage (aminoglycosides, sulfonamides).[1,2]

Uric acid is an end product of degradation of dietary protein in humans, primates, the Dalmatian dog, birds and certain reptiles (terrestrial chelonians, all lizards and snakes).[3] Specifically, uric acid is the end product of purine nucleotide breakdown. Briefly, dietary protein is degraded into individual nucleic acids. These nucleic acids are broken down by nucleases to individual nucleotides, which are further hydrolysed into individual purine and pyrimidine bases. Pyrimidines are catabolized into CO_2 and NH_3. Purines undergo further degradation by xanthine oxidase to uric acid.[1,2]

In reptiles uric acid is cleared from the blood via the renal tubules. This is different from most mammals where clearance is accomplished through glomerular filtration. In blood, uric acid is present both as free uric acid and urate salts, both of which are relatively insoluble in water. When the concentration of either of these forms becomes elevated in the blood (hyperuricemia) or body fluids (e.g., synovial fluid) the uric acid and salts crystallize forming insoluble precipitates deposited in tissues throughout the body.[1-3]

These precipitates or "gout tophi" are frequently grossly visible at necropsy. In reptiles the most common sites of deposition are the pericardium, kidneys, liver, spleen, lungs, subcutis and other soft tissues.[1,4] A definitive diagnosis of gout is made by demonstrating monosodium urate crystals within the joints or affected tissues. There are diseases that result in the deposition of crystals other than sodium urate, which result in similar gross and histologic findings. This condition is referred to as pseudogout.[1,2]

In this case the animal was being fed an appropriate diet and had adequate water available. Histologic examination of the kidneys revealed possible moderate interstitial

fibrosis (and euthanasia solution artifact). Within the oviduct there was a severe focal subacute ulcerative salpingitis with intralesional gram-positive bacteria. Other organs demonstrated multifocal acute vascular fibrinoid necrosis. These findings suggest that this animal may have been septic prior to euthanasia. It is possible a combination of decreased renal function and decreased water intake due to underlying sepsis resulted in the development of articular gout in this case.

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AFIP Diagnosis: Carpus and associated soft tissue: Arthritis, tenosynovitis, and myositis, granulomatous, multifocal to coalescing, severe, with reactive bone and numerous urate tophi (gout), frilled lizard (*Chlamydosaurus kingii*), reptile.

Conference Comment: The contributor gives a concise review of pathologic mineralization. There are two forms of gout in birds and reptiles: visceral and articular. The visceral form is more common and presents grossly as white or gray chalky patches on the pericardium, liver, mesentery, and peritoneum, and renal interstitial or subcapsular deposits. The articular form is much less common and is characterized by swollen joints with white deposits on tendon sheaths.[5]

Tophi are crystalline structures with spicules that radiate from the center in a "starburst" fashion and can be stained with Gomori's methenamine silver (GMS).[6] Since urates are water soluble[7], urate deposits are leached when tissues are formalin-fixed. It may be preferable to collect tissues in absolute ethyl alcohol for best visualization of tophi.[3]

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SLIDE 49
<u>**CONFERENCE 12 / CASE IV - N2003-72 (AFIP 2897595)**</u>

Signalment: Approximately 6 1/2 year old, male, cloud rat (*Phloeomys pallidus*).

History: This cloud rat was thin and dyspneic. Pneumonia was confirmed on radiographs and the animal was placed in an oxygen cage and started on a course of intraosseous antibiotics and antifungals and antimicrobial nebulization. The rat died 5 days later.

Gross Pathology: The cloud rat was in thin body condition. Multiple fibrous adhesions were present between the pleura and the thoracic wall, pericardium, and diaphragm. The lungs had an irregular contour and approximately 90% of the parenchyma was firm and diffusely pale tan-gray with multifocal red mottling. On section, the lungs were firm and tan-white with multiple pockets and cavitations containing thick, yellow-white, often pasty material.   The tracheobronchial/ bronchial lymph nodes were enlarged, firm, and diffusely light tan with poor distinction between cortical and medullary tissue.

Laboratory Results:
•   Antemortem: positive *Cryptococcus* titer using latex agglutination
•   Post-mortem:
•   <u>lung cytology</u>: numerous organisms consistent with *Cryptococcus neoformans*
•   <u>lung fungal culture</u>: *Cryptococcus neoformans* (biotyping of variant not performed)

Contributor's Morphologic Diagnosis: Lung: Pneumonia, necrotizing, fibrosing, histiocytic to granulomatous, chronic, diffuse, severe with myriad intralesional fungal organisms consistent with *Cryptococcus neoformans*.

Contributor's Comment: *Cryptococcus neoformans* is a saprophytic heterobasidiomycetous yeast that appears in tissue sections as uninucleate, thin-walled, spherical, oval and elliptical cells that vary in size from 3.5 to 8um or more in diameter.[1,2] The yeast are surrounded by a mucopolysaccharide capsule that are unstained to very lightly stained with hematoxylin and eosin staining, and vary in appearance from wide spherical halos ("soap bubble" appearance) to nearly undetectable lighter zones around the cells.[1,2] The capsular material is usually readily demonstrated with mucin stains (Alcian blue, Mayer's mucicarmine) and PAS staining. In tissues, *Cryptococcus* species reproduce asexually as blastoconidia with narrow-based, most often single, budding.[2,3] Chains of budding cells may be observed; pseudohyphae and branched, septate hyphae are rarely produced in tissues.[1,2] The host response to infection with *C. neoformans* usually depends on the immunologic status of the host, the presence of underlying disease and whether or not the

cryptococci are encapsulated.[2] Inflammation can vary widely from none to an intense, suppurative and necrotizing reaction with subsequent granuloma formation and eventually fibrocaseous lesions. The relatively nonantigenic polysaccharide capsule inhibits plasma cell function, macrophage phagocytosis and leukocyte migration.[4] In older fibrocaseous lesions, GMS staining may be needed to demonstrate organisms. It is often difficult to isolate fungus from these lesions; a presumptive histologic diagnosis can be confirmed with immunohistochemistry.

There are two recognized biovariants of *Cryptococcus neoformans* that cause disease in both humans and animals. *Cryptococcus neoformans* var. *neoformans* has nearly worldwide distribution. It is most frequently associated with bird droppings (the organism utilizes creatine in the droppings), especially those of pigeons, and soils contaminated with bird manure.[1] *Cryptococcus neoformans* var. *gatti* occurs mainly in tropical, subtropical, and temperate climates and in association with certain species of gum trees (*Eucalyptus camaldulensis* and *E. tereticornis*).[5] In humans, *C. neoformans* var. *neoformans* more often affects and causes more severe disease in immunocompromised individuals, whereas *C. neoformans* var. *gatti* is repeatedly isolated primarily from immunocompetent hosts.[5]

Pulmonary and cerebromeningeal cryptococcosis are the two main forms of disease in humans and several species of domestic and wild animals.[2,4,6] Infection occurs by inhalation of aerosolized fungal cells from the environment.[2] The clinical course of pulmonary cryptococcosis is subacute or chronic and frequently is complicated by concomitant extrapulmonary infection. Additionally, about 1% of human patients with first-infection cryptococcosis develop a primary pulmonary-lymph node complex.[2] *Cryptococcus neoformans* has neurotropism and frequently spreads to the central nervous system from the respiratory tract either hematogenously[2] or via direct extension through the cribriform plate.[3,4] Other sites are involved in disseminated infection and cutaneous lesions (primary or secondary) can occur in humans and animals.

Cryptococcosis is the most common systemic fungal disease of domestic cats.[3,4] It has been reported in 2 cheetahs.[4] Some studies have noted an increased incidence of FeLV or FIV in cases of feline cryptococcosis.[3] However, concurrent underlying diseases are often not detected in domestic and exotic felids with cryptococcosis.[3,4] Therefore, though immunosuppression has been suggested as a predisposing factor in cryptococcal infection, it is difficult to make valid conclusions about the relationship between cryptococcosis and immunosuppression in cats.[4] In dogs, nasal cryptococcosis is the primary form, although disseminated systemic infection occurs. An association between cryptococcal infection and immunosuppression has not been documented in dogs.

There are sporadic reports of cryptococcosis in captive nonhuman primates, especially in Old World species. Reports in New World monkeys include a squirrel monkey (pulmonary and lymph nodes),[2] Geoffrey's tamarin (disseminated disease) and a common marmoset with a 1 month history of wasting (intestinal and mesenteric lymphatics).[7] Intestinal involvement is not common in animals or humans but it has

been sporadically identified as a opportunistic enteric pathogen in AIDS patients with diarrhea.[7]

Respiratory and central nervous system infection has been reported in captive bred elephant shrews (*Macroscelides proboscideus*) and captive bred and wild-caught tree shrews (*Tupaia tana* and *T. minor*).[6] Environmental exposure to the organism may be enhanced by exhibit conditions and/or behavioral factors, and there is evidence to suggest that tree shrews may have a predilection for cryptococcosis.[6]

Cloud rats (*Phloeomys pallidus*) are large, nocturnal, arboreal Asian rats. Necropsy results show that six of eighteen adult (>1 year of age) captive cloud rats (including the case submission) housed at the same facility but in three different locations have died between 1989 and 2003 due to cryptococcal infection. Affected animals were between 4 and 9 years of age. The respiratory tract was the most commonly affected organ system. Some animals died without premonitory signs. The cloud rat exhibits contain trees and other substrates that might be suitable sites for proliferation of *C. neoformans*, and it is suspected that the rats were exposed to the fungus via contaminated substrate. The immunologic status of the affected animals was unknown and significant concurrent disease was not always present. Further investigation including immunologic studies would be needed to determine whether the cloud rat is a species that is particularly susceptible to cryptococcosis.

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AFIP Diagnosis: Lung: Pneumonia, necrotizing, chronic, diffuse, severe, with myriad yeast, etiology consistent with *Cryptococcus neoformans*, cloud rat (*Phloeomys pallidus*), rodent.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. The contributor gives a thorough review of cryptococcosis. Conference attendees noted there was variation in the degree of fibrosis present among slides.

Differential diagnoses for fungal infections that cause granulomatous pneumonia include *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Histoplasma capsulatum*. *Blastomyces*, *Coccidioides*, and *Histoplasma* are unencapsulated, unlike *Cryptococcus*. *Blastomyces* reproduces by broad-based budding, while *Cryptococcus* and *Histoplasma* reproduce by narrow-based budding. *Coccidioides* reproduces by endosporulation. Mature sporangia of *Coccidioides* are 10-80um in diameter with a double-contoured wall and are filled with 2-5um diameter endospores. *Histoplasma* is much smaller (2-4um diameter) than *Cryptococcus* and is located intracellularly within macrophages.[8,9]

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SLIDE 50
CONFERENCE 13 / CASE I - A02-335 (AFIP 2890560)

Signalment: 3 year-old, female, Rhesus macaque (*Macaca mulatta*), nonhuman primate.

History: This monkey was inoculated with SIVmac316 in June of 2001. In June of 2002, she was incoordinated and febrile and had dermatitis. Two days later she was bloated and had diarrhea. She was euthanized due to a poor response to treatment and a poor prognosis.

Gross Pathology: The mucosa of the stomach, small intestine and large intestine contains multiple red, raised, pedunculated, nodular masses (Fig. 1) ranging from 0.6-1.2 cm. The ileum and cecum are the most severely affected. There is moderate to marked enlargement of peripheral and mesenteric lymph nodes, with marked edema of the mesenteric lymph nodes. The spleen is enlarged and there is prominent lymphoid hyperplasia. There is mild ventral subcutaneous edema and a small amount (20 mls) of clear straw colored fluid in the abdomen. There is a 3-4 cm focus of thickened, crusty

skin in the right inguinal region, and a similar 2-3 cm focus lateral to the right eye. There is marked, multifocal to coalescent thickening of the meninges, particularly along meningeal vessels, due to the accumulation of thick, green/yellow suppurative material. The mandibular and parotid salivary glands are markedly enlarged and there are multiple small (< 1mm) red foci throughout the mandibular salivary glands.

Laboratory Results: There is sporadic positive immunohistochemical staining with anti-CMV antibody.

Contributor's Morphologic Diagnosis: Severe, multifocal, proliferative and suppurative colitis (inflammatory pseudotumors) with intranuclear and intracytoplasmic CMV inclusion bodies, superficial mucosal hemorrhage, and intralesional *Balantidium coli*.

Contributor's Comment: Simian cytomegalovirus (sCMV), like its human counterpart, causes few if any, symptoms in monkeys. However, in an immune-compensated or suppressed patient, such as during transplantation, or as in this case, simian immunosuppressive virus-induced AIDs, CMV infection can be fatal[1]. The common identifiable gross lesions can be found in the meninges, eye, lung, heart, intestine, testicle and skin. Microscopically, lesions can be found in organs of the central and peripheral nervous, lymphatic, vascular, digestive and reproductive systems. It was thought that the infected cells were of mesenchymal origin, but recent work in clinical isolates of human CMV has shown that the virus is also endothelial cell-tropic and leukocyte-tropic[2]. The histological hallmark of the lesion induced by the virus is intranuclear and intracytoplasmic inclusion bodies and cytomegaly[1]. Furthermore, since CMV infection is known to induce the host cells to express proinflammatory proteins, such as IL-8 and RANTES, and also other binding molecules, such as ICAM-1 and LFA-3, recruitment and aggregation of neutrophils at the site of the lesion is very common and characteristic of a CMV infection[3].

Inflammatory pseudotumor, or IPT, is a quasineoplastic lesion that has been found to occur in nearly every site in the body. In humans, it is most commonly found in the orbit and lung. Due to its gross and radiographic resemblance to a neoplastic mass, it has been an area of focus for human medicine so as to avoid unnecessary radical surgery[4]. In the case presented here, the quasineoplastic lesion, as seen grossly and microscopically, is the result of an influx of neutrophils to the site of infection and proliferation of the mucosal epithelium. In most cases, both humans and nonhuman primates, CMV infections of the intestines are erosive, resulting in enterocolitis, hemorrhage, or intestinal perforation. Inflammatory mass formation is rare[5].

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AFIP Diagnoses:
1. Colon: Colitis, proliferative, neutrophilic, acute, multifocal, moderate, with superficial mucosal hemorrhage, cytomegaly, and eosinophilic to basophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), nonhuman primate.

2. Colon: Intraglandular ciliated protozoa, numerous.
3. Colon: Intraglandular epithelial-attached bacilli.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Conference attendees discussed the presence of surface associated spirochetes in glandular epithelium, consistent with *Brachyspira pilosicoli*. Although the significance in this case is unknown, the incidence of intestinal spirochetosis in clinically normal rhesus macaques was 42% in one study.[6]

Cytomegaloviruses are classified in the subfamily *Betaherpesvirinae* and are highly host-specific, causing low-grade, inapparent infections in immunocompetent humans, nonhuman primates, pigs, mice, and guinea pigs, among other species. Other betaherpesviruses of veterinary importance include porcine herpesvirus-2 (inclusion body rhinitis) and caviid herpesvirus-1 (guinea pig cytomegalovirus).[9]

Inclusion body rhinitis is a disease of young piglets causing necrosuppurative rhinitis with intranuclear inclusion bodies and cytomegaly in nasal mucous, harderian, and lacrimal glands, and renal tubular epithelium. Immunosuppressed piglets may develop systemic infection, causing widespread petechiae and edema.[7] Guinea pig cytomegalovirus causes karyomegaly and intranuclear inclusion bodies in the salivary gland, although interstitial pneumonia and multifocal necrosis in the lymph nodes, spleen, liver, and kidney may also be present. Guinea pigs are used as models for human cytomegalovirus, especially congenital infections. Like humans, the guinea pig placenta is hemochorial with a single layer of trophoblasts separating maternal and fetal circulation, facilitating transplacental transmission.[8,9]

Balantidium coli is a natural inhabitant of the intestinal lumen of pigs, rodents, and primates. Although usually an incidental finding, the organism rarely invades the mucosa and can cause enteritis and colitis. The trophozoites are large (50-200um), with a macronucleus and ciliated periphery.[10,11]

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SLIDE 51
CONFERENCE 13 / CASE II - 02154-6 (AFIP 2897019)

Signalment: 16-year-old, male, Poodle, Dog (*Canis familiaris*).

History: Oral bleeding for one week.

Gross Pathology: Oral mass (3 x 2 cm) on the lingual frenulum.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Lingual frenulum: Pleomorphic liposarcoma.

Contributor's Comment: A moderately infiltrative mass, 1 cm in diameter, was present in connective tissue covered by a squamous epithelium (not present on all the sections); the epithelium was ulcerated, which explains the oral bleeding (on the slides, the ulceration seems to be accentuated by the surgery). The tumor was composed of polygonal cells arranged in sheets with little stroma. Tumor cells have indistinct borders and are moderately pleomorphic; some cells are rather small, with eosinophilic cytoplasm; other cells have abundant cytoplasm, filled with either numerous small vacuoles, or a single large vacuole. Anisokaryosis is focally severe and mitotic figures

are present (Fig. 1). Small necrotic foci and discrete lymphoplasmacytic infiltrates are present.

Oil red-O staining of frozen sections demonstrates that the vacuoles are lipid droplets (Fig. 2), which helps to identify the tumor cells as lipoblasts. Most of the cells have numerous, small, lipid droplets, thus we diagnosed a pleomorphic liposarcoma[1].

The differential diagnosis includes infiltrative lipomas, composed of mature lipocytes which invade surrounding tissues[2], anaplastic carcinomas and balloon-cell melanomas[3,4]. Immunostaining with an anti-human cytokeratin (MNF116, Dako), reacts with a great majority of carcinomas, and was positive in the oral epithelium, but negative in the tumor cells (Fig. 3); the vacuoles did not stain by Periodic-Acid Schiff (Fig. 4). No cytoplasmic granules were observed with Schmorl staining (Fig. 5).

Oral liposarcomas are rare in human pathology[5] and only a small proportion develop in the tongue[6,7]. They generally occur in old patients[6-10], have a high recurrence rate and almost no tendency for metastasis[6,8]. They are generally small (less than 3 cm in greatest diameter)[6,10]. Two histological types of lingual liposarcomas are described: well-differentiated[6,8,9] and myxoid[5,7]. Human lingual liposarcomas have low mitotic activity and a high recurrence rate with a long period between the first presentation and the first recurrence[6].

To our knowledge, lingual liposarcomas have never been described in veterinary pathology[11]. In dogs, liposarcomas are rare[12] and mainly described in the subcutis, thoracic and abdominal cavities[13]. One case was associated with a foreign body[14]. The tumor of this dog did not contain a foreign body. It seemed to exhibit a higher mitotic rate than its human counterparts. However, 8 months later, the dog is still alive and free of recurrence.

Liposarcomas of the tongue are possibly underdiagnosed in veterinary pathology. There is a risk of recurrence, with a long delay. Wide excision is recommended.

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AFIP Diagnosis: Tongue: Liposarcoma, poodle, canine.

Conference Comment: Most conference attendees identified the tissue simply as mucous membrane and underlying connective tissue. In addition to the differential diagnoses mentioned by the contributor, attendees who favored conjunctiva as the tissue site also included meibomian gland carcinoma as a possible diagnosis.

Pleomorphic liposarcomas may resemble pleomorphic malignant fibrous histiocytomas (MFH); however, unlike MFH, liposarcomas have little to no collagenous stroma. Pleomorphic MFHs are composed of spindle cells with variable morphology and are arranged in a storiform pattern, whereas pleomorphic liposarcomas have highly

variable morphology, varied cellular arrangement, and intracytoplasmic fat vacuoles in a small percentage of cells.[15]

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SLIDE 52

CONFERENCE 13 / CASE III - AO 40673 (AFIP 2734406)

Signalment: 3-month-old male Simmental-cross calf, *Bos Taurus*.

History: This calf was a member of a small group of cows and calves. This calf was the only calf showing clinical signs. Labored breathing was noticed in the evening and the calf was dead the next morning. Dams were vaccinated with IBR, BVD, PI3 and BRSV vaccines.

Gross Pathology: At necropsy, there was pneumothorax and marked pulmonary emphysema. Numerous large subpleural bullae were present throughout the lungs. Overall, the lungs were heavy and wet. The ventral portions of the middle lung lobes were dark purple and consolidated.

Laboratory Results: Fluorescent antibody tests for IBR, BVDV, and PI3 were negative and positive for BRSV.

Contributor's Morphologic Diagnoses:
1. Subacute fibrinopurulent bronchopneumonia with bronchiolitis, syncytial cells and intracytoplasmic inclusion bodies.
2. Diffuse pulmonary emphysema and pneumothorax.
(Bovine respiratory syncytial virus pneumonia)

Contributor's Comment: In sections of lung, bronchi and bronchioles are filled with degenerate neutrophils, erythrocytes and cell debris. There is necrosis of bronchiolar epithelium and numerous syncytial cells are present in the lumens. Pleomorphic eosinophilic intracytoplasmic inclusions are present in bronchiolar epithelial cells and in syncytial cells. Peribronchiolar alveoli are filled with degenerate neutrophils, erythrocytes, and fibrin. The lungs are markedly congested and septal lymphatics are filled with fibrinocellular exudate. Septal edema and emphysema are marked.

Respiratory syncytial viruses are pneumoviruses in the family Paramyxoviridae. Members of the genus *Pneumovirus* include human, bovine, ovine, and caprine respiratory syncytial viruses, pneumovirus of mice, and turkey rhinotracheitis virus. BRSV is distributed worldwide and antibody prevalence in the United States ranges from 65-81%. Clinical disease is most common in calves less than 6-months of age and is more severe if infections are concurrent with other respiratory viruses or bacterial agents. Secondary bacterial pneumonia is common. Consistent findings include emphysema, severe necrotizing bronchiolitis with syncytial cells, purulent and bronchointerstitial pneumonia. Inclusion bodies are evident in the early stages of infection. Bronchiolitis obliterans is a common sequela. Diagnosis is confirmed by fluorescent antibody test or immunohistochemistry. Virus isolation is difficult.[1,2]

The present calf died suddenly due to pneumothorax secondary to severe pulmonary emphysema. At necropsy, atypical interstitial pneumonia was considered in the differential diagnosis. Microscopically, inclusion bodies and syncytial cells were

prominent in bronchiolar epithelium and the diagnosis was confirmed by immunofluorescent antibody staining. Bacterial colonies were present in some sections.

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AFIP Diagnoses:
1. Lung: Pneumonia, bronchointerstitial, neutrophilic, acute, diffuse, severe, with necrosis, syncytia, and eosinophilic intracytoplasmic inclusion bodies, Simmental-cross, bovine.
2. Lung: Rare epithelial basophilic intranuclear inclusion bodies.

Conference Comment: Basophilic intranuclear inclusion bodies, consistent with adenoviral inclusions, were identified in the respiratory epithelium. In addition to bovine respiratory syncytial virus (BRSV), conference attendees discussed other causes of bovine bronchointerstitial pneumonia, including parainfluenza type-3 (PI-3) and bovine adenovirus.

Bovine respiratory syncytial virus is an important component of the bovine respiratory disease complex (BRD) that most often affects young cattle and predisposes to secondary infections, like *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*. Bronchoconstriction is an important feature of BRSV that leads to airway obstruction and terminal interstitial emphysema. Evidence suggests that virus-infected cells activate complement, which causes mast cell degranulation and histamine-induced bronchoconstriction. Virus specific IgE antibody is also implicated in the pathogenesis and resultant clinical signs.[3,4,5]

Differential diagnoses for any bovine pneumonia should include those agents associated with the BRD complex. This includes enzootic pneumonia of calves (a variety of etiologic agents), pneumonic mannheimiosis (*Mannheimia haemolytica*), respiratory hemophilosis (*Haemophilus somnus*), infectious bovine rhinotracheitis (bovine herpesvirus-1), mycoplasmosis, and adenovirus.[6]

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SLIDES 53, 54, and 55
CONFERENCE 13 / CASE IV - 0200584 and 0201878 (AFIP 2891625)

Signalment: Adolescent (1.7-3.8 kg) female Cynomolgus monkey(s), *Macaca fascicularis*.

History: Cynomolgus monkeys were exposed to spores of anthrax (Ames strain) by inhalation to determine the LD_{50} and pathology, in order to investigate this species as a model for inhalation anthrax subsequent to the reduced availability of Rhesus monkeys for this purpose.

In general, monkeys remained bright and alert for several days (2-7), then became lethargic and non-responsive, with death (or euthanasia) within a couple hours.

Gross Pathology: The gross photographs represent tissues from 3 monkeys (Figs. A-C); slides provided were from two monkeys (one of which was in these gross photographs). The most common gross lesions were mild splenomegaly, lymph node enlargement, and hemorrhages in various organs, particularly involving the meninges and the lungs (Fig. D). Mediastinal hemorrhage and/or edema affected 29% of the monkeys.

[1]The gross brain image annotated with superscript 1 (Fig. C) is from Vasconcelos et al. Pathology of inhalation anthrax in cynomolgus monkeys (*Macaca fascicularis*). Laboratory Investigation 83(8):1201-1209, 2003.

Laboratory Results: *Bacillus anthracis* was cultured from blood of all affected animals (collected when moribund or as soon as possible post mortem). Bacteria in histologic sections were usually strongly Gram-positive.

Contributor's Morphologic Diagnoses:
SPLEEN (0200584):
1. Lymphocytolysis (necrosis and apoptosis), severe, acute, diffuse, with hemorrhage and numerous bacteria characteristic of *Bacillus anthracis*.
2. Vasculitis, moderate, acute, multifocal.

BRAIN (0201878):

1. Meningitis, hemorrhagic and suppurative, mild to moderate (based upon gross), acute, multifocal, with bacteria characteristic of *Bacillus anthracis*.

Contributor's Comment:
Description:
0200584 (Spleen): There is severe loss of lymphoid tissue from the periarteriolar lymphoid sheaths, which are almost obliterated, and from splenic corpuscles (Fig. 1). The mantle zones of the splenic corpuscles are hemorrhagic (Fig. 2). The splenic corpuscles contain abundant nuclear debris ("nuclear dust") both extracellularly (Fig. 3, green arrows) and within macrophages. Some macrophages contain brownish pigment (hemosiderin). Only occasional splenic corpuscles still contain intact lymphocytes. The red pulp contains numerous large square-ended ("boxcar"-like) rods (bacteria, black arrow in Fig. 3) and abundant granular eosinophilic material. Fewer bacteria are visible within arteries and veins, which have an increased number of leukocytes (predominantly neutrophils). Many sections have arteries in which leukocytes are lined up along the endothelium (pavementing) or are beneath the endothelium (Fig. 4; the vessel lumen is at lower left). In an occasional section there is fibrillar eosinophilic material within splenic corpuscles, interpreted to be fibrin.
Special stain (Hopps): Bacteria are Gram-positive.

0201878 (Brain): The meninges have foci of hemorrhage and infiltration (minimal to mild) with leukocytes (moderate numbers of neutrophils and fewer lymphocytes) (Fig. 5). Within these areas there are few to numerous square-ended rods (bacteria, Fig. 6 arrow), which are also present intravascularly although usually in smaller numbers.

Significance:
    The recent use of anthrax as an agent of bioterrorism in the United States has resulted in increased use of monkeys as models for inhalation anthrax of humans. Rhesus monkeys are currently less available than Cynomolgus monkeys, which are a suggested alternative for investigations into the pathogenesis and immunology of this disease (1).

Pathogenesis:
    The pathogenesis of inhalation anthrax is thought to begin with macrophage phagocytosis of inhaled spores. The macrophages migrate to intrathoracic (bronchial or mediastinal) lymph nodes and the spores germinate and are released. The vegetative bacilli release 2 exotoxins known to be important to the pathogenesis, lethal toxin (LT) and edema toxin (ET) (reviewed in 2). Lethal toxin is composed of lethal factor (LF) and protective antigen (PA), whereas edema toxin is composed of edema factor (EF) and protective antigen (PA). It is thought that PA diffuses more rapidly from the bacilli and binds cells, forming pores. Subsequent binding of lethal factor or edema factor forms the respective toxins, which enter the cell. Anthrax strains harboring mutations to either the LF or EF protein genes are less lethal than the parent strain, whereas mutations in the PA gene abolish lethality (reviewed in 2).

Lethal toxin is a zinc protease to which macrophages are particularly susceptible (4, reviewed in 2). It inhibits mitogen-activated protein kinase (MAPK) *in vitro* by proteolysis of the N-termini of mitogen-activated protein kinase kinases (MAPKK), rendering them incapable of activating MAPK (3, and reviewed in 2). Depletion of macrophages, pre-treatment with an IL-1 receptor antagonist, or passive immunization against IL-1 all render mice resistant to lethal toxin (4). Sublethal doses of LT may induce macrophages to produce TNF-alpha and IL-1 (4), which may account for the shock-like death.

Edema toxin is considered responsible for the edema characteristic of anthrax septicemia. It is an adenylate cyclase, converting ATP into cAMP in a Ca^{++}- and calmodulin- dependent manner (2). Edema toxin action upon macrophages inhibits phagocytosis and the oxidative burst (reviewed in 2).

Whereas herbivores are extremely susceptible and have minimal lesions, carnivores are resistant and often have lesions at the site of entry (e.g. pharyngitis). Primates are considered to be of intermediate susceptibility.

Comparative Pathology:
The gross and microscopic pathology of anthrax in both Rhesus monkeys and Cynomolgus monkeys is very similar to that in humans (1, 5-9). Lesions are typical of a fulminant septicemia. Grossly, hemorrhages are common in the meninges, lung, and mediastinum, although they can occur anywhere in the body (1, 5, 7). Enlargement of intrathoracic lymph nodes is characteristic (1, 5, 7). Mild splenomegaly appears more common in non-human primates than in humans (1, 5, 8, 9). Animals that die peracutely (i.e. within 2-3 days) have fewer lesions than those that survive several days. Occasional non-human primates exhibit a virtual absence of gross lesions (1, 6, 7), suggesting the possibility that human cases might not be diagnosed if histopathology or bacteriologic cultures were not performed.

Microscopically, hemorrhages are common in intrathoracic lymph nodes, lungs, meninges, adrenal glands, mediastinum, and gastrointestinal tract, possibly secondary to necrotizing vasculitis, which has been reported in all three species (1, 7-9). Pulmonary edema (as well as less-frequent acute pulmonary inflammation), hemorrhagic meningitis, and mild suppurative mediastinitis and lymphadenitis are all characteristic microscopic findings in primates including humans (1, 5-9). Marked lymphocytolysis (apoptosis and/or necrosis) in the spleen and lymph nodes is very common in all 3 species, with some differences among reports as to whether a B- or T-lymphocyte predilection exists (1, 7, 9). There do not appear to be reports of lymphocytolysis occurring in the thymus, although this may be a reflection of the age of patients and/or animals involved (1, 5-9).

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AFIP Diagnoses:

1. Spleen: Lymphoid necrosis, diffuse, with perifollicular hemorrhage, fibrin, and myriad gram-positive bacilli, cynomolgus macaque (*Macaca fascicularis*), nonhuman primate.
2. Cerebellum, meninges: Meningitis, neutrophilic, acute, with vasculitis, and myriad bacilli.

Conference Comment: The contributor gives an excellent review of anthrax. Conference attendees noted that a few neutrophils extend beyond the affected meninges into the neuropil. Attendees also noted eosinophilic globules within the white matter, as noted by Vasconcelos, et al. and interpreted to be either necrotic oligodendroglia or macrophages.[1]

Herbivores are most susceptible to anthrax, primates have intermediate susceptibility, and carnivores and pigs are least susceptible. Under natural conditions, most birds, reptiles, and fish are resistant because their normal body temperature is outside of the optimal range for the anthrax bacillus. Ostriches are the only avian species reported to have been naturally infected with anthrax.[10,11,12]

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SLIDE 56
CONFERENCE 14 / CASE I - D01-7839 (AFIP 2789808)

Signalment: Tissue section is from a 10-year-old spayed female, mixed breed canine.

History: The dog was presented with heartworm disease of unknown duration. One week before presentation the dog was treated with a filaricide. There were no adverse effects during or following treatment. The day before presentation, severe respiratory compromise developed. The clinician suspected possible pulmonary embolism. The animal did not respond to treatment with dexamethasone sodium phosphate and prednisone. The dog expired following severe respiratory compromise and cardiovascular collapse.

Gross Pathology: Necropsy revealed adult heartworms in the pulmonary vasculature.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Dog, lung: Interstitial pneumonia, marked, diffuse, suppurative with thrombosis, microfilaria and adult *Dirofilaria immitis*.

Contributor's Comment: A large area of the lung section is characterized by necrosis, hemorrhage and infarction. In adjacent viable lung, alveoli contain neutrophils, macrophages and red blood cells. Within large pulmonary arteries, thrombosis is evident and associated with intravascular nematodes. A sagittal section of an adult *Dirofilaria immitis* illustrates coelomyarian muscles, gravid uterus and gut. Microfilariae are evident within the small vessels and capillaries of the lung.

Heartworm disease is a common infection of Canidae and Felidae of the southern and eastern coastal area of the United States. There are many genera of filarial parasites that can infect both man and animal. *Dirofilaria immitis* is the filarial parasite of most importance.

The adult worms of *Dirofilaria* lodge in pulmonary arteries and right ventricle of the heart interfering with blood circulation and causing many, and sometimes fatal, clinical manifestations. These manifestations are shortness of breath, weakness, cardiac enlargement, hepatomegaly, ascites and hypertrophic pulmonary osteoarthropathy. The lodged adult female worms discharge microfilariae into the bloodstream where mosquitoes ingest them during feeding. These microfilariae then mature into the infective state (L3 larvae). Given proper environmental conditions, the microfilariae are deposited into the skin of an animal during the mosquito's next feeding. The L3 larvae then molt and migrate to the pulmonary arteries and mature into adult nematodes.

Diagnosing dirofilariasis is possible with the following procedures: Knott's test, ECG, thoracic radiographs, IFA for microfilariae, ELISA for adult antibody (cats only), ELISA for adult antigen and arterogram. Treatment of infected animals requires adulticide, mircofilaricide and preventative for re-infection with microfilaria.

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AFIP Diagnosis: Lung: Pneumonia, necrosuppurative, diffuse, severe, with hemorrhage, fibrin, thrombi, microfilaria, and intravascular adult nematodes, etiology consistent with *Dirofilaria immitis*, mixed breed, canine.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, parasitology consultant to the AFIP. Identification of this filarid is based on its size, thick cuticle, paired uteri, and a very small intestinal diameter. On some oblique sections, the lateral internal ridges in the lateral chord area can be identified. Some slides show pyogranulomatous inflammation associated with microfilariae. There are aggregates of free and phagocytized debris from dead microfilariae.

Conference attendees discussed the presence of smooth muscle hypertrophy in smaller vessels. In addition to pulmonary hypertension-induced smooth muscle hypertrophy, the role of platelet-derived growth factor (PDGF) was discussed. Vascular injury disrupts the balance between growth inhibition and growth promotion, favoring smooth muscle cell growth. When endothelial cells are injured PDGF is released, mediated by thrombin, promoting the migratory and proliferative activity of smooth muscle cells.[4]

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SLIDE 57
CONFERENCE 14 / CASE II - 25683-03 (AFIP 2893040)

Signalment: Two-day-old, male, cross-breed calf, (*Bos taurus*).

History: Calf appeared to be normal at birth and nursed well. Unexpected death.

Gross Pathology: Heart and skeletal muscles were reported to be pale in appearance.

Laboratory Results: No ancillary tests were performed.

Contributor's Morphologic Diagnoses: Severe acute to subacute multifocally disseminated necrotic myocarditis.

Contributor's Comment: These sections of heart are characterized by randomly arranged broad areas of necrosis. There is modest diffuse interstitial hemorrhage throughout the necrotic areas. Nuclear pyknosis, karyorrhexis, loss of striations, and granular eosinophilic cytoplasm are noted in necrotic myocytes. Hypercontraction bands are present in low numbers of necrotic myocytes. Occasional aggregates of mineral can be seen in necrotic myocytes. There are foci of interstitial inflammation consisting of neutrophils and macrophages in some areas. Fibrinoid necrosis of tunica media of occasional arteries can be seen.

These lesions are consistent with what has been reported for nutritional cardiomyopathy due to selenium or vitamin E deficiency.[1] This case is somewhat unusual compared to what is described for the typical age of onset of this disease.[1] A report of a recent investigation indicated bovine fetal deaths with myocardial lesions and heart failure were associated with selenium deficiency.[2] Liver lesions of heart failure were also reported.[2] In the present case, there were histologic changes in the liver consistent with heart failure, mainly periportal fibrosis.

AFIP Diagnosis: Myocardium: Necrosis, multifocal and coalescing, with fibrinoid vasculitis and edema, cross-breed, bovine.

Conference Comment: Vitamin E is a fat-soluble vitamin and an important antioxidant, acting as a free radical scavenger. Selenium is an essential component of glutathione peroxidase, which catalyzes the breakdown of free radicals. A deficiency of either

vitamin E or selenium results in increased levels of free radicals, which cause membrane damage and cellular destruction.[3,4]

Common gross lesions of vitamin E/selenium deficiency in different species were discussed. Typical gross findings of skeletal muscle necrosis (white muscle disease) may be seen in most species, including cattle, sheep, goats, horses, pigs, dogs, mink, rats, mice, rabbits, guinea pigs, nonhuman primates, and humans. Mulberry heart disease and hepatosis dietetica are diseases of young swine. Nodular panniculitis and steatitis (yellow fat disease) are most common in cats, mink, and piscivorous birds. Intestinal lipofuscinosis (brown dog gut) is a characteristic finding in dogs. Chickens develop encephalomalacia (crazy chick disease) and turkeys develop encephalomalacia with hemorrhage (cherry red cerebellum).[4]

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SLIDE 58
CONFERENCE 14 / CASE III - 2-1000 (AFIP 2888622)

Signalment: 4 month old, female, DSH kitten.

History: This kitten presented with a 5-day course of vomiting and mucoid diarrhea. It was also febrile and had experienced significant weight loss. On physical examination the animal was emaciated and slightly icteric. Abdominal palpation revealed a firm, small abdominal mass.

Gross Pathology: On laparotomy, there was a focal enlargement of the distal ileum extending into the ileocecal junction and the ileocecal lymph node complex was moderately enlarged. No intussusception was noted. A block resection of the ileocecocolic region was submitted.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
Intestine/Cecum:
1. Enteritis/typhlitis, necrotizing and ulcerative, transmural, with extensive submucosal expansion by multifocal and coalescing nodules of pyogranulomatous inflammation & edema and extension into adjacent mesentery (some slides also have focally extensive regions of mucosal ulceration associated with a severe necro-suppurative process).
2. Angiitis, with areas of fibrinoid vascular wall necrosis.

Mesenteric lymph node:
1. Lymphadenitis, necrotizing and pyogranulomatous with extensive nodal effacement and areas of fibrinoid necrosis and angiitis.
2. Lymphoid depletion, diffuse, moderate-marked.

Contributor's Comment: The microscopic lesions varied somewhat in the different conference slides submitted, depending on what portion of the block resection submission (i.e. ileum, cecum and/or mesenteric lymph node) was represented.

The clinical presentation, gross lesions and microscopic findings are all consistent with an uncommon, but well described variant of the non-effusive form of FIP in which the disease manifests initially as a localized/segmental transmural swelling in the ileocecal region[1]. This process is readily detectable clinically as a palpable abdominal mass.

Immunohistochemical evaluation of these tissues revealed strongly positive staining for antigen in the cytoplasm of macrophages in pyogranulomas located in the submucosa of the intestine as well as mesenteric lymph nodes.

Feline Infectious Peritonitis (FIP) is a progressive, fatal disease caused by a coronavirus. The condition is described in numerous felids and develops predominantly in younger animals, although any age may be affected. Disease exists in two clinical forms, effusive (wet) and non-effusive (dry)[2]. The wet/effusive form manifests as a characteristic effusion in the thoracic or abdominal cavity associated with pyogranulomatous inflammation. This presentation occurs when there is a weak cell-mediated immune response and a strong humoral immune response by the host. The dry/non-effusive form is more variable with respect to lesions, often presenting more localized granulomas or pyogranulomas within solid abdominal organs, lungs, eye or CNS tissue. It develops when there is an inflammatory but non-protective cell-mediated immune response[3]. In general, cats with FIP virus (FIPV) infection that develop disease have significant depletion of both T and B cells in lymphoid tissue, whereas cats with FIPV infection and no disease show distinct lymphoid hyperplasia.[4]

The reason for the localized manifestation of this form of the disease (vs. the multi-organ pyogranulomatous disease process generally seen in cats affected with the non-effusive form of FIP) is not clear. The pathogenesis may involve a partial cell-mediated immune reaction that initially restricts the virus to macrophages in focal segments of the

intestine, but does not eliminate the virus - causing the development of a localized, chronic-active inflammatory process. In all cats reported with this condition, the lesions progressed to multisystemic FIP. This variant, therefore, does carry the same grave prognosis as other forms of the disease.

Submitting veterinarians often tentatively diagnosis this condition as lymphosarcoma, based on the presence of a relatively circumscribed firm mass in the ileocecal region. Although the prognosis for both conditions (FIP and intestinal lymphoma) is poor, the distinction is critical to prevent exposure and infection of other susceptible cats.

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AFIP Diagnoses:
1. Intestine: Enteritis, pyogranulomatous, transmural, diffuse, severe, with multifocal vasculitis, domestic shorthair, feline.
2. Lymph node and associated mesentery: Lymphadenitis and serositis, pyogranulomatous, diffuse, severe, with necrosis and vasculitis.

Conference Comment: In sections with intestine, conference attendees had either sections of ileum, with villus blunting and fusion, or colon. There is variation in vasculitis among slides, from fibrinoid necrosis to vasculitis with neutrophilic infiltrates and necrotic debris. Conference attendees discussed how both type III and type IV hypersensitivity reactions are involved in the pathogenesis of FIP.

Cats are likely infected by exposure to exogenous virus via the oro-nasal route or by mutation of an endogenous enteric coronavirus. The virus replicates in the tonsil, lymph nodes, and intestine. After primary replication in lymphoid tissues a viremia occurs resulting in infection of macrophages in many tissues. As the contributor mentioned, disease occurs when the host fails to mount adequate cell-mediated immunity. If there is no cell-mediated immune response, macrophages infected with the virus accumulate in the perivascular spaces and interstitium of serous surfaces resulting in the wet, or effusive, form of FIP. If there is a weak cell-mediated immune response, fewer macrophages accumulate and there is decreased production of virus, resulting in the dry form of FIP. In either the wet or dry form of the disease the pathologic changes are induced by excess formation of antigen-antibody complexes (type III hypersensitivity). These complexes are phagocytized by macrophages and deposited in vessel walls. Complement fixation followed by neutrophil chemotaxis and macrophage activation culminates in tissue destruction. This Arthus reaction permits the effusion of protein-rich fluid and is most pronounced in the serosal surfaces, liver, and kidney.[5,6]

Due to similarities with other immunological granulomatous diseases, it is suggested that type IV hypersensitivity also plays a role in the pathogenesis of FIP. Immunohistochemical findings in such lesions include the presence of CD4+ T lymphocytes uniformly distributed throughout the lesion and CD8+ T lymphocytes at the periphery.[7,8] A type IV hypersensitivity pattern has also been detected in focally

induced lesions of FIP, demonstrated by the progressive activation of CD4+ T lymphocytes and the presence of macrophages.[8]

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SLIDE 59
CONFERENCE 14 / CASE IV - UFSM-1 (AFIP 2897023)

Signalment: One-year-old, castrated male, Holstein, bovine.

History: A disease characterized by severe respiratory distress was diagnosed in a herd of 23 dairy cattle in a small farm in southern Brazil. The first clinical signs developed one day after a batch of sweet potatoes (*Ipomoea batatas*) was introduced into the animals' feed, and the disease ran a clinical course of 2-4 days. Clinical signs included extended head and labored breathing, rapid respiratory rate (120 breaths/min) and rhythmical flaring of the nostrils. Five cattle were affected and three of them died (including the case cited in this report).

Gross Pathology: Gross lesions were similar in the 3 necropsied cattle, being restricted to the lungs and consisting mainly of emphysema and edema. The lungs

were firm, rubbery, distended and failed to collapse when the thorax was open and were firm and rubbery (Fig. 1). Interstitial emphysema characterized by numerous air bubbles in the interlobular and subpleural spaces was prominent (Fig. 2). At the cut surface, the lobules and the interlobular and peribronchial spaces were distended by gelatinous, light yellow material (edema) and air bubbles (Fig. 3). Multifocal, small (1-2 mm) white foci were distributed throughout the cut surface of the lung. Abundant white froth was observed within the trachea and major bronchi. Mediastinal lymph nodes were enlarged and moist.

Laboratory Results: Mycological cultures of the damaged sweet potatoes that were fed to the cows yielded *Fusarium solani* and *F. oxysporum*.

Contributor's Morphologic Diagnosis: Interstitial pneumonia with pulmonary edema and emphysema, acute, severe, Holstein, bovine.

Etiologic diagnosis: Toxic pneumonia

Etiology: Moldy sweet potato toxins

Contributor's Comment: The slides submitted are representative of the lesions found in all three cases, although they were from a single animal (a 1-year-old-calf). The interlobular septa are markedly distended by edema and emphysema, and the alveolar septa are thickened by edema, mononuclear infiltrate, and few neutrophils. Hyperplasia and hypertrophy can be observed in the epithelium of terminal bronchioles and alveolar ducts, imparting an adenomatous appearance to these structures. Numerous desquamated pneumocytes (occasionally forming syncytia) can be seen in the airways and alveolar spaces. Some alveoli have hyaline membranes. Reactive hyperplasia is observed in the mediastinal lymph nodes (slides not included).

The diagnosis of interstitial pneumonia caused by the ingestion of moldy sweet potatoes in the cases described here is based on epidemiology, clinical signs, laboratory results, necropsy findings and histopathology, all of which are similar to the description of this condition by several authors[2,3,6,8,9].

The great majority of cases of interstitial pneumonia associated with the consumption of moldy sweet potatoes (*Ipomoea batatas*) are caused by contamination with the fungus *Fusarium solani*, although *F. fimbriata* and *F. oxysporum* are occasionally implicated[10]. It has been demonstrated that these *Fusarium* species have a stimulant effect on toxin production by sweet potatoes. Sweet potatoes, under stress caused by mechanical injury, insect invasion, treatment with exogenous chemicals, or microbial infection can produce 3-substituted furans; toxins that have the ability to cause lesions in lung cells of cattle, rats, rabbits and guinea-pigs[3,6,910]. These toxins are collectively referred to as "lung edema factor" and include 1-ipomeanol, 4-ipomeanol, 1,4-ipomeanol, 1,4-ipomeadiol and ipomeanine, which are responsible for the acute pulmonary edema and emphysema that occurs upon consumption of *Fusarium*-infected sweet potatoes. The pathogenesis of the poisoning by moldy sweet potatoes in cattle is

similar to that of other interstitial pneumonias involved in ARDS. It consists basically of the generation of free radicals within type I pneumocytes and bronchiolar epithelia, which result in the death of these cells. Upon its arrival in the lung, 4-ipomeanol is activated through mixed function enzymes (oxidases) into potent lung toxins[3]. In addition to the destruction of pneumocytes, 4-ipomeanol causes edema by the destruction of endothelial cells leading to the formation of hyaline membranes[1]. As time goes by, type II pneumocytes undergo cellular division, proliferate in great numbers and line the alveoli imparting the adenomatous histopathological appearance, characteristic of affected cattle. In those cattle that survive the more acute phase, there is accumulation of inflammatory cells and fibroblasts in the pulmonary interstitium.

Ingestion of moldy sweet potatoes has caused interstitial pneumonia in cattle in the USA[8], Japan[10], Australia[3], Uruguay[9] and Brazil[6]. In the USA there was an outbreak of interstitial pneumonia associated with the consumption of hay of the pink half-runner bean (*Phaseolus vulgaris*), contaminated by the fungus *F. semitectum*[5].

Clinical signs of interstitial pneumonia in cattle caused by the consumption of mold-damaged sweet potatoes are acute in onset and include tachypnea, tachycardia, hyperpnea and dyspnea. Loud expiratory grunting, frothing at the mouth, extension of the head and neck, and flaring of the nostrils will also be seen[2,3,5,6,10]. Signs usually occur within one day of exposure and death usually occurs 2 to 5 days later[10].

Gross lesions and histopathology are rather characteristic of the condition and, when associated to right epidemiological, clinical and laboratory data, allow for a definite diagnosis. There are, however, several other causes producing clinical signs and lesions in cattle remarkably similar to those described here and should thus be included in the differential diagnosis. These conditions were grouped in the past under the term "atypical interstitial pneumonia" (AIP), because many causes of these pneumonias were unknown. In recent years however, the term AIP tends to be replaced by the designation "acute respiratory distress syndrome" (ARDS) of cattle. The various causes of ARDS include[4] 1) ingestion of moldy sweet potatoes; 2) extrinsic allergic alveolitis (hypersensitivity pneumonitis) caused by exposure to the dust from moldy hay or other plant matter contaminated by *Micropolyspora faeni* or *Thermoactinomyces vulgaris*[4]; 3) acute bovine pulmonary edema and emphysema (ABPE), also known as "fog fever" which occurs in cattle which are changed from dry, sparse forages to green pastures, and is caused by the conversion of L-tryptophan present in the lush green forages to 3-methylindole; 4) reinfection syndrome, i.e., hypersensitivity to the lungworm *Dictyocaulus viviparus* infection; 5) poisoning by plant fungal toxins such as perilla (*Perilla frutescens*) ketone, stinkwood (*Zieria arborescens*), rape, kale and turnip tops (*Brassica* spp.), *Crotalaria* spp., *Acremonium loli* contaminated ryegrass[7]; and 6) poisonous gases such as nitrogen dioxide produced by anaerobic fermentation of green plant material ("silo gas"), zinc oxide, chlorine, and manure gases (mixture of hydrogen sulfide, ammonia, carbon dioxide, methane and carbon monoxide).

AFIP Diagnosis: Lung: Pneumonia, interstitial, acute, diffuse, severe, with interstitial edema and emphysema, hyaline membranes, and type II pneumocyte hyperplasia, Holstein, bovine.

Conference Comment: The contributor gives an excellent review of pneumonia caused by moldy sweet potatoes and the differential diagnosis for bovine interstitial pneumonia. Conference attendees discussed the prominent hyaline membranes in this case. Hyaline membrane formation, typical of the acute phase of interstitial pneumonia, is evidence of significant cellular injury caused by the mixture of protein-rich edema fluid and remnants of necrotic cells.[11]

Conference attendees discussed the importance of the P450 enzyme system in Clara cells in the pathogenesis of acute bovine pulmonary edema (fog fever, named for regrowth - "foggage" - after hay or silage has been cut). When cattle are moved from dry to lush pasture with high concentrations of tryptophan, the tryptophan is converted in the rumen to 3-methylindole (3MI) and disseminated throughout the body. This metabolite is transformed by the P450 enzyme system of the lung to 3-methyleneindolenine (3MEIN), which damages cell membranes of bronchiolar cells and type I pneumocytes, and increases alveolar permeability leading to edema and interstitial pneumonia.[12,13]

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SLIDE 60
CONFERENCE 15 / CASE I - 00-238 (AFIP 2738736)

Signalment: 3 year old, male, foxhound mix.

History: The dog was from a kennel of 120 dogs in New York. Twenty of the dogs were sick or had died recently. This dog presented with fever, generalized lymphadenopathy, multiple subcutaneous masses and multifocal facial and periocular alopecia. (This case was submitted in July 2000).

Gross Pathology: The dog exhibited marked muscle wasting and depleted fat stores. There were multiple patches of alopecia on the face and ears. There were three soft, tan subcutaneous nodules over the thorax. The liver, spleen and multiple peripheral and mesenteric lymph nodes were enlarged. The carpal, stifle and tarsal joints had a moderate amount of brown exudate.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Spleen: Histiocytic and lymphoplasmacytic splenitis, with numerous intrahistiocytic protozoa, consistent with *Leishmania* sp., lymphoid involution and extramedullary hematopoiesis.

Contributor's Comment: *Leishmania* sp. are intracellular, kinetoplastid protozoan parasites from the family *Trypanosomatidae* that parasitize cells of the mononuclear phagocytic system. (Fig. 1) Leishmania amastigotes have also been reported within fibroblasts, which may represent a survival mechanism to evade the immune system. Leishmania is endemic in many parts of the world. In some areas, dogs are a reservoir and therefore have a role in zoonotic transmission. Leishmania is spread by sandflies (*Lutzomyia* - new world, *Phlebotomus* - old world). Other biting insects such as *Stomoxys* and *Rhipicephalus* may act as mechanical vectors. Leishmania are phagocytized by macrophages. Within the phagolysosome, the organism transforms into a round amastigote that lacks a flagella but contains a single, large mitochondrion-

like structure (kinetoplast). A proton-transporting ATPase protects the amastigotes from the acidic environment and maintains an intracellular parasite pH of 6.5. Two additional virulence factors on the surface include lipophosphoglycans and gp63. Lipophosphoglycans bind C3b or iC3b. Organisms resist lysis by complement C5-C9, and are phagocytized by macrophages via complement receptors CR1 (LFA-1) and CR3 (MAC-1 integrin). Lipophosphoglycans may also scavenge oxygen radicals and inhibit lysosomal enzymes. Gp63 cleaves complement and some lysosomal antimicrobial enzymes. Amastigotes multiply by binary fission, which leads to mechanical rupture of the macrophage. The extent of lesions depends on the cell-mediated immune response. Parasite specific CD4+ helper T lymphocytes secrete interferon gamma and macrophages secrete TNF alpha, which activate phagocytes to kill toxic parasites via toxic metabolites of oxygen and/or nitric oxide. TNF alpha acts in an autocrine fashion to induce nitric oxide production. Nitric oxide is toxic to amastigotes by interfering with iron dependent enzymes responsible for DNA replication, the citric acid cycle and mitochondrial respiration. Parasite specific helper T cells secrete IL-4 which inhibits macrophage activation by interferon gamma and inhibits the secretion of TNF alpha, thereby depressing the immune response. Demodicosis is common in dogs with Leishmaniasis, and provides evidence of suppressed cell mediated immunity. There are three major forms of Leishmania, and they are each associated with a specific species of the organism: cutaneous, mucocutaneous and visceral. Dogs may develop concurrent cutaneous and visceral manifestations, while they are single entities in humans. Clinical signs include chronic wasting, generalized lymphadenopathy, cutaneous lesions ranging from alopecia to ulcers, hepatomegaly and splenomegaly. In the later stages, there is chronic renal failure secondary to immune mediated glomerulonephritis. Clinical pathology abnormalities typically include normocytic, normochromic anemia, hyperproteinemia, hypergammaglobinemia, hypoalbuminemia, thrombocytopenia, leukopenia and azotemia. Additional histologic lesions in this case are representative of described cases of canine Leishmaniasis and include proliferation of macrophages and plasma cells within the lymph nodes, kidneys, bladder, bone marrow, conjunctiva, skin, subcutaneous tissue and joint capsules, with varying numbers of intrahistiocytic protozoa. There are also infiltrates of varying numbers of macrophages and plasma cells within the myocardium, alveolar septa, testes, small intestine and colon. Other lesions include membranoproliferative glomerulonephritis, and depletion of the T-cell dependent areas in the spleen and lymph node. Histologic differential diagnoses include: 1) *Trypanosoma cruzi* (amastigotes are usually within pseudocysts in cardiac fibers, and are larger than *Leishmania*, with a larger more basophilic kinetoplast that is parallel to the nucleus), 2) *Histoplasma capsulatum* (lacks a kinetoplast, PAS +, GMS +), and 3) *Toxoplasma gondii* (PAS + cyst wall and GMS -).

The CDC identified the isolates as *Leishmania donovani*. It still remains a mystery how the foxhounds contracted the Leishmania. Wildlife, horses, humans and pets in the surrounding area are negative. The dogs have an extensive travel history throughout the east coast, including to southern areas where the vector is present. Additional cases of Leishmania in foxhounds in other areas have occurred recently. As a result, the American Foxhound Association has canceled all of their spring/summer shows. The

reason for the high incidence in foxhounds is unclear. Some clinicians involved in this outbreak feel that Leishmania may not be a new problem for the foxhounds, but has just been under diagnosed.

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AFIP Diagnosis: Spleen: Splenitis, histiocytic and plasmacytic, diffuse, moderate, with numerous intrahistiocytic amastigotes, etiology consistent with *Leishmania* sp., foxhound mix, canine.

Conference Comment: The contributor provides a thorough review of the pathogenesis and host immune response in animals with leishmaniasis.

Since visceral leishmaniasis was detected in foxhounds from a hunt club in New York in 2000, seropositive dogs have been detected in 60 foxhound kennels in 22 states and two Canadian provinces. Other breeds, to include wild canids living in close proximity to the infected foxhounds, have been sampled and none have generated positive titers. So far, data indicate that direct transmission between dogs, as well as a possible sand fly vector may be important means of transmission in foxhounds. There are species of sand flies in the United States capable of transmitting *Leishmania* sp., particularly *Lutzomyia shannoni* (a possible vector of *Leishmania mexicana* which is endemic in Texas and Mexico). No infected sand flies have been identified in association with this outbreak. A group of Beagles and Bassett Hounds that were housed with seropositive foxhounds, and traveled to the southeastern United States with them (where they presumably would have been exposed to the same vector, if present), were seronegative. It is not known why foxhounds are more susceptible than other breeds.[7-10]

Although no human disease has been detected in association with these cases of canine visceral leishmaniasis, this is an important consideration since dogs are reservoir hosts for human visceral leishmaniasis, a significant concern in immunosuppressed individuals.[8,10]

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SLIDE 61
CONFERENCE 15 / CASE II - S1138.03 (AFIP 2890233)

Signalment: Thirteen-month-old breeder hens (*Gallus domesticus*).

History: Severe dyspnoea and mortality in breeder hens in an intensive production system.

Gross Pathology: Severe, diffuse, acute caseous necrotic laryngo-tracheitis.

Laboratory Results: On histopathological examination there was severe diffuse epithelial hyperplasia and necrosis with infiltration of colonies of coccoid bacterial organisms. There was also severe multifocal to coalescing degeneration of epithelial cells with accumulation of eosinophilic intracytoplasmic and amphophilic intranuclear inclusion bodies as well as the infiltration of numerous heterophils, plasma cells and mononuclear leucocytes into the mucosa and submucosa.

On electronmicroscopic examination of uranyl acetate and lead citrate-stained sections of affected tracheal mucosa, numerous large (250 x 254 nm) brick-shaped to biconcave intracytoplasmic virus particles with dense cores as well as smaller (80-100 nm in diameter) intranuclear virus particles with dense cores and a vague icosahedral symmetry, could be observed (Figures 1 and 2 - see arrows).

Contributor's Morphologic Diagnosis: Severe multifocal to coalescing chronic active and necrotic laryngotracheitis with intracytoplasmic and intranuclear viral inclusion bodies.

Contributor's Comment: The intracytoplasmic and intranuclear inclusions are morphologically compatible with Avian pox virus and Infectious Laryngotracheitis virus (ILTV-Herpes virus) infections respectively, which represents a dual infection. Avian poxviruses (fowl, turkey, pigeon, canary, junco, quail, sparrow and starling) are members of the genus *Avipoxvirus* of the family *Poxviridae.* Psittacine poxvirus and mynah poxvirus probably represent different members of the genus. Fowl poxvirus represents the type species of the genus[1,3]. Avian poxviruses affect a wide range of birds of various families by naturally or artificially occurring infection. A substantial degree of host specificity exists among some avian species, especially those that infect wild birds. Both skin and respiratory epithelium may become infected with respiratory infections being the most severe with mortalities rising as high as 50% in chickens and turkeys. Infectious laryngotracheitis virus is classified as a member of the family *Herpesviridae* in the subfamily *Alphaherpes-virinae* and is taxonomically identified as Gallid herpesvirus[2]. Virus particles are also similar in shape to herpes simplex [2,4]. The chicken is the primary natural host of ILTV, and although the virus affects all ages, the most characteristic signs are observed in adult birds. Viral multiplication is limited to respiratory tissues with little evidence of viraemia. Severe epizootics of the disease cause high morbidity and variable mortality of 5-70%. Milder enzootic forms of the disease also occur. Gross lesions may be found throughout the respiratory tract, but they are most consistently found in the larynx and trachea.[2] Although ILT is routinely diagnosed by histopathology, a specific PCR test has been introduced recently and would appear to render more specific diagnoses in cases of suspected ILTV infection[5].

AFIP Diagnosis: Larynx and trachea: Laryngotracheitis, necrotizing, proliferative, heterophilic, lymphoplasmacytic, and histiocytic, diffuse, severe, with ulceration, abundant caseonecrotic exudate, epithelial eosinophilic intracytoplasmic and intranuclear inclusion bodies, and myriad luminal bacteria, etiologies consistent with avian poxvirus and gallid herpesvirus-1, chicken, avian.

Conference Comment: As mentioned by the contributor, avian poxvirus may affect both skin and respiratory epithelium. There are two forms of fowl pox: dry (cutaneous) and wet (diphtheritic). The dry form is more common, with nodular proliferative lesions on unfeathered skin of the head, neck, legs, and feet. Fibrinonecrotic lesions of wet pox often occur in the mouth, esophagus, trachea, pharynx, and larynx. These lesions begin as small white nodules and progress to form coalescing raised plaques with a diphtheritic membrane. Large (15-30um) eosinophilic, intracytoplasmic inclusion bodies, also known as Bollinger bodies, within epithelial cells are characteristic for fowl pox.[1,6]

Important differential diagnosis for lesions resembling wet pox includes hypovitaminosis A, trichomoniasis, and candidiasis. Vitamin A deficiency is characterized by small white nodules, often with a central depression, in the nasal passages, mouth, esophagus, and pharynx. These lesions are the result of squamous metaplasia and subsequent blockage of mucous glands and their ducts by necrotic

debris and secretions. *Trichomonas gallinae* causes caseous, proliferative lesions, which may be surrounded by a zone of hyperemia, in the buccal cavity, pharynx, esophagus, and crop. In pigeons, this condition is known as "canker". *Candida albicans* causes gray-white pseudomembranous patches in the mouth, pharynx, esophagus, and, most frequently, the crop.[7]

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SLIDE 62
CONFERENCE 15 / CASE III - 139-03 (AFIP 2887157)

Signalment: 11 year old, female spayed, mixed breed (Spaniel/Terrier) canine.

History: This otherwise healthy dog presented to our referral hospital for surgical resection of an oral mass that had been identified by the referring veterinarian during a routine physical examination appointment.

Gross Pathology: A 3 X 1 X 1 cm raised firm tan non-pigmented gingival mass from the buccal surface of the right cranial mandible was submitted in 10% buffered formalin. The mass caused distortion of the first premolar and canine tooth. On cut section, the mass was expansile, gelatinous and pale tan with gritty white foci throughout. (Fig. 1)

Laboratory Results: Elevations were seen in the following blood parameters: WBC - 17.0 (4-15.5), Absolute Polymorphs 13430 (2060-10600), and Absolute Monocytes 1020 (0-840). Clinical Chemistry revealed elevations in all of the following: Glucose 144 (70-138), BUN 30 (0-25), Total protein 9 (5-7.4), Phosphorus 6.3 (2.5-6.0), Sodium 165 (139-154), Potassium 5.7 (3.6-5.5), Chloride 129 (102-120), and Globulin 5.1 (1.6-3.6). Chest radiographs were within normal limits.

Contributor's Morphologic Diagnoses:
1. Oral Malignant Melanoma with Chondroid Metaplasia.
2. Severe Focally Extensive Subacute Ulcerative, Suppurative and Lymphoplasmacellular Gingivitis.

Contributor's Comment: Malignant melanoma can have desmoplastic, neurotropic or osteochondrogenic differentiation.[1] Osteochondroid differentiation is an extremely rare feature of malignant melanoma in humans and dogs.[1] Chondroid formation has been seen in some canine dermal malignant melanomas.[2] In veterinary medicine, a single canine osteoid-producing variant of melanoma was described in the gingiva of a 12 year old miniature Dachshund and three chondroid-producing variants have been reported, one involving the lip commissure in an 11 year old FS cocker spaniel and the others within the gingiva of two unknown breeds.[1,3,4] Only rare combined osteochondroid variants of melanoma have been described in people - 3 with osteoid and bone, 3 with osteoid, bone and cartilage and 4 with osteoid and cartilage.[1] Generally, these variant tumors can be distinguished from osteosarcoma or chondrosarcoma because of junctional activity, the presence of melanin and positive melanin A staining. Surprisingly, Melanin A staining of the tumor, including regions of prominent junctional activity, was negative. This does not rule out melanoma as a small percentage of canine oral melanomas will be melanin A negative.[4]

The origin of these matrices are not well determined but may result from pseudosarcomatous differentiation of neoplastic melanocytes or from metaplasia of the surrounding stroma during invasion by the melanoma.[1] Some authors strongly support the contention that the chondroid formation is a result of melanocytic histiogenesis.[1] By far the most prominent matrix component in this tumor is chondroid and as pigmented and pleomorphic spindle cells are seen interspersed within this matrix it is thought to be a direct differentiation product of the malignant melanocytes.

It is of interest in this case that there are proliferative changes in several other gingival elements that may have resulted from induction of the periodontal ligament and resembled benign canine epulidides.[5] There were focal regions where squamous epithelial clusters and aggregates of hypereosinophilic matrix product (not seen in every section) were interspersed throughout a loose fibrous connective tissue. Special stains of the matrix (blue with Alcian blue and Masson's trichrome and weakly congophilic with Congo red but without birefringence under polarized light) suggested this was possibly of dental origin (i.e. dentin) and was not consistent with amyloid or osteoid. The possibility that malignant melanocytes induced epulis-like changes by induction of the periodontal ligament needs to be considered in this case.

AFIP Diagnosis: Gingiva (per contributor): Malignant melanoma with osseous and cartilaginous metaplasia, spaniel/terrier cross, canine.

Conference Comment: Melanocytic neoplasms vary among sites and among species. In dogs, a large majority of melanocytic neoplasms of the skin and eye are benign, whereas digital and oral melanocytic neoplasms are frequently malignant. Benign and malignant cutaneous melanocytic neoplasms occur with nearly equal frequency in cats but are uncommon tumors in this species.[6,7,8]

Gray or white horses often present with a melanocytic neoplasm of the perineum, base of the tail, or external genitalia. Although melanomas are possible in any breed or color of horse, they are clinically detectable in 80% of gray horses over 15 years of age. Some authors believe that all gray horses will develop melanomas if they live long enough. Three growth patterns of equine melanocytic tumors are described. The first pattern are those that grow slowly over many years and do not metastasize, whereas the second pattern are those that present as a benign growth but suddenly begin to grow rapidly and assume malignant characteristics. The third pattern are those that grow rapidly and are malignant from the onset.[6,9]

Pigs are frequently used in melanoma research. Sinclair miniature pigs, used as models for human cutaneous melanoma, and Duroc pigs are genetically predisposed to develop these tumors. Melanomas are unique in pigs in that up to 90% spontaneously regress, making pigs a valuable animal model for studying immunopathogenesis.[6]

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SLIDE 63
<u>CONFERENCE 15 / CASE IV - N688/01 (AFIP 2890236)</u>

Signalment: Presumed 15 years old, female, bottlenose dolphin, *Tursiops truncatus*.

History: The animal had a history of cutaneous traumatic lesions caused by fighting with a male of the same species. The last report of a lesion on the caudal fin was a month before time of the death. The animal died suddenly after one day of acute symptoms and signs characterized by abdominal contractions and vomit; acute, severe, green-yellowish diarrhea and a bilateral, diffuse, moderate subcutaneous swelling behind the blowhole. A clostridial infection was suspected. Antibiotics, cortisone and fluid therapy was carried out with no positive results.

Gross Pathology: At necropsy, a bilateral, diffuse, moderate to severe subcutaneous swelling was observed just behind the blowhole (Fig. 1). Removing the skin and the blubber, in the corresponding area, severe and diffuse subcutaneous hemorrhages, edema and emphysema and slight multifocal necrosis of the blubber were present (Fig. 2). The superficial muscular fascial planes and the skeletal muscles were characterized by diffuse, severe superficial edema, hemorrhages and emphysema and deep severe, multifocal to coalescent, hemorrhagic muscular necrosis. Moreover severe periocular hemorrhages and bilateral mucopurulent conjunctivitis was found. The lungs showed moderate to severe, diffuse hyperemia and slight, bilateral, diffuse pulmonary edema. Mild superficial enteritis and multifocal superficial hyperemic gastric erosions were observed.

Laboratory Results:
Hematology (same day of clinical signs and death):
WBC 1.00 (10/mm)
Creatine kinase 5.000 (mg/dl)
K 6.54 (mEq/l)

CYTOLOGY of the subcutaneous effusion behind the blowhole revealed numerous bacterial cocci, either isolated or in chains, associated with occasional neutrophils and macrophages and abundant slightly stained proteinaceous material and vacuoles referable to adipose tissue (Fig. 3).

MICROBIOLOGY on sterile swabs from subcutaneous lesions: isolation and identification of *Streptococcus agalactiae.*

Contributor's Morphologic Diagnosis: Muscles, thorax/head, anterior, dorsal: myositis, hemorrhagic-necrotizing, purulent, severe, coalescent associated with numerous Gram-positive bacterial cocci and with thrombi formation.
Etiology: *Streptococcus agalactiae*

Additional histology reveals:
Skin and superficial muscles, thorax - head, anterior, dorsal: cellulitis and fasciitis, diffuse, moderate associated with numerous Gram-positive bacterial cocci.
Superficial lymph nodes: reactive hyperplastic lymphadenitis and diffuse severe hemorrhages and hyperemia.
Stomach: muscular part - superficial diffuse erosion and moderate superficial diffuse chronic gastritis.
Mesenteric lymph nodes: chronic, diffuse, moderate, lymphoplasmacytic enteritis; diffuse lymphocytic depletion, diffuse moderate histiocytosis of the sinuses and follicular hyalinosis moderate diffuse.
Liver: hydropic degeneration, severe, diffuse and slight diffuse biliary stasis.
Kidney: tubular degeneration, diffuse, moderate, associated with occasional tubular pigmentation.
Lungs: severe, diffuse hyperemia, associated with moderate, diffuse emphysema and diffuse bronchiolar epithelial erosion with disseminated dystrophic mineralization.

Contributor's Comment: The severe localized subcutaneous and muscular lesions associated with a severe *Streptococcus agalactiae* infection were considered responsible for a toxic-shock syndrome responsible for hypotension and septic-toxic multiple organ failure and consequential death of the subject.

In veterinary medicine *Streptococcus agalactiae* is one of the most important organisms in bovine mastitis; nevertheless its role in invasive cutaneous infections is well documented in man[1]. Particularly, after *Clostridium* sp., streptococci (mainly group A and group G) play an important role in the pathogenesis of necrotizing fasciitis (NF), a well-known soft tissue infection, primarily affecting the superficial fascial planes and often associated with cellulitis and myositis[2,3]. Few cases of NF have been associated with isolation of *S. agalactiae*[4,5].

Severe fascial and muscular necrotizing lesions, referred to as NF, have been recently recognized in veterinary medicine particularly associated with *Streptococcus canis.*[6] Therefore, the specific lesions we report could be considered a case of NF in a marine mammal associated, in this case, with *S. agalactiae.*

Necrotizing fasciitis is often secondary to traumatic / post surgical wounds in compromised patients (stressed, immune-depressed)[7,1]. In this subject the history of cutaneous lesions and eventual stress for its captive condition cannot be ruled out as

presumptive predisposing factors. The eye lesions might also have represented the portal of entry for the pathogen.

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AFIP Diagnosis: Skeletal muscle: Myositis, necrotizing, fibrinosuppurative, diffuse, severe, with hemorrhage and myriad cocci, bottlenose dolphin (*Tursiops truncatus*), cetacean.

Conference Comment: Classification of streptococci is based on numerous factors, including carbohydrate and protein antigen composition and hemolytic properties. The Lancefield system (groups A-H and K-V) uses antigenic differences in the cell wall carbohydrates for classification. Bacterial action on erythrocytes in culture media is another defining characteristic. Alpha hemolysis is characterized by a greenish-colored zone associated with partially digested erythrocytes and reduction of hemoglobin. Beta hemolysis is characterized by complete lysis of erythrocytes and a halo of clearing around the colonies. Some strains are nonhemolytic (gamma hemolysis). Beta hemolytic strains are typically the most pathogenic, while alpha hemolytic and nonhemolytic strains are found on the skin and mucous membranes of healthy animals.[8]

Streptococcus agalactiae is the only member of Lancefield group B and is alpha hemolytic. The contributor mentions the significance of this organism in bovine mastitis. Infections with this strain are rare in dogs and cats, but have been reported to cause septicemia, endometritis, and fading puppy syndrome.[8]

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SLIDE 64
CONFERENCE 16 / CASE I - 991622.4 (AFIP 2739904)

Signalment: Canine, Brie sheepdog, male, 14 week-old.

History: This dog was presented for polyuria-polydipsia, emaciation, prostration, vomiting, and terminal seizures.

Gross Pathology:
Remarkable lesions were:
- A diffuse thickening of the cranial bones and of the mandible (Figures 1 and 2).
- An elasticity of the same bones as well as the ribs.
- Both kidneys appeared retracted, deformed and fibrous (Fig. 3).
- Parathyroids were enlarged (Fig. 4).

Laboratory Results:
Clinical investigations revealed:
- BUN > 4g/l; creatinine > 200mg/l
- Aregenerative anemia (Hb=7,2g/100ml, reticulocytes=0/l).

Contributor's Morphologic Diagnosis: Congenital renal dysplasia (primitive lesion) bilateral, fibrous osteodystrophy, Brie sheepdog, canine.
Some mandibular sections contain a tooth with its alveolus.

Contributor's Comment: Renal lesions consist of immature (fetal-like structures) glomeruli throughout the cortex and dilatation of Bowman's spaces. There is a separation of renal tubules by immature connective stroma minimally infiltrated by inflammatory cells (lymphocytes and plasmocytes). Many tubules are atrophic or dysplastic. Some sections reveal focal calcifications in tubules as well as some luminal granular eosinophilic casts. No cartilage nodules are noted.

Bone tissue of the mandible shows osteopenia (trabecular paucity) with osteolysis and medullary fibrosis (immature connective tissue). The number of osteoclasts is greatly increased; the associated trabecular surface is particularly eroded (numerous Howship's lacunae). In this disorganized tissue, we can observe osteoid material surrounded by some activated osteoblasts (particularly in some sections around the tooth alveolus).

The diagnosis of renal dysplasia was made based on the macroscopic appearance of kidneys and histological appearance of mesenchymal tissue with primitive tubules. Although the dog is 14 weeks old, this lesion is interpreted as a primitive dysplastic lesion because immature glomeruli are not merely present in the subcapsular zone. Secondary hyperparathyroidism is confirmed by the hyperplasia of parathyroids and fibrous osteodystrophy.

AFIP Diagnoses:
1. Kidney: Dysplasia with diffuse severe fibrosis, fetal glomeruli, and tubular adenomatous hyperplasia, Brie sheepdog, canine.
2. Mandible: Osteodystrophy, fibrous, diffuse, severe.

Conference Comment: Renal secondary hyperparathyroidism, or renal fibrous osteodystrophy, is a complex syndrome caused by elevated parathyroid hormone levels in response to hypocalcemia. It is initiated by decreased glomerular filtration rate and the resultant inability to excrete phosphate, and inadequate production of 1,25-dihydroxycholecalciferol (calcitriol). Hyperphosphatemia, due to the mass law effect, results in concomitant hypocalcemia which stimulates the parathyroid glands to secrete parathyroid hormone (PTH). Parathyroid hormone acts principally on the bone and kidney. Parathyroid hormone stimulates bone resorption and mobilization of calcium stores, resulting in the characteristic marked softening of bones and replacement with fibrous connective tissue. The diseased kidney is unable to facilitate production of calcitriol, which normally suppresses PTH secretion, thus impairing this negative feedback system allowing the parathyroid glands to continue to produce PTH. In addition, hyperphosphatemia inhibits calcitriol production. With time, continued stimulation of PTH secretion causes parathyroid chief cell hyperplasia (renal secondary hyperparathyroidism).[7-9]

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SLIDE 65
CONFERENCE 16 / CASE II - 03-7103 (AFIP 2908326)

Signalment: 17- week old, female B6.129S6-*Cybb^{tm1Din}* mouse (*Mus musculus*).

History: This mouse was from a production colony maintained in conventional housing at The Jackson Laboratory. The mouse was submitted to the diagnostic laboratory because it was found to be thin with a submandibular mass.

Gross Pathology: There was a solitary, white, 1.4 x 1.0 cm, subcutaneous, multi-nodular mass containing pale yellow amorphous material. The lungs had multiple, randomly distributed, raised, white nodules of various sizes up to 2 mm in diameter.

Laboratory Results: Bacterial culture positive for *Staphylococcus* sp. coagulase negative.

Contributor's Morphologic Diagnoses:
1. Tongue, mandible and adjacent soft tissue: Myositis, osteomyelitis and periodontitis (some sections), severe, multifocal, pyogranulomatous with intra-lesional Splendore-Hoeppli material centered on bacterial colonies.
2. Lymph node: Lymphadenitis, severe, multifocal, pyogranulomatous with intra-lesional Splendore-Hoeppli material and plasmacytosis.
3. Lung: Pneumonia, moderate, multifocal to coalescing, fibrinopurulent, pyogranulomatous with intra-lesional Splendore-Hoeppli material rarely centered on bacterial colonies. Mild, multifocal, acidophilic, macrophage pneumonia.

Contributor's Comment: The B6.129S6-*Cybb*[tm1Din] mouse was generated by the targeted disruption of the gp91[phox] gene[1]. This gene is located on the X chromosome and encodes the β subunit of the phagocyte oxidase cytochrome b 558. Oxidase cytochrome b is a membrane component of NADPH oxidase, which generates superoxide, a critical component in the process known as the respiratory burst. The absence of respiratory-burst-derived oxidants in phagocytes results in defective microbicidal activity. Neutrophils and macrophages from *Cybb*[tm1Din] hemizygous male and heterozygous females mice lack respiratory burst oxidase activity[1]. The lesions in the *Cybb*[tm1Din] mouse have previously been described[2].

The lungs of this mouse also had acidophilic macrophage pneumonia, characterized by the presence of large numbers of alveolar macrophages many containing eosinophilic, Ym1 crystals[3]. Acidophilic macrophage pneumonia has previously been reported in *Cybb*[tm1Din] mice and is a prominent lesion in motheaten mice (*Hcph*[me]/*Hcph*[me])[2]. *Hcph* encodes the SHP-1 protein-tyrosine phosphatase, which is expressed primarily in hematopoietic cells. SHP-1 is a critical negative regulator in multiple signaling pathways in the hematopoietic and immune systems. Interestingly, in undifferentiated myeloid cells SHP-1 activity has been shown to decrease transcription of Cybb[4].

Pyogranuloma formation with intra-lesional Splendore-Hoeppli material is a background lesion found in some mice housed in our conventional facilities. While the lesion is often more extensive in immunodeficient mice such as the *Cybb*[tm1Din] mouse, it is also found in immunocompetent mice. The pathogenesis of pyogranuloma formation in these mice is unknown. However, we believe that the nidus for such lesions may be a hair foreign body secondary to grooming activity. Penetration and disruption of the oral mucosa facilitates the spread of bacteria into adjacent structures. Generally these lesions involve the cervicofacial tissues with spread to cervical lymph nodes and on occasion as this case demonstrates lymphogenous spread to the lungs. Coagulase-positive *Staphylococcus aureus* is the bacteria most commonly associated with botryomycosis, however in our mice coagulase-negative *S. hominus* and *S. xylosus* are most frequently recovered.

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AFIP Diagnoses:
1. Mandible, tongue, skeletal muscle, and tooth: Osteomyelitis, glossitis, myositis, and periodontitis, pyogranulomatous, focally extensive, severe, with Splendore-Hoeppli material, and colonies of cocci, B6.129S6-*Cybb*[tm1Din] mouse, rodent.
2. Lung: Bronchopneumonia, neutrophilic, multifocal, marked, with Splendore-Hoeppli material, and colonies of cocci.
3. Lung: Numerous intra-alveolar macrophages with eosinophilic intracellular crystals.

Conference Comment: Some slides also contain lymph node with a similar pyogranulomatous inflammatory infiltrate.

Botryomycosis is a term used to describe the chronic granulomatous infection caused by coagulase-positive staphylococci, most commonly *Staphylococcus aureus*. These lesions usually follow some type of skin trauma and begin as microabscesses around a small colony of organisms and may progress to large granulomas. The colonies are often imbedded within Splendore-Hoeppli material, which represents antigen-antibody complexes, and forms a characteristic, radiating corona of brightly eosinophilic, homogenous club-shaped bodies. Macroscopically, the bacterial colonies and Splendore-Hoeppli material are described as "grains", or tiny white granules present within purulent exudate.[5]

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SLIDE 66
CONFERENCE 16 / CASE III - 1795/02 (AFIP 2888772)

Signalment: 4-month old male fennec, *Vulpes zerda* (Zimmermann, 1780).

History: In a litter of five captive-born fennecs of a zoological park in Southern Germany, increased incidence of respiratory problems and weight loss were observed. Animals were treated with penicillin, streptomycin, dexamethasone and furosemide, without any clinical improvement. The clinician noted microclimatic problems (smell) in the kennel. Within one week, two animals were euthanized due to deterioration of their body condition and resistance to therapy. Both were submitted for necropsy. One year ago the same symptoms had occurred in a litter of fennecs of the same zoological park, but the patients had recovered after antibiotic treatment.

Gross Pathology: One animal was emaciated (800g) and exhibited purple, firm and atelectatic cranial lung lobes. Bronchi were filled with mucus and edema fluid. Caudal lung lobes were emphysematous. Other organs were unaltered. The second animal exhibited numerous 10-20 mm filiform nematodes in the trachea and bronchi.

Laboratory Results: Large amounts of parasite eggs compatible with *Capillaria* sp. were found in the feces. Microbiological examination of the first animal did not lead to isolation of pathogenic bacteria.

Contributor's Morphologic Diagnoses:
1. Lung: Pneumonia, pyogranulomatous and eosinophilic, focally extensive, severe, with adult nematodes and numerous bipolar plugged, embryonated eggs within alveoli consistent with *Capillaria aerophila*; alveolar hemorrhage, multifocal, moderate, fennec, *Vulpes zerda*, canidae.
2. Bronchi and bronchioli: Bronchitis and bronchiolitis, lymphoplasmacytic and histiocytic, multifocal, severe, chronic, with bronchiectasia, peribronchial fibrosis and intraluminal adult nematodes and nematode eggs, fennec, *Vulpes zerda*, canidae.

Contributor's Comment: A fennec is a small member of the genus Vulpes which populates the desert of Sahara and the peninisula of Arabia and Sinai. These small smart foxes are often kept in zoological parks.

Capillaria aerophila (Creplin, 1839), an aphasmid worm, is a lung parasite of foxes, badgers, martens, hedgehogs, cats, dogs and, occasionally, humans[1,6]. In Germany, about 72% of necropsied free-ranging red foxes (*Vulpes vulpes*) and 0.9% of stray cats are affected[4,6]. In the UK and Spain, a prevalence between 0.24 and 67.2% was reported[2,5]. *Capillaria aerophila* infestation is a common health problem in fox farms[6]. *Capillaria aerophila* were also found in arctic foxes, Tsushima leopard cats, raccoons, bobcats, coyotes, black bears and opossums.
Male *Capillaria aerophila* worms are about 16-25 mm and females are 25-32 mm in length. Adult parasites live in the trachea and bronchi, sometimes in the nasal cavity or the paranasal sinuses of the host[6]. Histologically, a characteristic feature of aphasmid worms is distinct hypodermal lateral cords (bacillary bands) and a row of esophageal gland cells called stichocytes that form a stichosome. In contrast to phasmids, females have only one genital tract[3]. Eggs are released into the environment via the respiratory and alimentary tract of the host and develop well in humid soil. The life cycle is not known but is considered as direct or, more likely, indirect. Earthworms have been considered as intermediate hosts. After ingestion of infected earthworms, larvae migrate via lymphatics and blood vessels to the lung within 7 days. The prepatent period is about 4 weeks and the patent period is 10 to 11 months long[6].

In general, mild infestations remain clinically inapparent or induce a mild bronchitis. With severe infestations, however, bronchitis, tracheitis and rarely rhinitis and sinusitis are observed. Secondary bacterial infections are common. Infected animals show coughing, sneezing, reduced general state of health, cachexia and anemia[6].

An aetiological diagnosis is feasible by demonstration of embryonated, bipolar and plugged eggs in feces and tracheal mucus. The outer egg wall is structured, which renders the differentiation between *Capillaria* sp. and *Trichuris* sp. possible. Another parasite in the paranasal sinus of foxes and sometimes of dogs is *Capillaria boehmi* (Supperer, 1953)[3,6].

Respiratory problems often occur in litters kept in kennels with inadequate microclimate. In the present case, all members were affected. It can be hypothesized that a massive accumulation of infectious larvae in the environment leads to the severe nematode load, whereas adults are often subclinically infected and serve as reservoir.

Other parasites in the respiratory tract of carnivores are *Crenosoma vulpis* (dogs, foxes), *Crenosoma globei* (raccoons), *Crenosoma mephiditis* (skunks); *Crenosoma striatum* (hedgehogs), *Filaroides osleri* and *Aelurostrongylus abstrusus* (cats).

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AFIP Diagnosis: Lung: Bronchopneumonia, chronic-active and eosinophilic, multifocal, severe, with hemorrhage, bronchiolar epithelial hyperplasia, and intraepithelial and intraluminal aphasmid adults and eggs, fennec fox (*Vulpes zerda*), canine.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. The contributor gives a concise review of the morphologic and clinical characteristics of *Capillaria aerophila*. An important feature of *Capillaria* sp. is its intraepithelial location in the host. Other intraepithelial nematodes include *Trichosomoides crassicauda*, *Gongylonema* sp., and *Anatrichosoma* sp.

Adult female *Trichosomoides crassicauda*, also known as the bladder threadworm, parasitize the urothelium of rats. Adult males are much smaller and live within the lumen of the urinary tract, or within the uterus of the female.[7] *Gongylonema* sp. affect ruminants, pigs, horses, nonhuman primates, and occasionally rodents. They are characteristically seen as thin, red, serpentine nematodes imbedded in the esophageal mucosa.[8,9] *Anatrichosoma* sp. are found in the nasal epithelium of nonhuman primates where infection is usually subclinical. Grossly, lesions may be characterized by white, serpentine tracks on the palms of the hands or soles of the feet caused by adult parasite migration through the epithelial/subepithelial layers of the skin, which has been described as creeping eruption.[10]

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SLIDE 67
<u>**CONFERENCE 16 / CASE IV - 0065 296 (AFIP 2900352)**</u>

Signalment: About 800-day old female Sprague-Dawley rat.

History: The rat was a control from a carcinogenicity study with a restricted access to rodent diet (17 g/day) and daily gavage with 0.4% methylcellulose. A small mammary gland mass was found on Day 722. The rat was sacrificed on Day 731 in good condition, at the terminal necropsy.

Gross Pathology: Principal findings included 15 x 15 mm, smooth, firm, pedunculated mass attached to the right side of the enlarged (20 x 5 mm) uterine body, a 25 x 25 mm mammary gland mass, small thymus, right pulmonary adhesions, enlarged dark-red adrenal glands, and bilateral cage sores.

Laboratory Results: Hematologic laboratory results were within normal limits**.**

Contributor's Morphologic Diagnoses:
1. Malignant uterine granular cell tumor (GCT).
2. Hypertrophy of the uterine body and cervix (*portio vaginalis uteri*).

Other principal findings (slides not included) were mammary gland adenocarcinoma, unilateral pheochromocytoma, hepatocellular basophilic focus, focal hepatic angiectasia, minimal foreign body bronchopneumonia and focal pleural fibrous adhesions consistent with the sequela of a gavage accident, focal C-cell hyperplasia of the thyroid gland, severe thymic and mild ovarian atrophy consistent with aging, hypercellular bone marrow and mild pododermatitis. Pituitary gland was not available for examination.

Contributor's Comment: An expansile, circumscribed, spherical, not encapsulated mass, except for a single layer of squamous epithelium covering the surface, was attached over a small area to the uterine body. The mass consisted of a uniform population of large cells with abundant granular cytoplasm, indistinct cellular borders, small, round, dark, centrally located nuclei with indistinct nucleoli, and eosinophilic collagenous matrix. Dissociation of cells, foci of hemorrhage, occasional apoptotic bodies and mitotic figures, proliferating blood vessels and a few mast cells were evident. The boundary between the tumor and uterine wall was irregular as the tumor infiltrated the outer layer of the myometrium. The large size of the tumor, cellular dissociation, occasional apoptotic bodies, mitotic figures and areas of hemorrhage suggested a malignant variant[6]. No regional or distant metastases were found.

Age-related endometrial stromal fibrosis and hypertrophy of the uterine body and cervix were the cause of the enlargement of the uterine body[4].

Histological methods for characterization of granular cell tumors (GCTs), including electron microscopy (for detection of basal lamina and desmosomes characteristic of Schwann cell); application of the immunohistochemical markers S-100, neuron-specific enolase, and glial fibrillary acid protein (antigens related to cells of neural crest), muramidase (histiocyte-associated antigens), desmin (muscle-associated antigens), and vimentin (connective tissue associated antigens); and periodic acid-Schiff (PAS) with diastase digestion, were not utilized. Granular cell tumors are usually positive for NSE, S-100 and vimentin, variably positive for desmin and muramidase, and negative for GFAP. Cytoplasmic granules are PAS positive and diastase resistant[3,11].

Granular cell tumors and granular cell foci occur spontaneously in various species, tissues, and organs, and were described for the first time in 1854 by Weber[1] and in 1926 by Abricossoff[2]. The first uterine GCT in a rat was characterized in 1991 by Nyska et al.[8]. In rats, GCTs are usually found in the meninges, uterus and vagina[9]. In humans, as in rats, they are rare, more common in middle aged females[7], and appear as solitary or multiple, small nodules in the dermis, subcutis, tongue, breast, in the respiratory tract, esophagus, ovary, cervix and vulva. The characteristics of GCT (dermal "myoblastoma") from a female patient[11] and of 13 vulvar tumors[1] paralleled those of the typical GCT of the rat.

The positive correlation between the occurrence of granular cell alterations and estrogen levels has been proposed[5]. For example, in a study using Donryu rats, known

for age-related increases in estrogen-to-progesterone ratio, granular cell foci were found in 11/855 rats in the endometrial stroma (lamina propria) of the uterine horns[9].

The etiology of the GCT is not known, but several authors suggest a neuroectodermal origin, common to both the GCT and Schwannoma. Thus the GCT may arise from neoplastic Schwann cells, and from the pituicytes (modified astrocytes) of the pars nervosa[2,10]. At first human granular cell tumor was thought to be a granular cell myoblastoma[2]; however, the histochemical and ultrastructural evidence indicated a relationship with the peripheral nerve sheath tumors and some are associated with small nerves[7]. It has been suggested that the PAS-positive, diastase digestion-resistant cytoplasmic granules are the secondary lysosomes[3] containing some insoluble polysaccharide[2].

AFIP Diagnoses:
1. Uterus: Granular cell tumor, Sprague-Dawley rat, rodent.
2. Portio vaginalis uteri[4]: Hypertrophy, marked.

Conference Comment: As mentioned by the contributor, a characteristic feature of granular cell tumors is the presence of PAS positive, diastase resistant cytoplasmic granules. Ultrastructurally, granular cell tumors contain densely packed lysosomes and phagosomes (myelin figures). Differential diagnoses for other tumors with granular cytoplasm include oncocytoma and rhabdomyoma. Features that differentiate these from granular cell tumors were discussed. The cytoplasmic granules in oncocytomas are due to the presence of numerous mitochondria, an ultrastructural feature that is diagnostic for that tumor. Rhabdomyomas are immunohistochemically desmin and myoglobin positive and are ultrastructurally characterized by intracytoplasmic myofibrils and Z lines.[12,13]

As mentioned by the contributor, the primary sites for granular cell tumors in rats are the meninges and uterus. In dogs, granular cell tumors primarily affect the tongue. In horses, granular cell tumors are the most commonly reported primary lung neoplasm. They are generally adjacent to, and often invade, bronchi and bronchioles.[14]

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SLIDES 68 and 69
CONFERENCE 17 / CASE I - 0300052 (AFIP 2891626)

Signalment: 644-day-old male Fischer 344 rat, *Rattus norvegicus*.

History: This rat was from the low-dose group of a 2-year carcinogenicity study in Fischer 344 rats. The rat exhibited clinical signs of opacity of the right eye, an ulcerated mass in the inguinal area, nasal discharge and posterior paralysis. The rat was sacrificed on day 602 of the study.

Gross Pathology: Macroscopic findings were: an ulcerated preputial gland mass, 20x20x15 mm; a mottled white right testicle; an opaque right eye; multiple pale nodules up to 3x3x3 mm in the lungs; and a 30x30x20 mm mottled red mass in the lumbar vertebra.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Chordoma with metastasis to the lung.

Contributor's Comment:
Description

The site of origin for the tumor was the lumbar vertebra (Fig. 1). The tumor (arrows) infiltrated the bone of the vertebra (SP=spinous process) and surrounding muscle. The tumor cells were arranged in masses and cords separated by fine connective tissue septae. Metastatic tumors were numerous in the lung filling blood vessels and extending into the parenchyma (Fig. 2). The metastatic cells had the same morphology as the neoplasm at the primary site. The tumor cells were polyhedral with clear or vacuolated cytoplasm, a distinct cytoplasmic border, and round to oval nuclei with central nucleoli (Fig. 3).

Discussion

Chordomas are believed to arise from residual notochordal tissue in the axial skeleton[1,2]. They have a predilection for the proximal and distal extremes of the axial skeleton and are most common in the lumbosacral spinal cord of Fischer rats[3]. Chordomas have been reported in humans, rats, mice, dogs, cats, ferrets, and mink.

An incidence of 0.05% has been reported[4] in the NTP's database of 115,000 Fischer 344 rats from 300 toxicity/carcinogenicity studies. Metastasis to the lung occurred in 56% of the cases and in 43% of the cases diagnosis was made from the metastatic lung site without the primary site being found. There was a higher incidence in treated versus control but no association with treatment was evident. Most of the chordomas occurred as a single tumor within a study. The incidence in males was three times that observed in females. Rats with chordoma died from 74 to 138 weeks of age.

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AFIP Diagnoses:
1. Vertebral body: Chordoma, Fischer 344 rat, rodent.
2. Lung: Chordoma, metastatic.

Conference Comment: Closely packed polygonal cells with distinct cell borders and multiple, large, clear intracytoplasmic vacuoles (physaliferous cells) are a characteristic feature of this tumor. Chordomas are typically composed of three zonal components: a central zone of trabecular bone, often with bone marrow elements; a zone of cartilage;

and lobules of physaliferous cells at the periphery. A mucinous matrix often surrounds the physaliferous cells. The mitotic rate is generally low. By immunohistochemistry, chordomas are positive for keratin and vimentin and variably positive for S-100 and neuron-specific enolase (NSE). These immunohistochemical markers differentiate chordomas from liposarcomas and myxoid chondrosarcomas, two differentials for this tumor.[5,6]

Although these tumors are uncommon in general, chordomas are the most frequently reported musculoskeletal neoplasm of ferrets. Chordomas are generally slow growing, locally invasive, often recur following excision, and occasionally metastasize. Chordomas can occur anywhere along the axial skeleton but predilection sites differ among species. In ferrets, they are typically located distal to the last caudal vertebra and most often expand the tip of the tail to form a club-shaped mass. Excision is generally curative in this location in ferrets; however, there are rare reports of chordomas arising within the cervical and thoracic vertebrae. In these locations, excision is difficult. Cutaneous metastasis and neurological signs from spinal cord compression can occur. In other species chordomas are more commonly located in the sacrococcygeal region.[5,6,7,8,9]

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SLIDES 70 and 71
<u>**CONFERENCE 17 / CASE II - A030390049/A030390049-2 (AFIP 2893493)**</u>

Signalment: 1-2 month old female, Holstein, Bovine.

History: Calf is from a commercial 2,000-head calf farm that has had approximately 20 calves in the past week appear weak one day and found dead the next. They are fed unpasteurized withheld whole milk usually from cows being treated for mastitis. This calf was reported "skinny", semi-comatose and the joints appeared slightly swollen.

Gross Pathology: None reported.

Laboratory Results: Bacterial isolation: *Listeria monocytogenes*.

Contributor's Morphologic Diagnoses: Acute multifocal necrotizing, purulent hepatitis and multifocal purulent interstitial nephritis.

Contributor's Comment: Scattered randomly through out the liver are numerous large and sometimes coalescing foci of necrosis with intense infiltrate of neutrophils and mononuclear cells. With special stains many small gram-positive bacilli are evident in the foci of necrosis and inflammation. In the kidney there are a few scattered cortical and medullary interstitial infiltrates of neutrophils. The histologic lesions are compatible with visceral listeriosis. Bacterial culture supports the diagnosis.

   The pathogen, *Listeria monocytogenes* is a gram-positive facultative intracellular bacillus found in the environment and soil. Ingestion and traumatic penetration of the mucosa or skin are considered routes of infection[1]. It affects many species and is a public health concern for humans[2]. In ruminants the sporadic disease occurs in three, seldom overlapping syndromes: septicemia with visceral miliary abscesses, encephalitis and infection of the pregnant uterus with abortion [3].

   In this case, the disease appeared to affect several herd mates and had a fairly sudden onset. The brain was not available for examination.

AFIP Diagnoses:
1. Liver: Hepatitis, necrotizing, acute, random, marked, with bacilli, Holstein, bovine.
2. Kidney: Nephritis, necrotizing, acute, multifocal, marked, with bacilli.

Conference Comment: The contributor mentions three syndromes associated with *Listeria monocytogenes* infection: encephalitis, abortion, and septicemia; in addition, conjunctivitis and mastitis have also been described. Human infections caused by *Listeria monocytogenes* are usually the result of food-borne outbreaks and manifest as encephalitis, abortion, or septicemia.[7]

The encephalitic form is also known as "circling disease", based on the clinical signs associated with brainstem lesions. Ruminants become infected when eating silage that is poorly preserved, which favors listerial survival. Rather than hematogenous spread, evidence suggests that the pathogenesis of the encephalitic form involves local invasion through a defect in the oral mucous membranes, followed by migration along the trigeminal nerve to the brainstem. The typical histopathological findings are meningoencephalitis centered on the pons and medulla, with microabscesses and lymphocytic leptomeningitis. Trigeminal neuritis and ganglionitis are also described. Ruminants are most commonly affected, but listerial meningoencephalitis has also been reported in pigs, horses, and dogs.[1,4,5,6]

The abortion syndrome usually occurs late-term and does not cause systemic illness in the aborting ruminant. This syndrome is thought to occur via hematogenous spread where the organism localizes in the uterus, causing metritis and abortions.[1,5]

The septicemic disease causes miliary abscesses in multiple organs, principally the liver. It occurs most frequently in neonates and may be a continuation of an intrauterine infection. Septicemic disease has also been reported in pigs, rabbits, guinea pigs, chinchillas, and birds.[1,4,5]

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SLIDE 72
<u>**CONFERENCE 17 / CASE III - 03-5830 01 (AFIP 2888628)**</u>

Signalment: Seven-year-old spayed female ferret (*Mustela putorius furo*).

History: This animal was owned by a small zoo for its entire life, and had no previous significant health problems. It presented to the referring veterinarian after a one-week history of diarrhea, with vomiting for one day. On physical examination, the animal was thin and dehydrated, with a palpable cranial abdominal mass, which was deemed non-operable upon exploratory laparotomy. The animal was subsequently euthanized.

Gross Pathology: At the root of the mesentery there was an approximately 5 cm diameter, firm, nodular, pale mass, with fibrous adhesions to several sections of intestine, and thickened, fibrotic mesentery. There were numerous, firm, 1 to 2 mm diameter, white masses throughout omentum and mesentery. A smaller, 1 cm diameter, pale pink, firm mass was present in the wall of a segment of colon.

Formalin-fixed sections of affected intestine and mesenteric lymph node were submitted for histopathologic examination.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Intestine: Mucin-producing intestinal adenocarcinoma with lymph node metastasis.

Contributor's Comment: There is diffuse infiltration and thickening of mesentery, subserosa and muscularis of multiple sections of intestine by a pleomorphic population of epithelial cells forming undulating ribbons and nests embedded between bands of proliferating fibrous connective tissue. In some areas, ribbons of cells form distinct tubules of varying diameter, some containing luminal pale eosinophilic material or granular, necrotic cellular debris. Most cells are polygonal, clustered in small groups or aligned along basement membranes, with scant basophilic cytoplasm, large round to ovoid nuclei, lacy basophilic chromatin and distinct nucleoli. Other cells are distended with pale basophilic mucin, with flattened, marginated nuclei, consistent with features of goblet cells. Sections of subserosa and muscularis are markedly expanded, with nests of cells between muscle bundles in muscularis and multifocal lymphatic invasion. Variably-sized lakes of wispy, pale basophilic mucin markedly expand invaded lymphatics and stroma, with suspended ribbons and tubules. There are scattered tubules in lamina propria of some sections. Moderate follicular aggregates of lymphocytes expand the mesentery. In the mesenteric lymph node there are ribbons and clusters of tubules formed by cells that invade subcapsular, cortical and medullary sinuses.

Primary intestinal neoplasia is generally uncommon in domestic species[1]. In ferrets, there are two brief references to intestinal tumors, but no published descriptions[2,3].

Mucus-producing or mucinous adenocarcinomas are seen with variable frequency in many species, including dogs, cats, pigs and non-human primates[1]. In humans, this type comprises approximately 15% of colorectal tumors, commonly arising from rectum[4]. The primary tumor does not appear to be represented in the submitted tissues. However, the differentiation of this mass to mucin-producing cells suggests a possible colonic origin. Diagnosis of mucinous adenocarcinoma requires that greater than 50% of the mass be composed of mucin in cysts or extracellular pools, and might be properly applied to some areas of this neoplasm. Lymphatic metastasis is well demonstrated in this case, with spread of the tumor to regional lymph nodes, which is typical. The marked scirrhous mesenteric response to this tumor is also common to intestinal carcinomas.

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AFIP Diagnoses:
1. Small intestine and mesentery: Mucinous adenocarcinoma, ferret, mustelid.
2. Small intestine: Enteritis, lymphocytic, diffuse, severe, with villous blunting and fusion, and lymphangiectasia.
3. Lymph node: Mucinous adenocarcinoma, metastatic.

Conference Comment: In addition to the intestinal adenocarcinoma, conference attendees discussed the enteritis, which is characterized by marked expansion of the lamina propria and transmigration of the epithelium by lymphocytes. The severity of the lymphocytic response is an unusual finding in association with intestinal neoplasia in ferrets (Williams BH, personal communication). It is possible that, given the additional findings of lymphangiectasia and villous blunting and fusion, there may be concurrent inflammatory bowel disease. The inflammation, neoplasia, or both could cause the lymphangiectasia.

As noted by the contributor, there are very few reports of intestinal adenocarcinoma in ferrets.[3,5,7] Gastric adenocarcinoma, however, has been documented in ferrets and is reported to be associated with *Helicobacter mustelae* infection. Much like *Helicobacter pylori* infection in humans, *H. mustelae* causes increased gastric epithelial proliferation, presumably due to chronic inflammation. This suggests that gastric adenocarcinoma in ferrets may be a potential model for studying *Helicobacter* sp.-associated gastric carcinogenesis in humans.[6,7]

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SLIDE 73
CONFERENCE 17 / CASE IV - 2784/03 (AFIP 2899509)

Signalment: Six-week-old calf, male, limousin, *Bos taurus*, bovine.

History: On an organic beef cattle farm two calves died in a short period at the age of 5-6 weeks without having any previous symptoms. This calf was found dead with foam in the mouth and nostrils. Earlier some of the newborn calves had been weak, some had died.

Gross Pathology: Normal body condition. Heart muscle mottled and pale. Multiple scars in the myocardium. Lung oedema. Marked, symmetrical enlargement of both lobes of the thyroid gland.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
Thyroid gland: diffuse hyperplasia, lack of colloid. Hyperplastic goiter.
(Myocardium, not submitted: myocarditis, multifocal, chronic, fibrotizing with multiple fibrotic scars.)

Contributor's Comment: Goiter is a non-neoplastic and noninflammatory enlargement of the thyroid gland. It is caused by iodine deficient diet, goitrogenic compounds that interfere with thyroxinogenesis or genetic enzyme defects in the biosynthesis of thyroid hormones. These factors result in inadequate thyroxine synthesis and decreased blood levels of thyroxine and triiodothyronine. This is detected by hypothalamus and pituitary gland. They increase the secretion of thyrotropin, which results in hypertrophy and hyperplasia of the follicular cells of thyroid gland. Paradoxically also dietary iodine excess can cause goiter.[1]

In cattle the most prominent clinical signs of goiter are abortion, stillbirth and weakness in newborn calves. Newborn animals show thyroid gland enlargement. The thyroid gland can be so large that it compresses the large blood vessels in the region of the neck, causing haemostasis in the neck and the cranium and pressure on the larynx and trachea resulting in dyspnoea and high heart and breathing rates. The neck can be oedematous and swollen. Calves seem to be more resistant than lambs, pigs and goats, and they may recover if they survive the calving and the first two days but often they need special veterinary care. In adults thyroid gland enlargement is rather rare. Loss of libido in bulls and failure to express estrus in cows can be observed.[1,2,3]

Gross pathology of the goitrous thyroid gland usually shows symmetrical enlargement of both lobes of the gland**.** In cattle, the isthmus is also wider. In hyperplastic goiter, the thyroid gland is darker due to hyperaemia and the lobular structure is more expressed.[3]

Histologically the follicles of the thyroid gland are irregular in size and shape because of varying amounts of colloid in lumen and some are collapsed due to lack of colloid. The follicles are lined by single or multiple layers of hyperplastic follicular cells which may form papillary projections into the lumen of some follicles. The epithelial cells are columnar, the cytoplasm is eosinophilic and the nuclei are small and hyperchromatic. The nuclei are often situated in the basilar part of the cell. The changes can be observed throughout the thyroid gland.[1]

The death of this animal was caused by cardiac failure caused by chronic fibrotizing myocarditis. However, the herd problem was iodine deficiency causing goiter.

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AFIP Diagnosis: Thyroid gland: Hyperplastic goiter, limousin, bovine.

Conference Comment: The contributor gives a concise review of the pathogenesis, clinical signs, and gross and histologic findings with diffuse hyperplastic goiter. Colloid goiter represents the involutionary phase of diffuse hyperplastic goiter. With resolution, T3 and T4 serum levels return to normal and stimulation of TSH is diminished. Follicles, however, continue to progressively distend with colloid because TSH-induced endocytosis of colloid is reduced. Grossly colloid goiter, like diffuse hyperplastic goiter, causes diffuse enlargement of the thyroid gland, but the thyroid gland is more translucent and lighter in color than hyperplastic goiter. This difference in gross appearance is due to reduced vascularity and to distension of macrofollicles with colloid. The follicular cells lining the distended follicles become flattened, inactive, and atrophic, with a smooth interface between the colloid and the luminal surface of epithelial cells.[1,4]

Goiter may be caused by iodine deficiency or, paradoxically, iodide excess. Some causes of iodine deficiency include goitrogenic plants (white clover, rape, kale), goitrogenic compounds (sulfonamides, thiouracil), or an iodine deficient diet. Iodide

excess causes goiter because high blood iodide interferes with thyroxinogenesis and leads to low blood thyroxine levels with a compensatory increase in pituitary TSH secretion. In addition, excess iodine interferes with the proteolysis of colloid in thyroid follicular cells and blocks the release of T3 and T4.[1,4]

Congenital dyshormonogenetic goiter is an inherited disorder of thyroid hormone synthesis and secretion in sheep (Corriedale, Dorset Horn, Merino, and Romney breeds), Afrikander cattle, and Saanen dwarf goats. The thyroid lobes are symmetrically enlarged at birth and these animals have clinical signs of hypothyroidism, including subnormal growth rate, rough sparse hair coat, myxedema, weakness, and sluggish behavior. Histologically, there is diffuse hyperplasia of follicular cells and follicles are lined by tall columnar epithelium; however, follicles are collapsed due to marked endocytosis and lack of colloid.[1,4]

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SLIDE 74
CONFERENCE 18 / CASE I - 2422/02 (AFIP 2888767)

Signalment: Bennett's Wallaby (*Macropus rufogriseus*), 4 years, female, born and kept in zoo.

History: The animal showed increasing anorexia and a decline of body weight over a period of 2-3 months; prior to death it was lethargic/apathic. One week prior to death, the wallaby's approximately 15 cm large neonate was found dead within the cage. During the last years, several individuals of this group of Bennett's Wallabies suffered from lumpy jaw. Post mortem examination of this adult was performed one day after death; the neonate was not submitted for investigation.

Gross Pathology: The animal was emaciated (body weight: 9.5 kg). Bilaterally, the first and second premolars showed moderate tartar as well as a chronic purulent

periodontitis, osteitis and ulcerative gingivitis (lumpy jaw). In the liver and spleen, multifocal to coalescing, well demarcated, whitish masses of 2 to 15 mm diameter were observed (Fig. 1). Few noncoalescing nodular whitish masses were found in femoral bone marrow and lactating mammary gland. The hepatic, splenic and cranial mesenteric lymph nodes were moderately enlarged and exhibited a homogeneous whitish cut surface. The lungs showed moderate alveolar edema and moderate acute congestion.

Laboratory Results: Microbiology: Liver, spleen, mammary gland: numerous acid-fast bacilli (*Mycobacterium avium* complex) (Fig. 2); liver, spleen, kidneys, lungs, small intestine, mammary gland: high amounts of *Escherichia coli* (+++).

Contributor's Morphologic Diagnosis: Liver, spleen, mammary gland, bone marrow: Hepatitis, splenitis, mastitis, osteomyelitis, pyogranulomatous and necrotizing, multifocal to coalescing, severe, chronic, with multifocal mineralisation and numerous intra- and extracellular acid fast bacilli and gram-negative rods, partly intrasinusoidal and intravascular, Bennett's Wallaby (*Macropus rufogriseus*), marsupial.

Etiology: Infection with *Mycobacterium avium* complex and *Escherichia coli*

Contributor's Comment: Infections of marsupials kept in captivity with bacilli of the *Mycobacterium avium* complex (MAC; *M. avium, M. intracellulare*) are reported from North American Matschie's tree kangaroos (*Dendrolagus matschiei*). In this species, an increased susceptibility to opportunistic MAC infections with mainly pulmonary manifestation has been observed in relation to a reduced cellular immune reactivity, although additional factors, such as genetic influence, stress, and environmental exposure are not excludable[1]. In the present case, a similar type of MAC infection with manifestation in liver, spleen, mammary gland, bone marrow (not seen in all slides), but not in the lungs is presented in a Bennett's Wallaby. Acid-fast bacilli (MAC) and gram negative rods (*E. coli*) were additionally found in the cisternae of the teat of the lactating mammary gland, indicating a possible relation between the bacterial infection of the mother and the death of the suckling neonate.

The source of bacterial infection in this case could not be determined. Acid-fast bacilli of the MAC are of environmental origin. In immunosuppressed mammalian hosts, including humans, systemic diseases due to an infection with bacilli of *Mycobacterium avium* complex occur. MAC organisms persist and replicate within mononuclear phagocytes of the reticuloendothelial system[2]. They tolerate the acidic conditions of the stomach, resist the membrane-disrupting activity of cationic peptides, and invade intestinal epithelial cells[3]. Experimental studies on phagosomes containing *M. avium* showed that phagosomes are arrested in their maturation, lack lysosomal markers and are not acidified. Infected macrophages undergo apoptosis. Tumor necrosis factor-alpha (TNF-alpha) is one of the key cytokines elicited by macrophages infected with pathogenic mycobacteria. The TNF-alpha release of macrophages is regulated in a strain-, cell type-, and stimulus-specific manner. The *M. avium* induced production of

TNF-alpha seems to be regulated by mitogen-activated protein kinases (MAPKs)[4, and references therein].

In contrast to the described cases in Matschie's tree kangaroos, the manifestation of mycobacteriosis in the Bennett's Wallaby was in the liver, the spleen, the mammary gland, the lymph nodes, and the bone marrow.

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AFIP Diagnosis: Spleen, liver, bone marrow, and mammary gland: Granulomatous inflammation, diffuse, marked, with granulomas, Bennett's Wallaby (*Macropus rufogriseus*), marsupial.

Conference Comment: Marsupials are generally more susceptible to mycobacterial diseases than eutherian (true placental) mammals. The contributor summarized the incidence of MAC in Matschie's tree kangaroos. Other reports of mycobacterial infection in marsupials include subcutaneous atypical mycobacteria in captive tiger quolls (*Dasyurus maculatus*)[6], cutaneous and respiratory infections by *Mycobacterium ulcerans* in koalas (*Phascolarctos cinereus*)[7], and *Mycobacterium bovis* infections in brushtail possums (*Trichosurus vulpecula*)[6]. Reduced cell-mediated immunity has been demonstrated in several marsupial species and may increase their susceptibility to mycobacterial infection. The cause of depressed cell-mediated immunity in these animals, however, is not known.[6]

This case demonstrates a striking amount of central necrosis in the granulomas, similar to that seen in cases of *Mycobacterium bovis* in white-tailed deer[8]. Conference attendees discussed the general pathology of granuloma formation. Interleukin-12 (IL-12) is produced by macrophages and induces T_H1 differentiation and interferon-gamma (IFN-gamma) secretion by T cells and natural killer (NK) cells. T_H1 cells secrete interleukin-2 (IL-2), IFN-gamma, and tumor necrosis factor (TNF). Interleukin-2 causes T cell proliferation. Interferon-gamma activates macrophages and causes further secretion of IL-12, enhances their ability to phagocytize microorganisms, and secretes polypeptide growth factors, such as platelet-derived growth factor and transforming growth factor-beta. These factors stimulate fibroblast proliferation and collagen synthesis, resulting in fibrosis if macrophage activation is sustained. Tumor necrosis factor-alpha exerts its effects on endothelium to facilitate the extravasation of lymphocytes and monocytes at the site of inflammation. All of these changes are characteristic of type IV hypersensitivity.[5]

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SLIDE 75
CONFERENCE 18 / CASE II - 43305 (AFIP 2840998)

Signalment: Eight-year-old female red ruffed lemur (*Varecia variegata rubra*).

History: The lemur initially presented for evaluation of a large cervical mass after a 48-hr period of lethargy and anorexia. Physical exam revealed a soft fluctuant subcutaneous swelling extending from the dorsal aspect of the skull to the ventral cervical region. CBC and serum biochemistries were unremarkable. Radiographs of the cervical region showed discrete areas of mineralization in the mass. Ultrasonography of the mass was non-diagnostic. Surgical exploration of the area revealed a multiloculated mass with each cyst-like structure containing hundreds of <1mm bead-like nodules. Cestode larvae were identified upon examination of a wet-prep of the nodules. After a series of treatments with praziquantel, amoxicillin and albendazole, the mass was reduced to mild thickening of the ventral cervical region. No regrowth of the mass was detected during periodic examinations over the next year. Approximately one year post treatment, the cervical mass recurred.   Despite surgical debulking and repeated anthelmintic treatment, the mass persisted and the lemur was finally euthanized.

Gross Pathology: The left ventrodorsal cervical region was moderately swollen from the caudal mandible to the thoracic inlet. Reflection of the skin revealed a gelatinous, multiloculated, infiltrative mass composed of thousands of soft round 1 to 2 mm coalescing cystic structures (cysticerci) joined by thin translucent membranous tissues. The mass encircled the trachea from the larynx into the thoracic inlet and dissected into adjacent subcutis, skeletal muscle and adventitia of the esophagus.

Laboratory Results: Microscopic examination of the cystic structures from the cervical mass revealed numerous cestode larvae. The morphology of the rostellar hooks and the characteristic exogenous budding identified the larvae as *Taenia crassiceps.*

Contributor's Morphologic Diagnosis: Subcutis and skeletal muscle: Severe regionally diffuse granulomatous and eosinophilic cellulitis and myositis with intralesional cysticerci (Etiology: *Taenia crassiceps*).

Contributor's Comment: *Taenia crassiceps* is a cestode parasite that is found commonly throughout North America, Europe and the former USSR and uses a variety of canids and occasionally felids as definitive hosts. Rodents are the most common reported intermediate hosts. Intermediate hosts become infected by ingesting oncospheres in the feces of a definitive host. Cysticerci develop in the subcutis and body cavities of the intermediate host and are subsequently consumed with body tissues by the predator definitive host. The extensive infections sometimes seen in intermediate hosts, such as this lemur, is a result of the ability of *T. crassiceps* to proliferate by budding both exogenously and endogenously. This ability also may explain the recurring and persistent nature of the infection. The enclosure in which this lemur was housed would not allow entry of any canid or felid but it is possible that a definitive host (most likely grey fox) defecated close enough to the enclosure so that the lemur could reach the feces. Cysticercosis has been reported in other non-rodent species, most recently in immunocompromised domestic dogs and humans.

AFIP Diagnosis: Skeletal muscle: Cysticerci, with granulomatous and eosinophilic myositis, red ruffed lemur (*Varecia variegata rubra*), nonhuman primate.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant for veterinary parasitology. Although measurement and description of hooks is necessary to definitively characterize the species, Dr. Gardiner believes this is *Taenia crassiceps* because this species has been found to asexually multiply in host tissue. Of interest, he notes the presence of degenerate cysticerci in the tissues surrounding the viable (at the time of fixation) cysticerci. Many times, only calcareous corpuscles and hooks are all that remain in tissue sections. An acid-fast stain may be used to highlight the hooks.

There are four basic types of second stage larval cestodes, which serve as the infective form for definitive hosts: cysticercus, strobilocercus, coenurus, and hydatid. A

cysticercus consists of a single bladder with one scolex, whereas a coenurus consists of a single bladder with many scolices, each having the potential to develop into a mature tapeworm. A strobilocercus is a cysticercus that has already begun to elongate and segment while in the intermediate host.

Hydatids contain thousands of scolices (protoscolices) and are formed by members of the genus *Echinococcus*. Often protoscolices are grouped into small clusters termed brood capsules. When brood capsules rupture, the scolices are released to form a sediment called hydatid sand within the fluid-filled cyst cavity. Unilocular hydatid cysts are the second stage larvae of *Echinococcus granulosus*, for which dogs and wild carnivores serve as definitive hosts. Many species serve as intermediate hosts, including sheep, cattle, horses, swine, and humans. The dog-sheep cycle has been identified as one of the most important in many geographic areas. Hydatid cysts reside in the body cavities and viscera and may become extremely large, especially in human beings.[4,5,6]

Contributor: Zoological Society of San Diego, San Diego, CA

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SLIDE 76
CONFERENCE 18 / CASE III - 211305 (AFIP 2910178)

Signalment: Adult, female, fish crow (*Corvus ossifragus*).

History: This fish crow was wild-caught in Maryland and used as a positive control in a West Nile virus (WNV) study*. It was humanely killed on day 10 post-infection.

*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.

Gross Pathology: None reported.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Heart: Pancarditis, necrotizing, lymphoplasmacytic and histiocytic, multifocal to coalescing, severe.
2. Pulmonary artery: Arteritis, chronic, multifocal, moderate with adult filarial nematode, etiology consistent with *Splendidofilaria caperata*.

Contributor's Comment: This case is interesting because it has two lesions of different etiologies. The first lesion within the heart was caused by WNV and is characterized by multifocal to coalescing areas of myocardial degeneration and necrosis with infiltrates of lymphocytes, plasma cells, macrophages, and an occasional heterophil. Similar inflammation was also observed multifocally within the endocardium and epicardium. The second lesion is a chronic pulmonary arteritis caused by a gravid, adult, female, filarial nematode embedded in the tunica media. *Splendidofilaria caperata* has been reported to inhabit the tunica media of the pulmonary artery in magpies and starlings in Colorado and crows in Ontario, Canada[4].

West Nile virus lesions are well characterized in birds[2]. In this study lymphoplasmacytic and histiocytic inflammation with necrosis often in a perivascular distribution was observed in just about every tissue in the body with the most severe lesions affecting the heart, skeletal muscle, and spleen. Meningoencephalitis was observed in every case but was mild. West Nile Virus infection of the brain in crows and magpies has been reported to be mild in comparison to WNV infection in many other bird species[2].

Other WNV lesions observed in this case included: hepatitis, splenic red pulp necrosis and lymphoid depletion, interstitial nephritis, ventriculitis (gizzard), dermatitis, myositis, coelomitis, and adrenalitis.

In the fall of 1999, WNV emerged for the first time in the western hemisphere in New York City during an outbreak of disease that involved humans, horses, and wild and exotic birds[1]. Since that time, the virus has rapidly spread to infect humans and animals in almost every state in the United States. West Nile Virus belongs to the family Flaviviridae in the Japanese encephalitis (JE) serocomplex group that includes Saint Louis encephalitis virus, Kunjin virus, and Murray Valley encephalitis virus[2]. WNV is somewhat unusual from the other flaviviruses in that it can be transmitted by several species of mosquitoes and ticks, orally through the consumption of infected birds and rodents[3], by direct contact[3], blood transfusion[5,7], organ transplantation[5,7], and intrauterine fetus infection[6]. There is even a strong suspicion of transmission to a human infant through breast milk[6]. West Nile Virus is also unusual in that it has a wide

host range including many species of birds, mammals, and reptiles. The National Wildlife Health Center maintains a list of animal species found positive for WNV in surveillance efforts. As of September 4, 2003, over 200 avian species, 29 mammalian species and 2 reptile species have tested positive for the virus. (http://www.nwhc.usgs.gov/research/west_nile/wnvaffected_text.html).

The opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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AFIP Diagnoses:
1. Heart: Myocarditis, necrotizing, subacute, diffuse, severe, fish crow (*Corvus ossifragus*), avian.
2. Ganglion, heart base epicardial fat: Periganglioneuritis, lymphoplasmacytic, focal, mild.
3. Pulmonary artery: Endarteritis, proliferative, lymphoplasmacytic, diffuse, moderate, with intramural nematodes.

Conference Comment: Conference attendees noted that myocardial inflammation multifocally extends into the epicardium and endocardium. Lymphoplasmacytic inflammation also surrounds an epicardial ganglion, although an association with WNV infection is uncertain.

The genetic variant of WNV isolated in the United States is highly pathogenic in many species of birds, especially corvids. Other species of birds, such as chickens, are more resistant, although chickens do maintain a level of viremia adequate to infect mosquitoes and serve as an amplifier host. The reservoir host for WNV remains unknown.[1,3,8]

Differences in the histologic lesions and amounts of tissue viral antigen among several species affected by this virus have been documented. In crows and other wild birds, there is a large amount of viral antigen within both the CNS and extraneural organs, including the heart and kidney, whereas the amount of viral antigen detected in horses is minimal and limited to the CNS. Histologic lesions in free-ranging crows tend to be mild or absent, whereas lesions may be severe in other species of birds, such as strigiform owls, consisting of encephalitis and myocarditis.[10] Horses tend to exhibit moderate or severe histologic lesions, again, limited to the CNS. These consist of polioencephalomyelitis with perivascular infiltrates and hemorrhage. Horses may be considered incidental, dead-end hosts based on the presence of moderate to severe lesions, shortened viremia, and limited tissue distribution of viral antigen.[8,9,10]

Contributor: United States Army Medical Research Institute of Infectious Diseases Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011

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SLIDE 77
CONFERENCE 18 / CASE IV - 1984-577 (AFIP 2835107)

Signalment: Female Parma (or white-fronted) wallaby (*Macropus parma*) of unknown age (likely an adult).

History: Not a collection animal. Neurologic signs for 1 week; unable to right herself; treated with SC fluids, dexamethasone, Flocillin, Tribrissen, vitamin E, ivermectin and Sulmet for coccidia; periods of improvement where she appeared almost normal; head tilt for 2 days prior to death.

Gross Pathology: This animal was in good flesh and had no gross lesions other than markedly thickened, pale green-tan friable esophageal mucosa.

Laboratory Results: Antemortem data unavailable. Immunohistochemistry on sections of brain is positive for *Toxoplasma gondii*.

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis, necrogranulomatous, chronic, multifocal, severe with disseminated protozoa consistent with *Toxoplasma gondii*.

Contributor's Comment: In all sections of brain examined there are multiple nodular infiltrates of lymphocytes, plasma cells and macrophages either associated or unassociated with foci of necrosis. In and around these lesions there are numerous approximately 15-60 um diameter protozoal cysts that are PAS and GMS positive. There are diffuse moderate meningeal and perivascular lymphoplasmacytic infiltrates.

Additional histologic findings include multifocal, mild to moderate, lymphoplasmacytic myocarditis, diffuse pulmonary edema and congestion and mild, random, multifocal lymphocytic hepatitis. Although these lesions are likely related to *Toxoplasma* infection, organisms were only seen in the brain. There was also a marked, diffuse, chronic bacterial esophagitis.

Toxoplasma gondii is a coccidian parasite with worldwide distribution. Virtually all vertebrate species are susceptible to infection.[1] Although infection may be common in many mammalian species, clinical disease is rare. Virulence of *T. gondii* strains, compromised immunity and species susceptibility may be factors in outbreaks of acute disease and death. Certain taxonomic groups and species of animals are highly susceptible to clinical and often fatal toxoplasmosis. These include Australian marsupials, New World monkeys, prosimians and slender-tailed meerkats.[1,3]

Clinical manifestations of toxoplasmosis vary with the species and organ system(s) affected. Signs may be localized as in ocular or CNS involvement or pneumonia or they may be generalized. As with other highly susceptible species, Australian marsupials often acquire overwhelming infection with *T. gondii* and may die peracutely without premonitory signs.[1,3,4,6]

Deaths due to toxoplasmosis have been reported in numerous species of Australian marsupials including wild and captive macropods (kangaroos, wallabies, wallaroos), captive koalas, wild bandicoots and possums and captive wombats and dasyurids. There are often no visible gross lesions at necropsy. In macropods, pulmonary congestion, edema and consolidation are commonly seen and may be the only significant necropsy findings.[2,4,5,6] Other macroscopic lesions reported in macropods include myocardial hemorrhages, often interspersed with pale streaks or foci,[3,5] cerebral malacia,[5] gastrointestinal ulceration[3,5] and splenomegaly and lymphadenomegaly.[4,5]

Microscopically, necrosis is the predominant lesion, especially in the CNS, lungs, lymph nodes, liver and muscle, with a variable inflammatory response.[1,3] Histologic lesions commonly seen in macropods include nonsuppurative meningoencephalitis +/- necrosis, glial nodules,[3-7] fibrinonecrotic pneumonia and/or pulmonary edema and

congestion,[2-7] necrotizing lymphadenitis,[4,5,7] necrotizing hepatitis [4,5,7] and myocarditis and myocardial, skeletal and smooth muscle necrosis +/- mineralization.[3,5,7] Necroulcerative gastroenteritis, caused by tachyzoites released following ingestion of infective *T. gondii* tissue cysts or fecal oocysts, also occurs.[1,3,5,7]

Although the distribution of *T. gondii* organisms in tissues is variable, organisms are often widely disseminated. Intracellular aggregates of either bradyzoites or tachyzoites, extracellular tachyzoites and encysted organisms have been identified in various tissues.[5,7] Tissue cysts are especially common in brain and striated and smooth muscle and often are not associated with lesions.[4,5] Free zoites and intracellular aggregates of tachyzoites are often more common in extensive areas of necrosis.[5]

Diagnosis of the toxoplasmosis is based on demonstration of organisms in tissues by light and electron microscopy and *T. gondii* specific immunohistochemical staining, bioassay, tissue antigen ELISA or PCR analysis.[1] With light microscopy, *T. gondii* tachyzoites are 4-6 um long and oval to crescent shaped; cysts are 10-100 um, round to elongate, thin-walled (less than 0.5 mm thick) structures containing a few to several hundred slender, PAS-positive bradyzoites.[5,8] Antemortem diagnosis is aided by serology. Detection of antibodies in serum suggest previous or current infection by *T. gondii*.[1,2] Non-species specific assays are available for use in exotic, non-felid animals. Paired IgG titers on serum taken 2-3 weeks apart and measured with the same test at the same time that show at least a fourfold rise in IgG are indicative of active infection.

Infection with *T. gondii* occurs via three possible routes of transmission. Carnivores are infected by ingestion of tissue cysts in raw muscle, liver or other tissues (raw meat diets or predation of infected mammals in birds). Transplacental infection is possible and was suspected in 3 wallaroos that died with toxoplasmosis.[6] Commonly, exposure of herbivores is through ingestion of hay or grain contaminated with feline feces containing infective sporulated oocysts.[1] In several reports of toxoplasmosis in captive macropods, which are herbivorous, there was evidence of domestic cat involvement or contamination of marsupial feed and/or exhibits by domestic cats was considered probable.[1,2,3,4,5,6,7] Activation of latent *T. gondii* infection by immunosuppression or stress (shipping, recent introduction, etc.) is also a possible source of organisms.[1,4] Reactivation of latent *T. gondii* infection in humans and mice is consistent with localized toxoplasmosis and the distribution of lesions in localized toxoplasmosis may reflect the common distribution of *T. gondii* tissue cysts in muscle and brain of latently infected hosts.[3]

The extreme susceptibility of certain groups and species of animals to toxoplasmosis may be explained by the following: arboreal habitat of New World monkeys and prosimians (no contact with food contaminated by cats), feeding ecology of herbivores/insectivores (absence or sporadic absence of meat in diet) and reduced evolutionary exposure to felids (prosimians and Australian marsupials).[1,3] There were no cats in Australia before settlement by Europeans, therefore it may be that marsupials were never exposed to *T. gondii* during the evolutionary process.[5]

AFIP Diagnosis: Brain: Meningoencephalitis, necrotizing, lymphoplasmacytic, multifocal, severe, with protozoal cysts, parma (white-fronted) wallaby (*Macropus parma*), marsupial.

Conference Comment: There is variation in the sections of brain provided by the contributor. This case was reviewed in consultation with Dr. J. P. Dubey, veterinary parasitology consultant to the Armed Forces Institute of Pathology.

  The contributor gives an excellent review of toxoplasmosis. Conference attendees discussed *Neospora caninum* as a differential diagnosis for this lesion. Immunohistochemistry or electron microscopy is needed to differentiate these two organisms.

  As noted by the contributor, Australian marsupials and New World monkeys are most susceptible to infection, whereas Old World monkeys, rats, cattle, and horses are highly resistant. Lesions vary among the different species affected. In small ruminants, toxoplasmosis most commonly causes necrotizing cotyledonary placentitis, with characteristic 1-2mm diameter white foci of inflammation, necrosis, and mineralization. In dogs, pulmonary lesions may be severe, causing necrotizing interstitial pneumonia. Often in puppies, toxoplasmosis is triggered by immunosuppression caused by infection with canine distemper virus. In disseminated infection, other lesions include necrotizing hepatitis, myocarditis, splenitis, myositis, encephalitis, and ophthalmitis. In humans, toxoplasmosis is a common complication in immunosuppressed patients, such as those with AIDS or organ transplants. It can cause disseminated and often fatal parasitemia in the human fetus during the first trimester of pregnancy.[9,10]

Contributor: Wildlife Conservation Society, Department of Pathology, 2300 Southern Blvd., Bronx, NY 10460

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SLIDE 78
CONFERENCE 19 / CASE I – 1650/02 (AFIP 2885739)

Signalment: Five-month-old male, European short-haired cat, body weight 1.9 kg.

History: Died within one hour following convulsions; no vaccinations, no history of previous illness.

Gross Pathology: An immature male short-haired cat in fair body condition. Severe pulmonary edema and acute emphysema; hyperplastic pulmonary lymph nodes; myocardial hypertrophy of the left ventricle, slight dilatation of the right ventricle; distinct lobulation of the liver.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Occlusive vascular endothelial proliferation in several organs (heart, intestine, liver, kidney, spleen, pancreas, lymph nodes, brain); endotheliosis; intravascular pseudoangiosarcoma.

Contributor's Comment: Provided to the conference are H & E stained paraffin sections of the myocardium.

Histological examination of the cat revealed identical unusual vascular lesions in all organs (listed above). Proliferation of cells of endothelial type filled the lumina of small arteries and veins, forming cords. Other vessels contain glomerulus-like whorls with small capillary spaces. The newly formed cells are strictly intraluminal and always in contact with endothelium. Their nuclei are dark, elongated and have an irregular shape. Few mitoses are found. Rarely, small thrombi are in the center of the cellular proliferations.

Immunohistochemical investigation for factor VIII-related antigen proved the histogenesis of the cells as endothelial origin. Factor VIII-related antigen is an

established cell marker for endothelial cells in human and animal tissues[3]; however, the etiopathogenesis remains unclear. It is reported that these lesions may be a neoplasm or hyperplasia of endothelial cells caused by a toxic aetiology.

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AFIP Diagnosis: Heart: Reactive angioendotheliomatosis, European short hair, feline.

Conference Comment: In humans, reactive (benign) angioendotheliomatosis is a rare condition characterized by intravascular proliferation of endothelial cells that is usually limited to the skin.[5,7] Published reports of a histologically similar lesion in animals is limited to few cases in cats,[1,2,4] although several additional cases in cats have been identified since those initial reports (personal communication, Schulman FY).

In humans, the disease has been associated with coexistent systemic disease and patients present clinically with erythematous macules, ecchymoses, and plaques that may resolve spontaneously.[6] In contrast, the disease in cats is multisystemic (commonly involving the heart and brain) and fatal. The pathogenesis in both humans and cats is unknown, but it is thought that immunologic factors may play a role.[6]

Immunohistochemistry performed at the Armed Forces Institute of Pathology confirmed that the lining cells in the intravascular proliferations are positive for factor VIII-related antigen, and further revealed actin-positive cells interspersed between the lining cells compatible with pericytes.

Contributor: Institute of Veterinary Pathology, Ludwig-Maximillians-University of Munich; Veterinaerstr. 13, 80539 Munich, Germany

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disorders erroneously considered vascular neoplasms. J Am Acad Dermatol **38**(2):143-175, 1998

SLIDE 79
<u>**CONFERENCE 19 / CASE II – XN2604 (AFIP 2893499)**</u>

Signalment: 16-day-old, female, Seal-point Siamese, cat.

History: Six deaths occurred in a litter of seven Seal-point Siamese kittens born at a breeding cattery in April 2003. One kitten was stillborn, partly decomposed and reported by the owner to be malformed. Four kittens failed to feed and died within 3 days of birth; two of these were reported by the owner to have cleft palates. One kitten that died at day 16 was submitted for necropsy. One surviving kitten was reported by the owner to be 30% heavier than the other kittens.

Gross Pathology: The 16-day-old, male, Seal-point Siamese kitten weighed 133 g and had a crown-rump length of 150 mm. The carcass was pale and the blood was pale pink and "milky". The liver was enlarged and mottled pale pink to red. The kidneys were pale pink and had multifocal ecchymotic haemorrhages, 1 to 4 mm in diameter, on the capsular surface and superficial cortex. There were pale pink streaks in the heart. Patchy atelectasis was evident in the lungs.

Laboratory Results: A cryostat section of the liver stained with oil red O had periacinar accumulation of lipid droplets.

Contributor's Morphologic Diagnosis: Kidney: Emboli, lipid, haemorrhagic, necrotising, infarction, cortical, subcapsular, cat.

Contributor's Comment: The kidney has multiple subcapsular vascular lipid emboli associated with haemorrhage and locally extensive necrosis of the cortical parenchyma. In some areas there is infarction of the renal cortex. There are fine vacuoles in glomerular capillaries and other blood vessels in the kidney that represent lipid droplets circulating in the blood. Fine lipid vacuoles were also present in blood vessels in the liver, brain and other tissues in this cat. Periacinar vacuolar change of hepatocytes was evident in the liver and lipid was demonstrated in the vacuoles by staining with oil red O. Capillaries in the heart were dilated and there was intravascular and interstitial vacuolation of the myocardium. Variation in the size of lipid vacuoles within adipocytes was evident in adipose tissue at multiple sites. The pale blood, hepatic vacuolar change with periacinar lipid deposits and vascular lipid emboli in the kidneys are consistent with familial hyperlipaemia.

     Familial hyperlipaemia, also known as primary hyperlipoproteinaemia or hyperchylomicronaemia, is an autosomal recessive condition in Siamese cats characterised by fasting hyperlipaemia, lipaemia retinalis, xanthomas in the skin and

other tissues and peripheral neuropathy.[1,2,3] In cats the condition is thought to be due to lipoprotein lipase deficiency and is thus analogous to type I hyperlipoproteinaemia of humans.[1] The activity of lipoprotein lipase in affected cats is reduced in comparison with control animals.[3] This appears to be a primary deficiency of lipoprotein lipase, since it is not due to defective activation of lipoprotein lipase by its serum cofactor apolipoprotein C-II or by the presence in plasma of a factor that inhibits lipoprotein lipase.[3] The primary gene defect, however, has not yet been determined.

Cats with familial hyperlipaemia present clinically with lethargy, inappetence, hind limb ataxia and anaemia.[2,3] Clinical signs are ameliorated or resolve when affected cats are fed a diet low in fat. Subcutaneous plaques may be present.[1] Splenomegaly with splenic rupture has been described.[1] Thrombosis of the aorta at the level of the bifurcation of the iliac arteries has also been observed.[3] Tyzzer's disease due to *Bacillus piliformis* has been identified in kittens with familial hyperlipaemia from an experimental colony.[4] On biochemical examination, the blood of cats with familial hyperlipaemia has elevated plasma concentrations of very low density lipoprotein, cholesterol and triglycerides.[2,3,7]

Histologically, in cases of feline hyperlipaemia, there is lipid accumulation within clear vacuoles in the liver, spleen, lymph nodes, kidney and adrenal glands, as well as other organs.[5] Ceroid also accumulates in hepatocytes, macrophages and other cell types, mainly in older cats. Lipid emboli may be present in blood vessels, including the caudal aorta.[3] There is degeneration and fibrous replacement of glomeruli and nephrons. Focal degenerative changes are evident in arteries, with haemorrhage and formation of lipid-rich granulomas (xanthomas).[5] Degenerative lesions in peripheral nerves are due to compression by lipid granulomata.[2] Xanthomas are also observed in the skin.[2] Ultrastructurally, there are numerous lipid vacuoles within hepatocytes, renal proximal convoluted tubular epithelial cells and macrophages in the liver, spleen and lymph nodes.[6] Lipid emboli lodge in glomerular capillaries and interlobular blood vessels in the kidneys.[6] There is fusion of podocyte feet and thickening of basement membranes in glomeruli, Bowman's capsule and some proximal convoluted tubules.[6] Xanthomas were not evident in this case, probably because the age at death was too young for lipid to have accumulated in sufficient quantities in the skin and walls of blood vessels to induce granuloma formation. In this case there are multiple lipid emboli in the kidney associated with multifocal haemorrhage and necrosis. There was no evidence of aortic thrombosis.

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AFIP Diagnosis: Kidney, subcapsular veins and glomerular capillaries: Fat emboli, multiple, with hemorrhage and granulomatous inflammation, Seal-point Siamese, feline.

Conference Comment: The contributor provides an excellent review of feline inherited hyperchylomicronemia. In addition to Siamese cats, this condition has been reported in domestic shorthair cats.[1,2,4-6]

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SLIDE 80
CONFERENCE 19 / CASE III – N53 (AFIP 2892820)

Signalment: 1 year-old male domestic rabbit (*Oryctolagus cuniculus*), lagomorph.

History: Onset of a large skin mass (4-5 cm in diameter) in the interscapular area.

Gross Pathology: Firm flattened 4-5 cm skin mass in the interscapular area.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Subcutis: Atypical mesenchymal cell proliferation with eosinophilic intracytoplasmic inclusions (Shope fibroma).

Etiology: Leporipoxvirus, Poxviridae.

Contributor's Comment: The lesion consists of a large subcutaneous multinodular mass that is partially delineated and encapsulated with a moderately abundant fibrous capsule and has an expansile growth towards subcutaneous skeletal muscle. It is composed of interlacing bundles and whorls of spindle cells consistent with fibroblasts remaining within a sparse collagenous stroma with multifocal areas of marked edema. The proliferation is either densely cellular or more loosely cellular with densely cellular areas predominating. Proliferating cells are spindle shaped to polygonal, large with

poorly defined cell borders, abundant glassy to fibrillar eosinophilic cytoplasm and large, centrally located, ovoid hypochromatic nuclei with one to 3 round eosinophilic nucleoli. There are numerous eosinophilic intracytoplasmic inclusion bodies varying in size (1 to 4um in diameter) and shape (round to ovoid to fusiform). There is a marked anisocytosis and anisokaryosis but the mitotic index is low (less than one mitotic figure per HPF). There are multifocal areas of necrosis associated with heterophilic infiltration and edema within the proliferation. There is also a rim of inflammatory cells at the periphery including mainly lymphocytes, plasma cells, macrophages and fewer heterophils.

The electron micrographs show fibroblasts (fibroma cells) with numerous intracytoplasmic viral inclusions that consist of masses of viral material, immature and mature poxviruses (Fig. 1). Viral material is better depicted in figure 2 (x 12000) and consists either of fibrillary, regular aggregates of a moderately electron dense material arranged in long strands or bundles, or of finely granular aggregates. Interspersed within this viral material are numerous immature virions characterized by a spherical shape (around 250 nm in diameter) with electron dense granular content and an outer envelope. Numerous matures virions are also present showing characteristic features of poxviruses: large size (around 300 x 200 nm), ovoid shape, dumbbell-shaped finely granular electron lucent central body (nucleoid) and granular electron dense matrix of viroplasm surrounded by an outer envelope.

All these features are consistent with Shope fibroma although the interscapular localization is not the most commonly involved site. The most frequent reported sites are legs and feet and, to a lesser extent, muzzle, periorbital and perineal regions[1].

Rabbit fibroma virus is a poxvirus closely related antigenically to myxomatosis virus and to the hare and squirrel fibroma viruses. It was first isolated from a cottontail rabbit (*Sylvilagus floridanus*) in the United States in 1932. The virus is transmissible to European rabbits (*Oryctolagus cuniculus*) and cottontails. The infection is considered as a benign, self-limiting disease in the wildlife population. The virus may persist for several months within lesions and mechanical transmission by arthopod vectors appears to be the primary means of spread.[1] These pox virus-induced lesions are not neoplastic but hyperplastic[3] and may regress spontaneously due to cell-mediated immunity.

Poxviruses in general have an affinity for epithelium, particularly epidermis, but leporipoxviruses produce fibroblastic nodules rather than epidermal nodules, and cytokines are probably involved in genesis of the lesion.[3]

In the present case, the overlying epidermis is not present but the lesions in the epidermis are commonly described as severe hyperplasia with projection of cords towards the dermis. Epithelial cells show ballooning degeneration, cytoplasmic vacuolation and presence of irregular eosinophilic intracytoplasmic inclusion bodies.[2] Their nuclei are often large, hypochromatic with one or several large nucleoli.

AFIP Diagnosis: Skeletal muscle and associated fibroadipose tissue, subcutis (per contributor): Atypical mesenchymal proliferation, with chronic-active inflammation and eosinophilic intracytoplasmic inclusion bodies (Shope fibroma), domestic rabbit (*Oryctolagus cuniculus*), lagomorph.

Conference Comment: There are four poxviruses that affect rabbits: myxoma virus, Shope fibroma virus, and hare fibroma virus, all in the *Leporipoxvirus* genus, and rabbitpox virus in the *Orthopoxvirus* genus. Arthropod vectors are the primary means of transmission of myxoma virus and Shope fibroma virus, whereas the mode of transmission of hare fibroma virus is unknown. Rabbitpox is spread by nasal discharge and inhalation or ingestion of airborne droplets. Characteristic eosinophilic intracytoplasmic inclusion bodies are present in the lesions caused by viruses of the genus *Leporipoxvirus*, but are uncommon in lesions of rabbitpox.[4]

Clinical disease caused by myxoma virus varies with species susceptibility. Rabbits of the genus *Sylvilagus* (wild rabbits of the Americas) are natural hosts of the virus and relatively resistant to infection, although young rabbits may succumb to generalized disease. Rabbits of the genus *Lepus* are highly resistant, whereas infection in *Oryctolagus cuniculus* (wild European rabbits) results in severe disease and high mortality. In susceptible species, initial clinical signs include edema of the eyelids, followed by blepharoconjunctivitis, mucopurulent nasal discharge, and edema of the base of the ears, perineal region, external genitalia, and lips. The disease rapidly progresses and rabbits may die within 48 hours of initial clinical signs. If rabbits survive longer, disseminated subcutaneous gelatinous swellings develop within several days, and 99% of affected rabbits die within 12 days of infection. Histologically, these lesions are characterized by a proliferation of undifferentiated mesenchymal cells, which become large stellate (myxoma) cells surrounded by a mucinous matrix interspersed with capillaries and inflammatory cells.[4,5] Other histologic findings may include vascular endothelial proliferation, reticuloendothelial cell proliferation, and lymphopenia.[1,2,4]

As described by the contributor, Shope fibroma virus is a benign, self-limiting disease that causes subcutaneous, freely moveable tumors most commonly located on the legs or feet, but may also occur on the face, perineum, and elsewhere. These lesions, characterized by localized fibroblastic proliferation, can persist for several months before regressing.[4]

Hare fibroma virus is a disease of European hares (*Lepus europaeus*) and, although European rabbits (*Oryctolagus cuniculus*) are susceptible, there are no reports of natural outbreaks. The disease causes skin nodules on the face, eyelids, and around the ears with similar histopathologic features as Shope fibroma virus.[4]

Rabbitpox is a relatively rare virus that is antigenically related to vaccinia virus. The natural source of the virus has not been determined. It causes high mortality in young rabbits and pregnant or lactating females. Lesions range from localized cutaneous

papules to confluent maculopapular lesions with necrosis and hemorrhage anywhere in the body, extensive facial and oral edema, orchitis, conjunctivitis, and death within 7 to 10 days of infection. Histologically, a typical nodule consists of a central zone of necrosis surrounded by mononuclear cells with edema and hemorrhage in adjacent tissues.[4]

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SLIDE 81
CONFERENCE 19 / CASE IV – 03-12819 (AFIP 2890206)

Signalment: 10 year-old, female, domestic shorthair cat.

History: One eye of this cat had changed color, was painful and blind and had a severe acute onset of buphthalmos. The cat was vomiting brown fluid and had a decreased appetite. The eye was enucleated.

Gross Pathology: The globe was 2.4 cm in diameter. The posterior segment was completely filled and obliterated by white, opaque, firm and solid tissue with the presence of a small amount of brown-red mucoid material in the center. (Fig. 1)

Laboratory Results: CBC and biochemistry panels were unremarkable.

Contributor's Morphologic Diagnosis: Feline ocular sarcoma with lens rupture and chronic, severe, diffuse keratitis.

Contributor's Comment: The posterior segment of the eye is filled (predominantly peripherally) by an unencapsulated, poorly demarcated, invasive, densely cellular

neoplasm which extends to the anterior chamber and the cornea. It consists of closely packed spindle cells with indistinct borders and small amount of eosinophilic amorphous cytoplasm. The cells are organized in wavy bundles and whorls supported by a moderate amount of collagenous matrix. Other areas show osseous and chondroid differentiation. The nuclei are elongated with finely stippled chromatin. There is fourfold anisokaryosis and anisocytosis. Mitoses are rare. There are large numbers of cells undergoing necrosis. The lens is ruptured and a fragment of its capsule is coiled and embedded within the tumor. There are moderate numbers of macrophages, neutrophils, lymphocytes and plasma cells and a few multinucleated giant cells throughout and surrounding the mass. At the periphery of the globe there are lymphoid nodules. There is moderate vascularisation throughout the cornea with squamous metaplasia of the corneal epithelium.

The cat developed neurologic signs one week after enucleation and was euthanized. Necropsy was performed and histology revealed an extensive infiltration of the brain by the ocular sarcoma. The other eye had lens rupture with phaecoclastic uveitis.

Ocular sarcoma is the second most common primary ocular neoplasm in cats[1] (the first being diffuse iris melanoma) and is often secondary to ocular trauma,[2,3,4,5] but uveitis without trauma may also be a risk factor.[3,5] Histological characteristic features include long-standing lens rupture and inflammation, and circumferential distribution of the tumor within the globe.[1] Morphologic studies showed convincing evidence of lens epithelial cell origin for this neoplasm.[5] Ultrastructural features of ocular feline sarcoma suggesting an epithelial origin include a thick basement membrane surrounding each cells as well as visible cell junctions.[5] A common consequence of this tumor is infiltration of the brain via invasion of the optic nerve[2] but local recurrence in the orbit and distant metastasis can also occur.[1,3]

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AFIP Diagnosis: Eye: Feline ocular sarcoma, with osteosarcomatous and chondrosarcomatous differentiation, domestic shorthair, feline.

Conference Comment: As mentioned by the contributor, the two important histologic features of this neoplasm are 1) evidence of long-standing lens rupture (i.e. lens capsule embedded within the sarcoma), and 2) circumferential distribution of the sarcoma within the globe. Previously, this neoplasm was termed "post traumatic sarcoma" to highlight its association with a history of trauma.[2] This term has fallen out of favor because a history of trauma has been documented in only half of the published cases. More recently the term "feline ocular sarcoma" has been proposed.[1]

Lens epithelium is one proposed cell of origin, although an *in vitro* study[6] raises the possibility that ciliary body epithelium may be the cell of origin. The immunohistochemical staining pattern of these tumors are similar to those of lens epithelial cells in a traumatized globe. Neoplastic cells stain positively for vimentin, muscle-specific actin, transforming growth factor-beta (TGF-beta), and basic fibroblast

growth factor (bFGF). Another morphologic feature of these tumors supporting lens epithelial origin is the presence of periodic acid-Schiff (PAS)-positive basement membrane surrounding the neoplastic spindle cells.[1]

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SLIDE 82
CONFERENCE 20 / CASE I – 4281 (AFIP 2886859)

Signalment: 6-year-old neutered-female Visla, canine, *Canis familiaris*.

History: The dog began having abnormal head movements 9 months previously, with noticeable neck stiffness, ataxia and circling noticed for 5 months before death. Over the 3 months prior to death, the animal developed whole body tremors, ataxia affecting all four limbs, and a reduced menace response. The animal was euthanized after treatment with corticosteroids did not result in improvement.

Gross Pathology: The entire fixed brain and fixed specimens from spleen, lung, liver, duodenum, pancreas and skeletal muscle were submitted for examination. Grossly, the brain was characterized by irregular, moderately symmetrical, demarcated regions in the periventricular white matter that were discolored gray-tan. (Figures 1 and 2) These extended slightly more forward in the left cerebral hemisphere, compared to the right side. Similar foci were present in the brainstem, pons and medulla. The specimen of spleen was thickened and contained numerous coalescing gray-tan foci.

Laboratory Results: Cisternal CSF contained 86 mg/dl protein and was Pandy positive, but the leukocyte count was not increased. Serologic tests for *Ehrlichia canis*,

Ehrlichia equi, Ehrlichia risticii, Rickettsia rickettsia, Toxoplasma, Neospora and *Cryptococcus* antibodies were negative. Canine distemper IgM antibody was not found. MRI demonstrated contrast-enhancing multifocal periventricular foci, but little alteration was evident on T1 or T2 non-contrast imaging. Fungal isolations were not done.

Contributor's Morphologic Diagnosis: Multifocal to coalescing granulomatous encephalitis, predominantly periventricular, with fungal organisms.

Contributor's Comment: Clusters of well-defined inflammation were found in the periventricular regions of the diencephalon and at other sites. Each consisted of a central region of debris in which fungal organisms were generally numerous. This core is surrounded by an inflammatory response that is dominated by macrophages and multinucleate giant cells. Perivascular cuffs away from the granulomas contain lymphocytes and plasma cells. Neutrophils and large areas of necrosis are not evident. Both slender, unbranching fungal pseudohyphae with parallel walls that are 3-5um in width and occasional yeast forms up to approximately 10um diameter are present, and one form dominated some of the lesions. Apparently viable multinucleate giant cells within granulomas frequently contained fungi. Organisms were better detected with PAS stain than H&E, and sections were stained with PAS. Similar organisms were found in numerous granulomas in the spleen and lung. The type of reaction and presence of pseudohyphae and yeasts is suggestive of *Candida* sp.

Although localized infection with *Candida* has been noted in dogs[1], only a few cases of generalized infection have been reported.[2,3] Tissue from one of these cases contained many organisms in granulomas[3], while in the other, organisms were not observed in lesions.[2] Brain involvement was mentioned in neither case, although it is thought to occur in dogs.[4] *Candida albicans* readily causes meningitis in dogs when experimentally injected into the brain, and was lethal to neutrophil-depleted animals.[5]

In contrast to the situation in dogs, CNS involvement is very common in human systemic candidiasis, itself a fairly common condition, and usually occurs in the context of systemic immunosuppression, hyperalimentation, or extreme prematurity.[6] When encephalitis occurs, it is usually multifocal and bilateral, although neutrophils are more numerous in people than in this dog. Occasional cases of unilateral brain vasculitis have been found in the context of localized skin infection.[7] This dog was unusual in that no underlying cause of immunosuppression was noted clinically, except for a short course of corticosteroids some months prior to death, after the onset of clinical signs. The disease affected multiple organs. In contrast to human cases, neutrophils are uncommon in lesions, and many of the surrounding macrophages appear viable. *In vitro* studies suggest that Candida is capable of intracellular growth or penetration of human cerebrovascular endothelial cells[8] and can do so without killing the cell or perturbing the electrical resistance of the endothelial monolayer.

AFIP Diagnosis: Cerebrum: Encephalitis, granulomatous, multifocal to coalescing, marked, with fungi, Visla, canine.

Conference Comment: *Candida* sp. is a dimorphic fungus, of which the yeast phase is a normal inhabitant of alimentary, upper respiratory, and genital mucosal surfaces of animals. They reproduce by budding and proliferate as blastoconidia (budding yeast-like cells), or are present as pseudohyphae (segmentally constricted at points of attachment of individual yeasts) and branched, septate hyphae. *Candida* sp. may cause infection in young or debilitated animals, or as a complication of antibiotic therapy. *Candida albicans* is the most commonly isolated species.[9,10,11]

In general, candidiasis of the oral cavity (thrush) is the most frequent manifestation of infection in mammals, and is characterized by a gray-green pseudomembrane over an intact mucosal surface.[10]

In piglets, candida most often invades the squamous mucosa of the stomach, usually along with the oral cavity and esophagus. In calves, lesions are seen most commonly in the rumen, but may also involve the omasum, reticulum, and abomasum. In foals, candidiasis most often involves the esophagus and squamous epithelium of the stomach, with ulceration adjacent to the margo plicatus.[13]

In birds, candida affects the mucosal surfaces of the mouth, esophagus, crop, and proventriculus. The characteristic lesions are raised white mucosal plaques with a catarrhal or mucoid exudate.[12]

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SLIDE 83
CONFERENCE 20 / CASE II – 03-256 (AFIP 2888698)

Signalment: 5-month-old Angus heifer.

History: The calf was found in lateral recumbency and a neurologic examination revealed tetraparesis, no response to deep pain in the left hind limb, and absence of the panniculus muscle reflex caudal to T3/T4. The calf was euthanatized.

Gross Pathology: A 6 cm section of spinal cord between T2 and T3 had focally extensive softening and gray to brown, streaking discoloration of the right lateral and ventral portions of the cord (Fig. 1).

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Myelomalacia with intravascular fibrocartilaginous emboli, spinal cord.

Contributor's Comment: This is a case of fibrocartilaginous emboli causing infarction of the spinal cord and has not been reported in the bovine. The spinal cord has extensive necrosis of gray and white matter with hemorrhage. Areas of white matter degeneration with axonal swelling and loss and macrophage accumulation surround the necrotic area (Fig. 2). Occasional blood vessels in the meninges and parenchyma are occluded by homogeneous lavender to basophilic material (Fig. 3). This material stains positive with alcian blue (Fig. 4) and a von Kossa stain (Fig. 5) and is consistent with

intervertebral disk material. The affected vessels have fibrinoid necrosis and neutrophil inflammation within the wall.

The pathogenesis of fibrocartilaginous emboli within the vasculature of the spinal cord is uncertain and five mechanisms have been proposed[1,2]. These are 1) dissection of the nucleus pulposus through a degenerate dorsal annulus fibrosis into areas of neovascularization associated with the degenerate annulus; 2) direct penetration of the spinal arteries or veins following rupture of the nucleus pulposus through the dorsal annulus fibrosis; 3) herniation of disk material through the vertebral end plate into the marrow cavity and venous sinuses; 4) herniation of disk material into persistent embryonal vasculature of the annulus fibrosis; and 5) herniation into anomalous vasculature or arteriovenous fistulae. Trauma is probably a factor in several of these mechanisms.

Fibrocartilaginous emboli occur most commonly in large breed dogs that are predisposed to type II intervertebral disk disease. Extrusion of the nucleus pulposus through a degenerating annulus fibrosis is the most likely mechanism of embolism in these cases[2]. The disease has not been reported in the bovine and the mechanism in this case is more likely to be extrusion of nucleus into anomalous vasculature.

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AFIP Diagnosis: Spinal cord, ventral gray and white matter: Infarct, focally extensive, with fibrocartilaginous emboli, Angus, bovine.

Conference Comment: Fibrocartilaginous embolism has been described in dogs, pigs, horses, cats, sheep, and humans.[3] As the contributor mentions, the origin of the embolus is most likely the nucleus pulposus, but the annulus fibrosis and vertebral growth plate cartilage have also been suggested as sources for emboli.[3,4] The contributor gives a concise review of the proposed pathogenesis of this disease.

This disease is described in large breed dogs but not in chondrodystrophic breeds, which are most prone to develop intervertebral disk prolapse. No age or sex predisposition has been identified in dogs.[3,4] In one report in pigs[3], genetic, performance, and behavioral characteristics were believed to be predisposing influences. These pigs had heavier market weights than usual and were excitable and prone to sudden movements with vigorous muscle contraction, all which are believed to contribute to increased pressure on intervertebral disks.[3]

In affected animals, the involved spinal cord segments are often brown-red and soft. Both gray and white matter may be affected, and the lesions are commonly asymmetrical. Microscopically, the diagnostic finding is occlusion of blood vessel(s) by an embolus of fibrocartilage. This appears grayish on H&E stain, magenta with PAS, tan with PTAH, and blue with Alcian blue stain. Since there is abundant collateral circulation to the spinal cord, numerous vessels must be occluded to produce an infarct. If some blood flow continues to the affected area, the infarct will become hemorrhagic.[4]

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SLIDE 84
CONFERENCE 20 / CASE III – 02-7876 (AFIP 2889973)

Signalment: 11-26 days of age mixed gender lambs (ovine).

History: Nursing lambs were being found dead with no clinical signs observed.

Gross Pathology: No lesions were found in 3 of 4 lambs submitted for necropsy. A fourth lamb had approximately 10 ml of clear pericardial fluid.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Necrosis and edema, brainstem and cerebellar peduncles, multifocally extensive (encephalomalacia).

Contributor's Comment: Microscopic lesions in these brain sections consist of multifocal areas of neuronal necrosis and rarefaction (edema) in the neuropil. Most of the slides submitted with this case are from only one half of the brainstem, however the lesions were bilaterally symmetrical. The areas affected were primarily in the anterior brainstem and cerebellar peduncles.

These microscopic brain lesions are diagnostic for focal symmetrical encephalomalacia, which in the literature is described as a chronic neurological manifestation of enterotoxemia. Focal symmetrical encephalomalacia has been produced in experimental enterotoxemia by infusion of epsilon toxin of *Clostridium perfringens* type D. While this is referred to as a chronic manifestation of enterotoxemia[1], the clinical syndrome in these lambs was peracute, with lambs found dead with no clinical signs observed.

The course of enterotoxemia is usually very short, often less than 2 hours and never more than 12 hours[1]. Lambs are often found dead, although some individuals may show clonic convulsions prior to death. Lambs that survive for a few hours may have green, pasty diarrhea, staggering, recumbency, opisthotonos, and convulsions.

The disease can be reproduced experimentally by injection into the duodenum of whole culture of *C. perfringens* type D, or by intravenous infusion of epsilon toxin[1]. *Clostridium perfringens* type D normally inhabits the alimentary tract of sheep, but only in small numbers. If there is passage of large quantities of starch granules into the duodenum when sheep overeat, or consume large quantities of milk, organisms can multiply and toxin production proceeds to the point where toxemia occurs. Lambs on well-fed, heavy-milking ewes are particularly susceptible.

The epsilon toxin increases the permeability of the intestinal mucosa to this and other toxins, thereby facilitating its own absorption[1]. A receptor for epsilon toxin has been identified on vascular endothelial cells, with a result of vascular damage and increased vascular permeability. Vascular damage results in perivascular and intercellular edema in the basal ganglia, thalamus, internal capsule, substantia nigra, subcortical white matter, and cerebellum[2]. Protein-rich fluid accumulations also occur in the pericardial sac and lung.

Hemoconcentration and hyperglycemia occur in enterotoxemia. The increase in blood glucose is proposed to be caused by mobilization of hepatic glycogen by hepatocyte-bound epsilon toxin, or release of catecholamines due to stimulation of the sympathetic division of the autonomic nervous system because of brain edema. There may be profound hyperglycemia and glucosuria in affected lambs[2].

Other histologic changes of enterotoxemia include degeneration and necrosis of the epithelium of the proximal convoluted tubules in the kidney, secondary to endothelial damage by the epsilon toxin[2].

Diagnosis of enterotoxemia can be challenging, as gross and microscopic lesions often are minimal and obscured by postmortem autolysis. Detection of epsilon toxin in intestinal contents by mouse neutralization testing and ELISA assays has been evaluated, with inconsistency in the various techniques for detection of the toxin[3].

Vaccination of pregnant ewes 3 weeks prior to lambing is a highly effective tool in prevention of enterotoxemia in newborn lambs to at least 12 weeks of age, whereas vaccination of neonatal lambs may not provide added protection[4]. Ewes and lambs in this flock of sheep were not vaccinated, as the owner was attempting to raise the sheep under natural conditions without the use of "artificial" measures of husbandry.

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AFIP Diagnosis: Thalamus, dorsal and lateral: Necrosis, multifocal, breed not specified, ovine.

Conference Comment: The contributor gives a thorough review of the disease caused by *Clostridium perfringens* type D, often also called "overeating disease" or "pulpy kidney disease".[5,7]

There are five strains of *Clostridium perfringens* (A, B, C, D, and E), each of which causes enterotoxemia, among other diseases. There are four principle lethal exotoxins elaborated by *C. perfringens* that are important in its pathogenicity – alpha, beta, epsilon, and iota. Alpha toxin acts on cell membranes and produces hemolysis or cell necrosis. Beta, epsilon, and iota toxins cause necrosis and increased vascular permeability. In addition to the epsilon toxin, *C. perfringens* type D also produces alpha toxin.[2,5,6]

Clostridium perfringens type A, which produces alpha toxin, is the most common cause of gas gangrene in both humans and animals. It also rarely causes acute intravascular hemolysis and icterus in calves and lambs and is known as "yellow lamb disease". Colitis X is a rapidly fatal diarrheal disease in horses with an uncertain etiology, but it has been associated with *C. perfringens* type A. Additionally, food-borne illnesses in humans are commonly linked to type A.[2,5,6]

Clostridium perfringens type B elaborates alpha, beta, and epsilon toxins and is a cause of enterotoxemia in lambs, calves, and foals. The syndrome in lambs is called lamb dysentery, affects lambs less than 2 weeks old, and causes acute hemorrhagic enteritis. Occasionally it also causes ulceration with subsequent intestinal perforation and peritonitis. In older lambs, this disease is known as "pine" (in England) and causes depression, unthriftiness, and reluctance to suckle. In calves, this organism causes acute hemorrhagic enteritis with mucosal necrosis in animals younger than 10 days of age. It affects foals within the first two days of life, and also causes hemorrhagic and ulcerative enteritis.[2,5]

Clostridium perfringens type C produces alpha and beta toxins and causes disease in adult sheep, goats, and feedlot cattle, and neonatal lambs, calves, foals, and piglets. In adult sheep, the disease is called "struck". The lesions include hemorrhagic enteritis with ulceration, and peritonitis with a large volume of clear yellow fluid in the peritoneal cavity. The disease in goats and cattle is similar to sheep. Calves, lambs, and foals are affected by hemorrhagic and necrotizing enteritis during the first few days of life, and piglets during the first 8 hours of life. Antemortem clinical signs in neonatal animals may not be observed.[2,5,6]

Clostridium perfringens type E produces alpha and iota toxins. It causes intestinal disease in calves, lambs, and rabbits.[2,5]

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SLIDE 85
CONFERENCE 20 / CASE IV – X26871 (AFIP 2888447)

Signalment: Adult female raccoon (*Procyon lotor*).

History: This wild raccoon was found recumbent in a driveway and was euthanized by an intramuscular injection of Ketamine® and an intrathoracic injection of Euthanyl®.

Gross Pathology: The animal was obese with normal muscle mass and very abundant subcutaneous and internal adipose tissue stores. There were several, multifocal to coalescing, firm, raised skin masses in the right shoulder region. They ranged in diameter from 2 – 5 cm, and the epidermis overlying the largest one was ulcerated. On cut surface, they were uniformly white and extended from the superficial dermis into the underlying subcutaneous tissues. The right prescapular lymph node was moderately enlarged. The stomach was empty and the descending colon contained normal formed feces.

Laboratory Results:
1. T-lymphocyte CD3 marker (Avidin biotin complex – Peroxidase NCL-CD3-12 Novocastra®) – Positive
2. B cell CD79a marker (Avidin biotin complex – Peroxidase HM47-A9 Neomarker®) – Negative
3. Class 2 MHC Marker (Avidin biotin complex – Peroxidase TAL.1B5 Dako®) – Negative

Immunohistochemistry was performed on sections of skin and brain. There was diffuse infiltration of class 2 MHC marker positive cells in the skin mass with more marked staining of the meningeal cellular infiltrate. However, it was not the neoplastic cells that were staining. The positive cells were interpreted to be within the inflammatory cell population admixed with the tumor cells, and, as a result, this test was considered negative. Using extensive cell conditioning and dropping the primary antibody dilution, much of the effects of prolonged fixation of the tissues in formalin on the CD3 stain were overcome. The entire sheet of tumor cells in the skin and the clusters of neoplastic cells within the meninges were CD3 positive. The cellular morphology was not ideal but adequate to make a diagnosis of a T cell tumor using this protocol. CD79a B cells were not detected in either tissue.

Contributor's Morphologic Diagnosis: T-cell lymphosarcoma with skin, lymph node and brain involvement.

Contributor's Comment: In this section of brain, the subarachnoid space and spaces of Virchow-Robin are moderately to markedly distended by a pleocellular cell infiltrate with a population of relatively uniform round cells predominating. The round cells have fairly well defined cell borders, moderate amounts of finely granular basophilic cytoplasm and round nuclei with a condensed to euchromatic chromatin pattern; up to three-fold anisokaryosis; and a prominent nucleolus. Single cell necrosis within the population is common. Mitotic figures are common (four mitoses observed in 10 random HPF). A variable number of lymphocytes, plasma cells, eosinophils and macrophages are admixed with the round cell population. The normal architecture of the right prescapular lymph node and skin of the right shoulder region was effaced by similar neoplastic and inflammatory cell populations. In the skin, the neoplastic lymphocytes had a perifollicular distribution in the superficial dermis, but there was no clear evidence of tumor cells infiltrating the overlying epidermis or follicular-adnexal epithelium.

Lymphoid tumors are among the most common neoplasms in domestic animals, but cutaneous lymphoma is rare in all species[1]. In the literature, there are two reports of lymphosarcoma in wild raccoons[2,3], neither of which describes cutaneous involvement. However, both of these cases also involved females, and the tumor in one case also involved the brain. The raccoons described in these two reports were in poor body condition, as opposed to the present raccoon which was obese. This would suggest that the neoplastic disease had not been a long term problem for this animal.

Cutaneous lymphoma is traditionally divided into epitheliotropic and nonepitheliotropic forms[4]. In the dog, either form can be of T-cell origin. However, in epitheliotropic tumors, the neoplastic cells have an affinity for the epidermis and adnexal epithelium; are typically CD8$^+$ T cells; and originate in the skin, metastasizing only late in the disease. Whereas in the nonepitheliotropic tumors, the neoplastic cells grow deeply in the dermis and subcutis; are predominately CD3$^+$ T cells; and may be multicentric in origin. Based on this information, the cutaneous lymphoma in this raccoon was considered to be of the nonepitheliotropic form.

T-cells secrete cytokines that activate and attract other inflammatory cells. This would account for the pleocellular population of inflammatory cells admixed with the neoplastic cells in this case.

The cause of lymphosarcoma in raccoons has not been identified. Viral, environmental and genetic etiologies are described for lymphosarcoma in domestic animals[4].

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AFIP Diagnosis: Cerebrum: Lymphoma, raccoon (*Procyon lotor*), procyonid.

Conference Comment: Based on the pattern of a dense infiltrate within the meninges and Virchow-Robbins space, conference attendees favored a neoplastic process over an inflammatory process. A parenchymal distribution would be more characteristic of an inflammatory process.

One inflammatory condition with a predominantly perivascular distribution was, however, discussed. Granulomatous meningoencephalomyelitis (GME) is characterized by a perivascular accumulation of well-differentiated lymphocytes, monocytes, plasma cells and, often, epithelioid cells within the white matter. These inflammatory cells are sometimes arranged in whorls around vessels and, as the disease progresses, these cells expand to form coalescing perivascular cuffs which compress, but usually do not infiltrate, the intervening parenchyma.[5,6]

Lymphoma infiltrating the central nervous system is described in cattle as part of enzootic bovine lymphoma. In adult cattle, lymphoma is associated with bovine leukosis virus (type C retrovirus) and has a predilection for several sites, one of which is the epidural fat. This B-cell lymphoma may infiltrate the lumbar spinal cord and spinal nerve roots, causing posterior paralysis. Other sites commonly associated with bovine leukosis virus-associated lymphoma include retrobulbar fat, abomasum, myocardium (most frequently the right atrium), and uterus.[4,5,7]

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SLIDE 86
CONFERENCE 21 / CASE I – PN375/02 (AFIP 2895485)

Signalment: 12 year old, male neutered, Persian, *Felis domesticus*, cat.

History: The cat developed progressive, diffuse, symmetrical alopecia of the neck, abdominal, axillary, inguinal regions and extremities. The skin was thin, greasy and glistening (Fig. 1). The footpads were fissured (Fig. 2). The cat was in poor body condition, lethargic and had severe abdominal distension.

Abdominal ultrasound identified a hypoechoic mass in the pancreatic region and multiple, hepatic focal areas characterized by peripheral hypoechoic and central hyperechoic features. Based on the ultrasound findings and on the owner's request the cat was euthanized.

Gross Pathology: A full necropsy was performed. In the abdominal cavity a 1 cm in diameter pancreatic mass was observed. Multiple, round, umbilicated, variably sized (0.5 to 3 cm in diameter) hepatic masses were evident. Hepatic and renal lipidosis were observed. Multiple small white nodules were found in the mesenterial fat. Small nodules were disseminated throughout the lungs.

Laboratory Results: Routine haematology and serum biochemistry were unremarkable. T3 and T4 and cortisol levels were in the normal range. Feline leukemia virus and feline immunodeficiency virus tests were negative. Skin scrapings revealed the presence of elevated numbers of budding yeasts with the typical morphology of *Malassezia* sp. No mites were observed. Wood's lamp examination and dermatophyte cultures were negative.

Contributor's Morphologic Diagnoses:
Haired skin (neck):
1. Severe, diffuse, follicular telogenization with follicular miniaturization.
2. Minimal to mild, chronic lymphoplasmacytic and neutrophilic perivascular dermatitis with mild acanthosis and multifocal parakeratosis with loss of granular cell layer.

Contributor's Comment: The epidermis of most areas evaluated was characterized by multifocal to diffuse parakeratosis with loss of normal superficial keratin layers, loss of granular cell layer and mild, diffuse, irregular acanthosis extending to the follicular infundibulum. In some sections a focal erosion can be seen. Occasionally (in few sections) mild keratinocyte basal and suprabasal dysplasia is present.

The major finding is the presence of hair follicles in telogen phase and complete absence of anagen follicles. Moreover, most follicles are reduced in size (follicular miniaturization) and in some area of the mid-dermis, residual follicles have a thickened fibrous sheath. Sebaceous glands are normal in number and in size or mildly hyperplastic. Occasional sweat gland dilation and stasis is present. The dermal collagen was characterized by increased fragility.

The additional histopathological findings of the tissue examined were consistent with pancreatic exocrine adenocarcinoma with secondary hepatic, mesenteric and pulmonary metastases. The cutaneous findings were interpreted as paraneoplastic alopecia associated with pancreatic adenocarcinoma.

In cats, paraneoplastic alopecia has been associated with pancreatic exocrine adenocarcinoma[1,2,3,4,5,6] or biliary duct adenocarcinoma[2,7]. The association of pancreatic and hepatic malignancies with this dermatosis is not clear. Several metabolic imbalances such as hypoproteinemia or deficiencies in biotin, zinc, fatty acids have been proposed[5].

The glistening appearance of the skin is considered a characteristic feature of feline paraneoplastic alopecia[1,2,3,5,7]. This feature is interpreted as secondary to the loss of the stratum corneum that is not related to trauma or excessive grooming[2,3,7]. Also, no abnormal production of insulin, glucagon, somatostatin or adrenocorticotropic hormone has been detected[2]. Both loss of stratum corneum and follicular atrophy have been hypothesized to originate from the release of circulating products from the tumor[2,3].

The differential diagnosis of bilaterally symmetrical alopecia in cats needs to include demodicosis, dermatophytosis, endocrine, immune mediated and neoplastic disease. In most cases, demodicosis and dermatophytosis are excluded by skin scrapings and cultures, as it was in this case.

In feline hyperadrenocorticism, clinicals findings of polyuria, polydipsia, insulin resistant diabetes and increased skin fragility are typical. In cases of paraneoplastic alopecia no skin fragility has been reported and the glistening appearance is characteristic. Histopathology in the two diseases has some similarities, however in cases of paraneoplastic alopecia a normal or mildly acanthotic epidermis and follicular miniaturization with no prominent follicular keratosis are distinctive[2].

Although the gross appearance of the lesions may be similar, clinical signs in this cat were not consistent with hyperthyroidism and T3 and T4 basal levels were normal. Other differential diagnoses need to include self-induced alopecia and telogen and

anagen defluxion. However, clinical and histopathological findings allow the exclusion of these disorders.

Malassezia sp. infection is rare in cats. However, the association of *Malassezia pachydermatis* dermatitis with paraneoplastic alopecia and internal malignancies has been documented in cats[5,6]. Thus, in cats *Malassezia* sp. generalized infection is considered indicative of internal disease. Frequently, as it was in this case, in cats with paraneoplastic alopecia no specific histopathologic changes associated with *Malassezia* overgrowth have been detected[5].

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AFIP Diagnosis: Haired skin: Follicular atrophy, diffuse, severe, with epidermal hyperplasia and minimal lymphocytic perivascular inflammation, Persian, feline.

Conference Comment: The contributor gives a thorough review, with differential diagnosis, for paraneoplastic alopecia in cats. As mentioned, the features of complete follicular atrophy on the ventrum and the characteristic smooth, glistening gross appearance of the alopecic skin are diagnostic for this syndrome. Reports of successful surgical excision of the neoplasm resulted in regrowth of hair.[8,9]

Rare *Malassezia* sp. were present in the stratum corneum in some sections. Finding *Malassezia* on histopathology is difficult because the organisms in the stratum corneum are often lost during processing. Generalized *Malassezia* dermatitis in cats is rare and their presence is considered a poor prognostic sign, as they are often associated with an internal malignancy or immune suppression.[6]

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SLIDE 87
CONFERENCE 21 / CASE II – 1548-03 (AFIP 2888029)

Signalment: 13-year-old male, canine, Shetland sheepdog.

History: Lip biopsy from a dog with thickened, inflamed lips, and mild inflammation on the nose.

Gross Pathology: Not applicable.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Discoid lupus erythematosus.

Contributor's Comment: This section shows the junction of the normal lip haired skin and the inflamed glabrous skin, with heavy upper dermal, interface, plasma cell-rich, mononuclear cell inflammation in the upper dermis and sometimes migrating into the basal epithelium. There is marked basal layer melanosis and many melanophages are in the upper dermis (pigmentary incontinence). Hypereosinophilic, separating, acanthocytes ("Civatte bodies") are occasionally seen in the epidermis, within and near small intraepidermal microabscesses. This dog is doing very well two months later with steroid therapy.

   The lip margin is the most common site in our biopsy submissions of discoid lupus, but the lesions also occur on the nasal planum as "Collie nose". Besides Collies and Shelties, German Shepherds and Siberian Huskies have a breed predilection.

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AFIP Diagnosis: Mucocutaneous junction (per contributor): Dermatitis and cheilitis, superficial, lymphoplasmacytic, diffuse, moderate, with numerous intracorneal pustules, Shetland sheepdog, canine.

Conference Comment: There was marked variation in submitted slides, with two distinct presentations in the slides presented in conference.

The first presentation is that of dermatitis and cheilitis with intracorneal pustules, rare intraspinous pustules, and acantholytic keratinocytes. The differential diagnosis discussed included pemphigus erythematosus, as it represents a crossover syndrome of pemphigus and lupus erythematosus, and pemphigus foliaceus, since pemphigus erythematosus is also described as a variant of pemphigus foliaceus. Both syndromes are characterized by subcorneal pustules and acantholysis. Lesions of pemphigus erythematosus are usually confined to the face and often have lichenoid inflammation. Pemphigus foliaceus may involve the dorsal muzzle, planum nasale, pinnae, periorbital skin, footpads, or trunk, although facial lesions of this syndrome are indistinguishable from pemphigus erythematosus. These two conditions may be differentiated using immunofluorescent or immunohistochemical testing. Immunoglobulins are found in the intercellular spaces of the epidermis in pemphigus foliaceous, whereas they are found both in intercellular spaces of the epidermis and along the basement membrane in pemphigus erythematosus.[1,3]

The second presentation has furunculosis and intraepithelial pustules containing bacteria, but acantholytic keratinocytes are not present. The differential diagnosis discussed for this second presentation included mucocutaneous pyoderma and discoid lupus erythematosus (DLE). Both of these affect the nasal planum and mucous membranes and have similar clinical and histopathologic features. Plasma cells often predominate in mucocutaneous pyoderma, whereas lymphocytes and macrophages predominate in DLE. Features of DLE include lichenoid interface dermatitis, hydropic degeneration of basal cells, thickened basement membrane zone, apoptotic keratinocytes, and marked mononuclear periadnexal and perivascular dermal infiltrate. Basal cell degeneration is not a feature of mucocutaneous pyoderma.[1,2,3]

Conference attendees discussed the clinical importance of these two different presentations within the same lesion and the limitations of evaluating a single section. If only one tissue section from this dog was evaluated, indicated therapy or prognosis could significantly differ, depending on which section was evaluated.

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SLIDE 88
CONFERENCE 21 / CASE III – G6535/7 or G6542/9 (AFIP 2892541)

Signalment: Tissue from a 1 year-old male tamarin (*Saguinus fuscicollis*).

History: The animal had nonspecific clinical signs. In the course of a febrile disease the animal exhibited severe sero-mucous nasal discharge and single hemorrhagic to papular skin lesions.

Gross Pathology: At necropsy skin lesions were distributed randomly over the body, but preferentially on the face, scrotal regions and on the soles and palms. The skin lesions presented as hemorrhagic lesions. Some developed into erythematous papules, and single papular lesions became incrusted. The oral mucous membranes had severe necro-ulcerative inflammation. Notable facial oedema was evident. Peripheral lymphadenopathy was marked and involved submandibular and axillary lymph nodes.

Laboratory Results:
Microbiology: lung, liver, spleen, heart: *Pseudomonas* sp.; lung: *Klebsiella ocoenae*; Oral mucous membranes: *Pseudomonas* sp., *Klebsiella ocoenae*, yeast (*Candida albicans*).
Histology: A spectrum of different focal epithelial lesions became obvious. These patterns were categorized as severe epidermal hemorrhage and vesiculation, epidermal acanthosis and acantholysis as well as full thickness epidermal necrosis and ulceration. In some locations hair follicles and sebaceous glands were involved. The histopathologic pattern depended on the stage of development, degree of severity and superimposed bacterial infections. Eosinophilic granular intracytoplasmic inclusion bodies were found in degenerate keratinocytes within single locations of epidermal origin.
Electron microscopy revealed virus particles with orthopox-like morphology within intracytoplasmic inclusions. Ultrastructurally, mature viral particles measured 140 x 260 nm. The enveloped viral particles were ovoid to brick-shaped with a pale central zone, presenting characteristic pox-like ultrastructural features.
Real time PCR: DNA from different tissues was analyzed with a set of orthopox specific real time PCR assays. The presence of orthopox virus was confirmed, excluding at the same time variola virus. Virus could be detected in different organs among them liver, spleen, lung, intestine, skin and mucosa.

Contributor's Morphologic Diagnosis: Skin: Dermatitis, vesicular, multifocal, severe, subacute, with epithelial ballooning degeneration, epithelial syncytia and eosinophilic variably sized intracytoplasmic inclusion bodies (Guarnieri bodies).

Contributor's Comment: A putative cowpox virus, member of the genus orthopoxvirus, was identified as the causative agent for this fatal infection. Cowpox virus is a rodent virus that may infect cats, cows and zoo animals and, through contact to

these, humans. Cowpox virus infections are endemic in cattle, although clinical cases in the European cattle population are rare. Field and experimental studies have indicated that cowpox has a broad host range and a wildlife reservoir in rodents and foxes. Furthermore, the virus is often isolated from domestic cats, which should be regarded as important vectors in urban areas[1,2,5]. The orthopoxviruses are epitheliotropic. Typical lesions are characterized by vesicle formation and intracytoplasmic inclusion bodies. The presence of abundant co-infecting bacteria is a common sequela to poxviral ulceration. Lesions in humans can often be found on the hands, forearms, face and neck. Predisposition, such as immunosuppression, may lead to a more severe or fatal course of infection like in a case of a glucocorticoid treated asthma patient after contact with a cat[3,4]. A careful evaluation of the epidemiology of cowpox virus infection suggests that cowpox has a low virulence and contagiousness for humans, although the situation for nonhuman primates is still unclear.

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AFIP Diagnosis: Haired skin: Dermatitis, vesicular, acute, multifocal, marked, with superficial dermal hemorrhage, and keratinocyte and sebocyte syncytia and eosinophilic intracytoplasmic inclusion bodies, saddle-backed tamarin (*Sanguinus fuscicollis*), nonhuman primate.

Conference Comment: Conference attendees discussed the presence of very large syncytia and the presence of intracytoplasmic inclusion bodies in both keratinocytes and sebocytes.

Cowpox virus is found in Western Europe and Asia. Among the range of species infected by this virus, cats are important in the zoonotic transmission of this virus. Cowpox in cats and has even been called "feline cowpox" or "catpox". The disease in cats is presumably due to exposure to infected rodent hosts, as cases increase in the autumn when the rodent populations peak.[6,8]

Cats present with a single primary cutaneous lesion on the head or forelimbs, which is the site of direct contact with the infected rodent. The primary lesion is characterized by an ulcerated nodule with crusts. After 7-14 days, multiple secondary lesions develop anywhere on the body as ulcerated erythematous nodules that eventually scab over. Systemic signs are rare but may be present if the cat is immunosuppressed. Cats with cowpox and concurrent feline immunodeficiency virus (FIV) or feline leukemia virus (FeLV) infection have been reported to have fatal complications.[6,7,8,9] Histopathology reveals typical orthopoxviral lesions: hydropic degeneration of epithelial cells (ballooning degeneration) and eosinophilic, intracytoplasmic inclusion bodies. Immunohistochemistry, culture, PCR analysis, rising antibody titer, and electron microscopy may be used to confirm the diagnosis. Ultrastructurally, orthopox virions are brick-shaped, 250 x 200 nm and have an irregular arrangement of surface tubules.[7,8,9]

Besides orthopoxviruses, only three other genera of poxvirus cause disease in humans: parapoxvirus, molluscipoxvirus, and yatapoxvirus. Parapoxviruses (orf, pseudocowpox, bovine papular stomatitis) cause erythematous papules on the hands, fingers, and forearms ("milker's nodules") in humans. Molluscipoxvirus causes molluscum contagiosum in humans, characterized by multiple discrete epidermal nodules that occur anywhere on the body except the soles and palms. Yatapoxviruses (Yabapox and tanapox) occur naturally in tropical regions. Yabapox produces large, benign tumors that regress in 2-3 months. Tanapox is a common skin infection in parts of Africa.[7,9]

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SLIDE 89
CONFERENCE 21 / CASE IV – 252/03 (AFIP 2899565)

Signalment: 2-year-old heifer, Ayrshire, *Bos taurus*, bovine.

History: Three heifers on a dairy farm had developed skin lesions during the winter. The lesions were pruritic, alopecic, circular, 2-3 cm in diameter, with moderate crust formation. The lesions were situated in the head and thorax region. There were also nodular skin lesions. Skin biopsies were sent for histologic examination.

Gross Pathology: See history.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Haired skin: Dermatitis and folliculitis, lymphocytic and eosinophilic, chronic, focal, with mild hyperkeratosis. Within stratum corneum and hair, fungal structures (hyphae and arthrospores), consistent with *Trichophyton* sp.
Dermatophytosis (ringworm)

Contributor's Comment: Dermatophytosis is most commonly caused by zoophilic dermatophytes, *Trichophyton* and *Microsporum* in animals[2]. Bovine dermatophytosis is almost exclusively caused by *Trichophyton verrucosum*. Other dermatophytes that have been isolated from bovine dermatophytosis are, for example, *T. mentagrophytes, T. quinckeanum, T. rubrum, T. megninii* and *M. canis*[4]. The disease occurs worldwide. It is not fatal but it causes high economic losses in cattle farming and it is zoonotic[4,5].

   Trichophyton verrucosum has been isolated from soil, dung and numerous fomites. The disease is commonly seen where the climatic conditions are optimal to fungi. Most cases appear in the winter among housed animals (crowding, contamination, high humidity and darkness in the buildings). It is transmitted by direct contact or by indirect contact by contaminated objects such as housing, fencing and grooming equipment. Latent carrier animals act as a reservoir of the infection. Young animals are more susceptible; they have not developed immunity against the disease and their skin physiology is different (alkaline pH). Other predisposing factors are poor condition and immunosuppression[1,2].

   Dermatophytosis is a superficial cutaneous mycosis. The infection involves the keratinized layers of the skin and the hair; dermatophytes do not invade living tissue. The fungi are keratinolytic. The infection with *Trichophyton* sp*.* may be initiated if the stratum corneum is altered by slight trauma or by continued moisture and maceration. The branching septate hyphae of the fungi colonize the surface stratum corneum, the follicular infundibulum and the hair shafts. The boring hyphae penetrate the hair cuticle and tunnel extensively through the cortex. The hyphae break up into round arthrospores within the hair (endothrix) or on its external surface (ectothrix). The dermatophyte produces disease by excreting irritant substances like trichophytin, causing an effect similar to irritant contact dermatitis. This results in increased epidermopoiesis in the surface epidermis and proximal external root sheath, histologically seen as hyperplasia and hyperkeratosis. Mononuclear cells and neutrophils infiltrate the dermis and epidermis. Later, subcorneal and intracorneal microabscesses are formed

and significant numbers of eosinophils may be seen, especially in bovine and canine[1]. Secondary bacterial infections may occur.

The classic gross lesion in dermatophytosis is an annular area of alopecia, stubbled hairs, and scaling or crusting, and dermatitis. The predilection sites are the head, neck and pelvis. The lesions may be pruritic and painful[2].

The infection is usually self-limiting and the duration of the disease is usually 1-4 months[1]. Reinfection is uncommon. Cell-mediated immunity is considered more important than humoral, antibody-mediated reactions, but it seems that the combination of both is needed for the elimination of the fungi[1,3]. Efficient vaccines have been developed. Preventive measures consist of hygiene and vaccination of cattle[3,4].

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AFIP Diagnosis: Haired skin: Dermatitis, perifollicular and perivascular, lymphocytic and eosinophilic, diffuse, moderate, with intracorneal pustules, epidermal hyperplasia, and intrafollicular hyphae and arthrospores, Ayrshire, bovine.

Conference Comment: The pathogenic genera of dermatophytes include *Microsporum*, *Trichophyton*, and *Epidermophyton*. Zoophilic dermatophytes (such as *Microsporum canis* and *Trichophyton verrucosum*) are animal pathogens. Anthropophilic dermatophytes (*Epidermophyton*) are adapted to human beings and rarely infect animals. Geophilic dermatophytes (such as *Microsporum gypsum*) are soil saprophytes and, under favorable conditions, may infect humans and animals.[1,6,7]

The most important causes of dermatophytosis in dogs and cats are *Microsporum canis*, *Trichophyton mentagrophytes*, and *Microsporum gypsum*. Cats are natural hosts for *M. canis* and are often asymptomatic carriers. Lesions in cats vary from small areas of alopecia or broken hairs to nodular and ulcerated lesions. Dermatophytic pseudomycetoma is a rare manifestation of *M. canis* infection that occurs almost exclusively in Persian cats. Grossly, lesions are nodular with fistulous tracts. Histologically, granulomatous inflammation with aggregates of compact mycelia is present in the deep dermis and subcutis. The most frequently isolated species in dogs is *M. canis*, which causes alopecia with scaling, crusting, erythema, folliculitis and furunculosis. *Microsporum gypsum* causes a rare manifestation of dermatophytosis in dogs that produces discrete, solitary cutaneous nodules called kerion. These are areas of intense inflammation and furunculosis.[1,7]

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SLIDE 90
CONFERENCE 22 / CASE I – 02-6330 (AFIP 2910172)

Signalment: Adult male SD rat *Rattus norvegicus*.

History: On 10/30/02 the rat presented to veterinary services for noisy breathing. Rat had cranial surgery 6 weeks prior to clinical presentation. Head submitted by clinical veterinarian for examination of sinuses.

Gross Pathology: Not reported.

Laboratory Results: Mycoplasma and SDA serology (ELISA) were both negative.

Contributor's Morphologic Diagnosis: Chronic focally extensive moderate neutrophilic, necroulcerative, exudative, and lymphoplasmacytic rhinitis with edematous lymphoplasmacytic adenitis and intralesional fungal ball composed of concentric layers of mycelium (hyphae and conidia) morphology consistent with *Aspergillus fumigatus*.

Contributor's Comment: Aspergillomas, or fungal balls, form in the paranasal sinuses of humans and have been reported in rats. Concentric layers of hyaline mycelium create the fungal ball. The superficial layers are viable and often have conidial heads. *Aspergillus* hyphae measure 3-6um in width, are regularly septate, have parallel walls, and progressive dichotomous branching at acute angles. Hyphal morphology is best demonstrated with GMS or PAS stains. The fruiting body of *A. fumigatus* is relatively characteristic with a golden-brown dome-shaped terminal vesicle, covered by uniseriate phialides which are elongated cells that produce columns of spherical conidia. Conidial heads are produced when the aspergilli are exposed to air such as in sinuses, pulmonary cavities, cutaneous infections and otomycosis. Note: conidial heads may not be present in all sections.

AFIP Diagnosis: Nasal cavity: Rhinitis, ulcerative and suppurative, multifocal, moderate, with aspergilloma, Sprague-Dawley rat, rodent.

Conference Comment: *Aspergillus* sp. are ubiquitous environmental saprophytes that are not usually pathogens, but can cause opportunistic infection. Infection occurs in debilitated or immunosuppressed animals, or those on prolonged antibiotic therapy. While *Aspergillus fumigatus* is most frequently diagnosed as the cause of Aspergillosis in mammals and birds, *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus* are also associated with disease in animals. Respiratory infections are common and inhalation of spores is the primary means of establishing infection. Since *Aspergillus* is angio-invasive, hematogenous spread can lead to infection in multiple sites. *Aspergillus flavus* and *A. parasiticus* can produce aflatoxins, the most significant of which is aflatoxin B1, a potent hepatotoxin.[3,4]

The manifestation of *Aspergillus* infection varies among species. In birds, it causes granulomatous pneumonia and air sacculitis. In horses, *Aspergillus nidulans* is most often associated with guttural pouch mycosis. *Aspergillus* sp. causes mycotic placentitis and abortion in cattle and mares, with mycotic dermatitis in aborted calves, and sometimes in aborted foals. Damage to the ruminal mucosa by lactic acidosis, mechanical injury, or administration of antibiotics may predispose to mycotic rumenitis, with which *Aspergillus* sp. are associated. *Aspergillus fumigatus* is the most common cause of canine nasal aspergillosis. Disseminated aspergillosis also occurs in dogs but most cases occur in German Shepherd Dogs, and is caused by *A. terreus*.[3-6]

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SLIDE 91
CONFERENCE 22 / CASE II – N98-75 (AFIP 2893182)

Signalment: Adult feral pig.

History: Tissue collected from one of several feral pigs slaughtered at a local meat processing plant. The pigs were heavily infested with various metazoan parasites.

Gross Pathology: The lungs were mottled pink and tan with irregular shaped, pale, hyperinflated areas in the caudal lung lobes and variably sized gray-tan nodular foci. On cut surface of these areas, the lumen of small to medium sized airways often contained one or multiple white, threadlike, 2-5 cm long nematode parasites.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
1. Lung, airways: Bronchitis, bronchiolitis and peribronchiolitis, lymphoplasmacytic and eosinophilic, multifocal, moderate with intraluminal adult nematodes.
2. Lung: Pneumonia, subacute to chronic and eosinophilic, multifocal, mild.

Contributor's Comment: Prominent hypertrophy of bronchiolar smooth muscle is evident in some sections. Several airways contain cross and tangential sections of male and female adult nematodes with morphologic features characteristic of swine lungworms (*Metastrongylus* sp.). Metastrongyles have the typical strongylid intestine composed of few multinucleate cells with microvillus border similar to true strongyles and trichostrongyles but they differ from these other strongyle subgroups in having coelomyarian musculature[1]. Other features seen in these sections include lateral chords and female reproductive tracts with eggs containing developing larvae.

There are three important species of Metastrongylus found in the bronchi and bronchioles of swine: *M. apri, M. pudendotectus*, and *M. salmi*[2]. They are common parasites of swine throughout the world, especially feral populations and farmed pigs kept on soil or pastures. The female worms lay thick-shelled eggs containing L1 larvae in the airways where most are coughed up and pass out in the feces. When ingested by the intermediate host, earthworms, the parasite continues its development to the infective L3 stage. These larvae can survive in the earthworm for several years and the life cycle is then completed only if the earthworm is eaten by a pig. L3 larvae migrate across the gut wall and travel via lymphatics to mesenteric lymph nodes where they develop into L4 larvae. These larvae migrate through the lymphatics and vessels to the pulmonary arteries where they penetrate into alveoli and then migrate to bronchioles and bronchi. The migration through alveoli may result in areas of bronchopneumonia[3]. Adult worms may be found in all lobes but often have a predilection for the ventro-caudal portions of the caudal lung lobe. Although lesions are not normally as severe as seen in ruminants infected with *Dictyocaulus*, lungworms in swine can produce a chronic catarrhal bronchitis and bronchiolitis with multifocal hyperinflated areas in the

diaphragmatic lung lobes[4].   Large lymphofollicular aggregates are evident grossly as 1-3 mm grayish subpleural nodules. Larvae and eggs may provoke a granulomatous inflammatory reaction in the alveoli. A persistent cough and poor growth rate are common clinical signs in infected pigs. Lungworms not only predispose pigs to secondary bacterial pneumonias, but may also play a role in the transmission of swine flu and hog cholera viruses

Other parasites discovered at necropsy in these feral swine included spargana in the subcutaneous tissues, *Stephanurus dentatus* (the kidney worm) in the perirenal connective tissue, and intestinal worms *Ascaris suum* (large roundworm) and *Macracanthorhynchus hirudinaceus* (thorny-headed worm).

Many different nematodes have adapted to live in the lungs of mammals including *Filaroides hirthi* in dogs, *Crenosoma vulpis* in foxes, *Aelurostrongylus abstrusus* in cats, *Dictyocaulus* sp. in ruminants and equids, *Muellerius capillaris* and *Prostrongylus rufescens* in sheep and goats, *Parafilaroides* sp. in sea lions, and *Filaroides* sp. *and Filariopsis* sp. in monkeys. *Angiostrongylus cantonensis*, the rat lungworm, is a cause of eosinophilic meningoencephalitis in people.

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AFIP Diagnosis: Lung: Bronchopneumonia, eosinophilic and lymphoplasmacytic, multifocal, moderate, with intra-airway adult metastrongyles, etiology consistent with *Metastrongylus* sp., breed not specified, porcine.

Conference Comment: This case was reviewed in consultation with Dr. Chris Gardiner, parasitology consultant to the Armed Forces Institute of Pathology, Department of Veterinary Pathology. The contributor provides a concise overview of *Metastrongylus*, its life cycle, and the comparative pathology of lungworms.

Smooth muscle hypertrophy and hyperplasia of the bronchial-associated lymphoid tissue are present on some slides. The possibility of a *Mycoplasma hyopneumoniae* infection was discussed in conference because of the peribronchiolar lymphocytes.

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SLIDE 92
CONFERENCE 22 / CASE III – Fi01-03 (AFIP 2890205)

Signalment: Fish, grayling (*Thymallus thymallus*), 55 cm, age unknown, female.

History: Wild fish from a small river, found dead.

Gross Pathology: Whitish mass, 6 cm in diameter, cranial of the caudal fin, not ulcerated, in cut section whitish color and firm consistence, severe infestation with *Saprolegnia* sp.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Skin: Iridophoroma.

Contributor's Comment: Chromatophoromas are pigment cell tumors arising from dermal chromatophores in the skin of fish, amphibians and reptiles.[1] The four chromatophore classes commonly found in fish are: melanophores with black or brown pigment (melanin), iridophores with colorless pigment (purines), erythrophores with red pigments, and xanthophores with yellow pigment. Erythrophores and xanthophores both contain carotenoids, pteridines and flavins.[2] Chromatophores are common fish tumors and large epizootics have occurred worldwide in marine and freshwater fish.[3,4] While the majority of chromatophoromas are benign and restricted to the dermis, some of the largest are malignant as evidenced by increasing anaplasia, invasion and occasionally metastasis to liver or gill.[5]

Iridophoromas are characterized by the presence of olive-green granular pigment, which is birefringent with polarized light. Ultrastructurally, in unstained sections, iridophoromas have stacked arrays of reflecting platelets.

Although the etiology for the majority of chromatophoroma epizootics is unknown, epidemiologic surveys from many studies are at least suggestive for a possible exposure to anthropogenic carcinogens.[5] However, other potential etiologies like oncogenic viruses, genetic predisposition or ultraviolet radiation have to be evaluated.

AFIP Diagnoses:
1. Scaled skin and skeletal muscle: Iridophoroma, European grayling (*Thymallus thymallus*), piscine.

2. Skin: Ulcer, focally extensive, with superficial zoosporangia, etiology consistent with *Saprolegnia* sp.

Conference Comment: Chromatophores are contractile pigment cells of cold-blooded vertebrates that produce rapid color changes of the skin used for camouflage, sexual attraction, and protection. Color change is induced by intracellular aggregation and dispersion of pigment granules. These cells originate in embryonic neural crest cells and migrate to all tissues. Neoplasms of chromatophores are reported in snakes and fish.[5,6,7]

The contributor mentions the four types of chromatophores in fish: melanophores, iridophores, xanthophores, and erythrophores. Iridophores are not true pigment-containing cells, but have birefringent intracytoplasmic particles that refract and reflect light, giving the appearance of color. Melanophores, xanthophores, and erythrophores contain true pigment.[5,7]

Saprolegnia is an oomycete, or water mold, and is an opportunistic pathogen that causes infection in fish secondary to immunosuppression or environmental stress, but can occur as a primary pathogen. The two species most commonly isolated from fish are *S. parasitica* and *S. diclina*. The typical gross appearance is a white to gray proliferative cotton-like growth on the skin or gills. Histologically, there may be epidermal erosion, ulceration, necrosis, and edema, with broad colorless aseptate hyphae.[8,9]

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SLIDE 93
<u>**CONFERENCE 22 / CASE IV – NIAH No.1 (AFIP 2888761)**</u>

Signalment: 6-year-old, female, Holstein, *Bos taurus*, bovine.

History: In December 2001, a 6-year-old Holstein cow developed a fever (41.3 C) a few days after parturition, and stopped milking with mammary consolidation. Despite treatment with antibiotics and ointment for mastitis, the cow died 15 days after parturition.

Gross Pathology: At autopsy, no lesion was seen in the skin of the udder or the teats, though the lower surface of the udder had turned dark green. Palpation of the udder revealed moderate consolidation. On the cut surface, the mammary tissue was partially autolyzed and half-liquefied. Dilation of lactiferous sinuses and ducts can be seen with mild autolysis, and the sinuses and ducts were filled with milk-yellow exudate. The other organs were not collected due to severe autolysis.

Laboratory Results: BHV-4 was isolated from the mammary tissue. The viral DNA was detected by nested PCR from the same tissue.

Contributor's Morphologic Diagnosis: Mamma: Galactophoritis, necrosuppurative, severe, with eosinophilic inclusion bodies in ductal epithelium and many bacilli, with squamous metaplasia, Holstein, bovine.

Contributor's Comment: Histologically, autolytic changes were not too severe to recognize microscopic lesions in some areas of the mammary tissues, though the autopsy was carried out 3 days after the cow's death. In such areas, most of the lactiferous ducts and sinuses were filled with debris containing degenerated epithelium, neutrophils, and clumps of bacilli. The epithelial cells were degenerating and desquamating. Some of them had large swollen nuclei with eosinophilic inclusion bodies surrounded by a clear halo. There was focal squamous metaplasia in the sinus and ductal epithelium. Intranuclear inclusion bodies were sometimes seen in these metaplastic cells. The tunica propria, and interlobular connective tissue was severely dilated with congestion, edema, and infiltrates of neutrophils and mononuclear cells. There was mild to moderate neutrophilic infiltration in mammary acini. No inclusion bodies were seen in the acinar cells.

Although a few investigators described isolation of BHV-4 or detection of the viral DNA from milk of cows with clinical mastitis, no one has reported either histopathological changes of mammary tissue associated with BHV-4 or in situ detection of BHV-4. In the present study, we detected intranuclear inclusion bodies in the mammary tissues of a cow with clinical mastitis. Immunohistochemistry could be successfully used to detect BHV-4 antigen, and electron microscopy revealed herpesvirus particles in the cells with inclusions. Infection of BHV-4 was also demonstrated by virus isolation and nested PCR technique.

It could not be definitively determined whether BHV-4 was a primary infection or was secondary to bacterial mastitis. The fact that inclusion bodies appear for only a transient period 2-3 days after experimental respiratory infection of BHV-1 may support the latter scenario. The possibility remains, however, that BHV-4 is a primary and persistent infection, as occurs in field cases of BHV-1 infection, in which inclusion bodies occasionally persist long enough to be found in bronchial or alveolar epithelium. The primary BHV-4 infection may facilitate secondary bacterial infection.

BHV-4 was associated with degeneration and desquamation of epithelial cells. These lesions were principally similar to those seen in endometria of cows naturally infected with BHV-4. It was not clear whether BHV-4 infection caused squamous metaplasia in the sinus and ductal epithelium. Although no bacteria were isolated, probably due to the treatment with antibiotics, suppurative inflammation in the present case was most likely caused by bacterial infection.

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AFIP Diagnosis: Mammary gland: Galactophoritis, necrotizing and suppurative, diffuse, severe, with numerous bacterial colonies and epithelial eosinophilic intranuclear inclusion bodies, Holstein, bovine.

Conference Comment: Bovine herpesvirus-4 is a gammaherpesvirus and has been isolated from cows with mastitis, abortion, metritis, vaginitis, enteritis, and pneumonia, as well as from healthy cattle.[5,6,7] It is reported to be an emerging cause of endometritis in cattle.[6] It has been isolated from a variety of animals, including lions, domestic cats, and pigs, although the pathogenic role in these animals is unknown.[8,9]

Other important bovine herpesviruses are alphaherpesviruses. Bovine herpesvirus-1 causes infectious bovine rhinotracheitis, infectious pustular vulvovaginitis and balanoposthitis. Bovine herpesvirus-2 causes bovine mammillitis, an economically important disease that causes lesions on the teat and udder, and on the muzzle of suckling calves. Bovine herpesvirus-5 causes encephalitis and is believed to result from direct spread from the nasal cavity, pharynx, and tonsils through migration along the trigeminal nerve.[7,10]

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SLIDE 94
CONFERENCE 23 / CASE I – 03-0975 (AFIP 2889972)

Signalment: 1.5-year-old, female, Jack Russell terrier, canine.

History: The dog presented to the Small Animal Clinic because of seizure activity for the previous 1.5 hours. Upon presentation the dog was immediately admitted to the Intensive Care Unit (ICU). At admission to the ICU the animal was in respiratory and cardiac arrest. Resuscitation efforts were initially successful but the dog soon arrested again and died.

Forty-five days prior to presentation to the Small Animal Clinic the dog had whelped. The bitch had been nursing her puppies and had been presented to the referring veterinarian once for hypocalcemia.

Gross Pathology: Both horns of the uterus were dilated to a diameter of 2 cm and the lumen of both contained a moderate amount of mucoid, red-brown material. Scattered along the length of both uterine horns were multifocal, mural, ellipsoidal enlargements that were approximately 4 cm in length and bulged into the uterine lumen. The mucosal surface of these enlargements was red-brown and covered with a friable, necrotic tissue and the mucoid material described above.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Uterus: Endometrial ulceration, necrosis and hemorrhage, diffuse, severe, with invasion by placental trophoblast-like cells (subinvolution of placental sites), Jack Russell terrier, canine.

Contributor's Comment: This animal died of acute respiratory and cardiac arrest most likely secondary to prolonged muscle rigidity (seizures). The cause of the muscle rigidity is believed to be hypocalcemia related to nursing (eclampsia). The uterine lesions are not related to the death of the dog.

Histologically, the uterine lesions consist of an irregular, ulcerated luminal surface covered with eosinophilic debris, collagen and erythrocytes. Subjacent to the eosinophilic debris are degenerating placental trophoblast-like cells that have eosinophilic fragmented cytoplasm. There are multifocal areas of hemorrhage scattered about within the eosinophilic debris, collagen and degenerating trophoblast-like cells. Scattered randomly within the lesion are moderate numbers of macrophages many of which contains a brownish granular pigment (hemosiderin). Multifocally covering and randomly distributed within the eosinophilic debris and degenerating trophoblast-like cells are small sheets to islands of viable placental trophoblast-like cells. These epithelial cells have mildly distinct cytoplasmic borders and variable amounts of finely to moderately vacuolated eosinophilic cytoplasm. Nuclei are oval to irregular, moderately basophilic and have finely clumped randomly distributed chromatin. Nucleoli are small to moderate in size, moderately basophilic and randomly placed within the nucleus. Villous projections lined by highly vacuolated cuboidal to columnar epithelial cells are prominent in some fields. Deep to the degenerating and viable placental epithelium are scattered normal uterine glands. There are small to moderate numbers of lymphocytes and macrophages scattered between the normal endometrial glands. There are numerous congested vessels and scattered vessels contain fibrin thrombi. The myometrium is moderately hypercellular.

Subinvolution of placental sites was first described as a clinical and pathological condition in 1966.[3] Subinvolution of placental sites is an important differential diagnosis in the postpartum bitch that has a persistent bloody uterine and vaginal discharge.[5] In the normal postpartum bitch, uterine hemorrhage usually ceases within 1 to 2 weeks

following parturition. Uterine bleeding in dogs with subinvolution of placenta sites (SIPS) can continue well into the postpartum period sometimes lasting up to 7 to 12 weeks following whelping.[5] There has been one reported case where a dog with SIPS spontaneously recovered.[6] The condition is usually treated with an ovariohysterectomy.[4]

The cause of SIPS is unknown and the condition has only been recognized in the dog. Several theories (reviewed in reference 5) have been proposed for the condition but none has received wide acceptance. Early theories proposed the presence of bacterial infections in the placental implantation sites that prevented normal uterine involution. Another theory proposes that the condition is caused by an imbalance in estrogen and/or progesterone. A more recent proposal suggests that there may be a failure of normal endometrial blood vessel thrombosis following parturition. In this theory the lack of normal thrombus formation is attributed to vascular damage caused by trophoblast-like cell migration along uterine blood vessels.

The histological appearances of normal postpartum involution[2] and subinvolution of placental sites[1] have both been described. Histologically, both lesions are similar but in dogs with subinvolution of placental sites the uterine lesions persist for longer than normal.

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AFIP Diagnosis: Uterus: Subinvolution of placental site, Jack Russell terrier, canine.

Conference Comment: Typical gross findings in cases of subinvolution of placental sites are multiple ellipsoidal enlargements of the endometrium visible from the serosal surface. These segmental thickenings are sites of previous placental attachment and the mucosal surface is characterized by hemorrhagic, irregularly thickened, rough, gray to brown plaques up to twice the size of a normal placental site from the same breed at the same stage after parturition. The endometrium between the sites is normal.[2,7-10]

The key histologic finding is the presence of syncytial masses of trophoblast-like cells in the endometrium, often surrounding blood vessels. These cells invade the myometrium and, in some cases, may perforate the serosa. Other characteristic histologic findings include a plaque that protrudes into the uterine lumen composed superficially of necrotic debris and regenerating endometrium. Deeper within the plaque there is collagen deposition, hemorrhage, and dilated endometrial glands.[2,7-10]

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SLIDE 95
CONFERENCE 23 / CASE II – 03N0808 (AFIP 2890687)

Signalment: Near term (one-month premature by breeding dates and crown-to-rump length), female Morgan fetus.

History: Fetus and intact amnion were found on the ground in the pasture one-month prior to term. The mare, a 6 year-old Morgan mare, showed no systemic illness and was current on all vaccinations. The mare had foaled once before with no complications.

Gross Pathology: The villous surfaces of the gravid and non-gravid placental horns were diffusely red to dark red with multiple, randomly distributed avillous foci up to 0.3cm in diameter. Attached to the umbilicus were multiple yolk sac remnants. There were multiple epicardial and endocardial petechial and ecchymotic hemorrhages that were most pronounced in the left ventricle. Petechial and ecchymotic hemorrhages were also noted in the left caudal lung lobe and liver.

Laboratory Results: Bacterial cultures of the fetal liver were negative.

Contributor's Morphologic Diagnosis: Placenta: Multifocal nonsuppurative placentitis with necrosis and abundant intralesional *Encephalitozoon* sp.

Contributor's Comment: Scattered throughout the chorioallantoic villi there are multifocal to coalescing foci of necrosis of the chorionic epithelium. (Fig. 1) Shed necrotic chorionic epithelial cells are frequently noted. (Figs. 2 and 3) Associated with foci of necrosis, as well as within unaffected regions, there are abundant numbers of individual or aggregates of protozoa both free and within chorionic epithelial cells. These organisms are oval in shape and measure approximately 1-2um. (Fig. 4) These organisms are variably positive when stained with the following histochemical stains: Gram stain, silver (Steiner's) stain, acid-fast stain and Giemsa stain. Ultrastructurally, organisms were identified as spores of the phylum Microspora based upon spore size, cross sections of a coiled polar filament, the presence of a thick wall and posterior vacuole, and by the presence of organisms within typical parasitophorous vacuoles. (Figs. 5 and 6) Based upon these findings a diagnosis of encephalitozoonosis was made.

   Encephalitozoon cuniculi is a protozoan of the Phylum Microspora. Recent molecular analyses of microsporidia have suggested a closer relationship to fungi rather than protozoa based upon the presence of a mitochondrial heat shock protein and alpha- and beta-tubulins that closely resemble those of fungi, as well as the presence of chitin and trehalose, both of which are components of fungi.[1] The organism is an obligate intracellular parasite and is most commonly recognized as a cause of nephritis and encephalitis in rabbits. *Encephalitozoon cuniculi* infections have also been described in birds, mice, rats, guinea pigs, hamsters, cats, dogs, wild carnivores, humans, and non-human primates.[2-7] Several species of *Encephalitozoon* have been implicated as causes of disease in immunocompromised people, and encephalitozoonosis has become an issue of increased concern amongst AIDS patients.[8]

   In most species infection is usually subclinical and organisms are found incidentally. When present, lesions in the brain usually consist of small granulomas located most commonly in the cerebral cortex. In more severe cases large areas of necrosis and lymphocytic perivascular cuffing are often present. Renal lesions are usually characterized by granulomas and lymphocytic infiltrates involving the renal tubules and interstitium. In addition, the organism has been associated with lesions in the liver, pancreas, adrenal, spleen and lung.[2-4] In blue foxes the organism has also been associated with the development of polyarteritis nodosa, and appears to be a lesion unique to this species.[4]

   Infection generally occurs by ingestion of spores. Once ingested, the spores are able to inject their sporoplasm, via the extruded polar filament, into an appropriate host cell. Once inside the host cell the sporoplasm undergoes asexual proliferation (merogony) with the formation of meronts. Meronts undergo differentiation into sporoblasts (sporogony) and eventually develop into spores, which are packaged within

a parasitophorous vacuole. Enlargement of the parasitophorous vacuole eventually leads to cell rupture and release of spores into extracellular spaces. Dissemination can occur by both direct extension into surrounding cells, or by introduction into the vascular system. Spores are typically shed in feces, urine and mucus.[1,9,10]

Both horizontal and vertical transmission of encephalitozoonosis occurs. Transplacental infections have been documented in numerous species, and lateral transmission among young within the same group is known to occur.[4] Placental lesions are uncommon findings in most species with encephalitozoonosis and have been reported in a single squirrel monkey and a Quarterhorse and consisted of granulomatous placentitis and necrotizing placentitis respectively.[11,12]

A number of methods for antemortem diagnosis of encephalitozoonosis have been described. Most common methods include detection of specific antibodies by IFA, ELISA, CIA, and serology. PCR has also been utilized for diagnosis.[13] Postmortem diagnosis is based upon the finding of characteristic histologic lesions and/or the demonstration of organisms within tissues. A variety of staining methods can be used to demonstrate microsporidia in formalin-fixed and paraffin-embedded tissues. Mature spores usually stain Gram-positive utilizing the Brown and Brenn method of tissue Gram staining. Silver staining, such as the Warthin-Starry method, is another useful histochemical means of identifying the organism, and will identify both mature and developing stages of microsporidia. Other histochemical methods such as the periodic acid-Schiff stain can be used, but are not optimal for the identification of organism within tissues.

Electron microscopy is considered the gold standard for diagnostic conformation and species identification. Electron micrographs reveal organisms within parasitophorous vacuoles and a distinctive polar filament measuring approximately 100-150nm in diameter. Spores measure approximately 2um in length and enclose an extrusion apparatus that consists of the polar filament, an anchoring disc (polar sac) and a complex stack of membranes known as the polaroplast. The posterior vacuole and cytoplasm occupy the remaining space within the spore wall.[2,3]

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AFIP Diagnosis: Chorioallantois: Degeneration and necrosis, multifocal to coalescing, marked, with loss of chorionic villi, mild subacute inflammation, and intratrophoblastic microsporidia, Morgan, equine.

Conference Comment: The contributor gives a comprehensive overview of encephalitozoonosis. In addition to the changes noted by the contributor, conference attendees identified squamous metaplasia of trophoblasts.

It is difficult to differentiate *Encephalitozoon cuniculi* by light microscopy from other small protozoan parasites, especially *Toxoplasma gondii*. All stages of microspora are gram positive and the spores are birefringent, both of which differentiate

Encephalitozoon from *Toxoplasma*.[3] The birefringent property of the spores of microsporidia is a result of chitin in the endospore layer.[13] Cysts of *T. gondii* are smaller (60um diameter) than the pseudocysts of *E. cuniculi* (60-120um diameter), and mature *Toxoplasma* organisms are larger (2 x 6 um) than those of *Encephalitozoon* (1.5 x 2.5um).[12]

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SLIDE 96
CONFERENCE 23 / CASE III – CASE 1 (AFIP 2890505)

Signalment: 2-year-old CD1 female mouse.

History: This control (untreated) mouse on a 2-year study was euthanized. Clinical signs included abdominal distension, pale appearance, blood in the cage pan, red vaginal discharge, staining of fur and perineal staining.

Gross Pathology: The uterus was enlarged bilaterally with multiple nodular foci in both horns that were clear, red or yellow. There were bilateral ovarian cysts, the left was clear and 10x12 mm, while the right was dark and 5x5mm.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses: Decidual reaction affecting the endometrium and myometrium.
Additional findings: angiectasis, thrombosis, cystic endometrial hyperplasia, abscess.

Contributor's Comment: Elderly female CD1 mice have a high incidence of cystic endometrial hyperplasia and often develop angiectasis and sometimes thrombosis in the uterus, but decidual reactions are rare[1,2]. Decidual reactions, sometimes referred to as deciduomas, are seen occasionally in mice and rats[3], often in younger animals than this case, and can present a diagnostic challenge in the differentiation from neoplasms. Decidual reactions occur in the endometrium, but in this case there is also extensive proliferation within the adjacent myometrium and this could be confused with a smooth muscle neoplasm. This proliferation in the myometrium resembles the submucosal proliferative lesion of the trigone of the urinary bladder of male mice[4]. The amount of this proliferative tissue varies between slides but some is present in both the endometrium and the myometrium on all slides.

   The clinical signs relating to blood loss and vaginal bleeding were considered to be due to angiectasis, thrombosis, cystic endometrial hyperplasia and abscess.

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AFIP Diagnoses:
1. Uterus: Decidual reaction with myometrial infiltration, CD1 mouse, rodent.
2. Uterus: Endometritis, neutrophilic, acute, diffuse, moderate, with intraluminal bacteria.

Conference Comment: Conference attendees discussed the presence of an apparently discrete extramyometrial nodule present in some slides. It was concluded that, due to the similarity in organization and histologic characteristics (to the decidual reaction), that this is part of the same process and extends into the myometrium and

serosa. We consider the bacterial endometritis to be secondary to the decidual reaction.

In addition to deciduomas, other proliferative lesions of the mouse uterus were discussed. Histiocytic sarcomas may involve multiple tissues, although a common site is the uterine wall. It is not clear if the uterus is the primary site but since the liver is invariably involved, hepatic origin has been proposed.[2] Histiocytic sarcoma in the uterus should be distinguished from schwannomas or poorly differentiated leiomyosarcomas.   Endometrial stromal sarcomas are common and may arise within endometrial stromal polyps or are found in the uterine wall. Endometrial adenomas and adenocarcinomas arise from the epithelial lining of the uterine mucosa or endometrial glands.[2,5]

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SLIDE 97
CONFERENCE 23 / CASE IV – E 2228/02 (AFIP 2885741)

Signalment: Dog (*Canis familiaris*) Picard, 10 months old.

History: When acquired, the dog was thought to be female. Later on, as it matured, the animal indicated discomfort whenever it sat down, and a prolapse of a penis-like mass from the vulva was observed. Veterinary examination revealed a "penis-anlage" in the fossa clitoridis (praeputialis) of the vulva. Surgical excision of this "anlage" and of the internal genitalia (designated as uterus and testes by the referring veterinarian) was performed. The excised tissues were placed in 7% formaldehyde and passed on for pathological examination.

Gross Pathology:
1. Clitoris: large (4 x 1 x 1 cm), with a knobby apical swelling, and a massive central os clitoridis.

2. Uterus: with two inconspicuous uterine horns (0.4 x 0.4 x 10.5 cm each) and a corpus uteri.
3. Gonads: testis-like organs (1.5 x 0.7 x 2 cm each)

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Gonads: Bilateral ovotestis (true, bilateral hermaphroditism), Picard, canine.

Contributor's Comment: Originally, the case had been diagnosed as unilateral ovotestis in conjunction with contralateral hypoplastic testis. But during production of slides for AFIP Wednesday Slide Conference, deeper sections of the gonadal tissue became accessible for histologic examination. In a great number of slides an ovotestis and an apparent hypoplastic testis can be seen: Ovotestis consists of a peripheral zone of ovarian tissue, in which the complete sequence of follicular maturation (primordial follicle up to the tertiary follicle) can be discerned, and a central zone of testicular tissue, where both interstitial cells and hypoplastic seminiferous tubules lined by Sertoli cells can be observed. The contralateral testis exclusively shows testicular tissue with hypoplastic seminiferous tubules and normal appearing interstitial cells. Moreover parts of a hypoplastic epididymic duct and ductus deferens, with attenuated epithelium each, are visible. However, in deeper sections of the gonad that had originally been designated as hypoplastic testis, a peripheral zone of ovarian tissue becomes evident, too. The uterus (not present in the sections) shows normal tissue.

Hermaphrodites have ambiguous genitalia with part or all of the genital organs of both sexes present. The intersexual condition is subclassified into true (hermaphroditismus verus) and pseudohermaphroditism, the distinction being based on the presence of both types of gonadal tissue in the true hermaphrodite. The pseudohermaphrodite has gonads of one sex and accessory reproductive organs of the opposite sex. The true hermaphrodite either appears as unilateral (present case: testicular and ovarian tissue on one side, testicular or ovarian tissue on the other side), bilateral (testicular and ovarian tissue on both sides), or lateral (testicular tissue on one side, ovarian tissue on the other side). Germinocytes can only be found in the ovaries and in the ovarial parts of ovotestes. The accessory reproductive organs differ as the case may be, and can develop in varying degrees into male or female direction during ontogenesis.

True hermaphroditism has only rarely been observed in domestic animals, occurring most often in swine. But it has been described in goats, dogs, cats and horses, too. The development of this malformation is poorly understood. Physiologically, embryonal development of the genital ridge either into an ovary or a testis depends on the absence or presence of an intact Y-chromosome. For the testicular differentiation, the SRY-gene (Sex Determining Region on the Y-Chromosome) is essential. It is responsible for the induction of testicular development, and the Sertoli cells to produce Müllerian Inhibiting Substance (MIS), leading to Müllerian duct-degeneration. Zygogenetic investigations have shown that in humans 80% of the hermaphrodites show a female karyotype 46,

XX (a small number of patients shows 46, XX/46, XY mosaicism, and a very small number 46, XY). The development of testicular tissue in the absence of a Y-chromosome contradicts the above cited theory that a genetically active Y-chromosome is essential for gonadal differentiation in male direction. Various mechanisms including translocated Y-chromosomal sequences (SRY) or a mutation that allows testis determination without SRY have been discussed.

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AFIP Diagnosis: Gonad: Ovotestes, Picard, canine.

Conference Comment: The contributor gives a concise overview of true hermaphrodism and pseudohermaphrodism. Another well-described anomaly of development is that of freemartinism. This is an abnormality of chromosomal sex, in contrast to true hermaphrodism and pseudohermaphrodism which are abnormalities of gonadal sex and phenotypic sex, respectively.[7]

Freemartinism is primarily described in cattle and, although rare, it also occurs in sheep, goats, and swine. A freemartin is a female born as a co-twin to a male and is an XX/XY chimera. The freemartin is sterile because anastomoses between the placental circulations allow androgenic hormones from the male fetus to influence the female fetus. This suppresses female genital development and allows male vestiges to develop. Common gross findings in freemartins are vestigial seminal vesicles (always present), stunted ovaries, a hypoplastic vagina, lack of communication between the vagina and uterus, and an enlarged clitoris.[6,8]

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SLIDE 98
CONFERENCE 24 / CASE I – 02N2678 (AFIP 2890694)

Signalment: 1-year-old, female spayed Gordon Setter.

History: This animal had a history of being a poor grower and general poor thrift (per owner). The animal presented to the referring veterinarian with a three-week history of limping on the right pelvic limb, swelling of the right pelvic limb paw, enlarged peripheral lymph nodes and a draining tract lesion of the left thoracic limb paw. Abdominal ultrasound revealed nodules within liver, spleen and kidneys and the owners elected euthanasia due to poor prognosis.

Gross Pathology: The body was in poor nutritional condition with generalized atrophy of body fat stores and skeletal muscle. Abundant numbers of coalescing granulomas and pyogranulomas were scattered throughout the peripheral and internal lymph nodes, liver, spleen, kidneys, pancreas, diaphragm and heart.

Laboratory Results:

CBC

WBC 13,900/ul
Bands 417/ul slight toxicity
Neut 10,564/ul slight toxicity
Lymph 1390/ul
Mono 1112/ul
Eos 417

Urinalysis (cystocentesis)

Turbidity: Cloudy	Ketones: Neg
Color: Yellow/red	Hemoprotein: 3+
SG: 1.012	WBC: 4-6/hpf
pH: 5.0	RBC: >100/hpf
Protein: 1+	Bilirubin: Neg
Glucose: Neg	

Blood Chemistry Panel

CK: 2.5 mg/dL (0.5 - 1.6)	Total Protein: 7.6 g/dL (5.4 – 7.4)
BUN: 56 mg/dL (8 – 31)	Albumin: 2.4 g/dL (2.9 – 4.2)
Chloride: 97 mm/L (105 – 116)	Globulin: 5.2 g/dL (2.3 – 4.4)
Phosphorus: 7.0 mg/dL (3.0 – 6.2)	

Fungal cultures
Pseudallescheria boydii and *Scedosporium apiospermum* cultured from lymph nodes and draining tract exudate.

Contributor's Morphologic Diagnosis: Heart: Severe, multifocal to coalescing, pyogranulomatous and necrotizing myocarditis with intralesional fungal hyphae and chlamydospores.

Contributor's Comment: Multiple, coalescing, intensely cellular, nodular foci of inflammation and necrosis expand and efface the normal myocardial architecture. (Fig. 1) These foci are characterized by a central accumulation of degenerate neutrophils and necrotic cellular debris surrounded by large numbers of epithelioid macrophages, and lesser numbers of Langhans and foreign body-type multinucleated giant cells, lymphocytes and plasma cells. (Figs. 2,3) Within regions of intense inflammation there is extensive myocardial necrosis characterized by loss of myocyte cross striation, cellular hypereosinophilia, and fragmented, hyperchromatic nuclei. Abundant numbers of fungal elements including parallel walled, septate, dichotomously branching hyphae, and round, chlamydospores up to 50um in diameter are present in areas of inflammation. Chlamydospores have a clear central space, occasionally containing an eosinophilic granular material, surrounded by a basophilic, 2-4um thick spore wall. (Fig. 4) Often these spores are surrounded by epithelioid macrophages and are occasionally noted within the cytoplasm of macrophages and multinucleated giant cells. Within the interstitium of regions of intense inflammation there are large numbers of plump reactive fibroblasts and there are scattered foci of hemorrhage throughout the section.

Pseudallescheria boydii, and its asexual form *Scedosporium apiospermum*, is a ubiquitous, saprophytic, filamentous fungus belonging to the family *Microascaceae*. The organism is most commonly isolated from soil, vegetation, fresh water and sewage.[1] In recent years this fungal organism has arisen as one of the more important emerging opportunistic fungal infections of immunocompromised people, especially post-organ transplant recipients and AIDS patients.[2-4] In humans the infection typically results in subcutaneous mycetoma formation, or a more serious condition referred to as pseudallescheriasis. Pseudallescheriasis encompasses a wide variety of diseases including infections of the upper respiratory tract and lungs, sinuses, soft tissues, arthritis, osteomyelitis, myocarditis, ophthalmologic disease and infection of the central nervous system. Infection is most often a result of penetrating trauma or surgical incision; however, primary respiratory infection is also possible. The organism, much like *Aspergillus*, is highly angioinvasive and dissemination may take place through blood vessels or lymphatics.[1]

In animals, infections with *P. boydii* or *S. apiospermum* have been described in the horse, cattle, dogs and a stranded northern elephant seal. The spectrum of disease in animals is quite similar to those seen in humans and includes cutaneous lesions and systemic infections. In the horse and dog, the organism has been reported as a cause of subcutaneous and abdominal mycetoma formation, septic arthritis, osteomyelitis, rhinitis, sinusitis, pneumonia and systemic infection.[5-13] Mycotic onychomycosis

caused by *P. boydii* or *S. apiospermum* has been described in seven horses, and keratomycosis has been described in a single dog.[14,15] Infection with *P. boydii* has been implicated as a cause of placentitis and abortion in cattle and horses, and the organism has been reported as a cause of granulomatous pneumonia in a single calf.[16,17] In a single report involving a stranded northern elephant seal, infection resulted in fungal granulomas in multiple organ systems.[18]

In tissues the organism is virtually indistinguishable from other fungi with branching, septate hyphae such as *Aspergillus* and therefore, fungal cultures are necessary to make a definitive diagnosis. Proper identification of *P. boydii* is crucial, as the organism is resistant to many of the more common antifungal drugs. In humans, treatment usually requires both surgical and antimicrobial therapy.[1,2]

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AFIP Diagnosis: Heart: Myocarditis, necrotizing, granulomatous, multifocal, severe, with numerous fungal hyphae with intercalary swellings[1], Gordon Setter, canine.

Conference Comment: The contributor gives a thorough review of *Pseudallescheria boydii* infection. As the contributor notes, this organism is difficult to distinguish from *Aspergillus* sp. If present, the identification of filamentous hyphae with terminal and intercalary vesicles (chlamydoconidia) may aid in differentiating these two organisms.[13]

Conference attendees noted rare protozoal cysts within myocytes in some sections. These cysts were not associated with inflammation. Our differential diagnosis included *Toxoplasma gondii* and *Neospora caninum*, but we were unable to further characterize this organism.

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SLIDE 99
CONFERENCE 24 / CASE II – E03-263 (AFIP 2895810)

Signalment: One-year-old, castrated male, Holstein *Bos taurus* steer.

History: Holstein (*Bos taurus*) steer ear tag 44 was less than 10 months of age and weighed between 500-600 pounds and necropsy was performed on 8/21 (7 DPI). Inoculated (1 ml) of Rinderpest virus was administered subcutaneously over the right prescapular lymph node on 8/14.
Daily temperature data is as follows:
DPI: Rectal temp (°F):

0	Not done
1	101.1
2	102.5
3	105.8
4	106.2
5	104.9
6	105.3
7	103.2 euthanasia and necropsy that day

Gross Pathology: Scattered vesicular oral lesions, some of them covered with necrotic debris. In the large intestine there are large areas of necrosis affecting most of the lymphoid areas**.** These areas are covered by a thick layer of necrotic debris mixed with fibrin.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Colon: Severe diffuse subacute necrosuppurative colitis with intracytoplasmic inclusion bodies and occasional syncytial cells.

Contributor's Comment: Colon: Diffusely, there is a severe, acute, necrotizing and suppurative colitis with lymphangitis. On the mucosal surface, there are thick accumulations of fibrin mixed with large numbers of degenerate neutrophils, necrotic cellular debris, and large colonies of bacterial rods. There is necrosis of the mucosal epithelium and a locally-extensive area of full-thickness mucosal ulceration. The submucosa is markedly expanded by edema and contains moderate numbers of neutrophils and fewer macrophages. There are numerous variable sized (up to 25um) bright eosinophilic intracytoplasmic inclusion bodies. There are few scattered syncytial cells. Submucosal lymphatics are dilated (up to 1 mm) and contain large numbers of degenerate neutrophils mixed with necrotic cellular debris. There is diffuse hyperemia and endothelial cells are reactive.

Rinderpest, also called cattle plague, is an acute to subacute contagious viral disease of cattle with high morbidity rate and high mortality. Clinically it is characterized by fever, necrotic stomatitis and gastroenteritis. It is caused by a virus from the family Paramyxoviridae, genus Morbillivirus.

Rinderpest virus infects a variety of hosts including cattle, zebus, water buffalo and many species of wild animals: African buffalo, eland, kudu, wildebeest, various antelope, bushpig, warthog, giraffe, as well as domestic sheep and goats are susceptible. Asian pigs seem more susceptible than African and European pigs. Rinderpest is rare among camelidae. Rabbits, hamsters, mice, giant rats, ferrets and susliks have been infected only experimentally. There is no age- or sex-linked predisposition.

The disease is characterized by erosions and necrosis in the gastrointestinal tract. These erosions and necrotic areas are the classic vesicular lesions in the mouth and the intestinal wall.

Differential diagnosis include:
Bovine viral diarrhea (BVD) virus (pestivirus)
Foot-and-mouth disease (picornavirus)
Infectious bovine rhinotracheitis (alpha herpesvirus)
Malignant catarrhal fever (gamma herpesvirus)
Vesicular stomatitis (rhabdovirus)
Peste des petits ruminants (small ruminants; paramyxovirus)
Bluetongue (small ruminants; reovirus)

The Global Rinderpest Eradication Programme (GREP) is an international effort, whose Secretariat is at the Food and Agriculture Organization of the United Nations, to eradicate one of the world's most devastating livestock diseases. This effort has been an international partnership with valuable input from the Office International des Epizooties, European Union, African Union-Inter African Bureau for Animal Resources, USAID and other donors in Europe, numerous non-governmental organizations and most importantly the countries and villagers themselves. Currently, Rinderpest is believed to be limited to a focus of infection in east Africa (Somali ecosystem), but further field work is required to ensure that foci of virus activity in southern Pakistan or southern Sudan have been truly removed. GREP is designed to coordinate responses and develop guidelines to address rinderpest issues, including outbreak and surveillance strategies in order to reach the goal of complete global eradication by the year 2010 with the following time table:

2003 - declaration of worldwide provisional freedom from rinderpest.

2006 - freedom from disease for whole world established.

2008 - freedom from sub-clinical infection established.

2010 - Global Declaration of complete freedom from rinderpest.

http://www.fao.org/news/1996/960803-e.htm
http://www.fao.org/news/2000/000607-e.htm
http://www.fao.org/ag/AGA/AGAH/EMPRES/grep/e_rinder1.htm

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AFIP Diagnosis: Colon: Colitis, necroulcerative, subacute, diffuse, severe, with lymphoid necrosis, crypt herniation, diphtheritic membrane, transmural edema, and eosinophilic intracytoplasmic inclusion bodies, Holstein, bovine.

Conference Comment: Rinderpest is transmitted directly or indirectly through infectious secretions and, in confined areas, by aerosol droplets. The rinderpest virus is inhaled or ingested and localizes in the palatine tonsils and regional lymph nodes where it replicates. The incubation period usually lasts 4-5 days, after which a 2-3 day period of viremia occurs that coincides with the onset of fever and the clinical syndrome. After the viremic stage, the virus replicates in lymphoid tissues, bone marrow, and the mucosa of the upper respiratory tract and gastrointestinal tract.[2,3,4]

Typical gross findings include erosions in the upper gastrointestinal and respiratory tracts; edema, hemorrhage, and necrosis of Peyer's patches; and hemorrhage and congestion that run transversely across the colonic mucosa to produce a "zebra striped" appearance. Since the virus is tropic for lymphoid tissues, diffuse lymphoid necrosis is characteristic. Mucosal epithelium of the upper gastrointestinal tract and crypt epithelium become necrotic, and syncytial cells may form. Eosinophilic intracytoplasmic and intranuclear inclusion bodies may be evident in infected cells.[2,3,4]

The contributor gives an important differential diagnosis list for rinderpest. In addition to bovine pestivirus and rinderpest, salmonellosis should be considered as a cause of Peyer's patch necrosis.[3]

Contributor: Department of Biomedical Sciences, Pathology Program, College of Veterinary Medicine, Cornell University, Ithaca NY, 14853
http://web.vet.cornell.edu/public/BioSci/new/vbs_ld.html

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SLIDE 100
CONFERENCE 24 / CASE III – 02-2069 (AFIP 2890853)

Signalment: 9 year-old spayed female Labrador Retriever dog (*Canis domesticus*).

History: The dog had a 3-year history of a liver mass that was non-progressive in size, glomerulonephritis, and had been treated for hypothyroidism with Soloxine for an unspecified period of time. Liver enzyme activity was persistently increased. Progressive pulmonary radiodensities were seen on thoracic radiographs. There had

been a recent history of *E. coli* sepsis on two occasions along with severe diarrhea with profound melena and weight loss. Megabacteria and fungal overgrowth were noted in the feces.

Gross Pathology:
External examination: Diffuse, severe muscle wasting
Pendulous abdomen with fluid wave

Integument/Subcutis: Subcutaneous mass (3x2x1.5cm) on the dorsal midline, cranial to the scapulae.

Peritoneal cavity: 1L serosanguineous effusion

Liver: Hepatomegaly, marked
Hepatic mass (hepatocellular carcinoma)

Digestive system: Stomach: Trichobezoar
Multifocal acute and chronic ulcers
Pancreas: Multifocal 1-3 mm raised white firm foci within the parenchyma
Small intestine: Multifocal 1-3 mm raised white firm foci within the mucosa and submucosa.

Respiratory system: Diffuse consolidation with gritty, hard parenchyma on cut surface

Urinary system: Multifocal renal cortical cysts

Cardiovascular system: Mild left AV valve endocardiosis

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
Pancreas: 1) Granulomatous pancreatitis, marked, multifocal with intralesional trematode eggs.
2) Interstitial fibrosis, mild to moderate, diffuse.
3) Nodular hyperplasia, mild, multifocal.

Contributor's Comment: The diagnosis of *Heterobilharzia americana* infection was made by recognition of spherical to oval ova containing miracidia in the pancreas and the intestine. Adult schistosomes live in the vascular system of the definitive host after migration through parenchymal organs. Ova pass through the mesenteric vessels, penetrate the intestinal mucosa and pass into the intestinal lumen. Miracidia are released into water and penetrate snails from the Lymnaeidae family and then from sporocysts. After they develop into cercariae, they leave the snail and are released into water to penetrate the skin of the main hosts, raccoons, dogs or nutria to complete the life cycle. Lesions can be found along the portal vein flow, affecting intestines,

pancreas and the liver.[1] There was marked pulmonary interstitial mineralization detected at postmortem examination. Schistosomiasis in canines has been associated with hypercalcemia, but not mineralization of organs. It is unlikely that schistosomiasis was responsible for pulmonary mineralization in this case since there was no evidence of hypercalcemia in this dog. Renal disease in this geriatric canine is the most likely cause for a transiently elevated the Ca:P ratio, indicating that this lung mineralization was most likely attributable to renal rather than parasitic causes. Interestingly, schistosomiasis in humans has been associated with hepatic neoplasia, but there was no evidence of hepatic involvement with schistosomes in this case.

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AFIP Diagnosis: Pancreas: Pancreatitis, granulomatous, multifocal, moderate, with nodular regeneration, interstitial fibrosis, and trematode eggs, Labrador Retriever, canine.

Conference Comment: The Schistosomatidae family, or blood flukes of mammals and birds, contains three genera of veterinary importance in mammals: *Schistosoma*, *Heterobilharzia*, and *Orientobilharzia*. These are important parasites in Africa, Asia, the southern United States, and tropical or subtropical regions in which the intermediate snail host is found.[4]

   Schistosoma bovis and *S. japonicum* are the most pathogenic of these flukes in cattle and sheep, and are found in the mesenteric veins. The main definitive hosts of *Heterobilharzia americana* are the dog, raccoon (*Procyon lotor*), and nutria (*Myocastor coypus*), although other hosts have been identified. *Schistosoma mansoni* is endemic in humans in Africa, portions of the Middle East, and Central and South America. A link between hepatocellular carcinoma and schistosomiasis in humans has been proposed, but frequent concomitant viral hepatitis complicates understanding the parasite's role. Woodchucks (*Marmota monax*) can be experimentally infected with *S. mansoni*, which makes them a potentially useful animal model to study the outcomes of chronic concurrent schistosomal and viral hepatitis.[4,5,6,7]

   A characteristic feature of these flukes is that the male and female are permanently coupled. The male is shorter than the female and has a distinctive gynecophoric canal, which encloses the more slender female. These trematodes live in veins and their eggs circulate and lodge in tissues, which cause the most severe lesions: microgranulomas (or "pseudotubercles") surrounding schistosome eggs in the liver, spleen, brain, gastrointestinal tract, urinary bladder, and other organs. The adult schistosomes can cause an eosinophilic endophlebitis, with intimal proliferation and thrombosis.[4,5]

Contributor: North Carolina State University - College of Veterinary Medicine

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SLIDE 101
CONFERENCE 24 / CASE IV – P03-9694 (AFIP 2908331)

Signalment: 11-year-old, Male intact, Cairn terrier, canine.

History: Bilateral glaucoma.

Gross Pathology: Two eyes (OS and OD). One shows lateral flattening and a local projection within the posterior limbal area, both eyes do not show a trabecular aspect of the ligamentum pectinatum but a solid ridge instead. Both eyes show asteroid hyalosis.

Microscopic Description:
Diffuse proliferation of cells containing large amounts of moderately coarse melanin granules in the iris, ciliary body, iridocorneal angle and to a lesser extent in the choroid, sclera, cornea and episcleral tissue. Occlusion of the corneoscleral meshwork with focal synechia anterior. Focal extensive retinal atrophy. Pigmentation and slight squamous metaplasia of corneal epithelium
(epidermidalization) and marked vascularization of corneal stroma with a mixed inflammatory infiltrate. Moderate lymphoplasmacytic inflammation of conjunctiva.

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
MD: eye:
1. Uveal melanosis, severe, diffuse, bilateral, consistent with melanocytic

glaucoma in the cairn terrier.
2. Secondary keratitis and retinal atrophy, anterior synechia

Contributor's Comment: The pathological changes in the eye of this Cairn terrier were bilateral. This condition, named melanocytic glaucoma, formerly pigmentary glaucoma, is known to occur occasionally in older cairn terriers (from the age of 10 and older). It has been described in the USA in 1984 by Covitz and others, in the UK in 1991 by Peterson-Jones.

The origin of the proliferating pigmented cells is not yet clear; they are described as being melanocytes or melanophores. The cells seem to show infiltrative behavior. However, metastasis has never been observed. The prognosis is poor, mainly because usually both eyes are affected and sooner or later glaucoma develops due to occlusion of the corneoscleral network.

Formerly the condition was named pigmentary glaucoma, referring to the disease in humans now known as pigment dispersion syndrome. This syndrome occurs rarely in young (20-40 years) myopic individuals, and is characterized by intensive pigment deposition on the posterior lens, iris surface, trabeculas, retina, depigmentation of the iris, Krukenberg's spindle (deposition of liberated melanin pigment on the posterior cornea in a vertical line) and radial thinning of the iris. Most of these features are not present in the dogs, suggesting a different condition with different pathogenesis.

On other slides of this dog (not provided), there are multiple round pale eosinophilic, crystalline birefringent structures in the corpus vitreum (asteroid hyalosis). This is seen as a reactive process in many eye pathologies.

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AFIP Diagnosis: Eye: Melanosis, uveal, diffuse, moderate, with peripheral anterior synechiae, retinal atrophy and detachment, and chronic keratoconjunctivitis (melanocytic glaucoma), Cairn terrier, canine.

Conference Comment: Canine glaucoma is classified as either primary, secondary, or congenital, based on possible cause. Congenital glaucoma is seen at birth or shortly thereafter, and is associated with an anterior segment anomaly. Primary glaucoma develops without concurrent ocular disease and is hereditary in some breeds. It may result from defective development of the iridocorneal angle (narrow-angle glaucoma) or a functional abnormality in the filtration angle (open-angle glaucoma).[5] Secondary glaucoma is associated with some antecedent or concurrent ocular disease that causes increased intraocular pressure. Melanocytic glaucoma of the Cairn terrier is an unusual form of secondary glaucoma. It affects middle-aged to older Cairn terriers and may be unilateral or bilateral. Melanocytes or melanomacrophages proliferate and are located in the filtration angle, episcleral and subconjunctival tissues, tapetal ocular fundus, and the meninges around the optic nerve head. Although the cause of this melanocytic proliferation is unknown, the disease process is similar to benign iris melanoma. The

pattern of melanocytic glaucoma differs from that of neoplasia, however, in that it is diffuse rather than nodular.[3,4,5]

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The Netherlands

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SLIDE 102
CONFERENCE 25 / CASE I – 01-A-89 (AFIP 2890553)

Signalment: Seventeen year old, female, rhesus macaque, *Macaca mulatta*, nonhuman primate.

History: This animal presented to necropsy as part of a terminal research project. A semi-purified diet had been fed from birth. Chronic, intermittent episodes of vomiting were reported. She was deeply anesthetized and exsanguinated via the distal aorta. A hyperosmolar paraformaldehyde/glutaraldehyde solution was administered via the left ventricle.

Gross Pathology: The distal ten cm of the esophagus were flaccid and dilated to 2.5 to 3 cm. The esophageal mucosa was mildly to moderately thickened throughout its length.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Esophagus: Esophagitis, proliferative, chronic-active, lymphoplasmacytic, eosinophilic, diffuse with gastric and intestinal metaplasia (Barrett's esophagus), glandular abscesses, hyperplasia of the muscularis mucosa and erosions.

Contributor's Comment: The esophageal mucosa is thickened and markedly hyperplastic with prominent downgrowth of epithelial cords. The stratified squamous epithelium of the distal portion of the cords is extensively replaced by glandular

epithelium characterized by columnar cells that resemble either intestinal absorptive cells or goblet cells. There is multifocal single cell necrosis of the glandular lining cells. The glands are frequently dilated and contain neutrophils and/or karyorrhectic debris. In other areas, the surface esophageal mucosa is completely replaced by columnar epithelium that resembles gastric foveolar cells, rarely with parietal cells admixed (not present in all sections). High numbers of plasma cells admixed with fewer lymphocytes, eosinophils, macrophages and neutrophils expand the superficial lamina propria and multifocally traverse the overlying surface epithelium and the glandular epithelium. Multifocally, the mucosa is eroded and there is luminal exudation of neutrophils. The muscularis mucosa is multifocally hyperplastic. The vasculature is diffusely, moderately ectatic.

Barrett's esophagus in humans is generally defined as the presence of metaplastic specialized columnar epithelium occurring in the distal esophagus. Typically, the metaplastic epithelium contains a mix of gastric cardia-type mucosa and intestinal goblet cells.[1,2,3] Incomplete intestinal metaplasia is most often seen in Barrett's esophagus and is characterized by goblet cell metaplasia. Less common is complete intestinal metaplasia or the added presence of intestinal absorptive cells.[2,3] Most cell types found in the gastric and intestinal mucosa (Paneth cells, goblet cells, parietal cells, chief cells, small intestinal absorptive cells, and gastric foveolar cells) may occur in Barrett's esophagus.[3]

Barrett's esophagus in humans is a sequel of chronic gastroesophageal reflux. Although the pathogenesis is unclear, it is thought to be a protective metaplastic response to prolonged mucosal injury. It is of particular clinical concern in humans because it is associated with a significantly increased risk of development of esophageal adenocarcinoma[1,3].

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AFIP Diagnoses:
1. Esophagus: Squamous metaplasia of submucosal ducts, focal, marked, with mild lymphoplasmacytic, histiocytic, and eosinophilic esophagitis, and marked smooth muscle hyperplasia, rhesus macaque, nonhuman primate.
2. Esophagus: Intestinal metaplasia, focal, marked.

Conference Comment: This case was reviewed in consultation with the Department of Gastrointestinal Pathology at the Armed Forces Institute of Pathology. There was variation in submitted slides, with two distinct presentations in the slides presented in conference.

The first presentation is that of squamous metaplasia of the esophageal submucosal ducts and pronounced hypertrophy of the muscularis mucosa. This is consistent with a condition in humans called pseudodiverticulosis. The squamous metaplasia leads to blockage of outflow, causing cystic dilation of the glands with atrophy. If extensive, these can resemble diverticula, hence the name. The Department of Gastrointestinal

Pathology noted that, in humans, these are not usually diagnosed with biopsy. These intramural lesions can be seen grossly, and are diagnosed endoscopically or radiographically.[4]

The second presentation is also that of pseudodiverticulosis, but with an abrupt transition to intestinal metaplasia with numerous goblet cells characteristic of Barrett's esophagus. The presence of goblet cells is reported to be the most useful feature for diagnosis of Barrett's esophagus since these are not normally present in the gastric mucosa. The two major components of Barrett's esophagus are metaplasia of the surface and pit epithelium and metaplasia of the mucous glands. The surface and pit epithelium may be lined by a combination of goblet and columnar cells. The mucous glands are usually composed of pure mucous cells, but may also contain parietal cells, Paneth cells, endocrine cells, or pancreatic acinar cells.[4]

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

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SLIDE 103
CONFERENCE 25 / CASE II – AR02-373 (AFIP 2888365)

Signalment: 5-month-old male nu/nu mouse (*Mus musculus*).

History: During a 2 week period, four 18-week-old male outbred *nu/nu* mice were found to be tachypneic, lethargic, hunched, and hypothermic, with varying degrees of abdominal distension. The mice were group housed in separate microisolator cages on ventilated racks with corn cob bedding in a barrier facility. These animals were part of a cancer study and had recently received subcutaneous flank injections of glioblastoma cells. No drug treatment had yet been given. Previous clinical history included several incidences of fighting among cage mates resulting in multifocal superficial puncture wounds of the caudal dorsum. At time of presentation the prognosis was poor and euthanasia was elected.

Gross Pathology: An adult male nude mouse in slightly thin body condition was examined. Weight at necropsy was 22.2 grams. No postmortem changes were evident. Diffusely the subcutis was expanded by abundant clear gelatinous material (edema) (Fig. 1), and the thoracic and abdominal cavities each contained ~0.5ml of clear, red-tinged fluid. The pancreatic lobules were markedly separated by clear fluid. Both kidneys were pale brown with a finely granular surface. Ingesta was present in the stomach. No masses were evident on either flank.

Gross Morphologic Diagnoses**:**
　　Nephropathy, Bilateral, Chronic, Moderate
　　Anasarca

Histologic Findings:
Kidney: The glomerular capillary tufts are segmentally expanded by abundant homogenous, pale eosinophilic infiltrate which is uniformly populated by deeply basophilic, pleomorphic, angular, and karyorrhectic nuclei. Bowman's capsule contains cells with similar, but distinctly vesicular, nuclei and pale amphophilic cytoplasm. Each corpuscle is circumferentially delineated by 2-5 layers of fibrous connective tissue. The cortical and medullary interstitium is diffusely hypercellular owing to large numbers of lymphocytes and plasma cells with occasional mast cells. Congo red stain for amyloid is negative.

Ultrastructural Findings: (Fig. 2)
Transmission electron microscopy revealed segmental expansion of the glomerular basement membrane and mesangium by large deposits of medium electron dense material containing linear arrays of denser fibrils. These fibrils were arranged in packets of long, wavy, roughly parallel rows suggestive of membranous lamellae, but lacked consistent periodicity. Occasional podocyte foot processes were blunted and fused.

Laboratory Results: None reported.

Contributor's Morphologic Diagnosis: Membranous Glomerulonephropathy, Diffuse, Chronic, Severe with Periglomerular Fibrosis and Moderate Subacute Lymphoplasmacytic Interstitial Nephritis, Kidney.

Contributor's Comment: This condition has previously been documented as a sporadic finding in athymic nude mice and associated with circulating antinuclear antibody.[1] In general, two types of immune-mediated glomerulonephritis are recognized, and classification is based on the immunologically perceived antigen. In immune complex glomerulonephritis, antigen-antibody complexes are passively deposited in the glomeruli. Conversely, autoimmune glomerulonephritis involves active binding of antibody to renal antigens, such as basement membrane or mesangial cells.[2] The findings of Pelletier et al suggest that the lesion in this case is secondary to deposition of circulating autoantigen-autoantibody complexes. The role of experimental injection of tumor cells in this case is unclear, but tumor-associated inflammation may have contributed to the pathogenesis.

AFIP Diagnosis: Kidney: Glomerulonephritis, membranous, global, diffuse, severe, with tubular ectasia and protein casts, nu/nu mouse, rodent.

Transmission electron micrograph: Glomerulonephritis, membranous, with intramembranous irregularly arranged, electron dense fibrils.

Conference Comment: Conference attendees discussed the ultrastructural appearance of different types of glomerular deposits. Amyloid deposits are characterized by irregular, non-branching fibrils that are 10-15nm in diameter.[4] Collagen consists of well-organized fibrils with periodic cross-banding. The width, periodicity, and arrangement of the fibrils depend on the type of collagen.[3] Immune complexes consist of electron dense granules within or on either side of the glomerular basement membrane.[4] While the appearance of the immune complexes in this case consisting of well-structured, parallel fibers is unusual, it is interesting to note that this pattern of glomerular deposits has also been reported in humans with systemic lupus erythematosus.[2,3]

Contributor: Wake Forest University School of Medicine
http://www.wfubmc.edu

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SLIDE 104
CONFERENCE 25 / CASE III – 01-2452 (AFIP 2888758)

Signalment: Porcine, mix breed, 36 day old, intact male.

History: Caesarian-derived, gnotobiotically maintained piglets were experimentally inoculated on day 1 of life with 4.3×10^6 units of infectious porcine circovirus-2 (PCV-2) by oral-nasal route[2]. Piglets were given 50 mg/kg/days 1-5 and 25 mg/kg/days 6 through termination of cyclosporin (Neoral ®) *per os*. This piglet was normal until day

35 of life at which time he became lethargic and icteric. He was afebrile. The pig was euthanatized on day 36 of life.

Gross Pathology: In addition to icterus, the liver was small and ascites was present.

Laboratory Results: Compared with virus infected non-cyclosporin-treated controls, virus infected piglets treated with cyclosporin had significant lymphopenia and hypoproteinemia which was characterized by lowered albumin alpha and beta-globulin and absence of gamma-globulin.

Contributor's Morphologic Diagnosis: Liver: Marked widespread to diffuse subacute hepatocellular necrosis with hepatocellular loss.

Contributor's Comment: This experiment was conducted in order to determine the effects of immunosuppression on the pathogenesis of PCV-2 infection in piglets, and the role of the immune response in lesion development[1]. PCV-2 is the suspected cause of Porcine Multisystemic Wasting Syndrome. Infection of cyclosporin-treated gnotobiotic piglets resulted in diffuse hepatocellular necrosis with minimal inflammatory response. There was no hepatic necrosis in piglets given virus alone. Significant amounts of virus were present in livers of PCV-2 infected, cyclosporin-treated piglets compared with PCV-2 infected, untreated piglets. Hepatocytes and Kupffer cells contained both intranuclear and intracytoplasmic viral antigen. Intracytoplasmic inclusion bodies in Kupffer cells in these slides are very difficult to distinguish from phagocytized debris. In non-immunosuppressed piglets, PCV-2 virus accumulates in the cytoplasm of macrophages, mononuclear cells, and histiocytes, disseminates via monocytes and serum/plasma viremia, causes minimal or no overt viral cytopathic effects, and induces angiocentric granulomatous inflammatory cell infiltrates that can lead to organ failure[2]. Viral antigen is rarely detected in hepatocytes in viral infected non-immunosuppressed piglets[2]. It is suspected that immunosuppression of these piglets by cyclosporin allowed for an increased, sustained viremia with infection of Kupffer cells, and secondarily, of hepatocytes. The lack of significant inflammatory response to the infection of the liver suggests that virus infection of hepatocytes overwhelmed cell function, leading to cell death[1].

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AFIP Diagnosis: Liver: Hepatocellular degeneration, necrosis, and loss, diffuse, with stromal collapse and cholestasis, mixed breed, porcine.

Conference Comment: The differential diagnosis for hepatic necrosis discussed by conference attendees included hepatosis dietetica, aflatoxicosis, cocklebur intoxication, gossypol intoxication, pyrrolizidine alkaloid toxicity, and porcine circovirus-2 (PCV-2) infection. Although there was not striking biliary hyperplasia, some attendees preferred a toxic etiology as they considered the large vacuolated cells to represent megalocytosis. After discussion, it was concluded that these large vacuolated cells probably represent a degenerative change.

Postweaning multisystemic wasting syndrome (PMWS) causes generalized lymphadenopathy, hepatitis, nephritis, and pneumonia in piglets. It is reported that immune activation is a key component in the pathogenesis of PCV-2-associated PMWS in pigs. Coinfection with other pathogens, such as porcine parvovirus and porcine reproductive and respiratory syndrome virus (arterivirus), have been found to be required to cause clinical PCV-2 disease in gnotobiotic piglets.[1,3,4] Other exacerbating factors include overcrowding, co-mingling of age groups, and other stressors.[3]

Typical gross findings include marked systemic lymphadenopathy, hepatitis with icterus and edema, and firm, mottled lungs that fail to collapse. Histologically, there is lymphoid depletion with infiltration by histiocytes and multinucleated giant cells with intensely basophilic botryoid intranuclear inclusion bodies. There is also granulomatous hepatitis, interstitial pneumonia, and nephritis.[3]

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http://www.vet.ohio-state.edu/level2/depart/depart.html

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SLIDE 105
CONFERENCE 25 / CASE IV – 30927 (AFIP 2910190)

Signalment: Adult, male African green monkey (*Chlorocebus aethiops*), nonhuman primate.

History: This 6.9 kg African green monkey was exposed to a lethal dose of ricin toxin by inhalation*. The animal died approximately 72 hours postexposure. The body presented for necropsy is that of an adult, male African green monkey (*Chlorocebus aethiops*). The carcass is in good body condition with adequate subcutaneous and cavitary fat. The pelage is within normal limits.

* 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.

Gross Pathology: Gross examination reveals a missing left index finger. There is approximately 30-60 ml of serosanguineous pleural effusion in the thoracic cavity, and the lungs are moderately congested, hemorrhagic, and edematous. (Figs. 1, 2) The stomach contains a small amount of ingesta. There is scant material in the small intestine, with normal-appearing fecal material within the colon and rectum. The gall bladder is full of bile and the urinary bladder contains urine.

Gross diagnoses:
1. Lung: Hemorrhage, congestion, and edema, diffuse, moderate, with serosanguineous pleural effusion.
2. Left hand: Missing digit #2 (index finger).

Laboratory Results: None reported.

Contributor's Morphologic Diagnoses:
Microscopic diagnoses:
1. Lung: Pneumonia, fibrinohemorrhagic, acute to subacute, diffuse, moderate, with edema, and multifocal septal necrosis, vasculitis, and fibrinous pleuritis, African green monkey (*Chlorocebus aethiops*), nonhuman primate.

The following sections are not provided, but diagnoses are included for completeness:
2. Mediastinum: Mediastinitis, neutrophilic and histiocytic, multifocal, moderate, with hemorrhage, fibrin and edema.
3. Lymph node, tracheobronchial: Lymphadenitis, neutrophilic, acute, multifocal, mild, with draining hemorrhage and edema.
4. Heart, left ventricle: Myocarditis, acute to subacute, multifocal, mild, with myocyte necrosis.

Contributor's Comment**:** Ricin is a highly toxic protein derived from the bean of the castor oil plant, *Ricinus communis*, composed of two polypeptide chains joined by a disulfide bond. The active A chain is the toxic moiety, and the B chain is the binding lectin moiety. Rapid uptake of the toxin occurs after B chain binding to glycoprotein cell-surface receptors.[3] Transport of the A chain moiety to cellular ribosomes results in catalytic inhibition of protein synthesis by inactivation of the 28s ribosomal subunit. Toxicity is dependent on dose and route of exposure.[1]

Ricin has been used as a weapon of terrorism and assassination and is considered a potential biological warfare threat agent to military operations.[2] Aerosol ricin exposure conducted in rats and rhesus monkeys results in signs of respiratory embarrassment within 8-24 hours depending on exposure dose. Grossly, lesions are confined to the respiratory tract and consist of serous to serosanguineous fluid within the thoracic cavity. Lungs are edematous and heavy, do not collapse, and are mottled red and

purple. Fibrin strands are occasionally attached to pleural surfaces. Histologically, there is multifocal to coalescing areas of intra-alveolar fibrin, edema, and hemorrhage, acute alveolitis, and necrosis of lower respiratory tract epithelium.[3]

Although it is suspected that ricin-induced pulmonary edema is due to increased pulmonary capillary endothelium permeability or 'vascular leak syndrome', the specific mechanism by which inhaled ricin crosses the respiratory epithelium to injure the vascular endothelium has not yet been determined.[3]

In this study, the pulmonary lesions and the related hemorrhage and inflammation in draining lymph nodes and mediastinum are consistent with reported inhalation ricin toxin exposure. The lesions in the draining lymphatics and surrounding tissues may have been induced by transported intact ricin molecules detached from cells, or ricin-stimulated effector cells or inflammatory cytokines.[3] The mild inflammation also seen in the heart is possibly related to cardiopulmonary insufficiency induced by the toxin.

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

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AFIP Diagnosis: Lung: Pneumonia, interstitial, necrotizing, acute, diffuse, severe, with hemorrhage, fibrin, and edema, African green monkey (*Chlorocebus aethiops*), nonhuman primate.

Conference Comment: The contributor gives a concise overview of ricin toxicosis. While all parts of the castor bean plant are toxic, the beans contain the most ricin and must be crushed or broken to release the toxic component. Ricin is 100 times more toxic parenterally than orally. Ingestion causes vomiting, abdominal pain, diarrhea, and gastrointestinal hemorrhage. After parenteral administration, ricin causes hemorrhage and necrosis in the heart, stomach, lungs, liver, kidneys, and pancreas.[5]

Two other toxic causes of pulmonary edema were discussed during the conference. Oxygen toxicity causes damage to type I pneumocytes and capillary endothelium, with hyaline membrane formation from cellular debris and proteinaceous exudate.[4] Paraquat poisoning is characterized acutely by hemorrhage, hyaline membrane formation, and loss of pneumocytes. With chronic insult, diffuse interstitial and intraalveolar fibrosis and type II pneumocyte hyperplasia develop.[4]

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