The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2005-2006

SLIDE 1
CONFERENCE 1 / CASE I – 04W10854 (AFIP 2979950)

Signalment: Adult male mule deer (*Odocoileus hemionus*).

History: A blind alert deer was found in southeastern Wyoming in October by several hunters. The animal was reluctant to stand and when it did so it stumbled into trees. They shot it in the chest. A fresh carcass was submitted for examination.

Gross Pathology: Gross examination revealed a well-nourished adult (~3 - 5 year old) buck deer with bilateral corneal opacity. Internal organs were unremarkable.

Laboratory Results: *Yersinia pestis* was cultured from conjunctival sac and cornea. Retropharyngeal lymph nodes were positive for chronic wasting disease using a proprietary ELISA test. Samples of liver and lung were cultured aerobically, with negative results.

Contributor's Morphologic Diagnosis: Anterior segment, eye: 1. Severe acute diffuse endophthalmitis with myriad intracellular and extracellular coccobacilli.
2. Severe acute diffuse stromal keratitis with intralesional coccobacilli, limbal neovascularization, and non-occlusive fibrinous thrombosis.
3. Retinal detachment, with acute bacterial retinitis.

Contributor's Comment: The mule deer had bilateral ocular infection due to *Yersinia pestis*. In addition to these changes there were acute necrotizing inflammatory lesions in lung, adrenals, lymph node, and liver with intralesional bacteria, and disseminated intravascular coagulation.

Plague is unusual in big game animals and ungulates are generally considered resistant to the disease. There is a published report of plague in a free-ranging mule deer in Wyoming,[1] an unpublished, laboratory-confirmed case in a mule deer in Montana,[2] and bilateral plague-associated necrotizing panophthalmitis in a black-tailed deer in California.[3] Ocular plague has been seen in Colorado (Dr. M. Miller, Colorado Division of Wildlife, unpublished observations).

Numerous organisms are present in the anterior and posterior chambers, in the drainage angle, and between the pigmented epithelial cells at the posterior aspect of the iris. Unilateral plague endophthalmitis without evidence of disseminated disease was

reported in a man who had recently handled poisoned rats.[4] He was treated successfully and with no loss of vision by intravenous and subconjunctival antibiotics.

Plague is an endemic disease of rodents in the western United States, with occasional spread into human and non-rodent populations via enzootic or amplification hosts. In addition to virulence factors common to the yersiniae that promote tissue invasion and resistance to successful phagocytosis, *Y. pestis* has additional virulence factors. These include pesticin, which serves as a plasminogen activator, murine toxin, and F_1- toxin, which forms a gel-like envelope around cells that protects them from phagocytosis. The florid inflammatory and necrotizing lesions present in this animal's eyes are presumably a consequence of these factors.

The animal's retropharyngeal lymph nodes were positive for chronic wasting disease. No lesions were present in the brain and the animal was in good nutritional condition. As CWD is common in mule deer in southeastern Wyoming, this may be an incidental finding.

AFIP Diagnosis: Eye: Endophthalmitis, suppurative, severe, with myriad coccobacilli, and moderate stromal keratitis, mule deer (*Odocoileus hemionus*), cervid.

Conference Comment: Conference attendees briefly reviewed the anatomy of the eye including the layers of the retina. Discussion was directed at developing a differential diagnosis for large, plaque-like colonies of bacteria. Residents at AFIP utilize the mnemonic "YACS" to develop a differential diagnosis when large colonies of bacteria are present in hematoxylin and eosin stained sections.
YACS stands for:

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

Attendees eliminated *Actinomyces sp.* and *Actinobacillus sp.* from the differential diagnosis based on the bacteria's morphology. *Actinobacillus sp.* is a Gram negative rod and *Actinomyces sp.* is a Gram positive rod which forms branching filaments. Gram stains were then used to eliminate *Staphylococcus sp*., *Streptococcus sp*., *Arcanobacter sp*., *Corynebacteria sp.* and *Clostridia sp*., all Gram positive bacteria, from the differential diagnosis. The only remaining bacterium from the differential diagnosis list was *Yersinia sp*., a Gram negative coccobacillus.

The contributor provides a concise summary of the virulence factors associated with *Y. pestis* infection. *Yersinia pestis*, the bacterium which causes "plague", is transmitted primarily by fleas and less commonly by the consumption of animals or inhalation of aerosolized droplets from animals with the pneumonic form of the disease. The three clinical manifestations of "plague" are the bubonic form, commonly associated with

swollen, ruptured and draining inguinal and axillary lymph nodes or “bubos”; the rapidly fatal pneumonic form and the septicemic form.

Participants briefly discussed *Y. pestis* as a potential biological weapon. The Centers for Disease Control (CDC) lists *Y. pestis* as a Category A agent. Category A agents have the greatest potential for inflicting high numbers of human casualties, can be manufactured and disseminated on a large scale, require significant efforts in public health preparedness, and are most familiar to the public.

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SLIDE 2
CONFERENCE 1 / CASE II – 1 (AFIP 2987474)

Signalment: 24-year-old male castrated Selle Français horse (*Equus caballus*)

History: The owner noticed blood droplets in the preputial opening of this horse and sent him to the veterinary hospital in Berne, Switzerland. The horse was in good body condition. He presented with a multilobulated, cauliflower-shaped, about 10 cm in diameter, partially ulcerated nodule on the penis (preputium). The clinical suspected diagnosis was a squamous cell carcinoma.

Gross Pathology: Provided.

Histopathologic Description: The subepithelial mucosa is expanded by numerous confluent granulomatous inflammatory foci that contain multiple tangential and cross sections of well preserved larval and adult nematodes and cellular debris surrounded by epithelioid macrophages, multinucleated giant cells and, at the periphery, by moderate numbers of plasma cells and lymphocytes admixed with fewer eosinophils. The nematodes are 10 to 25 μm in diameter with a smooth cuticle, platymyarian-meromyarian musculature, a long rhabditiform esophagus with terminal bulb, numerous deeply basophilic 2-3 μm internal structures, tubular digestive tract lined by low cuboidal

epithelium, tapered tail and pseudocoelom that are often visible. The nematodes are interpreted as *Halicephalobus gingivalis*.

Contributor's Morphologic Diagnosis: Posthitis, nodular to diffuse, severe, chronic, granulomatous, with numerous larval and adult nematodes, etiology consistent with *Halicephalobus gingivalis*, Selle Français, equine.

Contributor's Comment: Halicephalobus gingivalis (Micronema deletrix) has been sporadically associated with equine infections. The organism has been reported in human infections and has also been the cause of extensive encephalitis in a bighorn sheep. Halicephalobus infection has been reported in Japan, Egypt, Switzerland, the Netherlands, the United Kingdom, Colombia, and the United States. Inflammation of the central nervous system is a consistent feature of infection in human beings and almost always occurs in equine infections (1, 4, 8, 11, 12). Vulnerable organs include brain (1) and spinal cord (4), optic nerve (9), lungs, skin, kidneys, maxilla, nasal cavities, bone and joints, prepuce and testicles (10). The brain is the most commonly involved tissue, followed in descending order by the kidneys, oral and nasal tissues, lymph nodes, lungs, spinal cord, and adrenal gland. There are also reports of involvement in the heart, liver, stomach, ganglion, bone, and prepuce. (2: Dunn DG, et al, 1993).

Halicephalobus is a parasite of which, to date, only females have been identified. This order of nematode (Rhabditida) can be found free-living in soil and humus. To date, identification of Halicephalobus in soil or other environmental samples has required labour-intensive microscopic identification of small adult nematodes present in the material (6: Nadler et al. 2003). Infections have been postulated to be associated with skin wounds. Of interest here is that viable organisms were noted in the sperm and urine of 2 stallions (5: Kinde H et al, 2000), suggesting the possibility of a urogenital route of infection. There are eight members of the genus, all of which are free-living saprophytes found in soil and decaying organic matter, except for H. gingivalis (7).

The identification of the parasite within tissues is based on the following criteria: a smooth, thin cuticle; platymyarian-meromyarian musculature; a pseudocoelom; a rhabditiform esophagus composed of a corpus, isthmus, and bulb; an intestinal tract composed of single nucleated, low cuboidal cells; flexed ovary and uterus; and a tapered tail. The flexed ovary and pointed tail differentiate this organism from other rhabditiforms.
Other rhabditid parasites infecting the horse include Pelodera strongyloides, Strongyloides westeri and Cephalobus sp. These nematodes must be differentiated from H. gingivalis in verminous cutaneous or mucocutaneous lesions. Differentiation is based upon location and severity of lesions and parasite morphology. Pelodera causes a self-limiting dermatitis normally confined to the ventral abdomen and limbs. The life-cycle of Strongyloides westeri involves cutaneous penetration by larvae; adults and eggs are not found in the skin. Cephalobus sp. can be distinguished from H gingivalis by its blunt posterior end and differences in the shape of the stoma and esophagus (Cephalobus has a greater ratio of corpus to isthmus).

Other causes of equine verminous encephalitis may include Hypoderma bovis, Hypoderma lineatum, Strongylus vulgaris, and Draschia megastoma. Setaria spp. are reported to be a common cause of cerebrospinal nematodiasis in horses in Asia. Other causes of ataxia in horses include trauma, degenerative myelopathy, Wobbler's syndrome, neoplasia, and various infectious agents. Differential diagnoses include also other infectious causes of granulomatous inflammation in skeletal, urinary, or central nervous system.

The pathogenesis, life-cycle, and route of infection of H. gingivalis are poorly understood. The route of infection in humans seems to be from manure contaminated skin lacerations. Speculated routes of infection in the horse include: skin and mucous membrane penetration in recumbent animals by the free-living form of the parasite in the soil with subsequent invasion of the sinuses and bones of the head, and/or hematogenous spread to internal parenchymal tissues; prenatal or transmammary infection of suckling foals (13: Wilkins P.A. 2001). The nematode gains access to the brain via migration along vessels; continued migration within the brain causes necrosis and inflammation.

The typical clinical findings in horses include: lethargy, loss of condition, hyperesthesia, opisthotonos, nystagmus, ataxia, progressive neurologic signs, recumbency, and multiorgan dysfunction. Hyperglobulinemia and hyperfibrinogenemia (chronic inflammation); elevated creatine kinase (muscle injury from parasites, trauma, or recumbency); elevated creatinine (postrenal obstruction due to lower motor neuron disease if cauda equina neuritis). Abnormal clinical pathology values and clinical tests depending on organs affected and extent of disseminated lesions.

The typical gross findings are multifocal to diffuse, proliferative, firm, gray-white, granulomatous lesions.

The typical light microscopic findings include: Adult females, 15-20 mm in diameter, 250-430 mm in length, with a thin, smooth cuticle and tapered, pointed tail; platymyarian-meromyarian musculature; a pseudocoelom; a rhabditiform esophagus composed of a corpus, isthmus, and bulb; an intestinal tract lined by uninucleate, low cuboidal cells; and a single genital tube, with a dorsoflexed ovary and a ventroflexed uterus at the vulva.
Larvae, approximately 10 mm diameter, with a rhabditiform esophagus and tapered, pointed tail. Embryonated eggs are oval and average 15x35 mm.
Perivascular granulomatous inflammation with numerous adult and larval parasites; necrosis, vasculitis, and hemorrhage.
Normal tissue architecture may be replaced by dense collagen and fibroblasts, with infiltration of tissue by lymphocytes, plasma cells, epithelioid macrophages, multinucleate giant cells, eosinophils, and intralesional adult and larval nematodes.

H. gingivalis disseminated disease has been reported in a Grevy's zebra (Equus grevyi) (3).

AFIP Diagnosis: Prepuce (per contributor): Posthitis, granulomatous, nodular, severe, with numerous rhabditid adults, larvae, and eggs, etiology consistent with *Halicephalobus sp*., Selle Français, equine.

Conference Comment: Conference attendees briefly reviewed the histomorphological characteristics of metazoan parasites. Metazoan parasites can be broken down into six morphologically distinct categories: nematodes, acanthocephalans, trematodes, cestodes, arthropods and pentastomes. Below is a simple table to identify the parasite to its group:

GROUP	GENERAL SHAPE	BODY CAVITY	DIGESTIVE TRACT	STRIATED MUSCLE	SPECIAL DIAGNOSTIC FEATURES
Cestode	Flattened dorso-ventrally	--	--	--	1. calcareous corpuscles 2. scolex 3. tegument
Trematode	Flattened dorso-ventrally	--	+	--	1. suckers 2. tegument 3. blind ceca 4. yolk gland 5. hermaphroditic (except Schistosomes)
Acanthocephalan	Spherical in section, flat when viable	+	--	--	1. hypodermis 2. lemniscus 3. two muscle layers 4. proboscis
Nematode	Spherical	+	+	--	1. cuticle 2. musculature
Arthropod	Tend to be spherical	+	+	+	1. chitinized exoskeleton 2. jointed appendages 3. tracheal tubes
Pentastomes	Spherical	+	+	+	1. chitinized exoskeleton 2. digestive glands 3. sclerotized openings

Once the organism has been identified as a nematode, it must be further classified into one of the following groups: Aphasmids or Phasmids. Aphasmids lack a tiny pair of sensory papillae (the phasmids) on the caudal end; however, these are not readily identifiable in histologic sections. The histologic features that distinguish them from phasmid nematodes are hypodermal bands with associated nuclei, and prominent esophageal glands that form a stichosome. The Phasmids consist of the Rhabditoids, Oxyurids, Ascarids, Strongyles, Spirurids, and Filarids. Both the Rhabditoids and Oxyurids have a rhabditoid esophagus composed of a corpus, isthmus and bulb. The Strongyles have a cuticle, which occasionally is ridged, and all have an intestine composed of few multinucleated cells and a prominent brush border. Spirurids can be very diverse, but all adult females in this group produce embryonated eggs. Filarial nematodes are small and can produce either eggs or free, distinctive larvae; microfilariae.

This case was reviewed in consultation with Dr. Chris Gardiner, AFIP consultant in veterinary parasitology. Dr. Gardiner adds," The reaction is what we would expect in

cutaneous halicephalobiasis. The organisms are plentiful and are described well by the contributor. I would only offer this note. I'm not so sure that the species in question is *gingivalis.* I have, for a long time, suspected more than one species is involved in these cases. And, there have been lots of cases. The key in differentiating this parasite from others in histological section, is the characteristic rhabditiform esophagus and the dorsal reflection of the ovary….both can be seen readily in the section. The other parasites mentioned in the differential do not have these characteristics." Dr. Gardiner also comments on other cases where viable organisms were found in sperm, "… it leaves speculation if the infection can be passed by artificial insemination. I wonder if the parasites become "dormant" when frozen down with the sperm, and then become viable when the sperm is brought to room temperature."

The contributor provides a thorough description of the pathogenesis, clinical, gross and histologic lesions associated with *Halicephalobus* infection as well as the nematode's distinguishing morphologic features and an extensive differential diagnosis list. Interestingly, adult females are parthenogenetic and thus can produce viable larvae without the necessity of mating which can result in large numbers of larvae as are seen in this case. We are grateful to Dr. Gardiner for his comments on this interesting case.

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SLIDE 3
CONFERENCE 1 / CASE III – 05-255 (AFIP 2984136)

Signalment: 10 year-old, intact female Barn owl (*Tyto alba)*

History: This ten year-old Barn owl had the right wing amputated six years prior. The owl presented with a large growth at the amputation site. The animal died acutely soon after presentation.

Gross Pathology: Necropsy revealed an amputation site at the humeral-ulnar joint of the right wing. Along the medial aspect of the humerus there was a 3.5cm x 3cm x 2cm white-grey raised mass which contained a 1cm x 1.5cm firm red central ulcerated region exuding tan white material.

Laboratory Results: Cultures of the bone consisted of 30 colonies of *E. coli*, >100 colonies *Proteus mirabilis* and approximately 50 colonies of Enterococcus.

Histopathologic Description: Humeral bone: Most of the normal architecture of the humerus is obliterated by the presence of a proliferation of pleomorphic epithelial cells arranged in papillary projections, tubules and, less commonly, solid sheets. The cells have moderate amounts of lightly eosinophilic cytoplasm and large vesicular nuclei, often with a single nucleolus. There is little anisocytosis and anisokaryosis. Mitotic activity is rare. Epithelioid macrophages, some of which contain hemosiderin, are accompanied by necrotic bone, congested vasculature and hemorrhage.

Contributor's Morphologic Diagnosis: Humerus: Air Sac Adenocarcinoma.

Contributor's Comment: Neoplasms in barn owls are infrequent, although proventricular adenocarcinoma, papillary carcinoma of the thyroid gland, feather folliculoma, mucinous adenocarcinoma of the tongue, lymphoid neoplasia and cutaneous mast cell tumors have been reported (1,2). The histologic appearance of the

humerus is consistent with an air sac adenocarcinoma. There is no other report of this tumor in barn owls or other raptors. A humeral mucinous adenocarcinoma of the air sacs has been reported in a salmon-crested cockatoo (*Cacatua moluccensis*) (1).

The air sacs are extrapulmonary extensions of the parabronchi that assist in air movement but not in gaseous exchange. They are lined by a single layer of epithelium supported by mesothelial serosa. Involvement of the air sacs in primary neoplasia is uncommon.

AFIP Diagnosis: Bone, humerus (per contributor): Adenocarcinoma, papillary, favor air sac origin, barn owl (*Tyto alba*), avian.

Conference Comment: Conference attendees generated considerable discussion on the origin of this neoplasm and what role the previous surgery may have had on the presence of air sac epithelium, bone and keratinizing epithelium (skin) being present in the same section. There is speculation that the lamellations of keratin were produced by a portion of squamous epithelium which invaginated into the healing surgical site possibly creating a focus of continuing keratinization. Gram-positive bacterial colonies are multifocally scattered throughout the keratin suggesting a nidus of infection within the neoplasm.

We believe this to be the same case as reported in reference #1; more can be read about it there as well. Of note, a similar case involving the humerus of a Moluccan cockatoo was presented as a Wednesday Slide Conference case in 2002. Interested parties are encouraged to review slide 53, conference 14 from the 2002-2003 slide set or to review the case on-line by visiting the Wednesday Slide Conference Results page and clicking on the appropriate year, conference, and slide.

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SLIDE 4

CONFERENCE 1 / CASE IV – 04135 (AFIP 2983613)

Signalment: 16 year old, male *Macaca fascicularis*

History: This wild caught Indonesian cynomolgus macaque was found reluctant to move and non-weight bearing on the left leg 16 months after arrival. Despite supportive treatment, it was found dead on the following day.

Gross Pathology: Purulent osteoarthritis accounted for the lameness, involving the left tibiotarsal and stifle joint. Multiple variably sized abscesses were present in the liver and kidneys which contained pale greenish-yellow, opaque, viscous fluid. The left seminal vesicle was approximately twice normal size and similar fluid exuded the cut surface. The bladder contained 2 ml of similar fluid.

Laboratory Results: A pure growth of *Burkholderia pseudomallei* was cultured from the bladder, tibiotarsal joint, and liver.

Histopathologic Description: Examined is a section of seminal vesicle which has largely undergone abscessation. Abundant neutrophils, many degenerate, eosinophilic granular material, pyknotic nuclei, and finely granular basophilic debris fill the vesicular lumina. The epithelial lining is variably effaced and replaced by this suppurative inflammatory exudate which affects all lobules. Up to 90% of the epithelium is lost with few remaining recognizable tubular structures lined by cuboidal epithelial cells. Interlobular connective tissue is expanded by edema, finely fibrillar eosinophilic material (fibrin), and neutrophils.

Contributor's Morphologic Diagnosis: Seminal Vesiculitis, Necrosuppurative, Diffuse, Subacute, Severe

Contributor's Comment: Melioidosis is an infectious disease caused by the motile, gram-negative environmental bacterium *Burkholderia pseudomallei.* The disease is generally restricted to tropical areas between latitudes 20˚N and 20˚S, predominantly in Southeast Asia and Northern Australia. The organism is a saprophyte that can be isolated from soil and muddy water in endemic areas (1). Inhalation, ingestion and inoculation are recognized modes of acquisition for *B. pseudomallei* (2). Melioidosis has been reported in captive non-human primates, and in all cases the animals were imported from endemic areas with the exception of a chimpanzee that was thought to have acquired the infection in the laboratory (3,4,5,6,7). The time to development of overt disease ranged from 3 months to 10 years from the time of capture. Clinical signs were non-specific and varied depending on the primary system involved. The characteristic pathologic feature of melioidosis is abscessation of visceral organs, the subcutis, and regional lymph nodes (1). There is a high incidence of genitourinary infections in men in Australia, with prostatic abscesses occurring in 18% of males (2). The bacterium has a broad host range and melioidosis is commonly reported in sheep, goats and swine, and most mammals appear to be susceptible, although cattle, water buffalo, crocodiles, and birds are considered to be relatively resistant (8).

Melioidosis is considered an emerging disease with high impact on animals and humans. Due to the heightened awareness and threat of terrorist activity *Burkholderia pseudomallei* and *B. mallei*, the causative agent of glanders, have recently been classified as category B biological agents by the Centers for Disease Control and Prevention. Both organisms can be transmitted by aerosols, are naturally occurring in the environment in endemic regions, can be cultivated easily, and may cause severe, incapacitating or even fatal infections. Glanders and melioidosis have both been studied as potential agents of biological warfare, and *B. mallei* was used during both the first and second world wars (9).

AFIP Diagnosis: Seminal vesicle: Vesiculitis, suppurative, subacute, diffuse, severe, Cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: The contributor provides an excellent overview of melioidosis. Melioidosis affects several animal species as well as human beings and is commonly known as pseudoglanders due to its clinical and pathologic similarity to glanders. The lesions associated with pseudoglanders are typically granulomatous nodules with a caseous center often containing colonies of bacteria.[8] Although Gram negative, the coccobacilli are often difficult to identify in hematoxylin and eosin stained tissue sections. Methylene blue or Wright's stain can be used to help identify the bipolar morphology of the bacterium (9).

There are four clinical forms of melioidosis described in humans. The pulmonary form develops over one to two weeks following inhalation, and is characterized by non-specific symptoms of fever accompanied by sweating, rigor, cough, and chest-pain as well as ulcerative nodules within the nasal cavity. The septicemic form is characterized by several of the same symptoms and can rapidly culminate in multi-organ failure and death within 7-10 days. Local infections occur in cuts and abrasions and result in grey-white, firm and often ulcerated nodules surrounded by hemorrhage. Nodules can become caseous or calcified and local lymph nodes can become swollen. The chronic form is characterized by multi-systemic abscesses (9).

Glanders is primarily an infectious disease of horses which is also zoonotic and can be transmitted to carnivores through the ingestion of infected horse meat. Although the pathogenesis is poorly understood, it is believed that following ingestion, the bacteria penetrate the oropharyngeal or intestinal mucosa, enter and spread through the lymphatics to the blood and multiple organs. Ulcerative lesions within the nasal mucosa develop into a characteristic star-shaped scar. The lungs can similarly be affected and contain randomly dispersed, miliary, nodular granulomas. Farcy is the cutaneous form of the disease, characterized by subcutaneous nodules with thickened lymphatics most commonly occurring on the limbs and ventral abdomen (10).

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SLIDE 5
CONFERENCE 2 / CASE I – 04101477 (AFIP 2985398)

Signalment: 5-month-old, Jersey bull calf, *Bos taurus*

History: The animal was sick 3 months ago with pneumonia and high fever, with clinical improvement following antibiotic therapy. The animal began exhibiting clinical signs of CNS disease 4 days ago and was treated with antibiotics and Banamine. The animal has opisthotonus, increased salivation/foaming from the nose and mouth and is ataxic with a constant 104ºC temperature. No other animals on the farm are affected. The patient was euthanized for necropsy evaluation.

Gross Pathology: The animal was in good nutritional condition. Bilaterally, the hock, fetlock, and pastern joints were swollen. The cranioventral regions of the lung lobes were light yellow and distended by gas (emphysema). The caudodorsal regions were pale red/purple and palpably meaty with marked accentuation of lobular pattern by

edema of interlobular septa. The cut sections were diffusely dark red and wet. The hock, fetlock, and pastern joints contained moderate quantities of thick, stringy synovial fluid often admixed with white flakes. The synovial membrane of the right fetlock joint was prominent with edema. The renal cortices were diffusely wet with slightly raised bulging margins. There were no oral lesions observed.

Laboratory Results: PCR for MCF ovine herpesvirus-2 was positive. Pseudomonas sp. was cultured from the lungs and joints.

Histopathologic Description: Brain (cerebral hemisphere, hippocampus, brain stem and cerebellum) and spinal cord**:** The most conspicuous lesion in the brain sections is a severe vasculitis affecting predominantly meningeal blood vessels, but also affecting blood vessels that are embedded within deep regions of the brain. The vessel walls are expanded and effaced by intense infiltrates of macrophages, lymphocytes and plasma cells. The inflammatory cells are admixed with a coagulum of homogenous eosinophilic material and cellular debris that obscures recognition of the tunics of the blood vessels. The tunica intima is often indiscernible due to the intense cellular infiltrate, but when seen, the endothelium is markedly hypertrophied. Rarely there are thrombi. Occasionally, there are random aggregates of macrophages, plasma cells and lymphocytes present in the neuropil. Although the inflammation involves all regions of the brain, lesions are most severe in the cerebellum. Similar inflammatory foci are present in the spinal cord at all anatomical levels (cervical, thoracic and lumbar). The kidney, testicles and adrenal glands exhibit a severe vasculitis of similar character with inflammation extending into the surrounding parenchyma of these organs.

Contributor's Morphologic Diagnosis: Brain; Meningoencephalitis with vasculitis, necrotizing, lymphoplasmacytic, histiocytic, multifocal, chronic, severe

Contributor's Comment: The necrotizing vasculitis of predominantly medium-sized arteries of multiple organs suggested a presumptive diagnosis of malignant catarrhal fever (MCF). Other differentials included BVD and idiopathic vasculitis. The presumptive diagnosis of MCF was further confirmed by a positive PCR test for ovine herpesvirus-2.

Malignant catarrhal fever (MCF) is a highly fatal, sporadic disease affecting several ruminant species including cattle, bison and all species of Cervidae except fallow deer. There are two forms of disease, wildebeest (WA-MCF) and sheep-associated form (SA-MCF). The WA-MCF is caused by alcelaphine herpesvirus-1 (AHV-1, formerly bovine herpesvirus-3) while SA-MCF is caused by ovine herpesvirus-2. Both of these viruses are cell-associated lymphotropic viruses and belong to the subfamily gammaherpesvirinae and genus *Rhadinovirus.*

WA-MCF primarily occurs in Africa and occasionally in zoological parks while SA-MCF is seen throughout North America, Europe, and Australia. The principle reservoir for WA-MCF is the blue or white-bearded wildebeest in which AHV-1 is asymptomatic. Wildebeest calves become infected during the first 2-3 months of life and shed cell-free

AHV-1 in nasal and ocular secretions. Wildebeest are infected for life and transmit AHV-1 to their calves without showing clinical signs. The mucosa of the upper respiratory tract and/or tonsils is the most likely route of entry for AHV-1. Sheep are the reservoir for OHV-2, although goats can also be affected. Clinical signs of MCF are not observed in sheep and goats under natural conditions.

Both forms of disease can be transmitted with whole blood or lymphocytes but not cell-free filtrate. Viremia in WA-MCF usually starts about 7 days before the onset of fever and persists throughout the course of the disease. Virus replication occurs primarily in the lymphocytes, and the spleen and lymph nodes have high virus titers. There is wide variation in the presenting clinical syndromes; however, consistent signs include lymphadenomegaly with ocular and oral disease and exudative dermatitis. There is edema of the eyelids, conjunctivitis, corneal opacity with occasional hypopyon and congestion of nasal and buccal mucosa. Photophobia may be accompanied by extensive lacrimation. Nervous signs present in some cases include head pressing, hyperesthesia, trembling, nystagmus and incoordination. Gastroenteritis with and without bloody diarrhea may occur in acute cases, especially in deer. Laryngeal, pharyngeal and rarely the tracheal mucosa may develop erosions and ulcers that are also covered by pseudomembranes. Erosions and ulcers may also involve the tongue, dental pad, the tip of buccal papillae, esophagus and forestomachs. The kidneys have foci of interstitial nephritis. The meninges are wet and cloudy.

The pathogenesis of the vascular lesion is not well understood. A lack of circulating antibody and viral antigen and absence of antigen-antibody complexes and complement in the vessel walls are inconsistent with an immune-mediated pathogenesis. In one study, the vascular lesions in the brains of a cow with acute MCF demonstrated that the predominant infiltrating cell type in these lesions was CD8(+) T lymphocytes and that large numbers of these cells were infected with ovine herpesvirus 2.

Antemortem diagnosis can be done by PCR for OHV-2 on buffy coats from whole blood (EDTA). PCR is both sensitive and specific. Serological tests are not reliable because some animals fail to seroconvert before death or convert later in the disease process. Virus isolation is not routinely performed because OHV-2 does not replicate in cell culture. There is no treatment and no effective vaccine available.

AFIP Diagnoses: Meninges; cerebrum: Vasculitis and perivasculitis, lymphohistiocytic, necrotizing, subacute, severe, Jersey, bovine.

Conference Comment: The contributor provides a good review of the two forms of malignant catarrhal fever (MCF), the clinical signs associated with the disease and the typical gross findings. Conference attendees focused their discussion on the histopathologic presence of a vasculitis, predominantly within the meninges, but also within the vessels of the brain, and on developing a differential diagnosis list. Other viral diseases which cause vasculitis in cattle and wild ruminants include bovine

pestivirus (Bovine Viral Diarrhea), ovine orbivirus (Bluetongue), and adenoviral hemorrhagic disease. Common bacterial diseases include salmonellosis and thrombotic meningoencephalitis (TME) caused by *Histophilus somni* (formerly *Haemophilus somnus*). The differential diagnosis for erosive and ulcerative mucosal diseases in ruminants includes bovine pestivirus, ovine orbivirus, rinderpest, foot-and-mouth disease and vesicular stomatitis. The differential diagnosis for lymphoproliferative diseases includes enzootic bovine lymphosarcoma, caused by bovine leukemia virus, Jembrana disease in Indonesia, caused by a lentivirus, and East Coast Fever in Africa, caused by the protozoan *Theileria parva*.

Malignant catarrhal fever is characterized by lymphoproliferation, vasculitis, and erosive-ulcerative mucosal and cutaneous lesions, predominantly affecting the respiratory and gastrointestinal systems. Corneal edema and lymphocytic uveitis often occur concomitantly and can help differentiate MCF from other ulcerative mucosal diseases.

Clinically, MCF can be divided into four overlapping categories:

The **head-and-eye form** is the most common and results in pyrexia, copious serous to mucopurulent ocular and nasal secretions, an encrusted muzzle with occluded nostrils, dyspnea, oral mucosal hyperemia with erosions, sloughed buccal mucosal tips, photophobia, conjunctival hyperemia, chemosis, corneal opacity and hypopyon.

The **peracute form** results in severe oral and nasal mucosal inflammation and hemorrhagic gastroenteritis.

The **intestinal form** is characterized by pyrexia, diarrhea, hyperemic oral and nasal mucosa with profuse catarrhal and mucopurulent discharge and generalized lymphadenopathy.

The **mild form** results in mild oral and nasal mucosal erosions.

The typical light microscopic findings associated with MCF include vascular and perivascular necrosis and lymphocytic and lymphoblastic infiltrates in all tissues. Ocular lesions include corneal edema with or without vascularization, erosions, ulcerations and lymphocytic uveitis and ophthalmitis. Kidneys may have infarcts and nonsuppurative interstitial nephritis. Lymph nodes may be severely edematous with ectatic lymphatics and lymphocytic and reticuloendothelial cell proliferation. Central nervous system findings include perivascular edema, nonsuppurative meningoencephalomyelitis and lymphocytic perivascular cuffing. Gastrointestinal tract lesions include congestion, mucosal erosion, abomasal ulcers and submucosal eosinophilic inflammation. Other potential findings include lymphocytic stomatitis and pharyngitis with erosions and ulcers, cutaneous edema, and lymphocytic ulcerative dermatitis.

Other ruminants in North America which can become clinically affected include deer, elk and bison.

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SLIDE 6
CONFERENCE 2 / CASE II – 2004AC328 (AFIP 2986830)

Signalment: Three year old, neutered male ferret

History: A purpose-bred sable ferret, maintained in a laboratory animal facility, presented for weight loss and dehydration. Physical examination was unremarkable and the ferret was treated with fluids and nutritional supplementation. Blood glucose was slightly elevated; otherwise, serum chemical analyses were within normal limits. Hematologic evaluation revealed that the white blood cell count, hemoglobin and platelet count were slightly increased. The differential was within normal limits. The condition continued to worsen and dyspnea was noted. Radiographs revealed a diffuse pulmonary interstitial pattern. Despite treatment, the ferret's condition continued to deteriorate. The ferret was euthanized.

Gross Pathology: Extensive multifocal lesions (about 1.5 mm in diameter) affecting all lung lobes were reported. The spleen contained similar lesions.

Contributor's Morphologic Diagnosis: Lung: Extensive, marked, pyogranulomatous pneumonia with intralesional yeast, consistent with *Blastomyces dermatitidis*.

Contributor's Comment: Similar, less extensive, inflammation with yeast present was also observed in the spleen from this animal. Other tissues were unavailable for examination.
Blastomycosis commonly results from inhalation of the mycelial phase of this dimorphic fungus from infected soil of an endemic area.[1] At body temperature, the organism assumes the yeast form and may cause an asymptomatic infection, pneumonia, or disseminated disease. Cutaneous infection is also possible through wound contact with

infected soil. A case of focal blastomycosis in a veterinarian, associated with necropsy of an infected dog, has been reported.[2] Although not a contagious disease, outbreaks may be associated with exposure to a common environmental source. Within North America, the organism is prevalent in the Mississippi and Ohio River valleys. It is also present in Africa and Asia. Although different serotypes exist, *B. dermatitidis* is the species unique to the genus.

B. dermatitidis may infect a wide range of species, including human beings, dogs, horses, domestic and exotic cats, wolves, dolphins, sea lions, monkeys, prairie dogs, and bats. A least one other case of blastomycosis in a ferret is documented in the literature.[3] Blastomycosis has also been described in a laboratory animal (Rhesus monkey) without recent contact with soil.[4] In that case, as in this one, subclinical infection was presumed to have occurred prior to introduction to the facility, with subsequent exacerbation.

AFIP Diagnosis: Lung: Pneumonia, pyogranulomatous, diffuse, severe, with yeast, etiology consistent with *Blastomyces dermatitidis*, ferret, mustelid.

Conference Comment: Conference attendees identified the yeast as *Blastomyces dermatitidis* by its characteristic "broad-based budding," its size of 10-20 µm diameter and its double-contoured, 1-2 µm thick wall. Discussion centered on differentiating *Blastomyces dermatitidis* from the other common fungal causes of systemic or deep mycoses. Below is a simple chart to help with identification of the common systemic mycoses.

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

Common Systemic (Deep) Mycoses

Yeast	Size	Wall or Capsule	Reproduction

There are three clinical forms of blastomycosis:

1. Primary **pulmonary infection** is most common.
2. The **disseminated** form can involve the eyes, bone, skin, lymph nodes, subcutaneous tissues, external nares, brain and testes.
3. Local **cutaneous infection** follows traumatic implantation.

The pulmonary form occurs when spores are inhaled and deposited in the alveoli of the host. Alveolar macrophages phagocytose the spores which develop into the yeast form and multiply. Macrophages rupture and release the yeast initially inciting a neutrophilic response which progresses to granulomatous pneumonia.

Typical clinical signs include: fever, weight loss, anorexia, dyspnea, chronic cough, ocular and nasal discharge, swollen joints and lameness, lymphadenopathy and draining lymph nodes, blindness, and enlarged testicles. Clinical pathology findings can include neutrophilic leukocytosis, nonregenerative anemia (anemia of chronic disease), lymphopenia, decreased serum albumin, increased alpha2 globulins and hypercalcemia.
Blastomycosis affects primarily dogs but has also been reported in humans, non-human primates, cats, horses, hamsters, bottlenose dolphins, sea lions, a ferret and an African lion.

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SLIDE 7
CONFERENCE 2 / CASE III – 760-05 (AFIP 2985446)

Signalment: 8 year old, male castrate, Boxer dog (*Canis familiaris*).

History: This dog presented with a 4-week history of aphonia attributed to a right laryngeal mass. Physical examination revealed a laryngeal mass arising from the right larynx. Computed tomography (CT) scans revealed a contrast enhancing mass involving the right larynx that extended into the adjacent musculature laterally and craniodorsally. Regional lymph nodes appeared to be within normal limits. *En bloc* resection of the mass via laryngectomy was performed two weeks after initial presentation.

Gross Pathology: A 2.0 x 1.0 x 1.0 cm, pale tan to orange, circumscribed, smooth-surfaced, raised, ovoid mass was observed arising from the right ventrolateral surface of the right vocal fold. The mass projected into and occluded the right lateral laryngeal ventricle, and resulted in mild right lateral occlusion of the laryngeal vestibule and rima glottis (Figures 1, 2, 3 and 4).

Laboratory Results: Immunohistochemistry was performed on sections of the mass. There was strong immunoreactivity to vimentin (Figure 10) and moderate immunoreactivity to desmin (Figure 11). There was no immunoreactivity to cytokeratin AE1/AE3, pan-actin, smooth muscle actin, CD3, and CD79.

Histopathologic Description: There was a focally extensive, expansile, nonencapsulated, circumscribed mass within the right vocalis muscle (Figure 5). The mass comprised of sheets, lobules, and packets of cells supported by scant fibrovascular stroma (Figure 6). The cells were large and pleomorphic (ovoid, polygonal, spindle, strap-like) with well defined borders and moderate to large amounts of finely granular, deeply eosinophilic cytoplasm (Figure 7). A small proportion (5 to 15%) of cells contained many uniformly-sized, 1 to 4 micron diameter, clear, round intracytoplasmic vacuoles (Figure 8). PTAH-stained sections of the mass did not reveal cross-striations within the cytoplasm of the cells (not submitted). The nuclei were pleomorphic with finely stippled chromatin, single to multiple prominent basophilic nucleoli, and rare large, glassy, eosinophilic, cytoplasmic invaginations consistent with pseudoinclusions (Figure 9). Less than 1 mitosis per ten 400X fields was noted. A few cells were binucleated. There was marked anisocytosis and anisokaryosis (up to 3 fold). Moderate to large numbers of neutrophils, lymphocytes and plasma cells were scattered throughout the mass, along with moderate amounts of hemorrhage and fibrin deposition. The inflammation extended multifocally into surrounding skeletal muscle tissue and into overlying propria-submucosa, which was also mildly edematous. There was multifocal ulceration of overlying mucosa. There was moderate central osseous metaplasia of neighboring laryngeal cartilage. The mass did not extend beyond the natural tissue planes defined by neighboring laryngeal cartilage.

Contributor's Morphologic Diagnosis: Laryngeal rhabdomyoma.

Contributor's Comment: Rhabdomyomas and rhabdomyosarcomas are neoplasms of striated muscle. In humans and domestic animals, rhabdomyomas occur less commonly than their malignant counterparts.[1] Striated muscle neoplasms can be divided into two main groups (cardiac and extra-cardiac) based on anatomical location.

Cardiac striated muscle neoplasms are rare. *Cardiac rhabdomyomas* have been reported mostly in young animals in pigs, sheep, cattle, dogs and a fallow deer.[1,2,3] There is a general consensus that cardiac rhabdomyomas represent a congenital developmental abnormality (i.e., hamartoma) of cardiac muscle.[1,2,3] *Cardiac rhabdomyosarcomas* are extremely rare, and there are only a few cases reported in dogs.[1] Since mature cardiac muscle does not retain the capacity for cell division under normal conditions, cardiac rhabdomyosarcomas are thought to arise from

undifferentiated mesenchymal cells in the heart that retain the capacity to differentiate into striated muscle.[1]

Classification of extra-cardiac rhabdomyomas is similar in humans and animals, and is loosely based on age of occurrence and location[1]:

1. *Adult rhabdomyomas* occur in older individuals. In humans and animals, these tumors occur in the head or neck region as either lingual, pharyngeal, laryngeal, or subcutaneous masses.[1] There is a predisposition in males.[1] These neoplasms have been very rarely reported in dogs, cats, and horses, with the exception of laryngeal rhabdomyomas in dogs, which are relatively more common.[1]

2. *Fetal rhabdomyomas* occur in younger individuals. In humans, these tumors occur as subcutaneous masses in the head or neck.[1] There is a predisposition in males.[1] There is only one report in the veterinary literature of a congenital fetal rhabdomyoma occurred as a ventral cervical subcutaneous mass in an Appaloosa filly.[4]

3. *Genital rhabdomyomas* occur in the vagina or vulva of young to middle-aged humans.[1] Examples of this type of rhabdomyoma have not been reported in the veterinary literature.

Classification of extra-cardiac rhabdomyosarcomas is also similar in humans and animals[1]:

1. *Embryonal rhabdomyosarcoma* is the most common variant in humans and animals. Microscopically, they may present as either relatively primitive round myoblasts arranged in a compact fashion, or myotubular myoblasts embedded in myxoid matrix.[1,2] These have been reported in dogs, cats, pigs, cows, and birds, without obvious sex, age or site predilection.[1] A special variant of embryonal rhabdomyosarcoma is the *botryoid rhabdomyosarcoma*, which is a distinct entity in dogs (especially Saint Bernards) that affects the urinary bladder. [1,2] These are myotubular-type neoplasms that are grossly polypoid, botryoid ("grape-like") and exophytic masses which project into the urinary bladder lumen. [1,2] Similar botryoid rhabdomyosarcomas have been reported in two fillies, affecting the urinary bladder and the uterus respectively.[1,2]

2. *Alveolar rhabdomyosarcoma* is characterized microscopically by poorly-differentiated, round myoblasts supported by fairly dense fibrovascular septae.[1] The alveolar appearance is due to central degeneration, necrosis, and loss of the cells, resulting in a clear central area surrounded by myoblasts that line the fibrovascular septae.[1] These tumors are infrequently reported in the veterinary literature (dogs, horses and cows) without observed sex, age, or site predilection.[1]

3. *Pleomorphic rhabdomyosarcoma* is the least commonly reported variant in humans and animals, characterized microscopically by pleomorphic myoblasts without any similarities to the embryonal or alveolar variants.[1] These have been reported in dogs and horses.[1]

The diagnosis of rhabdomyomas and rhabdomyosarcomas is often difficult on light microscopy. Distinctive features of striated muscle may not be evident microscopically (i.e., cytoplasmic cross striations on H&E and PTAH stained sections); however, if present, they are reliably diagnostic.[1,2] Another feature of many tumors of myoid origin is the presence of eosinophilic granular cytoplasm, which may contain intracytoplasmic glycogen that is demonstrable on PAS staining.[1,2,3]

Transmission electron microscopy is useful for demonstrating specific ultrastructural characteristics of striated muscle, such as actin and myosin filaments that are organized into parallel myofibrils with electron dense Z-bands.[1,5,6] Other common but nonspecific ultrastructural features of striated muscle neoplasms include the presence of numerous mitochondria (often arranged between myofibrils), large euchromatic nuclei (sometimes possessing intranuclear cytoplasmic invaginations, or "pseudoinclusions"), numerous ribosomes (many arranged as polyribosomes), and accumulation of glycogen.[1,5,6]

Immunohistochemistry is often useful in diagnosing striated muscle neoplasms, which are typically immunoreactive for vimentin, desmin, muscle specific actin, sarcomeric actin, and myoglobin, with no immunoreactivity to cytokeratin and smooth muscle actin.[1,2,6,7] However, this immunohistochemical profile is not always consistent, and is dependent on the degree of differentiation of the neoplastic cells – for example, in normal developing muscle, the order of protein expression is as follows: vimentin, desmin, myosin, and myoglobin.[1]

Canine laryngeal rhabdomyoma and rhabdomyosarcoma is a rare distinct entity in dogs. Although most are histologically benign, they may cause death or result in euthanasia due to laryngeal obstruction.[1,5,6,7] Affected dogs typically present with dysphonia, aphonia, stridor, and dyspnea.[1,5,6,7] A few cases have been reported to be locally invasive, with one case report of hepatic metastasis after surgical resection.[1] Due to the limited number of cases, age, sex and site predilection have not been recognized. Canine laryngeal rhabdomyoma and rhabdomyosarcoma have been reported as laryngeal oncocytoma in the past.[1,5,6] Although histologically similar, many of these reported cases of oncocytoma were found to be striated muscle in origin, based on immunohistochemical labeling.[1,6] This is in contrast to true oncocytes (such as those found in renal oncocytomas and salivary gland oncocytomas), which are epithelial in origin.

In this case, the dog is alive 3 months post laryngectomy with no recurrence of the laryngeal rhabdomyoma.

AFIP Diagnosis: Skeletal muscle, laryngeal: Rhabdomyosarcoma, Boxer, canine.

Conference Comment: The contributor provides a thorough review of the classification of both rhabdomyomas and rhabdomyosarcomas. There was slight variation in slides among the conference attendees with some sections demonstrating infiltration of the neoplastic cells into the adjacent laryngeal muscle and fibrous connective tissue. In some sections, neoplastic cells have atypia characterized by round nuclei, prominent nucleoli and cytoplasmic invaginations. The diagnosis of rhabdomyosarcoma is based on the atypia, infiltration at the margins, which produces islands of neoplastic cells separated from the main mass, and the fairly high (2-3/hpf) mitotic rate.

Throughout the neoplasm is an extracellular, eosinophilic, homogenous material which separates neoplastic cells and resembles amyloid, prompting a differential diagnosis among conference participants of extramedullary plasmacytoma. The material stains with Periodic Acid Schiff (PAS); however, Congo Red results are equivocal. Interestingly, media from rhabdomyosarcoma cell cultures has stimulated *in vitro* aggregation and fibrillization of amyloid beta-protein (8).

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SLIDE 8
CONFERENCE 2 / CASE IV – CSU05-06#2 (AFIP 2985207)

Signalment: 8 month-old, male castrated, Persian, feline.

History: Cat presented with a sudden onset of neurological signs including: circling, disorientation, head pressing, muscle tremors, focal seizures and visual deficits. Blood chemistry revealed a low BUN, creatinine, total protein and albumin and increased bile acids. No shunts were identified grossly during abdominal exploratory. The cat was euthanized due to a worsening condition and poor prognosis.

Gross Pathology: Necropsy findings revealed a diffusely yellow, small and firm liver.

Laboratory Results: Brain: Predominately within the white matter, optic nerve tracts and less in the gray matter there are extensive areas of spongiform vacuolar degeneration and multifocal neuronal necrosis. Spongiform change is characterized by variably sized (7-200 micron in diameter), often coalescing, clear, distinct, round to oval vacuoles within the neuropil. Numerous blood vessels within affected areas are prominent with plump endothelial cells and are surrounded by an increased clear space that contains small amounts of eosinophilic proteinaceous fluid (edema) and variable numbers of neutrophils, lymphocytes, plasma cells and macrophages. Multifocally, within the meninges, there are variable accumulations of perivascular neutrophils with fewer numbers of lymphocytes and plasma cells.

Liver: There is a bridging midzonal pattern of hepatocytes that are expanded 2-3 times normal size by single or multiple, variably sized, clear, discrete cytoplasmic vacuoles which often peripheralize and flatten the nucleus (lipid). Diffusely sinusoids are collapsed. Portal areas contain moderate arteriolar hyperplasia with smooth muscle hypertrophy, variable biliary hyperplasia and patchy lymphoplasmacytic inflammation and scant hemorrhage.

Contributor's Morphologic Diagnosis: 1/Brain**:** a/White matter: Spongiform vacuolar degeneration, multifocally extensive, moderate with myelinolysis and neuronal necrosis. b/Meninges: Meningitis, suppurative, acute, patchy, moderate.

2/Liver**:** Hepatic lipidosis, midzonal, bridging, and moderate with arteriolar hyperplasia and smooth muscle hypertrophy.

Contributor's Comment: The histologic changes in the brain are consistent with hepatic encephalopathy. The cause of the liver failure is unknown but is most consistent with a toxic insult.

A number of theories have been proposed to explain the pathogenesis of hepatic encephalopathy. Patients develop an alteration in brain energy metabolism coupled with increased permeability of the blood brain barrier that facilitates the passage of neurotoxins into the brain. Neurotoxins include short chain fatty acids, mercaptans;

false neurotransmitters such as tyramine, octopamine, and beta phenylethanolamines; ammonia and gamma-aminobutyric acid.

Hyperammonemia is a critical, although not the sole factor in the development of hepatic encephalopathy. Ammonia is produced in the gastrointestinal tract by bacterial degradation of amines, aminoacide, purines and urea. In a healthy liver, ammonia is detoxified by conversion of urea and glutamine. With liver disease, there is inefficient conversion of ammonia and increased levels of ammonia can enter the systemic blood supply by portal shunts (1,3). In the brain, astrocytes are responsible for ammonia detoxification by conversion of glutamate to glutamine. When ammonia is high in the brain, it is thought that the accumulation of glutamine in the astrocytes causes swelling of the astrocyte (Type II astrocytosis) resulting in dysfunction of the astrocyte itself and brain edema (1,3).

Another hypothesis involves gamma-aminobutyric acid (GABA) which is a neuroinhibitory neurotransmitter produced in the gastrointestinal tract by bacteria. In liver disease there is an increase in the number of GABA complex receptors (2,3). When GABA crosses the increasingly permeable blood brain barrier it interacts with the GABA receptor complex leading to inhibitory nerve transmission. Benzodiazepines and barbiturates can bind to the GABA receptor complex and act in a similar fashion (2,3).

In liver disease, there is a shift from branched chain amino acids to aromatic amino acids (phenylalanine, tyrosine and tryptophan) which are neurotoxic, with decreased hepatic clearance. Aromatic amino acids are precursors for neurotransmitter synthesis. When levels are high within the brain, other substances such as tyramine, octopamine and phenylethanolamines are produced that act as false neurotransmitters (2,3). These substances compete with the normal neurotransmitters for the same receptor.

AFIP Diagnoses: 1. Brain, cerebral white matter: Spongiform change, multifocal, mild, Persian, feline.
2. Brain, cerebral cortex: Necrosis, multifocal, with mild gliosis, and acute meningitis.
3. Liver, hepatocytes: Degeneration, with vacuolar change (fatty type), midzonal, diffuse, moderate.
4. Liver: Portal venous hypoplasia and arteriolar reduplication, diffuse, moderate.

Conference Comment: This is a very complex case with no clear answers**.** The contributor provides an excellent review of the pathogenesis of hepatic encephalopathy. There was variation in brain sections among conference attendees. Some brain sections had intense perivascular neutrophilic infiltrates in both the meninges and cerebral cortex, with reactive changes in the associated vessels, all features characteristic of sepsis. Several slides had areas of liquefactive necrosis with occasional neutrophils predominantly within the grey matter of the cerebral cortex. The multifocal, and seemingly random, cerebral cortical necrosis with gliosis (predominantly gemistocytic astrocytes) could reasonably be secondary to vascular impairment. Gram

stains did not identify bacteria within necrotic areas or the meninges; however, this does not eliminate sepsis as a possible cause.

The presence of Alzheimer type 2 cells are a consistent finding in human and equine patients with hepatic encephalopathy; however, they are not a consistent feature in several other species, including cats. Alzheimer type 2 cells are astrocytes with a swollen, vesicular nucleus and swollen cytoplasm caused by accumulation of ammonia or other endogenous toxins (4). Alzheimer type 2 cells were not identified in the examined sections of brain.

This cat's brain has lesions consistent with hepatic encephalopathy, such as white matter spongiosis; however, it also has lesions which are not consistent with hepatic encephalopathy, such as neutrophilic inflammation and cortical necrosis.

Midzonal fatty degeneration of the liver is uncommon in domestic animals and is most likely due to a toxin. Midzonal degeneration and necrosis has been reported in cats exposed to hexachlorophene (5). Incidentally, cats exposed to hexachlorophene have been shown to develop spongiosis of the white matter and exhibit behavioral changes (6).

To complicate this case even further, there is arteriolar reduplication within sections of liver supporting the presence of a shunt or some form of hepatic microvascular dysplasia.

Clinical pathology and toxin screening results are not available but could help in further defining the underlying cause for this cat's interesting lesions.

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SLIDE 9
CONFERENCE 3 / CASE I – AFIP#2 (AFIP 2983842)

Signalment: Female, adult, BALB/c mouse

History: Lungs submitted from dead mice, 9 days post injection with vaccinia virus.

Gross Pathology: Lung lobes are non-collapsed and spongy.

Laboratory Results: None

Histopathologic Description: Multifocal individual mostly medium to small diameter bronchioles are lined by multiple piled layers of swollen epithelial cells which occasionally occlude the airway lumen. Epithelial cells are expanded by large, clear, indiscrete cytoplasmic vacuoles and most cells contain variably sized, but generally large, brightly eosinophilic, glassy, intracytoplasmic inclusion bodies. Scattered small numbers of lymphocytes and macrophages fill immediately adjacent alveolar spaces.

Contributor's Morphologic Diagnosis: Bronchiolitis, proliferative, multifocal, with intraepithelial, intracytoplasmic inclusion bodies

Contributor's Comment: Histologic lesions in the lung (and in sections of nasal cavity) are consistent with *Orthopoxvirus* infection. These are large DNA viruses of the poxvirus family to which vaccinia, variola, monkeypox, cowpox, ectromelia virus and others belong. Orthopoxviruses share antigenic cross-reactivity, but each is a distinct species.
Most information on *Orthopoxvirus* infections in mice is based on reports of ectromelia virus infection, specifically (1). These viruses are not highly contagious and can be experimentally transmitted via a number of routes, but the primary means of natural transmission is through cutaneous trauma, requiring direct animal contact. Other studies have indicated that transmission was facilitated by handling of animals.

Young mice suckling immune dams were protected by maternal antibodies from disease, but not from infection. The hypothetical model of infection (based on ectromelia virus infection; mousepox), involves invasion through skin, local replication, release to regional lymph nodes, primary viremia and replication in the spleen and liver. Between 3-4 days post exposure, secondary viremia ensues leading to replication in the skin, lungs, kidney, intestine and other organs. There is increasing evolution of lesions days 7-11 post exposure, including cutaneous rash.

The disease scenario differs markedly between mouse genotypes with susceptible strains such as C3H, A, DBA, SWR and BALB/c often dying acutely with minimal lesions or opportunity for virus excretion. Other mouse strains develop illness and progress to develop cutaneous lesions and virus shedding. Still others, such as B6 mice, are resistant to disease, but allow virus replication and excretion. In most cases,

liver necrosis is present and is sometimes the only lesion noted. Pathognomonic viral inclusion bodies in respiratory epithelium, along with the history of vaccinia virus vaccination allow for definitive diagnosis in this case.

AFIP Diagnosis: Lung: Bronchitis and bronchiolitis, acute, diffuse, moderate, with epithelial ballooning degeneration, eosinophilic cytoplasmic inclusion bodies and alveolar fibrin and edema, BALB/c mouse, murine.

Conference Comment: The contributor provides a detailed synopsis of Orthopoxvirus infections in mice. Although this case was caused by experimental infection with vaccinia virus it shares many characteristics with mousepox caused by ectromelia virus.

Ectromelia virus was discovered in England in 1930 and has been extensively studied as a model of the pathogenesis of exanthematous diseases. The study of ectromelia virus led to the concept of a primary and secondary viremia as well as the role of cell mediated immunity, particularly T-lymphocytes and macrophages, in the recovery from infection. [2]

Mousepox has a restricted host range and causes severe disease with a high mortality rate (50-100%). Voles are believed to be the natural host.
There are two recognized forms of mousepox: the rapidly fatal form with few, if any, cutaneous lesions and the chronic form with ulceration of the skin, particularly on the snout, feet and tail often resulting in loss of limbs or tail.

The typical gross findings in mice include conjunctivitis, alopecia, crusting and erythema of the skin and dry gangrene of the extremities and tail. The liver and spleen swell and develop multifocal hemorrhages and pinpoint white foci. Lymph nodes and Peyer's patches may be enlarged and hemorrhagic.

Typical light microscopic findings include intracytoplasmic eosinophilic A-type (Marchal) inclusion bodies within the epidermis, pancreas and intestine. Early in the disease there is epidermal hyperplasia, hypertrophy, spongiosis and ballooning degeneration with inclusion bodies. Later, the epidermis becomes necrotic and ulcerated. The liver develops multiple foci of coagulative necrosis, hepatocellular syncytia and vacuolar degeneration with minimal inflammation. There is splenic necrosis involving both lymphoid follicles and red pulp. Additionally, there may be intestinal mucosal erosions and bone marrow degeneration. The combination of hepatic and splenic necrosis along with cutaneous lesions and characteristic eosinophilic intracytoplasmic inclusion bodies is pathognomonic.[1]

Other important members of the Orthopox genus include (3):
Variola virus (smallpox in humans)

Monkeypox virus (lesions in nonhuman primates vary from mild to severe depending on the species, age and immune status, and range from cutaneous lesions to disseminated visceral infection; fatal infections in humans)
Cowpox virus (self-limiting lesions on the udder in cattle; fatal pneumonia in elephants and felids)
Buffalopox virus
Camelpox virus

Contributor: Utah Veterinary Diagnostic Laboratory (UVDL)
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SLIDE 10
CONFERENCE 3 / CASE II – AFIP1 (AFIP 2985003)

Signalment: One year old male ICR mouse (*Mus musculus*).

History: The mouse was submitted to necropsy because of observed jaundice. The mouse died shortly before necropsy was performed.

Gross Pathology: Liver (All lobes): Enlarged, soft, with nodular surface, mottled dark red and yellow.
Spleen: Splenomegaly, minimal.
Seminal Vesicle (Right), Prostate Gland: Discoloration, green/yellow, multifocal, moderate.

Laboratory Results: The liver was PCR positive for *Helicobacter hepaticus*. This animal came from an SPF colony, seronegative for a panel of 18 murine viral pathogens.

Histopathologic Description: There is multifocal, marked bile ductule hyperplasia, accompanied by a mixed inflammatory infiltrate of lymphocytes, plasma cells and neutrophils. The inflammation extends from the portal zones across the limiting plate into the hepatic parenchyma, with associated piecemeal necrosis of hepatocytes. Hepatocytes frequently exhibit multifocal, marked cytomegaly. Enlarged hepatocytes typically have karyomegaly, with frequent cytoplasmic invaginations into the nucleus.

On most sections there are small foci of cellular alteration, either vacuolated or clear cell foci, or both.

Contributor's Morphologic Diagnosis: Liver: Cholangiohepatitis, chronic, suppurative, multifocal, marked, with marked bile duct hyperplasia, piecemeal necrosis and marked hepatocytomegaly and karyomegaly.

Warthin Starry silver stain: Multiple elongate, curved bacilli are seen localized between hepatocytes (in bile canaliculi) (Figure). An unusually high number of spiral bacteria are seen in the liver of this case. Often the organisms are rare and thus difficult to find.

Additional lesions, not included on sections:
Prostate, seminal vesicle: Abscesses, multiple, well-developed, severe, with myriad intralesional Gram negative bacilli.
Kidney: Pyelonephritis, chronic, suppurative, multifocal, moderate.

Contributor's Comment: The hepatitis and spiral bacilli seen on silver stains are characteristic of *Helicobacter hepaticus* infection. Some of the hepatocellular anisocytosis may be attributable to the advanced age of the mouse.
Helicobacter spp. are significant pathogens of a number of species (reviewed in1), including mice. *Helicobacter* spp. colonize the cecum and colon of rodents and some are associated with liver disease in susceptible strains of mice and rats. Colony acquired infections are persistent, with long term shedding of the bacteria in the feces. Endemic infections are common. *Helicobacter hepaticus* was the first murine helicobacter identified, associated with hepatitis and hepatocellular neoplasia in mice on a long-term toxicology study (2, 3). *Helicobacter*-associated disease is dependent on age, sex (male>female), genetics and immune status of the host, and on bacterial virulence factors. Known *Helicobacter hepaticus* susceptible strains include A, C3H, SCID and other immunocompromised mice. *Helicobacter hepaticus* also causes proliferative typhlocolitis and rectal prolapse in immunocompromised mice. Additional helicobacters known to infect mice include. *H. bilis, H. rodentium, H. ganmani, H. typhlonius, H. muridarum, H. muricola* and *H. rappini (Flexispira rappini)* (1).

Mice are likely infected with helicobacters through ingestion of contaminated feces, and *Helicobacter* spp. are readily transmitted by contaminated bedding (4). *Helicobacter hepaticus* will persist indefinitely in the bile canaliculi. The pathogenesis of hepatitis and hepatocellular tumors is still under investigation, but hepatotoxins and immune mechanisms are likely.

While the typical lesions and presence of intralesional spiral bacilli on silver stains are strong evidence for *Helicobacter* infection, definitive diagnosis requires culture and/or PCR (1). Diagnostic specimens can be lesional tissue, cecal scrapings or fecal pellets.

Although this mouse was not of the recognized *Helicobacter hepaticus*- susceptible strains, the advanced age and concomitant urogenital tract infection may have conferred susceptibility to *Helicobacter*-induced hepatitis.

AFIP Diagnosis: Liver: Cholangiohepatitis, chronic-active, portal and random, moderate, with multifocal marked biliary hyperplasia, piecemeal hepatocellular necrosis, and nodular hyperplasia, ICR mouse, rodent.

Conference Comment: *Helicobacter* spp. are Gram negative microaerophilic curved to spiral motile bacilli. There are at least twenty species definitively identified in animals, many which are nonpathogenic.

Helicobacter hepaticus and *H. bilis* are most commonly associated with disease in mice. Typical clinical findings associated with infection include: wasting; mucoid, hemorrhagic and sticky feces; and rectal prolapse. Typical necropsy findings include typhlitis and colitis. Microscopic lesions include colonic and cecal crypt hyperplasia, variable leukocyte infiltration in the lamina propria of the colon and cecum and, infrequently, randomly scattered foci of hepatocellular necrosis with mixed leukocyte infiltrates (4).

In susceptible mice, *H. hepaticus* causes acute focal non-suppurative necrotizing hepatitis that progresses over the course of several months. Features include oval cell hypertrophy and hyperplasia, increased hepatocellular mitoses, prominent ductule formation and lymphoplasmacytic cholangitis and hepatitis. As the lesions progress, the liver becomes fibrotic and develops extensive bile duct hyperplasia, hepatocytomegaly and peribiliary lymphoid nodule formation (4). In this case, there was marked hepatocytomegaly and karyomegaly noted.

Mouse Hepatitis Virus Infection (MHV), caused by a coronavirus, was considered in the differential diagnosis; however, MHV infection results in virus-induced syncytia formation and acute hepatic necrosis but does not typically result in biliary hyperplasia (4).

H. hepaticus is significant to the research community for several reasons besides the effects of acute infection: it causes an increase in hepatocellular tumors in certain strains of mice and promotes experimental chemical hepatic carcinogenesis thereby confounding research efforts (4).

Contributor: Charles River Laboratories, www.criver.com

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SLIDE 11
CONFERENCE 3 / CASE III – 2003abbott20 (AFIP 2986815)

Signalment: An approximately 13-week-old, female, Sprague Dawley rat

History: This rat is one of ten female rats given a fibrate derivative in the diet for approximately 35 consecutive days. Control rats received the basal diet only. There were no clinical signs of toxicity, but body weight gain and food consumption were decreased in the treatment group. This rat survived to the scheduled necropsy.

Gross Pathology: Absolute and relative liver weights were increased, and hepatomegaly was noted in most of the animals in the treatment group.

Laboratory Results: Increased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase activities. Creatine kinase (CK) levels were not evaluated.

Histopathologic Description: Sections of skeletal muscle are from the gastrocnemius muscle. Some sections also include soleus muscle. There are multifocal areas of myofiber degeneration characterized by loss of striations, hyaline change, increased eosinophilia, presence of angular fibers and phagocytosis. Infiltration of primarily macrophages is observed associated with degenerate fibers. Evidence of regeneration (nuclear hypertrophy, internalization of nuclei, sarcoplasmic basophilia) is also present. The deep portions of the intermediate gastrocnemius and the soleus muscles are the most severely affected. Immunohistochemistry was performed against myosin from type 1 (slow, oxidative) skeletal muscle fibers or against myosin from type 2 (fast, glycolytic) fibers. Areas of densest type 1 muscle fiber concentration coincided with zones in this rat having the most prominent skeletal muscle degeneration evident in H&E stained sections. Areas lacking type 1 muscle fibers also lacked evidence of degenerative change in H&E sections.

Contributor's Morphologic Diagnoses: Skeletal muscle, caudal thigh: Degeneration, myofiber, multifocal, mild to moderate, with histiocytic cell infiltration and myofiber regeneration.

Liver: Hypertrophy, hepatocellular, centrilobular, moderate (tissue not provided)

Contributor's Comment: Fibrates are marketed as lipid-lowering agents in humans, indicated for the treatment of hypercholesterolemia, mixed dyslipidemia and Types IV and V hypertriglyceridemia. Fibrates and other types of lipid-lowering agents such as statins have been reported to cause myopathy in human patients characterized by myalgia, weakness, CK elevation and rarely rhabdomyolysis. The etiopathogenesis of these myopathic changes is poorly understood; as these various agents have very different chemical structures yet cause an indistinguishable myopathic syndrome in humans[1]. Fibrate-induced myopathy in the rat has been previously reported[2,3]. Interestingly, fibrates appear to selectively damage type 1 fibers in the rat[3]. Conversely, reports on effects of statins and fibrates in humans and statins in rats indicate that type 2 fibers are primarily affected[1,4].

Alterations in cholesterol content of sarcoplasmic membranes appear to be an important factor in the development of myofiber damage by lipid-lowering agents. Membrane fluidity varies inversely to cholesterol content and increases in fluidity can result in significant sarcolemmal changes[1]. Other mechanisms that have been implicated include deficiencies in selenoprotein synthesis, organic anion transporters, muscle energy metabolism, changes in intracellular ubiquinone concentrations and mitochondrial injury[4].

In addition, fibrates are classical inducers of peroxisome proliferation and cytochrome P450 4A1 enzymes. This appears to be mediated by peroxisome proliferator activated receptor alpha (PPAR α) resulting in hepatocellular hypertrophy evident histologically. This type of change is rodent-specific and generally reflective of an adaptive response to the drug[5].

AFIP Diagnosis: Skeletal muscle: Degeneration and necrosis, multifocal, moderate, with histiocytic myositis, Sprague Dawley rat, rodent.

Conference Comment: Conference attendees discussed the histologic features of myocyte degeneration, regeneration and necrosis. Slides are of muscle in cross-section making the evaluation of regeneration more difficult. Regenerative skeletal muscle fibers have reduced myofiber diameter, slightly basophilic sarcoplasm and proliferating satellite cells located between the plasmalemma and the basal lamina. In regenerating myocytes, satellite cells migrate to the center of the damaged myocyte, form longitudinal rows and begin to produce sarcoplasm. In cross-section, regenerative myocytes have slightly basophilic sarcoplasm and centrally located nuclei corresponding to the rows of internal nuclei seen in longitudinal section (6).

There are numerous causes of myositis and myopathies. Attendees discussed a variety of general differentials which include nutritional causes such as vitamin E/selenium deficiency; exertional rhabdomyolysis; injection site injury and reaction to toxic drugs or plants. Toxins include ionophores such as monensin, salinomycin, narasin and

lasalocid, which are commonly used as growth promoters in feedlot cattle and coccidiostats in poultry feed; gossypol from cottonseed meal and *Cassia* sp. plants (e.g. coffee senna). All have similar histological lesions including myocyte degeneration, necrosis, regeneration, variable degrees of histiocytic myositis and, in later stages, fibrosis. In horses, nutritional myopathies can be differentiated from ionophore toxicity by the presence of lesions in multiple stages of necrosis (multiphasic). Presumably, ionophore toxicity would be a one time event and result in uniform progression of myocyte necrosis with all lesions at the same stage (monophasic) (6).

The earliest published reports of muscle complications associated with fibrate administration date back to 1968 (7). Since then, there have been numerous reports of similar findings with statins and fibrates resulting in the moniker, Cholesterol-lowering agent myopathy (CLAM).

Contributor: Abbott Laboratories, Department of Pathology, AP13A/R 469, 100 Abbott Park Road, Abbott Park, IL 60064

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SLIDE 12
CONFERENCE 3 / CASE IV – C04-2863 (AFIP 2985457)

Signalment: 9-month-old, female C57BL/6 GM-CSF homozygous knockout (-/-) mouse (*Mus musculus*)
History: Mouse was used for breeding and underwent no experimental manipulation.

Gross Pathology: The mouse was received in good body condition. All lung lobes were mottled pale tan especially at the periphery, firm on palpation and sunk in formalin (Figure 1). The uterine horns were mildly dilated and thickened and contained small amounts of dark red material. In addition, there were multifocal to coalescing petechiae on the serosal surface of the ventral aspect of the left uterine horn covering a 0.5 x 0.5 cm area.

Histopathologic Description: Numerous alveoli throughout all lung lobes are filled with homogeneous and compact to granular, eosinophilic, acellular, PAS-positive material (Figures 2-4). Scattered alveoli also contain small numbers of large alveolar histiocytes with abundant, foamy cytoplasm. Alveolar septal walls are unaffected and acicular (cholesterol) clefts are not overtly apparent. Moderate to large numbers of lymphocytes and plasma cells expand the interstitium around blood vessels and bronchioles (Figure 5). Cystic endometrial hyperplasia was also present in the uterus (not submitted).

Contributor's Morphologic Diagnosis: Lung, alveolar proteinosis, multifocal to coalescing, chronic, moderate with perivascular and peribronchiolar lymphoplasmacytic infiltrates.

Contributor's Comment: Pulmonary alveolar proteinosis (PAP) is a rare condition characterized by the intra-alveolar accumulation of surfactant protein (1,2). There are three clinically distinct forms of the disease: congenital, secondary and acquired (1,2). Mutations in genes encoding surfactant proteins B or C, or the β chain of the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor result in fatal neonatal respiratory distress. Secondary PAP develops in association with various conditions in which there is functional impairment or reduced numbers of alveolar macrophages. Upwards of 90% of all cases are of unknown etiology and are classified as acquired. However, numerous reports have identified GM-CSF as playing a critical role in the pathogenesis of PAP since the initial description of the condition in 1958.

GM-CSF is a 23-kDa hematopoietic growth factor which modulates the function of mature myeloid cells, especially neutrophil and eosinophil granulocytes and monocytes/macrophages. In addition, GM-CSF is required for pulmonary surfactant homeostasis (2,3). This latter role was first recognized upon the generation of GM-CSF deficient mice which develop a characteristic pulmonary phenotype resembling PAP (3). In these mice, exons 1 and 2 and intron 1 of the GM-CSF gene are deleted; however, the mice are viable and have normal peripheral blood counts and bone marrow cellularity. Although normal at birth, all GM-CSF homozygous knockout (-/-) mice develop PAP by 3 weeks of age. The eosinophilic, PAS-positive, intra-alveolar material contains type C lamellar bodies, also present within phagolysosomes of alveolar macrophages, which are characteristic of surfactant. An additional feature of the pulmonary lesion in mice, which is not reported in human patients, is perivascular and peribronchiolar aggregates of lymphocytes (80% B and 20% CD4+ T) and plasma cells.

The relationship between the perivascular and peribronchiolar lymphocytes and plasma cells is not known since similar aggregates are commonly observed in various tissues in mice, especially older mice, and are not necessarily indicative of pathology (4). Both GM-CSF deficient mice and human patients are at increased risk for developing opportunistic bacterial and fungal infections (1,3), although such infections were not a feature of this case.

Although mutations in GM-CSF are not found in human patients with acquired PAP, all patients do have GM-CSF-neutralizing autoantibodies in bronchoalveolar-lavage fluid and serum (1,2). These autoantibodies inhibit the activity of endogenous GM-CSF resulting in a state of functional GM-CSF deficiency similar to that in GM-CSF -/- mice.

Supplemental GM-CSF has been shown to be therapeutic in both mice and humans. Overexpression of GM-CSF targeted to the lung by the surfactant protein C gene in GM-CSF -/- mice resulted in complete resolution of alveolar proteinosis (1,5) as did daily aerosolization of GM-CSF in GM-CSF -/- mice (1,6). In addition, some human patients treated with subcutaneous GM-CSF have responded favorably (1,6).

Based on these observations, it is now clear that GM-CSF is critical for catabolism of surfactant by alveolar macrophages (1). Surfactant, which reduces surface tension and prevents alveolar collapse, is composed of surfactant proteins A, B, C and D that are synthesized, stored and secreted by type II pneumocytes. Approximately 70-80% of surfactant is taken up by type II pneumocytes and recycled or catabolized. Alveolar macrophages internalize and catabolize the remaining surfactant. In GM-CSF -/- mice and humans with PAP, biosynthesis and secretion of surfactant in GM-CSF is normal. Despite increased uptake by alveolar macrophages, catabolism is severely impaired. This results in intra-cellular and intra-alveolar accumulation of surfactant, thereby reducing the surface area available for gas exchange.

Spontaneous PAP has also been reported in CB.17 scid/scid mice (7), germ-free scid/scid-beige mice (8), a 3.5-year-old male Shih Tzu (9) and a 1-year-old Cocker Spaniel (9). PAP can be experimentally induced by crystallized silica (referred to as lipoproteinosis) or amphophilic drugs (referred to as phospholipidosis), and is seen in goats with caprine arthritis-encephalitis virus-induced interstitial pneumonia and lungworm infestation (10).

AFIP Diagnoses: 1. Lung: Alveolar proteinosis, diffuse, moderate, C57BL/6 GM-CSF (-/-) mouse, rodent.
2. Lung: Lymphoid infiltrates, peribronchiolar and perivascular, diffuse, moderate.

Conference Comment: The contributor provides a thorough review of the pathogenesis of pulmonary alveolar proteinosis (PAP) and the role GM-CSF plays in its development.

PAP not only occurs in mice and humans but has been reported in dogs. In one report, a 3_ yr old Shih Tzu diagnosed with PAP responded well to repeated bronchoalveolar lavages during which thick, turbid, white fluid was retrieved from the lungs and shown to contain globules of inspissated mucus, protein, and cholesterol clefts (9).

Attendees noted that the lungs in the provided gross photograph (Fig 1) had not collapsed, and discussed other differentials for mouse lungs which, on gross examination, fail to collapse. The list includes *Pneumocystis carinii* infection, lymphoma and acidophilic macrophage pneumonia. *P. carinii* is an atypical fungus which causes pulmonary infections predominantly in immunocompromised animals and humans. Lymphoma is very common in mice and can be broken down into several classifications with higher incidence rates among specific strains. Acidophilic macrophage pneumonia (AMP) is characterized by an accumulation of macrophages within alveoli with brightly eosinophilic intracytoplasmic crystalline material. Additional information on both *P. carinii* and AMP can be found by reviewing Wednesday Slide Conference number 3, case 1 from 2004-2005.

The conference closed with the moderator emphasizing the importance of conducting an extensive literature review when working with animal models.

Contributor: http://www.mskcc.org; http://www.med.cornell.edu/; http://www.rockefeller.edu

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SLIDE 13
CONFERENCE 4 / CASE I – H05-0631 (AFIP 2987675)

Signalment: Ara (*Ara chloroptera*), male, 5 years old

History: The ara showed an irregular, soft mass on his right leg, measuring 7 to 10cm in diameter. Radiographs revealed lytic areas within this mass. The animal was euthanatized.

Gross Pathology: Necropsy revealed a slightly enlarged liver. The mass on the right leg consisted of several nodes with yellowish caseous centres and numerous miliary nodules.

Laboratory Results: Bacterial cultures of heart and liver were negative. Impression smears of liver and intestine were negative in the Ziehl-Neelsen stain, but few acid fast bacteria were detected in smears of the mass on the leg.

Histopathologic Description: Subcutaneous tissue of the right leg: Fibrous connective tissue diffusely infiltrated with macrophages, lymphocytes, heterophils and plasma cells. Scattered groups of epitheloid and multinucleated giant cells. Multiple foci are present with central caseous necrotic material consisting of degranulating heterophils, surrounded by epitheloid and multinucleated giant cells.

Contributor's Morphologic Diagnosis: Subcutaneous tissue of the right leg: Panniculitis, granulomatous, diffuse, severe, with intralesional acid fast bacteria.

Contributor's Comment: This ara was presented at the departement of zoo and wildlife medicine because of a mass on his leg. Radiographs revealed a lytic pattern and tuberculosis was suspected. The owner admitted that he himself had suffered from pulmonary tuberculosis two years previously. He first refused to have the bird euthanatized but later agreed for the sake of his other birds (a 55 year old Amazon and several budgerigars).

At necropsy, typical subcutaneous caseous lesions were found on the right leg. Furthermore, the liver was enlarged. Impression smears of liver and intestine revealed no acid fast bacteria whereas smears of the leg mass showed a small number of acid

fast bacteria. Histopathology revealed typical lesions consisting of tuberculous nodules with central necrosis, surrounded by epitheloid macrophages and multinucleated giant cells. For cultivation, parts of the mass on the right leg were sent to the national reference center for mycobacteria where *Mycobacterium tuberculosis* was identified.

This case is one of few rare reports describing an infection with *M. tuberculosis* in a bird living in a household with a person who also had active tuberculosis previously. It is likely that the ara contracted the disease from his owner. Moreover, the bird may have acted as a source of infection for other birds. So far, it is not clear whether humans can acquire the infection from birds; environmental sources are considered the principal origin of mycobacteriosis in humans (1, 2).

Avian mycobacteriosis has a worldwide distribution and the most common mycobacteria species that infect birds are Mycobacterium avium, of the serotypes 1,2,3 and 8 as well as Mycobacterium genavense. Since Mycobacterium avium and M. intracellulare show nearly equal growth characteristics and species-specific antigens, they are grouped together and termed the M. avium-intracellulare (MAI) complex. Some investigations on the etiological agents of mycobacteriosis in pet birds have been carried out recently. Mycobacterium genavense was found to be the main causative agent followed by M. avium intracellulare complex and to a much lesser extent, M. tuberculosis. Isolates like M. fortuitum, M. gordonae, M. nonchromogenicum were found in one or two species (2,3,4,5).

The route of infection depends on the organism. M. tuberculosis seems to be contracted directly from people by inhalation of aerosolized bacteria, whereas M. avium and M. genavense are contracted by ingestion of the organism. The latter two appear to be ubiquitous in the environment. The genetic susceptibility, the immune response and possible stressors like malnutrition, overcrowding or other diseases and the number of organisms to which the bird is exposed determine whether it will get infected by Mycobacteria or not. (2,5).

The clinical findings in birds infected with mycobacteria can vary extremely. Often, it seems to be a chronic wasting disease which may result in death. Some infected birds are found dead without previous abnormal appearance or behaviour. At clinical examination, an enlarged liver or a thickened intestine may be noticed. M. avium and M. genavense typically cause a widely disseminated disease. Mycobacteria spread from the intestine which is first colonized following ingestion and any organ system may be affected. The liver, spleen, lung, air sac, skin and bone marrow are most often involved. Less common are cutaneous, subcutaneous or ocular granulomatous lesions (2,3,4,5).

An antemortem diagnosis is only successful in biopsies of skin lesions or those taken during laparascopy. Post mortem impression smears stained according to Ziehl-Neelsen are a fast procedure with a clear diagnosis in positive cases (2,3,4,5). Cultivation of mycobacteria requires much time and experience. Histological lesions can also vary and according to some reports are divided into three groups. In the first group, only a proliferation of epitheloid cells laden with mycobacteria and associated

inflammatory cells such as heterophils and round cells are noted. In the second group, single epitheloid cells can be seen and in the third group, large expanses of epitheloid cells laden with mycobacteria, without accompanying inflammatory infiltrates, are found. There seems to be a variation of these findings depending on the host species. However, there is no diversity in the histology of lesions between the different species of mycobacteria (2,3,4).

AFIP Diagnosis: Subcutis, right leg (per contributor): Panniculitis, granulomatous, diffuse, severe, green-winged macaw (*Ara chloroptera*), avian.

Conference Comment: The contributor provides an excellent review of mycobacterial infections. Although *Mycobacterium avium* may be much more common in birds**,** especially under crowded conditions, this case demonstrates the susceptibility of psittacines to *M. tuberculosis*.

Following inhalation, mycobacteria are endocytosed by macrophages via mannose receptors which bind a cell wall glycolipid called lipoarabinomannan. Cord factor (trehalose dimycolate) is a glycolipid that inhibits or decreases PMN chemotaxis, phagosome-lysosome fusion, TNF secretion and microsomal enzyme function. By inhibiting phagosome-lysosome fusion the mycobacteria are able to survive and replicate within the phagosome. There are a number of virulence factors which contribute to infection by *M. tuberculosis* including Wax D and mycolic acids within the mycobacterial cell wall. Muramyl dipeptide and various glycolipids attract antigen–processing cells for antigen presentation to naïve T-cells.

Antigen presenting cells such as alveolar macrophages present mycobacterial antigen and MHC class II proteins, to naïve T-cells. Binding of the T-cell receptor with mycobacterial antigen and exposure to the cytokine interleukin-12 (IL-12), released by the antigen presenting cell, drives the differentiation from naïve CD4+ T-cell to T-helper 1 lymphocyte which, in-turn, secretes interferon gamma (IFN-∂). IFN-∂ "activates the macrophages" which promotes phagosome-lysosome fusion, exposing mycobacteria to a destructive acidic environment. Activation also results in increased production of inducible nitrous oxide synthase (iNOS) which generates free radicals capable of destroying mycobacteria. Activated macrophages also release tumor necrosis factor (TNF) which is chemotactic for monocytes and induces them to differentiate into epithelioid macrophages or histiocytes. These epithelioid macrophages are characteristic of the delayed type hypersensitivity response and, through the secretion of several mediators of inflammation such as TNF, IL-1 and additional IL-12, continue to amplify the T helper 1 lymphocyte response. Additionally, they also secrete platelet-derived growth factor (PDGF) which stimulates fibroblast proliferation and collagen synthesis. The end result is continued inflammation, lymphocyte activation, and fibrosis resulting in the formation of a granuloma.

The hallmark of disease caused by *M. tuberculosis* is the development of tubercles. Tubercles form when granulomas develop and coalesce. The center of a tubercle may contain caseous necrotic debris bound by epithelioid macrophages, multinucleated giant cells, fibroblasts, collagen, lymphocytes and plasma cells. Although rare in birds, but not other species, tubercles may become calcified.

Tuberculosis in man has been recognized since the age of the ancient Egyptians. Currently, it is the second leading cause of death due to infectious disease, behind the Human Immunodeficiency Virus (HIV), and there are approximately 8-10 million new cases each year.

The presence of classical granulomas should promote the investigator to consider mycobacteriosis as a cause. Mycobacteria are long, thin, non-motile and non-spore forming bacilli. The acid-fast reaction can help identify acid retention in the walls of the mycobacteria which makes them appear as tiny, red threads within macrophages and cellular debris, most commonly found at the edge of a lesion. Delayed type hypersensitivity, the tuberculin response and granuloma formation develop through a classic sequence of events involving multiple inflammatory cells, mediators of inflammation and growth factors and must be a process familiar to every student of pathology (6).

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SLIDE 14
CONFERENCE 4 / CASE II – 8005-1011 (AFIP 2985455)

Signalment: 15 week old, intact male, Labrador Retriever, *Canis familiaris*.

History: A two cm diameter mass on the right maxilla was removed by the referring veterinarian and submitted for histopathology. The mass recurred over the next two weeks. The patient was referred to Mississippi State University College of Veterinary Medicine, and a CT scan demonstrated that the mass invaded from the maxilla to the zygomatic arch and cribriform plate. The animal was euthanized March 9, 2005.

Gross Pathology: A four cm by two cm, firm mass expands the right maxilla, just cranial to the zygomatic arch. The surface of the mass is ulcerated and hemorrhagic. On cut surface, the mass is firm and fibromatous and bordered by thin cortical bone. The submandibular lymph nodes are enlarged. The other abnormalities noted on necropsy are mild pulmonary edema and endoparasitism (*Dipylidium* sp.).

Histopathologic Description: Maxillary mass: A poorly circumscribed neoplasm is composed of irregular cords of odontogenic epithelium in a pale, basophilic mesenchyme (suggestive of dental pulp) and small amounts of collagenous, fibrovascular supporting tissue. The morphology of the neoplastic epithelial cells varies widely. Well-differentiated cuboidal to columnar epithelial cells palisade along eosinophilic material with fine, tubular cavities (dentin) or hyaline material (enamel). The features of these cells include vacuolated, basophilic cytoplasm, round to oval apical nuclei, finely stippled chromatin, and small nucleoli. Poorly differentiated epithelial cells are pseudostratified and blend into the surrounding stellate stroma. The stroma is composed of spindle, stellate, and fewer polygonal cells with variably distinct margins and moderate, amphophilic to basophilic cytoplasm. The nuclei are round to oval and contain finely stippled chromatin and inconspicuous to small nucleoli. In one section, the mass compresses trabecular bone, and reactive bone formation is present. The gingival surface is ulcerated and covered by a moderate amount of beaded, eosinophilic, fibrillar material (fibrin) and a moderate number of degenerating neutrophils and macrophages and fewer lymphocytes and plasma cells. Immunohistochemistry demonstrates that the epithelial cells have positive cytoplasmic staining for cytokeratin; the mesenchyme stains positively for vimentin.

Contributor's Morphologic Diagnosis: Ameloblastic fibro-odontoma

Contributor's Comment: Odontogenic tumors are rare in animal species. Multiple classification schemes exist for these tumors. One scheme divides odontogenic tumors based on the inductive effects of the odontogenic epithelium on the surrounding epithelial tissues. In addition, tumors may be classified as epithelial, mesenchymal, or mixed odontogenic tumors. The World Health Organization divides tumors based on benign or malignant characteristics (1).

Ameloblastomas are locally invasive, slow growing odontogenic epithelial tumors that typically do not metastasize (1). These tumors occur more frequently in dogs and cattle than other species and may arise at any age. Ameloblastomas occur more commonly

in the mandible (2). Those tumors that occur within bone are central, whereas, gingival tumors are peripheral. The prominent features of ameloblastic basal cells are as follows: palisading epithelial cells, apical nucleus, cytoplasmic clearing, and intercellular bridging (3). Odontogenic epithelial cells may occur in follicular or plexiform patterns. The tumor cells originate from the dental lamina, outer enamel epithelium, dental follicles of unerupted teeth, oral epithelium, or odontogenic epithelium (2). Keratinizing ameloblastomas are a type of ameloblastoma with pronounced keratinization of the epithelial cells (3). Acanthomatous ameloblastoma, previously named acanthomatous epulis, also arises from odontogenic epithelium, with prominent acanthocytes. These tumors are common in dogs, and may be bilateral. The tumors contain sheets of odontogenic epithelium with central acanthocyte formation (3). Amyloid- producing odontogenic tumors arise from odontogenic epithelium, and have features similar to ameloblastomas. However, amyloid matrix is interspersed among the epithelial cells (3).

Ameloblastic fibromas are typically slowly expanding, well-circumscribed, non-invasive odontogenic tumors (1), which occur more commonly in cats (2) and cattle (3). These tumors consist of epithelium similar to but less well developed than that of ameloblastomas. The connective tissue found throughout the tumor is composed of cellular fibroblastic tissue similar to dental papilla (1).

Ameloblastic fibro-odontomas occur in dogs, horses, and cows. These tumors resemble ameloblastic fibromas, but contain dentin and enamel and a more differentiated enamel epithelium (2). Gardner argues that these only occur in animals with mature dentition; in immature individuals, the tumor may be a developing stage of an odontoma (1). A malignant ameloblastic fibro-odontoma with metastasis was recently described in a dog (4).

Odontomas, often classified as hamartomas, contain well-differentiated dental tissues, including enamel, dentin, cementum, and dental pulp or its precursor, dental papilla. Those tumors with organized tooth structures, or denticles, are compound odontomas. Tumors with disorganized tooth components are complex odontomas (1). Odontomas are more common in cattle and horses; they usually occur in the mandibular or maxillary arch, distorting the bone and nearby teeth (2).

Odontoameloblastomas are very rare tumors that contain ameloblastic epithelium and distinct areas of compound or complex odontoma (2). Their biological behavior resembles that of ameloblastomas (1).

This tumor demonstrates many features of an ameloblastic fibro-odontoma, but may be a developing compound odontoma because of the age of the animal and the presence of denticles in the mass initially presented for histopathology. Ameloblastic fibro-odontomas are described as encapsulated, well-circumscribed neoplasms. The tumor in this case appears on CT scan to be invading the adjacent bone and histologically is poorly circumscribed. These features of malignancy are accompanied by cellular atypia; however, metastasis was not noted.

AFIP Diagnosis: Maxilla, right (per contributor): Odontogenic tumor, favor Ameloblastic fibro-odontoma, Labrador Retriever, canine.

Conference Comment: The Department of Oral Pathology reviewed this case and agreed that the neoplasm is consistent with an ameloblastic fibro-odontoma.

Teeth develop from two embryonic tissues: buccal cavity squamous epithelium and embryonic mesenchyme. During early tooth development, a portion of buccal cavity squamous epithelium invaginates into the subjacent embryonic mesenchyme and forms the dental lamina which ultimately forms the enamel organ and ameloblastic cell layer. All other parts of the tooth including dentin, cementum, and pulp, derive from embryonic mesenchyme.
Dental epithelial and mesenchymal cells have an inductive influence on each other. The ameloblastic epithelium induces differentiation of the dental papilla mesenchyme into odontoblasts, which form dentin. The presence of dentin induces the ameloblasts to form enamel. After odontogenesis, ameloblasts disintegrate; thus, damaged enamel cannot be repaired. Odontoblasts remain viable for the life of the tooth.

The World Health Organization currently divides odontogenic tumors into four major groups (5):

1) Tumors of odontogenic epithelium **without** odontogenic mesenchyme
    a. Ameloblastoma
    b. Amyloid producing odontogenic tumor
    c. Canine acanthomatous ameloblastoma (formerly know as acanthomatous epulis)

2) Tumors of odontogenic epithelium **with** odontogenic mesenchyme
    a. Ameloblastic fibroma
        1. Ameloblastic fibro-odontoma
    b. Feline inductive odontogenic tumor
    c. Complex odontoma
    d. Compound odontoma

3) Tumors composed primarily of odontogenic ectomesenchyme
    a. Cementoma
    b. Cementifying fibroma

4) Tumors derived from the tissues of the periodontal ligament
    a. Fibromatous epulis of periodontal ligament origin

Since the submitted neoplasm has both epithelial and mesenchymal components exhibiting ameloblastic and odontoblastic differentiation, conference attendees agreed

that it belonged with the **tumors of odontogenic epithelium with odontogenic mesenchyme** group. There was debate; however, as to which of the four neoplasms from that group best resembled this neoplasm. There is both ameloblastic epithelium present and odontoblasts forming dentinal tubules as well as internalized epithelial cells reminiscent of stellate reticulum. Additionally, there are multiple abnormally shaped, tooth like structures or denticles within the mass separated by dense collagenous connective tissue. Most agreed the neoplasm was most consistent with either ameloblastic fibro-odontoma or a developing compound odontoma.

Although dental neoplasms may be locally destructive, as in this case, a good prognosis is warranted if excision is complete; metastasis is unreported.

Categorizing odontogenic neoplasms can be a challenge which requires a basic understanding of odontogenesis, dental anatomy, a categorizing scheme and experience. The moderator stressed the importance of practicing how to describe odontogenic neoplasms using the appropriate terminology (i.e. don't let Ames be the first place you've had to write and describe dentinal tubules and stellate reticulum) and the need for each resident to develop and add a standard odontogenic neoplasm description to their repertoire.

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SLIDE 15
CONFERENCE 4 / CASE III – ND1 (AFIP 2983853)

Signalment: Three bighorn (*Ovis canadensis canadensis*) rams

History: These rams were hunter harvested in the fall of 2004. All animals were considered clinically normal. Lung tissue was collected by a referring veterinarian and forwarded to the diagnostic laboratory.

Gross Pathology: Fixed tissue only was submitted. There were well-defined, multifocal areas of pallor, primarily subpleural, in the pulmonary parenchyma.

Histopathologic Description: Microscopic examination revealed multiple, unencapsulated, well-demarcated foci characterized by inflammation, fibrosis and numerous intralesional nematodes. Representative foci showed moderate to marked interstitial lymphocytic to plasmacytic inflammation in both alveolar septa and affected locations subjacent to the pleura. Alveoli contained various amounts of proteinic material, lymphocytes, macrophages, and numerous nematode eggs, nematode larvae and cross sections of adult nematodes. Granulocytes were randomly present in small numbers but were not a significant component of the exudate. Adults and larvae were present in terminal bronchioles and some bronchi as well. Larva demonstrated the pointed tail characteristic of protostrongylids. There was atelectasis of unaffected adjacent parenchyma. Proximate bronchioles and bronchi were accentuated by smooth muscle hypertrophy, increased amounts of peribronchiolar and peribronchial connective tissue, scattered lymphoplasmacytic inflammation and moderate epithelial hyperplasia.

Contributor's Morphologic Diagnosis: Marked, multifocal lymphoplasmacytic and histiocytic pneumonia with interstitial fibrosis and numerous intralesional nematode ova, larva and adults consistent with *Protostrongylus stilesi*.

Contributor's Comment: Lungworm infections are well-reported in bighorn sheep. *Protostrongylus stilesi* and *P. rushi* are the two species most commonly associated with the condition. *Muellerius capillaris* has been reported, but this appears to be uncommon. In many areas the nematode is considered ubiquitous within resident sheep populations. The parasite is recognized as an important part of the pneumonia complex reported in bighorns and various techniques have been attempted to reduce the worm load in susceptible groups.

Protostrongylids belong to the order Strongyloidea, superfamily Metastrongyloidea, and family Protostrongylus. Adult worms reside in alveoli, bronchioles and bronchi of sheep. Adult males are thread-like brown nematodes measuring 16 to 28 mm in length while females measure 25 to 35 mm. Larva and eggs can be found in lung tissue as well. Following the hatching of embryonated eggs, L1 larva pass up the trachea either through the mechanism of coughing or the action of the mucociliary apparatus. Once in the mouth, L1 larva are swallowed and pass in the fecal pellets. The L1 larva are very resistant to the environment, apparently being able to overwinter in fecal material. This may be in part due to the fact that L1 larva are found in higher concentrations in the core of a fecal pellet rather than the edges. When conditions are favorable, L1 larva leave the fecal pellet and penetrate the foot tissue of the intermediate host, a terrestrial gastropod. Studies have indicated that moisture is an important predisposing factor in the movement of L1 larva in that the intermediate host is more available in wet

conditions. Several different species of gastropod have been identified as suitable intermediate hosts. Larval development continues within the gastropod to the L3 or infective stage; this may take anywhere from two to six weeks. At this point the larva containing gastropod is ingested by a sheep and infection occurs. The larvae subsequently molt to an L4 stage, penetrate the intestinal wall, enter lacteals, undergo a final molt in draining lymph nodes, enter the lymphatics and reach the heart and lungs. Finally, the larva migrate from alveolar capillaries into alveoli, bronchioles and bronchi where they develop into adults. The prepatent period is five to six weeks and patency is long, reported to exceed two years. The life cycle is complicated by the ability of the nematode to move via the uterine artery into the fetal circulation in pregnant ewes. L3 larva have been found in the liver and lungs of fetal lambs.

AFIP Diagnosis: Lung: Pneumonia, lymphoplasmacytic, histiocytic and eosinophilic, chronic, multifocal, moderate, with bronchiolar smooth muscle hypertrophy, and myriad metastrongyle eggs, larvae, and few adults, etiology consistent with *Protostrongylus* sp., bighorn sheep (*Ovis canadensis canadensis*), ovine.

Conference Comment: The contributor provides an excellent review of lungworm infestations in bighorn sheep, with reference to domestic sheep as well.

In general, there are three well known and common parasitic pneumonias of sheep and goats. All three are caused by metastrongyles which can be differentiated from the true strongyles and trichostrongyles by having coelomyarian instead of platymyarian musculature. Although difficult to recognize, another distinguishing feature of adult metastrongyles are accessory hypodermal chords. Metastrongyle larvae can often be identified by a characteristic finger-like projection at the tip of their tail (6).

Dictyocaulus filaria commonly affects lambs and goat kids and causes partial obstruction of small bronchi, atelectasis, eosinophilic bronchitis, lymphoid and type II pneumocyte hyperplasia and an alveolar exudate. *Muellerius capillaris* also known as the “nodular lungworm” affects both sheep and goats and forms numerous subpleural nodules, especially in the dorsal part of the caudal lung lobes. Nodules are the result of an eosinophilic and granulomatous response to subpleural adults, eggs and larvae. *Protostrongylus rufescens* usually affects lambs and goat kids and can cause mucopurulent nasal discharge, anorexia, diarrhea and weight loss (7).

Multiple species are affected by lungworms. Below is a list of the more common lungworms in domestic animals and phocid pinnipeds. This list is intended to be used as a training aid and is by no means all inclusive.

Lungworms of domestic animals:

- *Aelurostrongylus abstrusus* – cats; catarrhal bronchiolitis, submucosal gland hyperplasia, granulomatous alveolitis, alveolar fibrosis
- *Capillaria aerophila* – dogs, cats, foxes; dogs and cats very mild infection
- *Crenosoma vulpis* – foxes, occasionally dogs; eosinophilic catarrhal bronchitis and

bronchiolitis
- *Filaroides hirthi*, *F. milksi* – dogs, mink; pyogranulomatous, eosinophilic pneumonia
- *Angiostrongylus vasorum* – dogs, foxes; inhabits pulmonary artery and right ventricle
- *Dictyocaulus filaria* – sheep and goats; catarrhal and eosinophilic bronchitis and bronchiolitis
- *Dictyocaulus viviparous* – cattle; pneumonia, bronchitis, pulmonary edema and emphysema
- *Dictyocaulus arnfieldi* – horses, donkeys; obstructive or eosinophilic bronchitis, edema, atelectasis
- *Muellerius capillaris* – sheep and goats; small nodular lesions on the lung surface
- *Protostrongylus rufescens* – sheep and goats; lambs and kids; lives in bronchioles, causes pulmonary nodules.
- *Metastrongylus apri* – pigs; growth retardation, bronchitis, catarrhal inflammation

Lungworms of phocid pinnipeds:
- *Otostrongylus circumlitis* - the only other lungworm of phocid pinnipeds (seals: harbor, ringed, spotted, ribbon, Baikal, grey, bearded, northern elephant)
    - Large roundworm found in large bronchi and bronchioles; causes
    - vasculitis
- *Parafilaroides* spp. – spp. based on host and geographic range
    - *P. gymnurus* – final host: phocid seals (harbor, ringed, harp, spotted, grey, bearded, Baikal); range: coastal waters of Canada, Europe, and Russia
    - *P. hydrurgae* – final host: leopard seal; range: Southern Ocean
    - *P. decorus*- final host: California sea lion, Stellar sea lion, northern fur seal; range: Pacific waters of the United States

P. hispidus – final host: phocid seals (ringed and grey); range: coastal
    - waters of Canada
    - *P. caspicus* – final host: Caspian seal; range: Caspian Sea

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

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SLIDE 16
CONFERENCE 4 / CASE IV – B7968-03 (AFIP 2984962)

Signalment: 5 years, male, Petit Basset Griffon Vendéen, *Canis familiaris*, dog

History: Nausea, vomiting and marked salivation for less than 2 weeks at presentation to the clinician. Clinically, mandibular glands were bilaterally tender and enlarged, and the dog had a low-grade fever (39.1^{o}C). Both glands were removed and antibiotic treatment was instituted. The condition was markedly improved after a few days but the case was lost for long term follow-up.

Laboratory Results: Leukocytosis (17.2x10^{9}WBC/l, 85% neutrophils, no left shift). Hematocrit 36% (N 43-52).

Histopathologic Description: A piece of one mandibular salivary gland for examination. There is a sharply delineated area of parenchymal coagulative necrosis with preserved lobular architecture. Fibroblast-rich immature granulation tissue, inflammatory edema and hemorrhage occur in the necrotic area and multifocally in adjacent lobules. Mild to moderate infiltrates of polymorphonuclear leukocytes appear multifocally, even within neighboring glandular acini. Several epithelial ducts with squamous metaplasia and pleomorphic irregular epithelial hyperplasia occur in the lesions and sometimes show prominent mitotic activity and mild nuclear atypia. Necrotic acinar cells occur multifocally intimately associated with metaplasia and hyperplasia, indicating metaplasia of glandular cells. Only in some sections, focal necrotizing arteritis with fibrinoid necrosis and even thrombosis, is evident in the interstitial tissue bordering the necrotic parenchyma.

Contributor's Morphologic Diagnosis: Necrotizing sialometaplasia.

Contributor's Comment: Necrotizing sialometaplasia in humans is a self-limiting benign inflammatory disorder, mainly of the minor salivary glands, and affecting major

glands only rarely (1). It is also termed salivary gland infarction. Major morphologic features include squamous metaplasia of ducts and acini, lobular coagulation necrosis with preservation of lobular architecture, granulation tissue, and, in minor salivary glands, overlying pseudoepitheliomatous mucosal hyperplasia (1). The cause is not established but the necrosis is of ischemic type and underlying blood vessel injury is often proposed. Several factors have been proposed to explain vascular changes (1). This morphologic entity also occurs in dogs and cats, but involvement of mandibular glands is characteristic in these species (2,3,4,5) and the disease has much more severe clinical signs than the human analogy (5). In one large series of salivary gland specimens submitted to a diagnostic laboratory, the diagnosis was established in 9/160 dogs and 11/85 cats (2). In those cases, though an ischemic basis was considered likely, arterial thrombosis was only found in one specimen, whereas three canine cases described by others (3,5) had vascular thrombi. The metaplastic and hyperplastic epithelial changes are considered as a reparative response (1,2). Since they, as in the present case, often show pseudocarcinomatous features, the diagnosis may not be straightforward but can be confused with salivary gland malignancies in both humans and animal species (1,4,5).

AFIP Diagnosis: Salivary gland, mandibular: Coagulative necrosis (infarct), multifocal, with ductular hyperplasia and squamous metaplasia, Petit Basset Griffon Vendéen, canine.

Conference Comment: The contributor provides an excellent and concise review of necrotizing sialometaplasia. In humans, four criteria must be met for a diagnosis of necrotizing sialometaplasia:

1) lobular necrosis of salivary tissue
2) squamous metaplasia conforming to duct outlines, acinar outlines, or both
3) maintenance of salivary lobular morphology
4) time-variable prominence of granulation tissue and acute and chronic inflammation (5).

Necrotizing sialometaplasia most commonly affects terriers, is extremely painful and often causes nausea, inappetence and vomiting. Most importantly, in chronic cases, squamous metaplasia and cellular atypia of salivary ducts can appear very similar to salivary gland neoplasia, especially if occluded ducts are surrounded by abundant fibrous tissue making them resemble islands of neoplastic cells.

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SLIDE 17
CONFERENCE 5 / CASE I – 04A486 (AFIP 2987468)

Signalment: 17 day old male Indian Rhesus macaque (*Macaca mulatta*).

History: This animal was born within the Tulane National Primate Research Center colony. Following culture of *Campylobacter coli* from a rectal swab taken at 2 days of age, the infant was transferred to the nursery for care at 3 days of age. Fluid stool, poor appetite and severe dehydration were observed clinically at 17 days of age and the animal died despite receiving subcutaneous and oral fluid supplementation.

Gross Pathology: The body was in good condition with adequate fat stores, but subcutaneous tissues were tacky and the eyes sunken interpreted as dehydration greater than 15% of body weight. The stomach contained approximately 20 ml of curdled ingesta mixed with a clear watery fluid. The small intestine was markedly dilated with clear watery fluid mixed with small amounts of ingesta (Figure 1). The colon was empty and the luminal surfaces were dry. The esophagus contained a small amount of ingesta similar to that found in the stomach. The oral and nasal cavities contained a small amount of curdled ingesta.

Laboratory Results: *Campylobacter coli* was isolated from a rectal swab 1 day prior to transferal to the nursery. *Campylobacter coli* and *Escherichia coli* were isolated from intestine at necropsy. No Shigella, Salmonella, or Yersinia were isolated at necropsy. The *E. coli* culture was submitted to the Gastroenteric Disease Center, Pennsylvania State University, University Park, PA, USA (http://ecoli.cas.psu.edu) for typing. The results are presented in the table below.

Abbrev	Description	Result
O Type	Somatic antigen	**O55**
H Type	Flagellar antigen	**H7**
LT	Heat labile toxin	Negative
STa	Heat stable toxin a	Negative
STb	Heat stable toxin b	Negative
SLT1	Shiga-like toxin I	Negative
SLT2	Shiga-like toxin II	Negative
CNF1	Cytotoxic necrotizing factor 1	Negative
CNF2	Cytotoxic necrotizing factor 2	Negative
EAE	Intimin	**Positive**
BFP	Bundle forming pili	Negative

TABLE

Histopathologic Description: Colon and small intestine are submitted. The small intestine has moderate autolytic changes, however lesions are still evident. There is marked villus atrophy and fusion with crypt regeneration and increased numbers of mitotic figures in the crypts (Figure 2). Segments of intestinal epithelium have numerous plump short rods adherent to their apical surfaces (Figure 3). These cells are often attenuated and are sometimes vacuolated. Adherent bacteria are most frequently seen in the ostia to the crypts. The bacteria are Gram negative (Figure 4) and readily demonstrable by Giemsa staining (Figure 5). The colon also has moderate autolytic change, but within the lumen are numerous teardrop-shaped to oval protozoa 5-20 um in length and 3-7 um in width, with a single eccentric nucleus which colonize the surface and superficial glandular lumina (Figure 6). The morphology of the protozoa is consistent with a trichomonad species. There is little to no tissue reaction to the trichomonads.

Contributor's Morphologic Diagnosis: Small intestine: Villus atrophy and fusion with epithelial degeneration and colonization of epithelium by short rods.

Colon: Marked colonization by trichomonads.

Contributor's Comment: The marked villus atrophy and fusion, in the presence of adherent gram negative rod bacteria, is characteristic of attaching and effacing *E. coli* (AEEC) infection. This is consistent with culture results of an *E. coli* strain carrying the

intimin (*eae*) gene. Sestak and colleagues found up to 25% of animals at the Tulane National Primate Research Center (TNPRC) were carriers of *E. coli* strains with enteric virulence genes, regardless of the presence or absence of diarrhea (1). However, enteropathogenic *E. coli* (EPEC) infections are considered significant causes of diarrhea and wasting in macaques with AIDS (2). The historically high incidence of previous episodes of diarrhea in the healthy TNPRC cohort (32%) (1) may reflect previous clinical disease associated with EPEC strains which are endemic to the colony.

E. coli causes urinary tract infections, septicemia, and enteric disease (3). Classification of *E. coli* strains is partly dependent upon the somatic (O), flagellar (H), and capsular polysaccharide (K) antigens which are associated with specific clinical syndromes but are generally not virulence factors themselves. For enteric E. coli infections, a number of toxins and colonization factors have been identified and are associated with specific clinical syndromes, outlined in the table below. Some of these factors are carried on pathogenicity islands in the bacterial chromosome (e.g. type III secretion systems, Tir, eae, espB), while others are carried on virulence plasmids (e.g. ST, LT, BFP) or have phage-delivered virulence sequences (e.g. Shiga toxin).

Bonnet macaques (*Macaca radiata*) have been shown to be susceptible to experimental inoculation with enterohemorrhagic *E. coli* with an O157:H7 strain, resulting in attaching-effacing lesions with epithelial degeneration and neutrophilic infiltration without fecal blood (5). Examination of fecal samples from healthy and diarrheic new world monkeys (Black tufted ear marmoset, *Callithrix penicillata*; Common marmoset, *C. jacchus*; White-fronted marmoset, *C. geoffroyi*; Saddlebacked tamarin, *Saguinus fuscicollis*; and a Brown howler monkey, *Alouatta fusca*) yielded several *E. coli* strains carrying intimin genes with and without the bundle forming pilus (BFP)(6, 7). Intimin-positive *E. coli* were also isolated from cotton-top tamarins (*Saguinus oedipus*) and associated with active colitis (8). *E. coli* carrying the intimin gene with the BFP are considered “typical” enteropathogenic *E. coli* (EPEC), whereas those without BFP are termed “atypical” EPEC (9). In the case presented here, BFP was not expressed and it would be classified as atypical EPEC.

The serotype of this case (O55:H7) is unlike any EPEC isolates reported from New World monkeys (6, 7), but is instead a strain associated worldwide with infantile diarrhea which is believe to have given rise to the O157:H7 enterohemorrhagic *E. coli* strain (10). We have previously found intimin expression in macaques within our colony (1), indicating EPEC is endemic. However, we cannot exclude the possibility that, in the setting of the nursery, a nosocomial infection occurred.

The overgrowth of trichomonads in the colon of this monkey was not associated with lesions in the colon. However, trichomonads, tentatively identified as *Tritrichomonas mobilensis*, are opportunistic pathogens and have been reported to invade mucosal sites during AIDS (11).

Abbrev	Name	Virulence factors	Disease association and pathogenesis comments
ETEC	Enterotoxigenic *E. coli*	Heat-stable toxins (STa, STb) Heat-labile toxins (LT-I, LT-II) colonization fimbriae (K88, K99)	diarrhea in neonates (human, pig, calf, lamb) diarrhea in non-immune adults (traveler's diarrhea)
EHEC	Enterohemorrhagic *E. coli* (typified by O157:H7) EHEC is a subset of *E. coli* strains producing Shiga toxins (STEC)which are also termed vero cytotoxins (VTEC)	Shiga(-like) toxins (Stx1 and Stx2) Heat stable toxin (EAST1) in many strains Intimin (eae) chuA (*E. coli* heme utilization gene) enterohemolysin (pathogenetic effects uncertain)	bloody diarrhea which may be followed by hemolytic uremic syndrome (acute renal failure, thrombocytopenia, microangiopathic hemolytic anemia) Shiga-like toxin effects predominate (hemorrhage and edema of lamina propria), but attaching/effacing lesions occur early
*EAEC or EAggEC	Enteroaggregative *E. coli*	fimbriae (AAF/I, AAF/II) and a heat stable toxin EAST1 may play a role in pathogenesis	diarrhea in children and healthy adults with excess mucus production
*DAEC	Diffusely adherent *E. coli*	AIDA-I, an outer membrane protein	watery diarrhea in children 1-5 yrs and possibly nosocomial infections
EPEC	Enteropathogenic *E. coli*	bundle-forming pilus (BFP) a type III secretion system (sep, esc) and Tir (translocated intimin receptor) Intimin (eae) EPEC-secreted proteins (EspA, EspB, EspD) A heat-stable toxin (EAST1) in some EPEC strains	diarrhea in neonates (humans, rabbits, calves, pigs, dogs) but only very rarely in older hosts attaching and effacing histopathology with cytoskeletal rearrangement increased chloride secretion and enterocyte permeability due to pathologic signal transduction in the enterocyte and malabsorption contribute to diarrhea
*EIEC	Enteroinvasive *E. coli* Very similar to Shigella spp.	pInv plasmid encoding mxi, spa (type III secretory apparatus) and IpaA, IpaB, IpaC, IpaD invasiveness factors	Initially watery diarrhea which becomes scant and bloody Lesions similar to Shigella, colonic ulceration
NTEC	Necrotoxigenic *E. coli*	cytotoxic necrotizing factor (CNF1, CNF2)	extraintestinal infections, diarrhea isolated from humans, cattle, pigs, sheep, and dog

*EAEC, DAEC, and EIEC are human pathogens only. EPEC and EHEC together cause attaching/effacing lesions and are also termed attaching and effacing *E. coli* (AEEC)

References: 3, 4

AFIP Diagnoses: 1. Small intestine: Villus atrophy and fusion, diffuse, marked, with enterocyte degeneration and myriad adherent short bacilli, Rhesus Macaque (*Macaca mulatta*), primate.

2. Colon, lumen and glands: Myriad trichomonads.

Conference Comment: The contributor provides a comprehensive review of *E. coli* infection in non-human primates and how different strains of *E. coli* are classified.

A simplified pathogenesis for AEEC begins with the loose attachment to enterocytes by a plasmid-encoded adhesin (EPEC adhesive factor, EAF) which binds via a cell surface glycoprotein receptor, possibly fibronectin. The next step involves the translocation of bacterial proteins into the cytosol of the host cell resulting in cytoskeletal reorganization, effacement of microvilli and formation of a pedestal of cytoplasm that protrudes from the enterocyte and cups the bacteria. Intimate attachment is mediated by a surface-exposed bacterial outer membrane protein called intimin which binds to a receptor called the translocated intimin receptor (Tir) which was originally translocated into the host cell along with other bacterial proteins and has since undergone structural changes. Finally, invasion of host cells is mediated by EAF and the attaching and effacing (eae) gene. Invasion is generally more profound with EPEC strains than with EHEC strains (11).

Causes of diarrhea in non-human primates include:

- *E. coli* (ETEC, EPEC, EHEC)
- Shigellosis [*Shigella flexneri* (most common)*, S. dysenteriae*, *S. boydii*, and *S. sonnei*]: lesions limited to large intestine; colonic edema, hemorrhage and ulcers +/- pseudomembrane
- Campylobacteriosis (*Campylobacter. jejuni* and *C. coli*): similar to shigellosis but also affects small intestine; necrosis, edema, hemorrhage, villus blunting and fusion
- Salmonellosis (*Salmonella enteritidis* and *S. typhimurium)*: fibrinonecrotic enteritis, typhlitis and colitis
- *Yersinia enterocolitica*: multifocal intestinal mucosal necrosis, ulcers, hemorrhage, marked neutrophilic inflammation, and large, lobulated bacterial colonies
- *Y. pseudotuberculosis*: acute, severe, fibrinonecrotic enteritis, and mesenteric lymphadenitis with necrosis in the liver, lung, and spleen
- *Proteus vulgaris* and *P. morgani*
- *Cryptosporidium parvum*: protozoa usually line apical border of small intestine enterocytes
- Pinworms: *Oxyuris* sp., *Trypanoxyuris* sp., *Enterobius* sp.

- *Balantidium coli*: ulcerative colitis
- Stress, dietary, inflammatory bowel disease

An excellent review article titled “Attaching-effacing Bacteria in Animals” was recently published in the Journal of Comparative Pathology (11).

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SLIDE 18
CONFERENCE 5 / CASE II – 2 (AFIP 2987460)

Signalment: 11-year-old female Franche Montagne Horse (*Equus caballus*)**.**

History: The mare was submitted to the horse clinic, veterinary hospital of the University of Berne, Switzerland with symptoms of therapy resistant fever for 4 weeks, intermittent severe diarrhea and severe ascites. The animal was euthanized.

Gross Pathology: Gross examination revealed approximately 10 liters of clear, yellow fluid in the thoracic cavity and 20 liters of similar fluid in the abdominal cavity (pleural and abdominal effusion). The parietal and the visceral pleura showed areas of numerous, raised, firm reddish nodules (from 0.1 cm up to 1.5 cm diameter). The lung had a thick, fibrotic surface and multifocal to confluent reddish nodules. Inside the abdominal cavity, the ascending colon, the liver, the mesenterium and the diaphragm had thick surfaces and multifocal to confluent reddish nodules.

Laboratory Results: Direct microscopic examination of abdominal fluid revealed mesothelial cells and mitotic figures.

Histopathologic Description: Mesenterium: Expanding the tissue is an unencapsulated, poorly demarcated, infiltratively growing neoplasm composed of nests and bundles of neoplastic mesothelial cells which occasionally palisade along or form papillary projections on the serosa supported by a large amount of fibrovascular stroma. Neoplastic cells are cuboidal to polygonal to spindle shaped with distinct cell borders. They have a moderate amount of pale eosinophilic fine fibrillar cytoplasm and one round to oval nucleus with finely stippled chromatin and up to two prominent nucleoli. There is moderate to severe anisocytosis and anisokaryosis and occasional multinucleated neoplastic cells. There is less than one mitotic figure per 10 high power fields. Large numbers of lymphatics are dilated and filled by neoplastic emboli. Rarely, on some slides, blood vessels are filled with neoplastic cells, which form papillary projections in the lumen and are admixed with fibrin. Multifocally endothelial cells lining blood vessels are plump (reactive). Multifocally there are foci of necrosis, increased clear space (edema), hyperemia, extravasated erythrocytes (hemorrhage) and multifocal infiltration by few degenerate neutrophils, lymphocytes and plasma cells.

Lung: the serosal surface is severely thickened due to fibrous tissue and infiltration by neoplastic mesothelial cells; multifocally the underlying interstitium is severely thickened by proliferation of fibrous tissue that is infiltrated by the same neoplastic cells. Neoplastic cells also fill alveolar spaces and alveolar capillaries. Multifocally tumor emboli are found also in larger blood vessels, occasionally accompanied by fibrin thrombi. Within alveolar spaces there are scattered foamy macrophages, edema fluid and fibrin.

Contributor's Morphologic Diagnoses: Lung and mesenterium: Malignant mesothelioma, multifocal, Franche Montagne, equine.

Contributor's Comment: Mesotheliomas are primary tumors arising from the mesothelial cells lining the body cavities (thoracic, peritoneal and pericardial cavities) (4). Cases of involvement of the tunica vaginalis of the testis have been reported also. They are rare neoplasms of low grade malignancy with rare metastasis (usually via exfoliated cells). In domestic animals they have greatest frequency in cattle and dogs. They occur most frequently as a congenital neoplasm in fetal or young cattle. Actually, cattle and sheep develop tumors in fetal, newborn and young animals whereas in others species, adult or aged animals are affected. In cattle and goats the location is most commonly peritoneal; in swine mainly pleural. In dogs, the pleura is the main site of development after the pericardium and the peritoneal cavity. Mesothelioma arising from the tunica vaginalis is one of the most common tumors of the male Fischer 344 rat.

Mesotheliomas are rarely seen in horses in which they are reported as mostly malignant, usually pleural and pericardial but also abdominal (1, 2, 3, 5). Mesotheliomas are also reported in buffalo, mice, fowl, hamsters, and cats.
In humans, an association between malignant mesothelioma and asbestos exposure has been well established. In domestic animals, they generally occurs spontaneously. Ferruginous bodies (fine fibers coated with amorphous protein and ferritin) which are suggestive of asbestos exposure, have been identified in the lungs of urban dogs with mesothelioma (this association is confirmed in humans). However there is little evidence for a relationship between asbestos inhalation and mesothelioma. Concerning newborn cattle, a congenital origin has been found for mesotheliomas.

The clinical signs associated with mesothelioma are typically anorexia and depression, dyspnea, respiratory distress, cardiac insufficiency, cardiac tamponade, decreased lung sounds, and/or abdominal distension (accumulation of large amounts of fluid, resulting in ascites) depending upon the location of the tumor.
On gross examination, tumors are formed either by small nodules, sessile or pedunculated, from a few millimeters to 10 centimeters in diameter or by villous projections arising from the serosal surface. Depending on the amount of hemorrhage, the color varies from gray-white to red. Some fibrous or sclerosing forms have been reported as well as milky or blood-tinged effusion. Ascites if the peritoneum is involved and adhesions may also be present.

Mesothelioma can be papillary epithelioid or sarcomatoid or, most commonly, a combination of these two patterns (biphasic). Generally cells are pleomorphic (polygonal to spindle-shaped), occasionally multinucleated and display anisokaryosis with a prominent nucleolus. Neoplastic cells can line cystic spaces and tubular structures which may contain a mucinous matrix. Cells line papillary structures in several layers. Mitotic figures are rare. In the epithelioid form, cuboidal epithelioid cells cover papillary projections made of spindle-shape cells and conjunctivo-vascular stroma. Sometimes cells can form tubules or rosettes and mitoses are usually not

numerous. Sclerosing mesotheliomas are composed of large anaplastic cells with sometimes multinucleated giant cells. (6)

Mesotheliomas are of low grade malignancy so there is limited invasive growth. Metastases to drainage lymph nodes are rare and distant metastases very rare. It is not always possible to determine whether widespread involvement of the peritoneal cavity and the tunica vaginalis is the result of secondary spread or of multicentric origin. (6)

Concerning the epithelioid pattern, the differential diagnosis must include metastasis from an adenocarcinoma located in another site, mammary gland or ovary for examples. Lipomas, liposarcomas and more rare tumors such as ganglioneuromas can also develop from the serous membranes.
Ultrastructurally, neoplastic mesothelial cells have a prominent basal lamina, many long slender, well-developed microvilli present on all surfaces, prominent desmosomes, abundant rough endoplasmic reticulum, and mitochondria.
Immunohistochemical stains may be helpful in differentiating mesothelioma from other neoplasms. Mesothelioma are positive for vimentin, cytokeratin 5, LP 34; they are negative for CEA (carcinoembryonic antigen), CD 15 (LEU M1), BER EP4 (tumor glycoprotein) (markers for neoplasm of epithelial origin), S100, HMB 45 (markers for neoplasm of melanocytic origin).

The mucicarmine stain with and without hyaluronidase can help distinguish mesothelioma from adenocarcinoma. The presence of mucicarminophilic, hyaluronidase resistant material within cytoplasmic vacuoles supports adenocarcinoma. By immunohistochemistry, neoplastic cells of mesothelioma are typically positive for both keratin and vimentin. Carcinomas are usually keratin positive and vimentin negative.

Calretinin was also used as an immunohistochemical marker in the diagnosis of mesothelioma in an equine species (7: Stoica G. et al. 2004).

Immunohistochemically, in the present case, the neoplastic cells are multifocally positive for keratin and occasionally positive for vimentin. Thus, histomorphology and immunohistochemistry support mesothelioma. The calretinin immunohistochemical stain is running. The widespread involvement of the peritoneal cavity and the involvement of the lung parenchyma render this case particularly rare and interesting.

AFIP Diagnoses: 1. Mesentery (per contributor): Malignant neoplasm, with vascular invasion, and marked sclerosis, Franche Montagne horse, equine.

2. Lung, alveolar capillary and arterial lumina: Malignant neoplasm, with thrombi, infarcts, edema, and marked interlobular and pleural fibroplasia.

Conference Comment: This neoplasm has proven to be a challenge to both describe and diagnose. Conference attendees debated everything from tissue identification and

cell shape to immunohistochemical interpretations. The section of mesentery was simply identified as fibroadipose tissue and the arrangement of neoplastic cells did not easily fit into any of the three recognized patterns for mesotheliomas (i.e. papillary epithelioid, sarcomatoid or biphasic). Several attendees recognized the numerous pulmonary vessels filled with tumor cells and considered a round cell neoplasm such as lymphoma. Others favored widespread carcinomatosis as a differential. All agreed neoplastic cells express strong cytoplasmic immunopositivity for pancytokeratin; however, both the AFIP's vimentin and calretinin results are equivocal. All agreed that electron microscopy to identify desmosomes and microvilli on the cells would be very helpful in differentiating carcinoma from mesothelioma. Regardless, the contributor provides an excellent, detailed review of mesotheliomas and a challenging neoplasm to spark much debate.

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SLIDE 19
CONFERENCE 5 / CASE III – 05040376-2,3 (AFIP 2985397)

Signalment: Yearling mixed breed bovine

History: During late winter/early spring of 2005, increased morbidity and mortality was reported in range cattle on the Navajo Reservation in northeast Arizona. Clinical signs included: staggering, stupor, blindness and tongue paralysis. Yearlings and two year olds were principally affected. Tansy mustard (*Descurainia pinnata*) was in exuberant bloom on the range.

Gross Pathology: Field necropsies of affected animals revealed no significant gross lesions.

Laboratory Results: Toxicological analysis:

Sample: Tansy mustard, leaves and stems
Selenium 0.48 ppm
Sulfur 9410 ppm
Nitrate 2392 ppm

Histopathologic Description: Multiple sections of cerebrum, cerebellum and brainstem are examined and significant lesions are restricted to the cerebral cortex. There are disseminated foci of laminar cortical necrosis of neurons characterized by shrunken, hypereosinophilic neurons that exhibit either karyorrhexis, karyolysis or complete loss of nuclear detail. Regionally, the foci of necrosis are accompanied by mild to marked populations of astrocytes (astrogliosis) and rarefaction of the neuropil. Both sulci and gyri are affected.

Contributor's Morphologic Diagnosis: Brain, cerebral gray matter: Severe laminar cortical necrosis with prominent astrogliosis

Contributor's Comment: *Descuraninia* spp. plants are native to most regions of North America and are present in open prairie, fields and along roadways. Several species of plants in this family are potentially toxic to animals and *Descuraninia pinnata* (tansy mustard) associated with "tongue paralysis of cattle" is the most well known, *see review by Burrows* (1).

Cattle are the principle species affected in reported outbreak situations; however, sheep, horses and goats may also be affected. Toxicities under range conditions follow winters of over-abundant moisture. In this case, abundant plant growth was reported because of above normal autumn and winter rainfall.

Historically, intoxicated cattle present initially with varying degrees of blindness, walking in circles or become difficult to drive. Later tongue paralysis develops, and the early reports of outbreaks utilized this as the syndrome name. Following tongue paralysis, the animals have difficulty obtaining feed or water and become weak and depressed. Spasms or tremors of muscles of the head, ear or neck (including head bobbing) have also been described in cattle, but are more prominent signs in horses and goats. Animals die from weakness and inanition; however, many recover with removal from contaminated pastures and with medical treatment. In the currently reported outbreak, many animals that were removed from the pastures and/or treated with thiamine clinically improved.

Early suspicions for the toxic principle for *Descurainia pinnata* were centered around selenium accumulation; however, selenium levels have not been found to be elevated in this plant. Elevated sulfur levels have been previously described in tansy mustard (2) and confirmed by analysis of leaves and stems from the pasture plants implicated in this

case. Sulfur is known to cleave and render thiamine inactive and the positive response to thiamine treatment suggests that sulfur-induced thiamine inactivation may play at least some role in the pathogenesis of the disease. The central nervous system clinical signs associated with this plant have not yet been experimentally produced, so the exact mechanism remains a mystery.

AFIP Diagnosis: Brain, cortical grey matter: Necrosis, laminar, multifocal, with gliosis, mixed breed, bovine.

Conference Comment: Several saline tolerant plant species are known to accumulate sulfur. A short list includes: *Kochia scoparia* (burning bush, fireweed), *Descurainia pinnata* (tansy mustard), *Melilotus officinalis* (yellow sweet clover), *Elymus trachycaulus* (slender wheatgrass) and *Helianthus annuus* (sunflower)(2). Inorganic sulfur compounds are capable of cleaving thiamine and rendering it inactive and high levels of sulfates in the diet or drinking water have been shown to increase the incidence of polioencephalomalacia in cattle and sheep(4).

Thiamine (Vitamin B1) deficiency is a common cause of progressive encephalopathy that affects young ruminants and carnivores at any age. Thiamine deficiency is also known as polioencephalomalacia (PEM), cerebrocortical necrosis, Chastek paralysis (in carnivores), forage poisoning and blind staggers.

Ruminants depend on ruminal microbial synthesis of thiamine. Deficiency may be caused by grain overload with the subsequent overgrowth of thiaminase producing bacteria such as *Bacillus thiaminolyticus, Clostridium sporogenes* and *Bacillus aneurinolyticus*. During absorption, thiamine is phosphorylated to produce thiamine pyrophosphate which has three main functions: 1) it regulates the synthesis of ATP; 2) acts as a cofactor for transketolase, an important enzyme in the Pentose Phosphate Pathway (PPP), which is the primary metabolic pathway for central nervous system glucose metabolism; and 3) it maintains neural membranes and normal nerve conduction (3). Additionally, thiamine pyrophosphate is a cofactor for alpha-ketoglutarate dehydrogenase, pyruvate dehydrogenase, branched-chain alpha-keto acid dehydrogenase, and probably contributes to most oxidative decarboxylations and metabolic transformations. Transketolase is also apparently important in the metabolism of oligodendrocytes (4).

Other causes of polioencephalomalacia related to thiamine deficiency in ruminants include the ingestion of thiaminase-containing plants such as bracken fern (*Pteridium aquilinium*), horsetails (*Equisetum arvense*), and nardoo (*Marsilea drummondi*), also molasses in feedlot cattle, and the administration of thiamine antagonists such as amprolium.

Carnivores, unlike ruminants, have a dietary requirement for thiamine and deficiency can be caused by reduced intake. Additionally, foods high in thiaminase, such as tuna,

salmon, and meats that have been exposed to excessive heating and preservation by sulfur dioxide can all result in thiamine deficiency.

In ruminants, the characteristic microscopic lesion of polioencephalomalacia is laminar cortical necrosis. Once laminar cortical necrosis is recognized, it should bring to mind a differential diagnosis which includes:

1. Polioencephalomalacia (thiamine deficiency)
2. Sulfur toxicosis (accumulation in plants or high levels in water, resulting in thiamine deficiency)
3. Lead poisoning (also see basophilic stippling of erythrocytes and increased nucleated red blood cells, >5/100 WBC)
4. Salt poisoning/Water intoxication (following water deprivation)
5. Hypoxia (due to peri-mortem agonal breathing)

Interestingly, approximately one-fourth of chronic alcoholics admitted to general hospitals suffer from thiamine deficiency. In humans, thiamine deficiency predominantly affects the peripheral nerves, heart and brain. Polyneuropathy associated with thiamine deficiency is known as dry beriberi and results in myelin degeneration; whereas, the condition affecting the cardiovascular syndrome is called wet beriberi and results in a flabby, dilated heart. The most advanced form is known as Wernicke-Korsakoff syndrome and is characterized by ataxia, confusion, apathy, listlessness, disorientation, nystagmus, ophthalmoplegia, retrograde amnesia, the inability to acquire new information, and confabulation (3). Some would argue that these are also the classic signs of an overwhelmed pathology resident and that perhaps they should all start thiamine supplementation.

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SLIDE 20
CONFERENCE 5 / CASE IV – 05-13561B (AFIP 2984526)

Signalment: 57 day fetus, intact male, Shih Tzu, *Canis familiaris*, canine

History: The breeding kennel had three late term abortions in the last month. The submitted fetus had been aborted approximately seven days before the expected whelping date. The kennel had a history of *Brucella canis* and has had an extensive serologic testing and culling program in progress for the last 4 years.

Gross Pathology: There were no gross abnormalities.

Laboratory Results: Microbiology: Aerobic cultures of the kidney and liver were negative. There was a moderate, pure growth of *Brucella canis* from the lung on aerobic culture. There was a heavy growth of *Brucella canis* from placenta on aerobic culture. The bacterial species was confirmed by the Minnesota Department of Health by biochemical assays.

Serology: Tube agglutination for *Brucella canis* on serum from the dam was considered suspect at 1:200.

Virus isolation: Virus isolation for canine herpesvirus was negative after two passes on MDCK.

Gram stain: The bacteria were Gram negative.

Contributor's Morphologic Diagnosis: Necrotizing placentitis, moderate, acute, diffuse with myriad intracellular bacteria Shih Tzu, *Canis familiaris*

Contributor's Comment: *Brucella canis* was discovered as a canine pathogen in 1966 by L. Carmichael in Beagles.[3,4] Since then it has been documented around the world. In the United States the estimated prevalence is 0.5-5%.[3] *Brucella* species are Gram negative, aerobic, slow growing coccobacilli.[2,4] *B. canis* is a mucoid strain, similar to *B. ovis*, which does not express the O antigens that the smooth strains, such as *B. abortus* and *B. melitensis*, do.[3,4] Because of this, the antigens in commercial test kits for *Brucella* will not cross-react with *B. canis*.[3] Dogs can be infected with *B. abortus*, *B. melitensis*, and *B. suis* on an individual animal basis but *B. canis* is an epidemiologically important pathogen in kennels.[4]

Clinical signs in dogs range from asymptomatic to reproductive failure. In bitches, the most common clinical sign is late term abortion, with the majority after 50 days.[3,4] Other signs include weak born pups that die a few days to one month after birth and rarely pups that live with a persistent infection.[3,4] After abortion, the bitch will normally have a brown to green vaginal discharge for 1-6 weeks.[1,4] In males, the most common clinical signs are epididymitis and testicular atrophy with secondary, lick-induced, scrotal dermatitis.[2,3] Some dogs may develop prostatitis.[2,3] Prepubertal dogs that are infected

may have generalized lymphadenopathy.[4] Other diseases caused by *B. canis* include diskospondylitis, osteomyelitis, meningoencephalitis, granulomatous dermatitis, and recurrent anterior uveitis.[2]

The aborted feti are usually partially autolyzed and show changes consistent with a bacterial infection. Combinations of the following are seen in aborted feti: subcutaneous edema, subcutaneous hemorrhage of the abdominal wall, pneumonia, endocarditis, hepatitis, and ascites. Our case was highly unusual in that there were no gross or microscopic abnormalities in the feti, only in the placenta. The placenta had a majority of the trophoblasts filled with abundant, basophilic, small (approximately 1 micron in diameter), coccobacilli with occasional bacterial colonies extending into the mesenchyme. There was also multifocal villus necrosis and infrequent lymphocytic vasculitis. These findings are consistent with published reports.[3] Other infectious agents that can cause abortion in the bitch include canine herpesvirus, *Toxoplasma gondii*, and *Neospora caninum*.[1] Canine herpesvirus mainly manifests as pups born live which become ill 1-2 weeks after birth and die.[1] Classical gross lesions include subserosal petechia and ecchymoses and microscopically there should be rare, basophilic, intranuclear inclusion bodies.[3] *Toxoplasma gondii* and *Neospora caninum* are protozoal pathogens that rarely cause abortion in dogs, more commonly in sheep and cattle respectively. On microscopic exam, cysts should be seen within the brain.[3]

After an animal becomes infected with *Brucella canis* the bacteria invade macrophages and spread to lymphoid tissues (lymph nodes and spleen) where they replicate.[2] Bacteremia occurs 1-4 weeks post infection and persists for a minimum of six months then intermittently up to 5 years.[2] The bacteria subsequently spread to the target organs (genital tracts) and can cause clinical signs.

Transmission of *Brucella canis* is through mucous membranes, most commonly oral, conjunctival, and vaginal. The bacteria are shed in the aborted fetus, placenta, vaginal discharge, and semen in the highest concentrations but are also secreted in the urine of male dogs and milk.[2] Bacterial transfer can also occur through fomites such as vaginoscopes, artificial insemination equipment, and syringes, but this mode is less likely since the bacteria do not live long outside the host.[2] The bacteria can also be transmitted in utero but most of the pups die and do not become persistently infected.[2]

The diagnosis of *Brucella canis* is not simple. The gold standard is culture, either of blood from live animals or tissues from necropsy specimens. Other antemortem diagnostics include multiple forms of serology: tube agglutination with or without 2-mercaptoethanol, agar gel immunodiffusion, enzyme linked immunosorbent assays, and immunofluorescence. Serology is not always sensitive or specific and definitive diagnosis is through blood culture.[4]

AFIP Diagnosis: Chorioallantois: Placentitis, necrotizing, acute, multifocal, moderate, with myriad intratrophoblastic coccobacilli, Shih Tzu, canine.

Conference Comment: The contributor provides an excellent review of *Brucella canis* infection as well as other causes of abortion and stillbirth in dogs.

All *Brucella* species are closely related, pathogenic, gram-negative, intracellular rods with a predilection for the reproductive tract and all cause similar diseases characterized by:

- Reproductive failure in young females with persistent lifelong infection and shedding from reproductive tissues and mammary glands
- Epididymitis and orchitis in males
- Bronchopneumonia in aborted fetuses

The ungulate placenta, fetal fluids and testes contain a polyhydric alcohol known as erythritol which has been shown to stimulate growth of *Brucella*. Erythritol is not present in the human placenta. Virulent strains of *B. abortus* release 5' guanosine monophosphate and adenine which inhibit PMN degranulation contributing to intracellular survival. *B. abortus, B. melitensis and B. suis* have surface antigens A and M present on the LPS protein complex. *B. canis and B. ovis* possess an R surface antigen. Since all *Brucella* sp. are intracellular bacteria, acquired immunity is mainly cell mediated.(5)

Below is a simple list to help remember the different species of *Brucella* and what species they most commonly affect:

- **Cattle:** *B. abortus*. Placentitis, abortion, mastitis, epididymitis, orchitis
- **Swine:** *B. suis* (also *B. abortus* and *B. melitensis*). Sterility, abortion, arthritis
- **Sheep & goats:** *B. melitensis*. In goats similar to *B. abortus* in cattle with occasional mortality; less severe in sheep
- **Sheep:** *B. ovis*. (also *B. abortus*) Epididymitis, rarely placentitis and abortion
- **Dogs:** *B. canis*. Prostatitis, epididymitis, testicular atrophy, abortion, osteomyelitis, diskospondylitis
- **Horse:** *B. abortus*, *B. suis*. Suppurative bursitis (poll evil and fistulous withers)
- **Wildlife:** Described in a wide variety of mammals, including ungulates and marine mammals; especially bison, elk, reindeer and other ungulates (*B. abortus*)

Humans are susceptible to all species (most commonly *B. abortus*, *B. melitensis* and *B. suis)*, as well as RB51 and strain 19 vaccines. Transmission is by infected milk products or direct contact with infected tissues. Symptoms include prolonged or recurrent fever, weakness and impotence. Brucellosis in humans is known as undulant fever, gastric fever, Malta fever, Gibraltar fever, and Mediterranean fever. *Brucella abortus* is also commonly associated with olecranon bursitis in people.

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SLIDE 21
CONFERENCE 6 / CASE I – POO-208 (AFIP 2739286)

Signalment: Piglet, six-weeks-old, mixed breed, castrated male.

History: This piglet was submitted from a 300 sow farrow to finish operation farm in southern Taiwan. Piglets were initially healthy when weaned at 4 weeks of age; at the early post-weaning period, 20% of the nursery piglets lost condition and became sick, showed poor growth rate, unthriftiness, dyspnea and pallor. Eventually piglets became emaciated with rough hair and some died or were culled; an increased mortality rate was reported in the weaner facility.

Gross Pathology: The piglet showed gauntness with poor nutritional state; pallor of skin and mucous membranes were noted upon external examination. At necropsy, enlarged lymph nodes were the most obvious lesion, particularly in inguinal, mesenteric, bronchial and mediastinal lymph nodes. Lungs were diffusely mottled and firm; few fibrinopurulent strands were adhered in the peritoneal cavity.

Laboratory Results: Positive for Type II porcine circovirus by polymerase chain reaction (PCR) and transmission electron microscopic examination of bronchial lymph node.
Negative for porcine reproductive and respiratory syndrome virus (PRRS) by pulmonary virus isolation.
Negative for swine influenza virus (SIV) by tracheal endothelial cellular virus isolation.
Negative for pseudorabies virus (PRV) by immunohistochemical staining of section of lymph node using pseudorabies monoclonal antibody.
Positive for *Streptococcus* sp. from peritoneal fluid.

Contributor's Morphologic Diagnoses:
1. Lymph node (mesenteric): Lymphadenitis, granulomatous, diffuse, moderate, with intrahistiocytic polymorphous eosinophilic to basophilic cytoplasmic inclusion bodies.
2. Tonsil: Tonsillitis with lymphocytolysis and rare intrahistiocytic basophilic

cytoplasmic inclusion bodies.

Contributor's Comment: This case is consistent with post weaning multisystemic wasting syndrome (PMWS). In the submitted histologic sections of lymphoid organs, grape-like clusters of basophilic inclusion bodies are observed in epithelioid macrophages. Occasional multinucleated giant cells can be seen in the germinal centers of lymphoid follicles, paracortex and medullary and subcapsular sinusoids. There was evidence of mild lymphocytic depletion and increased pyknosis in lymphoid follicles. Variable eosinophilic and neutrophilic infiltration was also seen. Other lesions in this piglet were interstitial pneumonia, fibrinous peritonitis and lymphohistiocytic infiltrates within Peyer's patches.

Transmission electron micrography of cytoplasmic inclusions from the lymph node showed paracrystalline arrays of circovirus-like viral particles; PCR test from fresh lymph node also confirmed Type II porcine circovirus (PCV) in this tissue.

Porcine circovirus belongs to the family Circoviridae which also includes chicken anemia virus and psittacine beak and feather disease. Circoviruses are the smallest animal viruses using genome size and virion diameter as qualifiers. Another interesting feature of this family is that these are the only animal viruses that possess a circular single-stranded DNA genome. These three viruses do not share common antigenic determinants or DNA sequence homology.

PCV is a non-cytopathic virus that was isolated in 1974 from a pig kidney cell line (PK15). The line was shown to be persistently infected with porcine circovirus. At the time of its discovery, it could not be shown to cause disease in pigs. According to antibody titers, the virus has a high prevalence in both European and Canadian swine populations. Presently PCV is thought to play a role in postweaning multisystemic wasting syndrome (PMWS) in pigs. Postweaning multisystemic wasting syndrome was first descried in 1996 and to date, cases have been confirmed in the western provinces and the province of Quebec in Canada, in California, Indiana, Iowa, Spain, Ireland, UK, Japan, Korea and also in Taiwan.

Pigs with PMWS are generally affected two to three weeks post weaning. Signs may be exacerbated by stress. In acute outbreaks mortality may reach 10% but will be lower in endemically infected herds. The most common clinical findings of PMWS are wasting, dyspnea and enlarged lymph nodes. Pallor, jaundice and watery diarrhea are seen in some cases. Histopathological findings may include interstitial pneumonia and basophilic intracytoplasmic inclusion bodies may be found in lymph nodes, tonsil, and spleen.

In Taiwan, postweaning multisystemic wasting syndrome (PMWS) characterized by progressive weight loss and dyspnea was first diagnosed in 1997. Since then the disease has become widespread here in Taiwan and is quite commonly found in nursery and pre-growers. The disease was first found in the major pig producing prefectures of southern Taiwan in weaning pigs, and is most often seen in pigs 5-10

weeks old. From the cases of Pig Research Institute Taiwan, we find that now it quite commonly exists in many pig farms. This disease is seen in herd sizes from small farms to large farrow to finish operations. In our cases, PMWS is commonly diagnosed in herds complicated with other respiratory diseases, including mycoplasmosis, salmonellosis, and PRRS which therefore have masked damages caused by the disease. In an acute outbreak, the postweaning mortality rate peaks at 10~15% (monthly calculation) and the worst case may reach 25%, especially during the winter.

The lack of specific gross lesions makes this disease syndrome extremely ambiguous, especially when farms experience this important disease concurrent with other infections such as salmonellosis and PRRS. Because damage was masked by the concurrent pathogens, pig producers here all seem to attribute the losses to other pathogens. As more information was found regarding the exact role of PCV in the pathogenesis of PMWS, which recently has been reproduced by co-infection with parvovirus, pig producers and veterinarians in Taiwan are just starting to be aware of the damage caused by the syndrome. Researches here are focused on the epidemiology and molecular features of PMWS.

AFIP Diagnoses: 1. Lymph node, mesenteric (per contributor): Lymphoid depletion, diffuse, moderate, with sinus histiocytosis, multifocal draining neutrophilia, and intrahistiocytic intracytoplasmic botryoid inclusion bodies, mixed breed, porcine. 2. Tonsil: Lymphoid depletion, diffuse, moderate, with intrahistiocytic intracytoplasmic botryoid inclusion bodies, mixed breed, porcine.

Conference Comment: The contributor provides an excellent review of porcine circovirus-2 (PCV-2) and postweaning multisystemic wasting syndrome (PMWS) and stresses the importance of the virus in co-infection with other common porcine viruses such as porcine parvovirus (PPV), and porcine reproductive and respiratory syndrome virus (PRRSV). Conditions currently associated with PCV-2 include PMWS, porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex, reproductive failure, and most recently, granulomatous enteritis, necrotizing lymphadenitis and possibly exudative epidermitis (5).

A proposed pathogenesis for PMWS involves coinfection by PCV-2 and porcine parvovirus (PPV) which localize in the regional histiocytes of the oropharynx. In the host, PPV spreads via cell-associated viremia, replicates in lymphoid tissues, and stimulates lymphocyte and macrophage proliferation thereby resulting in PPV protective immunity. PCV-2 slowly replicates in macrophages but immunity to PCV-2 is not established. Due to the immunoproliferative response to the PPV infection, PCV-2 replication within macrophages is heightened and dissemination occurs as a macrophage-dominant pantropic granulomatous response recognized clinically as PMWS.

The clinical signs of PMWS include wasting and unthriftiness, dyspnea, lymphadenopathy and, less frequently, pallor, diarrhea and icterus.

Typical gross findings include a failure of the lungs to collapse and lobular atelectasis, lymphadenopathy (especially of the superficial inguinal lymph nodes), icterus, hepatic atrophy, and enlarged kidneys with cortical white foci and peripelvic edema.

The characteristic histologic lesions of PMWS are granulomatous inflammation with large, grape-like amphophilic or basophilic intracytoplasmic inclusion bodies within histiocytes and multinucleated giant cells (6).

Circoviruses of veterinary importance include: the agents of postweaning multisystemic wasting syndrome and psittacine beak and feather disease, chicken anemia virus and pigeon circovirus.

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SLIDE 22
CONFERENCE 6 / CASE II – 05-9006 (AFIP 2985005)

Signalment: 2 days old, gender not identified, quarter horse, *Equus caballus*, equine

History: On the affected farm 7 out of 8 neonatal foals died. One foal was necropsied by the submitting veterinarian. At necropsy, this 2 day old foal had no perirenal fat, no milk curd in the stomach, and ecchymoses in the lung, heart, and kidneys. The kidneys were described by the submitting veterinarian as being “trashed”.

Gross Pathology: Both kidneys had 1-3 mm, coalescing suppurative foci that were almost entirely limited to the renal cortex (Fig. 1).

Laboratory Results: Foal serum IgG – 800 mg/dL
Actinobacillus equuli was isolated from the kidney, lung, liver, and spleen.

Histopathologic Description: Randomly distributed throughout the renal cortex and rarely in the medulla are accumulations of degenerating and intact neutrophils. These foci of neutrophils replace approximately 50% of the renal cortical parenchyma; occasional clusters of coccobacillary bacteria are present in the foci. Occasional glomeruli are partially obliterated by neutrophilic infiltrates. Hemorrhage and congestion is present in the remaining normal renal parenchyma.

Contributor's Morphologic Diagnosis: Nephritis, suppurative, multifocal, coalescing (embolic)

Contributor's Comment: In horses, the most common cause of embolic suppurative nephritis is *Actinobacillus equuli*, which is acquired *in utero*, during parturition, or shortly after birth. If foals survive for several days, microabscessation is seen in the kidneys and other organs, and polyarthritis is present.[1]

Actinobacillus equuli may cause mortality rates approaching 100% in neonatal foals. Infections occur worldwide, but appear to be declining. The infection in foals is sometimes referred to as sleepy foal disease, with foals that are sleepy or comatose at birth or soon afterwards. These so-called sleepers may be aroused but quickly revert to a comatose state. Foals infected with *A. equuli* often have low plasma immunoglobulin G.[2] In foals, serum IgG concentrations above 400 mg/dL are associated with protection against septicemic disease and concentrations above 800 mg/dL are sufficient to reduce the risk of infectious disease in most environments.

Actinobacillus equuli is a member of the Pasteurellaceae family. It has been associated with arthritis, bronchitis, pleuritis, pneumonia and peritonitis. The organism can be isolated from the oral cavity of healthy horses.[3] *Actinobacillus equuli* has recently been reclassified, with nonhemolytic isolates named *Actinobacillus equuli* subsp. *equuli*, and hemolytic isolates named *Actinobacillus equuli* subsp. *haemolyticus*.[4] Culture supernatants of hemolytic strains of *A equuli* are toxic to equine neutrophils. An RTX toxin/hemolysin from a hemolytic isolate of *A. equuli* has been characterized.[5]

Embolic suppurative nephritis occurs when bacteria alone or in small clumps, and small septic emboli lodge mainly in glomerular and peritubular capillaries. Larger emboli can lodge in afferent vessels of the kidney and produce septic infarcts, which may be unilateral. Developed abscesses are generally cortical rather than medullary but in bacteremia caused by Gram-negative enterobacteria, microscopic suppurative foci may be scattered in the medulla.[1]

AFIP Diagnosis: Kidney: Nephritis, suppurative, embolic, severe, with colonies of coccobacilli, Quarter Horse, equine**.**

Conference Comment: The contributor provides a thorough review of this classic disease of foals.

Like other Gram-negative bacteria, *Actinobacillus equuli* contains endotoxin, a lipopolysaccharide (LPS), which is a major component of the cell wall and has widespread effects, including those on macrophages, endothelium, platelets, Hageman factor, neutrophils, complement and mesenchymal cells. LPS-LPS binding protein complexes in the blood bind to CD-14 receptors and Toll-like receptor protein-4 (TLR-4) found on macrophages, neutrophils, dendritic cells, NK cells, mucosal epithelial and endothelial cells. Signals from TLR-4 initiate a number of cytokines and activate leukocytes and vascular cell walls (6). Macrophages become activated and produce interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-A). Once activated, the endothelium synthesizes adhesion molecules, other cytokines, growth factors, eicosanoids, nitric oxide, and thrombogenicity is increased. Reactive oxygen species produced in the respiratory burst of activated macrophages have toxic effects, such as cell membrane lysis and extracellular membrane degradation. Endotoxin damages endothelium directly or indirectly. Damage to the endothelium and exposure of subendothelial collagen, will initiate the coagulation cascade by the intrinsic pathway. The extrinsic pathway is activated by thromboplastin-like procoagulant activity (PCA) expressed by endothelial cells and activated macrophages. Endotoxin stimulates platelets to make phospholipid more available for coagulation, and to synthesize and release thromboxane A2, which induces platelet aggregation. Endotoxin also activates factor XII (Hageman factor), and the alternate pathway of complement. Neutrophils are directly primed by endotoxin to increase their response to mediators; they are attracted by IL-1, complement and leukotrienes, and activated by TNF-A and complement. Endotoxin also stimulates mesenchymal cells to release proteolytic enzymes. Besides the “sleepy foal” or comatose state, affected foals may present with fever, diarrhea and hot, painful, swollen joints.

Necropsy findings vary from no gross lesions in cases of acute death to severe enteritis, fibrinopurulent polyarthritis and polysynovitis, multiple small visceral abscesses and kidneys with multiple, grey, evenly distributed small cortical abscesses and medullary hemorrhage. In the conference, it was also pointed out that another target organ besides the kidney can be the adrenal gland. Additional information on a case of *A. equuli* causing adrenalitis can be found in the WSC archives; see Conference 28, Case 3, 1995.

Conference attendees briefly discussed other frequent causes of foal septicemia. A short list includes: *Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., and *Salmonella* spp. Additionally, failure of passive transfer is often associated in cases of neonatal

septicemia; however, in this case the foal's immunoglobulin levels were at normal levels.

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SLIDE 23
CONFERENCE 6 / CASE III – 05-21199 (AFIP 2988328)

Signalment: 4 year old female Thoroughbred horse

History: This mare had a 3 furlong workout, became wobbly while still on the track, collapsed, and died.

Gross Pathology: The mare was in good, athletic body condition. Approximately 1 liter of frank blood was free within the thorax and 0.5 liter of blood was present within the pericardial sac. Multiple poorly demarcated pale yellow linear subendocardial plaques were present in the left ventricle and on the left side of the interventricular septum. The liver had an enhanced lobular pattern.

Histopathologic Description: Multiple sections from the left ventricle and interventricular septum were examined and each had marked endocardial thickening due to fibrosis, endocardial fibrillar degeneration and mineralization, endocardial mucinosis, and infiltration of few foamy macrophages. Subendocardial Purkinje fibers were swollen and degenerate, with variably extensive cytoplasmic vacuolation. In the

liver, few small (150-250 um) random to periportal foci of necrosis were infiltrated by low numbers of lymphocytes, plasma cells, and degenerate neutrophils.

Contributor's Morphologic Diagnoses: 1. Endocardial fibroelastosis with Purkinje cell degeneration
2. Mild necrotizing hepatitis and cholangitis (likely related to bacterial showering from intestine and of no clinical significance)

Contributor's Comment: Endocardial fibroelastosis (EFE) has been described in humans (1), cats (2), horses (3,4), and cattle (5). Primary EFE is unaccompanied by other cardiac lesions, whereas secondary EFE develops in conjunction with congenital cardiac anomalies, myocarditis, glycogen storage diseases, and carnitine deficiency (1,2,6). Primary EFE is an inherited disorder in humans and Burmese cats (6). In humans, both forms of EFE commonly result in sudden death in very young children (1). The pathogenesis of primary EFE is not clear, but may be related to impairment of drainage from myocardial lymphatics (6).

Equine endocardial fibroelastosis is an uncommon condition that has previously been associated with sudden death following exercise in 2 young Thoroughbreds (3). An additional case report described left-sided heart failure in a 4 month old Thoroughbred foal with similar endocardial lesions (4). No additional gross or histologic lesions were evident in heart in these previous cases, with the exception of mitral valve insufficiency in the foal that was assumed to have resulted from abnormal distensibility of the left atrium (4). This lesion in horses is presumed to be congenital, due to the young age of the 3 animals involved in these case reports and the absence of other predisposing inflammatory or degenerate lesions in endocardium or myocardium (3).

Entrapment and subsequent degeneration of Purkinje fibers within the fibroelastic tissue, with resultant conduction disturbances, has been hypothesized to be the cause of sudden death in humans and animals with endocardial fibroelastosis (6). Vacuolation and degeneration of Purkinje fibers was evident in the horse presented here and has been described in affected Burmese cats (6), although this lesion was not described in the other 3 equine cases described in the literature. Mineralization within foci of endocardial fibroelastosis, as identified here, was also not described in previously reported cases of EFE. Endocardial mineralization without proliferation of fibroelastic tissue has been reported in acute vitamin D toxicosis and lymphosarcoma in horses, although foci of metastatic mineralization were also described in other anatomic sites in affected animals (7,8).

In other species affected with EFE and in this case, hearts in the early stages of EFE may have a normal gross appearance (6). In advanced stages of the disease, the endocardium appears grossly thickened and opaque, with lesions mainly involving the left ventricular endocardium. Other cardiac-related causes of sudden death during or subsequent to exercise in equine athletes that present with anatomic lesions include rupture of great vessels (9), myocarditis, and myocardial interstitial fibrosis involving the cardiac conduction system (10). Both myocarditis and myocardial fibrosis are

presumed to result in interference with normal conduction pathways, leading to potentially fatal arrhythmias. Conduction disturbances due to electrical or biochemical (electrolyte) aberrations generally produce no specific morphologic lesions that can be identified at necropsy. In humans, an excess of circulating catecholamines during exercise, in combination with hypoxia-induced myocardial sensitization related to variations in heart rate and systolic blood pressure, is thought to promote potentially fatal arrhythmias (11).

AFIP Diagnosis: Heart: Endocardial fibroelastosis, diffuse, moderate, Thoroughbred, equine.

Conference Comment: Conference attendees agreed that bundles of Purkinje fibers were completely surrounded by fibrous tissue but debated whether the vacuolated and lacey appearance of the Purkinje fiber's cytoplasm indicates they are undergoing degeneration or if the vacuoles indicate glycogen storage and are a normal finding. There was additional debate over whether the lesion was predominantly fibrotic or fibroelastic. Haphazardly arranged aggregates of deeply eosinophilic fibers were suspected to be elastic fibers. Subsequent staining with modified Russell-Movat pentachrome, confirms they are elastic fibers as they are black and are surrounded by yellow collagen fibers.

In addition to an excellent review of EFE, the contributor also provides a list of other cardiac-related causes of sudden death in equine athletes. Although many questions still remain to be answered concerning equine EFE, increased reports of the disease warrants inclusion in the differential diagnosis for unexplained sudden death, especially in young equine athletes.

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SLIDE 24
CONFERENCE 6 / CASE IV – 05-2007 (AFIP 2984970)

Signalment: Six-week-old, male, Jersey calf, bovine

History: Sudden death in pen raised calves in a dairy beef operation.

Laboratory Results: *Salmonella dublin* was isolated from lung, and small intestine

Histopathologic Description: Random foci of hepatocellular necrosis occur throughout the liver section. The foci are roughly nodular in outline and contain variable numbers of macrophages and neutrophils. In the lung, there is expansion of the interstitial space by mononuclear cells and a few neutrophils. Alveolar spaces contain fibrillar eosinophilic material consistent with fibrin commingled with erythrocytes, neutrophils and macrophages in small numbers. Scattered foci of hemorrhage involving large numbers of adjacent alveoli and a few bronchioles are present. The interlobular septa are widened by proteinic fluid and contain dilated vessels with similar fluid.

Contributor's Morphologic Diagnoses:
Acute, multifocal, severe, necrotizing hepatitis (Etiology: *Salmonella dublin*)
Acute, diffuse, moderate, interstitial pneumonia (Etiology: *Salmonella dublin*)

Contributor's Comment: *Salmonella typhimurium* and *Salmonella dublin* are the most common agents causing salmonellosis in the bovine. Both adults and calves may be infected. Enteritis and septicemia may occur in both calves and adults and cows may abort. Clinical signs include, fever, inappetence, and depression. In calves with *Salmonella dublin* infection clinical signs of pneumonia and septicemia predominate. When enteritis is present, there is the production of foul smelling, liquid feces that may contain blood and mucus.[1]

Salmonella typhimurium has been reported as the most common serotype recovered from sick cattle. However, *Salmonella dublin* is a common isolate in Europe. In the United States, *Salmonella dublin* was once common only west of the Rocky Mountains. However, in recent decades it has been moving eastward and isolated with increasing frequency from veal and dairy beef operations.[2]

Carrier animals are an important source of infection. Carrier animals harbor the infection in lymph nodes and visceral organs and shed bacteria intermittently in feces and milk. A carrier state appears to be more common in heifers infected between one-year-of-age and first calving and for cows if infected around parturition. In Denmark, the risk was higher in late winter to early spring. The risk of a carrier state was highest in herds currently experiencing an outbreak of clinical disease.[3]

The *spv* gene is a virulence factor commonly found in Salmonella isolates that are host adapted to animals including *S. dublin*, *S. choleraesuis*, *S. gallinarum-pullorum* and *S. abortusovis*. Salmonella species with a broad host range such as *S. typhimurium* and *S. enteritidis* have only a variable proportion of isolates that carry this virulence factor. The gene encodes a MetR/LlysR-type transcriptional activator. The gene is strongly expressed following uptake into eukaryotic cells. It apparently does not affect colonization or invasiveness in the bowel. Rather, it increases the growth rate of the bacteria in the intracellular compartment.[4]

The lesions of *Salmonella dublin* infection in calves are characteristic of an acute septicemia. Random foci of hepatocellular necrosis with infiltrates of macrophages (typhoid nodules) are seen in the liver. Histologic changes in the lung are characterized by acute injury to the interstitium with exudation of fibrin and inflammatory cells into the alveolar spaces.

AFIP Diagnoses: 1. Liver: Hepatitis, histiocytic and lymphocytic, necrotizing, random, moderate, Jersey, bovine.

2. Lung: Edema and hemorrhage, diffuse, acute, marked, with intraalveolar fibrin.

Conference Comment: There are over 2,300 serovars of *Salmonella* belonging to the genus *Salmonella***,** family *Enterobacteriaceae***.** *Salmonella* are Gram-negative, flagellated, facultatively anaerobic bacilli that possess three major antigens: H antigen or flagellar antigen; O or somatic antigen; and Vi antigen, a superficial antigen present in very few serovars (e.g. *S. typhi*) (5). *Salmonella* live in the intestinal tract of vertebrates and are passed into food, water and the environment through fecal contamination. In animals, salmonellosis manifests itself in three basic forms: 1) the enteric form characterized by watery diarrhea which may contain mucous, fibrin or blood and has a characteristic “septic-tank” odor; 2) the septicemic form characterized by fever, inappetence and depression and 3) the abortion form. Some serovars, such as *S. dublin* in cattle, have a higher incidence of causing abortion than others. Most

species of *Salmonella* are host adapted to either humans, specific animals or to both (e.g. *S. typhimurium, S. enteritidis*).

Salmonella infection often results in a carrier state where the host does not suffer from the disease but sheds large numbers of the bacteria into the environment. Active carriers are oftentimes animals that have recovered from the disease and continue to shed bacteria in their feces.

Salmonella are ingested by the host and subsequently adhere to and penetrate intestinal enterocytes, multiply intracellularly, and invade deeper tissues where they are phagocytosed by macrophages and neutrophils. They are transported within phagocytic cells via the lymphatics to regional lymph nodes and ultimately to the reticuloendothelial system of the spleen and liver.

Intestinal infection results in mucosal degeneration, neutrophilic inflammation of the mucosa and lamina propria, thrombus formation within vessels of the lamina propria and shortening of intestinal villi. Young animals are more susceptible to infection due to immature intestinal microflora, which normally inhibit *Salmonella* growth in mature animals (1).

Virulence factors associated with *Salmonella* include: a heat-labile enterotoxin that is related to cholera toxin; at least three cytotoxins which induce intestinal cell damage, lipopolysaccharide (LPS), variably active flagella which affect functional motility and invasiveness, siderophores or iron chelators which bind to host iron, and both plasmid and chromosomal virulence genes (1).

Typical gross necropsy findings include enteritis which can range from catarrhal to fibrinous and hemorrhagic with characteristic Peyer's patch necrosis. Differentials for Peyer's patch necrosis and fibrinohemorrhagic enteritis include *Salmonella* sp. enteritis, bovine pestivirus infection (BVD) and bovine morbillivirus infection (Rinderpest).

Contributor: Arizona Veterinary Diagnostic Laboratory

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SLIDE 25
CONFERENCE 7 / CASE I –05-17970 (AFIP 2988330)

Signalment: 2 year old spayed female American Cocker Spaniel.

History: This dog was presented to the referring veterinarian 48 hours after the onset of diarrhea with occasional vomition while at a boarding kennel. Physical examination identified icterus and melena. The dog's condition deteriorated rapidly while hospitalized for blood work, and she died 4 hours after presentation.

Gross Pathology: Mucous membranes were icteric and melenic feces were adherent to the perineum. The liver was diffusely yellow and atrophic, and normal architecture was effaced due to micronodular hyperplasia. The gallbladder was distended and the bile duct was patent. Vascular (venous) shunts were present between the vena cava and ovarian veins (right and left), and within the mesentery adjacent to the pancreas. Approximately 60 ml of clear yellow fluid was free in the abdominal cavity, and 100 ml of similar fluid was present in the thoracic cavity. Lungs were diffusely congested and edematous.

Laboratory Results:
WBC 38.47 x 10 9 / L (6.0-17.0)
Lymph 13.46 x 10 9/L (0.6-6.8)
Mono 1.84 x 10 9 /L (0.1-1.7)
Granulocytes 23.17 x 10 9/L (3.0-13.6)
RBC 3.15 x 10 12/L (5.5-8.5)
MCV 62.0 fl (58.0-73.0)
Hct 19.5% (35.0-55.0)
MCH 21.2 pg (19.5-24.5)
MCHC 34.3 g/dl (28.0-40.0)
RDW 12.1 (8.0-12.0)
Hb 6.7 g/dl (10.0-18.0)

Plt 109 x 10 9 /L (120-600)

Albumin 8 g /dl (25-44)
ALP 267 U/L (20-150)
ALT 81 U/L (10-118)
Amylase 851 U/L (200-1200)
Total bilirubin 198 umol/L (2-10)
BUN 18 mmol / L (2.5-8.9)
Calcium 2.15 mmol/L (2.15-2.95)

Phosphorus 3.14 mmol/L (0.93-2.13)
Creatinine 247 umol/L (27-124)
Glucose 1.3 mmol/L (3.3-6.1)
Sodium 136 mmol/L (138-160)
Potassium 3.6 mmol/L (3.7-5.8)
Total protein 25 g/L (54-82)
Globulins 0 g / L (23-52)
Na:K 37.8

PT, APTT elevated to twice normal values

Abdominal fluid (harvested at necropsy):
Cellularity 2.3 x 10 9 / L
Protein T.S. <20 g/L
Segmented neutrophils 73%
Macrophages 27%
Sheets of mesothelial cells present with moderate numbers of neutrophils and a few mononuclear cells. No bacteria seen.

Leptospira serology:
Suspicious titers on thoracic fluid (1:20) & abdominal fluid (1:40) for *L. bratislava*.
Leptospira PCR negative of liver and kidney.

Histopathologic Description: In the liver, there was marked, generalized dissecting fibrosis with hepatocellular nodular hyperplasia, and piecemeal and single cell necrosis to total loss of hepatocytes. Moderate to marked cholangiolar proliferation and canalicular cholestasis were generalized. Most hepatocytes were vacuolated or contained red-brown granular cytoplasmic pigment. Hepatocellular anisokaryosis and anisocytosis were moderate. A mild infiltrate of primarily plasma cells was present in the large tracts of periportal fibrous tissue.

Lesions were also present in kidney and brain, although these tissues are not included in the slide set. Cortical tubular degeneration in kidney was characterized by epithelial loss, flattening, and attenuation. Amorphous to slightly granular eosinophilic debris is present in lumina of affected tubules, and the mesangial matrix was mildly increased in some glomeruli. In brain, neuronal vacuolation with occasional neuronal necrosis was present in mesencephalon, particularly in the rostral colliculus and reticular formation. Occasional Alzheimer type II astrocytes, with abundant pale cytoplasm and vesicular nuclei, were present in cortex.

Contributor's Morphologic Diagnoses:
1. Micronodular hepatic cirrhosis
2. Acute renal tubular necrosis (nephrosis)
3. Mild spongiform encephalopathy

Contributor's Comment: Chronic hepatitis with micronodular cirrhosis in Cocker Spaniels has been recognized since 1993, although documentation in the literature is limited to few descriptive reports (1, 2). In the most complete report, affected dogs ranged in age from 1.5 to 11 years, although the majority of dogs were relatively young, with a mean age of 5.6 years (1). Male dogs were over-represented. Most animals had a short history of clinical disease, as in this case, and demonstrated a variety of clinical signs expected with liver disease and including anorexia, vomiting, lethargy, depression, and weight loss. Ascites was consistently evident on physical examination. Disease progression was rapid in most of these dogs, as in the Cocker Spaniel described here, with survival limited to one to several weeks. Gross and histologic lesions described are similar to those in our dog and included small livers with multiple foci of nodular regeneration, lymphoplasmacytic portal hepatitis with bridging fibrosis, destruction of limiting plates, piecemeal necrosis, biliary proliferation, and hepatocellular cytoplasmic vacuolation. Acquired extrahepatic portosystemic shunts, as seen in this dog, were not reported in other cases. Results of hepatic copper evaluation were based mainly on histochemical staining and were equivocal. Other studies have also identified an increased incidence of chronic hepatitis in Cocker Spaniels, supporting a hereditary basis for this disorder (3).

Cirrhosis is a common end-stage lesion in chronic hepatitis due to a variety of etiologies, including hepatitis associated with genetic factors and hepatic copper accumulation. An excellent review of canine chronic hepatitis, with breed predispositions and association with hepatic copper levels, is present in Ettinger and Feldman's Textbook of Veterinary Internal Medicine (4).

Spongiform lesions in brain of this dog were consistent with hepatic encephalopathy. Leptospirosis remained a differential diagnosis for renal lesions, although this could not be confirmed, and may have been a major contributing factor in this dog's rapid demise.

AFIP Diagnosis: Liver: Hepatocellular degeneration, necrosis and loss, chronic, diffuse, severe, with parenchymal collapse, bile stasis, nodular regeneration, moderate dissecting fibrosis, biliary hyperplasia, and mild lymphoplasmacytic portal hepatitis (cirrhosis), Cocker Spaniel, canine.

Conference Comment: The histologic changes within this section of liver are profound and all the features of an end-stage, or cirrhotic, liver are identifiable.

In general, hepatic parenchyma responds to chronic injury in three ways: 1) by forming regenerative nodules, 2) by undergoing fibrosis and 3) by biliary duct hyperplasia. Regenerative nodules form under the influence of transforming growth factor – alpha (TGF-A) and hepatocyte growth factor which stimulate hepatocyte stem cells, or oval cells, to replicate. Stimulated oval cells differentiate and undergo rapid replication in an attempt to compensate for lost hepatic mass. The end result to prolonged regeneration is the formation of nodules of hepatic parenchyma. Unfortunately, regenerative nodules

lack normal architecture which disrupts the flow of both blood and bile and promotes vascular shunt formation. Fibrosis occurs following hepatocellular and extracellular matrix injury when Ito cells (a.k.a. hepatic stellate cells) undergo a change from their normal role of lipid and vitamin A storage to a myofibroblastic–type cell capable of producing collagen and extracellular matrix proteins. Fibrosis has a detrimental impact on liver function and can occur in several recognized patterns which may help determine the type of injury sustained. Biliary hyperplasia is most prominent in diseases that cause obstruction of bile flow. The mechanism is unknown; however, following hepatic injury proliferation of bile ducts is most profound in portal and periportal areas (5).

The contributor also provides a complete hematology and serum chemistry analysis. With regards to hepatic injury, there are three general categories of parameters frequently measured to diagnose hepatic disease: 1) leakage enzymes, 2) inducible (or cholestatic) enzymes and 3) function tests. Leakage enzymes are found in hepatocyte cytosol and mitochondria and literally “leak out” when cell membranes are damaged. The levels measured in the serum depend on the duration and severity of the injury. In this case, alanine aminotransferase (ALT) is the only leakage enzyme provided and is within the normal reference range. This is most likely due to either decrease liver mass or decreased amounts of enzyme per cell - leading to decreased amounts of the enzyme being leaked which then is measured within the "normal" range. Induced enzymes are membrane bound and require enzymatic induction for elevations to occur in the serum. In this case, alkaline phosphatase (ALP), a sensitive indicator of cholestasis, is elevated to almost twice normal. This elevation is most likely due to cholestasis but could also have been induced by the administration of corticosteroids. In dogs, there is a corticosteroid induced ALP isoenzyme. This isoenzyme can be differentiated from the hepatic isoenzyme by utilizing levamisole, since the corticosteroid isoenzyme is largely resistant to levamisole inhibition. Function tests for the liver include bilirubin measurement and bile acid tests. Hyperbilirubinemia can be either prehepatic (e.g. hemolytic disease), hepatic (e.g. decreased functional mass), or posthepatic (e.g. cholestasis). Total bilirubin in this case is nearly 20 times normal and may be due to a combination of both hepatic and posthepatic causes. Bile acid testing is also another commonly used method of detecting decreased liver function. Bile acids are made in the liver and stored in the gallbladder. Following ingestion of a meal, the gallbladder contracts and expresses the bile acids into the small intestine where they are responsible for solubilizing lipids and fats. Most are reabsorbed from the small intestine via a process known as enterohepatic circulation. Anything that disrupts the cycle can result in increased serum bile acids. The most common causes are portosystemic shunts, loss of functional hepatocellular mass and cholestasis.

Other important serum chemistry findings in this case include azotemia and hyperphosphatemia, possibly due to concurrent renal failure but differentiation is impossible without a urine specific gravity. The hypoproteinemia and hypoalbuminemia could be the result of concurrent renal disease or decreased production by the liver. Likewise, increased clotting times may be due to decreased production of clotting factors by the liver and possibly disseminated intravascular coagulation (DIC).

Hypoglycemia may be due to anorexia or prolonged contact of the serum with erythrocytes in vitro (6).

Important hematologic findings include normocytic, normochromic anemia; mild thrombocytopenia; and a leukocytosis characterized by mature neutrophilia, lymphocytosis and mild monocytosis.

Special stains were used to help identify the nature of cytoplasmic granules noted within hepatocytes. Rhodanine identifies most of the hepatocyte cytoplasmic granules as copper; whereas Kupffer cells often contain deposits of iron stained with Perl's Prussian blue. All attendees agreed that the copper accumulation may be the result of the liver's inability to manufacture and export it as ceruloplasmin or to excrete it in the bile, rather than excess copper being the cause of liver damage.

A short list of the most common causes for end–stage liver include:

Long term administration of anticonvulsants

Abnormal metabolism or accumulation of copper or other metals (West Highland White Terriers, Bedlington Terriers)

Chronic aflatoxicosis, especially Aflatoxin B1

In ruminants: ingestion of pyrrolizidine alkaloid containing plants such as *Senecio* spp. (tansy ragwort, groundsel); *Crotalatia* spp.; *Heliotropium* spp.

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SLIDE 26
CONFERENCE 7 / CASE II – N2005-310WCS (AFIP 2987445)

Signalment: 3 year old, female brown pelican (*Pelecanus occidentalis*)

History: This was one of twelve brown pelicans moved from an indoor winter holding facility to an outdoor pond. One month later, this bird presented with lethargy, progressive weakness, and anorexia. Despite hospitalization and intensive treatment, the bird died twelve days after presentation. A total of seven of the twelve birds subsequently died with similar clinical signs, lab results, and necropsy findings.

Gross Pathology: The bird was in thin body condition, with moderate muscle wasting over the keel. There was extensive, multifocal to coalescing, tan discoloration throughout all major skeletal muscles, including the ocular muscles. The heart contained numerous, multifocal to coalescing, pale yellow streaks that were most prevalent within the region of the left ventricle. Several, 0.3 cm diameter, ulcers were present on the mucosal surface of the stomach.

Laboratory Results:
WBC: 27.2 x 103/UL (Mean: 12.55; SD 5.114)
CPK: 65,000 U/L
AST: 934 IU/L (Mean: 266; SD 256)
ALT: 106 IU/L (Mean: 45; SD 43)
Vitamin A, liver: 111.1 ug/g DRY
Vitamin E, liver: 39.91 ug/g DRY
West Nile Virus PCR and viral isolation, heart, kidney, brain: Negative
Heavy metal screen, liver (another bird from affected group): Acceptable levels of all heavy metals

Gas chromatography-mass spectrometry organic chemical screen, liver (another bird from affected group): No toxic compounds detected

Histopathologic Description: Skeletal muscle: The majority of the submitted tissue is affected by various degrees of myofiber degeneration and necrosis, with hypereosinophilia, loss of striations, variations in fiber size, sarcoplasmic vacuolation and/or floccular degeneration, and rare mineralization. A mild mononuclear inflammatory infiltrate is present within the more acutely affected areas, and fatty infiltration and fibrosis characterize the more chronically affected regions. A few aggregates of macrophages contain fine granular yellow pigment (lipofuscin, presumptive) within their cytoplasm.

Heart: There is moderate multifocal myocyte degeneration, necrosis, and replacement by fibrous connective tissue. A mild mononuclear inflammatory infiltrate is present. There is mild multifocal fatty infiltration. Mild multifocal hemorrhage is present within the more acutely affected areas.
Contributor's Morphologic Diagnoses: Skeletal muscle: Myofiber degeneration and necrosis, subacute to chronic, multifocal, moderate, with myofiber drop-out, replacement by fibrous connective tissue and fatty infiltration

Heart: Myocyte degeneration and necrosis, subacute to chronic, multifocal, moderate, with myofiber drop-out, replacement by fibrous connective tissue, mild multifocal fatty infiltration, and minimal hemorrhage

Contributor's Comment: The morphologic findings within the heart and skeletal muscle of these brown pelicans are consistent with a degenerative myopathy. Differentials based on morphology include hypovitaminosis E, ionophore toxicity, and exertional myopathy. Although these pelicans were supplemented with a paste that purportedly contained vitamin E, nutritional analysis of the paste at two laboratories indicated that none of the submitted samples contained vitamin E. Additionally, nutritional analysis was performed on fish normally fed to the pelicans. Vitamin E levels within herring and spearing were at the low end of the normal range, and those in capelin were low. Combined, these findings were highly suggestive of hypovitaminosis E as the cause of the myopathy in these birds. Although vitamin E levels in the liver were within the normal range reported for most adult animals (between 20 and 40 ug/g dry wt.), this is most likely due to the fact that this bird had been on vitamin E injections from the time it was admitted to the hospital (twelve days prior to death). There was no exposure to ionophores in these birds.

The histories of these birds suggest that increased activity (recent release to an outdoor enclosure) may have led to the acute onset of clinical signs within multiple birds.

Vitamin E deficiency results in oxidative damage to actively contracting muscle fibers due to a decrease in free radical scavenging[1]. Similar findings are also present in cases of selenium deficiency. Selenium is a vital component of the antioxidant enzyme glutathione peroxidase, a lack of which also results in oxidative damage. Myopathies due to vitamin E and selenium deficiencies are most often seen in young herbivores fed poor-quality grass hay with little to no access to pasture[2]. Affected animals often have generalized weakness, with preferential involvement of muscles involved in suckling and mastication, as well as locomotory musculature. Myopathies in birds, including brown pelicans, have also been reported[3]. In the case of pelicans, fish diets are frequently high in polyunsaturated fatty acids, which when stored over time have an increased propensity to form lipid-derived oxidation products, increasing the need for vitamin E.

It is interesting to note that although other piscivorous birds in the collection (including white pelicans) were receiving the same vitamin paste and diet, none have presented to date with similar clinical signs or lesions. It could therefore be hypothesized that brown pelicans are more sensitive to hypovitaminosis E or have higher vitamin E requirements than other birds, and further investigation may be warranted.

AFIP Diagnoses: Heart; skeletal muscle: Myofiber degeneration and necrosis, multifocal, mild to severe, with replacement fibrosis and fatty infiltration, brown pelican (*Pelecanus occidentalis*), avian.

Conference Comment: The contributor provides an insightful glimpse at one of the many challenges faced when working with wildlife species as well as a concise review of vitamin E and selenium deficiency.

In general, vitamin E is a fat soluble vitamin and the alpha-tocopherol isomer is the most available and active form. It is found in vegetables, grains, nuts, fish, meat and dairy products. After absorption, vitamin E is transported via chylomicrons through the blood and accumulates in fat depots, liver, and muscle. Vitamin E is an important antioxidant that sequesters free radicals before they can initiate peroxidation of the polyunsaturated fatty acids of cell membranes. Fish often have decreased levels of vitamin E and increased levels of polyunsaturated fat which antagonizes the absorption of vitamin E. Selenium is found in varying amounts in the soil and is an essential component of the enzyme glutathione peroxidase which prevents peroxides from causing membrane damage.

A simplified pathogenesis for vitamin E/selenium deficiency follows:
Deficiency of vitamin E and/or selenium results in increased levels of oxygen free radicals which disrupt cellular metabolism and cause extensive membrane damage. Membrane damage results in an influx of calcium ions from the extracellular compartment into damaged cells greatly increasing cellular demand for energy to move calcium into mitochondria and away from calcium-sensitive myofilaments. Once mitochondria become overloaded with calcium, the end result is energy depletion. Excess calcium still in the cytosol causes myofibril hypercontraction, degeneration and subsequent cellular destruction.

In a production situation, deficiency may be severely affected by the climate and housing conditions, poor quality hay, grain, or rancid food sources such as fish or cod liver oil. Additionally, iron injections given to neonatal pigs can cause cell membrane lipid peroxidation and depletion of vitamin E/selenium.

Below is a helpful comparative pathology list of conditions associated with vitamin E and/or selenium deficiency in a variety of species:

- Muscle necrosis (skeletal, cardiac): most species, including cattle, sheep, goats, horses, pigs, dogs, mink, rats, mice, rabbits, guinea pigs, nonhuman primates, birds, reptiles and humans
- Dietetic microangiopathy (Mulberry Heart Disease), hepatic necrosis (hepatosis dietetica), encephalomalacia - conditions of vitamin E deficiency noted in swine and reported in elephants
- Hepatic necrosis (hepatosis dietetica): pigs, experimentally induced in rats, mice
- Glossal necrosis: young, suckling ruminants
- Myelin sheath and spinal cord degeneration, Equine Motor Neuron Disease: horses and other equids
- Encephalomalacia, neuronal necrosis, axonal degeneration: pigs, birds, dogs, rats, monkeys, humans
- Steatitis (yellow fat disease): may result from a combination of membrane damage and production of ceroid, an acid fast, PAS-positive, yellow pigment which may act as

an irritant foreign material, inducing inflammation; most strikingly affects subcutaneous adipose tissue, but also intracavitary fat; cats, mink, pigs, rabbits, birds, reptiles; not noted in ruminants
- Intestinal lipofuscinosis (brown dog gut): dogs
- Testicular degeneration and aspermatogenesis: rats, guinea pigs
- Fetal resorption: rats
- Hemolytic anemia: pigs, cattle, nonhuman primates, humans
- Pigmentary retinal degeneration: dogs, nonhuman primates, humans
- Cataracts: rabbits
- Exudative diathesis: poultry, pigs
- Cherry cerebellum: turkeys
- Decreased hatchability due to inadequate pipping musculature: birds

Vitamin E and selenium deficiency affects multiple species and is probably the prevailing nutritional myopathy recognized in veterinary medicine. Anytime myodegeneration, necrosis and regeneration are present vitamin E/selenium deficiency should be considered in the differential diagnosis (2).

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SLIDE 27
CONFERENCE 7 / CASE III – CP96-322 (AFIP 2594648)

Signalment: 7 month old, male, BXH-2 mouse

History: This animal died on the 13th day of quarantine

Gross Pathology: The spleen, liver and mesenteric lymph nodes were enlarged. The liver had gray mottling and the spleen was uniformly enlarged.

Contributor's Morphologic Diagnoses: Liver: myeloid leukemia
Lymph node, mesenteric: myeloid leukemia
Spleen: myeloid leukemia

Bone marrow: myeloid leukemia

Contributor's Comment: This strain of mice has a high incidence of myeloid leukemia which results in poor production of offspring and early death of adult mice. Transmission of the virus occurs not via the germ line but in utero or by the milk.

AFIP Diagnosis: Liver: Leukemia, favor myeloid origin, BXH-2 mouse, rodent.

Conference Comment: This case once again illustrates the importance of understanding and researching the specifics of the animal model you are working with, especially when it involves a specialized strain. Of interest in this case is the two-step relationship between a recessive mutation which causes myeloproliferation and a retroviral induced clonal expansion of leukemic cells resulting in myeloid leukemia (5). Although the strain of mouse supports a diagnosis of myeloid leukemia, the interpretation of cellular morphology in this case is equivocal. Immunophenotyping, including use of CD79a and CD3 antibodies, may prove especially helpful in such cases to help rule out lymphoid neoplasia.

More importantly, we used this case as a stepping-off point for a discussion on hematopoietic neoplasia and, more specifically, the two broad categories of lymphoid neoplasia and myeloproliferative disorders and the plethora of classifications and subtypes of each. While it is beyond the scope of this conference report (and this pathology resident) to recreate what Duncan and Prasse have so expertly done, a table (a.k.a. breakdown list or cheat sheet) created directly from the Duncan and Prasse text outline has been included below to help residents remember the separate categories and a few key features of each of these neoplasms (6).

HEMATOPOIETIC NEOPLASIA	
LYMPHOPROLIFERATIVE DISORDERS -lymphoid neoplasms originate from clonal transformation of lymphocytes with various degrees of differentiation and function	MYELOPROLIFERATIVE DISORDERS -all leukemias that originate in the bone marrow and involve cells **other than lymphocytes. Leukemia** is a clonal disorder where a single neoplastic cell gives rise to a homogenous progeny which can replace normal hematopoietic cells and result in myelophthisic disease.
Lymphoma; solid tissue mass, in organs other than bone marrow -**Multicentric**, multiple lymph nodes and organs -**Alimentary**, g.i. tract and regional lymph nodes -**Mediastinal**, young animals, T-cell origin -**Cutaneous**, epitheliotropic or dermal, solitary or generalized	**Acute myeloid leukemia** (AML) "blast cells' Subgroups: -*Acute undifferentiated leukemia* (AUL) -large numbers of blast cells, can be labeled with anti CD-34 -*Acute myeloblastic leukemia* (AML) **M1** If >90% of nonerythroid cells in BM are myeloblasts **M2** If 30-90% of nonerythroid cells are myeloblasts

	-*Promyelocytic leukemia* (**M3**) Only in pigs; Neoplastic cells have few azurophilic cytoplasmic gran. -*Myelomonocytic leukemia* (**M4**) both neutrophilic and monocytic cell lines; most common type of AML in dogs, cats, horses -*Monocytic leukemia* (**M5**) >80% of the nonerythroid nucleated cells in the bone marrow -*Erythroleukemia* (**M6**) coproduction of erythroblasts and myeloblasts, only reported in cats and poultry -*Megakaryocytic leukemia* (**M7**) >30% of the nucleated BM cells are megakaryoblasts
Acute lymphocytic leukemia (ALL), arises from undifferentiated lymphocytes in the bone marrow -neoplastic lymphocytes are large, round, indented nucleus, dispersed chromatin, one+ nucleoli or nucleolar rings, thin dark blue granular cytoplasm	**Myelodysplastic syndrome** (MDS) a preleukemic condition characterized by: **<30% blast cells in the BM, abnormal erythrocyte morphology** and **cytopenia affecting more than one cell line**
Chronic lymphocytic leukemia (CLL), differentiated lymphocytes that "home" to secondary lymphoid organs e.g. spleen -neoplastic cells resemble benign small Lymphocytes	**Chronic myeloid leukemia** (CML) "mature cells" Subgroups: -*Chronic granulocytic leukemia* (CGL) -*Chronic eosinophilic leukemia* -*Chronic basophilic leukemia* -*Polycythemia vera*; an unregulated proliferation of mature erythrocytes -*Essential thrombocythemia* -*Chronic myelomonocytic leukemia*; unregulated production of neuts and monos -*Chronic monocytic leukemia*
Large granular lymphocyte (LGL) **lymphoma and leukemia**, arise from bone marrow and "home" to epithelial sites, especially intestine. **LGL lymphoma**-arise in abdominal l.n., intestine, liver and spleen **LGL leukemia**-may be the primary abnormality in the dog -Both have variably sized cells with chunky, pink cytoplasmic granules often near the site of nuclear indentation	**Mast cell leukemia** Rare form of leukemia characterized by circulating mast cells which must be differentiated from a solid mast cell tumor with a leukemic blood profile.
Plasma cell tumors (Plasmacytoma and Plasma Cell Myeloma a.k.a. multiple myeloma) both are clonal proliferations of **B-lymphocytes** **Plasmacytoma-**solitary or multiple masses in older dogs, benign, skin and mucous memb.	

Plasma Cell Myeloma-clonal neoplasm of plasma cells, arises in bone marrow, often associated with monoclonal gammopathy, osteolysis in dogs, rare in cats
Must satisfy two of the four diagnostic criteria for a diagnosis:

1) Radiographic evidence of osteolysis
2) Large clusters of plasma cells in the bone marrow
3) Monoclonal gammopathy on serum electrophoresis
4) Bence-Jones proteinuria (free immunoglobulin light chains which readily pass the glomerulus)

Individuals are encouraged to review WSC case 1 (slide 04-113), conference 5, 2004-2005 for an interesting case of large granular lymphocyte leukemia in a mixed breed dog.

Additionally, a review article has recently been published which outlines the myelodysplastic syndromes: Weiss DJ. Recognition and classification of dysmyelopoiesis in the dog: a review. *J Vet Intern Med.* 2005;19:147-154.

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SLIDE 28
CONFERENCE 7 / CASE IV – TAMU-2 (AFIP 2984013)

Signalment: Nine-year-old, castrated male, Maltese *Canis familiaris*

History: The dog was taken to the groomer who called later to tell the owner that the dog "was not doing well". He was depressed, possibly seizuring, and salivating in his cage. The dog had been left in a drying cage with hot air blowers for 3 hours. On presentation, the dog had tachycardia (220 bpm) and had hyperthermia (107.3 F) with widespread cutaneous petechiae and ecchymoses. He began to improve, but then began to vomit and had abdominal pain. Although he was on fluids, diuretics, and mannitol, he became anuric. He developed a bloody, mucoid diarrhea and was euthanized in multiple system failure and DIC.

Gross Pathology: At necropsy, it was noted the dog weighed 7.15 kg while at presentation he weighed 5.21 kg. This obese dog had petechiae and ecchymosis widespread on skin and over viscera with melena as well. Approximately 500 ml and 400 ml of effusion were in the thorax and abdomen respectively. Two, 3 mm calculi were in the urinary bladder. The liver was slightly pale and friable.

Laboratory Results: On presentation, HCT=59%, corrected to 30% before euthanasia (reference range 37-55%); The dog had 42 nucleated erythrocytes per 100 nucleated cells in the hemogram and circulating leucocytes were degenerating; [Na] on presentation 164 mmole/L corrected to 154 before euthanasia (reference range 139-147 mmole/L). On presentation: Alkaline phosphatase 510 u/L, Alanine aminotransferase 459 u/L (reference ranges 24-147 u/L and 10-130 u/L, respectively). Prothrombin time 10.1 seconds, partial thromboplastin time 31.4 seconds (reference ranges 6.0 – 7.5 seconds and 7.1 – 10.0 seconds, respectively); AT III 73.4% (control >114%). Fibrin degradation products ++ and platelets were clumped. Brain cholinesterase 0.074 pH/hr (normal 0.15 – 0.25). Anticoagulant screening and pesticide screening were negative.

Histopathologic Description: The liver presented has submassive, often bridging, periacinar (zone 3) hepatocytic necrosis, hemorrhage and dissociation of cords. Many apoptotic hepatocytes and acidophilic bodies are in the affected area. Edema and scattered megakaryocytes are noted.

Contributor's Morphologic Diagnosis: Submassive periacinar hepatic necrosis.

Contributor's Comment: The impressive liver lesion while typical of exertional heat stroke (EHS) or ambiental heat stroke remains unexplained as to mechanism. In humans, the lesion development varies between individuals and heat "dose", and lesions often occur with a delay from the heat insult. The liver lesion is severe enough to have prompted the practice of liver transplantation in cases of EHS.(5,9,13,14) The periacinar pattern of necrosis has resulted in the hypothesis that this is hypoxic

necrosis, and the term, “ischemic hepatitis”, has been applied to the lesion.(13) Indeed, shunting of blood from viscera, the dehydration, and the hypovolemia associated with heat stress all would lead to hypoxia; however, the contributor feels it is more than this. The disassociation of cords would support a diagnosis of shock, but the apoptotic features make me want to invoke a reperfusion hypothesis (see below). Interestingly, the lesion is redolent of severe cases of rabbit calicivirus infection.

The liver lesion of heat stroke in dogs is not a well-characterized.(1,3,4,6,8) There are no good descriptions of the hepatic lesion in dogs with lethal exertional or ambiental heat stroke. The delayed and progressive failure is an important feature because in dogs found acutely dead in cars from heat stroke (e.g. when the owner puts dog in the trunk of the car for a long ride on a hot day (10), the liver lesion is not present; rather, the lesions are restricted grossly to a “parboiled” carcass, with typical agonal hemorrhages, visceral congestion, and without hemorrhagic diathesis. The veterinary literature comments on renal complications, hemoconcentration, increased circulating nucleated RBCs, hypoglycemia, and hemorrhagic diathesis in animals that die. Many animals that die with a delay from outset of signs have not been completely examined but one case refers to “centrolobular necrosis(4).” The bloody mucoid stool may not be specific and is typically seen in severe shock due to a variety of causes in dogs. The dog of this report also had clinical and histologic renal involvement. Regarding the apoptosis, hyperthermia has been shown to cause hepatic apoptosis in humans, rats, and dogs (7,11,15); such that, liver blocks from dogs treated with one hour of hyperthermia have been used for apoptosis controls (7), cool or what! Experimental hyperthermia in dogs reflects much of what we see in this dog.(2,12) No doubt the obesity and maybe the temperament (excitability) of this Maltese may have exacerbated the hyperthermic event of this dog. Unfortunately, no clinical chemistry was performed in the end stages of this dog’s process. It is presumed the liver enzymes would have been much more severely affected just prior to death.

Other interesting features added spice to this case. The owner’s lawyer insisted we were negligent in our weighings (the Maltese weighed almost 2kg more at necropsy). However, anuria during correction of dehydration, ascites, and hydrothorax are presumed to explain the weight gain. (Veterinarians are warned to exercise care in hydration of these patients.) Seizuring, perhaps related to hypoglycemia or brain edema, is reported in severe, canine heat stroke, but the groomer’s comments of salivation and seizuring urged us to pursue a diagnosis of organophosphate intoxication. No organophosphates were found. It must be concluded that other processes besides organophosphate exposure must be able to lower brain cholinesterase. Because of pending litigation, we tested to be able to say that the hemorrhagic diathesis was not due to anticoagulant exposure.

AFIP Diagnosis: Liver: Necrosis, centrilobular and midzonal, acute, diffuse, Maltese, canine.

Conference Comment: A great lesion with an interesting story to accompany it. The contributor provides thoughtful insight into heatstroke, a commonly encountered condition occurring more frequently in summer when dogs are negligently kept locked in sweltering cars while their owners run errands. As the contributor alluded to, perhaps this dog was kept alive with supportive therapy longer than most, allowing this rarely observed lesion to develop.

Conference attendees discussed the high number of nucleated erythrocytes in the peripheral blood. Although commonly associated with lead toxicosis, chronic hypoxia and extramedullary hematopoiesis; in this case, the extremely high numbers were attributed to an acute breakdown of the blood-bone marrow barrier with systemic dissemination of nucleated red blood cells. Other important laboratory findings include elevated levels of alkaline phosphatase (ALP) and alanine aminotransferase (ALT), inducible and leakage enzymes associated with hepatocellular damage; prolonged prothrombin time (OSPT), partial thromboplastin time (APTT) and elevated fibrin degradation products (FDPs), all supportive of disseminated intravascular coagulation (DIC). Additionally, antithrombin III levels are reduced suggesting either consumption due to DIC or loss via a compromise in glomerular integrity and subsequent proteinuria.

There is some variation in slides which prompted discussion of the pattern of hepatocellular necrosis. Robbins and Cotran defines necrosis of entire lobules as submassive and of most of the liver as massive; whereas, Thomson's defines massive necrosis as that of an entire hepatic lobule or contiguous lobules without reference to a submassive pattern (16,17).

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SLIDE 29
CONFERENCE 8 / CASE I – MK05-4177 (AFIP 2992272)

Signalment: Adult, intact, male, rhesus macaque, *Macaca mulatta*

History: This monkey was challenged with simian immunodeficiency virus (SIV) approximately 4.5 years ago and treated with a regimen of antiretroviral therapy. The animal had a 4 month history of dry skin and a recurring nail infection, both of which responded to medical treatment, as well as a 4 month history of poor appetite. Lesions on his back and in his mouth were observed 11 days prior to necropsy.

Gross Pathology: On necropsy the monkey was in poor nutritional condition with numerous bony protuberances and scant adipose stores. Multifocal, varying sized proliferative lesions with central areas of ulceration were observed randomly scattered about the oral and gingival mucosa. There were two ulcerated areas on the skin of the left lateral thoracic region, one measuring 1.5 cm and the other measuring 1 cm in diameter. The entire left lung field was mottled dark purple, dark grayish red and red

and palpated meaty. The left caudal lung lobe had pleural adhesions. The right lung field was mottled reddish gray, red, and pink, and a small amount of crepitus was noted upon palpation. Lesions were not observed in any other organs.

Laboratory Results: PCR on mesenteric LN was negative for *Mycobacterium* spp.

Histopathologic Description: The samples submitted were sections of lip with a mucosal side and a haired skin side, although not all slides may have had haired skin present. The lesions of interest were in the oral mucosa; however, some sections showed involvement of the haired skin as well.

Involving the oral mucosa was a focally extensive area of ulceration that peripherally had epithelial hyperplasia. Associated with the ulcerated areas were inflammatory cells. The inflammatory cell population was largely comprised of neutrophils and macrophages admixed with cellular debris. Multiple epithelial cells at the margin had slightly expanded and vacuolated cytoplasm. Numerous other cells, individually or as syncytia, were expanded and contained amphophilic to basophilic intranuclear inclusion bodies.

Contributor's Morphologic Diagnosis: Oral cavity: Stomatitis, ulcerative, acute to subacute, multifocal, moderate to severe with intranuclear inclusion bodies.

Contributor's Comment: The most likely cause of the oral lesions in this case is Herpes Simplex Virus (HSV), which is common in HIV infected patients. The most common site of infection is the mucocutaneous junction of the lip, although lesions can be found elsewhere on the oral mucosa, conjunctiva, skin, esophagus, and external genitalia (7, 11, 13). Histologically, HSV infections appear with necrosis, ballooning degeneration, multinucleated giant cells, and intranuclear inclusion bodies (7).

There are three main subfamilies within Herpesviridae that infect monkeys: alpha-herpesviruses (simplex virus, simian varicella, B virus), beta-herpesviruses (cytomegaloviruses), and gamma-herpesviruses (lymphoproliferative viruses) (5). Most of the herpesviruses that infect nonhuman primates belong to the alpha-herpesviruses, which generally cause mild disease in the natural host but can cause life-threatening disease in unnatural hosts. The lymphoproliferative herpesviruses, such as Epstein-Barr virus, rhesus lymphocryptovirus, and rhadinovirus are generally associated more with lymphoproliferative disorders as opposed to ulcerative dermatitis (2, 5, 9). Herpes B, an alpha-herpesvirus, is a common virus of rhesus macaques that can cause lesions on the dorsal surface of the tongue and at the mucocutaneous junction; however, the gross lesions are generally only associated with an acute primary infection and are rarely noted in chronically infected animals (6). Another alpha-herpesvirus, simian varicella (Medical Lake Macaque virus), causes a chicken pox-like disease that forms a papule to a vesicle in gross appearance (4, 12, 14). Histologically, infected cells have enlarged nuclei with discrete intranuclear inclusions, and so should be considered as the possible etiologic agent in this case; however, there is not much evidence in the

literature that rhesus macaques get infected. The most common herpesvirus associated with SIV infections of rhesus monkeys is the rhesus monkey cytomegalovirus (CMV) which causes a systemic infection causing areas of necrosis in multiple organs and producing large intranuclear basophilic inclusions (11, 13).

Monkeys that have been infected with SIV can display a variety of clinical signs and lesions, largely dependent on the duration and stage of infection. This particular monkey had been infected for three and a half years by the time of death; therefore the gross findings were reflective of the chronicity of the infection. Lymphoid tissues are generally the principal target organs for SIV, and with prolonged SIV infections we expect to see depletion of follicles, paracortices, and periarteriolar lymphoid sheaths (3, 8, 10). In this animal, moderate to marked lymphoid depletion was noted in all lymph nodes as well as the spleen. In addition, this animal also had a markedly involuted thymus, which is a consistent sign among monkeys with late stage SIV infections regardless of age (8).

The hallmark sign of AIDS in either humans or macaques is severe immunosuppression resulting from an impairment primarily of cell-mediated immunity, especially CD4+ T cells (3). Immunosuppression then leads to secondary opportunistic infections. In this animal, the ulcerative lesions on the oral mucosa are associated with a secondary, herpesvirus infection.

In SIV infected animals, opportunistic infections are the hallmark of the development of AIDS, and in many cases some of the first signs are cutaneous lesions (11, 13). There are several etiologies that are commonly associated with oral and cutaneous lesions. Of the bacterial infections that complicate SIV infection and simian AIDS, *Staphylococcus aureus* is the most common (11, 13). These lesions are characterized by folliculitis, abscesses, and cellulitis. Mycobacterial disease, usually caused by *Mycobacterium tuberculosis* and *M. avium intracellulare*, is also known to complicate SIV or HIV infection (11, 13). The usual lesions are a tuberculosis chancre that begins as a painless nodule and forms an ulcer; however, skin lesions with tuberculosis are rarely encountered in human practice (11).

There are several secondary fungal infections that can cause disease in HIV/SIV infections. The most common fungal infection associated with AIDS is *Candida*, the cause of thrush (oral candidiasis) (11, 13). *Candida* is a part of the normal flora of the oropharynx and gastrointestinal tract, and so is a common source of secondary infections. The lesions appear as white plaques, usually on the tongue or oral mucosa, that when scraped off leave a bleeding ulcer behind. Histologically, pseudohyphae and spores are evident in the stratum corneum (13). Cryptococcosis is another fungal infection that is transmitted by inhaling spores from the environment, and disseminates readily in immunocompromised patients (13). The lesions associated with cryptococcosis are papules, nodules, pustules, and ulcerations that mainly involve the head and neck. Lastly, coccidioidomycosis can cause disseminated cutaneous involvement in AIDS patients (13). In addition, *Pneumocystis carinii* has been commonly identified as an opportunistic pathogen (13).

Parasitic infections must also be included when considering secondary infections associated with SIV. The most common parasitic infection of patients with HIV is *Sarcoptes scabiei*, which generally cause hyperkeratosis and plaque formation distributed diffusely (11, 13).

AFIP Diagnosis: Lip: Cheilitis, ulcerative, multifocal, subacute, marked, with epithelial syncytia and amphophilic to eosinophilic intranuclear inclusion bodies, rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: The contributor provides a concise list of herpesviral diseases of nonhuman primates as well as an excellent review of opportunistic infections in immunocompromised primates. Immunohistochemistry with herpes simplex virus I and II antibodies revealed strong nuclear positivity.

Herpesviruses are composed of an icosahedral shaped nucleocapsid surrounding a double strand of DNA and protein. The nucleocapsid is contained within a lipoprotein envelope which is coated with numerous small glycoprotein peplomers. Herpesvirus virions are fragile and do not survive for long outside the host. Transmission occurs through close or intimate contact, respiratory droplets or transplacentally. Virions attach to host cell receptors via glycoprotein peplomers and the nucleocapsid portion enters the cell through either fusion of the envelope to the cell membrane or by phagocytosis. Once inside the host cell, the DNA-protein complex enters the nucleus, interrupts host cell macromolecular synthesis and initiates the production of its own proteins for construction of more virions. Newly assembled mature virions bud from the nucleus, accumulate within vacuoles in the cytoplasm and are ultimately released by exocytosis or cytolysis. Eosinophilic intranuclear inclusion bodies, visible with the light microscope, are characteristic of herpes virus infections. In general, the alphaherpesviruses typically produce focal lesions in the skin and mucosa of the respiratory or genital tracts. In neonates, alphaherpesvirus infections cause necrosis in a variety of organs and, in pregnant animals, can lead to abortion with necrotic lesions found throughout the fetus. Betaherpesviruses are not typically lytic and cause the formation of greatly enlarged cells (hence the name cytomegalovirus). Whereas gammaherpes viral infections are associated with lymphoproliferative disease and neoplasia. An important characteristic of herpes virus infections is their ability to become latent with intermittent or persistent recrudescence and viral shedding. Alphaherpesviruses are typically latent in nerves; betaherpesviruses in secretory glands, lymphoreticular organs and the kidney; and gammaherpes in lymphoid tissue (15).

A list of common veterinary herpesviral infections is included below. However, herpesviruses have also been found in insects, reptiles, amphibians, mollusks, and exotic mammals, including marine mammals (15).

<u>Alphaherpesviruses</u>

- Bovine:
  - BHV1: Infectious bovine rhinotracheitis
    Infectious pustular vulvovaginitis
    Infectious balanoposthitis
  - BHV2: Bovine mammillitis virus/ Pseudo-lumpy skin disease
  - BHV5: Bovine herpesvirus encephalitis (no inclusion bodies)
- Equine:
  - EHV1: Equine herpesviral abortion, rhinopneumonitis, neurologic disease
  - EHV3: Equine coital exanthema
  - EHV4: Equine rhinopneumonitis, abortion
- Porcine herpesvirus-1: Pseudorabies, Aujeszky's disease
- Canine herpesvirus-1: Canine herpes
- Feline herpesvirus-1: Feline viral rhinotracheitis (FVR)
- Avian:
  - Avian HV1: Infectious laryngotracheitis
  - Avian HV2 (Gallid herpesvirus-2): Marek's disease
  - Anatid HV1: Duck plague
- Nonhuman primate:
  - Herpesvirus simiae (Cercopithecine herpesvirus 1; B virus): Herpes B
  - Herpesvirus tamarinus (Cebid herpesvirus 1; Herpes T): localized disease in squirrel monkeys; generalized disease in marmosets, tamarins, owl monkeys
  - Herpesvirus simplex, type 1: oral lesions in humans, apes, monkeys
  - Herpesvirus simplex, type 2: genital lesions in humans, apes, monkeys
  - Simian varicella: Simian varicella in macaques, African green monkeys, Patas monkeys
  - Herpesvirus papio 2: Oral and genital lesions in baboons

Betaherpesviruses

- Caviid herpesvirus 1: Guinea pig
- Porcine herpesvirus-2: Porcine cytomegalovirus disease/Inclusion body rhinitis

Gammaherpesviruses

- Bovine:
  - Alcelaphine herpesvirus 1: Bovine Malignant Catarrhal Fever
  - BHV4
- Equine:
  - EHV2
  - EHV5
- Nonhuman primates
  - Herpesvirus saimiri (Cebid herpesvirus 2): Squirrel monkey natural host, lymphomas in marmosets, owl monkeys, African green monkeys, howler monkeys and spider monkeys
  - Herpesvirus ateles: Spider monkey natural host, lymphomas in marmosets and owl monkeys
  - Rhesus rhadinovirus: Related to Human herpesvirus-8 (Kaposi sarcoma), possible association with retroperitoneal fibromatosis
  - Rhesus lymphocryptovirus: Lymphoma and epithelial proliferation in immunodeficient hosts

- Marmoset lymphocryptovirus: Gastrointestinal lymphoma

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SLIDE 30

CONFERENCE 8 / CASE II – E1191/05 (AFIP 2987121)

Signalment: 4 years and 10 months old, male, Rottweiler-mix dog (Canis familiaris)

History: The dog was presented to the clinic with a history of recurrent cough for three and a half years associated with fever and diarrhea. Antibiotics, immunostimulating drugs, and a special diet were given. Upon clinical examination, an intense inspiratory sound was present. Radiographically, an interstitial-bronchiolar shadow was found. Endoscopically, hyperemic and slightly edematous mucosal surfaces were observed in the nasopharynx, tonsils, trachea, and bronchi. In deeper airways a mucopurulent exudate was noticed. A foreign body was not detected. Predominantly on the right side, the submucosal tissue appeared granular. Cytological examination of bronchial smears revealed hyperplastic ciliated epithelium, few squamous epithelial cells interpreted as metaplastic cells, numerous goblet cells, few segmented neutrophilic granulocytes and macrophages, as well as mucus. A lobectomy of the right middle lobe was performed, and the tissue was submitted for histopathological examination.

Gross Pathology: The formalin-fixed, submitted lung lobe measured 8 x 5 x 5 cm. The pleura was glossy and smooth. On cut surface multiple cavities ranging from 0.5 to 1.0 cm in diameter were present. The cavities contained viscous, turbid, yellow fluid. Along the dorsal surface of the lobe, a moderate alveolar emphysema was observed.

Laboratory Results: White blood cell counts on four different occasions revealed varying abnormalities: leukocytosis (33,73 x10^3/µl; ref. range: 5 – 16), mild lymphocytosis (33%; ref. Range 10 – 30), mild granulocytosis (86%; ref. range: 66 –77), and slightly elevated numbers of eosinophilic granulocytes (9%; ref. range 1 4) and monocytes (9%; ref. range: 0 –6).

Microbiological results: A deep airway swab: alpha-hemolyzing and non-hemolyzing streptococci, coagulase-negative staphylococci were isolated.

Histopathologic Description: In the submitted lung tissue single, severely distended bronchi and bronchioles are present. Lumina contain variable amounts of mucopurulent exudate. The ciliated columnar epithelium is hyperplastic and infiltrated with numerous neutrophilic granulocytes and single lymphocytes. In the submucosa, a mild to moderate, diffuse infiltration with lymphocytes and plasma cells is present admixed with few neutrophilic granulocytes and macrophages. In addition, numerous hyperplastic lymphoid follicles and moderate fibrosis are seen. The smooth muscle layer of numerous arteries is moderately hypertrophic. Adjacent alveoli are collapsed and mild alveolar histiocytosis is found.

Contributor's Morphologic Diagnoses: Lung: bronchitis, lympho-plasmacellular and mucopurulent, diffuse, severe, chronic with severe bronchiectasis, epithelial hyperplasia, peribronchiolar follicular hyperplasia, and peribronchiolar atelectasis; moderate hypertrophy of vascular smooth muscles; canine.

Contributor's Comment: Bronchiectasis is an infrequent and debilitating condition of humans and animals, including dogs, cats, cattle, sheep, pigs, mice, and horses[3,11,13]. It is characterized by an irreversible, abnormal dilatation of bronchi. Most often bronchiectasis represents an acquired lesion subsequent to some types of bronchitis. In rare cases it is a congenital malformation. The circumscribed destruction of the airway wall results in focal or diffuse chronic airway dilatation. Accumulation of exudate in the lumen and partial rupture of bronchial walls occur frequently[3].

Grossly, bronchiectasis is characterized by prominent lumps resulting from distension of bronchi and concurrent obstructive atelectasis of the surrounding parenchyma. Bronchiectasis is often mistaken for pulmonary abscesses, because on cut surface dilated bronchi are filled with purulent exudate[14].

Morphologically, two types of bronchiectasis are distinguished, saccular or cylindrical. Bronchiectasis is regarded as saccular when only a small localized portion of the bronchial wall is destroyed with development of thin-walled circumscribed outpouchings. This type of bronchiectasis is less common. It can result from focal necrotizing bronchitis and is described in sheep, cattle and occasionally in horses with chronic obstructive pulmonary disease. Cylindrical bronchiectasis is characterized by destruction of a large bronchial segment or along its entire length. It is localized frequently in the cranio-ventral parts of the lung, which had been affected by chronic suppurative bronchitis[3]. Bronchiectasis rarely occurs as a congenital malformation.

In cattle, sheep, and pigs, bronchiectasis is acquired after severe broncho-pneumonia or is associated with severe parasitic bronchitis in sheep, goats and pigs[3]. Pathogenically, suppurative bronchitis causes damage to and weakening of the bronchial wall by neutrophilic lysosomal enzymes and oxygen radicals. The accumulation of inflammatory exudate results in obstruction of lower airways followed by alveolar atelectasis. Therefore, inspiration causes traction on the walls of the airways resulting in additional expansion of the lumina. The airflow is less rapid and muco-ciliary clearance is ineffective due to ciliary damage contributing to the accumulation of exudate in the lumen.

In dogs, bronchiectasis usually results from chronic inflammation associated with chronic bronchitis, foreign body or neoplasm[3]. In dogs, atelectasis is a less likely a sequelae of airway obstruction, because there is a very effective collateral ventilation. This may explain that bronchitis is a less frequent cause of bronchiectasis in this species or is limited to only one or two lobes, more often the middle lobes[3]. Other pulmonary lesions including allergic lung disease may also contribute to bronchiectasis in dogs[9,17]. An increased risk of bronchiectasis has been reported in American Cocker Spaniels, West Highland White Terriers, Miniature Poodles, Siberian Huskies, English Springer Spaniels, and dogs over 10 years of age[9].

In cats bronchiectasis was more frequently found in older male individuals and mostly restricted to a single caudal lung lobe. Concurrent respiratory diseases in cats included chronic bronchitis and bronchiolitis, neoplasms, bronchopneumonia, endogenous lipid pneumonia, and emphysema[17]. Heaves, a condition in horses associated with chronic

airway inflammation and mucous accumulation[13], may be accompanied by bronchiectasis.

Bronchiectasis with congenital conditions has been described in several breeds of dogs with primary ciliary dyskinesia (immotile cilia syndrome)[1,2,4,5,6,7,8,10,12,15,18,19]. Littermates with this condition have been recognized in English pointers[15], English Springer Spaniels[6], and Old English Sheepdogs[18]. Breeding studies suggest that it is an autosomal recessive trait. In humans, approximately 50% of the patients with primary ciliary dyskinesia have Kartagener's syndrome (situs inversus or partial lateralizing abnormality, sinusitis, and bronchiectasis). Similarly, about 50% of dogs with primary ciliary dyskinesia are affected by situs inversus[1,3]. These dogs usually develop bronchiectasis as a part of abnormalities associated with a basic ciliary defect. A variety of other clinical syndromes are seen with ciliary dyskinesia such as sinusitis, rhinitis, and recurrent bronchopneumonia. Due to the impaired mucociliary clearance subsequent bacterial infections are seen[1]. A common finding in dogs with primary ciliary dyskinesia is communicating hydrocephalus[2,5,6,7,18]. Males are infertile because the tails of spermatozoa are modified cilia, and therefore sperms are often dysmotile[1,5,15,18].

The basic ciliary defect is usually associated with one of several ultrastructural abnormalities of cilia throughout the body, including absence of one or both of the inner and outer dynein arms, microtubular transposition, random microtubular orientation, and partial microtubular deficiency[15].

Definitive diagnosis of primary ciliary dyskinesia usually requires ultrastructural analysis of respiratory cilia, but the fact that several body systems are affected often permits a probable diagnosis to be established using other diagnostic techniques. Interestingly, it has also been noted that some dogs with primary ciliary dyskinesia have no ultrastructural ciliary lesions[5,7].

AFIP Diagnoses: Lung: Bronchiolitis, lymphoplasmacytic, diffuse, chronic, severe, with bronchiolectasis, bronchiolar epithelial hyperplasia, vascular smooth muscle hypertrophy and interstitial fibrosis, Rottweiler-mix, canine.

Conference Comment: The contributor provides an excellent review of bronchiectasis. In this case, the damaged airways present in the sections examined are bronchioles prompting the use of the term bronchiolectasis.

Conference attendees discussed the pathogenesis of bronchiectasis and the complex role of ciliary clearance in the development of respiratory disease. In short, bronchiectasis occurs as a result of the accumulation of exudate in the lumen and partial rupture of bronchial walls. Proteolytic enzymes released from phagocytic cells during chronic inflammation degrade and weaken smooth muscle and cartilage that normally help maintain bronchiolar diameter. The result is destruction of the bronchial and, in this case, bronchiolar walls (20).

Dogs with primary ciliary dyskinesia (PCD) have congenitally impaired mucociliary clearance. This results in the congestion of upper and lower airways with mucus rendering them more susceptible to repeated bacterial infections. Initial signs include a mucoid to mucopurulent nasal discharge, sneezing and coughing (7).

Findings in dogs with PCD include chronic rhinitis characterized histologically by infiltration of the nasal mucosa with plasma cells and neutrophils. Additionally, submucosal mucous glands can become hyperplastic. Other lesions of the upper airways include hypoplastic nasal turbinates, frontal sinusitis, atresia of the frontal sinuses and rhinoliths. Lower airway lesions range from mild bronchitis and bronchiolitis to severe bronchopneumonia, bronchiectasis and ventral lung lobe consolidation (7).

The rhythmic, coordinated beating of cilia is a complex process whose mechanics go beyond the scope of this text. As students of pathology; however, it is important to familiarize ourselves with the ultrastructure of cilia in order to recognize the more common causes of ciliary dyskinesia. In short, cilia are covered by a ciliary membrane which surrounds the axenome or core. The core is composed of nine outer microtubule doublets surrounding two single, centrally located microtubules (remember the 9, 2+2 memory aid i.e. nine outer doublets plus two inner microtubules). Each outer doublet is made up of an A-microtubule and a B-microtubule. The A-microtubule has paired inner and outer dynein arms which project toward the B-microtubule of the adjacent doublet. Additional structural proteins include nexin links which bridge the gap between adjacent doublets and radial spoke linkages which project inward from the A-microtubule toward the center and terminate in a bulbous spoke head (7). In humans with PCD, the most common abnormalities are absence of inner and/or outer dynein arms, transposition of microtubules and defective radial spokes (18). The most commonly reported lesions in dogs include dynein arm deficiency, abnormal microtubular patterns, random orientation, and electron-dense inclusions in the basal body. The basal body, located at the base of the cilia, is derived from a centriole and has nine peripheral microtubule triplets that give rise to the doublets of the axenome. The basal body is anchored to the cell by a striated rootlet and has a lateral projection called the basal foot process (7).

In this case, a picture is worth a thousand words and students are encouraged to study pictures of cilia ultrastructure and become familiar with the complex anatomy. References 7 and 18, as well as several of the others, have excellent diagrams and electron micrographs to help in the understanding of ciliary anatomy, function and dysfunction.

Although ultrastructural analysis can be helpful in diagnosing PCD when lesions are identified in a large percentage of cilia from different locations; in some dogs with PCD no ultrastructural lesions are observed (7).

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SLIDE 31
CONFERENCE 8 / CASE III – #8568 (AFIP 2985462)

Signalment: 16 month, female, Multimammate rat, *Mastomys natalensis*

History: A 16 month old female multimammate rat, *Mastomys natalensis*, that was part of a breeding pair, was initially noted to have a small subcutaneous lesion in the right axillary region that slowly progressed to a 2.5 centimeter in diameter firm, multilobular mass. Due to the rat's recent poor reproductive performance, surgical intervention was not considered. The rat was euthanized and submitted for pathological examination.

Gross Pathology: Within the subcutaneous tissue there is a firm, circumscribed, multilobular, tan mass approximately 2.5 cm in maximum diameter.

Histopathologic Description: Within and expanding the subcutis of the ventral skin and extending to the cut border is a 2x1 cm, unencapsulated, but well delineated, lobulated, infiltrative, and densely cellular neoplasm. The tumor is composed of epithelial and spindle-shaped cells arranged in a solid and closely-packed acinar pattern, as well as a stellate to spindloid pattern, respectively. The neoplastic epithelial cells are arranged predominantly as a solid mass of disorganized lobules occasionally forming small acini supported by a highly vascular fibrous stroma. Irregular structures suggestive of ducts of varying size are interspersed throughout the neoplasm. There are variably sized cystic structures lined by pleomorphic acinar cuboidal epithelium filled with eosinophilic proteinaceous secretions and some necrotic cellular debris. Morphologically the neoplastic epithelial cells range from cuboidal to columnar with marked pleomorphism, eccentric heterochromatic nuclei, occasional prominent nucleoli, a high nucleus to cytoplasm ratio, and a moderate mitotic rate of zero to four mitotic figures per 40-power field.

There are scattered interspersed regions of neoplastic spindle-shaped cells projecting into the solid mass where the cells form streams or bundles. The neoplastic spindle cells exhibit indistinct cell borders, oval to spindle-shaped nuclei with blunted ends, one or more distinct nucleoli, clear, crisp cytoplasmic vacuoles, and occasional multinucleate cells. Mitotic figures vary from 1-6 per 40-power field. Additional features of the neoplasm include multiple foci of necrosis with scattered neutrophils surrounded by fibroplasia.

Immunohistochemical staining of the epithelial neoplastic cells was negative for alpha smooth muscle actin (SMA), vimentin, and cytokeratins 5/6 (C5/6). Cytokeratin CAM 5.2 antibodies resulted in a strong positive staining of tumor cells except for an area of faint staining in one nodular region. Tumor cells were variable in their staining for desmin with the majority of cells exhibiting negativity. The large bands of fibroblasts-like cells stain differently from the main tumor and are positive for SMA, negative for C5/6, and variably positive for CAM 5.2, vimentin and desmin.

Contributor's Morphologic Diagnosis: Mammary gland carcinoma with epithelial to mesenchymal transition

Contributor's Comment: Multimammate rats (*Mastomys natalensis*), sometimes referred to as multimammate mice, are widely distributed rodents of the family Muridae native to African savannas and sub-Saharan regions.[1] Over their 3 year life span these rats breed as monogamous pairs with 6-12 pups per litter. As described in their common name, they typically possess 12, and as many as 18 pairs, of mammary glands. Other unique physiological characteristics include submaxillary salivary glands rich in nerve growth factor[2], prostate glands in both males and females[3], and the absence of a gall bladder. In addition to being susceptible to *Yersinia pestis*, *Mastomys* are natural hosts for leishmaniasis, Lassa virus, and leptospirosis and consequently are commonly used in research involving these agents. Multimammate rats spontaneously develop gastric carcinoids and serve as an animal model for human Zollinger-Ellison syndrome.[4]

Spontaneous mammary gland tumors are common neoplasms in rodents. Variations in cell types of origin, frequency of occurrence and malignancy exist among species and individual strains. Although neoplasia is rare in guinea pigs (*Cavia porcellus*), mammary tumors are represented most frequently by fibroadenomas and ductal adenocarcinomas.[5] In a study of 634 female Djungarian hamsters, (*Phodopus campbelli)* greater than 11% were found to have mammary adenocarcinomas.[6]

Mammary tumors are the most frequently occurring neoplasms in most stocks and strains of rats. In Sprague-Dawley stocks the incidence can approach 50% whereas Fisher 344 rats show an incidence of 15%.[7] Benign fibroadenomas in aged females are the predominant (80-90%) tumor type seen in female Sprague-Dawley rats. In addition to sex, age and genetics, dietary and endocrine factors have been shown to play a role in their occurrence.[8] A reduction of food intake by 20% has been shown to decrease the incidence of mammary tumors in Sprague-Dawley rats by 80%.[9] Ovariectomy markedly reduces tumor incidence and, in one study, prolactin levels were 25 times higher in rats with mammary tumors than in 6-month-old female controls.[8,10] Adenocarcinomas of the mammary gland occur infrequently in the rat.[8]

A variety of factors have been shown to cause mammary tumors in mice; viruses, chemical carcinogens, radiation, genetics, immune status, diet, and hormones.[11] Such tumors are relatively rare in BALB/c, C57BL, and AKR mice, while C3H, DBA/2, and A mice have a high prevalence for spontaneous mammary tumor development.[12] Unlike

in humans where metastasis typically involves bone and draining lymph nodes, metastatic lesions secondary to murine mammary tumors are primarily pulmonary in nature.[13,14]

Classification of spontaneous mammary tumors in mice has evolved through several schemes over the last 45 years. In an initial attempt malignant murine mammary tumors were classified as carcinoma, carcinoma with squamous differentiation, and carcinosarcoma. Carcinomas were then further subdivided into adenocarcinoma types (A, B, C, Y, L, and P) with types A and B most common. Type A is represented by adenomas along with tubular and alveolar carcinomas. Type B tumors exhibit regions with varying degrees of differentiation within the same mass. Local invasion and pulmonary metastasis characterize both types.[15] A later system classified tumors according to tissue type (alveolar, ductal, and myoepithelial). Both systems have proven to be insufficient in defining the numbers of mammary tumors recently seen with the emergence of genetically engineered mice. As a result a panel of veterinary and medical pathologists convened in 2000 to produce a scheme based on descriptors and modifiers.[16]

Nomenclature for Histopathological Description of Mammary Gland Pathology in Genetically Engineered Mice

Descriptors

Descriptors	**Definition**
Glandular	Tumor is composed of glands
Acinar	Tumor is composed of small glandular clusters with small lumens. While this is a subclass of glandular, it is very characteristic of MMTV-induced tumors
Cribriform	Tumor is composed of sheets or nests of cells forming lumens with round, punched out spaces
Papillary	Tumor has finger-like projections composed of epithelium covering a central fibrovascular core
Solid	Tumor is composed of solid sheets of epithelial cells with little or no glandular differentiation
Squamous	Tumors composed solely of squamous cells with or without keratinization, absence of glandular pattern
Fibroadenoma	Tumor is composed of a proliferation of both myxoid fibrous stroma and glands
Adenomyoepithelioma	Tumor is composed of myoepithelium and glands
Adenosquamous	Tumor has both glandular and squamous differentiation
Not Otherwise Specified (NOS)	Tumor does not have any of the other common descriptor

Modifiers

Modifiers	Definition
***Biological potential*:**	
Carcinoma	Neoplasm originating from epithelium with proven malignant biological behavior
Adenocarcinoma	Neoplasm originating from glandular epithelium with proven malignant biological behavior
Adenoma	Neoplasm originating from glandular epithelium without proven malignant biological behavior
Mammary Intraepithelial neoplasia (MIN)	Spectrum of intraluminal epithelial proliferations with cytologic atypia including in situ carcinomas
Hyperplasia	Any increase in cell number without cytologic atypia
Tumor	Any space occupying mass with unknown biological potential
***Property*:**	
Atypia	Cells with abnormal nuclear morphology
Necrosis	Cell death generally not applied to programmed cell death (apoptosis)
Fibrosis	Increased or abnormal deposition of connective tissue
Secretory	Tissues or glands producing and exporting lipid or protein
Metaplasia	A change from one adult cell type to another adult cell type
***Topographic*:**	
Diffuse	All of the mammary gland is involved in the process
Focal	One area of the mammary gland is involved in the process
Multifocal	Multiple foci are in the mammary gland
***Inducer (Etiology)*:**	
Gene-induced	Tumors that have morphological or cytological patterns characteristic of specific transgenes or specific mutations. (myc-type, ras-type, erb B2- type)
MMTV-induced	Tumors known to be induced by the mouse mammary tumor virus (MMTV)
Chemically-induced	Tumors known to be induced by a chemical carcinogen
Hormone-induced	Tumors known to be induced by exogenous hormones
***Biological/Experimental context*:**	
Biological	Parity, pregnancy, lactation, involution, hormones
Experimental	Promotor, exogenous hormones or chemicals

Source: Cardiff RD et al. (2000)

Recently in 2004, mouse mammary tumors were characterized by morphology and immunohistochemistry for expression of terminal differentiation and categorized as ‘simple’ carcinomas, ‘complex’ carcinomas and carcinomas with epithelial to mesenchymal transition (EMT) markers.[17]

Mammary neoplasia, common in mice and rats, has been shown to be rare or absent in surveys of spontaneous disease in *Mastomys natalensis*.[18,19] In this instance a solitary

mammary tumor was present with no evidence of pulmonary or lymph node metastasis. Immunohistochemical staining for different components of the cytoskeleton such as intermediate filaments (e.g. cytokeratins and vimentin) and actin microfilaments can be used to determine mesenchymal from epithelial cell types of origin.

Keratins consist of a group of 19 polypeptides of molecular weights between 40-67 kilodaltons and serve as reliable markers for epithelial cell differentiation. Subsets of keratins are expressed variably as cells differentiate. Carcinomas generally express cytokeratin patterns reflective of the cell type of origin. Non-epithelial tumors are most frequently cytokeratin negative although few mesenchymal neoplasms may show faint or focal positivity to cytokeratins 8/18/19. In humans, breast myoepithelial cells express cytokeratins 5/14. In luminal and secretory mammary epithelia expression of cytokeratins 8/18 predominate.[20] Therefore antibody binding to selected cytokeratins can aid in differentiating myoepithelial from ductal or lobular mammary carcinomas.

In this case, negative staining for vimentin rules out the likelihood of a mesenchymal precursor. Lack of staining for both alpha smooth muscle actin and cytokeratins 5/6 in the presence of a strong positive antibody response to cytokeratin CAM 5.2 suggests a secretory rather than a myoepithelial immunophenotype. Desmin is a marker for intermediate filaments of skeletal, cardiac, and smooth muscle. Although shown to be expressed in a percentage of human non-myogenic tumors such as malignant fibrous histiocytoma, fibromatosis, and lung carcinomas a variable positive staining for desmin in this case suggests the presence of compartments of less differentiated cells.[17,21] Cytokeratin CAM 5.2 staining recognizes cytokeratins 8/18 which are associated with ductal and lobular cell types. A strong positive staining along with the presence of ducts within the tumor suggests a ductal cell type of origin. The subpopulation of neoplastic spindle-shaped cells with strong positive staining for SMA, negative staining for C5/6, and variably positive staining for CAM 5.2, vimentin and desmin suggest mesenchymal differentiation. The presence of two populations of cells, one epithelial and one mesenchymal, suggest that this tumor may be of mammary stem cell origin.

AFIP Diagnosis: Mammary gland (per contributor): Adenocarcinoma, Multimammate rat (*Mastomys natalensis*), rodent.

Conference Comment: The contributor provides an excellent and in-depth review of the naming scheme and immunohistochemical findings associated with rodent mammary neoplasms.

Epithelial to mesenchymal transition (EMT) and its role in tumor metastasis is a concept which has recently sparked some debate. The process of epithelial to mesenchymal transition was first described in 1982 with the discovery that epithelial cells in culture may acquire mesenchymal features. EMT has been described as a feature of embryonic development as well as the development of malignant and chronic fibrotic disorders and in cancer progression, specifically with tumor invasiveness and intravasations and extravasations of metastatic cells. More specifically, EMT involves a

complex, extreme manifestation of epithelial plasticity where polarized epithelial cells embedded in organized stratified or single cell layers convert into single fibroblastoid cells capable of locomotion. This occurs in a series of events which include the release of cells from epithelial polarity, remodeling of epithelial cell-cell and cell-matrix adhesion contacts and of their actin cytoskeleton (22).

The crux of the debate lies in whether or not EMT is truly a reflection of a change in cell lineage or whether it merely reflects a change in the phenotype of cells actively involved in metastasis. It has been argued that the metastatic spread of cancer from the primary site to form secondary tumors in distant organs constitutes the most stringent test of the EMT hypothesis and that for this to occur the neoplastic cells would have to undergo reverse EMT or mesenchymal-to- epithelial transition once they reached the target organ and formed a secondary tumor (23).

It is important to be clear, however, that this skepticism about EMT in malignancy in no way challenges the role of epithelial mesenchymal interactions in development, healing and remodeling, carcinogenesis, and metastasis, for which there is substantial evidence. The skepticism is directed towards transmutation of one lineage into another, not between biologically important interactions between them (23).

While it is important to familiarize ourselves with the current concept of epithelial to mesenchymal transition and the debate which currently surrounds the idea, it is beyond the scope of this text to completely decipher the intricacies of this complex process. Certainly it qualifies as a topic better left to oncologists rather than your garden variety anatomic pathology resident.

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SLIDE 32
CONFERENCE 8 / CASE IV – MK01-2477 (AFIP 2992271)

Signalment: Adult, male, rhesus macaque, *Macaca mulatta*

History: This animal was challenged with simian immunodeficiency virus, SIV, seven days prior to necropsy, and was started on 9-R-(2-phosphonomethoxypropyl)adenine (PMPA, or tenofovir) antiviral treatment a day after he was challenged with SIV. He was then found lethargic the day before necropsy, and found dead in his cage the following morning. A total of six days elapsed between initiation of the PMPA therapy and death of the animal.

Gross Pathology: At necropsy, this animal appeared well muscled with a moderate amount of body fat and in good hydration. Upon reflecting the abdominal skin, hemorrhage and subcutaneous edema were evident. There were also multifocal petechial and ecchymotic hemorrhages present in the skin of the medial legs, thighs, and arms. Both kidneys were mildly pale with soft cortices, and there was a moderate amount of peri-capsular hemorrhage adjacent to the left kidney. Approximately 100 ml of serosanguineous fluid was present in the thoracic cavity, and approximately 50 ml of clear amber fluid was present in the abdominal cavity. In addition, the liver had an accentuated lobular pattern with an expansile mass present in the right lateral lobe. This mass measured 6 X 5 X 3.5 cm and had a prominent vascular pattern. The lungs were diffusely edematous and exuded serosanguineous fluid from cut surfaces. A moderate amount of white froth was present in the trachea. Within the intestinal tract and colon there were multifocal submucosal and subserosal hemorrhages present. All other tissues appeared grossly to be normal.

Laboratory Results: Samples for bacterial culture were collected from the clear amber fluid present in the abdominal cavity as well as from samples of the right caudal lung lobe and spleen. Bacterial cultures yielded *E. coli* and *Morganella morganii*, with this growth likely representing post-mortem bacterial overgrowth. This animal also had extremely elevated creatinine and BUN levels, which is consistent with the extensive renal lesions.

Histopathologic Description: The sample submitted was from sections taken from the same kidney, most of which contained sections through the cortex, medulla, and renal pelvis. Diffusely there was necrosis of proximal convoluted tubules. Most proximal tubules had lost their tubular epithelium with pink, granular, cellular debris filling the tubular lumens. In other proximal tubules, the epithelium was flattened and attenuated. Some proximal tubules were undergoing regeneration with epithelial hypertrophy and hyperplasia. The glomeruli were uniform in size, and many glomeruli contained eosinophilic, homogenous, condensed material within the lumen of the

capillaries. This material was consistent with fibrin or fibrinoid deposits. There were no inflammatory cell infiltrates present.

Contributor's Morphologic Diagnoses:
1. Kidneys: tubular necrosis of the proximal convoluted tubules, bilateral, severe, acute
2. Kidneys: glomerular capillaries – fibrin thrombi, diffuse, severe
3. Liver: nodular hyperplasia

Contributor's Comment: The cause of death in this animal was due to severe acute renal tubular necrosis brought on by acute toxicosis. Given the short time period of infection, the cause of the renal lesions and the eventual death of the animal was not related to the SIV infection, but rather to the treatment with an antiviral agent, PMPA (9-R-[2-phosphonomethoxypropyl]adenine). PMPA, an acyclic nucleotide analogue, is a reverse transcriptase inhibitor that has worked well in clinical trials on both SIV and human immunodeficiency virus (HIV) infections in reducing viremia and improving overall lifespan (7). Since PMPA is only bioavailable parenterally and not orally, tenofovir disoproxil fumarate (TDF), which was approved by the Food and Drug Administration (FDA) in 2001 for the treatment of HIV, is a prodrug form of PMPA that is bioavailable orally (2) and has been used as part of a highly active antiretroviral therapy (HAART) to treat HIV infected humans (3, 6, 11).

Although other closely related acyclic nucleoside analogue antivirals, such as cidofovir and adefovir, have been associated with nephrotoxicity, tenofovir was not expected to have similar problems (3, 6, 7, 10, 11). Human trials with TDF failed to show evidence of clinically relevant renal toxicity; however, since August of 2003 there have been at least 22 cases of TDF-associated nephrotoxicity with more occurring every year (1).

There are several mechanisms of toxicity from antiviral agents that could explain the nephrotoxicity, including mitochondrial injury and transporter defects; however, tenofovir was not expected to have similar nephrotoxic results as cidofovir and adefovir in humans. This is mainly due to decreased interaction with human organic transporter 1 and minimal mitochondrial DNA toxicity in vitro (5, 10). The actual mechanism of renal injury caused by tenofovir has not been confirmed, although there are several hypotheses. One possibility is that drug interactions are responsible for the nephrotoxicity since tenofovir primarily is excreted by the kidneys via both glomerular filtration and active tubular secretion (3). Several other drugs, including the antiviral drugs acyclovir, cidofovir, ganciclovir, and valacyclovir, also compete for active tubular secretion, which potentially could raise concentrations of tenofovir in the serum (7, 10). In addition, dose-dependency is likely a factor, since nephrotoxicity closely corresponds with both dose and duration of therapy in animal models (7). Either way, once tenofovir reaches high concentrations in the renal tubules, cell function could be disrupted. Besides the drug-related pathways of nephrotoxicity, there are also patient-related factors that must be taken into consideration when considering risk factors. For instance, patients that have previous renal impairment, are dehydrated, are elderly, have electrolyte depletion or are acidotic are more likely to develop nephrotoxicity as the result of antiviral therapy then are young, healthy patients (5).

To date, there have been several clinical manifestations of kidney disease resulting from tenofovir use in humans infected with HIV. Many cases of tenofovir-associated renal toxicity are characterized by Fanconi's syndrome, which is typified by proximal renal tubular dysfunction, hyperaminoaciduria, glucosuria, and phosphaturia (1, 7, 10). In addition, other manifestations have included diabetes insipidus and acute renal failure (5). Often renal tubular epithelial cells and casts are observed in human cases (5). Given the results from subsequent human cases, it is not surprising that this case in 2001 demonstrated acute proximal tubular necrosis after PMPA treatment. In human cases of acute renal failure caused by nephrotoxicity, most toxicity is wholly or partially reversible; however, a large proportion of the cases will retain some renal damage (4). The case presented, on the other hand, was likely given a dose for which recovery was not possible. This is supported by the rapid onset of renal failure—this animal was lethargic within five days after PMPA treatment was initiated.

In addition to acute proximal tubular necrosis, this animal had fibrin thrombi within the glomerular capillaries indicative of disseminated intravascular coagulation (DIC). Phosphotungstic Acid Hematoxylin (PTAH) staining of the renal sections was positive for fibrin within glomerular capillaries. This animal also had multifocal hemorrhage in the subcutis, skeletal muscle, myocardium, intestine, colon, and adrenal glands, which is consistent with DIC (8, 9). There were no fibrin thrombi evident in other vessels, including the alveolar capillaries. The major mechanisms leading to DIC are widespread injury to endothelial cells and the release of thromboplastic substances into the circulation. Although viremia can lead to DIC, in this case the DIC was not likely secondary to viremia given the short period of time the animal had been infected with SIV. It is more likely that the DIC was secondary to the antiviral treatment with PMPA. Drug therapy can cause blood stasis, leading to excessive platelet activation and a state of hypercoagulation.

AFIP Diagnosis: 1. Kidney, tubules: Necrosis, acute, multifocal, with scattered regeneration, rhesus macaque, primate.
2. Kidney, glomerular capillaries: Thrombi, multiple.

Conference Comment: This is an interesting case of an anti-retroviral drug inducing acute tubular necrosis. The contributor provides an excellent review of anti-retroviral drugs currently in use for the treatment of HIV infection and their nephrotoxic side-effects.

Conference attendees debated the presence of regenerating tubular epithelial cells and the presence of multiple fibrin thrombi occluding glomerular capillaries. Tubular epithelial cell regeneration is characterized by the presence of mitotic figures lining the tubular basement membrane and plump epithelial cells with slightly basophilic cytoplasm. In this case, the proximal convoluted tubules appeared most effected which

is often the case with nephrotoxins. The presence of fibrin was confirmed with phosphotungstic acid hematoxylin (PTAH) staining.

Discussion focused on the myriad causes of nephrotoxic tubular necrosis and covered the basic categories of chemical and plant toxins associated with renal disease. Below is a short list of the chemical and plant toxins which most commonly result in tubular necrosis (12):

Pharmaceuticals

- Aminoglycosides-concentrate in lysosomes. When released they result in inhibition of the sodium-potassium-ATPase pump resulting in an intracellular influx of hydrogen and sodium ions and water; also inhibit phospholipase which results in the intracellular accumulation of phospholipids, altered mitochondrial function and inhibition of protein synthesis
- Oxytetracycline-large concentrations inhibit mammalian protein synthesis; tubular obstruction caused by desquamated necrotic tubular epithelium
- Amphotericin B-directly disrupts cell membranes, interferes with cholesterol-lipid interactions and causes potassium ion loss, hydrogen accumulation and cellular swelling and necrosis
- Sulfonamide-induced tubular necrosis-dehydrated animals; mechanical damage and direct toxicity due to crystal formation in tubules
- Monensin-altered ion transport
- Nonsteroidal anti-inflammatory drugs (NSAIDs)-decreased synthesis of renal prostaglandins resulting in arteriolar constriction->reduced renal perfusion->acute tubular degeneration and necrosis
- Cisplatin-directly damages tubules and causes vasoconstriction via renin-angiotensin mechanism->reduced renal blood flow->tubular degeneration and necrosis

Heavy metals

- Inorganic mercury-mercuric ions concentrate in rough endoplasmic reticulum->loss of brush border->mitochondrial swelling->cell death
- Cadmium, inorganic-induces tubular epithelial apoptosis
- Lead-damages mitochondria and epithelial cell membranes
- Thallium-a rodenticide, damages cell membranes
- Arsenic, inorganic-pesticide, damages cell membranes

Plant toxins

- *Amaranthus retroflexus* (Redroot pigweed)-acute tubular necrosis in cattle; perirenal edema in pigs
- *Quercus sp.* (Oak)-toxins are metabolites of tannins; pale swollen kidneys with petechial hemorrhages in cattle, also perirenal edema, abundant clear fluid in body cavities, renal fibrosis.
- *Halogeton, Sarcobatus* (Greasewood), *Rheum, Rumex*-oxalate containing plants, calcium oxalate precipitate in renal tubules->obstruction->necrosis

- *Cestrum diurnum, Solanum sp., Trisetum sp.*-all contain Vitamin D analogs; ingestion results in hypercalcemia->tubular epithelial cell absorption of calcium->mitochondrial calcification and dysfunction->cell death; also vasoconstriction->ischemia

Household toxins

- Ethylene glycol-antifreeze, toxic metabolites cause acute tubular necrosis; calcium oxalate crystals form in tubules and interstitium->obstruction

The focus then shifted to a discussion and review of Virchow's triad of events that predispose to thrombus formation (8). Those events are:

1) Endothelial injury, to include endothelial dysfunction
2) Alterations in normal blood flow such as turbulence and stasis
3) Hypercoagulability, genetic and acquired causes

The conference concluded with a brief overview of the fate of a thrombus once it has formed (8). Once formed, a thrombus can:

1) Undergo **propagation** by accumulating more platelets and fibrin, ultimately obstructing the vessel
2) **Embolize** or dislodge and travel to another location
3) Undergo **dissolution** via fibrinolytic activity
4) Undergo **organization,** by inducing inflammation and fibrosis; and **recanalization**, thereby reestablishing blood flow

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SLIDE 33
CONFERENCE 9 / CASE I – N05-169-6 (AFIP 2988200)

Signalment: A 3-year old female intact, Yorkshire, Sus scrofa, porcine

History: Animal was found dead in its pen on a dirt lot. Diarrhea was noticed in other pigs and one died approximately 1 week earlier.

Gross Pathology: A mature intact female Yorkshire hog was presented for necropsy in good body condition. External examination revealed numerous bright red rhomboid lesions located in the skin of the ventral neck, ventral abdomen, snout and all four limbs. The inguinal lymph nodes were palpable and enlarged 2 to 3 times normal size (5x8cm). A thick, red mucoid exudate exuded from both nostrils and a small quantity of tan mucoid exudate was present in the vaginal opening. Both the thoracic and abdominal cavities contained excessive amounts of serosanguineous fluid. The spleen was markedly enlarged and dark red. The liver was black and mottled. The omentum was attached to the abdominal wall by yellow strands of fibrin. The jejunum was moderately distended with gas and there were scattered 2 to 5 cm red foci in the mucosa of the jejunum and colon as well as on the serosa of the spiral colon. The joint capsules of the carpal joints were thickened and contained a serosanguineous fluid.

Laboratory Results: Microbiological examination: Heart Blood, no pathogen detected; Joint swab, *Erysipelothrix rhusiopathiae* growth was heavy.

Histopathologic Description: Sections of the skin show areas of necrosis involving the basal portion of the epidermis and upper dermis. Minimal neutrophilic infiltrates are present in the necrotic areas. The epidermis is thickened near areas of necrosis and some sections show mild hyperkeratosis, small foci of ulceration and separation of the epidermis and dermis. There is marked capillary congestion and some arterioles show myodegeneration and perivascular infiltrates of neutrophils and fibrin. Some vessels contain fibrinous and cellular thrombi. Occasional colonies of rod-shaped bacteria are

present in perivascular areas. Postmortem autolytic changes prohibited definitive microscopic evaluation of visceral organs.

Contributor's Morphologic Diagnosis: Dermatitis, necrotizing, ulcerative, mild to moderate, multifocal, with vasculitis, marked capillary congestion and vascular thrombosis, skin, porcine, due to *Erysipelas rhusiopathiae*

Contributor's Comment: *Erysipelothrix rhusiopathiae* is of wide geographic distribution and outbreaks occur in a wide range of animals including pigs, lambs, and birds. Under natural conditions, erysipelas is transmitted by ingestion of the organisms, contamination of skin lesions, or by the bites of infected flies. Erysipelas occurs in humans mainly as an occupational disease of meat and poultry plant workers, veterinarians, and rendering-plant workers (2).

In swine, the disease is an acute highly fatal septicemia or a chronic disease characterized by endocarditis, polyarthritis and skin necrosis. The organism can persist for months in tissues of diseased pigs and these animals become the primary source of infection for healthy swine. Healthy swine may be carriers and shed organisms in feces (1). While the disease can be a highly fatal septicemia, recovery can occur after treatment with appropriate antibiotics. Thus the disease in sows can be controlled in modern commercial farrow-to-finish operations when proper vaccination protocols are followed (3).

The pathogenesis of the early septicemic phase of the disease involves changes in capillaries and venules in visceral organs and synovial tissues. Thirty-six hours after subcutaneous exposure to virulent *E. rhusiopathiae*, there is swelling of endothelial cells, adherence of monocytes to vascular walls and wide spread thrombosis. This disseminated coagulopathy leads to fibrinous thrombosis, diapedesis and vascular invasion by bacteria. Lesions in skeletal muscle are associated with vascular lesions and consist of segmented hyaline necrosis of muscle fibers that may be followed by fibrosis, calcification and regeneration. Lesions in the central nervous system consist of vascular damage leading to degeneration of neurons and malacic changes in the cerebrum, brain stem and spinal cord. (2)

The most common lesion in chronic swine erysipelas is a proliferative, non-suppurative arthritis, occurring mainly in the hock, stifle, elbow and carpal joints. Vegetative valvular endocarditis is a less common finding.

AFIP Diagnosis: Haired skin and subcutis: Vasculitis, acute, multifocal, moderate, with fibrin thrombi and superficial dermal necrosis, Yorkshire, porcine.

Conference Comment: The contributor provides an excellent review of swine erysipelas lesions and pathogenesis. Conference attendees debated the presence of superficial dermal necrosis and bacteria within dermal vessels.

Erysipelothrix rhusiopathiae is a Gram-positive, non spore forming, facultative anaerobic bacillus with a worldwide distribution. Swine are the most important reservoir and approximately 30-50% of healthy swine harbor the organism in their tonsils or lymphoid tissue, periodically shedding the bacteria in their feces. Young pigs (3 months -1 year) and pregnant sows are most susceptible.

As the contributor noted, there are two forms of disease: the acute febrile septicemic form and the chronic form. Clinical findings for the acute septicemic form include pyrexia, depression, lethargy, anorexia, lameness, abortion, stillbirth and purple discoloration of the skin. Chronic cases may develop the classic "diamond skin" lesions, lameness and swollen joints as well as signs of cardiac insufficiency and decreased growth rate. Typical necropsy findings for acute disease include: pink to purple congested skin; edematous, congested lungs; petechiae and ecchymoses of the epicardium, atrial myocardium, renal cortex; hemorrhagic gastritis; and congestion of the liver and spleen. Chronic disease may result in slightly raised, necrotic diamond shaped skin lesions; arthritis and synovitis with serosanguineous effusion into joint cavities; hyperemic and proliferative synovial membranes and joint capsules; articular cartilage erosion; ankylosis; spondylitis; and vegetative valvular and mural endocarditis.

In this case, the cutaneous vasculitis with fibrin thrombi and dermal necrosis strongly supports the diagnosis. Attendees also considered porcine dermatitis and nephropathy syndrome (PDNS) in the differential diagnosis. PDNS is believed to be caused by coinfection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 (PCV2). The cutaneous lesion of PDNS consists of severe necrotizing vasculitis affecting the dermis and subcutis, with epidermal necrosis, ulceration and dermal hemorrhage (4).

In sheep, erysipelas is almost always a percutaneous infection with the bacteria gaining entry through the umbilicus, docking and castration wounds, shear wounds, cuts, and abrasions. Lesions may be confined to the skin or localize in the joints resulting in fibrinopurulent polyarthritis and osteomyelitis (1). Additionally, calves also develop polyarthritis and marine mammals develop an acute septicemia with lesions similar to swine erysipelas. Turkeys can develop acute and chronic forms of disease with hemorrhage, splenomegaly, polyarthritis, and endocarditis. In humans, *E. rhusiopathiae* causes erysipeloid, a localized skin lesion found in people who handle infected shellfish or turkeys. Conversely, human erysipelas is an infectious skin disease caused by *Streptococcus pyogenes* and is characterized by a rapidly spreading rash than can develop a "butterfly" shaped erythematous lesion on the face (5).

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SLIDE 34
CONFERENCE 9 / CASE II – P05-5907 (AFIP 2991567)

Signalment: Five year-old, female, Holstein Friesian cow.

History: This animal was losing weight and had malaise in October 2004. On 11-26-2004 it was taken into the clinic at the Faculty of Veterinary Medicine with polyuria and polydipsia. Later, in June 2005, over a period of a week, problems reoccurred with reduced appetite, malaise and dehydration. The clinicians suspected *Quercus* poisoning.

Gross Pathology: The animal was in poor body condition. The carcass exuded a uremic fetor. In the heart, two large valvular cysts were present on the left atrioventricular valve. Both kidneys showed irregularity of the external surface and adhesion of the renal capsule. On cut section multiple areas of firm pale tissue (fibrosis) were observed. The abomasal mucosa was thickened, with an irregular multinodular surface (hyperplasia).

Laboratory Results: Blood urea was measured at 50 mmol/l in June 2005.

Histopathologic Description: Abomasum. The tunica mucosa is markedly hyperplastic, with large numbers of mucus producing epithelial cells (mucoid hyperplasia) and absence of chief cells. There is an increase in the number of mitotic figures in the abomasal gland epithelium. In the abomasal lumen and sometimes in the glands multiple cross sections and occasional longitudinal sections of nematodal organisms are present. These nematodes are round in cross section, and they have a cuticle with external cuticular ridges. Usually a single, roughly circular section through the intestinal canal is seen, with a wall consisting of a single layer of columnar cells (morphologically consistent with Ostertagia sp. larvae). In some specimens a uterus can be seen (females), in others a vas deferens is present (males). The lamina propria contains moderate multifocal infiltrates of mainly eosinophils, lymphocytes and plasma cells and forming of lymphoid follicles.

Contributor's Morphologic Diagnosis: Chronic marked nodular hyperplastic abomasitis with intralesional nematodal parasites morphologically resembling *Ostertagia* sp. larvae (ostertagiosis).

Contributor's Comment: *Ostertagia ostertagi* is perhaps the most common cause of parasitic gastritis in cattle. Ostertagiosis is characterized by weight loss and diarrhea and typically affects young cattle during their first grazing season. Pathological changes are maximal when the parasites are emerging from the gastric glands. Parietal cells, which produce hydrochloric acid, are replaced by rapidly dividing, undifferentiated, non-acid-secreting cells. The end result is a thickened hyperplastic gastric mucosa, creating macroscopically visible nodules where the parasites are located. These nodules can coalesce, giving the mucosa the aspect reminiscent of Moroccan leather.[1]

This case is somewhat atypical in that the animal is a five year-old cow. Moreover, there was concomitant renal failure with a marked chronic interstitial nephritis and protein-losing nephropathy. Both the renal and the abomasal lesions may have contributed to the illness of the animal. Perhaps the animal's immune reactions to the *Ostertagia* larvae were depressed due to the chronic renal disease.

AFIP Diagnosis: Abomasum: Mucous neck cell hyperplasia, diffuse, moderate, with mild, subacute, eosinophilic abomasitis and trichostrongylid nematodes, etiology consistent with *Ostertagia sp*., Holstein Friesian, bovine.

Conference Comment: Conference attendees discussed the anatomical location of the section and the diffuse absence of parietal cells. Some felt the section may be from the pyloric region of the abomasum and that the complete lack of parietal cells was, in part, due to anatomic location. All agreed there was diffuse mucous neck cell hyperplasia and eosinophilic abomasitis.

Ostertagia spp. infection has been called the most important parasitism in grazing sheep and cattle in temperate climate zones throughout the world. It results in tremendous production loss, as well as clinical manifestations of anorexia, diarrhea, wasting, and some deaths.

The life cycle is direct with eggs being consumed with fecally contaminated feed. In the rumen the L3 larvae exsheath, penetrate the abomasal glands and undergo two molts to the L5 stage. L5 larvae emerge and mature on the mucosa approximately three weeks following ingestion (Type I ostertagiasis). During larval development there is interstitial inflammation and mucous neck cell hyperplasia which replace parietal cells. Parietal cells produce hydrochloric acid (HCl) and their loss results in a decreased hydrochloric acid production and a subsequent increase in abomasal pH. Under basic conditions, pepsinogen, produced by chief cells, is not converted to pepsin which results in decreased protein digestion. Increased plasma pepsinogen levels result from leaky intercellular junctions allowing backflow of pepsinogen between poorly differentiated

mucous neck cells. Increased blood gastrin levels may have a central effect on the satiety center resulting in decreased appetite and motility. Diarrhea is probably the result of the elevation in abomasal pH. Plasma proteins are lost into the gastrointestinal tract contributing to weight loss and hypoproteinemia. Some larvae may persist in mucosal glands in a stage of arrested development (hypobiosis) for up to four months before the synchronous maturation, emergence and molting to the L5 stage (Type II ostertagiasis) (2).

Typical necropsy findings include thickened abomasum with multifocal to coalescing nodules containing larval or adult nematodes (6-15mm long), multifocal erosions, erythema, catarrhal exudate and foul smelling abomasal contents. Additional findings include subcutaneous and mesenteric edema, ascites, liver atrophy and a dilated gallbladder due to inanition.

Typical light microscopic findings include mucous neck cell hyperplasia, decreased parietal cells and lymphoid nodules within the mucosa. The lamina propria may be infiltrated by variable numbers of eosinophils, neutrophils, lymphocytes, plasma cells and globule leukocytes.

Ostertagia spp. are trichostrongyle nematodes with evenly-spaced, external longitudinal cuticular ridges, platymyarian musculature and a large intestine composed of few multinucleated cells.

The mnemonic “HOT” is useful for remembering the most important abomasal nematodes:
“H”: *Haemonchus contortus* in sheep and goats and *H. placei* in cattle, also known as the “barber pole worm”.
“O”: *Ostertagia* spp.
“T”: *Trichostrongylus axei* infects cattle, sheep, and goats

All of these are trichostrongyles; the key to identifying *Ostertagia* spp. in histologic section is the evenly-spaced external cuticular ridges.

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

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SLIDE 35
CONFERENCE 9 / CASE III – B05-425 (AFIP 2985163)

Signalment: 31-year-old, neutered male with body weight of 2.1kilograms, Capuchin monkey, *Cebus* species

History: A 31-year-old capuchin monkey was presented to the University of Georgia Veterinary Teaching Hospital's Exotic animal service. Animal was bright, alert, responsive but in very poor body condition (Score: 1.5/5) weighing about 2.1kg (Gross photo: 1). Animal had decreased appetite, lethargy, and infrequent shivering or shaking of the body. A well circumscribed movable mass of approximately 4x3cm was palpated in the ventral neck. Ultrasonography revealed extensive cystic changes in the liver and both kidneys. Fine needle aspirates of these masses failed to indicate neoplastic disease and these cystic changes were considered to be benign geriatric changes. The entire mass from the ventral neck area was surgically excised. The excised mass was submitted in formalin for histopathologic examination.

Gross Pathology: The mass was firm, solid, tan, and measured about 3.0 X2.0 cm (Gross photo: 2).

Laboratory Results: Blood chemistry and urinalysis showed evidence of mild renal insufficiency. Creatinine was mildly elevated (1.4mg/dL), mild proteinuria (26 mg/dL) and low urine specific gravity (1.010) suggested a mild renal insufficiency. Thoracic radiographs, other biochemistry parameters and hematology were unremarkable.

Histopathologic Description: Thick fibro-vascular bands of varying size are compressing and separating numerous cords and follicles lined with epithelial cells. The cells are cuboidal to polygonal, with indistinct borders and scant amphophilic to basophilic cytoplasm. Nuclei are oval to elongated, hypo- to hyperchromatic with inconspicuous nucleoli. Cellular and nuclear polymorphism is moderate and mitoses average 24 per 10 HPFs. There are multifocal variably sized necrotic areas and hemosiderin laden macrophages throughout the tumor. Approximately _ of all follicles are filled with blood and cellular debris, while a few of them are filled with colloid. Immunohistochemically the neoplasm was positive for thyroglobulin and cytokeratins AE1/AE3 (IHC photos: 1 & 2).

Contributor's Morphologic Diagnosis: Thyroid gland – Follicular adenocarcinoma

Contributor's Comment: Reported frequency of thyroid neoplasia in new-world primates is very low. A review of the literature revealed only 2 cases of thyroid

cystadenoma in black-tailed marmosets (1). One case of nodular thyroid hyperplasia in a cynomolgus monkey has been described (2). Other endocrine tumors described in new-world primates include pituitary adenomas, pheochromocytoma, adrenal ganglioneuroma, adrenal cortical adenoma, parathyroid chief-cell adenoma and pancreatic islets-cell adenoma. To our best knowledge this is the first case of thyroid gland adenocarcinoma described in a new-world primate.

AFIP Diagnosis: Thyroid gland: Medullary thyroid carcinoma, Capuchin monkey, primate.

Conference Comment: Conference attendees debated several features of this neoplasm including the presence or absence of capsular invasion or penetration; the homogenous, eosinophilic, acellular material which often separates cells; and whether or not the neoplastic cells could be of medullary (C-cell) origin.

We chose to run several immunohistochemical stains to further characterize the neoplasm and a Congo red stain to see if stromal amyloid was present. In our lab the neoplastic cells are negative for both thyroglobulin and thyroid transcription factor-1 (TTF-1) and positive for calcitonin, chromogranin, and synaptophysin. The Congo red stain results are equivocal. Additionally, the case was reviewed by pathologists in the Department of Endocrine and Otorhinolaryngic Pathology. Based on the morphologic features and immunohistochemical staining characteristics, we favor a diagnosis of medullary thyroid (C-cell) carcinoma.

As noted in the contributor's description, this neoplasm was characterized by a pleomorphic polygonal cell population with a high mitotic rate, but neither capsular penetration nor vascular invasion was present in our sections. Attendees discussed the importance of clear capsular or vascular invasion in the diagnosis of malignancy in human thyroid follicular tumors. However, the same criteria do not apply for human medullary tumors; in fact, most human medullary carcinomas are nonencapsulated. There are insufficient reports of thyroid neoplasia in non-human primates to judge the significance of encapsulation in this case. Some attendees felt the homogenous, eosinophilic, acellular material may represent amyloid. Stromal amyloid deposits are common in C cell neoplasms of humans and animals (3).

Medullary carcinomas occur commonly in many strains of rats and their incidence increases with the age of the population. C-cell neoplasms also commonly occur in bulls, where they are known as ultimobranchial neoplasms (3).

Calcitonin-secreting C-cells originate from neural crest cells and migrate into the 5th (ultimobranchial) pharyngeal pouch. The ultimobranchial body migrates and fuses with the midline primordium that forms the thyroid gland in mammals. In submammalian species the ultimobranchial gland remains separate.

Ultimobranchial tumors may derive from:

1. Undifferentiated stem cell remnants of the ultimobranchial bodies (negative for calcitonin, somatostatin, and neurotensin)
2. Parafollicular C-cells (positive for calcitonin, somatostatin, and neurotensin)

In rats, the chronic administration of vitamin D3 has been reported to increase the frequency of medullary carcinomas (3). Likewise, there is a suggested relationship between long-term excess dietary Ca^{2+} and a high incidence of ultimobranchial tumors in bulls.

Typical clinical findings of C-cell carcinoma include normal or slightly lowered levels of circulating calcium; palpable nodules in the anteroventral cervical region of bulls and horses and; in bulls, an increased incidence of vertebral osteosclerosis with ankylosing spondylosis deformans, osteophytes, vertebral fractures and degenerative osteoarthrosis.

Grossly, C-cell carcinomas are firm, multinodular uni- or bilateral tumors that typically involve the entire gland. They can be yellow, tan or white with lobules separated by dense, white fibrous connective tissue. Additionally, they can be hemorrhagic and have areas of necrosis.

Microscopically, they are usually infiltrative neoplasms which may be solidly cellular or form small nests, occasional ducts, follicles or cysts with variable amounts of stromal amyloid. The neoplastic cells are polygonal to spindled with eosinophilic, finely granular cytoplasm and vesicular, oval to elongate nuclei with a variable mitotic rate. Calcitonin is the most reliable immunohistochemical marker for canine and bovine C-cells. Metastasis is usually to the cranial cervical lymph nodes with pulmonary metastasis occurring less frequently.

The differential diagnosis includes follicular adenomas or carcinomas of the thyroid gland, parathyroid adenomas or carcinomas, and chemodectoma (carotid body tumors, aortic body tumors).

The ultimobranchial (C-cell) tumors of aged bulls may present as part of a multiple endocrine neoplasia (MEN) syndrome and include pheochromocytomas or pituitary adenomas. An autosomal dominant pattern is suggested for development in Guernsey bulls.

Interestingly, in humans **a**pproximately 15-20% of C-cell carcinomas are part of an inherited syndrome of multiple endocrine neoplasia (MEN). Forms of familial medullary thyroid carcinoma syndromes include:

- Medullary carcinoma.
- MEN IIA characterized by C-cell hyperplasia-medullary carcinoma, adrenal medullary hyperplasia-pheochromocytoma and parathyroid hyperplasia-adenoma.

- MEN IIB characterized by C-cell hyperplasia-medullary carcinoma, adrenal medullary hyperplasia-pheochromocytoma, gastrointestinal and ocular ganglioneuromas and skeletal abnormalities (3).

Additional information on multiple endocrine neoplasia syndromes in animals can be found in two fairly recent Veterinary Pathology articles (4, 5):

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SLIDE 36
CONFERENCE 9 / CASE IV – NADC (AFIP 2984288)

Signalment: 6-8 month old, female, Dorset cross, *Ovis aries*, ovine

History: The animal was inoculated intravenously with a previously untested blood stabilate collected from a sheep infected with *Ehrlichia ruminantium* (Gardel strain). During the following 6 weeks this animal and all similarly inoculated sheep were clinically normal and were negative by PCR for *E. ruminantium* on multiple blood samples. Due to the apparent failure of the blood stabilate, the sheep was then re-inoculated intravenously with a bovine blood stabilate infected with the same Gardel strain of *E. ruminantium*. Eleven days after the second inoculation the animal's temperature increased to 39.5C and the fever persisted until the animal died on day 17. The animal was also observed circling in the pen on days 13 through 16 after the second inoculation.

Gross Pathology: There was mild cerebral, pulmonary and perirenal edema along with a mild generalized lymphadenopathy.

Laboratory Results: *E. ruminantium* was identified by PCR on multiple blood samples following the second inoculation.

Histopathologic Description: Cerebrum. Within the meninges and neuropil many blood vessels are markedly congested, contain increased numbers of leukocytes and are surrounded by abundant clear space. There is perivascular hemorrhage surrounding fewer vessels. Infrequently within the cytoplasm and adjacent to the nucleus of endothelial cells are 2 to 10 micron, oval, amphophilic colonies or morulae containing numerous 1-2 micron round organisms. There are increased numbers of glial cells with many glial cells and neurons partially to completely surrounded by clear space.

Contributor's Morphologic Diagnosis: Cerebral edema, mild, diffuse, with intraendothelial rickettsia consistent with *Ehrlichia ruminantium*.

Contributor's Comment: Heartwater is caused by the endotheliotropic organism *E. ruminantium*, formerly named *Cowdria ruminantium*, which infects sheep, goats, cattle, and some species of wild ruminants.[1] The disease is transmitted by multiple species of *Amblyomma* ticks and is present in sub-Saharan Africa and in the Caribbean Islands.[1] The most important vectors are *A variegatum* (Sub-Saharan Africa and Caribbean) and *A. hebraeum* (southern Africa).[1] Because the agent is infectious but not contagious, introduction of infected ticks and subclinically infected animals are the most likely mechanisms for introducing heartwater into new geographical areas.[2]

Heartwater is a large potential threat to United States livestock. In the US, there are susceptible domestic and wild host species (white-tailed deer), and two experimentally proven vectors, *A. maculatum* and *A. cajennense*, that are present in many areas of the US.[2,3] Exotic *Amblyomma* ticks, which could potentially be infected with *E. ruminantium*, have frequently been found on reptiles imported into the US.[4] Cattle egrets, which are hosts for immature *Amblyomma* ticks and are known to fly from the Caribbean to the US, are another possible mechanism for introducing the disease.[2]

Peracute and acute disease with high mortality rates are common in naïve susceptible animals, but clinical signs and mortality can be variable dependent on the virulence of the isolate and host factors (species, breed, and age of the animal).[5] Interestingly, calves less than 3 weeks of age and lambs less than 1 week of age are innately resistant to disease and this resistance is not related to maternal immunity.[5]

Fever, depression, anorexia, dyspnea, numerous central nervous system signs such as ataxia, and sudden death with few clinical signs are commonly seen in heartwater.[2] Diarrhea may also be a prominent sign in cattle.[2] Typical gross lesions are pulmonary edema, hydropericardium, hydrothorax, cerebral edema, lymph node edema, and splenomegaly.[1] Not all lesions are usually present in one animal and some animals

have minimal lesions.[1] Microscopically, edema, hemorrhage and vascular congestion may be present in multiple tissues.[6] Colonies of organisms within capillary endothelial cells are most easily observed in the brain, lung and kidney.[1] Additional lesions that may be present in the brain include focal areas of malacia, lymphocytic perivascular cuffs, and glial nodules.[1,6] The precise mechanism by which *E. ruminantium* causes increased vascular permeability is unclear.[1]

Laboratory diagnosis is by PCR, histopathology and brain smears. Brain smears are the traditional method of testing in which a 3 to 4 cubic millimeter portion of cerebral gray matter is crushed between two slides, and then smeared to create alternating thick and thin areas on the slide.[1] The slide is stained with Giemsa stain and thinly stretched capillaries are examined for colonies of organisms.[1]

AFIP Diagnosis: Brain, cerebrum: Congestion and edema, diffuse, mild, with intraendothelial rickettsia, etiology consistent with *Ehrlichia ruminantium*, Dorset cross, ovine.

Conference Comment: The contributor provides an excellent review of heartwater disease caused by *Ehrlichia ruminantium*, formerly *Cowdria ruminantium*. Conference attendees were able to locate the organism within endothelial cells but had difficulty characterizing its morphologic features. All agreed that cerebral edema was a feature and that increased clear space surrounding vessels was not an artifact. Cerebral edema was characterized by increased clear space surrounding vessels with thin fibrous connective tissue tags spanning the perivascular space.

E. ruminantium is a Gram-negative, pleomorphic rickettsia, and colonies containing from one or two to several thousand individual organisms are found in the cytoplasm of endothelial cells. Most organisms are coccoid, except for colonies containing very large organisms in which pleomorphic forms may be seen (1).

E. ruminantium belongs to the family Anaplasmataceae and therefore grows in an intravacuolar compartment, bound by a lipid bilayer membrane, within the cytoplasm of infected host cells. Conversely, species in the family rickettsiaceae grow freely in the cytoplasm of their eukaryotic host cells (1).

The susceptibility of different breeds of cattle varies with Zebu breeds (*Bos indicus*) being more resistant than European breeds (*Bos taurus*). Sheep are more susceptible than cattle and Angora goats are particularly susceptible (1).

The pathogenesis of heartwater is not well understood. Initial replication of the organism seems to take place in the reticuloendothelial cells and macrophages of the regional lymph nodes followed by dissemination via the bloodstream and endothelial cell invasion in various organs and tissues. Increased vascular permeability with transudation is responsible for effusion into body cavities and tissue edema (1). A toxin

has been suggested as the cause of brain edema resulting in increased cerebrospinal fluid pressure and the subsequent development of neurogenic myocardial degeneration and pulmonary edema (7).

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SLIDE 37
CONFERENCE 10 / CASE I – 26974 (AFIP 2988265)

Signalment: Adult, male paca *Agouti paca*.

History: This is one of five pacas (*Agouti paca*) that developed lameness (Fig. 1). They were kept in captivity within an environmental reservation, and they were fed fruits and vegetables with a small amount of corn and a commercial rabbit food, according to FAO's recommendations (1995). They received water free of fluoride or any other chemical or microbiological contaminant. The animal was submitted for euthanasia due to poor prognosis.

Gross Pathology: Cranial bones were markedly thickened (Figs. 2-4). Long bones had a very thick cortex and replacement of trabecular bone by compact bone, resulting in reduction of the marrow cavity. The vertebrae had exostoses that projected into the

vertebral canal (Fig. 5), compressing the spinal cord. Radiologically, in addition to these gross lesions there was an increased radiodensity of the whole skeleton (Fig. 6).

Histopathologic Description: Marked increase in the amount of compact bone tissue, characterized by narrowing of the Haversian canals and large number of cement lines giving a mosaic appearance to the tissue (Fig. 7). In all long bones, the trabeculae were thickened and coalescing, with substitution of trabecular bone by osteonic bone, and retention of cartilage (chondroid core) in the metaphysis. There were several pyknotic osteocytes within enlarged lacunae, and a large number of empty osteocytic lacunae (Fig. 8) with multifocal disintegration of the bone matrix, characterizing osteonecrosis (not seen in all sections submitted).

Contributor's Morphologic Diagnosis: Bone. Osteopetrosis, generalized, severe. Osteonecrosis, multifocal, moderate. *Agouti paca*.

Contributor's Comment: Osteopetrosis is a metabolic disease of bone, characterized by osteomegaly or increased amount of bone tissue per unit of volume. The most common cause of osteopetrosis is nutritional hypercalcitoninism that induces a reduction in bone resorption and consequently an increase in the amount of bone.

Osteopetrosis has been diagnosed in cattle both as a congenital condition[1,2] or as a metabolic condition secondary to nutritional imbalance[3]. Osteopetrosis has also been described in other species including mice, rats, rabbits, man and the manatee, in which it is physiologic (reviewed by 1). In man, osteopetrosis is a rare genetic disease also known as Albers-Schonberg disease and marble bone disease[4], which is due to an imbalance in bone remodeling due to a defect in bone resorption due to impaired osteoclastic activity (reviewed by 4). In mice, osteopetrosis is associated with lack of colony-stimulating factor 1 activity (*op/op* mice), which is associated with defective macrophage and osteoclast differentiation[5]. To our knowledge these are the first cases of osteopetrosis diagnosed in paca (*Agouti paca*).

AFIP Diagnosis: Long bone, epiphysis and metaphysis: Osteosclerosis, diffuse, severe, paca (*Agouti paca*), rodent.

Conference Comment: Attendees received a section of long bone with epiphysis and metaphysis; others will have received a similar slide or a slide with a section suspected of being intervertebral disc and adjacent bone or facets.

Conference attendees briefly discussed osteopetrosis as a clinical term used to describe osteosclerotic changes in bone. Osteopetrosis is an acquired or congenital disease of excess bone due to a failure of resorption; not increased formation. Osteosclerosis is an increase in bone mass resulting from the failure of osteoclasts to resorb and model primary trabeculae or, more simply, increased density of normal appearing bone. Osteosclerosis affects bones that elongate by endochondral

ossification from a growth plate. Grossly, affected bones are dense and have no medullary cavity. Microscopically, retained primary trabeculae form spicules of bone with central cores of calcified cartilage which fill the medullary cavity (6). The contributor listed several species in which osteopetrosis occurs. In addition, avian leukosis virus causes osteopetrosis in poultry (7).

In this case, osteosclerosis occurs in both the epiphysis and metaphysis. There is retention of longitudinal cartilage septa with retained cartilage cores and the presence of osteons with minimal porosity in the medullary bone indicating a defect in remodeling. As described by the contributor, there is marked mosaic patterning in the compacted regions of the epiphysis and metaphysis. Additional features include fibrous tissue on the articular surface, thin, irregular articular cartilage and the presence of very little marrow. In some sections there is also periosteal new bone formation indicating a defect in modeling as well.

The terms modeling and remodeling can be confusing. Modeling describes the change in shape or contour of a bone in response to normal growth, altered mechanical use, or disease. Modeling allows the shape or size of bone to change and enables the medullary cavity to enlarge and the overall shape of the bone to be maintained while the bone is growing. Remodeling is the constant replacement of old bone with new bone which allows for the repair of accumulated microscopic injury (microfractures). It is interesting to note that in mice, rats and several other small species, cortical bone is not remodeled (6).

The cause of osteosclerosis in this case is not apparent. The paca is described as an adult; however, sexual maturity and skeletal maturity do not necessarily coincide. Assuming the paca is skeletally mature, and this lesion does not represent a heritable trait, consideration was given to hypervitaminosis D. Chronic hypervitaminosis D (e.g. prolonged ingestion of vitamin D containing plants) results in hypercalcemia which causes chronic lowering of parathormone (PTH) and elevation of calcitonin, effectively stopping bone resorption (6).

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SLIDE 38
CONFERENCE 10 / CASE II – 05-06 Case#1 (AFIP 2985156)

Signalment: A newborn Peruvian Paso breed equine.

History: A history of an uneventful parturition, progressive weakness and eventual collapse.

Gross Pathology: Generalized sclerosis of axial and appendicular skeleton (Fig 1), multiple rib fractures, and brachygnathia inferior.

Radiographic Results: Regionally extensive increases in radiodensity (sclerosis) affecting all bones. In the vertebral column, this creates a "butterfly-like" effect (Fig. 2).

Histopathologic Description: The submitted slides consist of longitudinal sections through one of either the fifth, sixth, or seventh thoracic vertebrae. In each of the sections, the medullary spaces of the primary and secondary ossification centers are nearly obliterated by a disorganized meshwork composed of interweaving trabeculae of retained primary spongiosa. Each of the trabeculae consists of a central core of cartilage that is segmentally capped by superficial plates of osteoid or bone. Within the center of the primary ossification center, there are radiating trabeculae of predominantly woven bone with small amounts of lamellar bone and occasional islands of cartilage. There is segmental and irregular thickening of the growth plates, which occasionally project uneven tongues of cartilage into both the primary and secondary ossification centers. Increased numbers of osteoclasts are present, both within, and outside of, Howship's lacunae. Osteoclasts are often characterized by increased nuclear number, nuclear disorganization, and intracytoplasmic vacuolization (Fig 3), as compared to an age-matched control (Fig. 4). There is a reduction in marrow tissue in which the remnant medullary spaces contain decreased numbers of hematopoietic cells admixed with occasional bands of fibroblastic connective tissue, numerous lakes of hemorrhage, and strands of fibrin.

Contributor's Morphologic Diagnoses: Vertebrae: Medullary hyperostosis, chronic, regionally extensive, severe with retention of spongiosa and failure of bone resorption.

Contributor's Disease Diagnosis: Equine osteopetrosis

Contributor's Comment: Osteopetrosis is a clinically and genetically heterogeneous group of relatively rare skeletal disorders characterized by regional to generalized osteosclerosis, or increased skeletal mass. They are generally considered to be inherited diseases and have been reported in both humans and in many domestic species, including the calf, foal, cat, dog, mouse, rabbit, and sheep.[4] In those species in which it has been determined (human and calf), the pattern of inheritance is typically autosomal recessive. In osteopetrosis, the increase in bone mass is the result of an imbalance between the two arms of osteogenesis. That is, there is either increased bone production from osteoblasts or diminished bone resorption by osteoclasts. In mammalian species in which the pathogenesis is known, osteopetrosis typically occurs by the latter mechanism.

Due to osteoclast dysfunction, there is diminished bone modeling and remodeling, failure to resorb the primary spongiosa and the periosteal sleeve, and resultant accumulation of primary and/or secondary spongiosa. Additional clinical characteristics of the diseases include hematopoietic dysfunction, due to obliteration of medullary tissue, and cranial nerve dysfunction, including blindness and deafness, due to defective nerve foramina development.[3]

In osteopetrosis, the diminished osteoclast activity may be the result of one of the following three mechanisms:[1]

1. Decreased number of osteoclasts.
2. Lack of osteoclast function.
3. Lack of the appropriate microenvironment.

In the osteopetrosis of domestic animals, as normal-to-increased numbers of mature-appearing osteoclasts are recognized microscopically, it is assumed that the defect is in osteoclast function.[4,5] Although in the majority of the species, the specific derangement has yet to be clarified.

In previous reports of foal osteopetrosis, four of the five animals were, like the animal in this report, of the Peruvian Paso breed, strongly implicating a genetic etiology.[1] The final foal was an Appaloosa. As in other domestic species, the specific cellular defect in these foals is also uncertain. Based upon quantitative histomorphometric data, as compared to an age-matched control, the foal in this case had 25-300% greater numbers of osteoclasts depending upon the region of the bone examined. This finding supports the hypothesis that the defect involves some manner of osteoclast dysfunction and is similar to findings in the previous equine reports.

Among domestic animals, osteopetrosis is best characterized in the calf. The disease has been reported in numerous breeds, including the Angus, Hereford, and Simmental breeds, and is a genetic condition with an autosomal recessive pattern of inheritance.[4]

In the calf, cat, and chicken, osteopetrosis-like lesions are reported in association with infection with bovine pestivirus (BVD), Feline leukemia virus, and Avian leukosis virus, respectively. In humans, osteopetrosis has been classified according to its clinical phenotype (juvenile vs. adult forms and "malignant" vs. "intermediate" forms), its pattern of inheritance and its genotype. The most common genetic mutations in humans involve a dysfunctional membrane bound proton pump, which is responsible for acidifying the extracellular digestion chamber (w/in the Howship's lacunae) or a dysfunctional Cl^- anion exchanger that no longer acts to maintain electroneutrality.[3,5]

AFIP Diagnoses: Bone, thoracic vertebrae (per contributor): Osteosclerosis with failure of modeling of primary trabeculae, Peruvian Paso, equine.

Conference Comment: The contributor provides an excellent review of heritable equine osteopetrosis. Conference attendees debated the orientation of the section and the unexpected presence of additional growth plates in the developing neonatal vertebrae, which are interpreted as those of the vertebral arches. In this case, there is a total lack of modeling of the primary trabeculae in spite of high numbers of osteoclasts indicating an error in bone resorption.

In the Guides for Toxicologic Pathology, hyperostosis is defined as hypertrophy of bone, an abnormal increase in non-neoplastic skeletal bone mass which may be proliferative or non-proliferative (6). Attendees briefly discussed reserving the use of the term hyperostosis to denote increased bone mass due to increased dimension of bone; whereas, osteosclerosis more accurately denotes increased bone mass due to increased density of bone with normal dimensions.

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SLIDE 39
CONFERENCE 10 / CASE III – H04-2593 (AFIP 2988634)

Signalment: 12-year-old, female (neutered), Staffordshire Bull Terrier, *Canis familiaris*, Canine

History: Sudden onset polyuria/polydipsia. The dog was euthanized 6 weeks after onset of clinical signs due to significant weight loss (despite a good appetite) and no response to symptomatic treatment.

Gross Pathology: The tissue submitted is from a 2.5 cm diameter hard nodule on the cranial mandible that displaces two incisors.
Additional findings on post mortem examination:
Lung – disseminated white foci (up to 5 mm in diameter) throughout both lungs (gross photo)
Mammary region – multilobular dark red/black mass (4 x 2 cm) with a blood-filled central cavity
Thyroid glands – disseminated white foci (up to 1 mm in diameter)
Liver – multifocal tan nodules (up to 1.5 cm in diameter) that are slightly raised from the surface with a depressed darker tan centre (umbilicated); see gross photo
Brain – multifocal dark brown/black areas (up to 5 mm in diameter) throughout the brain parenchyma (gross photo)
Pituitary gland – enlarged with a bulbous appearance and disseminated white foci (1 mm in diameter)
Pancreas – multinodular appearance with a firm texture. Multifocal dark red/brown areas (1-3 mm in diameter); see gross photos
Kidneys – focal hemorrhage (from biopsy); multifocal subcapsular depressions (chronic glomerulointerstitial nephritis)

Laboratory Results: Urine specific gravity: 1.005
Initial hematology revealed mild dehydration (slightly raised erythrocyte count, PCV and hemoglobin concentration)
Biochemistry revealed no significant abnormalities.
Low dose dexamethasone test revealed normal basal cortisol levels and levels <28nmol/l at 3 and eight hours post dexamethasone administration (normal response).
Urinalysis revealed ++protein
Renal Tru-Cut biopsy revealed moderate chronic glomerulointerstitial nephritis.

Histopathologic Description: Oral cavity/mandible, within which there is a subepithelial binodular mass composed of irregular islands of well-differentiated cartilage bordered by less differentiated chondroplastic tissue, which merge into poorly differentiated mesenchymal tissue. Multifocally, there are islands of irregular mature spongy bone and there is evidence of ossification of some of the cartilage. Occasional mitoses are seen in the poorly differentiated cells.
Multifocally, within the cartilaginous and mesenchymal areas, and frequently within dilated vessels in the supporting stroma, there are lobules of neoplastic epithelial cells that occasionally form tubular structures; in the periphery of the lobules, the cells

occasionally palisade. These neoplastic cells have poorly defined borders, moderate amounts of eosinophilic cytoplasm and round to oval nuclei with vesicular chromatin and multiple small nucleoli. Mitoses are variable in number, but in areas average 15-20 per high power field; occasional bizarre mitoses are seen. Apoptoses are common. There is moderate anisokaryosis. In most sections there is a mild superficial subepithelial plasma cell-dominated infiltration.

Contributor's Morphologic Diagnosis: Mandible: Multilobular tumor of bone with metastatic adenocarcinoma, Staffordshire Bull Terrier, Canine.

Contributor's Comment: Multilobular tumor of bone (formerly called multilobular osteochondrosarcoma, multilobular osteosarcoma, multilobular chondroma, multilobular osteoma, chondroma rodens, calcifying aponeurotic fibroma, cartilage analogue of fibromatosis and juvenile aponeurotic fibroma) is an uncommon tumor that is primarily observed arising from the flat bones of the skull of older, medium-sized to large dogs (occasionally seen in man, cats, horses and ferrets) [1,2]. The gross appearance is of a hard nodular mass with discrete borders that generally ranges from 2 to more than 10 cm in diameter[3]. Histologically, the tumor is composed of numerous contiguous lobules of cartilage and/or bone bordered by septae of spindle cell mesenchyme[2]. Multilobular tumors generally exhibit slow progressive growth and may cause clinical signs by compressing adjacent structures. Recurrence following excision is common and metastasis can occur.
Multilobular tumors of bone have been graded using criteria including pushing/invasive borders, size of lobules, organization, mitotic index, cellular pleomorphism and necrosis[1,4]. Using these criteria, this mass was considered to be grade I.

In this animal, there was no history of clinical signs associated with the bone tumor. The extensive infiltration of the kidney by a metastatic simple mammary carcinoma, in addition to a pre-existing chronic interstitial nephritis likely resulted in the clinical signs of renal failure.

Simple carcinomas are the most common malignant mammary tumor in the dog[5], and these tumors commonly spread by lymphogenous and hematogenous routes. Histologically, neoplastic epithelial cells (which were similar to those seen in the mammary tumor) were seen infiltrating every tissue examined in this case, including the multilobular tumor of bone presented here.

AFIP Diagnosis: Mandible (per contributor): Carcinoma with vascular invasion, and associated chondro-osseous and fibrous proliferation (fracture callus) Staffordshire Bull Terrier, canine.

Conference Comment: This case is a descriptive challenge. Sections contain at least two separate nodules expanding the submucosa of gingival epithelium. One nodule contains numerous islands of neoplastic epithelial cells; whereas, the other contains

anastomosing bands of fibrous perichondrium which forms islands of well differentiated hyaline cartilage, several foci of chondrus to osseous metaplasia and, infrequently, areas of endochondral ossification.

Conference attendees agreed that the islands of neoplastic epithelial cells most likely represent metastasis from a primary carcinoma. Discussion focused on the bony, cartilaginous and fibrous nodule. Some felt this component of the lesion most likely represented a multilobular tumor of bone; others felt it was more representative of a callus formed in response to a pathologic fracture of the mandible.

Histologically, multilobular tumor of bone appears as a multilobular mass with lobules demarcated by fibrovascular septae and arranged in a characteristic trilaminar pattern consisting of a central area of cartilage or bone that may be calcified or ossified; a middle zone of plump, spindle-to ovoid-shaped cells; and a peripheral zone of fibrous tissue (1). The debate hinges on the lack of a prominent middle zone, also known as a cambium layer, surrounding the islands of cartilage and bone. Additionally, multilobular tumors are described as having a characteristic pattern of numerous contiguous lobules bordered by thin septae of spindle cell mesenchyme (5). It was noted that in our case the lobules were not contiguous and that they are uncharacteristically large and separate from each other, more suggestive of reactive callus formation than multilobular tumor of bone. Regardless, the contributor provides an excellent review of multilobular tumor of bone and an interesting lesion guaranteed to spark much discussion.

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SLIDE 40
CONFERENCE 10 / CASE IV – 05-1136 (AFIP 2984020)

Signalment: Female Great Dane, 15 weeks, canine

History: The dog presented with a 3 day history of generalized pain, reluctance to walk followed by lateral recumbency. Clinically the dog was tachycardic, anorexic and pyrexic. It went into cardiopulmonary arrest and did not respond to resuscitation.

Gross Pathology: The specimen is a puppy in good body condition and fair post mortem condition. The thorax contained approximately 35 cc of serosanguineous fluid. Approximately 60% of the lungs were mottled red and pink and oozed mucopurulent material when squeezed. There was a 1 mm space with white exudate between the metaphysis and physis of the humeri and femurs. In these bones the epiphysis and physis were easily separated from the metaphysis. The stool in the colon was soft and unformed. A moderate number of ascarids were present within the intestines. Multiple areas of the small intestinal mucosa ranging from 5 to 10 mm in diameter were red.

Laboratory Results: ALP 281 IU/L (range 10-150), Phosphorus 7.6 mg/dl (range 2.1-6.3), Calcium 11.6 mg/dl (range 8.2-12.4), Band neutrophils 1188/uL (range 0-300), Segmented neutrophils 5544 (range 3,000-11,500)

Histopathologic Description: Specimen is epiphysis, physis and metaphysis. There is neutrophilic inflammation with necrosis of the underlying tissue and inflammatory cells (necrosuppurative osteomyelitis) that is most intense in the region of the primary trabeculae. Viable osteoblasts are not present and presumed to have undergone liquefactive necrosis. Viable osteoclast-like cells are present in the exudates. The cartilage cores in this region have only slight amounts of osteoid on their surfaces and many are fractured or missing (liquefactive necrosis/osteoclastic osteolysis). Fibrinoid vascular necrosis with necrosuppurative and fibrinous inflammation in the adjacent marrow/periosteum involve vessels deeper in the metaphysis, periosteum and within the epiphysis near the physis.

Contributor's Morphologic Diagnosis: Acute necrosuppurative metaphysitis with metaphyseal infractions and acute fibrinoid vascular necrosis.

Contributor's Comment: The histologic lesions are characteristic of canine hypertrophic osteodystrophy (HOD). Lesions of hypertrophic osteodystrophy are found in long bones, most often the radius, ulna and tibia of young large-breed dogs (1-5). The primary lesions in HOD appear to be osteoblast and osteoclast necrosis, hemorrhage and neutrophilic inflammation where primary trabeculae would form leaving "naked" apparently elongated and retained calcified cartilage (1-5). This area appears as a radiodense region. Beneath this zone trabeculae are fractured and undergo marked osteoclastic resorption with marked suppurative inflammation (1-5). This area is seen as a radiolucent region. Later stages involve the formation of woven bone on the periosteum adjacent to the affected site and the replacement of reabsorbed trabecular bone with fibrous tissue (1-5). There has also been documentation of mineralization of soft tissues and vessels in some cases (1,2,4,5). Venous thrombosis in the diaphysis has been documented; however vasculitis has not (4). The

pathogenesis is not completely understood, but possibilities include vaccine induced or bacterial or viral agents, although the lesions appear to be sterile in nature (1-5). Patients are presented with a reluctance to walk from the extreme pain caused by inflammation, necrosis and fractures (1-5). This pain eventually leads to the anorexia, depression, and weight-loss (1-5). Other clinical findings include a decrease in total protein and albumin, an increase in AP and high and imbalanced calcium and phosphorus levels (1-5). The high levels of calcium and phosphorus may be the result of bone resorption and may lead to the soft tissue mineralization (5). Prognosis is good as the disease is self-limiting.

In the current case, the line of infraction has resulted in marked separation of the physis from the metaphysis in many histologic sections. This is an artifact of processing. This fracture space was visible grossly, but it was less than 1 mm wide. The cause of the widespread acute fibrinoid vasculitis in the bone is not apparent but suggests an Arthus reaction in the absence of visible infectious agents. Soft tissue mineralization was present in renal tubules, renal arteries, myocardium, and smooth muscle in the lung. The clinically reported cardiopulmonary arrest was suspected to be due to marked acute mucopurulent bronchopneumonia from which *Bordetella bronchiseptica* was cultured.

AFIP Diagnosis: Long bone: Osteomyelitis, necrosuppurative, acute, diffuse, severe, with microfractures and acute fibrinoid vascular necrosis, Great Dane, canine.

Conference Comment: The contributor provides an excellent review of hypertrophic osteodystrophy (HOD). Conference attendees discussed the neutrophilic inflammation and necrosis as most intense in the region of the primary trabeculae with a lesser amount present in the periosteum. There is a paucity of osteoid on the surface of the cartilage cores and many are either fractured or missing.

The name, hypertrophic osteodystrophy, comes from chronically affected cases where dogs may develop a markedly thickened metaphysis, widened periosteum, and deposition of extraperiosteal bone and cartilage; all of which are recognized clinically as painful, swollen joints.

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SLIDE 41
CONFERENCE 11 / CASE I – 03 RD1618 (AFIP 2942334)

Signalment: Bailey: 4-year-old neutered male domestic longhaired cat; *Felis catus*

History: The problem began with a rapid onset in early November 2003. Bailey was examined 11/12/03 and 12/10/03. An intraocular mass, probably vascularized, was noted medially displacing the iris forward and lens caudally. There was dyscoria and a low intraocular pressure. On the first exam, the fundus appeared normal. On second exam, the lesion was not visible due to a small pupil opening. The owners declined an attempt to excise the mass only. Enucleation of the left eye was performed.

Gross Pathology: There is a solid white mass distorting the ciliary body and cradling the lens.

Contributor's Morphologic Diagnosis: Feline iridociliary adenoma

Contributor's Comment: Feline iridociliary adenomas are the second most common primary neoplasm of the eye, following melanoma. This tumor is far less common than the iridociliary adenoma of dogs; however, it is more common than iridociliary epithelial tumors in humans. The morphology of the feline tumor is considerably different than the canine tumor. As in this case, the tumors in cats are usually made up of solid sheets of polygonal bland epithelial cells with a regular delicate vascular supply. Although the tumors may be large and extended to all corners of the globe, they always originate from the epithelial tissues which line the posterior chamber, iris and ciliary body. Feline iridociliary adenoma is a tumor which is commonly submitted for a second opinion because the feline tumor has a different morphology than the more common and familiar canine variant. A PAS stain is useful because a delicate basement membrane delineates individual cells or small clusters of cells highlighting the epithelial nature of this tumor.

AFIP Diagnosis: Eye: Iridociliary adenoma, Domestic Longhair, feline.

Conference Comment: Conference attendees debated whether the tumor is present in the anterior chamber or the posterior chamber of the eye. Iridociliary adenomas

originate from the iridociliary epithelium, which is of neuroectodermal origin. In this case, the neoplasm pushes the iris forward which partially fills the anterior chamber. Attendees speculated that the neoplasm may have effectively closed the filtration angle resulting in glaucoma. However, there is no loss of retinal ganglion cells suggestive of glaucoma.

Attendees included melanoma and extramedullary plasmacytoma in their differential diagnosis because of their frequencies and the distinct nesting and packeting arrangement of the cells. Keys to diagnosing iridociliary adenoma are recognition of the delicate fibrovascular stroma and the presence of a basement membrane. A Periodic Acid Schiff (PAS) procedure identifies the basement membrane and highlights the nest and packet pattern. Epithelial tumors require a basement membrane for support and survival. The only primary intraocular epithelial tumors are iridociliary adenoma and iridociliary adenocarcinoma.

In dogs, iridociliary adenomas range from papillary to solid, but almost all retain at least some regions with cuboidal epithelial cells. Pigment may be present, which can cause confusion, both clinically and histologically, with uveal melanocytoma. Additionally, the fibrovascular stroma is generally much more prominent in dogs than in cats (1). Anaplastic features and scleral invasion differentiate iridociliary adenocarcinomas from adenomas (3).

Although generally not required for diagnosis, immunohistochemistry may be useful in some cases. Iridociliary adenomas are almost always positive for vimentin, S-100 protein and neuron specific enolase (NSE) (1). Iridociliary adenocarcinomas are often positive for cytokeratin, but the normal iridociliary epithelium and cells of iridociliary adenomas are usually negative (3).

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SLIDE 42
CONFERENCE 11 / CASE II – 05-488 (AFIP 2988013)

Signalment: 10-year-old, female Miniature horse

History: Several days of not doing well. Off feed one morning and colic by evening.

Gross Pathology: Four well-delineated areas of jejunum, 6-10 cm in length, are thick and ulcerated and contain white to yellow plaques. Transmural necrosis and suppurative inflammation is present.

Laboratory Results: Leukopenia, acidosis, thickened small intestine on ultrasound

Histopathologic Description: Extensive necrosis, edema and suppurative inflammation of the submucosa with thrombosis of veins and lymphatics. Numerous gram-positive cocci are present throughout the submucosa forming dense colonies. The inflammation extends through the muscularis but is less severe than in the submucosa. The inner muscular layer has multifocal necrosis. The mucosa has focal necrosis and hemorrhage consistent with infarction.

Contributor's Morphologic Diagnoses: Transmural necrotizing, suppurative enteritis with thrombosis, infarction and gram positive cocci

Contributor's Comment: This horse had a bacterial infection of the intestinal wall caused by gram-positive coccoid bacteria. The organism was not cultured but is probably *Streptococcus equi* or *S. zooepidemicus*. The inflammation and probable organism suggest bastard strangles as the disease (1).

Strangles is caused by *Streptococcus equi* and produces suppuration of the lymph nodes of the head and neck. Some horses develop more extensive systemic disease, referred to as bastard strangles, with abscesses in lymph nodes of the mesentery and mediastinum, liver, kidney, intestine and elsewhere. This horse had no lesions in any other organs or lymph nodes, which would be unusual for a case of bastard strangles.

AFIP Diagnoses: Small intestine: Vasculitis, necrotizing, multifocal, severe, with thrombi, infarcts, and colonies of cocci, Miniature horse, equine.

Conference Comment: Conference attendees agreed that the central pathologic event occurring in this case is vasculitis, which was most likely the result of bacteremia, with subsequent thromboembolism and infarction. Some felt the pale areas of infarction were more representative of arterial rather than venous infarction.

Strangles is caused by *Streptococcus equi* subsp. *equi* and is characterized by suppurative rhinitis and mandibular and retropharyngeal lymphadenitis. Transmission occurs through feed, aerosolized droplets or exudate containing the bacterium. *S. equi* penetrates the nasopharyngeal mucosa and spreads via lymphatic vessels to the mandibular and retropharyngeal lymph nodes. In some cases there is hematogenous

dissemination of bacteria resulting in abscesses of multiple organs including the lungs, liver, kidney, spleen, brain and joints; such cases are commonly known as “bastard strangles”. Gross findings include mucopurulent nasal discharge with hyperemic respiratory mucosa, conjunctivitis, and purulent lymphadenitis. Occasionally, retropharyngeal and mandibular lymph nodes rupture leading to suppurative cellulitis and draining cutaneous ulcers. Sequelae to strangles include: bronchopneumonia due to aspiration of exudate; laryngeal hemiplegia (“roaring”) resulting from enlarged retropharyngeal lymph nodes compressing the recurrent laryngeal nerves; facial paralysis and Horner’s syndrome due to compression of cranial nerves; purpura hemorrhagica, believed to be caused by the deposition of *S. equi* antigen-antibody complexes in arterioles, venules, and capillaries resulting in vasculitis; infection of the paranasal sinuses and guttural pouches and cellulitis due to ruptured abscesses (2).

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SLIDE 43
CONFERENCE 11 / CASE III – 05 RD0943 (AFIP 2985166)

Signalment: Chili Dog: 8-year-old, neutered male Catahoula Leopard dog, *Canis familiaris*

History: In May 2005, Chili Dog presented to the ophthalmologist with a three-week history of right eye abnormality. The veterinarian categorized her findings as buphthalmos, conjunctival hyperemia, severe corneal edema, dyscoria, ventro-medial cyclodialysis, lens luxation, central cupping of the optic nerve head, heterochromia OS, and normal gonioscopy OS. The dog also had glaucoma, OD. The right globe was enucleated.

Gross Pathology: A white-tan mass partially fills the anterior chamber (Fig 1).

Histopathologic Description: The tissue submitted is the formalin fixed right globe from an 8 year old, male castrated, Catahoula Leopard dog. Grossly and histologically, the globe is enlarged, associated with a basophilic cellular infiltration involving the inferior iris leaflet, ciliary body, and peripheral choroid, which is adherent to the lens. Histologically, the mass is composed of fascicles of neoplastic spindle cells, which contain oval nuclei and wispy eosinophilic cytoplasm. Neoplastic cells involve the inferior iris leaflet, ciliary body, and peripheral choroid, which is adherent to the anterior lens capsule and forms a cavitated area (cyclodialysis), with a broad band of neoplastic

cells spanning to the end of Descemet's membrane. Furthermore, neoplastic cells line Descemet's membrane peripherally. Neoplastic cells are noted within the superior iris leaflet. There is corneal stromal neovascularization and suppurative inflammation. There is a preiridal fibrovascular membrane, associated with peripheral anterior synechiae, in addition to angle recession. There is fibrous metaplasia of lens epithelium (anterior subcapsular cataract). There is full thickness retinal atrophy, with sparring, in addition to gliosis and atrophy of the optic nerve head. Choroidal pigmentation is minimal.

Contributor's Morphologic Diagnoses:
1. Spindle cell tumor of blue-eyed dogs
2. Glaucoma
3. Preiridal fibrovascular membrane, associated with peripheral anterior synechiae and posterior synechiae
4. Anterior subcapsular cataract
5. Cyclodialysis
6. Iridocorneal angle recession

Contributor's Comment: Spindle cell tumors of the iris occur in dogs that have blue irides or partially blue irides. These tumors usually occur as flat masses with diffuse but irregular involvement of the anterior uvea. More than one half of the cases diagnosed in the pathology lab are recognized as being neoplastic by the clinical ophthalmologist. Morphologically, the spindle cells range from bland appearing, elongate cells with small, oval nuclei to highly pleomorphic and anaplastic cells with multinucleate cells and karyomegaly. Mitotic figures are highly variable. Neoplastic cell organization suggestive of peripheral nerve sheath tumor is commonly seen. Antoni A and Antoni B cellular organization is often prominent. However, less than half of the tumors tested stained positive with S100. More work is needed before a cell of origin can be identified for these spindle cell tumors of blue-eyed dogs. Extension into the posterior uvea, vitreous base, or through the sclera into the episcleral connective tissue is fairly common; however, metastasis has not been recognized. We have seen two cases that have recurred within the scleral shell after an evisceration procedure. Almost all affected eyes have glaucoma secondary to tumor infiltration in the iridocorneal angle or interruption of aqueous flow for other reasons. Siberian Husky is the most common breed affected. However, that is almost certainly because it is the dog breed most commonly seen with blue eyes (1, 2).

AFIP Diagnosis: Eye, anterior uvea: Spindle cell neoplasm of blue-eyed dogs, Catahoula Leopard Dog, canine.

Conference Comment: Conference attendees debated whether the lesion is proliferative or neoplastic. All agreed the lesion represents a nearly pure population of one cell type that overlays the corneal endothelium and obliterates the filtration angle,

which are convincing characteristics of a neoplasm. The loosely arranged streams and bundles of spindle cells suggest nerve sheath origin.

Some attendees favored melanoma but did not have the benefit of immunohistochemistry prior to the conference. In this case, neoplastic cells are immunopositive for glial fibrillary acidic protein (GFAP) and negative for S-100 protein. GFAP immunoreactivity eliminates melanoma as a differential and supports the diagnosis of spindle cell tumor of blue-eyed dogs.

The cell of origin comprising spindle cell tumor of blue eyed dogs has not been determined; however, immunohistochemistry and cellular morphology suggest peripheral nerve sheath origin. Metastatic spread has not been described (3,4).

Spindle cell tumor of blue-eyed dogs occurs in dogs with poorly pigmented uveal tissue. In this case, careful examination reveals an exceedingly thin choroid with very little pigment. Within the retina there is loss of the neurons from the ganglion cell layer as well as blending of the inner and outer nuclear layers. Blending of the nuclear layers occurs as a result of the loss of dendrites and axons which make up the inner and outer plexiform layers. Retinal degeneration, a thin choroid, and obliteration of the drainage angle by neoplastic cells indicate glaucoma.

Readers are encouraged to review WSC Conference 1/ Case 1 from the 2003-2004 academic year for another example of spindle cell tumor of blue eyed dogs.

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SLIDE 44
CONFERENCE 11 / CASE IV – N05-145 (AFIP 2984015)

Signalment: 2.5 year old, spayed female Golden Retriever (*Canis familiaris*)

History: The patient presented for a 3 week history of vomiting and intermittent diarrhea, with development of anorexia and lethargy. On ultrasound of the abdomen, small intestinal loops were fluid-filled and dilated, with no apparent motility, and mesenteric lymph nodes were moderately enlarged. The ileus was unresponsive to prokinetic therapy or enteral feeding. Thoracic radiographs revealed alveolar infiltrates in the left and right cranial, and right middle lung lobes, suggestive of aspiration pneumonia. Due to the patient's lack of response to therapy, the dog was euthanized.

Gross Pathology: The patient is in poor body condition, with markedly depleted adipose stores and prominence of bony protuberances. There are multifocal loose fibrous adhesions affecting several loops of mid-jejunum. Diffusely, the small intestinal serosa is discolored gray to red. The wall of the jejunum is flaccid and thin, and the contents are thick, pasty, and bright green. Mesenteric lymph nodes are moderately prominent.

Diffusely, the right middle lung lobe is dark red, firm, and consolidated, with fibrinous adhesions to the mediastinal adipose tissue. The remaining lung lobes are congested and moderately edematous. The heart appears slightly rounded. The mitral valve is mildly expanded by raised, 1-2 mm, glistening tan nodules (valvular endocardiosis).

Laboratory Results: Microbiology: Bacterial culture of the small intestine yielded light growth of *E. coli* and moderate growth of group D *Streptococcus*, which were judged to not be of clinical significance. Cultures of small intestine for *Nocardia* species, *Mycobacterium*, and *Bartonella* were negative. Fungal cultures of the small intestine were negative.

Virology: No viral etiology was isolated in specimen-infected MDCK cells from sections of small intestine, and no viral particles were found on electron microscopy of cell culture.

Histopathologic Description: Jejunum: Diffusely, throughout the small intestine, there is a highly cellular infiltrate within the wall of the small intestine which completely effaces the tunica muscularis. The infiltrate is often most intense in the circular muscular layer, and slightly less intense in the outer longitudinal muscular layer. The cellular infiltrate is composed of a mixture of lymphocytes and foamy macrophages in a loose, fibrillar stroma. There are scattered individual apoptotic cells and scattered cellular debris within the infiltrate. Scattered lymphocytes are present in the serosa. The muscularis mucosa remains unaffected in all sections examined. The mucosal lamina propria contains a mild to moderate cellular infiltrate composed predominantly of lymphocytes and plasma cells, with scattered eosinophils. Multifocally there are regions of mild edema in the lamina propria. The intestinal lumen contains degenerate cellular debris and numerous mixed bacteria.

Contributor's Morphologic Diagnosis: Small intestine, jejunum and ileum: Severe, diffuse lymphohistiocytic mural myositis; moderate, diffuse lymphoplasmacytic and eosinophilic enteritis.

Contributor's Comment: Various special and immunohistochemical stains were applied to sections of the intestinal lesion. No etiologic agents are identified on Gomori's methenamine silver (GMS), Ziehl-Neelsen acid fast (AFB), or Periodic acid Schiff (PAS); scattered mast cells are demonstrated by AFB. Immunohistochemistry for lysozyme demonstrates strong intracytoplasmic immunoreactivity within the large histiocytic population within the mural infiltrate, as well as within scattered mature plasma cells and neutrophils. Approximately 30% of the small lymphocyte population exhibits positive immunoreactivity for CD79a, and moderate numbers of the small lymphocytes exhibit immunoreactivity for CD3, indicating a mixed lymphocytic population.

The cells comprising the infiltrate are well differentiated, with minimal anisocytosis and anisokaryosis, and it is a mixed cell population of histiocytes and lymphocytes, more characteristic of an inflammatory rather than a neoplastic process. Additional diagnostics failed to reveal an underlying etiologic agent, leading to consideration of an immune-mediated myositis.

Chronic intestinal pseudo-obstruction (CIPO) has been described as a phenomenon in humans in which enteric neuromuscular disease or visceral myopathy lead to a functional obstruction of the intestine with ileus, with no apparent underlying mechanical cause (1). A small number of case reports describe CIPO in dogs, with infiltration of the tunica muscularis by mononuclear infiltrates, with variable amounts of fibrosis and atrophy. There is replacement of much of the muscular tunic by connective tissue containing lymphocytes, macrophages, and occasional plasma cells and neutrophils (2, 3). Clinical signs include progressive weight loss, vomiting, ileus, and abdominal distention. The myositis centered on the muscular tunic in this dog is consistent with chronic idiopathic intestinal pseudo-obstruction syndrome. Lesions in this patient were widespread through the gastrointestinal tract, with less severe, presumably earlier lesions extending to the stomach and to the cecum and colon; the jejunum and ileum were most severely affected. There is not a remarkable amount of fibrosis in the intestinal sections in this patient, possibly related to duration of illness.

The underlying cause of CIPO in dogs has not been definitively established, although immune-mediated mechanisms remain under investigation. Full-thickness biopsies are critical in diagnosis of the disease. The therapeutic benefit of immunosuppression is uncertain, but may be beneficial, particularly in early stages of the disease.

AFIP Diagnosis: Small intestine, tunica muscularis: Myositis, lymphohistiocytic, diffuse, severe, Golden Retriever, canine.

Conference Comment: The contributor provides an excellent review of an obscure but interesting condition. As noted by the contributor, the inflammation is centered on and limited to the inner and outer layers of the tunica muscularis. Attendees agreed that such target organ specificity suggests an immune mediated disease. Likewise, the inflammatory cells are predominantly a mixture of lymphocytes and histiocytes which does not support an infectious or neoplastic process. Like the contributor, attendees did not recognize fibrosis and atrophy yet previous journal articles list them as prominent features of this disease.

Interestingly, a recent journal article describes autoimmune enteric-leiomyositis as a rare cause of CIPO in human infants. The article describes the histological hallmark as a dense T-lymphocyte infiltrate and degeneration of smooth muscle fibers within the muscularis propria of the intestinal wall. The article also points out the need for full thickness biopsies of the small intestine for definitive diagnosis (4).

Attendees debated whether or not enteritis is present. Most considered the mucosa, muscularis mucosa and submucosa as within normal limits. The moderator specifically noted that there is not a universally accepted reference range for numbers of lymphocytes, plasma cells and eosinophils in the intestinal mucosa and, that these cell types are normal inhabitants of the intestinal mucosa and submucosa. Without architectural damage to the intestine, one should not diagnose enteritis based upon these cell types alone; however, the presence of neutrophils should be recognized as an important indicator of inflammation.

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SLIDE 45
CONFERENCE 12 / CASE I – 475380 (AFIP 2992505)

Signalment: Two-month-old, male, Asaf lamb.

History: The lamb was submitted for postmortem examination with a history of mild fever (40.8°C), mild dyspnea, and coughing. Several other lambs in the herd showed similar clinical signs and were treated with antibiotics.

Gross Pathology: On postmortem examination mucopurulent discharge around the nostrils with multifocal erosions on the oral mucosa, lips, tongue, and hard and soft palate were seen. Small amounts of blood-tinged contents were found in the gastrointestinal tract, especially in the small intestine. In the respiratory tract, multifocal hemorrhages and ulcerations on the laryngeal mucosa with flakes of mucopurulent discharge were present. The lungs were partially consolidated, especially the anteroventral lung lobes**.**

Laboratory Results: Peste des petits ruminants was diagnosed by immunofluorescence, PCR, Agar Gel Precipitation Test (AGPT) and IHC.

Histopathologic Description: Small intestine, the mucosa is heavily infiltrated by neutrophils, histiocytes and lymphocytes. Many crypts are filled with cell debris and neutrophils. The villi are shortened and blunted and Peyer's patches consistently show areas of lymphoid depletion. Typical syncytial cells and occasionally intranuclear and intracytoplasmic eosinophilic inclusions in the epithelial cells or in syncytial cells are seen. Multifocal coccidian oocysts in the epithelial cells with no significant infiltrate are also present.

Contributor's Morphologic Diagnosis: Small intestine: necrotizing enteritis, acute, diffuse, severe, with crypt abscesses and loss, villous atrophy and fusion, lymphoid depletion, syncytial cells and intranuclear & intracytoplasmic eosinophilic inclusions, "Asaf" lamb, etiology consistent with peste des petits ruminants virus (PPR). Coccidian oocysts were also seen, consistent with concomitant ovine coccidiosis

Contributor's Comment: The histological changes in the lungs (not submitted) were broncho-interstitial, necrotizing, subacute, diffuse, severe, pneumonia with type II pneumocyte and bronchiolar epithelial hyperplasia, syncytial cells, and eosinophilic intranuclear and intracytoplasmic inclusion bodies.

Peste des petits ruminants (PPR) is a contagious viral disease of sheep and goats. PPR is similar clinically and pathologically to rinderpest in cattle and frequently manifests as diarrhea, stomatitis, oculonasal discharge, and pneumonia. The causative pathogen is a morbillivirus of the family Paramyxoviridae. In natural infections, the virus causes disease in goats and sheep, but not cattle or swine. Goats are considered to be more susceptible than sheep. 1,2,3 Morbilliviruses are important pathogens of humans and animals in addition to PPR, classic members of this genus cause rinderpest in cattle, distemper in dogs, and measles in humans. Phocine and cetacean morbilliviruses have been described recently. 2,3,4

PPR is an economically important disease that was first reported in the Ivory Coast of Africa in 1942, and has since spread east to parts of Asia, including India and Pakistan. The African and Asian strains seem to be antigenically distinct. There is current concern that the virus may pose a serious threat to endangered wild goats and sheep in the Himalayas through contact with infected domestic sheep and goats. Transmission occurs by inhalation of aerosols from closely associated animals, by direct contact through licking and nuzzling, and occasionally through fomites such as water troughs and feed bunks recently used by infected animals. 5

Pathological changes associated with PPR include erosive stomatitis and enterocolitis, similar to that of rinderpest in cattle, and proliferative and necrotizing broncho-interstitial pneumonia. Lymphoid depletion or necrosis occurs in the spleen, Peyer's patches, and lymph nodes. In fatal cases, pneumonia may not be as severe in younger goats (less than 4 months) than in older animals (over 6-7 months), probably because young lambs succumb due to dehydration caused by diarrhea before pulmonary lesions can fully develop. 3,5

Coccidiosis is a contagious disease, especially in young kids and lambs, with a world wide distribution. The disease is caused by one or more of approximately 12 different species of protozoa, *Eimeria*, which parasitize cells lining the intestinal tract. An infected animal sheds thousands of microscopic coccidial oocysts in its feces daily. Transmission of coccidiosis to other kids or lambs occurs when infected animals shed the organisms in feces, resulting in contaminated feed or water. When first passed, the oocysts are harmless to other goats. However, under favorable conditions of warmth and moisture, each oocyst matures (sporulates) in 1 to 3 days to form 8 infective sporozoites. If a young kid swallows the sporulated oocyst, the sporozoites are released and rapidly penetrate the intestinal cells. From here, the coccidian passes through several periods of multiplication during which large schizonts are formed. The intestinal cell of the goat is destroyed and thousands of small forms called merozoites break out and invade other intestinal cells. Eventually, sexual stages are reached and new oocysts are produced. The entire life cycle from oocyst to new oocyst takes 2-3 weeks.

In the case of a young kid suddenly exposed to many sporulated oocysts, it may become severely ill in1-2 weeks. Young kids may die rapidly due to a severe attack of coccidiosis. While others, those stronger or less heavily infected, will develop a chronic disease characterized by intermittent diarrhea and poor growth.

AFIP Diagnoses: 1. Small intestine: Enteritis, necrotizing, acute, diffuse, moderate, with crypt abscesses, villous blunting and fusion, syncytia, and intracytoplasmic and intranuclear eosinophilic inclusion bodies, etiology consistent with peste des petits ruminants virus, Asaf, ovine.

2. Small intestine: Intraepithelial coccidia, multifocal, numerous, etiology consistent with *Eimeria* sp.

Conference Comment: The contributor provides an excellent review of Peste des petits ruminants (PPR) and *Eimeria* sp. infection in sheep. Attendees were able to diagnose the disease based on the presence of enteric syncytial cells which occasionally contain characteristic eosinophilic intracytoplasmic and intranuclear inclusion bodies. While several viruses cause the formation of syncytial cells and either intracytoplasmic or intranuclear inclusion bodies, only morbilliviruses cause the formation of syncytial cells and both eosinophilic intracytoplasmic and intranuclear inclusion bodies. The paucity of lymphoid cells and follicles supports the diagnosis as morbillivirus infection commonly results in profound lymphoid depletion. All agreed that the *Eimeria* sp. probably contributed very little, if any, to the necrosis within this section of small intestine.

As mentioned in the contributor's comments, PPR is caused by a morbillivirus, family Paramyxoviridae. Paramyxoviruses are single-stranded RNA viruses which are helical in shape and range from 100-300 nm in diameter. Below is a simplified taxonomic chart adapted from the International Committee on Taxonomy of Viruses (ICTV) outlining the most important Paramyxoviridae and the diseases they cause in animals:

Family: Paramyxoviridae
- Subfamily: Paramyxoviridae
  - Genus: Respirovirus
    - Species: Bovine Parainfluenza Type 3 virus (PI3)
      Sendai virus (mice)
      Simian virus 10
  - Genus: Rubulavirus
    - Species: Porcine rubulavirus
      Simian parainfluenza type 5 & 41
      Mumps (human)
  - Genus: Morbillivirus
    - Species: Canine distemper virus
      Rinderpest virus (mostly cattle)
      Peste-des-petits-ruminants virus (mostly sheep)
      Cetacean Morbillivirus (dolphins, porpoises, whales)
      Phocine distemper virus (seals, sea lions)
      Measles virus (primates)
  - Genus: Henipavirus (Note: There is a lot of new information concerning these two diseases as both have been transmitted to humans.)
    - Species: Hendra virus (affects mostly horses)
      Nipah virus (affects mostly pigs)
  - Genus: Avulavirus
    - Species: Newcastle disease virus (END, poultry)
- Subfamily: Pneumovirinae
  - Genus: Pneumovirus

Species: Bovine respiratory syncytial virus (BRSV)
Murine pneumonia virus
Genus: Metapneumovirus
Species: Turkey rhinotracheitis virus

For additional information concerning morbilliviruses please see Wednesday Slide Conference 25, Case 4, 2004-2005, Canine Distemper Virus in a ferret.

The life cycle of *Eimeria* and *Isospora* sp. is both host-specific and direct. Unsporulated oocysts are shed in the feces and sporulate in the environment to become infectious. Following ingestion, sporozoites excyst, invade intestinal epithelial cells, form trophozoites and undergo asexual multiplication (schizogony, merogony) within a schizont or meront. Merozoites are released and eventually form sexual stages (micro- and macrogametes), which unite to form oocysts (9). Some common coccidia species of domestic and wild mammals and birds include the following (8,9,10):

Animal	Coccidia	Organ affected
Cattle	*E. bovis*	1st gen schizont – Jejunum 2nd gen schizont – Cecum and colon
Sheep	*E. ahsata* *E. bakuensis* *E. ovinoidalis*	Small intestine Small intestine Ileum/Large intestine
Goats	*E. christenseni* *E. arloingi* *E. ninakohlyakimovae*	Small intestine Small intestine Large intestine
Equine	*E. leuckarti*	Small intestine
Swine	*I. suis*	Small intestine
Canine	*I. canis*	Ileum, colon occasionally
Feline	*I. felis*	Small intestine, colon occasionally
Mice	*E. falciformis*	Colon
Rabbit	*E. stiedae* *E. intestinalis* *E. flavescens*	Bile ducts Ileum & cecum Ileum & cecum
Birds		
Chickens	*E. acervulina* *E. necatrix* *E. maxima* *E. tenella*	Duodenum Mid-intestine Mid-intestine Ceca
Turkey	*E. adenoeides* *E. meleagrimitis* *E. gallopavonis*	Ceca Mid-intestine Colon, rectum
Geese & ducks	*E. truncata* *E. anseris*	Kidney Mid-intestine

For additional information concerning *Eimeria* sp. parasites please reference Wednesday Slide Conference 19, Case 3, 2004-2005, *Eimeria* sp. in a calf.

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SLIDE 46
CONFERENCE 12 / CASE II – 050309 (AFIP 2983861)

Signalment: 3-year-old, female, Boer cross, goat, caprine.

History: This 3-year-old goat had been at USAMRIID for 16 months. On February 23, 2005, the animal was observed with labored breathing, discharge from the nose, panting, encrusted nares, increased breath sounds bilaterally, and muffled heart sounds; the goat ate readily when provided grain. During physical examination the following clinical data were measured: body temperature - 102.4; pulse – 132;

respiratory rate - 60. Broad-spectrum antibiotic therapy was initiated with 2 ml of Naxcel® (ceftiofur) once daily for 5 days; and 1.5 ml of LA-200® every other day for three treatments. After treatment, the goat was returned to the herd with noticeable clinical improvement. On March 3, 2005, the goat was removed from the herd and returned to treatment barn with increased breath sounds, the right side worse than the left, and moist lung sounds, especially in the ventral lung fields. The goat was restarted on Naxcel antibiotic therapy per protocol above. The animal again seemed to respond clinically to antibiotic therapy, was observed eating in the treatment stall, and returned to the herd after the second course of treatment. On March 10, 2005, the animal was found dead in the pasture during morning rounds.

Gross Pathology: The carcass of the goat was in good nutritional condition with abundant body fat stores. The subcutaneous tissues over the ventral abdomen were moderately edematous. The abdominal cavity contained approximately 100 ml of clear yellow fluid. Several strands of fibrin extended between the liver lobes and adjacent omentum, intestines, and rumen. Fibrinous adhesions were present between the liver and diaphragm. The right side of the thoracic cavity contained approximately 50 ml of clear yellow fluid that congealed after exposure to air. The left side of the thoracic cavity contained approximately 100 ml of serosanguineous fluid. The pericardial sac was markedly distended with approximately 200 ml of cloudy, orange fluid. The pericardial wall was edematous and fibrous, measuring up to 5 mm in thickness. The internal surface of the pericardium and the surface of the epicardium were diffusely covered by a dense mat of villous, yellow to pink, fibrinous material, measuring up to 1.5 cm in thickness. Numerous fibrinous adhesions were present between the pericardium and the epicardium. The epicardium was diffusely fibrous and edematous, measuring up to 1 cm in thickness. The ventral and cranial aspects of all lung lobes were red, consolidated, and had numerous fibrous adhesions to the pericardium. Fibrous adhesions were also present between the right cranial lung lobe and the body wall. Multiple yellow-green, caseous nodules measuring up to 2 cm in diameter were scattered throughout the lungs and were most numerous in the areas of consolidation. Mediastinal lymph nodes were enlarged and edematous, measuring up to three times normal. The mesenteric, gastrosplenic, and retropharyngeal lymph nodes were congested, edematous, and similarly enlarged. The right inguinal and caudal cervical lymph nodes were enlarged approximately twice normal size. Reduced amounts of ingesta were present in the forestomachs. All other organs were unremarkable.

Primary Gross Diagnoses
1. Heart and pericardium: chronic diffuse fibrinosuppurative epicarditis and pericarditis (“bread and butter pericardium”; “shaggy heart”)
2. Lung: multiple pulmonary abscesses, with fibrinosuppurative pleuropneumonia and multiple fibrous adhesions
3. Abdominal cavity: fibrinous serositis, with adhesions
4. Haired skin, subcutis, ventral abdomen: interstitial edema, diffuse, mild

Laboratory Results: *Corynebacterium pseudotuberculosis* was cultured from the pericardial exudate and one of the pulmonary abscesses. Bacterial cultures of the

thoracic fluid, abdominal fluid, and cerebrospinal fluid were negative for aerobic bacterial growth.

Histopathologic Description: The tissue Gram stain demonstrated many gram-positive bacteria morphologically consistent with *C. pseudotuberculosis* within inflammatory lesions in the lungs, epicardium, and pericardium. The Congo red stain demonstrated varying amounts of amyloid deposition in the liver, spleen, kidneys, rumen, reticulum, and multiple lymph nodes; the amyloidosis was attributed to severe and prolonged inflammation within the thoracic organs**.**

Contributor's Morphologic Diagnoses: 1. Heart: diffuse chronic (organizing) fibrinosuppurative epicarditis, severe, with gram-positive bacillary bacteria, granulation tissue, multiple chronic abscesses, and multifocal myocardial edema with acute to subacute inflammation
2. Pericardium: diffuse chronic (organizing) fibrinosuppurative pericarditis, severe, with Gram positive bacillary bacteria, granulation tissue, and multiple chronic abscesses (histoslides not submitted)
3. Lung: multiple chronic abscesses, with Gram positive bacillary bacteria, diffuse chronic pleuropneumonia, congestion, and multiple fibrous adhesions (histoslides not submitted)
4. Liver: diffuse centrilobular fibrosis, mild to moderate, with congestion and multifocal proliferative endophlebitis (histoslides not submitted)
5. Spleen; kidney; liver; rumen; reticulum; and inguinal, cervical, and mesenteric lymph nodes: amyloidosis, diffuse, mild to moderate (histoslides not submitted)

Contributor's Comment: The pericarditis and epicarditis in this goat were sufficiently severe to have caused death of the animal; the inflammatory lesion in the heart and pericardial sac most likely developed secondarily from pulmonary infection with *C. pseudotuberculosis*. Centrilobular hepatic fibrosis, pulmonary and hepatic congestion, and the proliferative vascular changes in the hepatic veins (histoslides not submitted) reflect the effects of increased venous pressure secondary to congestive heart failure. Right-sided heart failure developed in this animal as a result of the restrictive pericarditis and epicarditis secondary to chronic fibrinosuppurative inflammation.

Corynebacterium pseudotuberculosis, the cause of caseous lymphadenitis in sheep and goats, was formerly known as *C. ovis*. Infection with *C. pseudotuberculosis* may be more severe in goats than sheep, and usually results in the development of lymph node abscesses in the head and neck regions that may resemble melioidosis or pseudoglanders[1]; we have observed lymph node abscesses in these anatomic areas as incidental findings during necropsy of other animals in this goat herd. Bacteria may occasionally spread from infected lymph nodes to the lungs, and pulmonary lesions are most common in older animals[1]. Chronic subpleural pulmonary abscesses and/or bronchopneumonia with secondary pleuropneumonia may develop in animals that have had previous episodes of "caseous lymphadenitis" due to *C. pseudotuberculosis*. Bacterial spread to other organs is uncommon, but has reported to occur in the kidney, spleen and liver[1].

Infection with *C. pseudotuberculosis* in sheep typically results in enlargement and abscessation of the superficial and visceral lymph nodes. Infection is spread to other animals directly through oral and nasal secretions, and ruptured peripheral lymph node abscesses[2]. Affected lymph nodes become enlarged and filled with caseous, inspissated, green to chalky-colored material; on cross-section, the encapsulated abscesses have a characteristic "onion ring" appearance due to the concentric lamellations of the material. Hematogenous spread may lead to internal lymph node abscessation and pneumonia[2]. Other pathogenic corynebacteria and their associated disease conditions in animals include: 1) *C. renale*, *C. pilosum*, and *C. cystitidis* as causes of cystitis and pyelonephritis in cattle; 2) *C. bovis* and *C. ulcerans* as causes of mastitis in cows; and 3) *C. kutscheri* as the cause of caseopurulent lesions in the lungs, liver, and lymph nodes in rats[3].

Corynebacteria are small, pleomorphic gram-positive bacteria with varied morphology, including coccoid, club, and rod forms; in stained smears they may be found singly, arranged in palisades of parallel cells, or angular clusters (Chinese letters)[2]. Many members of the group are found as commensals on the mucous membranes, and some species, such as *C. pseudotuberculosis*, can survive for several months in the environment[2]. The pathogenicity of some corynebacteria is known to be related to the elaboration of certain exotoxins. For example, the virulence of *C. pseudotuberculosis*, a facultative intracellular bacterium capable of surviving and replicating in phagocytes, is related to its cell wall lipid and to the production of the exotoxin phospholipase D[2]. Other virulence factors in the corynebacteria group are related to the use of bacterial attachments and production of proteases. *Corynebacterium renale*, the urinary tract pathogen causing cystitis and pyelonephritis in cattle, possesses fimbriae which facilitate attachment to the urothelium and produces urease to hydrolyze urea[2].

Fibrinous pericarditis (and by extension epicarditis) usually occurs by hematogenous routes, but lymphatic spread or direct extension of the inflammatory or infectious process to the adjacent tissue is also possible[4]. There are a multitude of infectious causes of fibrinous pericarditis in domestic farm animals, including: 1) in cattle, contagious bovine pleuropneumonia, clostridial infections, pasteurellosis, sporadic bovine encephalomyelitis, and neonatal coliform infections; 2) in swine, Glasser's disease, pasteurellosis, porcine enzootic pneumonia, and salmonellosis and streptococcosis in piglets; 3) in sheep, pasteurellosis and streptococcosis (lambs); and 4) in horses, streptococcal infections[4].

Ventral subcutaneous edema and effusions in the abdominal, thoracic, and pericardial spaces were prominent gross findings in this animal. While the inflammatory process in the pleural and pericardial spaces was likely the major contributing factor to fluid accumulation in these areas, the ventral subcutaneous edema and abdominal effusion suggest that other mechanisms may have resulted in extravascular fluid sequestration at these anatomic sites. The primary factors that generally govern fluid balance between the vascular and interstitial spaces are vascular hydrostatic pressure and plasma colloid osmotic pressure[5]. Given the gross and histologic findings, this goat

may have had derangements in both mechanisms: 1) right-sided congestive heart failure likely resulted in generalized increase in venous pressure and subsequent systemic edema via activation of the renin-angiotensin-aldosterone axis; and 2) reduced plasma osmotic pressure may have been present due to decreased production of albumin in the liver due to hepatic amyloidosis and increased loss of albumin into extensive areas of chronic suppurative inflammation in the pleural and pericardial areas. Unfortunately, albumin levels were not measured in this animal before death, and decreased plasma osmotic pressure cannot be confirmed.

AFIP Diagnosis: Heart: Epicarditis, pyogranulomatous and fibrinous, chronic, diffuse, severe, Boer cross, ovine.

Conference Comment: The contributor provides a thorough review of *C. pseudotuberculosis* infection in sheep and goats as well as differential diagnoses and diseases in other species caused by *Corynebacteria* sp.

Conference attendees did not have the benefit of the animal's history of antibiotic administration or Gram stains. Expecting to see large colonies of "Chinese characters" coccobacilli associated with *C. pseudotuberculosis* infection, attendees initially placed it lower on the differential diagnosis list. All agreed that the repeat administration of antibiotics is probably responsible for the absence of large bacterial colonies from these lesions and may have contributed to the protracted development of the "bread and butter" lesions of the pericardium and epicardium.

A Brown-Brenn stain revealed the scattered clusters and individual Gram-positive coccobacilli within the epicardium.

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

**The opinions, interpretations, conclusions, and views expressed herein are those of the author(s) and do not reflect the official policy of the Department of the Army, the Department of Defense, or the U.S. Government.

SLIDE 47
CONFERENCE 12 / CASE III – G 6288 (AFIP 2991429)

Signalment: 6-year-old, intact female, cynomolgus monkey (*Macaca fascicularis*), non-human primate.

History: Six animals of different age and sex died in a group of 35 cynomolgus monkeys housed indoor-outdoor within a short time period from late summer to late autumn. The animals became lethargic and febrile and died with unspecific clinical symptoms after one or two days of illness. The monkeys were considered to be serological negative for Herpes B, Herpes simplex I and II, Varicella Zoster virus, Cytomegalovirus, Epstein-Barr virus, SIV, and HTLV. All six deceased monkeys exhibited similar clinical and pathomorphological findings.

Gross Pathology: Main gross necropsy findings occurred in the respiratory tract. The pharyngeal lymphoid tissue was enlarged, heavily inflamed and showed multifocal caseous abscess formations. The lung showed multinodular to diffuse red to grey areas of consolidation in affected lobes and a severe multifocal granulomatous pneumonia. Necropsy findings included marked splenomegaly and mild hepatomegaly with numerous white foci up to 1 mm in diameter distributed throughout the parenchyma. The liver was friable with blunted margins.

Laboratory Results: Routine bacteriology was negative.

Histopathologic Description: The main histologic finding was multifocal granulomatous pneumonia accompanied by a severe purulent bronchitis. Granulomas were diffusely distributed within the lung tissue but were always found in close association to larger lung vessels. The granulomas were sharply demarcated and consisted of central necrotic areas, hemorrhage and mild infiltration of inflammatory

cells. They were surrounded and infiltrated by activated alveolar macrophages. The spleen contained coalescing foci of necrosis of both white and red pulp. The lesions consisted of amorphous cellular debris and were infiltrated by few lymphocytes and neutrophils. In general, they resembled the inflammatory reaction occurring in the lung. The submandibular lymph nodes were grossly enlarged and necrotic. Caseous granulomatous inflammation occurred within the pharyngeal tissue. Furthermore, a suppurative leptomeningitis was found.

Immunohistochemistry was performed on paraffin embedded sections of altered tissue using a specific monoclonal anti *Francisella tularensis* antibody. A widespread distribution of *Francisella* antigen within the pharyngeal lesion, the inflamed lymph nodes and the spleen could be demonstrated. Identification of the causative agent was achieved by culture, enzyme-linked immunosorbent assay (ELISA) and molecular techniques.

Special stains like Gram, Giemsa, PAS-reaction, Grocott and Ziehl-Neelsen staining failed to demonstrate intralesional microorganisms in any of the affected organs.

Contributor's Morphologic Diagnoses:

1. Lung: Pneumonia, granulomatous, multifocal, severe, Cynomolgus monkey
2. Spleen: Splenitis, granulomatous, multifocal to coalescing, severe
3. Liver: Hepatitis, necrotizing, focal, mild
4. Pharynx: Pharyngitis, pyogranulomatous to caseous, severe
5. Lymph node: Lymphadenitis, necrotizing, focal, mild

Etiologic agent: *Francisella tularensis* subsp. *holarctica*

Contributor's Comment: The pathologic findings in our cases correspond to the histologic features in naturally acquired and experimentally induced tularemia in nonhuman primates (Baskerville et al. 1978, Calle et al. 1993, Emmons et al. 1970, Hoelzle et al. 2004, Nayar et al. 1979, Posthaus et al. 1998). *Francisella tularensis* is a zoonotic gram-negative bacterium which causes the disease tularemia. It is widespread in North America as well as in parts of Europe and Asia. The organism is able to infect different species of mammals, and even some species of birds and reptiles. Rodents and lagomorphs are highly susceptible and are considered to be the main reservoir hosts in many areas of the world. Hematophagous arthropods like mosquitoes and ticks have been suggested as main vectors (Ellis et al. 2002). Transmission is often associated with an arthropod vector, but the infection can also be acquired orally, via the respiratory route, by bites of infected vertebrates or from direct contact with infected tissue. Six classic forms of tularemia are described in human medicine. The predominant manifestations of human disease are the ulceroglandular, glandular, occuloglandular, pharyngeal, typhoidal and the pneumonic form; however, overlapping of the different symptoms is observed frequently (Lamps et al. 2004). The presented case mostly resembles the pharyngeal form with pneumonic manifestation. The case illustrates that in addition to classical nodal and pulmonary involvement tularemia

usually affects the liver and spleen with hepatosplenomegaly and necrotic lesions within the altered organs.

The source of infection of this epizootic is still under investigation. The indigenous rodent population appeared to be unusually high. Starting several weeks prior to the epizootic, many dead mice had been found in the facility. A murine tularemia epizootic was the most likely source of infection for the nonhuman primates. The most probable route of infection is assumed via ingestion of infected mice or food contaminated by infected mice, feces or urine.

AFIP Diagnoses: 1. Lung: Pneumonia, granulomatous, multifocal, moderate, cynomolgus monkey (*Macaca fascicularis*), primate.
2. Adipose tissue and attached skeletal muscle (pharyngeal region): Pyogranuloma, with muscular atrophy and replacement fibrosis.

Conference Comment: Tularemia is endemic worldwide and primarily causes disease in wild rabbits and rodents. In the United States, the main reservoir is wild rabbits and contact with infected wild rabbits can result in fatal infection to humans.

F. tularensis is a small, pleomorphic, gram-negative, intracellular coccobacillus that is surrounded by a thick, lipid-rich capsule. Isolates are antigenically similar, but subdivided according to virulence, and epidemiologic and biochemical characteristics into three subspecies or biovars:

1. *F. tularensis* subsp. tularensis (Type A): most virulent; found in North America; associated with tick-borne tularemia in rabbits and zoonotic disease.
2. *F. tularensis* subsp. palaearctica (Type B): less virulent; found worldwide except for Australia and Antarctica; associated with waterborne disease of rodents.
3. *F. tularensis* subsp. mediasiatica: found in central Russia.

Disease susceptibility varies with species infected. Rodents and lagomorphs are most susceptible, and usually suffer fatal septicemia. Other herbivores, ruminants and birds are susceptible, but mortality is unusual. Carnivores are least susceptible, require a large infective dose, rarely develop bacteremia, and only occasionally manifest overt disease.

F. tularensis is transmitted through arthropod bites, penetration of the skin, cuts, abrasions, exposure of mucous membranes, ingestion or inhalation. *Dermacentor* spp. and *Amblyomma* spp. ticks pass the organism transstadially and transovarially, functioning as both vectors and reservoirs. Mosquitoes, fleas, horseflies, deer flies (especially important in N. America) and lice can also transmit the disease. Carnivores may be infected by ingesting infected carcasses and there are numerous reports of zoonotic infections resulting from dog and cat bites.

Following infection, bacteria are phagocytosed by local macrophages. Intrahistiocytic replication occurs in local lymph nodes. After 3-14 days, bacteria disseminate to the spleen, liver, lymph nodes and bone marrow resulting in septicemia. Consistent gross necropsy findings include numerous small, white foci on the surface of an enlarged liver, spleen, lymph nodes, and, less often, kidneys. Microscopically, these small white foci are identified as coalescing areas of caseous to lytic necrosis, surrounded by lymphocytes, neutrophils and macrophages. Additional microscopic findings include vasculitis and thrombosis resulting from bacterial invasion and damage to the vascular endothelium. In acute septicemia, an initial neutrophilia is often followed by neutropenia.

Francisella tularensis can be grown in culture, but the potential risk of human infection requires extra caution. The preferred diagnostic test is serology. A four-fold rise in antibody titer between acute and convalescent serum is considered diagnostic; however, cross-reaction with *Brucella* antigen can occur. IFA is available. *F. tularensis* bacteria are visible on smears stained with new methylene blue.

Participants briefly discussed *F. tularensis* as a potential biological weapon. The Centers for Disease Control (CDC) lists *F. tularensis* as a Category A agent. Category A agents have the greatest potential for inflicting high numbers of human casualties, can be manufactured and disseminated on a large scale, require significant efforts in public health preparedness, and are most familiar to the public.

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SLIDE 48
CONFERENCE 12 / CASE IV – 13125-05 (AFIP 2988628)

Signalment: Two-weeks-old Angus (*Bos taurus)* bovine

History: Owner's fall calving cows were obese and were experiencing dystocia. Some calves were born weak, failing to thrive probably due to dystocia (as per submitting veterinarian). Owner had treated calves without examination by a veterinarian. The following antibiotics were administered to this calf; ceftiofur, penicillin, spectinomycin sulfate, danofloxacin, tilmicosin, and florfenicol.

Gross Pathology: Discrete areas of dark discoloration 2.5 cm or larger were observed in the forestomachs. Small areas of cranioventral consolidation were noted in the lung.

Contributor's Morphologic Diagnoses: 1. Severe subacute diffuse necrosuppurative, erosive, ulcerative and transmural rumenitis with myriad intralesional fungi
2. Severe subacute necrotic vasculitis with thrombosis and intralesional fungal hyphae

Contributor's Comment: Two predominant types of fungi are seen in these sections. Those which are approximately two to three microns wide and septate with parallel sides are limited to the keratinized layer. These hyphae are branching with buds and free chlamydospores and were presumed to be *Candida sp.* The fungi, which are non-branching with non-parallel sides and approximately eight microns wide, are observed in all levels of the sections as well as in thrombosed blood vessels and muscular layers. These fungi are considered to belong to the class of fungi, *Zygomycetes*. Included in this class are *Absidia*, *Mucor*, and *Rhizopus*.[1] Spread to other organs occurs via a hematogenous route from the initial site of infection.[1]

Mycotic infection of the forestomachs of ruminants is generally the result of opportunistic infection secondary to other predisposing factors.[1,2,3,4] This case is somewhat unusual in that at least two species of fungi were identified in the sections. The predisposing factor is this case was believed to be the wide spectrum of antibiotics that were administered over the course of nine days.

In general, other predisposing factors include ruminal acidosis secondary to carbohydrate engorgement, rumen stasis, and reflux of abomasal contents into the omasum.[1,2] Rumen stasis and abomasal reflux may occur secondary to acid base disequilibrium, toxemia, fever, parturient hypocalcemia, and peritonitis.[2]

AFIP Diagnosis: Rumen: Rumenitis, necrosuppurative, erosive, subacute, transmural, diffuse, moderate, with vasculitis, thrombi, and fungal hyphae, Angus, bovine.

Conference Comment: There is some variation among slides. Participants may receive sections with an ulcerated rumen mucosa; whereas, other sections may simply have erosions. Some sections do not contain *Candida*. The contributor provides an excellent case of mycotic rumenitis. Attendees identified the larger hyphae as Zygomycetes class fungi and debated whether or not the few *Candida* organisms are pathologic or simply a commensal organism identified within the superficial epithelium of the rumen.

Zygomycetes are ubiquitous saprophytic molds associated with water, soil, decaying matter, and substrates high in carbohydrates. Zygomycetes are opportunistic invaders; predisposing factors include antibiotic therapy, ruminal acidosis (grain overload), reflux of acidic abomasal contents and erosive viral disease such as infectious bovine rhinotracheitis (IBR) or bovine pestivirus (BVD-mucosal disease). Focal or disseminated infections can occur in cattle of all ages.

The pathogenesis for mycotic rumenitis is another “classic” which every pathology resident must understand and be able to rapidly regurgitate. Briefly, antibiotic therapy, grain overload (or another predisposing factor) leads to the disruption of the normal rumen flora and proliferation of *Streptococcus bovis*. *S. bovis* utilizes the carbohydrates to produce an abundance of lactic acid which decreases the rumen pH resulting in ruminal acidosis, ruminal atony, chemical rumenitis and a favorable environment for growth of Zygomycetes class fungi (*Mucor, Rhizopus*, *Absidia* spp.). The inflamed mucosa becomes ulcerated allowing the fungal hyphae the opportunity to penetrate and invade the vasculature resulting in thrombosis, infarction, acute necrosis, and direct or hematogenous spread (1).

Typical clinical findings include anorexia, depression, rumen atony, foul-smelling feces and increased cardiac and respiratory rates due to acidosis. Clinical pathology findings include hemoconcentration due to the movement of fluid from the circulation into the rumen.

Gross findings include multifocal, well-circumscribed necrotic foci surrounded by zones of hyperemia in the rumen, reticulum, and omasum. Necrotic areas are red to black, firm, leathery, and thickened up to 1 cm. Usually the ventral portion of the rumen is most severely affected; however, up to 70% of the rumen may be involved. Severe cases may develop fibrinohemorrhagic peritonitis and, within the liver, necrotizing thrombophlebitis of portal triads.

Microscopically there is transmural, coagulative necrosis with necrotizing vasculitis, a fibrinous exudate and variable amounts of inflammation. *Zygomycetes* are described as rarely septate, thin-walled, 3-25 um wide hyphae with non-parallel walls, non-

dichotomous, irregular branching, and focal bulbous dilatations. Frequently, hyphae are collapsed, folded and twisted and resemble a ribbon.
Hyphae may be angiotropic, and perineural invasion is common.

Candida albicans (Class Blastomycetes, Family Cryptococcaceae) typically form round to oval budding yeasts (blastospores), masses of branching, septate hyphae (3-6 microns in diameter), and pseudohyphae (budding yeast that remain attached to each rather than breaking free) (5). Infection of the squamous epithelium in young people and animals is called "thrush". *C. albicans* is also an opportunistic invader of aged and immunosuppressed people and animals.

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SLIDE 49
CONFERENCE 13 / CASE I – H05-0326 (AFIP 2983869)

Signalment: Seven month old, mixed sex, *Cairina moschata*, Muscovy duck

History: An estimated 160 out of 500 commercial 2 to 4 month old Muscovy ducks presented with green tinged diarrhea, inappetence, polydipsia, fever, and leg weakness over a period of 17 days. A total of 48 ducks died. Other poultry and other age groups of ducks on the property were not affected (1).

Gross Pathology: All birds examined had similar lesions. There were multiple circular pale cream foci 2 to 3 mm diameter randomly scattered throughout the parenchyma of the liver and spleen. There was marked splenomegaly. The distal colon and rectum contained abundant green tinged liquid (1).

Histopathologic Description: Liver and spleen (H&E). There is randomly distributed multifocal to locally extensive well demarcated coagulative necrosis with an infiltrate of granulocytes and macrophages, and mineralization.
Liver and spleen (Warthin-Starry). Areas of coagulative necrosis contain occasional groups of spirochetes.

Contributor's Morphologic Diagnosis: Liver and spleen: Subacute marked multifocal to locally extensive coagulative necrosis and caseogranulomatous hepatitis / splenitis with mineralization.
Liver and Spleen (Warthin-Starry). Coagulative necrosis with spirochetes (consistent with *Borrelia anserina*).

Contributor's Comment: Avian borreliosis (avian spirochetosis) affects a wide range of bird species. The causative organism, *Borrelia anserine,* is an actively motile coiled spirochete 0.25 x 8 – 20 μm that can be propagated in duck or chicken embryos or chicks. Occurrence is worldwide, but reflects the temperate and tropical range of the vector *Argas persicus* (the cosmopolitan fowl tick), or other *Argas* spp. An infected Argas tick transmits *Borrelia anserina* to the avian host at every feeding, resulting in an acute systemic bacterial infection. There are many strains of *Borrelia anserina* with diverse serotypes and variable virulence. Young birds are more susceptible than adults. The disease course is one to two weeks and severity ranges from mild to fatal. Gross lesions range from splenomegaly with petechiae or ecchymoses, to multifocal necrosis. Spirochetes may be demonstrated in areas of caseous necrosis by Warthin-Starry stain. Ante-mortem diagnosis is by demonstrating spirochetes in Giemsa stained blood smears. Young birds develop a massive bacteremia that persists for several days. In adult birds the bacteremia is minimal and transient. Serologic diagnosis is problematic due to the existence of diverse serotypes (2).

AFIP Diagnoses: 1. Liver: Necrosis, coagulative, multifocal, with fibrin thrombi, Muscovy duck (*Cairina moschata*), avian.
2. Spleen: Granulomas, multifocal and coalescing, with mineralization.

Conference Comment: The contributor provides an informative review of avian borreliosis. Attendees discussed the differential diagnoses for well-circumscribed foci of coagulative necrosis in the liver. (e.g. in cattle, *Fusobacterium necrophorum*; in turkeys, *Histomonas meleagridis;* in reptiles, *Entamoeba invadens*; and primates, *E. histolytica*). With the aid of Warthin-Starry 4.0 tissue stain, *Borrelia* spp. spirochetes became readily identifiable within areas of coagulative necrosis.

Other important veterinary diseases caused by *Borrelia* spp. include: Lyme disease due to infection with *Borrelia burgdorferi*; bovine borreliosis caused by *Borrelia theileri*; porcine spirochetosis due to *Borrelia suilla* (ulcerative dermatitis); and ulcerative gingivitis or trenchmouth" in humans, non-human primates, and rarely puppies, caused by *Borrelia vincentii* (3).

Ixodes deer ticks transmit *B. burgdorferi* to dogs. In dogs, Lyme disease is characterized by six to eight weeks of sudden onset anorexia, vomiting, lethargy, and weight loss with or without a concurrent or recent history of lameness. Some dogs will develop signs of acute, progressive renal failure (i.e. uremia, azotemia, proteinuria, peripheral edema and body cavity effusions). The characteristic microscopic renal lesions associated with Lyme disease are membranoproliferative glomerulonephritis, lymphoplasmacytic interstitial nephritis and renal tubular degeneration, necrosis and regeneration (4).

In humans, Lyme disease affects multiple organ systems and can result in skin lesions, fever and lymphadenopathy followed by joint and muscle pain, cardiac arrhythmias, meningitis, chronic arthritis and encephalitis. Also in humans, relapsing fever, caused by *Borrelia recurrentis* and transmitted by soft ticks or lice, is characterized by chills, fever, headache and fatigue, followed by disseminated intravascular coagulation and multiorgan failure (5).

Borrelia spp. evade the immune system and prevent recognition by host antibodies by varying or shedding their surface antigens or outer membrane proteins. In Lyme disease, as the antibody response to one surface protein is mounting, the bacteria expresses an alternate protein thereby escaping immune recognition (5).

Interestingly, *Borrelia coriaceae* has, until recently, been considered as a potential etiology for epizootic bovine abortion (EBA). A recently published journal article; however, identifies the etiologic agent as a deltaproteobacterium of the class *Proteobacteria*. The only other mammalian pathogen in this class is *Lawsonia intracellularis* (6).

The contributor notes that spirochetes are identifiable in Giemsa stained blood smears. Other organisms found in avian blood smears include Apicomplexan parasites such as:

- *Leucocytozoon* spp. (gamonts or gametes in blood smears identifiable with Wright or Giemsa stain)
- *Haemoproteus* spp. (schizonts in visceral endothelial cells; gametocytes develop in circulating erythrocytes; birefringent pigment granules in infected erythrocytes)
- *Plasmodium* spp. (avian malaria; schizogony in peripheral blood; gametocytes in mature erythrocytes, contain birefringent pigment granules known as malaria pigment)

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SLIDE 50
CONFERENCE 13 / CASE II – 105.126 (AFIP 2987114)

Signalment: 7-year-old, castrated male, Ferret, *Mustela putorius furo*, ferret

History: Lethargy of unknown duration. Abdominal mass identified during ultrasound. Animal was euthanized.

Gross Pathology: A 5.5 x 7.0 x 4.0 cm soft to firm, tan/white to yellow, lobular, hemorrhagic and necrotic mass is present within the left caudal abdomen. This mass displaces adjacent abdominal viscera. The left adrenal gland is not identified. The right adrenal gland contains a 0.5 x 0.5 x 0.5 cm tan to yellow, soft, nodular cortical mass. Approximately 10 mls of sanguinous peritoneal fluid is present. The lungs appear grossly normal.

Laboratory Results: WBC: 3.800 x 10_ (normal 5.900–15.400)
Hematocrit: 25% (normal 36-51%)
BUN: 58 (normal 12–43)

No other significant CBC or chemistry abnormalities were identified.

Histopathologic Description: Within the abdomen, an expansile solid mass with prominent fibrous septae and regionally extensive areas of hemorrhage and necrosis are noted. Neoplastic cells form numerous cystic structures that contain a lightly basophilic to amphophilic staining acellular mucinous-type material. Neoplastic cells are cuboidal to polygonal with scant darkly eosinophilic staining cytoplasm and

prominent nuclei that often contain one or more nucleoli. The cytoplasm of neoplastic cells often contains variably sized clear to wispy vacuoles. Anisocytosis and anisokaryosis are moderate. Approximately two mitotic figures are present per high powered field.
Several pulmonary blood vessels contain variably sized intraluminal bundles of metastatic neoplastic cells that are identical to the cells identified within the abdominal mass.

Contributor's Morphologic Diagnoses:
1. Abdominal mass: Adrenal cortical carcinoma with myxoid differentiation
2. Lungs: Multifocal intravascular metastatic carcinoma
3. Right adrenal (not provided): Adrenal cortical carcinoma

Contributor's Comment: Adrenal cortical carcinoma is the second most common neoplasia within the domestic ferret. Common clinical signs include vulvar enlargement, bilaterally symmetrical alopecia and polyuria/polydipsia. Clinical signs are consistent with overproduction of estrogenic steroids. Plasma cortisol levels are consistently within normal limits. Females are affected more commonly than males and a mean age of 5 years has been reported_. Adrenal cortical carcinoma with myxoid differentiation is a variant of adrenal tumors that appears more malignant than typical well differentiated adrenal cortical carcinomas. The clinical signs in ferrets that have the myxoid variant are similar to those seen in non-myxoid adrenal carcinomas_. Immunohistochemistry results in positive cytoplasmic staining for vimentin, synaptophysin and _-inhibin within neoplastic cells_. This pattern of staining is indicative of a zona reticularis origin. The origin of the neoplastic cells is uncertain; however possible explanations include differentiation from neoplastic cells of the zona reticularis, degeneration of neoplastic cells and possible origination from ectopic rests of gonadal stromal granulosa or Sertoli cells_. A similar neoplasm of adrenocortical myxoid variation is rarely observed within people_. These tumors in people share similar clinical pathology including overproduction of sex hormones. The adrenal myxoid variant is also present in both carcinomas and adenomas of people, whereas it is only identified in the carcinomas of ferrets___.

AFIP Diagnoses: 1. Adrenal gland: Adrenal cortical carcinoma with myxoid differentiation, ferret, mustelid.
2. Lung, vascular lumina: Carcinoma, adrenal cortical, metastatic.

Conference Comment: In ferrets, adrenocortical carcinoma is second only to pancreatic islet cell tumors in frequency.

Four main differential diagnoses for a cellular proliferation of the adrenal glands includes (4):

- Adrenocortical carcinoma: identified microscopically by cellular atypia, invasion beyond the adrenal capsule and/or evidence of distant metastasis

- Cortical adenomas: do not extend beyond the capsule, are well-demarcated, and variably encapsulated
- Nodular hyperplasia: unencapsulated nodular proliferation confined to the adrenal cortex
- Pheochromocytoma

As noted by the contributor, this newly described variant of adrenocortical carcinoma is recognized microscopically as having multiple cystic spaces lined by neoplastic polygonal cells and containing abundant mucinous product. Neoplastic cells are positive for vimentin, synaptophysin and _-inhibin. Inhibins are peptide hormones produced by the granulosa cells in female ovarian follicles and by Sertoli cells in the male seminiferous tubules. They are selectively expressed by cells of sex cord stromal derivation. While anti-_-inhibin can be used to identify sex cord stromal tumors and trophoblastic tumors, it is also helpful to differentiate adrenocortical tumors.

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SLIDE 51
CONFERENCE 13 / CASE III – 203-06 (AFIP 2988627)

Signalment: 1 year old, male, intact ferret

History: Acute onset of lethargy with fever (107.8 F) that did not respond to antibiotic therapy. One day later exhibited signs suggesting abdominal pain. Two days after onset, feet were swollen, the fever persisted and lethargy was worse. No response to additional treatments (fluids and steroids). There was no improvement on day 3 and the

ferret was euthanatized. The referring veterinary practitioner performed a necropsy and submitted fresh and fixed tissues for analysis.

Gross Pathology: There were no gross lesions identified in submitted fresh and fixed tissues. No gross lesions were observed at necropsy by the referring veterinarian. Bloodwork was not completed.

Laboratory Results: No bacteria were isolated using routine aerobic and anaerobic culturing of muscle, lung and liver. Virus isolation using several tissues on MDCK, A72-180, BHK-21, and bovine turbinate cells did not demonstrate viral pathogens.

Histopathologic Description: Skeletal muscle of the esophagus and hindlimb muscles: The primary change is a severe suppurative myositis. Granulocytes, mostly neutrophils, with few mononuclear leucocytes dissect around muscles within fascia and within muscles between muscle bundle and myofibers. Occasional myocytes are shrunken or swollen (necrotic) and infiltrated with neutrophils. Increased numbers of granulocytes line lumina of small blood vessels in the connective tissue surrounding the esophagus and muscles.

Similar, but less intense lesions were identified in sections of lumbar and forelimb muscles and right and left ventricular myocardium. Other minor changes included septal capillary neutrophilia in lung, splenic lymphoid nodular hyperplasia, hepatic lipidosis, and pigment in renal tubular epithelium. These were the only tissues examined microscopically.

Contributor's Morphologic Diagnosis: Skeletal muscle, multiple sites; Inflammation, suppurative, acute, severe with occasional myonecrosis.

Contributor's Comment: Disseminated Idiopathic Myositis (DIM) appears to be a newly recognized disease in ferrets that was first described clinically in 2003. Clinical signs include high fever, lethargy/weakness, reluctance to move, pain on handling, decreased appetite, increased respiratory and heart rate, nasal discharge, and enlarged lymph nodes. Microscopic lesions are as described above.

AFIP Diagnosis: Skeletal muscle, hindlimb (per contributor) and esophagus: Myositis, neutrophilic, acute, multifocal, with scattered myonecrosis, ferret, mustelid.

Conference Comment: Conference attendees agreed that the specificity of the neutrophils for the skeletal muscle of the esophagus is most likely immune-mediated. The inflammation is composed primarily of nondegenerate rather than degenerate neutrophils and the esophageal mucosa is completely spared. In other cases, non-muscular organs such as the brain, liver, lung (bronchopneumonia) and spleen (extramedullary hematopoeisis) have also been affected. As of the time of this write-up, additional information for this disease has still not been published.

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SLIDE 52
CONFERENCE 13 / CASE IV – B01361/D (AFIP 2988203)

Signalment: 7 year old, male, Rottweiler, *Canis familiaris*, dog.

History: The dog was presented for physical examination with a history of ascites, and right cardiac failure was diagnosed. Thoracic examination by radiography and echocardiography revealed a mass within the lumen of the right atrium. The patient underwent open-heart surgery with dissection and removal of a 5 cm diameter, bright red, cerebriform, soft mass from the interatrial septum. Microscopic examination of the lesion was performed. After 9 months, the dog was submitted again to cardiac surgery for a relapse of the mass within the lumen of the right atrium and main vessels of the heart base. The animal was suppressed during surgery.

Gross Pathology: The lesion evident during second surgery within the right atrium was a multinodular, whitish to tan brown, smooth, lardaceous and soft mass. The neoformation was attached to the inter-atrial wall and it was growing within the pulmonary arteries and the *senum venosum* lumen (Fig. 1, kindly provided by Dr. R. Bussadori).

Laboratory Results: **H**ematobiochemistry was not available to be submitted since the case was referred for a second opinion to the surgeon.

Histopathologic Description: The section is characterized by a poorly demarcated, irregular shaped, unencapsulated, densely cellular, infiltrative, multi-lobular mass. Fine septa and small disseminated capillaries are observable. The cells are discrete, round to polyhedral (15-20 mm diameter), and arranged in packets and short cords. Occasionally, cells form lacune filled with erythrocytes. The cytoplasm is scant to moderate, lightly eosinophilic, finely granular, often vacuolated and chromaffin granules are evident with Grimelius specific staining (Fig. 2). The nuclei are round to oval (10-12 mm diameter), usually central, vesicular with marginated chromatin and a single paracentral nucleolus is often seen. Anisokaryosis and anisocytosis are mild to moderate and mitotic figures are rare (< 1 x high power field). Small lymphocyte aggregates are rarely seen within the lesion. Multifocal necrosis and extensive hemorrhages are also present; the latter is associated with disseminated pigment laden macrophages. Diffuse signs of degeneration are evident, particularly in the infiltrative areas.

Contributor's Morphologic Diagnosis: Intracardiac chromaffin paraganglioma.

Contributor's Comment: A paraganglioma was diagnosed arising from the inter-atrial groove, as confirm also by immunohistochemistry: a diffuse cytoplasmic positivity with anti-NSE and anti-Chromogranin A was observed (Fig. 3-4).

Primary cardiac neoplasms are rare in domestic animals, with an incidence varying in dogs between 0.19% and 3.1%, and the heart is one of the rarest locations of paragangliomas. However, the latter are second to hemangiosarcomas among tumors affecting the heart of dogs (1, 2, 3, 4, 5). Paragangliomas are neuro-endocrine neoplasms. They arise from paraganglionic tissue, which is a collection of neuroepithelial cells of the autonomic nervous system. These cells are of neural crest origin and are classified as chromaffin (catecholamine secreting) and non-chromaffin (non-catecholamine secreting). Paragangliomas arising from the adrenal medulla and the aorticosympathetic ganglia are innervated by the sympathetic nervous system and secrete catecholamines. They commonly occur in the adrenal medulla where they are referred as pheochromocytomas (1). In contrast, tumors from elsewhere generally do not secrete catecholamines and are innervated by parasympathetic fibers (1, 6). These nonchromaffin paragangliomas, also called chemodectomas, originate mainly from the aortic body in the thorax, carotid body in the neck, and the glomus jugulare of the middle ear. Aortic body tumors are the most frequent type in domestic animals and are also referred to as heart base tumors (2, 4). Paragangliomas are frequently benign, slow growing, and locally invasive. They are highly vascularized tumors that infiltrate the coronary circulation. The main feeding vessels typically originate from the left coronary system. (2, 4, 7, 8) There are no proven histological criteria to differentiate benign from malignant cardiac paraganglioma and there is no direct correlation between tumor size and malignant potential.

The neoplasm found in the heart of this subject showed a strong cytoplasmic chromaffin reaction and therefore we classified it as an extra-adrenal intra-cardiac chromaffin paraganglioma. In human medicine, 95% of all reported chromaffin paragangliomas are in the adrenal medulla and only 2% are above the diaphragm, accounting for 0.3% of all mediastinum neoplasms. In the mediastinum, 75% are found in the posterior compartment. Less than 50% are functional (1, 7, 8). The middle compartment contains only a few small clusters of chromaffin tissue that could potentially provide a site for tumor development. These neuro-endocrine cell nests are located close to the sympathetic fibers of the middle mediastinum or they could be considered as ectopic chromaffin tissue (1, 7, 9).

In our opinion, this is a very rare location for paraganglioma in domestic animals. Less than 50 cases of cardiac paragangliomas are described in human medicine (10). They can involve the left atrial wall, left ventricle and, less commonly the inter-atrial septum. More rarely they can originate in the right atrium (1, 9). These tumors are locally invasive and can produce symptoms secondary to pericardial involvement or through invasion of the conduction system (8).

AFIP Diagnosis: Heart, right atrium (per contributor): Cardiac paraganglioma, Rottweiler, canine.

Conference Comment: This case prompted the review of the distribution and classification of normal paraganglia as well as the appropriate name for neoplasms having typical neuroendocrine or "zellballen" histologic features which arise in or near the heart base.

Paraganglia are neuroendocrine organs composed of neuroendocrine cells and supporting (sustentacular) cells. They are of neural crest origin. Paraganglia are anatomically widely dispersed, but can be broadly divided into two groups.
The sympathetic paraganglia are distributed along the sympathetic chains in the para-axial regions of the trunk. The most familiar example of a sympathetic paraganglion is the adrenal medulla. Neuroendocrine neoplasms arising in the adrenal medulla are called pheochromocytomas. Neoplasms arising from sympathetic paraganglia in other sites have been called chromaffin paragangliomas or extra-adrenal pheochromocytomas.

The second group is the parasympathetic paraganglia, distributed along and innervated almost exclusively by parasympathetic nerves of the cranial and thoracic branches of the glossopharyngeal and vagus nerves. Examples of this type are the chemoreceptor organs, including the aortic and carotid bodies. Neoplasms arising from these organs have been called aortic or carotid body tumors, chemoreceptor tumors, chemodectomas, or nonchromaffin paragangliomas. In veterinary medicine, neoplasms of chemoreceptor origin occur primarily in dogs, with infrequent occurrence in cats and cattle. Brachycephalic breeds like the Boxer and Boston Terrier are predisposed.

Conference attendees agreed that the neoplasm has the typical features of a neuroendocrine tumor and an infiltrative growth pattern. However, because there are no consistent histologic differences between paragangliomas of sympathetic origin and those of parasympathetic origin, we are unable to classify this tumor further based on the hematoxylin and eosin section. The contributor's comments on the significance of a positive chromaffin reaction are thought provoking. In most references, the term chromaffin paraganglioma denotes a tumor of sympathetic paraganglion origin which produces catecholamines. Chromaffin positivity was based on identification of cytoplasmic granules which stained with the Grimelius stain. In our lab, the neoplastic cells are positive with the Churukian-Schenk stain, which, like the contributor's Grimelius stain, is another argyrophil stain. However, the neoplastic cells are negative with Fontana-Masson stain, an argentaffin stain, a finding that suggests this may be a nonchromaffin paraganglioma. Additionally, all paragangliomas may produce catecholamines to some extent, detectable by methods more sensitive than the chromaffin reaction (11). In some cases, the chromaffin reaction may not reliably separate these tumors (12). Both types can be positive for the immunohistochemical

markers neuron specific enolase, chromogranin A and synaptophysin. In the present case we cannot definitively rule out chemodectoma in this anatomic location, so we prefer the more inclusive diagnosis of cardiac paraganglioma.

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SLIDE 53
CONFERENCE 14 / CASE I – 104/04 (AFIP 2974022)

Signalment: 1 year, female, Haflinger, *Equus caballus*, equine.

History: 48 hours after feeding a new batch of concentrates, two yearlings showed weakness, increased heart and respiratory rates, mucosal congestion and intense sweating. Both were treated symptomatically. They went down in sternal and eventually in lateral recumbency and died within 3 days. All other horses in the stable had refused the new feed and remained free of symptoms.

Gross Pathology: Myocardium pale and patchy, pulmonary edema.

Laboratory Results: Serum: CK 6431 IU/l; EHV I and IV negative. Analysis of a sample of the ration revealed heavy contamination with salinomycin (295mg/kg).

Contributor's Morphologic Diagnoses: Myocardium: Degeneration and necrosis, multifocal, with histiocytic and lymphocytic inflammation; subacute, moderate to severe (depending on location; histoslides from two different locations).
Skeletal muscle: Acute degeneration of single muscle cells.

Contributor's Comment: Salinomycin, monensin, lasalocid and narasin are ionophore antibiotics used in veterinary medicine mainly as coccidiostatics and growth promoters. Ionophores interfere with the membrane transport system for Na+ and K+, resulting in disturbances in intracellular calcium homeostasis. Mitochondrial failure, energy exhaustion and failure of calcium ion retrieval from the cytosol eventually lead to myofibrillar hypercontraction and degeneration (1). Typical findings in cases of ionophore antibiotic poisoning are degenerative cardiomyopathy and/or myopathy (2, 3). Poisonings occur when target species are fed exceedingly high levels of ionophores due to incorrect mixture of premixes or when ionophore antibiotic containing feed is given to monogastric animals. Horses have a very low tolerance for the drugs (1, 3). The LD50 of salinomycin for horses is 0.6 mg/kg bodyweight (2). The ration in this case contained 295 mg/kg, which is even too high for the piglets it was intended for. The recommended dosage as a feed additive for piglets is 30-60 mg/kg feed and 15-30 mg/kg for fattening pigs (2).

AFIP Diagnosis: Heart: Myocardial degeneration and necrosis, subacute, multifocal, moderate, with histiocytic inflammation, Haflinger, equine.

Conference Comment: The contributor provides an excellent review of ionophore toxicosis. Attendees discussed the pathogenesis of ionophore toxicity and how ionophore antibiotics affect sodium, potassium and calcium homeostasis.

Briefly, ionophores are cation transporters that embed in plasma membranes and facilitate the movement of sodium and potassium ions from the extracellular fluid into the intracellular compartment. In response to the influx of sodium and potassium, hydrogen ions are exchanged to the extracellular fluid. Salinomycin, in particular, is a potassium ionophore that interferes with potassium transport across mitochondrial membranes, resulting in low intracellular energy production. The Na^{+}/Ca^{2+} exchange

mechanism may also be disrupted allowing a fatal accumulation of intracellular calcium (4). Calcium pumps are responsible for pumping cytosolic calcium into the sarcoplasmic reticulum in anticipation of the next action potential. Increased cytosolic calcium causes myofibril hypercontraction, ATP depletion and, ultimately, failed oxidative phosphorylation resulting in mitochondrial swelling, disruption and cell death (5).

Attendees discussed other causes of cardiomyocyte degeneration and necrosis in a variety of species. A brief list of causes and specific features of each follows:

- Nutritional myopathy (Vitamin E/selenium deficiency): generally more mineralization
- Exertional myopathies: Primarily affects type II fibers. Often results in a striped or streaking appearance of dead and dying myocytes mixed with less severely affected or more normal appearing myocytes
- Muscular dystrophy
- *Cassia* sp. (coffee senna) toxicity: also see diarrhea
- Gossypol toxicity: hepatopathy and cardiomyopathy
- Coyotillo toxicosis (*Karwinskia humboldtiana*): skeletal and cardiac muscle lesions in sheep and goats
- White snakeroot toxicity (*Eupatorium rugosum*): skeletal and cardiac muscle lesions in sheep, cattle, horses and swine
- Hairy vetch toxicosis (*Vicia villosa*): myocardial necrosis, with dermatitis, conjunctivitis, abortion, and granulomatous lesions in multiple organs

Readers are encouraged to review conference 7, case 2 from this year (2005-2006) for a case of Vitamin E deficiency in a brown pelican.

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SLIDE 54
CONFERENCE 14 / CASE II – 05-24297 (AFIP 2984846)

Signalment: 8 week-old, male, Miniature Schnauzer/Poodle cross, canine

History: The puppy had a history of lethargy and anorexia. It developed seizures and incessant barking.

Gross Pathology: The stomach contained no ingesta. Intestinal contents were scant and normally formed. The liver was mildly swollen and yellow.

Laboratory Results: An FA test for canine distemper was negative. No bacteria were isolated from the lung, liver, spleen, and kidney. Normal flora was cultured from the intestine.

Histopathologic Description: The lung was congested. Diffuse mineralization of bronchiolar adventitia and alveolar septa was present. Many alveolar spaces were distended. Occasional septa were fragmented. They contained variable numbers ranging from a few to several macrophages and traces of proteinic material. In tissues not submitted, additional findings were severe hepatic lipidosis, brain edema, and mild pancreatitis.

Contributor's Morphologic Diagnosis: Pulmonary mineralization (metastatic calcification)

Contributor's Comment: Pathologic calcification is divided into dystrophic and metastatic on the presence of necrosis or normal tissue, respectively. Metastatic calcification is the result of persistent hypercalcemia. The causes of hypercalcemia are numerous. Some of the more common causes are hyperparathyroidism, vitamin D toxicosis, secondary hyperparathyroidism associated with severe renal disease, and cholecalciferol rodenticide toxicosis. The lesions occur primarily in the interstitial tissues of the lungs, kidney, stomach, and blood vessels. The deposits of calcium salts may be amorphous or crystalline. In the kidney, the deposits occur in basement membranes[1].

Additional history obtained from the veterinarian and owner revealed that the bitch received four times the recommended dosage of a vitamin mineral supplement. The bitch and nursing puppy were both on premium quality rations. They were in confinement with no exposure to rodenticides. No lesions were observed grossly or microscopically in the thyroid, parathyroid and adrenal glands. Mineralization was detected in the gastrointestinal tract, kidney, or blood vessels. In other cases of hypervitaminosis D evaluated at our laboratory, the lung appears to be the primary site of mineralization. Confinement of mineralization to the lung may be vitamin D dose related. We have attributed sudden death in recently weaned puppies during stress of elective cosmetic surgery and routine examinations to severe lung lesions caused by hypervitaminosis D. We have also seen mineralization of lamina propria of the urinary

bladder. The cause of death in this case was canine fatty liver syndrome. This is however a significant pulmonary lesion that could have resulted in respiratory insufficiency.

A presumptive diagnosis of hypervitaminosis D was made based on the history and laboratory findings. Hypervitaminosis D with resultant soft tissue mineralization and/or hypercalcemia has been reported in several species of domestic animals[2,3,4,5,6].

AFIP Diagnosis: Lung: Mineralization, interstitial, multifocal, moderate, with emphysematous change, intra-alveolar edema and histiocytosis, Miniature Schnauzer-Poodle cross, canine.

Conference Comment: As the contributor described, alveolar septa are frequently blunted and fractured and, in many areas, there is failure of the alveoli to collapse. Attendees discussed the mineralization of endothelial basement membranes resulting in alveolar edema and the compensatory removal of the excess fluid via phagocytosis by alveolar macrophages.

In this case, excessive vitamin and mineral supplementation are believed to have resulted in high levels of Vitamin D and a subsequent increase in intestinal absorption of calcium and phosphorus. Because of Mass Law interactions between serum calcium and phosphorus, ionized calcium concentration is reduced as a result of precipitation of calcium and phosphorus (7). The basement membrane of the alveolar septa is a common site for the deposition of calcium and phosphorus precipitate.

Other causes of hypervitaminosis D include the consumption of cholecalciferol containing rodenticides and the ingestion of plants containing vitamin D analogs (e.g. *Solanum malacoxylon*, *Cestrum diurnum*, and *Trisetum flavescens*). The margin of safety for vitamin D is narrow and most animals require only low amounts of supplementation.

Typical findings associated with acute, high dose exposure to vitamin D include gastric, small intestinal and myocardial hemorrhage. In cases of intermittent or chronic low dose exposure there is often mineralization of the lungs, kidneys, stomach and arteries. Chronic low dose exposure can also result in osteosclerosis.

Besides vitamin D toxicosis, there are many other causes of hypercalcemia. A short list of the more common causes encountered in veterinary medicine follows:

- Neoplasia (lymphoma, plasma cell myeloma, adenocarcinoma of the apocrine gland of the anal sac, tumors metastatic to bone)
- Primary hyperparathyroidism (hyperplasia, adenoma, adenocarcinoma) elevated levels of circulating parathormone cause increased intestinal absorption of calcium and phosphorus as well as increased renal activation of vitamin D
- Granulomatous inflammation (canine blastomycosis, bovine paratuberculosis)

- Hypoadrenocorticism (increased tubular resorption of calcium)
- Osteolytic lesions of bone
- Immobilization
- Metabolic acidosis
- Renal failure in horses (rarely canine renal failure associated with familial disease)

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SLIDE 55
CONFERENCE 14 / CASE III – 05-12090 (AFIP 2984844)

Signalment: 7 day-old, female, Holstein, bovine

History: Several calves developed severe diarrhea at 2 to 3 days of age. This calf was submitted dead on arrival at the diagnostic laboratory. The calves were treated by the herdsman without an attending veterinarian. They received injectable cephalosporin and sustained release sulfamethazine boluses.

Gross Pathology: The eyes were sunken and there was marked loss of skin elasticity. The ventral portions of the cranial and middle lung lobes were consolidated. The abomasum was partially filled with fluid, clotted milk, and remnants of medication boluses. Segmental congestion was present in the lower small intestine and colon. Intestinal contents were fluid and reddish brown.

Laboratory Results: No viruses were detected by direct electron microscopic examination of negatively stained feces. E. coli was isolated from the liver and it was resistant to sulfas and most antimicrobial agents.

Histopathologic Description: In the kidney, many collecting tubules near the corticomedullary junction were dilated and contained residual basophilic material often surrounded by degenerate and necrotic epithelial cells. Similar basophilic material was also present in large renal vessels. The material was not birefringent in polarized light. In sections of ileum and colon there were erosions and many denuded villi. Crypts were dilated and contain neutrophils and cellular debris. Blood vessels in the lamina propria were congested. Moderate depletion of gut associated lymphoid tissue was noted. The lung was characterized by acute aspiration pneumonia.

Contributor's Morphologic Diagnosis: Sulfonamide nephrosis

Contributor's Comment: Sulfonamide nephrosis is rare today with use of sulfonamides that are relatively highly soluble at the pH normally occurring in the kidney. In this case, the dosage of sulfamethazine was estimated to be 50% higher than recommended. Additionally, the calf was severely dehydrated and no replacement fluid therapy was used. This product is recommended for calves greater than one month of age that are ruminating.

Sulfonamide nephrosis is attributed to mechanical damage and local toxic effects.[1] In this case, material also crystallized in the renal vessels. This is interpreted to be a result of the severe dehydration and overdosage. Potentiated sulfonamides (not including sulfamethazine) have been associated with idiosyncratic drug reactions in dogs and humans. In dogs, fever, arthropathy, blood dyscrasias, hepatopathy, skin eruptions, uveitis, and keratoconjunctivitis have been reported.[2]

AFIP Diagnosis: Kidney: Tubular degeneration and necrosis, acute, multifocal, moderate, with intratubular amphophilic granular material, Holstein, bovine.

Conference Comment: The contributor provides an interesting case of tubular necrosis. Attendees discussed the cumulative effect severe dehydration, the calve's immature kidneys, and massive overdose may have played in the development of tubular necrosis. The moderator mentioned that sulfonamide precipitate is lost in processing and intreprets the amphophilic, granular material found within the tubules as sloughed cellular, and perhaps mineralizing, debris forming clumps where the sulfonamide precipitate had once been.

Readers are encouraged to review conference 8 case 4 from this year (2005-2006) for another case of renal tubular necrosis in a rhesus macaque and a review of causes of renal tubular necrosis.

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SLIDE 56
CONFERENCE 14 / CASE IV – UFSM-1 (AFIP 2992236)

Signalment: 18 month-old, castrated male, mixed breed, bovine

History: Twenty four yearling calves were placed in a 20-hectare paddock in November 2004. Fifteen hectares of this paddock were occupied by eucalyptus woods. Early in April of 2005, abundant amounts of the mushroom *Ramaria flavo-brunnescens* were growing in the soil within the eucalyptus woods (Fig. 1). The first calf became ill in mid-April and died in April 25, 2005. From this date up to May 5, 2005, 12 calves became sick. The clinical signs presented by the affected calves included anorexia, depression and marked drooling of saliva (Fig. 2); there was a striking smoothness of the dorsum of the tongue, ulcers in the in the dorsum of proximal third of the tongue and loss of the long hairs at the tip of the tail (Fig. 3). In some calves there were ulceration of the proximal third of dorsum of the tongue and others presented hyphema. Other signs variably present were loosening and loss of the hooves and of the corneal portion of the horns (Fig. 4). The clinical course ranged from 8 to 15 days after which affected calves either died or were euthanatized. Six of those were necropsied. Photographs were taken from multiple affected calves in this outbreak.

Gross Pathology: At necropsy, the calf of this report was in poor nutritional state and markedly dehydrated. Multiple small papillomas were distributed in the skin of the head and there was a focal screwworm lesion the labial commissure. Most of the long hairs of the tip of the tail were absent. Fat deposits were depleted and there was serous gelatinous atrophy of the fat in the coronary groove of the heart and around the kidneys. The dorsal surface of the tongue was smooth (atrophy of the lingual papilla) and there is a round ulcer (3 cm in diameter) in the dorsum of proximal third of the tongue (Fig. 5). In the final third of the esophagus the mucosa is ulcerated and covered by fibrinous exudate (Fig. 6). The esophageal lesion tapers off toward the more proximal parts of the esophageal mucosa.

Contributor's Morphologic Diagnosis: (1) Esophagus, fibrinonecrotic esophagitis, marked, with thrombosis and clusters of intralesional bacteria, mixed breed, bovine.

(2) Skin of the tip of the tail (slide not included) follicular telogenization and associated alopecia.
(3) Tongue (slide not included) ulcerative glossitis.
(4) Tongue (slide not included) atrophy of lingual papilla.
Etiological diagnosis: toxic esophagitis
Etiology and name of condition: Poisoning by the mushroom *Ramaria flavo-brunnescens*, "mal do eucalipto" (eucalyptus sickness).

Contributor's Comment: The mushroom *Ramaria flavo-brunnescens*, family Clavariaceae, grows exclusively in soils among Eucalyptus woods. It has been recognized in the south and southeast regions of Brazil[2] and Uruguay[7]. It is also widely distributed in North America and occurs in Australia and China, but is not well known in Europe[2]. In south Brazil, the growth cycle of the mushroom is from mid-autumn to early-winter (April-June), being especially abundant in the warm weather following rainy periods[7].

Spontaneously occurring outbreaks of poisoning due to the ingestion of *R. flavo-brunnescens* have been reported in cattle[7], sheep[6,7] and buffaloes[5] and the toxicosis was experimentally reproduced in cattle and sheep by oral feeding of the mushroom[3,6,8]. Due to the consistent association of the mushroom with Eucalyptus woods, the sobriquet "mal do eucalipto" (Portuguese for "eucalyptus sickness") was coined to designate the toxicosis[7]. The toxicosis was first documented in 1958[1], although its etiology was not established at that time.

The clinical signs and pathological aspects of the natural and experimentally-induced toxicosis in cattle were detailed elsewhere[7,8]. Affected cattle have anorexia, depression and marked drooling of saliva. A striking smoothness of the dorsum of the tongue due to atrophy of lingual papillae is characteristic. There is often focal to multifocal fibrinonecrotic lesions at the margins of the tongue and similar linear lesions in the esophageal mucosa. Hyphema and corneal opacity lead to blindness in some cases. There is a loosening and loss of the hooves, of the corneal portion of the horns, and of the long hairs at the tip of the tail[7]. The clinical course ranges from 8 to 30 days after which affected cattle recover or death occurs[7,8].

Experimental trials in calves with reproduction of typical lesions have been done, but the lesions were of less intensity than the naturally-occurring disease[3,8]. In a study which was carried out to determine the morphological and pathogenic aspects of the epithelial changes occurring in the hooves, tail, horns and tongue of calves experimentally poisoned by *R. flavo-brunnescens*[3] the histopathologic changes were marked in those structures where hard keratinization occurred and where normally there is a high uptake of sulfur in the form of cystine during the keratinization process. Toxicosis appears to alter the metabolism of sulfur-containing amino acids in keratinocytes, particularly cystine, with resultant strength loss in the molecular structure of hard keratin and loosening of the hooves, hairs and horns, and flattening of the lingual filiform papillae. The lesions of cattle poisoned by *R. flavo-brunnescens* bare similarities to those of chronic selenium poisoning in cattle[4]. It is interesting to notice that in the first report of

the disease[1] selenium toxicosis was suspected as a cause, although not confirmed.

Due to erosive and ulcerative lesions in the upper digestive tract, the disease has been confused with viral diseases such as BVD/mucosal disease and foot and mouth disease. The esophageal lesion such as the one of this report needs to be differentiated from those caused by uremia in cattle.

AFIP Diagnosis: Esophagus: Esophagitis, necrotizing, ulcerative, diffuse, severe, with chronic-active inflammation and intra-mucosal thrombi, mixed breed, bovine.

Conference Comment: The contributor provides a thorough summary and wonderful gross photographs of a very interesting toxicosis. Attendees struggled with tissue identification as the mucosa is entirely ulcerated in most sections and replaced by a necrotic coagulum. Discussion centered on what etiology could cause such widespread ulceration, especially since the entire lesion is at the same stage of development. Consideration was given to selenium and thallium toxicosis as well as bovine pestivirus (Bovine Viral Diarrhea-Mucosal Disease) and bovine aphthovirus (Foot-and-Mouth Disease). Some suspected ingestion of a caustic substance, such as an acid, due to the even distribution, stage, and severity of necrosis and ulceration.

As mentioned by the contributor, the hooves can loosen and, in fact, slough. Histological changes occur in the laminar epidermis of the hooves and consist of vacuolization of keratinocytes in the epidermal laminae and tight junctions between the stratum lamellar and stratum medium without increased thickness of either, suggesting absence or incomplete and irregular keratinization of the keratinocytes of the epidermal laminae. Deranged keratinization may induce separation of these two structures, resulting in hoof loss (3). For a more detailed review of the histopathologic changes associated with *Ramaria flavo-brunnescens*, readers are encouraged to review reference number 3.

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SLIDE 57
CONFERENCE 15 / CASE I – 457966A (AFIP 2992393)

History: Five ewes were observed to have loss of appetite, poor body condition and progressive worsening respiratory distress. One of the ewes was euthanized, the clinician performed the necropsy and submitted lung samples to the pathology department at Kimron Veterinary Institute in Bet-Dagan.

Gross Pathology: The lungs were enlarged, heavy and failed to collapse when the thorax was opened. According to the clinician there were multifocal areas of consolidated lung tissue of various sizes. The lesions were grayish-white with a firm consistency.

Laboratory Results: Marked leukocytosis (neutrophilia and lymphocytosis).

Histopathologic Description: There are multifocal areas of epithelial cell proliferation on a fine connective tissue stroma showing papillary aspects or forming acinar structures. Adjacent to this proliferation, randomly compressed alveoli are present as well as multifocal areas of lung tissue infiltrates with histiocytes and lymphocytes. There are also areas of peribronchiolar lymphoid infiltrates and bronchioles filled with neutrophils and macrophages as well as multifocal areas of type-2 pneumocyte and smooth muscle cell proliferation can be seen.

Contributor's Morphologic Diagnoses: 1. Lung: Pulmonary carcinoma, adenomatosis, ovine.
2. Lung: Bronchointerstitial pneumonia, suppurative with alveolar atelectasis, subacute, moderate.

Contributor's Comment:

JAAGSIEKTE

(Synonyms: Sheep pulmonary adenomatosis, Ovine pulmonary carcinoma)
Jaagsiekte (JS) is a contagious bronchiolo-alveolar carcinoma of the lungs caused by a Retrovirus.

Diseases of livestock caused by members of the family *Retroviridae*:

DISEASE	HOST ANIMAL (uncommon host)*	SUBFAMILY
Jaagsiekte	Sheep, goat *	Oncovirinae
Enzootic bovine leukosis	Cattle, sheep*	
Maedi- visna	Sheep, goat *	Lentivirinae
Caprine arthritis-encephalitis	Goat, sheep *	
Equine infectious anemia	Horse, donkey	
Non- pathogenic, "foamy viruses" in cell culture	Various animals	Spumavirinae

Brief history and Etiology: First recognized in South Africa more than a century ago. A detailed macroscopic pathology was published by Hutcheon in 1891, with evidence of infectivity and attempts to control it, followed by reports from England, Germany, and France.

The disease is now known to have an almost worldwide distribution and to have considerable economic impact in countries with substantial sheep populations. In South Africa, a survey over a 40-year period indicated that JS is by far the most common neoplasm reported in sheep (64 percent). (7)

Even though JS was known to be contagious long before its neoplastic nature had been elucidated, many years of research in various countries failed to reveal its causal agent, although filtration experiments suggested a viral aetiology.

The first successful experimental transmission of the disease by co-habitation was reported by De kick. An ovine herpes virus was the first candidate virus isolated, but transmission and molecular hybridization experiments proved that this virus is a passenger and not directly involved in the aetiology of the disease.

The possibility of a retroviral aetiology was first suggested by the observation in Israel of typical retrovirus particles in adenomatous lesions. (1) Retroviruses were also found in cell cultures established from JS-affected lungs, and reverse transcriptase (RT) activity in extracts of tumor tissue. No transmission attempts were made in these studies and the results were difficult to interpret in view of the possible presence of maedi-visna lentivirus in the material studied.

Several lines of evidence (transmissions studies, serological studies, electron microscopy, genomic clone and sequence of a JSRV and genomic organization typical of type D and B retroviruses shown and the evidence that JRSV was found to be

consistently, specifically and absolutely associated with JS) cumulatively implicated a retrovirus called Jaagsiekte Sheep Retrovirus (JSRV) in the aetiology of JS.

The above evidence did not prove unequivocally that infection with JSRV is sufficient to induce JS, and the possibility existed that JSRV was acting as a helper virus, complementing a hitherto undiscovered acutely transforming retrovirus.

In 1996, Palmarini and co-workers constructed an infectious molecular clone derived from an integrated proviral exogenous JSRV sequence (called JSRV-21) isolated from a spontaneous case of JS. Concentrated stocks of JSRV-21 obtained from transfected 293T cells were used for intratracheal inoculation of new born-lambs. Two of the lambs developed JS (confirmed at the histological, immunological and molecular level), thus proving conclusively that JRSV is both necessary and sufficient to induce the disease.

Jaagsiekte sheep retrovirus exists in two closely related but molecularly distinct forms. The first is an infectious exogenous retrovirus which is transmitted horizontally from one animal to another and is the aetiological agent of JS.
The second is a group of endogenous retroviral loci that resemble exJSRV at the nucleotide level, but are transmitted/ inherited vertically through the germ line. These endogenous sequences will be referred to as enJSRV. enJSRV are widely distributed in ungulates, with 15 to 20 copies of closely related sequences being found in the genomes of all domestic sheep and goats. Three different loci have been cloned and completely sequenced. No sheep population that lacks enJSRV has been identified, and the possible role of these sequences in disease, as well as their importance to the host organism, has yet to be elucidated. Jaagsiekte is associated absolutely with its aetiological agent exJSRV, whereas enJSRV by themselves are not associated with disease. (1,2,3,7,9)

Epidemiology: Jaagsiekte occurs in sheep as a sporadic or endemic disease in all continents of the world where sheep occur, except in Australia. It displays an extremely variable prevalence between countries. Natural disease with a low prevalence does occur in goats in some countries, but it has not been diagnosed in this species in southern Africa (it has been experimentally transmitted to kids in South Africa). It would appear that goats are less susceptible to infection by JSRV than are sheep. Transmission studies in Europe have provided similar results and in Ireland, during the epidemic of JS in the 1930s and 1940s, JS was not seen in goats despite close contact with sheep suffering from the disease. Jaagsiekte is endemic throughout South Africa and Namibia, and observations suggest that more animals die as a result of the disease in winter or after sudden drop in temperature, possibly as a result of secondary bacterial pneumonia.

Outbreaks of JS occur when affected sheep are introduced into clean flocks. In Ireland it was observed that approximately 5.5 to 8 months elapsed between the introduction of an infected animal into a "clean" flock. It seems that there are breed differences in susceptibility: Iceland- Gottorp breed were far more susceptible than Adalbol breed. In the U.K, there is higher prevalence in Scotland than in England. In South Africa; Merino

and Karakul sheep and their cross-breeds are apparently more susceptible than are English breeds. When these genetic differences were recognized, farmers were urged to breed from resistant families and breeds, and this led to a decrease in the prevalence of the disease. A survey in South Africa indicated that the annual mortality due to JS in infected flocks varied from less than 1 to 24 per cent (average 3.6 percent).

The sex of the animal does not seem to play a role in its susceptibility. Although sheep of all ages are susceptible to experimental infection, neonatal lambs are more susceptible than are lambs more than a few days old. It is likely, therefore, that affected sheep contract the disease at a very early age from infected dams, some of which are asymptomatic. Experimental results suggest that intra-uterine transmission does not occur. Therefore, embryo transfer has a potentially important role in creating "clean" flocks, while enabling the introduction of new genes. (1,3,4,7,9)

Pathogenesis: Experimental transmission of JS suggests that the respiratory system is the natural route of infection. The primary lesion in JS is a well-differentiated multifocal tumor that originates from the transformation of type- 2 pneumocytes and the non-ciliated bronchiolar (Clara) cells. Transformed cells, in which replication of the virus has been observed, proliferate and form clusters that invade and eventually obliterate the alveolar lumen, resulting in death due to hypoxia. The transformed cells retain their secretory function, producing large amounts of surfactant-containing, clear, viscous fluid which accumulates in the air passages and aggravates respiratory distress. It also leads to coughing and the formation of aerosol droplets containing the virus or tumor cells, which can infect other animals. As mentioned, natural cases of JS are often complicated by secondary infections, and in such cases the immediate cause of death is usually an acute or chronic pneumonia.

A characteristic histological feature of JS is the presence of large numbers of macrophages, usually in the areas adjacent to the adenomatous lesions. Various studies of their possible role in the pathogenesis of the disease have failed to provide a clear-cut answer. On the one hand, it has been clearly demonstrated that JS tumor cells produce a chemotactic factor which stimulates proliferation of macrophages; while on the other hand, activated alveolar macrophages produce substances which stimulate the proliferation of type-2 cells. After chemotactic stimulation, macrophages have even been shown to produce both growth- stimulatory and inhibitory factors, depending on their interaction with other lymphoid cells. No evidence for activity of the alveolar macrophages against tumor cells has been found.

Another factor in the pathogenesis of JS about which little is known is the apparently close association of the disease with lentivirus infection. Lentiviruses remain latent in alveolar macrophages and replicate only after these cells are activated. The presence of large numbers of activated macrophages in JS- affected lungs may therefore create ideal conditions for lentivirus replication, possibly aided by an immunosuppression in sheep, the degree of which correlates well with the latent period before the appearance of JS signs in co-infected animals. It is possible that the lentivirus, in addition to causing

a mild interstitial pneumonia, may also predispose animals to secondary infections, including JS. (2,7)

Morphology and morphogenesis of the virion:
Mature virions have an average diameter of 110 nm and, when negatively stained, appear as pleomorphic, enveloped particles with spikes on the surface, typical of most retroviruses. The morphology of positively stained JSRV is distinct from that of any other retrovirus. It possesses a slightly eccentric nucleoid with an electron-dense perinucleoidal space. The virus has a density of 1.17 to 1.18 g/ml, similar to that of mouse mammary tumor virus (MMTV) and Mason-Pfizer monkey virus (MPMV), respectively type B and type D retroviruses. Replicating JSRV particles are occasionally seen in tumor cells. Immature particles, 75 nm in diameter, assemble in the cytoplasm and are then transported to the cell membrane, where budding of mature particles takes place. The morphogenesis is similar to that of types B and D retroviruses, but is quite distinct from that of type C oncoviruses (e.g. bovine leukemia virus) and lentivirus (e.g. maedi-visna virus), where the virions are assembled at cell membrane in a crescent shape before budding as an immature particle. (5,7)
Oncogenic potential of exJSRV: The mechanism of transformation in JS has not yet been elucidated. Owing to the recent discovery that the JSRV provirus had integrated into a part of the sheep genome that does not align with any known cellular sequences (including proto-oncogenes), the possibility of insertional mutagenesis being the transforming mechanism is strongly indicated. JSRV clone was able to induce JS by itself, in the same time frame as field isolates; exJSRV is not a helper virus for an acutely transforming retrovirus. It was concluded that the genome of exJSRV itself harbours its oncogenic potential.

Hecht and his co-workers suggested three possible alternatives:
1. One of the exJSRV protein products may still lead to cellular transformation.
2. One of the viral products may act as a transcription factor to stimulate a cellular oncogene; exJSRV may harbour a more potent activator than those found thus far.
3. Binding to the type-2 pulmonary epithelial cell receptors by their retroviral ligands could result in signal transduction, thereby stimulating the production of growth factors, followed by cell proliferation and subsequent neoplasia. (6,7)

Clinical signs, Pathology and Histopathology: In natural disease, the incubation period has been estimated approximately five to six months, with two- to four-year old sheep being most commonly affected. Clinical disease is therefore rarely seen in lambs under the age of six to nine months.

The onset of clinical signs is insidious in natural cases. Initially, while the habitus is normal and the condition of the animal is good, the respiratory rate is more rapid than normal after an animal has been driven. As the disease progresses, affected animals have reduced appetite, lose body weight, and lag behind the flock when it is driven. Marked respiratory distress is evident on exercise, the respiratory movements being short and jerky. Tachypnoea and dyspnoea eventually become evident even at rest. Spasmodic bouts of coughing occur and there is a great increase in the amount of

secretion from the lungs. This is regarded as almost pathognomonic for JS. Moist rales are heard on auscultation of the thorax, sometimes even without the aid of a stethoscope. There is generally no fever, but pyrexia may occur as result of pneumonia following secondary bacterial infection. Average duration of clinical disease is two months, with a range of a few days to six months. There are cases on record where affected animals have survived for more than a year. Presence, nature and extent of secondary bacterial pneumonia and the environmental conditions under which the animal is kept are important factors in the variations in the duration of clinical signs. Once clinical signs manifest, the disease is irreversible. (1,3,7,8,9)

No consistent hematological abnormalities have been reported in animals suffering from JS. However, increased blood levels of IgG occur in cases with advanced lesions, and these have been ascribed to the presence of numerous plasma cells in the lungs.

The presence of secondary lesions in the lungs often obscures those due to the primary condition and renders the diagnosis of the disease tenuous on gross pathological evidence alone. In advanced cases of JS, the lungs are three or more times their normal weight and almost fill the thoracic cavity. They do not collapse when the thorax is opened. Both lungs are usually involved, but not necessarily to the same extent. The cranial and middle lobes and the cranial part of the caudal lobes are commonly affected, but any part of the lung may be involved. Nodules and areas of diseased tissue of various sizes (less than one millimeter in diameter) are sometimes scattered throughout the normal lung tissue. The lesions consist of very dense tumorous tissue that is grayish-white with a relatively firm consistency due to fibroplasia, which in some cases can be extensive.

The distribution of lesions suggests that the primary lesion or lesions grow by expansion. Intrapulmonary spread of the infection with the development of new foci probably occurs both acrogenously and via the lymph and blood stream. Each new focus is seen grossly as a small grayish-white, semi-transparent nodule which at first is barely visible to the naked eye. As it expands it may coalesce with neighboring nodules and, in this way, large lesions eventually develop. Intra- and extrathoracic metastasis of the tumor may occur. In South Africa extrapulmonary metastasis is rare, whereas it frequently occurs in sheep of the Awassi breed in Israel, where metastases both within and outside the thorax may be found in 30 to 50 percent of cases. This variation may also be linked to different viral strains. The most frequent metastasis sites are the bronchial and mediastinal lymph nodes (approximately 10 percent), peritoneum, skeletal musculature, liver, spleen, kidneys, heart and mesenteric lymph nodes. Spread to visceral and parietal pleura may also occur. (1,3,7,8,9)

Ovine pulmonary carcinoma (OPC) lesions are those of well-differentiated, multicentric, bronchioloalveolar carcinoma. The tumor grows as a simple cuboidal to columnar epithelium, (early stages of tumor development where alveolar type-2 cell proliferation is the initial stage), on a thin connective tissue stroma forming acinar or papillary architecture. These masses tend to compress adjacent alveoli and may be associated with a moderate lymphohistiocytic alveolar infiltrate.

Continued proliferation obscures this pattern, and fibroplasia often occurs in more disorganized and degenerative regions. The papillary proliferations involve both alveoli and bronchioles in many nodules.

Lesions of OPC must be differentiated from progressive pneumonia (lentivirus). While both can have type-2 cell hyperplasia (papillary proliferations of cuboidal and columnar epithelium) and interstitial fibrosis in the absence of significant interstitial pneumonia , the ovine lentivirus-induced lesion has a prominent peribronchiolar and perivascular lymphoid inflammation and lacks the papillary alveolar ingrowths of epithelium present in early OPC lesions. (Dual infections with JSRV and ovine lentivirus are present in many flocks!). In some cases, the connective tissue stroma is very prominent and may proliferate to form thick layers between the tumor acini or beneath the pleura. This stroma generally consists of mature collagen but may appear myxomatous. There may be evidence of exudative pneumonia due to secondary invaders, represented by varying amounts of a fibrinous or purulent inflammatory reaction which may obscure the primary lesion. Abscesses and pleuritis may be present.

Ultrastructural characterization of the component epithelial cells shows a predominance of alveolar type-2 cell morphology with microvilli, desmosomes, and intracellular lamellar bodies, whereas the columnar cells have secretory granules and glycogen compatible with origin from secretory bronchiolar epithelial cells (Clara). Cytoplasmic dense bodies suggestive of Clara cell differentiation can also be present in some cases, and these cells may coexist with cells of type-2 cell morphology. Most neoplastic cells originate from granular type-2 pneumocytes, are well differentiated on the periphery of the lesion, contain many free polysomes and show nuclear margination, indicating that they are in a state of rapid protein synthesis and cell division. Intracytoplasmic JSRV particles, when seen, are often associated with centrioles, suggesting that the virus replicates in actively dividing cells. Nuclei and mitochondria are often abnormal in the malignant cells. In the cytoplasm, numerous secretory granules contain varying amounts of osmiophilic material and myelinoid bodies. These granules are associated with surfactant production and are responsible for the large amount of lung secretion characteristic of JS. (1,3,6,7,8,9)

Differential diagnosis, Diagnosis and Control: In the early literature, clinically and pathologically, JS was often confused with various forms of chronic pneumonia. Pasteurellosis, in South Africa is the most commonly encountered lung condition in sheep, and is also the most common secondary complication in cases of JS. Other organisms causing lung lesions include *Arcanobacterium* (*Actinomyces*) *pyogenes, Corynebacterium pseudotuberculosis, Mycoplasma* spp., and lungworms. In countries were maedi (synonyms: zwoegerzidkte, bouhite Montana progressive pneumonia, and Marsh's progressive pneumonia) occurs, which is caused by a lentivirus and characterized by chronic, nonneoplastic inflammatory lesions must also be differentiated from JS. Today it is known that the two diseases are closely associated in many countries and are often present as co-infections in the same animal, which adds to the existing confusion. (1,3,7,9)

The clinical diagnosis of JS cannot be made with certainty, especially in the first case to be encountered in a flock. A history of long-standing, progressively worsening respiratory distress syndrome in a flock, coupled with the results of clinical examination of affected animals, should alert one to the possible presence of JS. Up-ending an affected animal by its hind legs (often called the wheel-barrow test) may, in cases with extensive lung pathology, induce the flow of a clear viscous fluid from the nostrils, supporting a diagnosis of JS.

The diagnosis of JS must, at least initially, be confirmed by histopathological examination of the lung lesions. Several specimens of diseased tissue from different locations should be collected because lung neoplasia may be obscured, both macroscopically and microscopically, by secondary pneumonia.

The absence of circulating antibodies means that classical serological methods used as an indirect means to confirm infection cannot be used in the case of JS. The absence of a humoral response to exJSRV probably precludes the possibility of effective vaccination. Tools such as the complete nucleic acid sequence and an infectious clone are now available. With the complete genome sequence of both exogenous and endogenous viruses known, it should be a formality to identify those genes/ antigens that can be used to screen specifically for the presence of the exJSRV.

Eradication is not economically feasible. The prevalence in a flock can be reduced to less than 1 per cent (under southern African conditions) by strict isolation and removal of animals showing early clinical signs. If the lambs of infected ewes are eliminated together with their dams, the prevalence of JS can be further reduced. The probability that the etiological agent does not cross the placental barrier enhances the possibility of building up a clean flock by either embryo transfer or by removing lambs from their dams by caesarian section or immediately after birth and subsequently raising them in a disease-free environment. As natural infection by droplet inhalation, it requires close contact and retroviruses are relatively unstable when the atmosphere is dry and temperatures high. Good management practices can contribute considerably to the control of the disease. (7)

AFIP Diagnoses: 1. Lung: Carcinoma, multicentric, breed not specified, ovine.
2. Lung: Pneumonia, bronchointerstitial, suppurative and histiocytic, chronic, diffuse, severe with peribronchiolar lymphoid infiltrates.

Conference Comment: The contributor provides a thorough overview of jaagsiekte. Attendees discussed the etiology of the bronchointerstitial pneumonia and considered a secondary bacterial infection as well as concurrent infection with ovine lentivirus (Maedi) as plausible causes. In particular, interstitial pneumonia with peribronchiolar lymphoid infiltrates is a characteristic finding in cases of ovine lentiviral pneumonia (Maedi). As noted by the contributor, jaagsiekte and maedi can occur in the same flock and often complicate the diagnosis.

The family *Retroviridae* includes many viruses of veterinary importance. All retroviruses contain reverse transcriptase (RNA-dependant DNA polymerase) which enables reverse transcription of virion RNA into double-stranded DNA. The retrovirus genome encodes three major genes: The group-specific antigen (gag) gene; the reverse transcriptase or polymerase (pol) gene; and the virion peplomer proteins or envelope (env) gene. Some retroviruses, such as the lentiviruses, also encode several other accessory genes (10).

A list of some of the more important retroviruses in man and animals and the genus they belong to follows (10):

Retroviridae

- *Alpharetroviruses*
  - Avian leukosis, carcinoma and sarcoma viruses
  - Avian myeloblastosis viruses
  - Rous sarcoma virus
  - Duck spleen necrosis virus
- *Betaretrovirus*
  - Mouse mammary tumor virus
  - Ovine pulmonary adenomatosis virus (Jaagsiekte)
- *Gammaretrovirus*
  - Feline leukemia virus
  - Feline sarcoma virus
- *Deltaretrovirus*
  - Bovine leukemia virus
  - Simian T lymphotropic viruses
  - Human T lymphotropic viruses 1 and 2
- *Epsilonretrovirus*
  - Walleye dermal sarcoma virus
- *Lentivirus*
  - Human immunodeficiency viruses 1 and 2 (HIV)
  - Simian immunodeficiency viruses (SIV)
  - Maedi/visna virus
  - Caprine arthritis-encephalitis virus (CAEV)
  - Feline immunodeficiency virus (FIV)
  - Equine infectious anemia virus(EIA)
  - Bovine immunodeficiency virus (BIV)
- Spumavirus
  - As mentioned by the contributor, these are called “foamy viruses” and are not known to cause disease but can contaminate cultured cells

For a more complete listing of retroviruses in animals, readers are encouraged to review reference 10.

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SLIDE 58
CONFERENCE 15 / CASE II – WN05/945 (AFIP 2996888)

Signalment: Adult, male, breed unknown, *Bos taurus*, cattle.

History: A nodular enlargement of the mediastinal lymph node was detected during routine meat inspection at an abattoir.

Gross Pathology: Mediastinal lymph node: A circumscribed 15 mm diameter nodule enlarges one pole of the 10 mm diameter node. On section, this nodule contains multiple gritty off-white foci, 0.5 to 2 mm in diameter.

Laboratory Results: Microbiology: Mediastinal lymph node: A single colony of *Nocardia pseudobrasiliensis* was isolated.

Histopathologic Description: *On each glass slide there are two (2) tissue sections: the smaller section is undecalcified and the larger section is decalcified.*

Mediastinal lymph node (**undecalcified** smaller section): Within the circumscribed, sparsely encapsulated nodule at one pole of the node, normal node architecture is almost completely replaced by multiple mineralized foci bordered by a prominent zone of syncytial cells and epithelioid macrophages, and separated by irregular fibrous septa heavily infiltrated by plasma cells and lymphocytes. There are occasional small foci of neutrophilic infiltration associated with the mineralized foci. The margins of many of the mineralized foci comprise a mass of branching filaments of variable diameters, with the coarsest having an irregular staghorn-like appearance.

Mediastinal lymph node (**decalcified** larger section): Similar changes as in the undecalcified section, except that the branching filamentous structures bordering the mineralized foci are less coarse and therefore less distinct following decalcification.

Gram stain (**undecalcified** smaller section): No bacteria are detected. The mineralized foci and the surrounding coarse branching filaments have identical orange-brown staining. *This is consistent with the coarse mineralized filamentous structures representing mineralized 'gravestones' of an 'actinomycete' that is no longer demonstrable by the Gram stain.*

Contributor's Morphologic Diagnosis: Mediastinal lymph node: Granulomatous lymphadenitis, with mineralization and mineralized filamentous bacterial casts. Nocardiosis (*Nocardia pseudobrasiliensis*).

Contributor's Comment: The striking feature of this lesion is the staghorn-like mineralized filamentous structures at the periphery of the mineralized foci within the lymph node granuloma. Their filamentous mineralized morphology is different from the radiating club-like structures seen in the typical 'club colonies' of actinomycosis or actinobacillosis.

The significance of the single *Nocardia* sp colony isolated from this lymph node case is arguable. However the morphology of the mineralized filamentous structures surrounding the mineralized foci within the granuloma are consistent with them being 'gravestones' of an 'actinomycete' that is not club-forming.

Nocardia pseudobrasiliensis was designated as a new species in 1996, after a new taxon of *Nocardia brasiliensis*-like organisms was found in 1995 associated with cutaneous and disseminated human disease (1). It is an uncommonly recognized human pathogen, with approximately one case per year being detected in Queensland, Australia.

AFIP Diagnosis: Lymph node, mediastinal (per contributor): Lymphadenitis, granulomatous, multifocal to coalescing, marked, with mineral and filamentous bacilli, breed not specified, bovine.

Conference Comment: There are three main species of Nocardia: *N. asteroides, N. brasiliensis* and *N. otitidiscaviarum; N. asteroides* is the species most commonly isolated.

Nocardia are saprophytic actinomycetes that are present in most environments and produce a suppurative to granulomatous opportunistic infection. *Nocardia* sp. are Gram-positive, non-motile, aerobic, filamentous rods that are partially acid-fast. They are also facultative intracellular pathogens that survive within phagocytic vacuoles of macrophages and neutrophils by inhibiting phagosome-lysosome fusion, neutralizing phagosome acidification, resisting oxidative burst, and altering lysosomal enzymes. Most commonly, *Nocardia* sp. cause an opportunistic infection as a result of wound contamination and spread via direct extension or hematogenous dissemination. Typically, *Nocardia* sp. infections present as ulcerated nodules, abscesses and draining tracts on the distal limbs and head, especially at sites of previous wounds and injury. Occasionally, *Nocardia* sp. infections can present as a subacute to chronic respiratory infection with mucopurulent oculonasal discharge, dyspnea, diarrhea and hyperthermia. In dogs, coinfection with canine distemper virus is commonly reported.

Attendees discussed the presence of filamentous bacteria surrounding mineralized debris within the section of lymph node**.** Although present on H&E stained sections, the filamentous bacteria were best seen with a GMS stain performed at the AFIP. Although *Nocardia* sp. rarely form granules, as the contributor notes their morphology in this case is different from the radiating club-like structures seen in the typical 'club colonies' of actinomycosis or actinobacillosis.

Attendees developed a differential diagnosis based on those organisms which cause granulomatous to pyogranulomatous inflammation. Other etiologies considered include: actinomycosis, *Rhodococcus equi*, foreign body reactions, deep mycotic infections, mycobacteriosis, botryomycosis, or other chronic bacterial or fungal infections.

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SLIDE 59
CONFERENCE 15 / CASE III – 1042/05 (AFIP 2988212)

Signalment: Adult, female, Nelore, cow.

History: This cow is from a Nelore herd of 85 cows, 24 calves, and 3 bulls kept on pasture with mineral supplement ad libitum. Between April 2004 and June 2005, four cows and one bull developed clinical disease characterized mostly by progressive weight loss. The cow examined in this case calved four months ago and began losing weight two months after parturition.

Gross Pathology: The cow had a marked cachexia, the apparent mucosal surfaces were pale, and there was an ulcer in the tongue approximately 2 cm in diameter. Large numbers of *Haemonchus contortus* were found in the abomasum. There was a fibrous adhesion between the liver and the diaphragm. The pancreas was whitish, firm, and had a rough surface (Figs. 1 and 2). On the cut surface of the pancreas a large number of parasites of the genus *Eurytrema* were observed (Fig 3).

Histopathologic Description: Pancreas. Several intra-ductal trematodes, and accumulation of operculated eggs into dilated ducts and interstitium. Multifocal accumulation of epithelioid macrophages and multinucleated giant cells associated surrounding eggs in the interstitium (Fig. 4). Multifocal interstitial lymphocytic infiltration. Mild accumulation of inflammatory cells into the pancreatic ducts. Moderate multifocal to coalescing periductal and interstitial fibrosis with ductal hyperplasia. The parasites have no body cavity, tegument with few spikes and 5 to 15 µm squared crystal-like structures adhered to the tegument (Fig. 5), distinct oral sucker apparently located at one of the extremities of the body (Fig. 6), and both male and female genital organs in one single parasite, including the vitellaria, testes with mature spermatozoa (Fig 7), and an elongated and tortuous uterus filled operculated eggs with a yellow or brown shell.

Lymph node. Multifocal accumulation of a few eggs into the sub-capsular and paracortical sinuses with minimal inflammatory reaction (Fig. 8), and moderate lymphoid hyperplasia.

Contributor's Morphologic Diagnosis: Pancreas. Pancreatitis, granulomatous, chronic, multifocal to coalescing, severe, with intraductal trematodes (*Eurytrema* sp.), Nelore, bovine.

Contributor's Comment: The parasite described in this case has morphological features compatible with those described for trematodes in histological sections. (1) Considering its gross morphology and location, the parasites were recognized as belonging to the genus *Eurytrema*. *Eurytrema pancreaticum* and *E. coelomaticum* are trematode parasites of ruminants, pigs, and man. The adult form is usually located in the pancreatic ducts, or occasionally in the biliary ducts. *Eurytrema* sp. has a broad geographic distribution, including Europe, Asia, and South America. Two intermediate hosts are required for the life cycle of this parasite. In Brazil, the first intermediate host is the snail *Bradybaena similaris*, and the second intermediate host is a grasshopper of the genus *Conocephalus*. Cattle become infected by accidentally ingesting the second intermediate host. (2)

Eurytrema pancreaticum has been recognized as more pathogenic for cattle than *E. coelomaticum*. However, severe chronic pancreatitis, such as in the present case, has been described as a result of *E. coelomaticum* infestation. (2) Considering its morphology and geographical location (3,4), the parasite in this case was considered to be *E. coelomaticum*.

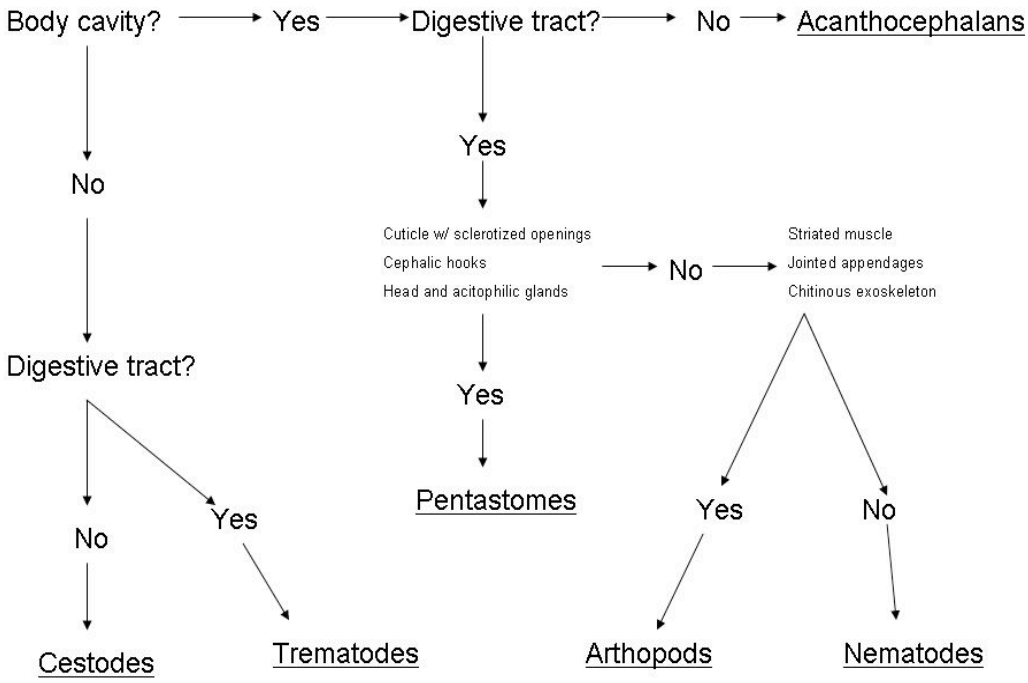
Eurytrema sp. is a frequent incidental finding at necropsy or in slaughterhouses in Brazil. (5) The frequency of *Eurytrema* sp. in slaughtered cattle in some areas of Brazil may reach 70%. The official Brazilian meat inspection service has reported more than 400,000 rejected pancreases out of more than 55 million cattle slaughtered over a 10-year period

In spite of being an incidental finding at necropsy, *Eurytrema* sp. often causes a chronic pancreatitis in ruminants that may be clinically associated with a wasting condition despite good nutritional supply (2,6), as reported in the present case.

AFIP Diagnoses: 1. Pancreas: Ductal ectasia, multifocal, marked, with intralumenal adult trematodes, periductal fibrosis, acinar atrophy and granulomatous pancreatitis centered on trematode eggs, Nelore, bovine.
2. Lymph node, sinuses: Trematode eggs, multifocal, few.

Conference Comment: The contributor provides an excellent overview of *Eurytrema* sp. infection in cattle**.** There is some variability among slides with respect to the sections of the parasites and the degree of granulomatous inflammation surrounding trematode eggs. Attendees discussed the parasite's morphology and how it was identified as a trematode. Below is a simple algorithm (adapted from ref. 1) to aid in

classification of metazoan parasites in tissue section.



This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP. In North America, *Eurytrema procyonis* can be found within the pancreatic duct of small carnivores, including raccoons, cats, and foxes. Clinical signs and histologic lesions are similar to those seen in ruminants infected with *E. pancreaticum*.

Readers are encouraged to review case 4, conference 29 from the 1995-1996 Wednesday Slide Conference for another case of *Eurytrema* sp. in the pancreas of an 18-month-old Holstein ox. http://www.afip.org/vetpath/WSC/WSC95/95wsc29.htm

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SLIDE 60
CONFERENCE 15 / CASE IV – 04 H 6608 (AFIP 2984847)

Signalment: 7.8 year old, female, German Shepherd, *Canis familiaris*, dog

History: One year and 8 months previously this dog had presented with limping and a painful bony mass on the right distal tibia. The only hematologic abnormality was a very mild hypercalcemia. Thoracic radiographs were within normal limits. At the time of first presentation, a fine needle aspirate (FNA) and biopsy specimen of the right tibia was done and revealed macrophages and branching septate fungal hyphae with parallel sides suspected to be *Aspergillus sp*. Similar findings were obtained from a FNA of a prescapular lymph node consistent with systemic mycotic disease.

Culture of the bone yielded rapid growth of an unidentified fungus that was submitted to the University of Texas Health Science Center Fungus Testing Laboratory (San Antonio, TX). Growth consistent with a basidiomycete was reported along with a susceptibility to itraconazole. Antifungal therapy with itraconazole (Sporanox®) resulted in some decrease in pain and limping after two months of treatment. Slow progression of the swelling of the right tibia over the next 18 months in spite of intermittent treatment occurred with progressively worsening ataxia. Computer tomography of the brain revealed lesions in the thalamic region and enlarged lateral and third ventricles suggesting disseminated disease. Because of worsening CNS signs, euthanasia was requested and necropsy performed.

Gross Pathology: Lungs were heavy, dark red, rubbery, and failed to collapse. In the heart the mitral valve had rounded, nodular, to roughened margins. The endocardium of the pulmonary outflow track was roughened, granular, and pale in a small patch (granulomatous endocarditis and myocarditis). The liver was slightly enlarged and pale (steroid hepatopathy). The spleen had siderocalcific plaques. Bone changes within left and right tibias consisted of irregular thickening of the periosteum and cortices with multiple irregular pale nodules within the marrow (granulomatous osteomyelitis). In the brain, the left to mid-thalamic area had a 2 cm dark tan circular lesion that pressed dorsally into the left ventricle (malacia and encephalitis).

Laboratory Results: Previous culture of the bone revealed a basidiomycete (University of Texas Health Science Center Fungus Testing Laboratory (San Antonio, TX)). Further characterization of the culture was done with genetic testing revealing a 94% correspondence with the basidiomycete fungus *Oxyporus populinus*, a decay shelf mushroom typically found on damaged maple trees.

Contributor's Morphologic Diagnoses: Bone: osteomyelitis, granulomatous and necrotizing, chronic, multifocal, severe, with intralesional fungal hyphae consistent with basidiomycosis
Artery: arteritis, granulomatous, chronic, multifocal, with intralesional fungal hyphae consistent with basidiomycosis

Contributor's Comment: This animal had been diagnosed with basidiomycosis over one year previously and treated with itraconazole but without resolution and with slow progression. Basidiomycetes are fungi that include puffballs, shelf fungi, rusts, smuts, and mushrooms. The hyphae detected at necropsy were assumed to be the same organism as that originally isolated. After euthanasia, histopathology revealed dissemination of the infection to many organ systems including heart, blood vessel walls, thyroids, adrenals, and kidneys, as well as bone. No organisms were detected in liver, lung, and spleen. Basidiomycete hyphae in this case appeared to have an affinity for growth within endocardium, myocardium, arterial walls, arterial adventitia, and endocrine organs (adrenals, thyroids) with relatively scant inflammation, possibly due to the recent steroid therapy. Inflammation was most abundant in bone and periosteal vessels. The brain lesion was an area of malacia, likely due to ischemia from blood vessel damage, but no hyphae or macrophages were found within brain.

The hyphae in this case are generally uniform in size bearing more or less parallel walls and frequent septa and occasionally perpendicular or dichotomous branching. In some areas the hyphae are slightly bulbous with undulating walls between septa. No clamp connections were noted on the hyphae by histopathology.

Basidiomycosis due to *Schizophyllum commune* has recently been reported in a dog from Japan, apparently the first report of its kind in a dog.(1) Basidiomycetous fungi, especially *Schizophyllum commune*, is reported to be more and more frequently isolated from humans with pulmonary and sinus disease as well as occasional systemic disease with brain involvement.(2,3) Infections in humans have also been reported due to a basidiomycete *Coprinus cinereus*, notably as endocarditis.(3) The infections tend to be associated with immunosuppression; in human cases this is thought to be due in part to HIV infections.(2)

Disease due to basidiomycetes may be under-diagnosed due to misdiagnosis as *Aspergillus sp*., which the hyphae in sections closely resemble. Identification of cultured specimens as basidiomycetes can be done based on tests such as the benomyl test and morphologic characteristics. *Schizophyllum*, for example, has characteristic clamp connections on hyphae in culture. These characteristic structures, however, may or

may not be present or detectable on hyphae within tissue sections, adding to the difficulty with diagnosis. (2)

Molecular analysis of DNA can be done to more specifically identify isolates. The present case, for example, has 94% correspondence to *Oxyporus populinus*, a shelf mushroom found on damaged maple trees. Further testing of the isolate is being done, and if the tentative species is verified, this would be the first reported case of systemic disease due to this organism in any species.

AFIP Diagnosis: Bone, distal right tibia (per contributor): Osteomyelitis, granulomatous and necrotizing, multifocal to coalescing, severe, with myriad fungal hyphae, German Shepherd Dog, canine.

Conference Comment: The contributor provides an interesting case of fungal osteomyelitis. There is some variability among slides, with some sections of bone marrow having higher numbers of neutrophils and necrotic debris. Attendees discussed the presence of fractured and scalloped trabeculae with empty osteocyte lacunae forming sequestra of necrotic bone. Attendees agreed that *Aspergillus* sp. is the top differential diagnosis based on both fungal morphology and frequency of occurrence. This case serves as a valuable example of why additional diagnostic tests, such as culture, are important in accurately identifying both fungal and bacterial microorganisms.

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SLIDE 61
CONFERENCE 16 / CASE I – 05-0027 (AFIP 2992349)

Signalment: 11-year-old, female common trumpeter (*Psophia crepitans*)

History: Possible exposure to snow and cold 01/26/05, with suspected frostbite. Treated symptomatically since 01/27/05. Right leg appears to have dry necrosis up to proximal metatarsus. Left leg appears to have necrosis of all toes to metatarsal-

phalangeal joint, which is swollen. Due to poor prognosis, euthanasia performed on February 16.

Gross Pathology: There are very mild, scabbed abrasions bilaterally on the medial carpi. All four toes of the left leg are dry, hard, and interphalangeal joints are not movable. The left metatarsal-phalangeal joint is distended twice that of normal and the dorsal and lateral surfaces have several abrasions with crusted blood and erythema. The cranial half of the plantar surface is hard. The joints of the second and fourth digits move slightly, but those of the first and third do not articulate. All four toes of the right leg are dry, hard, and all interphalangeal and the metatarsal-phalangeal joints are not movable. The diameter of the distal _ of the tarsometatarsus is smaller than that of the proximal 5 cm and that of the left leg. The distal portion is dry and hard. The transition between the hard tissue and the normal proximal tissue is an irregular 0.5-1.0 cm band of swollen, whitish tissue.

Laboratory Results: Blood taken at clinical presentation showed an increased CPK (4930 U/L; ref range 793-1008 U/L). Subsequent bloodwork showed an increasing leukocytosis with a heterophilia, lymphopenia, and monocytosis, with decreasing hematocrit.

Histopathologic Description: Focally extensive bone necrosis. Large granuloma with necrotic debris and bacteria at center, surrounded by multinucleated giant cells and macrophages with several fungal hyphae. Large area of infiltrate of lymphocytes, fewer heterophils. Multiple multinucleated giant cells dissecting under the necrotic layer of skin. Muscle atrophy, multiple ossified tendons. Viable skin with edema present below the necrotic epidermis in areas of slide.

Contributor's Morphologic Diagnosis: Leg (bone, skin, tendon): Necrosis, focally extensive, with secondary surface bacterial and yeast infection, common trumpeter (*Psophia crepitans*), gruiform.

Contributor's Comment: Cold injury is becoming increasingly more prevalent with the expanding epidemic of homelessness and the increased number of people involved in outdoor winter sports. Historically, military personnel were the people most susceptible to conditions promoting cold injury to the extremities. One manifestation of cold injury, trench foot, was seen commonly in the wars of the 20th century. This condition is caused by immersion of the feet in cold water at temperatures between 33 and 50 degrees Fahrenheit for longer than 12 hours (2). Another manifestation, frostbite, occurs when the tissues are exposed to temperatures below freezing. Ninety percent of human frostbite cases occur in the hands and feet and the pathophysiology of these changes is similar to that of thermal burns and ischemia/reperfusion injury (1).

Clinically, frostbite can be classified as superficial or deep (1,3). Only the skin and subcutaneous tissues are involved in superficial frostbite, whereas deep frostbite additionally involves the muscles, tendons and bones. The pathogenesis of frostbite involves three mechanisms that may take place simultaneously (1,3). One mechanism

involves direct cellular damage by freezing. Ice crystals form in the tissues extracellularly which damage the cell membrane. This causes fluid to leave the cells resulting in cellular dehydration. As freezing continues, ice crystals form within the cells, expanding and causing the cells to burst.

Another mechanism involves the vascular response to cold. When body temperature begins to fall, vessels go through cycles of vasoconstriction and vasodilation until the temperature becomes so low that vasoconstriction becomes constant. This causes local hypoxia, acidosis and increased blood viscosity. This eventually leads to thrombosis of the vessels and ischemic injury to the tissues.

Local thrombosis and endothelial damage trigger the release of prostaglandins PGF2alpha and thromboxane A2. These potent inflammatory mediators precipitate further vasoconstriction and thrombosis. During rewarming, the tissue levels of these mediators increases. Subsequently, repeated freezing and thawing of tissues causes progressively more damage.

The result of these physiologic mechanisms of damage does not fully manifest until 22 to 45 days after the initial injury and can be seen as a distinct line of demarcation between hard, gangrenous tissue and viable tissue (3). The clinical course of frostbite in this common trumpeter took place over 21 days. Manifestation of visibly damaged tissue had progressively moved up the leg during this time. Additionally, histology showed that tissue that appeared to be viable was, in fact, affected and undergoing degenerative and necrotic changes.

AFIP Diagnoses: 1. Bone, skin and tendon, distal leg: Necrosis, coagulative, diffuse, common trumpeter (*Psophia crepitans*), avian.
2. Bone, proximal leg: Periosteal and endosteal new bone growth, focally extensive.
3. Skin, proximal leg: Epidermal hyperplasia and hyperkeratosis, diffuse, marked with focally extensive ulcer and superficial fungi.
4. Leg, proximal: Synovitis and cellulitis, heterophilic and histiocytic, multifocal, moderate.

Conference Comment: Attendees discussed the presence of necrotic bone and the lack of viable osteocytes within lacunae. There is periosteal and endosteal new bone growth within some sections of the less severely affected proximal bone. The presence of fungal hyphae within sections of the necrotic epidermis and dermis (best seen with GMS stain) was attributed to opportunistic invasion and growth following tissue death.

The contributor provides an excellent review of the pathogenesis of frostbite injury. Interestingly, birds with webbed feet have a vascular network, the rete mirabile, which transfers heat from the arteries in their legs and feet to the veins in an attempt to help prevent heat loss in the distal limbs and maintain the temperature of the feet closer to the ambient temperature.

As mentioned by the contributor, prostaglandins (PGF2alpha) and thromboxanes (TXA2) are responsible for the cycle of vasodilation and vasoconstriction that occurs in blood vessels undergoing cold injury. More specifically, PGF2alpha and TXA2 are arachadonic acid metabolites produced by the cyclooxgenase pathway. PGF2alpha is responsible for vasodilation and is an important potentiator of edema. TXA2 is a potent platelet aggregating agent and causes vasoconstriction (4).

Contributor: Smithsonian's National Zoological Park
http://nationalzoo.si.edu/default.cfm?ref=index.htm

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SLIDE 62
CONFERENCE 16 / CASE II – 04-0309 (AFIP 2992355)

Signalment: Northern water snake *(Nerodia sipedon)*, adult, female

History: It was in the exhibit with three cagemates. Apparent regurgitation observed one week previously.

Gross Pathology: The body of this northern water snake is in fair to poor nutritional condition as evidenced by scant amount of fat storages. There is a generalized marked pallor of all organs, oral cavity and muscle. The lungs are markedly pale and there are five, up to 6.0 x 0.3 x 0.3 cm, white, rounded parasites attached to the mucosa (Fig. 1). There are many, up to 6 x 1 x 1 mm, dark, C-shaped parasites with a thickened white wall in multifocal hepatic serosal areas surrounded by mild hemorrhage (Fig. 2). There is the same type of parasites in the renal parenchyma, in fewer numbers and with hemorrhagic areas up to 10 mm in diameter (Fig. 3). There is digested fish in the stomach. There is yellowish, soft digesta in the intestinal tract. The gallbladder is filled with green bile. The splenopancreas is diffusely pale. The thyroid is enlarged (12 x 8 x 3 mm). The brain is fixed with the head. The heart, bones, trachea, ovaries, adrenal glands, eyes, skin and ears are unremarkable.

Laboratory Results: Heart blood culture: Salmonella IV 45:g251
Parasite identification: *Kiricephalus coarctatus*

Histopathologic Description: Liver: Multifocally, there are numerous up to 12 mm in diameter cross section and oblique sections of pentastomid parasites that are expanding the hepatic capsule and parenchyma, and are surrounded by minimal inflammatory reaction composed of eosinophils, heterophils, lymphocytes, macrophages and hemorrhage. There is multifocal degeneration and necrosis with numerous macrophages and few eosinophils, heterophils and foreign body type multinucleated giant cells. Diffusely, there is mild fatty change.

Contributor's Morphologic Diagnosis: Liver, hepatitis, eosinophilic, subacute, mild, multifocal, with many pentastome nymphs, macrophages, lymphocytes, heterophils and diffuse lipid degeneration.

Contributor's Comment: This animal has pentastomes in several organs and probably was infected by ingestion of an intermediate host, such as a mouse or rat. Parasites are present within the lung, as a primary site (Johnson-Delaney, 1996) as evidenced in Fig. 4; and in the oral cavity (Fig. 5) due to the life cycle, where eggs are coughed up by the host, swallowed and passed in the feces (Johnson-Delaney, 1996). The parasites are found in the kidney, causing mild inflammation and edema (Fig 6), within the serosa of the oviduct causing hemorrhage (Fig. 7) and within the biliary duct with minimal inflammation.

The species is identified as *Kirecephalus coarctatus,* and this genus is the most common found in colubrids, such as this snake (Johnson-Delaney, 1996). The sepsis with Salmonella IV 45:g251 is probably a secondary bacterial infection due to the many organs affected by these parasites (Murray, 1996).

The infestation of this snake was probably caused by ingestion of the intermediate host, a mouse or rat. Crocodiles and lizards can be infected by ingestion of fish (Flach et al, 2000; Adams et al., 2001). Pentastomes have zoonotic potential as humans are incidental hosts.

AFIP Diagnoses: 1. Liver: Pentastome nymphs, multiple, Northern water snake (*Nerodia sipedon*), reptile.
2. Liver: Granulomas, multiple.

Conference Comment: The contributor provides a beautiful example of pentastome nymphs in the hepatic parenchyma of a snake. Attendees noted that the granulomas were scattered throughout the hepatic parenchyma but were not exclusively focused on the nymphs. Consideration was given to the possibility that the granulomas were preexisting in this snake from an unrelated inflammatory lesion.

Pentastomes, also known as "tongueworms", are a phylum of highly specialized arthropod parasites. Most pentastomes parasitize reptiles but a few genera occur in birds and mammals. The typical lifecycle of pentastomes starts with the adults in the

respiratory tract of the reptile host where they mate and the female lays her eggs. The eggs are coughed up by the reptile, swallowed, move through the digestive tract and are expelled in the feces. In the environment, the eggs develop into larvae, are ingested by the intermediate host (usually a bird or rodent) and develop into nymphs. The intermediate host is ingested by a reptile and the nymphs are released, burrow through the intestine and migrate to the lungs where they develop into adults. The larval stage of the parasite is capable of parasitizing a wide range of hosts (5,6,7).

Morphologic features which help identify pentastomes in tissue section include two pairs of hooks surrounding the mouth, striated musculature, a thin cuticle, and a multicellular intestine bordered by two acidophilic glands for most of the pentastome's length. The most characteristic and unique feature of pentastomes are the sclerotized openings in the body wall. The openings appear as eosinophilic rings when stained with H&E; however, they are black and easily visible when stained with Movat pentachrome stain (5).

A short list of pentastomes found in reptiles follows (7):

- *Armillifer* sp. in pythons and vipers
- *Porocephalus* sp. in boas and rattlesnakes
- *Kiricephalus* sp. in colubrid snakes
- *Sebekia* sp. in crocodilians
- *Raillietiella* sp. in lizards and snakes

Additionally, *Linguatula serrata* occurs in the nasal and paranasal sinuses of dogs and cats, where it causes bleeding, catarrhal inflammation, and some impediment to respiration (6).

For a simple algorithm to help identify parasites in tissue sections, readers are encouraged to review WSC 15, case 3, 2005-2006.

This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP.

Contributor: Smithsonian's National Zoological Park
http://nationalzoo.si.edu/default.cfm?ref=index.htm

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SLIDE 63
CONFERENCE 16 / CASE III – 6611/0411 (AFIP 2983550)

Signalment: Snowy owl, adult, unknown sex (*Nyctea scandiaca*).

History: There was an increase in mortality of captive owls in a zoological garden. Different species of the family Strigidae were affected. Formalin fixed organs (liver, spleen, kidney, lungs) of a snowy owl (*Nyctea scandiaca*) and the carcass of a long-eared owl were submitted for pathological examination.

Gross Pathology: The long-eared owl was in a moderate nutritional condition. The liver was green-brown, discoloured and contained multiple miliary yellowish-white foci. Similar lesions, but more confluent, were found in the spleen. The content of the intestine was fluid and of a dark reddish-brown colour. The lungs were congested and within the kidneys there was a moderate deposition of white, dry, crystalline masses.

Laboratory Results: Virology: Herpesvirus was detected in cell culture.
Microbiology: Unspecific.

Histopathologic Description: In the liver, there are multiple randomly distributed foci of coagulation necrosis. Hepatocytes near the foci show degeneration with pyknotic nuclei and swollen, homogenous, eosinophilic cytoplasm. Also in these areas few amphophilic intranuclear inclusion bodies (Cowdry type A) can be seen in hepatocytes. Only very few inflammatory cells (lymphocytes) are present. Vessels and sinuses are moderately congested.
Additionally, the spleen shows similar lesions but more severe with confluent foci of necrosis and intranuclear inclusion bodies in reticular cells.

Contributor's Morphologic Diagnoses: Liver, hepatitis, necrotizing, acute, multifocal and randomly distributed, moderate with intranuclear pale amphophilic inclusion bodies in hepatocytes, snowy owl (*Nyctea scandiaca*), avian, etiology consistent with herpesvirus strigis infection.
Spleen (not submitted): Splenitis, necrotizing, acute, multifocal to coalescing, severe with intranuclear amphophilic inclusion bodies in reticular cells.

Contributor's Comment: The histopathological features are consistent with Hepatosplenitis infectiosa strigum, an infection with the herpesvirus strigis which was confirmed by cell culture.

The current taxonomy of the family of avian herpesviruses includes three subfamilies: alpha-, beta- and gammaherpesvirinae. Herpesvirus strigis is a betaherpesvirus. All avian herpesviruses are presently grouped into 11 different serotypes based on results of cross neutralisation tests. Herpesvirus strigis and herpesviruses from falcons and pigeons are serologically closely related (5).
Herpesvirus strigis is a pathogen for several species of owls in the order Strigiformes. Natural infections have been observed in the Eagle Owl (*Bubo bubo*), Long-eared Owl (*Asio otus*), Great horned owl (*Bubo virginianus*) and the Snowy owl (*Nyctea scandiaca*). Experimental susceptibility was established in the Little Owl (*Athene noctua*) and the Tengelmalms Owl (*Aegolinus funereus*). Also the Old World Kestrel (*Falco tinnunculus L.*) was found susceptible to the virus. On the other hand the Tawny Owl (*Strix Aluco*) and the Barn Owl (*Tyto albo Scopoli*) seem to be resistant (2).

Owls affected by Hepatosplenitis infectiosa strigum show nonspecific signs of illness like apathy, lassitude and anorexia. Usually the birds die after two to five days of illness. In captivity mortality approaches 100 per cent and any birds surviving infection should be considered to be latently infected and likely to shed virus. Another supposed source of infection is pigeons which are fed to owls in captivity (1, 3, 4).

Gross pathological findings are numerous necrotic foci in the liver, spleen and bone marrow. Additionally diphtheroid lesions can be found in the mucosa of beak, oesophagus, proventriculus, and intestine. Histologically, these organs show necrotic lesions with intranuclear inclusion bodies of Cowdry Type A, particularly in hepatocytes and with lower frequency in reticular cells of the spleen (1, 4).

AFIP Diagnosis: Liver: Hepatitis, necrotizing, acute, random, moderate, with eosinophilic hepatocellular intranuclear inclusions, Snowy owl (*Nyctea scandiaca*), avian.

Conference Comment: The contributor provides an excellent review of herpesvirus infections in owls. In general, the alphaherpesviruses typically produce focal lesions in the skin and mucosa of the respiratory or genital tracts. An important characteristic of herpes virus infections is their ability to become latent with intermittent or persistent recrudescence and viral shedding. Alphaherpesviruses are typically latent in nerves; betaherpesviruses in secretory glands, lymphoreticular organs and the kidney; and gammaherpes in lymphoid tissue. Below is a list of avian herpesviruses and the diseases they cause:

- Avian alphaherpesviruses:
  - Avian HV1: Infectious laryngotracheitis
  - Avian HV2 (Gallid herpesvirus-2): Marek's disease

    - Psitticid HV1
        - Parrot herpesvirus
        - Pacheco's disease virus in psittacines
- Unclassified avian herpesviruses
    - Acciptrid HV1 (Bald eagle herpesvirus)
    - Anatid HV1 (Duck plague herpesvirus, duck viral enteritis)
    - Ciconiid HV1 (Black stork herpesvirus)
    - Columbid HV1 (pigeon herpesvirus)
    - Falconid HV1 (Falcon inclusion body disease)
    - Gruid HV1 (cranes)
    - Strigid HV1 (owl hepatosplenitis herpesvirus)
    - Phalacrocoraid HV1 (cormorant herpesvirus)
    - Perdicid HV 1 (bobwhite quail herpesvirus)
    - Budgerigar herpesvirus - reduced egg hatchability, no clinical disease in adults
    - Proliferative cutaneous pedal lesions in cockatoos and macaws also thought to be related to an unclassified herpesvirus

Additionally, the following viruses are known to cause hepatocellular intranuclear inclusion bodies in birds:

- Herpesvirus in parrots, pigeons, owls, hawks and ducks
- Adenovirus in chickens, goslings and bobwhite quail
- Parvovirus in geese
- Papovavirus in budgerigars (Budgerigar fledgling disease)

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SLIDE 64
CONFERENCE 16 / CASE IV – 47473 (AFIP 2994636)

Signalment: East African Bongo (*Eurycerus isaaci*), Third trimester fetus, male

History: A 3-year-old, primiparous East African bongo had dystocia and delivered, with assistance, a stillborn male, third trimester fetus on 24 February 2005. The dystocia was due to a left shoulder lock. The dam recovered uneventfully, and the placenta and fetus were submitted for necropsy.

Gross Pathology: Examined were the placenta and a third trimester, male, East African bongo fetus.

The placenta weighed 3,770 g (normal range 1,300 – 1,600 g) and had the normal number and arrangement of cotyledons for this species, which is between 125-150 cotyledons arranged in four rows. The cotyledons ranged in sized from 3.2 x 3.0 x 0.2 cm to 15.0 x 6.0 x 0.2 cm. The placenta had several abnormalities. The most striking lesion was four partially demarcated, variably sized (up to 975 g), irregular, smooth, fleshy, lobulated, red to purple masses that expanded and were covered by the intercotyledonary chorioallantoic membrane, which contained numerous small blood vessels (Figs. 1 and 2). Approximately 50% of the total cotyledons had diffusely tan maternal surfaces. The tissue at the chorioallantoic interface was thickened, firm, and translucent to opaque white. The intercotyledonary areas also were translucent to opaque with multifocal, thick, opaque, yellow to white foci.

The fetus (21.5 Kg BW wet, 79 cm crown to rump length) was wet, covered in dirt, had intact fetal hooves, and appeared flaccid and large. Remarkable findings besides the non-aerated lungs were limited to several connective tissue abnormalities. The scapular and humeral regions of the thoracic limbs had marked asymmetrical muscling, with the left limb larger than the right. Concurrent skeletal involvement was excluded by radiographic examination. The sternum was concave, and the ribs curved inward (pectus excavatum). The manubrium at the left thoracic inlet had a 5.0 x 4.0 x 3.0 cm, firm, whitish grey to tan mass that was contiguous with the costo-sternal junction and had a similar consistency to cartilage. The trachea was flattened laterally throughout the entire length. A blind ended, 49.0 x 29.0 x 1.0 cm, smooth, tan, membranous mass filled one third of the abdominal cavity and was contiguous with the serosa of the forestomach (malformed omentum).

Histopathologic Description: The chorioallantois of the intercotyledonary placenta was expanded by multiple, poorly demarcated, variably sized, mesenchymal masses that were pleomorphic in cellular and stromal composition, of which the different morphologies were discrete or more often blended indiscernibly (Fig. 3) (this pleomorphism may be reflected by slight variations between slides). The more discrete regions had either moderate cellularity with scant vascularity (arbitrarily referred to as area #1) (Fig. 4) or had scant cellularity with pronounced vascularity (arbitrarily referred to as area #2) (Fig. 5). Area #1 was comprised of a pleomorphic population of spindle to stellate to oval shaped cells arranged in intersecting fascicles and bundles supported by minimal intervening, pale eosinophilic, fine fibrillar stroma. Cells sometimes assumed a storiform pattern or were contiguous with or whorled around vascular clefts, which variably contained erythrocytes. Most cells had distinct cell borders, tapering or blunt ends, a small to moderate amount of intensely eosinophilic, granular to amorphous, sometimes lightly vacuolated cytoplasm and a peripherally placed, thin to

plump, hyperchromatic to open-face oval nucleus. Some cells had cytoplasmic cross striations, multiple nuclei, and central nuclear rowing. Area #2 was comprised of scattered oval to spindle shaped cells supported by a large amount of pale eosinophilic, fine fibrillar stroma. Cells sometimes were contiguous with and whorled around variably sized, usually blood-filled and often distended blood vessels, which infrequently contained unorganized fibrin thrombi. Cells had indistinct cell borders, a small amount of pale eosinophilic cytoplasm, and an oval to polygonal, basophilic nucleus with variably distinct fine chromatin stippling. Mitotic figures were rare. There were small, scattered foci of hemorrhage, and the stroma multifocally was expanded by edema.

In the chorionic villi and membrane of multiple cotyledonary and intercotyledonary regions, the stroma was replaced by sparsely cellular, pale eosinophilic connective tissue, and the surface was lined by low cuboidal epithelium. Some of these and other villi contained basophilic, granular material (mineralization) that partially to completely disrupted the villous stroma and epithelium. Some villi contained small numbers of scattered, non-degenerate neutrophils.

IHC: Pancytokeratin (AE1/AE3), CK 8/18 (UCD10.11), CK 7/8 (CAM 5.2), Mixed LMW>HMW CK (MAK 6), desmin, muscle-specific actin (HHF35), myoglobin, smooth muscle actin, CD 31 (PECAM), Von Willebrand factor VIII

Special stains: phosphotungstic acid-hematoxylin, Masson's trichrome

Contributor's Morphologic Diagnoses:
1. Placenta: developmental anomaly, probable stem cell tumor, with mild, multifocal fibrin thrombi
2. Placenta: moderate, multifocal, fibrosis and mild mineralization
3. Placenta: mild, multifocal, acute placentitis

Ancillary Findings in Fetus:

1. Thoracic limbs (humoral and scapular regions): skeletal muscle asymmetry (probable unilateral hypertrophy)
2. Sternum: pectus excavatum and focal cartilaginous and adipocyte proliferation
3. Omentum: diffuse malformation with adipocyte proliferation
4. Forestomachs: moderate, multifocal, mural adipocyte infiltration
5. Trachea: diffuse, lateral flattening

Contributor's Comment: On gross examination, the placental masses resembled chorangiomas, as described in humans.[1,2] Chorangiomas (chorioangiomas) are ill-defined, benign placental tumors that have been reported in humans and animals and are hypothesized to be of fetal blood vessel origin or a hamartoma of primitive chorionic mesenchyme. Chorangiomas can occur in the placenta, usually towards the fetal side, or the umbilical cord and can present as one to multiple, usually well-circumscribed, thinly encapsulated, fleshy, red masses that sometimes have tan foci indicative of infarction. Chorangiomas can have multiple histological appearances, depending on the contribution of vascular and stromal components and the degree of vascular

engorgement, and sometimes assume a malignant appearance, but metastasis has not been reported. The appearance of the stromal components is not well described, but chorangiomas have been referred to as hemangioblastoma, fibroangiomyxoma, fibroma, myxoma, and pericytoma. In veterinary medicine, placental masses of similar description only have been documented in five bovine placentas, of which one case had concurrent fetal cutaneous and lingual hemangiomas.[3-5]

The gross similarity to chorangioma of the masses in this case, was not fully supported by findings on histology, special stains, and immunohistochemistry or by comparison of histology from this case with that from 21 human cases of chorangioma and with limited histological descriptions in the literature. Namely, the tumor in this case contained undifferentiated mesenchymal cells, endothelial cells, pericytes, smooth muscle cells, and skeletal muscle cells, of which the latter have not been described previously in chorangioma. An alternative diagnosis of hamartoma is not considered appropriate, because it implies the abnormal proliferation of tissue element(s) that normally are present at that site, which, in this case, would not include skeletal muscle cells. Neither is teratoma an appropriate term, because it implies the presence of cells from at least two, usually three different embryonic layers, and in this case, all cells were of mesoderm origin. Thus, combining information of placental tumor morphology, immunophenotype, and embryogenesis (as described below), the placental developmental anomaly in this East African bongo is proposed to be a placental stem cell tumor. This proposal would suggest that there is an alteration in the local microenvironment or a dysregulation of autocrine- or paracrine-mediated proliferation and differentiation of dormant fetal multipotent cells into cells of endothelial, pericyte, smooth muscle, and skeletal muscle type. This leads to the question of whether this stem cell tumor represents a variant of chorangioma and therefore, whether chorangioma and chorangioma-like tumors as described in human and veterinary medicine actually are stem cell tumors of which the final cell type is dictated by the local microenvironment. It also raises the question of whether the presence of multiple musculoskeletal and omental anomalies noted in this East African bongo fetus represent additional regional anomalies in morphogenesis or have an unrelated pathogenesis. The additional histological findings of placental fibrosis and mineralization were non-specific alterations and are not uncommon findings in ruminant placentas in the experience of this institution. The inflammation was attributed to the prolonged parturition and considered insignificant.

Ancillary diagnostics included histochemical stains and immunohistochemistry (IHC). Phosphotungstic acid-hematoxylin (PTAH) confirmed the presence of cross-striations in some cells having morphologic features suggestive of skeletal muscle (Fig. 6). Masson's trichrome stain was consistent with IHC results as discussed subsequently.

The IHC panel included markers for epithelium, specifically pancytokeratin (AE1/AE3) and low molecular weight keratins (CAM 5.2, MAK 6, UCD10.11), mesenchyme (vimentin), endothelium (CD31-PECAM and Von Willebrand factor (VWF) VIII), and muscle (desmin, muscle-specific actin-HH35, myoglobin, α-smooth muscle actin, and skeletal myosin). For each antibody, the external and internal controls were

appropriately immunoreactive, except in the case of myoglobin, and were weakly immunoreactive in the case of UCD10.11. Internal controls were of normal tissue from this fetus, including non-affected placenta, liver, skeletal muscle, lymph node, peripheral nerve, and adipose tissue. To facilitate interpretation of IHC, cells within the mass were divided into three main regions: blood vessel endothelium, blood vessel wall and pericytes, and interstitial cells. Table 1 provides a summary of results for antibodies immunoreactive with cells in the mass. Overall, immunophenotypic characterization showed the masses had endothelial, smooth muscle, skeletal muscle, undifferentiated muscle, and fibrous components. Additionally, it showed that area #1 often contained less mature cells than area #2 and contained skeletal muscle cells, unlike area #2.

During embryogenesis, embryoblastic-derived mesenchyme and vessels contribute to the formation of the placenta.[6] Later in placental development, hemangioblastic progenitor cells differentiate from local fetal mesenchyme and develop into fetal capillaries and hematopoietic stem cells and subsequently are involved in villous differentiation and placental maturation. Fetal mesenchyme is derived from progenitor cells in the somites, which are condensations of mesoderm that later become compartmentalized into the dermatomyotome and sclerotome. Cells from the dermatomyotome can remain undifferentiated or can become endothelium (also from the sclerotome), epidermis, muscle (skeletal, cardiac or smooth muscle), muscle satellite cells, adipose, and cartilage.[7] Determination and differentiation of the progenitor cells occur through a complex cascade of events that involves a network of signaling proteins and transcriptional factors to mediate the sequential transcription of distinct genes during different stages of development. The regulatory factors have a paracrine or autocrine affect, so that cell determination and differentiation is determined by the signals it receives from neighboring cells.

Recent studies suggest that at least some satellite cells of skeletal muscle are not derived from somite cells but from the endothelium and/or pericytes of embryonic vessels, such as the dorsal aorta.[7] Cells competent to generate satellite cells can express both myogenic markers, including desmin, and endothelial markers, including CD31 (PECAM) and α-smooth muscle actin, but not VWF VIII. They can differentiate, depending on the local regulatory factors, into skeletal muscle during embryonic and postnatal growth and regeneration, into vascular components, or perhaps into other mesenchyme. Cells competent to generate satellite cells migrate to their destination, likely through the circulation, early in development. These cells, however, do not acquire the competence to differentiate until later in development and upon maintenance of interaction with neighboring cells. These progenitors may remain demonstrable only in the bone marrow; therefore, they may be related to or representative of the multipotent mesenchymal cells that have been identified in the bone marrow and are capable of producing chondroblasts, adipocytes, skeletal muscle, osteoblasts, and possibly endothelial cells.[7,8] Thus, it is possible that the placental masses and perhaps fetal anomalies in this bongo are due to alterations in the local regulatory factors that control determination and differentiation of fetal multipotent cells.

Antibody	Area #1: Cellular, Scant Vascularity			Area #2: Vascular, Cell Poor		
	Vessel Endothelium	Vessel Wall and Pericytes	Interstitial Cells	Vessel Endothelium	Vessel Wall and Pericytes	Interstitial Cells
Vimentin	All	All	Most	All	All	All
CD 31	All	Negative	Negative	Most	Negative	Negative
VWF VIII	Most	Negative	Negative	All	Negative	Negative
Desmin	Negative	Few	Few-Half	Negative	All	Rare-Few
MSA	Negative	All	Most	Negative	All	Rare
SMA	Negative	All	Half	Negative	All	Half-Most
Skeletal Myosin	Negative	Negative	Rare	Negative	Negative	Negative

Table 1: Comparison of the presence and relative number of immunoreactive cells of the blood vessel endothelium and wall (including pericytes) and the interstitium of areas #1 and #2. The terms refer to the subjective assessment of the number of immunoreactive cells in that region of tissue. MSA: muscle specific actin; SMA: α-smooth muscle actin.

AFIP Diagnoses: 1. Placenta (per contributor): Atypical mesenchymal proliferation with striated muscle differentiation, East African bongo (*Eurycerus isaaci*), ruminant. 2. Chorioallantois: Fibrosis, multifocal, mild, with mineralization, and focal villar coagulative necrosis.

Conference Comment: The contributor provides a very interesting case, gross photographs, diagnostic criteria and a detailed write-up on the differentials considered. Attendees were surprised to find skeletal muscle cells ("strap cells") in the section of placenta and debated their origin. Most agreed that they may have come from aberrant fetal mesoderm that localized to the placenta.

Attendees discussed embryonal rhabdomyosarcomas as another neoplasm in which skeletal muscle cells are found. Embryonal rhabdomyosarcomas can arise in a variety of sites which do not normally contain skeletal muscle. Two types can be identified histologically: the round cell type and those composed predominantly of primitive myotubes. Although rare, the identification of strap-like cells with cross striations (best recognized with PTAH stain) supports rhabdomyocyte differentiation (9). Additionally, immunohistochemistry for desmin or myoglobin and other muscle markers can also be used to establish muscle differentiation of the tumor (10).

The origin of all fetal organs can be traced back to the primary germ layers which originate from the inner cell mass of the developing embryo. Below is a simple chart that identifies the three layers of the inner cell mass and the tissues derived from each germ layer (11):

Inner cell mass:

Ectoderm
- Epidermis, hair, hooves
- Nervous system

Mesoderm
- Somites, muscle tissues (smooth, striated, cardiac)
- Circulatory organs, heart, blood and lymph vessels
- Connective tissue (bone, cartilage, ligaments and tendons)

Endoderm
- Glands, liver
- Inner lining of digestive tract

Contributor: Zoological Society of San Diego, Department of Pathology
http://cres.sandiegozoo.org/staff/div_pathology.html

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SLIDE 65
CONFERENCE 17 / CASE I – AFIP 2005-#2 (AFIP 2985000)

Signalment: An approximately one-year-old, fighting rooster, *Gallus domesticus*

History: This animal was part of a confiscation of 14 adult chickens and 20 chicks from an illegal fighting rooster premise. Adults ranged in age from 1-3 years, up to 4 years of age. This animal was in poor body condition with multiple lacerations. Euthanasia was performed.

Gross Pathology: The bird was in poor body condition with a prominent keel and marked atrophy of pectoral musculature. The skin of the head was irregularly thickened and both eyes were closed. There were multiple, firm, 1-2cm diameter, raised, pale yellow nodules and focal hemorrhages in the skin of the head and comb, with scab formation and small amounts of moist seropurulent exudate, and loss of feathers. Multiple lacerations were present in the skin on the head, legs and pectoral region. There were small amounts of yellow caseous material focally in the subcutis overlying the keel. On internal examination, there were small amounts of caseous yellow material in the pericardial sac. No gross lesions were identified in other visceral organs.

Laboratory Results: Microbiology: Skin, keel: *Staphylococcus aureus*
Parasitology fecal floatation: *Eimeria* sp. oocysts, *Capillaria* sp. eggs

Histopathologic Description: Multifocally, there is marked hyperplasia, ballooning degeneration and necrosis of epidermal and follicular epithelial cells. The lumina of feather follicles contain variable amounts of necrotic cellular debris, sloughed epithelium, clusters of coccoid bacteria and smaller numbers of rod-shaped bacteria. Throughout the affected foci, epithelial cells contain intracytoplasmic eosinophilic A-type inclusion bodies (Bollinger bodies), and there is loss of nuclei in some infected epithelial cells. There are few foci of epidermal ulceration, focal necrosis of the superficial dermis and congestion of dermal vessels. Fibrin, necrotic cellular debris, extravasated red cells, degenerate heterophils, necrotic epithelium and large clusters of coccoid bacteria are found in superficial epidermal layers and lining the ulcerated areas. Large perivascular and interstitial infiltrates of lymphocytes and histiocytes, smaller numbers of heterophils and scattered focal hemorrhages are present throughout the superficial dermis and around feather follicles. There is separation of the epidermal layers and of the epidermis from the dermis (cleft formation) in multiple foci.

Other significant findings in this case were focally extensive bronchopneumonia.

Contributor's Morphologic Diagnoses: Skin, head: Epithelial hyperplasia, ballooning degeneration and necrosis, focally extensive, severe, chronic with intracytoplasmic eosinophilic A-type inclusions, etiology consistent with avipox virus

Dermatitis and folliculitis, lymphohistiocytic and heterophilic, focally extensive, severe, chronic with serocellular crusts, intraepidermal vesicles and epidermal ulceration

Contributor's Comment: Avian poxviruses (APVs) are members of the genus Avipoxvirus within the subfamily Chordopoxvirinae in the family Poxviridae (1). They are large linear dsDNA viruses that are found worldwide in all species of poultry and in more than 200 species of birds (2). Detection and differentiation of APV strains have been carried out by the use of polymerase chain reaction combined with restriction enzyme and nucleotide sequence analyses. Currently 10 defined species in the Avipoxvirus genus are described (1).

Avian poxviruses are immunogenically and antigenically distinguishable from each other, but varying degrees of cross relationships do exist (3). There is a substantial degree of host specificity in some avian poxviruses, especially those that infect wild birds (3). Fowlpoxvirus (FPV) is the type species of the genus (1). Unlike most DNA viruses, FPV matures and multiplies in the host cell cytoplasm of replicating cells and has an affinity for epithelial cells. FPV contains homologues of the poxvirus A-type inclusion (ATI) proteins. These inclusions protect mature virions from environmental insults, thus prolonging survival of the virus in nature (3).
Infection occurs through mechanical transmission of the virus to the injured or lacerated skin, through aerosol transmission by feathers and dried scabs containing poxvirus particles and by insect bites (3). Replication of viral DNA in epidermal epithelium at 12-24 hours post-infection is followed by appearance of infectious virus and epithelial hyperplasia 36-48 hours post-infection (3). One of the characteristic features of avian poxvirus infection is cellular hyperplasia of affected tissue. The virus produces a hormone analogous to epidermal growth factor which induces cell division and results in proliferative lesions (3).

The disease may occur in one of two forms, cutaneous or diphtheritic, or both (3). In the diphtheritic form, slightly elevated, white opaque nodules or yellowish patches develop on the mucous membranes of the mouth, esophagus, tongue, or upper trachea (3). Histopathologic changes of tracheal mucosa include hypertrophy and hyperplasia of mucus producing cells, with subsequent enlargement of epithelial cells that contain eosinophilic intracytoplasmic inclusions (3). The severity of the pox lesions vary depending on the susceptibility of the host, virulence of the virus, and distribution of the lesions among other factors (3).
A differential diagnosis in this case would be Marek's disease (alphaherpesvirus). The inflammatory lesions in skin sections in Marek's disease are perivascular and localized

around infected feather follicles (3). No gross or histopathologic lesions consistent with Marek's disease were identified in the sciatic nerve, brain or visceral organs.

AFIP Diagnoses: 1. Skin: Epidermal and follicular hyperplasia, ballooning degeneration and necrosis, focally extensive, marked, with eosinophilic intracytoplasmic inclusion bodies, chicken (*Gallus domesticus*), avian, etiology consistent with avipoxvirus.
2. Skin: Dermatitis and folliculitis, lymphohistiocytic and heterophilic, chronic, focally extensive, severe, with serocellular crust, multifocal ulcers and colonies of cocci.

Conference Comment: The contributor provides a thorough review of avian poxvirus infection. Attendees noted the perifollicular and perivascular distribution of inflammation and discussed these as similar features of Marek's disease (Gallid Herpesvirus-2); however, the microscopic lesions of cutaneous Marek's disease are predominantly lymphoproliferative inflammation surrounding feather follicles and inclusion bodies, when present, are intranuclear.

This case has all the classic, light microscopic features of poxviral infection: marked epithelial hyperplasia; ballooning (hydropic) degeneration and pathognomonic eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) located within epithelial cells.

Attendees developed a differential diagnoses for gross findings associated with the cutaneous (dry pox) and diphtheritic (wet pox) forms of disease:
Dry pox: Proliferative, hyperkeratotic and ulcerative cutaneous lesion
- *Trichophyton megninii* and *T. simii*: dermatophytic fungi.
- *Knemidokoptes gallinae*: mite in the basal shafts of feathers on the epidermis.

Wet pox: Caseous inflammation (yellow/white) in the pharynx/esophagus/crop:
- Vitamin A deficiency: Pustules on the mucosa of the mouth, pharynx, esophagus and crop in young birds, inflamed eyelids; squamous metaplasia of the nasal mucosa; keratinization of intestinal enterocytes, decreased goblet cells and blunting of villi (severe deficiency).
- Infectious laryngotracheitis: Alphaherpesvirinae (Gallid herpesvirus 1); mucohemorrhagic or caseous exudates of the trachea; intranuclear inclusion bodies in epithelial cells.
- *Trichomonas gallinae*: Raised caseous lesions in the mouth, pharynx, esophagus and crop.
- *Capillaria annulata* or *Capillaria contorta*: Thickened inflamed mucosa progressing to a sloughing mucosa.
- *Candida albicans*: White-grey pseudomembranous patches on the mouth, pharynx, esophagus and crop; pseudohyphae and budding yeast.
- Aspergillosis: Yellow-grey nodules in trachea, lungs and air sacs

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SLIDE 66
CONFERENCE 17 / CASE II – 00-20410 (AFIP 2790169)

Signalment: 4 year old female Persian cat, (*Felis catus*)

History: Had single mass removed 2 months ago - now 3 masses about 1 cm in diameter in chain over shoulder area.

Contributor's Morphologic Diagnosis: Focal pyogranulomatous dermatitis and cellulitis with intralesional fungal hyphae and spores (dermatophyte pseudomycetoma)

Contributor's Comment: Dermatophytosis is a superficial infection of the keratinized layers of the skin, hair and nails by fungi of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*. The production of keratinases enables the fungi to utilize the usually chemically-resistant keratin proteins as a nutrient source. The fungi grow only within the non-viable keratinized tissues and appear unable to invade the living tissues of a healthy host. Accidental penetration of the dermis, usually from spontaneous or traumatic rupture of an infected hair follicle, results in an intense inflammatory reaction and disappearance of the fungal elements. On rare occasions, the dermatophytes persist in living tissue, inciting a chronic granulomatous or suppurative inflammatory reaction that is distinctive only because of the contained fungi. Even more rarely the fungi persist in well-structured discrete granulomas resembling mycetomas.

All reported cases of dermatophyte pseudomycetomas in cats have been caused by *M. canis*, and all affected cats have been Persians. This suggests that an underlying genetic defect related to the immune system may predispose Persian cats to this disease, but the precise defect has not been determined. Dermatophyte pseudomycetomas appear as firm, raised nodular areas that may ulcerate and drain. Lesions usually involve the dorsum of the trunk or base of the tail. Pseudomycetomas have developed in asymptomatic carrier cats, in cats with known superficial dermatophyte infection, and in cats with no previous history of dermatophytosis. Fungal

elements are not often found in hair shafts or on skin overlaying pseudomycetomas. Cats with dermatophyte pseudomycetoma have a much poorer prognosis for cure than do cats with strict superficial infection. Surgical excision alone is not curative; lesions tend to recur at the surgical site.

Microsporum canis is the most common dermatophyte of the cat, and the cat is considered the natural host for this organism. Granulomas caused by traumatic implantation of non-dermatophytic fungi in the dermis or subcutis are designated as eumycotic mycetomas.

AFIP Diagnoses: 1. Haired skin and subcutis: Panniculitis and dermatitis, pyogranulomatous, nodular, focally extensive, marked, with multiple aggregates of fungal hyphae, Persian, feline.
2. Skin: Folliculitis and perifolliculitis, chronic-active, with intrafollicular arthrospores and fungal hyphae.

Conference Comment: The contributor provides a brief review of dermatophytosis in cats and, more specifically, pseudomycetoma development in Persian cats. There is some slight variation among slides with hair follicles containing arthrospores and fungal hyphae not present in all sections. Conference attendees also noted variation in the degree of folliculitis and perifolliculitis in the adjacent epidermis and dermis. The hyphae present within nodules are strongly PAS-positive and argentophilic with Grocott's methenamine silver.

Pseudomycetomas may originate by dermal invasion from infected hair follicles or subsequent to traumatic implantation of infected hairs. Within the deep dermis and subcutis of affected Persian cats, the hyphae are irregular in shape and size and have bulbous dilations. In this case, normal appearing dermatophyte hyphae, and sometimes arthrospores, are present in hair shafts above or adjacent to the pseudomycetoma.

Dermatophytes more commonly cause “ringworm”, an infection of the hair follicle and hair with keratinophilic fungi. Dermatophytosis can cause folliculitis or furunculosis and the presence of dermatophytes within hair shafts of furunculosis lesions, as seen in kerion, should not be confused with pseudomycetomas.

Attendees discussed the differences between eumycotic mycetomas and pseudomycetomas. Some authors have referred to this lesion as a mycetoma while others prefer pseudomycetoma. According to Ajello, Kaplan and Chandler, there are fundamental differences between the granules of the eumycotic mycetomas and the deep mycelial aggregates formed by dermatophytes. Included are the following:

"1. General absence of a developmental sequence of granule formation in eumycotic mycetomas in contrast to a sequential development ranging from individual mycelial

filaments to small clusters of filaments and, finally, to large aggregates of mycelium in pseudogranules produced by the dermatophytes.
2. Striking and abundant Splendore-Hoeppli reaction material which surrounds pseudogranules in all stages of their development in contrast to varying amounts or absence of such precipitate around eumycotic granules.
3. Mycelium of the pseudogranules is less abundant and not as intricately interwoven and compact as in the eumycotic granules.
4. Cement is not present in pseudogranules; it may or may not, depending on the etiologic agent, be present in the granules of eumycotic mycetomas.
5. The pseudogranules of the dermatophytes appear to have an endogenous origin with mycelial elements entering the dermis through a break in the follicular epithelium."

Gross, Ihrke, Walder and Affolter provide a similar description:

"Eumycotic mycetomas are opportunistic fungal infections that form variably pigmented tissue grains or granules composed of dense aggregates of organisms plus host-derived material. Similar to the tissue grains seen with actinomycosis, and dermatophytic and bacterial pseudomycetomas, these tissue grains are composed of fungal aggregates imbedded in amorphous eosinophilic material presumed to be antigen-antibody complexes formed as a result of the Splendore-Hoeppli reaction."

Attendees noted that dermatophyte hyphae and arthrospores were identified within and surrounding hair shafts but never hair bulbs. The conference moderator pointed out that each hair has a keratogenous zone located above the hair bulb where keratinization occurs. The zone is conical and is known as Adamson's fringe. Below this fringe the cells of the hair root are nucleated and non-keratinized; above this fringe the cells become keratinized. It is now accepted that dermatophytes can infect hair only to the level of Adamson's fringe, but not below it, because these fungi are able to live only in cells that have cornified completely, such as the stratum corneum and nail plate (6).

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SLIDE 67
CONFERENCE 17 / CASE III – S05-300 (AFIP 2991414)

Signalment: Dog, Rottweiler, female, 10 years

History: A mass on the subcutis of the thorax was submitted by a referring veterinarian.

Gross Pathology: The submitted sample was formalin fixed, white, soft skin tissue, with clear mucoid fluid.

Laboratory Results: The neoplastic cells were stained strongly positive for vimentin and moderately but variably positive for Alcian blue.

Histopathologic Description: The submitted tissue is unencapsulated, composed of spindle cells in varying cellularity. Cells have a round to ovoid, hyperchromatic nucleus with vesicular chromatin and visible nucleoli, and varying amounts of cytoplasm with indistinct cell borders. Aggregation of cells in islets and whirl structures, with proliferated vascular tissue at the centers is noted. Extracellular myxoid substance accumulation is also noted, especially in loose, low cellularity areas. Adipose tissue is involved by tumor cell infiltration. Mitotic figures are rare.

Contributor's Morphologic Diagnosis: Myxosarcoma, haired skin, canine.

Contributor's Comment: Myxosarcoma of fibroblast origin is rare, occurring mostly in middle-aged or older dogs and cats, distinguished by its abundant myxoid matrix rich in mucopolysaccharides (1). Cases in the eye, brain, and atrium of dogs have been reported (2). Histopathologically, this case is an unencapsulated proliferation of stellate to spindle shaped fibroblastic cells, loosely arranged in an abundant myxoid matrix. Varying cellularity is noted in different areas of the submitted tissue, and mitoses are rare. Nuclei of tumor cells tend to be small and hyperchromatic.

AFIP Diagnosis: Skin: Peripheral nerve sheath tumor of the skin and subcutis of dogs (hemangiopericytoma), myxoid type, Rottweiler, canine.

Conference Comment: The contributor provides a good case for discussing the controversy which surrounds naming subcutaneous spindle cell neoplasms with similar morphology.

In this case, we prefer the diagnosis of peripheral nerve sheath tumor of the skin and subcutis of dogs (PNST), myxoid type, as the tumor is composed of wavy spindle cells arranged in bundles, palisades and perivascular whorls. The cell of origin for these neoplasms remains controversial. Some pathologists prefer to restrict the term peripheral nerve sheath tumor to those neoplasms that arise and spread within peripheral nerves. Others contend that there is a subset of PNSTs that arise in the skin and subcutis, presumably from small peripheral nerves.(3)

The WHO classification of peripheral nerve sheath tumor of the skin and subcutis of dogs, formerly canine cutaneous spindle cell sarcoma, is a general term that includes neoplasms split into two categories:

- Benign PNST of the skin and subcutis:
    - Neurofibroma - composed of Schwann cells and perineural cells
    - Schwannoma - solely of Schwann cell origin

- Malignant PNST of the skin and subcutis
    - Neurofibrosarcoma
    - Malignant Schwannoma

- Canine hemangiopericytoma is also included with PNSTs due to similar histomorphologic features.

Gross et al. address the controversy and state, ”Recent studies indicate that hemangiopericytomas have been overdiagnosed in both humans and dogs and that ‘hemangiopericytoma’ is often used to denote the histologic pattern created by a variety of spindle cell tumors with a whorling pattern, rather than a specific tumor of pericytes.” Additionally, “differential diagnoses for true hemangiopericytoma include other spindle cell tumors with whorling pattern, such as PNSTs… and that differentiation of hemangiopericytoma from PNSTs can be difficult, as cytologic features may be strikingly similar.”(4)

Regardless of the cell of origin, the biological behavior of PNSTs is well known. They can be locally aggressive, are often difficult to completely excise and are prone to local recurrence; however, metastasis is rare and generally follows multiple recurrences.

Light microscopic characteristics are of a well-circumscribed dermal or subcutaneous tumor composed of spindle to ovoid cells with small amounts of eosinophilic cytoplasm. Typically there are areas of densely packed neoplastic spindle cells in short interlacing streams and bundles (Antoni A-like); intermixed with areas of fewer, more loosely and haphazardly arranged neoplastic cells (Antoni B-like). Nuclear regimentation (Verocay bodies) is common and neoplastic spindle cells often whorl (fingerprint pattern) around capillaries and or collagen. Additionally, clefts and lipid-laden macrophages are often present within and surrounding the neoplasm.

Gross et al. have added a further subclassification to benign PNSTs: Myxoid peripheral nerve sheath tumors, which are similar to PNSTs but have an abundant background of

mucin. “Myxosarcoma may need to be distinguished from myxoid liposarcoma and myxoid peripheral nerve sheath tumors…In contrast to myxosarcoma, myxoid peripheral nerve sheath tumors are composed of spindle cells arranged in concentric whorls or palisades; and the tumor cells have a basement membrane demonstrable by electron microscopy.” Additionally, “In contrast to benign peripheral nerve sheath tumors, formation of a lobular pattern and large, concentric whorls of the tumor cells are not seen with myxomas and low-grade myxosarcomas.”(4)

Unfortunately, immunohistochemistry of benign peripheral nerve sheath tumors in dogs and cats is unreliable. Although canine and feline benign PNSTs consistently express vimentin, they vary significantly in their expression of glial fibrillary acidic protein (GFAP) and S100 protein. Likewise, immunohistochemistry is not useful in the definitive diagnosis of hemangiopericytoma, as specific markers for pericytes do not exist.(4)

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SLIDE 68
CONFERENCE 17 / CASE IV – PN1/05 (AFIP 2983579)

Signalment: 2 years old, female, spayed, Shar Pei, dog.

History: The dog developed idiopathic mucinosis at 1.5 years of age (as did its two siblings). Steroids were not administered. The dog had also a history of recurrent fever treated with several antibiotic compounds (not specified by the referring veterinarian). The dog was brought to a private practitioner a week after vaccination for an acute onset of severe depression, fever, vomiting, diarrhea, dyspnea and skin lesions. The dog had severe bilateral inguinal cutaneous edema, cyanosis, erosions and hemorrhagic bullae. Lesions extended to both hind limbs. Despite intravenous antibiotics (enrofloxacin) and steroid therapy, the dog underwent cardiorespiratory arrest two hours after admission.

Gross Pathology: The dog had severe facial deformity (Fig. 3). Vesicles and bullae (Fig. 4) were present on all limbs and lateral trunk. These lesions contained abundant clear viscous material (Fig. 5). Cutaneous lesions described by the referring veterinarian were confirmed at necropsy (Fig. 6). Severe hind limb swelling extended to both hocks. On cut sections, the dermis, panniculus and skeletal muscles were diffusely hemorrhagic and necrotic (Fig. 7). Inflammation, hemorrhage and necrosis extended to the retroperitoneal area. Multiple splenic infarcts and diffuse hepatic discoloration were observed.

Laboratory Results:
Cytology: Cytologic specimens obtained from the inflammatory exudate of the muscles of hind limbs were characterized by a prevalence of degenerated neutrophils (karyolysis) with elevated numbers of intracytoplasmic large, round, 1-2 micron bacteria (cocci).
Bacteriology: *Staphylococcus aureus* was isolated in pure culture from the muscular lesions and from the exudate. The antibiogram revealed partial resistance to penicillin, ampicillin, amoxicillin, enrofloxacin, erythromycin and complete resistance to chloramphenicol, doxycycline, tetracycline and sulphonamides.
Special Stains: Alcian-PAS stain at pH2.5 was performed on tissue sections of the Shar Pei in areas of mucinotic skin not involved by the inflammatory process and compared with a normal mongrel dog and a “normal” Shar Pei (Fig. 1). Severe diffuse accumulation of mucin (non-sulfated mucopolysaccharides) extending to the deep dermis, panniculus and dissecting muscles was observed in the dog. The accumulated material was PAS negative. In some areas diffuse erosions of the skin were covered by elevated numbers of Gram positive bacteria (Fig. 2).

Histopathologic Description: Haired Skin: Diffuse epidermal necrosis is present and associated with multifocal (varies among biopsies) dermo-epidermal separation (vesicles/bullae). The dermis is characterized by abundant deposition of mucin, severe, diffuse edema and multifocal areas of haemorrhage. Perivascular accumulation of degenerated neutrophils (karyorrhexis) and lesser numbers of macrophages and mast cells is present. Vessels are characterized by hyperaemia, vascular wall necrosis, intraluminal fibrin thrombi in small veins of upper dermis, and fibrinocellular thrombi in the deep vessel (not always present).

Contributor’s Morphologic Diagnoses:
Haired skin:
1) Severe, acute, perivascular to interstitial, hemorrhagic and neutrophilic dermatitis with acute necrotizing vasculitis and intravascular thrombi with epidermal necrosis and dermo-epidermal vesicles.
2) Severe, diffuse, cutaneous mucinosis, Shar Pei, dog.

Contributor’s Comment: Additional microscopic findings were: severe, diffuse purulent and necrotizing myositis; multiple splenic infarcts with areas of diffuse fibrosis; and moderate diffuse, hepatic amyloidosis with hepatic cord atrophy. Intravascular

thrombi containing elevated numbers of bacteria were observed in lungs, meninges and lesional skin. These findings were consistent with septic DIC.

Lesions in this dog appeared to derive from the association of idiopathic mucinosis and a type II necrotizing fasciitis.

Mucinosis refers in general to abnormal accumulation of glycosaminoglycans in the dermis (11). The accumulated mucin consists of non-sulfated mucopolysaccharides, with prevalence of hyaluronic acid, that are metachromatic and stain with Alcian blue at a pH greater than 1.0 (11). Cutaneous mucinosis can be localized or generalized both in dogs and humans (7, 8). In humans, generalized forms have been associated with hypothyroidism, diabetes, acromegaly or discoid lupus erythematosus (4). Localized forms are classified as primary (cutaneous focal mucinosis, lip focal mucinosis, myxoid cysts, follicular mucinosis) and secondary forms such as pretibial myxedema of hyperthyroidism, *granuloma anulare*, and cutaneous neoplasms (4). In dogs, most forms of localized or diffuse mucinosis are idiopathic or associated with hypothyroidism and systemic lupus (7, 8). Idiopathic generalized mucinosis is a genodermatosis that has been described prevalently in Shar Peis (3). Cutaneous wrinkles typical of this breed derive from the variable accumulation of dermal mucins, which is maximal in puppies and juvenile dogs and decreases with age (3). Idiopathic generalized mucinosis represents the pathologic condition caused in Shar Peis by an excessive and uncontrolled production of dermal glycosaminoglycans that has not been associated with abnormal thyroid function. Idiopathic mucinosis develops more frequently in Shar Peis under two years of age and generally responds to corticosteroid treatment (3). In severe cases, the accumulation of dermal mucins may lead to severe facial deformity and formation of grossly visible mucinotic bullae with ulceration and secondary bacterial infections (3, 7, 8). Microscopically, the main lesion of idiopathic mucinosis of Shar Peis is a variably severe dermal thickening with disruption of normal dermal architecture with collagen bundles separated by abundant pale blue material (3). In the more severe cases subepidermal vesicles are visible. Confirmation of the mucinous nature of the infiltration is based on positivity to Alcian blue at pH2.5 and PAS negativity (3). An involvement of mast cells in the pathogenesis of cutaneous mucinosis has been hypothesized (7) and cases of idiopathic mucinosis associated with cutaneous mastocytosis/mast cell tumors have been described in Shar Peis (3, 5).

The lesions observed in the skin, muscles and deep fascia of the inguinal region and hind limbs were consistent with a necrotizing fasciitis leading to toxic shock that closely resembled type II fasciitis of humans. Fasciitis is an acute to hyperacute, rapidly progressing inflammation of soft tissues and muscular fascia (9). In humans, fasciitis is classified into three types. Type I fasciitis is causes by a mixed bacterial population composed by variable combinations of *Bacteroides spp., Clostridium spp., Proteus spp., Streptococcus spp and Staphylococcus spp.* (9). Type II fasciitis is a monomicrobic infection caused more commonly by group A Streptococci or by *S. aureus* (9). Type III fasciitis is the fulminant form of the disease caused by marine organisms such as *Vibrio vulnificans* and *V. parahaemolyticus* (9). Predisposing factors in man are related to trauma, surgery, diabetes, immune depression and odontogenic chronic infections (1).

Necrotizing fasciitis presents as a rapidly advancing, painful erythema, edema progressing to necrosis, blistering and ulceration. In humans the abdominal wall, perineum and extremities are commonly involved. Histopathology is characterized by infiltration of skin, soft tissues and fascia with neutrophils, areas of necrosis, hemorrhage and thrombosis (9). Differential diagnoses may include any type of panniculitis; however, the rapid and aggressive clinical behavior is characteristic and leads to toxic shock syndrome if the adequate antibiotic therapy is not instituted (9).

In this dog, the findings of the monomicrobic infection with *S. aureus*, the gross and microscopic lesions, and the rapidly progressive clinical course were consistent with a type II fasciitis. This dog presented a severe hereditary form of mucinosis that affected other two siblings with similar severity and may have concurred to the secondary bacterial infection leading to dermatitis and fasciitis. Antibiotic resistance may have arisen from the cyclical antibiotic therapy administered during the recurrent febrile episodes and may have lead to toxic shock syndrome**.** A secondary infection with *Staphylococcus spp.* and *Enterococcus sp.* in a Shar Pei with idiopathic mucinosis has been described, partially supporting this hypothesis (5).

The febrile episodes and the hepatic amyloidosis could have also contributed to immune depression facilitating the entrance and diffusion of bacteria from the skin. Recurrent fever in association with hepatic amyloidosis was consistent with the condition named “Familial Shar Pei Fever” that has been described as an inherited autosomal recessive disease (10) and in some reports referred to as familial renal amyloidosis (2). This syndrome is characterized by episodic self limiting fever lasting 12-36 hours and commonly associated with swelling of the tibiotarsal joints (“Swollen Hock Syndrome”). Dogs generally develop renal (not present in this dog) and hepatic amyloidosis (present in this case). Approximately 26% of dogs will develop renal failure (2, 10). Other causes of death associated with this syndrome have been multiorgan thromboembolism, pulmonary embolism, DIC, and streptococcal toxic shock syndrome (10). Increased systemic levels of IL-6 have been recognized as the trigger of the production of acute phase reactant proteins by the liver (10).

AFIP Diagnoses: 1. Skin and subcutis: Vasculitis, necrotizing, with dermal and epidermal necrosis, hemorrhage, dermal-epidermal separation, and acute inflammation, Shar Pei, canine.
2. Skin, dermis: Mucinosis, diffuse, severe.

Conference Comment: The contributor provides an interesting case and a wonderful review of cutaneous mucinosis in Shar-Peis complicated by concurrent necrotizing vasculitis. As stated by the contributor, *Staphylococcus aureus* was isolated from the muscle and exudate and grown in pure culture. *S. aureus* is a Gram-positive cocci which frequently grows in grapelike clusters and is a normal inhabitant of the skin and mucous membranes. *S. aureus* is also a very common cause of numerous suppurative diseases, the most common cause of food poisoning in humans and, due to superantigen production, results in diseases such as Toxic Shock Syndrome (TSS).

Below is a list of the more common virulence factors produced by *S. aureus* and how they contribute to disease:

- Alpha toxin: the most potent membrane-damaging toxin produced by *S. aureus*; creates a pore in the cell membrane through which cellular contents leak; not produced by all strains; systemic release causes septic shock
- Beta toxin: type C sphingomyelinase, damages membranes rich in lipid
- Leukocidin: forms a pore in affected cell membranes; also hemolytic
- Exotoxins
    - Superantigens; stimulate T-cells non-specifically without normal antigenic recognition
        - Enterotoxins; six types, responsible for food borne illness
        - Toxic shock syndrome toxin (TSST)
    - Exfoliatin toxins (ETA, ETB); associated with scalded skin syndrome in neonates (12)

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SLIDE 69
CONFERENCE 18 / CASE I – 11353-04 (AFIP 2988322)

Signalment: 4-month-old, domestic shorthair cat, female, *Felis domesticus*

History: Several kittens in the litter died with lethargy and dyspnea and the mother had mild respiratory signs.

Gross Pathology: The kitten was somewhat thin. The lungs were diffusely pink-red and somewhat rubbery and edematous, had no consolidated areas, but they did not float well. The pericardial sac contained a small amount of gelatinous fluid. Other organs were grossly normal.

Laboratory Results: Chlamydophila PCR, liver and lung: positive
FeLV and panleukopenia fluorescent antibody: negative
Virus isolation: negative
Bacteriology, lung and liver: no bacterial growth
Giardia ELISA: negative

Histopathologic Description: The lungs have diffuse, severe, interstitial hypercellularity with an influx of histiocytes, a few small lymphocytes and early fibroblast proliferation. Some alveolar macrophages are shedding into the narrowed alveoli (interstitial pneumonia). Essentially no neutrophils are in the airways but there is diffuse type II pneumocyte hyperplasia. The liver has extramedullary hematopoiesis and occasional foci of acute hepatic necrosis and small foci of hepatocyte degeneration and individual cell necrosis. Rare packed clusters of minute intracellular basophilic organisms are consistent with *Chlamydophila*. They do not stain with Gram stain but do stain nonspecifically with a Giemsa stain. Some slides also contain a section of very congested spleen with some EMH and possible minute foci of necrosis and fibrin.

Contributor's Morphologic Diagnoses: *Chlamydophila felis* infection with 1) Severe, diffuse interstitial pneumonia and 2) Moderate, multifocal, hepatic necrosis with intracellular *Chlamydophila* colonies and extramedullary hematopoiesis.

Contributor's Comment: Chlamydophila infection is the cause of "Feline Pneumonitis." This is a particularly severe case as it usually causes conjunctivitis and upper respiratory infection (1). The organism has not been found in the liver previously in a natural infection but has been isolated in the liver and spleen in experimental infections with a report of a spontaneous case of peritonitis (2,3). Chlamydia (Chlamydophila) have also been found by EM and histochemical staining in the gastric mucosa of healthy colony cats (4). The interstitial pneumonia in this case is similar to

that caused by feline calicivirus infection when the virus causes lower respiratory infection, rather than the usual upper respiratory tract infection (4). No virus was found by virus isolation in this case. This pattern, with foci of liver necrosis, would also make feline viral rhinotracheitis herpesvirus also a differential diagnosis. The liver involvement is suggestive of the lesions of *Chlamydophila psittaci* in birds and liver lesions were not found in experimental cases of feline chlamydiosis (3). We presume that the infection is due to *Chlamydophila felis*, previously *Chlamydia psittaci* or *C. psittaci felis.* Our PCR primer is probably "genus" specific (Chlamydia and Chlamydophila) rather than specific for *C. felis*. The old genus Chlamydia has been split in a new genus of Chlamydia that still includes *C. trachomatis*, with *C. suis* and *C. muridarum*, and a new genus of Chlamydophila that now includes *Cph. psittaci* (birds and humans) and the new species of *Cph. caviae* (guinea pig conjunctivitis), *Cph. felis*, *Cph. pecorum* (abortion, conjunctivitis, enteritis, pneumonia, arthritis in ruminants), and *Cph. pneumoniae* (koala, human, and horse respiratory disease). (1)

AFIP Diagnoses: 1. Lung: Pneumonia, interstitial, histiocytic, diffuse, moderate, with edema, domestic shorthair, feline.
2. Liver: Degeneration and necrosis, multifocal, mild.
3. Spleen, white pulp: Lymphocytolysis, multifocal, mild.
4. Liver; spleen: Extramedullary hematopoiesis, multifocal.

Conference Comment: As mentioned by the contributor, a recent taxonomic change in the family Chlamydiaceae created two genera, *Chlamydia* and *Chlamydophila*. The genera are distinguished by rRNA sequences and the detectable production of glycogen. The genus *Chlamydia* contains three recognized species, *C. trachomatis*, *C. muridarum*, and *C. suis*. The genus *Chlamydophila* contains six species, *C. pecorum*, *C. pneumoniae*, *C. psittaci*, *C. abortus*, *C. felis* and *C. caviae*.

Chlamydiaceae are obligate, intracellular, gram-negative organisms which contain DNA and RNA and form their own cell wall. They differ from bacteria in that they do not synthesize ATP; instead, they utilize ATP from host cell mitochondria.
Chlamydia exist in two forms: elementary bodies and reticulate bodies. Elementary bodies are small (0.3um) particles with rigid cell walls that can survive outside the host cell but are metabolically inactive and incapable of replication. They attach to the host cell by adhesins on their surface and enter the host cell via phagocytosis. Elementary bodies shed their cell wall and grow larger (0.6-1.5um), forming reticulate bodies that replicate by binary fission. Reticulate bodies are metabolically active, utilizing ATP from host mitochondria, but are incapable of infecting other cells. Reticulate bodies condense and reform elementary bodies that are released during cell lysis to infect other cells.

Chlamydiae persist as commensal flora on the conjunctiva and respiratory, gastrointestinal, and genitourinary mucosae, often with no clinical signs. They are shed in saliva, milk, urine, and feces.

Although difficult to visualize on H&E stained sections due to their small size; special stains can help identify the organisms. Typically, Chlamydiae stain purple with Giemsa, blue with Castaneda and red with Macchiavello/Gimenez. Ultrastructurally, elementary bodies are round and dark with a bilayered cell wall and a unique, dense core of condensed chromatin (nucleoid). Reticulate bodies are larger with more dispersed chromatin. Intermediate forms (intermediate bodies) resemble reticulate bodies but contain a central electron dense core. All three forms occur together, within membrane-bound vacuoles (phagosomes). Host mitochondria are closely associated with these vacuoles.

Listed below are the Chlamydiaceae and the diseases they cause in animals and man:
Chlamydophila felis: endemic in cats; causes conjunctivitis with blepharospasm, hyperemia, chemosis, ocular discharge and rhinitis. Persistent genital and gastrointestinal infection may occur.
Chlamydophila psittaci: a common avian pathogen, a.k.a. Parrot fever, Ornithosis,
Chlamydophila abortus: is endemic in ruminants causing abortion, especially in ewes, a.k.a. Enzootic Abortion of Ewes (EAE). Also affects rabbit, guinea pig, mice and human.
Chlamydophila caviae: specific pathogen of guinea pigs causing conjunctivitis.
Chlamydophila pecorum: causes reproductive and urinary tract disease in Koalas.
Chlamydophila pneumoniae: a human pathogen causing bronchitis and pneumonia, also affects frogs and snakes.
Chlamydia trachomatis: sexually transmitted human disease. In women, can infect the cervix, urethra and fallopian tubes resulting in Pelvic Inflammatory Disease (PID); in men, causes epididymitis. Also causes inflammation of the rectum, throat, and conjunctiva. Ocular infection in humans causes blindness and is called “trachoma”. A problem in developing countries primarily, affects 84 million people worldwide (5).
Chlamydia muridarum: pneumonia in mice and hamsters
Chlamydia suis: causes conjunctivitis, enteritis and pneumonia in swine.

Contributor: Arkansas Livestock and Poultry Commission
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SLIDE 70
CONFERENCE 18 / CASE II – P05-171f (AFIP 2991413)

Signalment: 28-day-old, male, nursery pig, swine.

History: Three nursery pigs from a farrow-to-finish commercial farm located in central Taiwan were culled and submitted for pathological examination in July, 2005. The pigs were weaned at 28 days of age and moved to a nursery house. These submitted nursery pigs had moderate digestive distress with watery diarrhea and weight loss that had been unresponsive of a variety of antimicrobial agents. Of 100 pigs in the group, half were affected, and there was low mortality. The farmer had noticed diarrhea in suckling pigs since early May 2005, and diarrhea was found to affect nursery and growing pigs at the end of June 2005.

Gross Pathology: The piglet was in poor body condition and appeared dehydrated. The stomach contained some intact feed. The jejunum and ileum were distended with yellow and frequently foamy fluid. The walls of the small intestine were thin and translucent. Large intestinal contents were pasty and lacked formed feces.

Laboratory Results: Coronavirus particles were demonstrated in intestinal contents of the pig by negative-contrast transmission electron microscopy.

Histopathologic Description: Extensive villous atrophy was present throughout the small intestinal mucosal circumference. The normal jejunal villous height: crypt depth ratio was reduced from 3:1 to 1:1. Epithelial cell necrosis of villi was accompanied by infiltration of inflammatory cells. The inflammatory cells were mainly lymphocytes, plasma cells and eosinophils. Immunohistochemistry testing for transmissible gastroenteritis virus (TGEV) was positive in infected-epithelial cells of villi.

Contributor's Morphologic Diagnosis: Enteritis, severe, acute to subacute, with villous atrophy, jejunum and ileum, swine. Etiology: TGE virus (a coronavirus)

Contributor's Comment: Transmissible gastroenteritis (TGE) is a highly contagious, enteric viral disease of swine characterized by vomiting, severe diarrhea, and high mortality (often 100%) in piglets less than 2 weeks of age. Although swine of all ages are susceptible to this viral infection, the mortality in swine over 5 weeks of age is very low. The disease is most frequently diagnosed and causes the most loss when occurring in herds at farrowing time [(1)]. TGE is recognized as one of the major causes of sickness and death in piglets. Swine producers are especially apprehensive about this disease because (1) mortality is high in newborn pigs; (2) there is no effective, practical treatment; (3) entrance of the virus into a herd in winter months is difficult to

prevent because of the probable role of birds, especially starlings in the USA. The viral etiology, TGE, is in the genus *Coronavirus* of the family *Coronaviridae*. TGE is enveloped and pleomorphic, with an overall diameter of 60-160nm. It has a single layer of club-shaped surface projections that are12-25nm in length and widely spaced.

An epizootiologic feature of TGE is seasonal appearance. i.e., during the winter months, usually from the middle of November to about the middle of April in the USA. However, our laboratory found some cases of TGE in mid-summer in the past two years [(1)]. There is evidence for existence of TGEV in nonporcine hosts. Cats, dogs, and foxes have been suggested as possible carriers of TGEV from one herd to another since they can shed virus in their feces for variable period [(6)].

Porcine epidemic diarrhea virus (PEDV) is another coronavirus of pigs that causes a disease similar to TGEV; it is less severe, and newborn animals are not always affected; this disease has been documented only in swine in Europe, Asia (China, Japan) and Canada [(2, 5)].

TGEV is ingested, infects the mucosa of the small intestine, and causes a rapid and extensive loss of functional epithelial cells. The pathogenesis of diarrhea in TGEV-infected pigs includes altered sodium transport in the jejunum, resulting in accumulation of electrolytes and water in the intestinal lumen, and loss of extravascular protein. The ultimate cause of death is probably dehydration and metabolic acidosis coupled with abnormal cardiac function resulting from hyperkalemia. A marked shortening or atrophy of the villi occurs in the jejunum and to a lesser extent in the ileum, but it is often absent in the proximal portion of the duodenum. Both virus production and villous atrophy are greater in newborn pigs than in 3-week-old pigs [(2, 3, 6)].

Collection and preservation of appropriate clinical specimens is necessary for reliable diagnosis. Although villous atrophy is a consistent lesion in severely affected pigs, it frequently occurs in other enteric infections as well (rotavirus, PED, coccidiosis, and sometimes, *E. coli*). Laboratory diagnosis of TGE may be accomplished by one or more of the following procedures: detection of viral antigen (direct or indirect IF method), detection of viral nucleic acid (nucleic acid hybridization probes), microscopic detection of virus (negative-contrast transmission EM; immune EM), isolation and identification, or detection of a significant antibody response (VN test; ELISA test; indirect immunoperoxidase test adapted to detect immunoglobulin class-specific antibody; radioimmunoprecipitation; and a modified autoradiographic test) [(3, 4, 5)]. Antiviral agents have not yet been developed for the specific treatment of TGE. The only treatment presently available is to alleviate starvation, dehydration, and acidosis. The following measures are suggested: provide a warm (preferably above 32ºC), draft-free, and dry environment and provide water or electrolyte or nutrient solution freely accessible to the thirsty TGEV-infected pigs. Such measure will tend to reduce mortality in pigs that are infected at more than 3-4 days of age. Antibacterial therapy might be beneficial in 2- to 5-week-old pigs, especially if there is a concurrent infection with pathogenic strains of *E. coli*. Cross-sucking, or putting infected or susceptible litters onto TGE–immune sows was found useful in various field outbreaks.

Diagnostic criteria for TGE includes: 1. Sudden onset with high mortality in sucking pigs, all ages of pig affected. 2. Severe atrophy of the villi in the small intestine. 3. Identification of the virus or of the viral antigen.

Differential diagnosis includes: 1. Colibacillosis 2. PED 3. Rotavirus infection 4. All other conditions causing an epidemic-like episode of diarrhea with or without vomiting.

AFIP Diagnosis: Small intestine: Villar blunting and fusion, diffuse, marked, 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. (7,8,9)

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 villar atrophy than is 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. In contrast to TGE, diarrhea is not severe. Coccidiosis causes diarrhea without blood in piglets 5-15 days of age, with peak incidence at 7-10 days of age. (10)

Grossly, TGE causes distension of the small intestine with gas, 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. (7,8,9)

Below is a simple chart listing some of the coronaviruses of veterinary importance, species affected and diseases they cause:

CORONAVIRUSES:

Bovine coronavirus (winter dysentery)	Bovine	Gastroenteritis, thought to be a coronavirus – still some debate
Canine coronavirus	Canine	Enteritis
Feline coronavirus (FIP)	Feline	Peritonitis, pneumonia, meningoencephalitis, panophthalmitis; granulomatous vasculitis
Feline enteric coronavirus	Feline	Diarrhea in kittens
Mouse hepatitis virus (MHV)	Mouse	Hepatitis, enteritis, encephalomyelitis; syncytia formation
Porcine transmissible gastroenteritis (TGE)	Porcine	Gastroenteritis, usually piglets less than 10 days old
Porcine hemagglutinating encephalomyelitis virus	Porcine	Vomiting, wasting and encephalomyelitis (usually no diarrhea)
Porcine epidemic diarrhea	Porcine	Gastroenteritis (western Europe, similar to TGE)
Rat coronavirus	Rat	Rhinitis, tracheitis, pneumonitis in young
Rat sialodacryoadenitis virus	Rat	Sialodacryoadenitis, porphyrin released from damaged harderian gland, squamous metaplasia of ducts
Avian infectious bronchitis	Chickens	Tracheobronchitis, nephritis
Bluecomb (turkeys)	Turkeys	Enteritis, cyanosis of the comb
Rabbit coronavirus	Rabbits	Enteritis, myocarditis

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SLIDE 71
CONFERENCE 18 / CASE III – 04-27 (AFIP 2983603)

Signalment: A 6 year old female owl monkey (*Aotus trivirgatus*)

History: This sample of lung was obtained from an 840g, 6 year old female owl monkey. The monkey was splenectomized and inoculated with various Plasmodium agents over a two year period. Aside from a reported history of mild anemia dating back to 10/03, the monkey was otherwise healthy. The monkey was found dead and no overt clinical signs were observed prior to death.

Gross Pathology: The lungs were multifocally consolidated. The remainder of the gross examination was unremarkable.

Histopathologic Description: The kidneys contained a moderate collection of lymphocytes, with glomerular adhesions, and tubular necrosis. Few mineralized calculi were found throughout the distal tubules. The liver contained lymphocytes and occasional eosinophils, and hepatocellular degeneration and necrosis was evident. Malarial pigment phagocytized by Kupffer cells was moderately distributed throughout the hepatic parenchyma. The lungs contained a mixed population of inflammatory cells. The alveolar walls were moderately thickened and malarial pigment was diffusely distributed throughout the lungs. The heart contained a focal collection of lymphocytes with occasional eosinophils. The stomach and intestinal tract were moderately autolyzed.

Contributor's Morphologic Diagnoses: 1. Kidney, interstitial lymphocytic nephritis, multifocal, moderate with minimal tubular necrosis, glomerular synechiae, few mineralized calculi, and intrahistiocytic and extracellular yeast.

2. Lung, mixed cell interstitial pneumonia, diffuse, moderate with fibrosis, pulmonary edema, phagocytized malarial pigment, and intrahistiocytic and extracellular yeast.
3. Liver, lymphoplasmacytic hepatitis, multifocal, mild with individual hepatocellular degeneration/necrosis, phagocytized malarial pigment, and intrahistiocytic yeast.
4. Heart, lymphocytic myocarditis, focal, minimal.

Contributor's Comment: On hematoxylin and eosin (H&E), the lungs, kidneys, liver, and lymph nodes contained macrophages distended with multiple round organisms measuring 2-4 µ in diameter. The organism also had a thin cell wall that enclosed a clear space with a single central basophilic structure. The organism was visibly stained with PAS and GMS. The morphologic characteristics of the organism were consistent with *Histoplasma capsulatum* var *capsulatum*, in which the cell wall appears as a distinct pale blue ring outlining the darker blue of the cell protoplasm. (2,3).

Histoplasmosis is a soil borne organism that is highly prevalent in certain regions of North and South America. There are two reported cased of systemic infections encountered in wild caught owl monkeys from South America. Generally, clinical signs are inapparent and diagnosis is based on histologic evaluation of intracellular and/or extracellular organisms in internal organs. (1,4).

AFIP Diagnosis: Lung: Pneumonia, interstitial, lymphohistiocytic, diffuse, marked, with edema, alveolar histiocytosis, hemosiderosis, and myriad intrahistiocytic yeast, etiology consistent with *Histoplasma* sp., owl monkey (*Aotus trivirgatus*), primate.

Conference Comment: *Histoplasma capsulatum* is a dimorphic fungus that grows as a nonparasitic, mycelial form in the soil and a parasitic, budding yeast in animals with body temperatures between 30 and 37°C. The mycelial form produces spherical microconidia ranging in size from 2-4 µm, and club shaped macroconidia which range from 8 to 14 µm. In tissue, *H. capsulatum* is a 2-4 µm spherical or oval yeast that reproduces by narrow-based budding. Histopathological processing often causes the cytoplasm of *H. capsulatum* to shrink from the cell wall, creating a clear halo.

Histoplasma infection is initiated when the microconidia are inhaled. Inhaled particles must be about 2 µm in diameter or smaller to reach the alveoli. Larger particles are trapped and removed from the respiratory tract by the mucociliary elevator. The small size of histoplasmal microconidia allows them to by-pass the mucociliary elevator and colonize the lung. Once in the alveoli, the microconidia convert to the yeast form and are phagocytized by pulmonary macrophages. In most instances, *H. capsulatum* causes a self-limiting pneumonia. In susceptible animals, however, the organism is spread by macrophages within the lymphatics and then the circulatory system. These animals develop a disseminated disease which can affect many organs including the central nervous system, intestine, adrenal glands, skeletal system, heart, and kidneys. It has also been hypothesized that the mycelia or conidia of *H. capsulatum* can infect the intestine after being ingested. (5)

Contributor: Centers for Disease Control and Prevention, Division of Parasitic Diseases, www.cdc.gov/ncidod/dpd/professional/default.htm

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SLIDE 72
CONFERENCE 18 / CASE IV – NIAH NO.1 (AFIP 2988335)

Signalment: Six-week-old, female, chicken, white leghorn, *Gallus gallus* var. *domesticus*

History: Six-week-old specific-pathogen-free chickens were inoculated intranasally with $10^6 EID_{50}$ highly pathogenic avian influenza virus (H5N1), and examined pathologically to confirm the virus-induced lesions. The virus (A/duck/Yokohama/aq10/2003) was isolated from duck meat, which had been imported from China to Japan, at the animal quarantine laboratory. All the inoculated chickens died from 2 to 6 days post-inoculation (dpi). The tissue sample of a chicken that died at 5 dpi was presented.

Gross Pathology: Grossly, dead chickens showed subcutaneous congestion in the comb and wattle, mucosal congestion in the palpebral conjunctiva, and subcutaneous congestion in the tibiotarsal joint. In addition, necropsy findings included diffuse whitish foci in the spleen and petechial hemorrhage in the adipose tissue at the base of heart.

Histopathologic Description: The major histopathological changes in the chicken included severe necrotic myocarditis in the heart, moderate nonsuppurative meningoencephalitis in the brain, mild focal necrosis in the pancreas, and severe dermatitis in the comb. In addition, the chicken had multifocal hepatocytic necrosis in the liver; a large number of hemosiderin-laden macrophages in the lung and spleen; and moderate depletion of lymphocytes in the spleen, thymus and the bursa of Fabricius.

Immunohistochemical examination using monoclonal antibody against type A influenza virus matrix protein revealed virus antigen-positive cells, as follows (+++, ++, and + signify a large, moderate, and small number of viral antigen-positive cells, respectively): cardiocytes in the heart (++), macrophages in the lung (+), exocrine cells in the pancreas (+), endocrine cells in the adrenal gland (+), ganglion cells in the ganglion associated with the adrenal gland (+), neurons, glial cells, and ependymal cells in the brain (+++), epithelial reticular cells in the thymus (+), and epidermal cells, macrophages and vascular endothelial cells in the subcutis (+++). No viral antigen-positive cells were detected in the trachea, liver, spleen, kidney, sciatic nerve, or femoral bone marrow.

Contributor's Morphologic Diagnosis: Heart: Myocarditis, necrotizing, lymphohistiocytic, subacute, multifocal, severe, chicken.

Contributor's Comment: An H5N1 influenza virus, A/duck/Yokohama/aq10/2003, was isolated from duck meat processed for human consumption, imported to Japan from China in 2003 (1). This virus was antigenically different from other H5 viruses, including the Hong Kong H5N1 viruses isolated from humans in 1997 and 2003. Sequence analysis revealed that six genes (PB1, PA, HA, NA, M, and NS) of the virus showed 97% nucleotide identity with their counterparts from recent H5N1 viruses, but that the remaining two genes (PB2 and NP) were derived from other unknown viruses. This duck meat isolate was highly pathogenic to chickens upon intravenous or intranasal inoculation. The histopathological and immunohistochemical analysis confirmed that the virus has properties of highly pathogenic avian influenza virus and pantropism in domestic chickens. The pathogenicity investigation of H5 influenza viruses for domestic ducks (2) indicated that A/duck/Yokohama/aq10/2003 caused neurological sings, grew in multiple organs more rapidly than A/chicken/Yamaguchi/7/04(H5N1) isolated from a dead bird during the HPAI outbreak in Japan (3). A/duck/Yokohama/aq10/2003 also replicated well in the lungs of mice and spread to the brain, but was not as pathogenic in mice as H5N1 human isolates. However, viruses isolated from the brain of mice previously infected with the virus were substantially more pathogenic and possessed some amino acid substitutions relative to the original virus. These studies show that poultry products contaminated with influenza viruses of high pathogenic potential to mammals are a threat to public health even in countries where the virus is not enzootic and represent a possible source of influenza outbreaks in poultry.

AFIP Diagnosis: Heart: Myocarditis, necrotizing, histiocytic, multifocal, moderate, white leghorn chicken, avian.

Conference Comment: Avian influenza is a contagious viral infection and/or disease of many avian species including poultry, ratites, shore birds and migratory waterfowl. The highly pathogenic form is characterized by severe depression, decreased egg production, edema, hemorrhage, frank necrosis and high mortality. Highly pathogenic

avian influenza (HPAI) is reportable to the World Health Organization for Animal Health (OIE).

Avian influenza (Orthomyxoviridae) is a type A influenza virus. The type designation is based on envelope matrix (M) antigens and the nucleoprotein (NP) within the virus. Avian, swine, equine, and most significant human influenza are type A viruses. Subtypes are described based on envelope glycoproteins. Fifteen hemagglutinin (HA) and nine neuraminidase (NA) antigens are currently used. Each virus has only one type of hemagglutinin antigen and one type of neuraminidase antigen on its surface. Standard nomenclature for naming viruses includes virus type, host of origin, geographic origin, strain number, year of isolation, and subtype designation. For example: A/Ck/TX/309402/04(H5N2).

Migratory waterfowl (especially ducks) are relatively resistant to clinical disease and are considered an important reservoir for other species. Clinical disease is most important in turkeys and chickens. Transmission occurs from infected to susceptible birds through both direct and indirect contact (aerosol, contaminated fomites, feces) via the respiratory tract or conjunctiva. (4) After inhalation and attachment of influenza virions to cilia, virulent strains replicate in the respiratory and intestinal epithelium and disseminate to the viscera and central nervous system. Hemagglutinin antigen (HA) is responsible for virus attachment to various sialic acid residues on the apical surface of respiratory epithelial cells. (5) Neuraminidase is responsible for release of the virus by cells via its action on neuraminic acid.

Influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species. For example, until 1998, only H1N1 viruses circulated widely in the U.S. pig population. However, in 1998, H3N2 viruses from humans were introduced into the pig population and caused widespread disease among pigs. Most recently, H3N8 viruses from horses have crossed over and caused outbreaks in dogs.

Avian influenza A viruses may be transmitted from animals to humans in two main ways:
•Directly from birds or from avian virus-contaminated environments to people.
•Through an intermediate host, such as a pig.

Influenza A viruses have eight separate gene segments. The segmented genome allows influenza A viruses from different species to mix and create a new influenza A virus if viruses from two different species infect the same person or animal. For example, if a pig were infected with a human influenza A virus and an avian influenza A virus at the same time, the new replicating viruses could mix existing genetic information (reassortment) and produce a new virus that had most of the genes from the human virus, but a hemagglutinin and/or neuraminidase from the avian virus. The resulting new virus might then be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or neuraminidase) not previously seen in influenza viruses that infect humans.

This type of major change in the influenza A viruses is known as antigenic shift. Antigenic shift results when a new influenza A subtype to which most people have little or no immune protection infects humans. If this new virus causes illness in people and can be transmitted easily from person to person, an influenza pandemic can occur.

It is possible that the process of genetic reassortment could occur in a human who is co-infected with avian influenza A virus and a human strain of influenza A virus. The genetic information in these viruses could reassort to create a new virus with a hemagglutinin from the avian virus and other genes from the human virus. Theoretically, influenza A viruses with a hemagglutinin against which humans have little or no immunity that have reassorted with a human influenza virus are more likely to result in sustained human-to-human transmission and pandemic influenza. Therefore, careful evaluation of influenza viruses recovered from humans who are infected with avian influenza is very important to identify reassortment if it occurs. (6)

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SLIDE 73
CONFERENCE 19 / CASE I – RP11124 (AFIP 2994575)

Signalment: Adult male mallard duck (*Anas platyrhynchos*).

History: Wild duck found on zoo grounds unable to fly. Clinical findings included a fracture of the left humerus, emaciation and dyspnea. Because of a poor prognosis euthanasia was elected.

Gross Pathology: Multifocal to coalescing yellow nodules (granulomas) were found in multiple sites including the lung, liver, coelomic cavity and left humerus (with pathologic

fracture). Impression smears demonstrated myriad beaded acid-fast bacilli within these lesions.

Laboratory Results: *Mycobacterium avium* was isolated from the lung, liver and humerus.

Histopathologic Description: Provided is a section of trachea and adjacent soft tissue. There is transmural inflammation that partially fills the tracheal lumen and results in focal loss of the osseous tracheal ring and tracheal mucosa. Lesions are composed of granulomas with abundant central cellular debris (predominantly degenerate heterophils) bounded by thin layers of macrophages that are frequently multinucleated. Macrophages, in turn, are bounded by small to moderate amounts of dense fibrous connective tissue containing variable numbers of macrophages, lymphocytes, plasma cells and non-degenerate heterophils. Within the tracheal lumen, debris is admixed with small to moderate numbers of septate, acute angle branching fungal hyphae. In some sections, hyphae are associated with light yellow pigmented conidiophores suggestive of *Aspergillus* sp. A Ziehl-Neelsen acid-fast stain shows myriad acid-fast bacilli within macrophages and admixed within central debris in granulomas outside the trachea. Multifocally in some sections there are colonies of Gram-positive cocci admixed with debris both inside and outside the tracheal lumen. There is squamous metaplasia of remaining tracheal mucosa.

Contributor's Morphologic Diagnosis: Trachea: tracheitis, necrotizing, heterophilic and granulomatous, chronic and active, transmural, marked with intralesional fungal hyphae, intralesional acid-fast bacilli, and intralesional cocci bacteria

Contributor's Comment: This case represents a combined occurrence of mycobacteriosis (avian tuberculosis) and probable tracheal aspergillosis based on the morphology of fungal hyphae and the observation of distinctive conidiophores. Mixed infections with these common infectious agents can sometimes present a diagnostic challenge as it is easy to focus on the prominent fungal hyphae, while overlooking the acid-fast bacilli. The presence of multicentric granulomas with intralesional acid-fast bacilli (subsequent isolation of *M. avium*) was useful in avoiding this error in this case. The presumptive aspergillosis is interpreted as secondary lesion to debilitation associated with the disseminated mycobacteriosis. Speculatively, the tracheal mycobacterial lesion may also have disrupted airflow or local mucociliary clearance with subsequent settling of fungal spores within the tracheal lumen. The possibility that the tracheal mycobacterial lesion began within a cervical air sac with extension into the tracheal wall cannot be excluded.

Mycobacteriosis ("avian tuberculosis") is a relatively common disease in a wide variety of avian species (1). Although usually associated with members of the *M. avium-intracellulare complex* (MAI complex), infections with organisms such as *M. genavense* are increasingly recognized, and rarely other mycobacteria, including *M. tuberculosis* are identified as avian pathogens (1, 2). Many of the mycobacteria implicated in avian mycobacteriosis are environmentally ubiquitous and infections are

frequently thought to be opportunistic. Infected birds may serve as sources of heavy environmental contamination (1). Mycobacteriosis can cause widespread mortalities in collections of zoo birds (3) as well as sporadic morbidity and mortality. Lesions can vary considerably from classic multicentric granulomas in a variety of tissues to diffuse histiocytic infiltrates without discrete granuloma formation (1, 4). Antemortem clinical diagnosis of avian mycobacteriosis is challenging and is a focus of current research (5).

Aspergillosis is common in a wide range of avian species and like mycobacteriosis is caused by organisms that are common environmentally. The most common isolates from affected poultry are *Aspergillus fumigatus* and *Aspergillus flavus* (6). Risk factors for the development of aspergillosis include environmental conditions that increase exposure to infective spores as well as less easily defined factors such as “stress” and debilitation associated with other underlying conditions. In the absence of fungal culture, presumptive diagnosis of aspergillosis can be made by identifying characteristic conidiophores in histologic section. Diagnostic challenges for the pathologist are cases of aspergillosis without widespread respiratory lesions. These might include isolated lesions in the trachea (“aspergillomas”) that are easily overlooked if the trachea (especially the syrinx) is not opened or carefully examined during necropsy or pectoral muscle infarcts associated with lesions in the great vessels or brachiocephalic arch (7).

AFIP Diagnosis: Tracheitis, necrotizing, heterophilic and granulomatous, transmural, focally extensive, severe, with ulceration, osteolysis, squamous metaplasia, fungi, colonies of cocci and many acid fast bacilli, mallard duck, avian.

Conference Comment: The contributor provides a brief review of both avian mycobacteriosis and aspergillosis. Attendees identified the colonies of cocci, fungal hyphae and conidiophores within the section but did not have the benefit of special stains prior to the conference. Acid-fast bacilli are readily identifiable with both the Fite-Faraco and Ziehl-Neelsen stains and fungal hyphae are clearly visible in Gomori's methenamine silver-stained sections.

Attendees recognized conidiophores and felt their morphology was distinctive for *Aspergillus* sp.. Aspergillosis is a common opportunistic infection and should be the top differential when fungi are identified in lesions lining airways. Conidiophores are specialized structures from which asexual spores, or conidia, arise. The end of the conidiophore forms a terminal vesicle which develops sterigmata (phialides) from which chains of conidia are produced. (8) Only if the *Aspergillus* is growing in a cavity with airspace can it form a “fungus ball” in which conidia and conidiophores are frequently observed. (9)

The moderator stressed the need to run acid-fast stains on all granulomatous lesions in birds and reptiles in order to detect mycobacterial infections which might otherwise be attributed to an opportunistic infection.

Readers are encouraged to review Wednesday Slide Conference 4, case 1, 2005-2006, for a case of avian mycobacteriosis in an Ara.

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SLIDE 74
CONFERENCE 19 / CASE II – 05L-1248C (AFIP 2988622)

Signalment: 14 year old, female, *Equus caballus*, equine

History: The mare was presented for investigation of a visible pale coloured ocular mass in the anterior chamber of the right eye. Additionally, an intraocular retrolental mass was identified clinically and ultrasonographically. The affected eye was blind. There were no direct or consensual light responses in the left eye. The globe was enucleated.

Gross Pathology: Bulbus 4.6 cm anterior posterior axis, 4.9 cm naso-temporal axis. The dorso-medial segment of the anterior chamber is partly filled with a spongy, pale pink-whitish, soft and fragile tissue mass (1.6 x 2.6 x 0.5cm), which is adherent to the

anterior surface of the iris, the corneal endothelium and protruding into the chamber angle.

The posterior eye segment reveals a comparable retrolental, cup shaped mass (≈ 3cm in diameter) extending from the posterior iridial surface and ciliary body into the posterior eye chamber towards the retina, by posterior displacement of the yellowish, liquid vitreous. In the ventro-medial segment, there is focal moderate acute subretinal haemorrhage.

Laboratory Results: Immunohistochemistry:

Antibodies	Ciliary body (npe)	Retinal cells	Tumour cells
GFAP	-	++	±
NSE	+	+	±
Neurofilament	-	±	+
Synaptophysin	-	+	++
Retinal S-protein	±	++	±
Vimentin	++	++	++
Pan-Cytokeratin	-	-	-

+ - +++ positive immune reaction
± focally positive immune reaction (single cells)
- no immune reaction

Special stains: PAS/Alcian Blue: multifocally Alcian blue positive areas between sheets of tumour cells, focally centrally in rosettes; multifocally linear PAS-positive structures at the apical cell junction (resembling outer limiting membrane)
Van Kossa Stain: multifocal areas of calcification within necrotic tumour tissue

Histopathologic Description: The tissue masses in the anterior eye chamber and anterior uvea are composed of undifferentiated spindloid to spherical cells with scant homogeneous eosinophilic cytoplasm and large, pleomorphic, hyperchromatic nuclei with 1-4 nucleoli of variable size (anisocytosis, anisokaryosis). Mitotic figures are frequent (2-4 per high power field).

The cells resemble primitive neuroectodermal tissue, are arranged in sheets, nodules or cords, form rosette like structures with or without a central cavity (Flexner-Wintersteiner / Homer-Wright rosettes) and surround well differentiated, thin walled (venous) blood vessels of variable caliber.

Within the endophytic neoplastic mass, apoptotic cells are numerous and there are extensive areas of cellular debris (necrosis) and calcification. Tumour cells focally invade the anterior iridial stroma (infiltrative growth) and expand subretinally (exophytic growth) causing focal retinal detachment. The non-pigmented epithelium of the ciliary body reveals mild to moderate cytoplasmic vacuolation and variable cellular size (atrophy). In the vitreous, small numbers of erythrocytes and macrophages containing

intracytoplasmic granular brown pigment (melano-macrophages) and focally macrophages with intracytoplasmic erythrocytes (erythrophagocytosis) are seen. Epiretinally, appearing bandlike (outer limiting membrane) and vitreally/ subretinally appearing globular, there are large amounts of basophilic material (condensed chromatin). The retina shows mild to moderate atrophy, predominantly of the neuronal and outer granular layers. Focally there are moderate amounts of subretinal amorphous eosinophilic material (subretinal oedema) and a focal mild hypertrophy of the retinal pigment epithelium (rpe). The conjunctiva shows a mild subepithelial infiltration with lymphocytes, plasma cells and neutrophils.

Contributor's Morphologic Diagnosis: Eye: Tumour, primary intraocular, endo- and exophytic, primitive neuroectodermal, (non-teratoid medulloepithelioma-/retinoblastoma-like), with transplantation metastasis into the anterior eye chamber, atrophy of non-pigmented epithelium of the ciliary body, retinal atrophy and detachment, vitreal/subretinal haemorrhages and oedema, chronic neutrophilic conjunctivitis, equine, female, 14 years

Contributor's Comment: Primary intraocular tumours of neuroectodermal origin are divided into two groups: tumours deriving from mature neuroepithelium, i.e. adenomas, adenocarcinomas of the (unpigmented/pigmented) ciliary epithelium and neoplasms derived from primitive medullary epithelium (during differentiation of the optic vesicle or optic cup) including medulloepitheliomas and retinoblastomas[1].
In animals, medulloepitheliomas in general are rare, congenital tumours, deriving from primitive neuroectoderm of the optic cup. They have multipotentiality and a retained ability to differentiate into retina, ciliary epithelium, vitreous or neuroglia[2]. The majority of medulloepitheliomas are reported in young animals, classified as benign or malignant, nonteratoid or teratoid. Nonteratoid medulloepitheliomas are solely composed of neuroepithelial cells, whereas teratoid medulloepitheliomas include neuroectodermal tissue and heteroplastic elements, e.g. hyaline cartilage, spindle cells and a myxomatous matrix[3,4]. Medulloepitheliomas are described in goldfish, cockatiels, llamas, feline, canine, and equine species[4].

The entity of retinoblastomas in animals is still under debate[3]. One confirmed case of a retinoblastoma in a dog has been described[5]. The morphological and immunohistological features described in this case are consistent with a primitive neuroectodermal tumour, exhibiting the characteristics of both, medulloepithelioma and retinoblastoma.

Due to the fact, that the distinction between medulloepithelioma and retinoblastoma can be very difficult, primitive primary neuroectodermal intraocular tumours may exhibit features of both entities and the diagnosis requires a series of criteria[6,7], a final decision in this case could not be made solely on a morphological and immunohistological basis.

AFIP Diagnosis: Eye: Primitive neuroectodermal tumor with features of medulloepithelioma and retinoblastoma, *Equus caballus*, equine.

Conference Comment: The contributor provides an example of an ocular primitive neuroectodermal tumor (PNET) with areas that have medulloepithelioma and retinoblastoma-like features. The majority of the neoplasm is composed of sheets of primitive neuroectodermal tissue with a high mitotic index. Multifocally, neoplastic cells form large rosettes or tubular structures that have a pseudostratified, columnar arrangement, rest along segments of PAS positive basement membrane and have a vague inner limiting membrane, features consistent with medulloepithelioma. Also present are many, small, Flexner-Wintersteiner rosettes. Rosettes are considered a characteristic feature of both medulloepitheliomas and retinoblastomas making interpretation, beyond neuroectodermal origin, difficult. Within medulloepitheliomas, rosettes are generally interpreted as the neoplasm's attempt to recapitulate portions of the optic vesicle or cup. In retinoblastomas, rosettes are interpreted as neuronal cells forming photoreceptors in an attempt to recapitulate portions of the retina. In this case, the contributor also provided TEM findings supportive of retinoblastoma. Within the luminal protrusions of a small rosette, cilia and basal bodies are present.

Medulloepitheliomas are uncommon; however, they are the most common primary intraocular neoplasm in horses. As mentioned by the contributor, medulloepitheliomas are derived from the embryonic neuroectoderm lining the inner layer of the optic cup and have the potential to differentiate into iridociliary epithelium, retina, vitreous and neuroglia. (7)

Retinoblastomas are neuroepithelial tumors that usually arise in the posterior retina and are the most common primary intraocular malignancy of children. In approximately 40% of human cases, retinoblastoma occurs in those who inherit a germ-line mutation of one retinoblastoma (RB) allele. Germ line associated retinoblastomas can be bilateral and may also be associated with pinealoblastoma, referred to as "trilateral" retinoblastoma. Since there has been only one confirmed case of retinoblastoma in a dog; with the remainder of animal cases occurring in fish, transgenic mice, and primates, the biologic behavior of these tumors in animals is unknown. In humans, retinoblastomas may invade along the optic nerve and choroid and those cases which involve the pineal gland are associated with a dismal outcome. (1,8)

The diagnosis of retinoblastoma in human medicine is based on histomorphology, immunohistochemical demonstration of retinal S-antigen, GFAP and carbonic anhydrase (CA), and identification of mutations in the RB1 gene. Histologically, this horse tumor has features of both medulloepithelioma and retinoblastoma. Based on immunohistochemistry performed at the Armed Forces Institute of Pathology, the tumor is negative for GFAP, S-100 protein, NSE, and NFP. It diffusely stained lightly to moderately strong for CD99.

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SLIDE 75
CONFERENCE 19 / CASE III – 360/05 (AFIP 2983553)

Signalment: Six-year-old male Maine Coon cat (*Felis catus*).

History: The animal was found dead without any previous clinical signs.

Gross Pathology: Except for acute congestion of liver, spleen, lung and kidneys as well as an acute alveolar edema of the lung, the animal was grossly unremarkable.

Histopathologic Description: Unfortunately the tissues contain artefacts due to the frozen condition in which the animal was sent to the institute. Remarkable histopathologic changes were found in the brain of the cat (cerebellum submitted). There are lightly basophilic inclusions within the cytoplasm of nerve cell bodies, neuronal processes, and scattered within the neuropil. Most of the inclusions can be found in Purkinje cells. The inclusions are of variable size ranging from 2 to 10 μm in diameter. They are non membrane-bound spherical structures containing a deeply hematoxyphilic central core with a spiculated outline and a surrounding zone of less staining material. The inclusions stain positively with PAS and Alcian blue (slides not submitted).

Contributor's Morphologic Diagnosis: Cerebellum: Multifocal polyglucosan inclusions (Lafora bodies Type II) within neuronal perikarya, neuronal processes and scattered within the neuropil, most numerous in Purkinje cells.

Contributor's Comment: Lafora bodies are composed of polyglucosans (polymers of sulphated polysaccharides) and regarding composition and staining characteristics they are similar to corpora amylacea.[2] The inclusions stain positively with PAS, Alcian blue, and methenamine silver.[5] However within the Lafora bodies there are differences in internal structure and staining characteristics and based on these varieties some authors recognize three types of Lafora bodies[4]:

1. Type I - Small (3 to 10 μm in diameter), fine, evenly stained granules. This is the most common type and is usually found in middle and deep layers of the cerebral cortex and in glial cells of the cerebellum. Ultrastructurally, these bodies consist of branching fibrillar structures without a limiting membrane. The branching filaments measure about 8 - 10-nm in diameter[4].
2. Type II - These larger bodies (13 to 30 μm in diameter) have a strongly PAS-positive homogeneous core with a more faintly staining radiating periphery. This form is commonly found in Purkinje cells of the cerebellum and in the midbrain. Electron microscopy reveals osmiophilic granules in a central core surrounded by fibrillar material. Rough endoplasmic reticulum in affected neurons may be dilated with increased numbers of coarse ribosomes free in the cytoplasm. Such changes suggest abnormalities in protein synthesis[4].
3. Type III - These bodies range from 5 to 20 μm in diameter and are occasionally found in the midbrain. These structures exhibit a dense peripheral ring of PAS-positive material[4].

In humans Lafora's disease is a severe progressive myoclonic epilepsy caused by inherited recessive mutations in the EPMA2A (chromosome 6q24) or EPMA2B (chromosome 6p22.3) genes[1].

In domestic animals Lafora bodies have been found as incidental changes within the central nervous system in dogs, cats and various other species[5]. Commonly they were found in aging animals (> 8 years old)[6] but occasionally they occurred in young aminals[5]. In some cases Lafora bodies were associated with neurological signs including depression, somnolence and seizures in advanced stages of the disease[4].

When the inclusions are associated with clinical signs the Lafora bodies are often located within the neuronal perikarya, whereas the incidentally found polyglucosan bodies were more often located in the neuropil than in the cytoplasm of the nerve cell bodies[5]. In this cat, inclusions can be found within the neuronal perikarya, although the owner reported no clinical signs.

AFIP Diagnosis: Cerebellum, Purkinje cells, molecular and granule cell layers, and white matter: Polyglucosan (Lafora) bodies, numerous, Maine Coon, feline.

Conference Comment: The contributor provides a brief review of Lafora's disease (LD) and more specific ultrastructural details of the three types of Lafora bodies.

Lafora bodies are carbohydrate-rich intracytoplasmic neuronal inclusions that are widespread in the CNS of humans and other animal species with Lafora's disease. Lafora bodies are most commonly found in thalamic neurons and cerebellar Purkinje cells and less commonly in glial cells. They may be located in the perikaryon, dendrites and axons and/or occasionally free within the neuropil. Lafora bodies may also be found in the heart, liver, voluntary muscles and skin, especially the sweat glands, which are often biopsied in humans to diagnose LD in its early stages.

Lafora bodies are basophilic to amphophilic and range from 5-20 um in diameter. They generally have a dense core, are occasionally concentrically laminated, and may have a radiating or sunburst pattern in their outer zone.

Lafora's disease in humans is a rare, fatal, neurometabolic disorder with an autosomal recessive mode of inheritance. Patients with LD present in late childhood or adolescence, after a period of normal development, with myoclonus and seizures which become intractable within two years of onset. Sadly, patients undergo progressive cognitive decline resulting in a vegetative state and continuous myoclonus prior to death. Death typically occurs within 10 years of onset of clinical signs.

Several mutations in the EPM2A or EPM2B gene have been identified as the cause of LD in humans. The EPM2A gene encodes for the protein laforin which may detect accumulation of polyglucosans and initiate elimination mechanisms to prevent further formation. The EPM2B gene encodes for the protein malin, an ubiquitin ligase that binds laforin and together they may destroy or prevent the production of polyglucosans; however, the relationship between laforin and malin remains uncertain.

Lafora's Disease in animals has been referred to as neuronal glycoproteinosis and has infrequently been reported in dogs, a fennec fox (7), a Maine Coon cat (5), and a Hereford/Angus crossbred cow (9). Animals may present with seizures or myoclonic contractions. Recently a research group at the Hospital for Sick Children in Toronto, Canada identified a genetic mutation in the EPM2B gene as responsible for LD in a group of Miniature Wirehaired Dachshunds. More than 5% of this breed in the UK have LD (8).

The differential diagnosis for Lafora bodies includes:

- Lafora-like bodies have similar histomorphologic characteristics and are found primarily in the neuropil but rarely within perikaryon. Lafora-like bodies are associated with aging but not with neurological signs.
- Corpora amylacea have similar morphology and staining patterns but are typically found within axons and astrocytic processes and not within the perikaryon of neurons. Corpora amylacea have been identified in multiple tissues and are also associated with aging.

- Amylopectin bodies are deposits of abnormal polysaccharides found in tissues of patients with glycogen storage disease IV or glycogen branching enzyme deficiency. Clinical signs may be present at birth or within the first few months of life. Humans with this disease develop liver dysfunction with hepatosplenomegaly, progressive cirrhosis, and chronic hepatic failure followed by early death. Glycogen storage disease type IV has been described in Norwegian Forest cats and amylopectinosis has been described in neonatal Quarter horses. Polysaccharide deposits can be found in the liver, heart, tongue and CNS. Inclusions are PAS (+), diastase resistant and black when stained with iodine.

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SLIDE 76
CONFERENCE 19 / CASE IV – 15652-03 (AFIP 2935879)

Signalment: 16-yr-old, male castrate, mixed breed dog, *Canis familiaris*

History: The dog had an inflamed left eye that became enlarged and bulging over a 2-3 month period with corneal hyperemia. The left eye pressure was 30 and the right eye

20. The eye with a pale retrobulbar mass was submitted from the enucleation surgery. The dog is doing fine 5-months after surgery.

Gross Pathology: Pale, firm, conical mass surrounding the eye and extending posteriorly. Gross photo submitted of the formalin-fixed tissue.

Histopathologic Description: The tumor cells are somewhat pleomorphic with large, vesicular and sometimes nearly clear, nuclei with a prominent nucleolus and are polygonal and round to elongated and plump with a moderate amount of eosinophilic and pale amphophilic cytoplasm and rare to absent mitoses. The cells are cohesive with mostly indistinct cell borders and are in nests and sheets with wide infiltration throughout the muscle, fibrous fascia, and fat with local dense fibroplasia or scirrhous reaction. The compact nests of the large cohesive, polygonal cells do have distinct cell membranes. There are occasional small nests of compact swirling tumor cells (meningothelial pattern) but no psammoma bodies (laminated tumor cell whorls with central mineralization).[8] The retina is atrophic with loss of the ganglion cell layer and partial loss of the inner nuclear layer cells and nests of tumor cells are also infiltrating the outer sclera. Some blocks in the present case show occasional foci of osseous metaplasia within the tumor

Contributor's Morphologic Diagnosis: Orbital (retrobulbar) meningioma

Contributor's Comment: This unusual tumor, although rare, is the most common primary orbital tumor in dogs[1] with other tumors being nasal carcinoma, mast cell tumor, and osteosarcoma.[1] They are usually benign to locally infiltrative, but at least one in a dog metastasized to the lungs.[1] The tumor cells are reportedly vimentin positive and are usually negative for cytokeratin except for some epithelioid types.[2,3] The diagnosis is largely based on the morphology and location. Primary optic nerve meningiomas arise from the arachnoid cap cells inside the dura of the optic nerve.[1,4,5] Secondary tumors infiltrate within the dura from the brain and have been occasionally seen in dogs.[4,6,7] Differentiation requires demonstration of normal tissue behind the tumor in the orbit, or CT scans for the presence or absence of tumor in the brain. The tumor is usually tightly wrapped around the optic nerve and back of the eye and tapers down as it approaches the optic nerve foramen and brain.[3] The tumor cells are variably sized and invade the local fatty fascia and muscle, usually with some large cells with plentiful eosinophilic, glassy, cytoplasm.[3] There is often a myxoid component with cartilage and bone metaplasia.[1, 2,3] The larger tumor cells have an eosinophilic cytoplasm and their tight clusters can be mistaken for invasive, anaplastic carcinoma.[3]

We believe that this is probably a case of primary orbital meningioma since there are no CNS signs and the dog is clinically normal 5-months later.

AFIP Diagnosis: Retrobulbar tissue: Meningioma, mixed breed dog, canine.

Conference Comment: Meningiomas of the optic nerve have been reported in people and dogs. These tumors grow within the space between the optic nerve and the surrounding muscle cone, causing slowly progressive compression of the optic nerve which can result in amaurosis - blindness without an apparent lesion to the eye. Some tumors infiltrate through the sclera into the choroid, along the optic nerve into the subretinal space, or through the optic foramen into the skull. Extensive growth within the orbit may cause substantial orbital remodeling and even expansion. (2)

Of the seven convincing cases of canine optic nerve meningiomas reported in the literature; three were euthanized upon diagnosis, one was euthanized 3 months after removal of the retrobulbar tumor because of blindness caused by meningioma involving the optic chiasm and three were removed but recurred. Of the three that recurred, two had pulmonary metastasis. (1,6,7,9,10,11,13)

In this case, the contributor provided an update on the dog; it was euthanized nine months after the left eye and retrobulbar meningioma were removed. At the time of euthanasia, the right eye was inflamed and the dog was going blind. Chest radiographs a few months earlier did not identify metastases. A necropsy was not performed.

In general, meningiomas are usually discrete tumors with smooth surfaces and broad dural attachments which can arise anywhere along the meninges. They can be soft or firm, and may be gritty when cut. There are nine histologic variants of meningiomas seen in domestic animals. While all of them, except the anaplastic (malignant) variant, have similar biologic behavior i.e. they grow slowly and cause clinical signs by compressing the underlying nervous tissue, the subtypes are used to help identify all of these histologically different tumors as meningiomas and not another primary CNS or metastatic tumor. (8) The nine histologic variants of meningiomas are listed below:

- Meningothelial meningioma: composed of solid, moderately cellular lobules of polygonal cells
- Fibrous (fibroblastic) meningioma: composed of spindle cells arranged in long interlacing fascicles
- Transitional (mixed) meningioma: features of both meningothelial and fibrous meningiomas
- Psammomatous meningioma: a meningioma with psammoma bodies
- Angiomatous meningioma: a meningioma characterized by numerous large or small blood vessels
- Papillary meningioma: composed of meningothelial cells arranged in papillary structures on vascular cores
- Granular cell meningioma: composed of oval-to-polygonal cells with abundant granular eosinophilic cytoplasm, granules are PAS (+) and diastase resistant
- Myxoid meningioma: vacuolated neoplastic cells are separated by moderate-to-abundant myxomatous matrix which stains well with PAS, Alcian blue, and mucicarmine
- Anaplastic (malignant) meningioma: exhibits several features of malignancy e.g. frequent mitoses, high cellularity, uninterrupted patternless growth, extensive necrosis, brain invasion and metastasis

In cats, meningiomas often arise from the tela choroidea of the third ventricle and are easily separated from the brain parenchyma; whereas, in dogs meningiomas frequently interdigitate with the superficial brain parenchyma and are more difficult to remove. (8)

Contributor: Diagnostic Laboratory, Arkansas Livestock and Poultry Commission http://www.arlpc.org

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SLIDE 77

CONFERENCE 20 / CASE I – 04N123 (AFIP 2983857)

Signalment: 2 year old, female, Yorkshire Terrier, *Canis familiaris*, canine

History: Progressive (several months duration) neurological signs including head tilt to left, hindlimb ataxia, circling to left, and not able to eat without syringe feeding. There was temporary improvement with corticosteroids.

Gross Pathology: Grossly, no lesions are appreciated on visual examination of the unsectioned brain. However, cross sections of the fresh brain reveal some prominence of vessels in the thalamus. After fixation, coronal sections of the brain reveal multiple small cavitations and dark discoloration of the white matter on the left frontal lobe of the cerebrum as well as extensive yellow-tan discoloration in the left side of the thalamus (malacia). No other gross lesions are noted in other organs.

Laboratory Results: CBC, serum chemistry, bile acids, cervical radiographs, CSF tap, and CT scan revealed no obvious problems. Serologic tests for canine distemper, Toxoplasma, Neospora were negative. MRI revealed demyelination of deep white matter of left and right frontal lobes of cerebrum, left thalamus and right rostral brainstem.

Histopathologic Description: The left frontal lobe of the cerebrum is characterized by multifocal to coalescing patchy areas of rarefaction of the white matter. Rarefied areas contain sporadic aggregates of spindle to stellate astrocytic cells (fibrillary astrocytosis) as well as large gemistocytic astrocytes and glial cells. Vessels in the white matter are surrounded by aggregates of lymphocytes and plasma cells. Lesions are similar but less severe in sections from the right frontal lobe, the white matter of the cerebrum adjacent to the caudate nucleus and the cortical white matter adjacent to the hippocampus. Some thalamic lesions are similar to those described in the frontal lobe. Other lesions in the thalamus include necrosis and loss of white matter with replacement by a glial scar, characterized by a mixture of fibrillary astrocytes, gemistocytes, and abundant lymphocytes, plasma cells and glial cells. Adjacent to the glial scar there are thick perivascular cuffs of lymphocytes and plasma cells.

Contributor's Morphologic Diagnosis: Necrotizing leukoencephalitis, multifocal, severe with astrocytosis and glial scar (thalamus), brain, canine, Yorkshire terrier.

Contributor's Comment: The overall lesions and distribution within the brain (primarily the cerebral white matter and mesencephalon) along with a history of progressive CNS disease and the clinical MRI findings would be compatible with necrotizing encephalitis of Yorkshire Terriers first reported in 1993 (1). The brain of this dog was negative for canine distemper and rabies via virology. Yorkshire Terriers with this disease commonly have progressive clinical lesions associated with both cerebral and brainstem lesions including lameness, falling, circling, head pressing, depression, proprioceptive

deficits, strabismus, nystagmus, cranial nerve deficits, occasional seizures, paresis and ataxia (1,2,3,4). Spinal reflexes are generally normal (1,2,4).

CBC, serum chemistry and radiographs are generally normal (1,2,3,4), and fluorescent antibody testing for distemper is negative (1). CSF tap usually reveals pleocytosis of monocytic and lymphocytic cells along with increased protein (1,5). MRI and CT scan often reveal asymmetric lesions in the white matter of the cerebrum, diencephalon and mesencephalon consistent with malacic to cavitated lesions, and variable asymmetric ventriculomegaly with no mass effect (2,3,4,5).

Gross lesions include variably dilated lateral ventricles, often unilateral multifocal grayish discoloration of cerebral white matter and brainstem, variable atrophy of the cerebral hemispheres, malacia/cavitation often involving thalamus and/or cerebral white matter, and rare dilatation of third ventricle (1,2,3,4,5).

Histopathologic lesions primarily have an asymmetric cerebral white matter and brainstem (mesencephalon) distribution pattern. Lesions include necrosis and cavitation with astrocytosis, gemistocytes, fibrillary astrocytes, perivascular cuffing of lymphocytes at the periphery of necrotic areas, variable hemorrhage, some macrophages, microgliosis, neovascularization, gitter cells, sclerosis, swollen axons, and astroglial scarring (1,2,3,4,5). Occasional involvement of the dorsal funiculus of the cervical spinal cord is also seen (3). No evidence of viral, bacterial, or protozoal diseases is detected histologically or by culture or immunohistochemical stains.

Necrotizing encephalitis of Yorkshire Terriers can be differentiated from distemper by the lack of inclusion bodies and negative virology testing and immunohistochemical stains (1,2,4,5). Another differential is granulomatous meningoencephalitis which has a prominent perivascular nodular histiocytic infiltration that is lacking in the Yorkshire Terrier encephalitis (3). Also, necrotizing encephalitis of Yorkshire Terriers can be differentiated from necrotizing encephalitis of Pugs and Maltese because those diseases have a cerebral distribution with seizures as the predominant sign and lack brainstem involvement/signs (1,3).

AFIP Diagnosis: Brain, cerebrum: Leukoencephalitis, necrotizing, nonsuppurative, multifocal, chronic, severe, with gemistocytic astrogliosis, Yorkshire Terrier, canine.

Conference Comment: The contributor provides a thorough review of necrotizing encephalitis of Yorkshire Terriers.

Attendees described the distribution of the lesions as primarily within the white matter. Discussion focused on the profound astrocytic response to injury and the presence of reactive astrocytes with gemistocytic morphology.

The common astrogliotic response to CNS injury is manifest by the development of visible cytoplasm within the cell. Reactive astrocytes have a central nucleus and multiple, long, slender processes best demonstrated by glial fibrillary acidic protein (GFAP) staining. Positive immunohistochemical staining for GFAP is evident within 1-3 days and will remain prominent for approximately a month. Gemistocytes are broad and polygonal with a peripherally located nucleus, abundant, smooth, eosinophilic cytoplasm and small vacuoles sometimes located along the margins. (6) In this case, the extremely large numbers of gemistocytes suggest chronicity, and in combination with white matter sclerosis, form a glial scar.

Without the benefit of laboratory or virology testing results, attendees considered the chronic form of canine distemper the top differential. Readers are encouraged to review case 2, conference 4, 1992-1993 for a case of canine morbilliviral encephalitis (canine distemper) in a German Shepherd Dog.

As mentioned by the contributor, Pugs and Maltese dogs suffer from their own form of necrotizing encephalitis; however, their lesions vary in distribution pattern and predominantly affect gray rather than white matter. Readers are encouraged to review case 1, conference 15, 1995-1996 for a case of necrotizing meningoencephalitis in a 5-year-old Maltese terrier.

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SLIDE 78
CONFERENCE 20 / CASE II – WN05/1091 (AFIP 2996889)

Signalment: 8-week- old, Large White, *Sus scrofa*, pig.

History: An intensive piggery included five sheds into which pigs were weaned at three weeks of age, and where they remained as a batch for a further six weeks until sold. On 4 April 2005, the water supply to one of these sheds was turned off at 7 am. This shed contained 432 pigs, in 12 pens of 36 pigs each. This batch of pigs had been in the shed for 5 weeks. The temperature within the shed was usually 31^0C, and the pigs were observed to be very thirsty when their water was restored at 2 pm that afternoon.

The following day, four pigs were dead and 20 were sick, lying about, depressed and head shaking. Two live pigs were submitted to the laboratory. Pig 1 was recumbent, with head trembling and frothy fluid around the mouth. When placed on its feet, it stood immobile with its head down or pressing against nearby objects. Occasionally it had a seizure, with its head thrown back and legs paddling. Pig 2 was recumbent and comatose, with the margins of its ears dark blue and cold. Both animals were euthanized by barbiturate overdose and necropsied at 4 pm.

Gross Pathology: Pig 1**:** No gross lesions were detected.
Pig 2**:** The jejunum was congested and distended with watery contents.

Histopathologic Description: Pig 1: Forebrain: In the dorsolateral cerebral cortex, adjacent to the sulci and, in some cases, also adjacent to the gyri, there is laminar (predominantly middle laminar) necrosis. Mildest changes are of vacuolation (edema) of the neuropil with capillary endothelial hypertrophy. In extensively-affected areas a central eosinophilic zone of 'compact necrosis' containing contracted dark neurons is bordered by a zone of neuropil vacuolation. Virchow-Robin spaces around medium-sized vessels in the cortex and the leptomeninges of the sulci are infiltrated by small to large numbers of eosinophils and mononuclear leukocytes.

Pig 2: Forebrain: As for Pig 1 but more severe.

Contributor's Morphologic Diagnosis: Brain: Necrosis, cerebrocortical, eosinophilic, acute, laminar, severe.

Contributor's Comment: Acute water deprivation syndrome (also designated salt poisoning, or water intoxication, as disease develops after water supply is restored). The history, clinical signs and histological changes are pathognomonic for this syndrome.

In pigs with water deprivation syndrome, there is commonly marked depression and cortical blindness, leading to typical seizures that begin with a twitching of facial muscles, jerking upwards motions of the head, retropulsion to a dog-sitting position, falling to the side, and paddling with the limbs (1). The two characteristic histological changes are of laminar necrosis and eosinophilic infiltration of the cerebral cortex (1). The lesions observed in these cases are acute, and probably of no more than 26 hours duration (the time interval between water supply restoration and necropsy). The degree

of eosinophilic infiltration of Virchow-Robin spaces and the leptomeninges of the cerebral cortex varies between these two pigs: more severe in Pig 1 than in Pig 2 (possibly partly due to a difference in lesion age between the two pigs). However within the same pig the numbers of eosinophils also vary amongst different areas of the cortex, possibly reflecting relative differences in age of the lesion in these areas. Mononuclear leukocytes are prominent in the perivascular cuffs in affected areas, regardless of the number of eosinophils present.

AFIP Diagnosis: Brain, cerebrum: Necrosis, cortical, laminar, sub-acute, multifocally extensive, with mild eosinophilic meningoencephalitis, Large White, porcine.

Conference Comment: The pathophysiology of excessive dietary sodium intake without access to adequate free water is well documented in pigs. With developing hypernatremia, brain cells have a compensatory mechanism of rapidly accumulating electrolytes (sodium, potassium and chloride) to prevent the movement of intracellular fluid into the hyperosmolar cerebrospinal fluid and plasma. This mechanism prevents intracellular water from following concentration gradients that would cause cellular dehydration and, consequently, brain shrinkage. Such a situation could lead to disruption of cerebral blood supply in the calvarium and cerebral infarction, inhibition of energy pathways, or cerebral edema.

When pigs are allowed access to water again, they often overindulge and sodium concentration in extracellular fluid is lowered rapidly through dilution effect. Intracellular osmolarity is now greater and water moves into neurons along a concentration gradient. Cerebral edema and the clinical signs of salt toxicity, or water deprivation syndrome, are then manifested. In known cases of water deprivation, access to water or replacement with intravenous fluids should be gradual. Despite these measures, the prognosis is generally poor.

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SLIDE 79
CONFERENCE 20 /CASE III – 37236 (AFIP2996898)

Signalment: 7 yr old, female, Jersey, bovine.

History: Sudden onset of depression, ataxia, hypermetria and stupor progressing to bilateral blindness with constricted pupils. Death occurred 36 hours after symptomatic treatment.

Gross Pathology: Brain: On cut surface of thalamus there was a moderately well circumscribed approximately 20mm diameter, grey, mottled soft, depressed area. There were multiple bilateral locally extensive dark red poorly circumscribed soft foci in the cerebral cortex at the level of the midbrain and basal ganglia. There was multifocal thickening and opacity of the meninges with prominent blood vessels.
Other organs: The lungs were firm, multinodular and had a yellow/red mosaic appearance on cut surface affecting 90% of the lung mass. There was marked thickening of the interlobular septae and subpleural space. The endometrium was diffusely thickened, tan and friable with few visible caruncles and no foetus.

Histopathologic Description: Tissues examined: Brain, spleen, liver, heart, lung, kidney, mesenteric lymph node, uterus, abomasum, small and large intestine. The most significant lesion within all of the tissues relate to the severe thromboembolic necrotising vasculitis. This is particularly well demonstrated in the submitted section of cerebrum and thalamus. In these sections there is a generalised severe vasculitis with occasional thrombi and fibrinoid necrosis of vessel walls. These are most severe where meningeal vessels run deeply into the sulci. Surrounding these are perivascular infiltrates of inflammatory cells mostly comprised of macrophages with lesser numbers of neutrophils. There are locally extensive areas of necrosis, oedema and fibrin deposition surrounding blood vessels with large numbers of 4-8 µm in width, non-septate, non-dichotomous branching organisms (Zygomycete). These are also visible within affected vessels/thrombi with PAS staining technique.

Contributor's Morphologic Diagnoses: 1. Thalamus, cerebrum: Meningoencephalitis, necrotising, thromboembolic, acute, severe, locally extensive with malacia, vasculitis with fungal hyphae, Jersey, bovine, etiology Zygomycete (probably *Mortierella wolfii*).
2. Uterus: Endometritis, necrotising, thromboembolic, severe, subacute, diffuse with hyphae.
3. Lung: Interstitial Pneumonia, necrotising, thromboembolic, pyogranulomatous, severe, subacute, diffuse, lobular, with hyphae.
4. Liver. Vasculitis, necrotising, severe, acute, multifocal with perivascular hepatocyte necrosis and hyphae.
5. Spleen. Splenitis, Fibrinonecrotic, severe, acute, focal with hyphae.

Contributor's Comment: The aetiological agent is likely to be *Mortierella wolfii*, a common cause of bovine abortion in New Zealand. These are usually sporadic and have been associated with feeding of poorly ensiled silage. Pathogenesis is still poorly understood but infection is believed to gain entry by ingestion which results in haematogenous spread of the organism to various tissues including the lungs and placenta, the latter resulting in abortion. Approximately 20% of affected cows develop fatal thromboembolic pneumonia. Neurological symptoms are a rare presentation and

there is only one unpublished report of gross lesions in the brain with natural infection in cattle. However, fetal lesions will sometimes include encephalitis, hepatitis and splenitis.

AFIP Diagnosis: Brain, cerebrum: Vasculitis, necrotizing, thromboembolic, acute, multifocal, severe, with fungal hyphae, Jersey, bovine.

Conference Comment: The contributor provides an excellent case of a necrotizing mycotic vasculitis with striking cerebral lesions. Conference attendees agreed that the lesions strongly resemble those of thrombotic meningoencephalitis (TME) caused by *Histophilus somni*. Only after fungal hyphae were recognized within the blood vessels and cerebrum did attendees consider another cause. Attendees considered the morphology to be most consistent with a *Zygomycete*.

Zygomycetes are ubiquitous saprophytic molds associated with water, soil, decaying matter, and substrates high in carbohydrates. Zygomycetes are opportunistic invaders; predisposing factors include antibiotic therapy, ruminal acidosis (grain overload), reflux of acidic abomasal contents and erosive viral disease such as infectious bovine rhinotracheitis (IBR) or bovine pestivirus (BVD-mucosal disease). Focal or disseminated infections can occur in cattle of all ages.
Zygomycotic hyphae are described as 3-25 µm wide, rarely septate, with thin non-parallel walls, non-dichotomous irregular branching, and focal bulbous dilatations. Frequently, hyphae are collapsed, folded and twisted and resemble a ribbon. Hyphae may be angiotropic, and perineural invasion is common.

In this case, hyphae stained very poorly with GMS, which was considered unusual in the experience of those in attendance. The hyphae are easily visualized; however, when stained with PAS.

For additional lesions caused by Zygomycetes, readers are encouraged to review conference 12, case 4, 2005-2006 for a case of mycotic rumenitis in a calf.

Readers are also encouraged to review conference 2, case 4, 1989-1990 for a case of thrombotic meningoencephalitis (TME) in a bovine. As a point of clarification, TME is formerly known as thromboembolic meningoencephalitis (TEME) and is caused by *Histophilus somni*, also formerly known as *Haemophilus somnus*.

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SLIDE 80
CONFERENCE 20 / CASE IV – 04-2379-5 (AFIP 2983612)

Signalment: Three month-old pig.

History: Pig coming from a feeder pigs operation in which 1.5% of the animals had a posterior paralysis.

Gross Pathology: Gross lesions were not observed.

Laboratory Results: A teschovirus was isolated from the spinal cord.

Histopathologic Description: Microscopic lesions were confined to the central nervous system. They were located mainly in the gray matter of the medulla and ventral horns of the spinal cord. In the spinal cord sections submitted, there is a perivascular cuffing and neuropil infiltration by lymphocytes, plasma cells and histiocytes. Glial nodules mixed with lymphocytes and plasma cells, and neuronal degeneration are also present. Less severe focal lesions are sometimes present in dorsal horns.

Contributor's Morphologic Diagnosis: Nonsuppurative poliomyelitis

Contributor's Comment: Lesions of nonsuppurative polioencephalomyelitis affecting mainly the medulla and ventral horns of the spinal cord are very suggestive of a teschovirus (enterovirus) encephalomyelitis (1, 2). Lesions caused by teschoviruses are principally located in the gray matter of the brain stem from the hypothalamus through the medulla, and ventral horns of the spinal cord (2). In the present case, the presumptive diagnosis was confirmed by the culture of a porcine teschovirus from the spinal cord. The porcine teschoviruses (formerly porcine enteroviruses CPE group I) comprise at least eleven distinct serotypes (3). The most severe form of polioencephalomyelitis caused by these viruses is called Teschen disease, and is produced by highly virulent serotype 1 strains (4). Milder forms of the disease called Talfan disease, enzootic paresis and North American encephalomyelitis are caused by

less virulent serotype 1 strains or other serotypes of porcine teschoviruses (4). All forms of the disease can affect pigs of all ages.

The hemagglutinating encephalomyelitis virus (HEV) causes a nonsuppurative polioencephalomyelitis localized mainly in the pons, medulla and dorsal horns of the upper spinal cord (5). Affected pigs are usually less than three weeks of age (2, 5). The infection causes two clinical syndromes which may coexist in the same herd. One of them is characterized by anorexia and vomiting with some of the affected pigs becoming emaciated. This syndrome is called vomiting and wasting disease (2, 5). The other syndrome is characterized by nervous signs and lesions which may resemble those caused by teschoviruses. Demonstration of the viruses is essential for the differential diagnoses.

Several viruses cause a nonsuppurative encephalomyelitis in pigs, but the location of the lesions observed in the present case is very suggestive of teschovirus infection.

AFIP Diagnosis: Spinal cord: Poliomyelitis and radiculoneuritis, nonsuppurative, multifocal, sub-acute, moderate with mild non-suppurative meningitis, porcine.

Conference Comment: Attendees noted lesions affecting predominantly the ventral horns of the spinal cord gray matter and agreed that the pattern supports the diagnosis of Teschen disease. Although not visible in all sections, attendees also noted non-suppurative inflammation affecting adjacent ventral nerve root ganglia.

As mentioned by the contributor, there are a number of viruses which can cause non-suppurative encephalomyelitis in pigs. Below is a list of viral agents which can cause inflammatory disease of the nervous system in pigs, with additional information adopted from references 1 and 9:

- Porcine Enteric Picornaviruses
  - Genus: *Teschovirus* (10 serotypes) polioencephalomyelitis associated with highly virulent PTV serotype 1 (Teschen disease); and less virulent PTV serotype 1 (Talfan disease and benign epizootic paresis)
    - CNS lesions especially numerous in the ventral columns of the spinal cord, cerebellar cortex and brain stem; neurons show progressive diffuse chromatolysis, focal gliosis and perivascular lymphocytes, especially over the cerebellum

  - Genus: *Enterovirus* (2 serotypes) no neurological disease has been observed

- Encephalomyocarditis Virus (family: *Picornaviridae*; genus: *Cardiovirus*): Congestion with meningitis, perivascular infiltration with mononuclear cells and some neural degeneration, nonsuppurative encephalitis and myocarditis has been described in swine fetuses with natural EMCV infection

• Porcine Adenovirus: Meningoencephalitis with perivascular infiltration and microglial nodule formation

• Pseudorabies (porcine herpesvirus 1): nonsuppurative meningoencephalitis and ganglioneuritis in the gray and white matter, also cervical and thoracic spinal cord, mononuclear perivascular cuffing and meninges thickened by mononuclear cells; intranuclear inclusion bodies in neurons, astrocytes and oligodendroglia

• African Swine Fever (family: *Asfarviridae*, genus: *Asfivirus*): Intense congestion in the meninges, choroid plexus, and encephalon

• Classical Swine Fever (family: *Flaviviridae*, genus: *Pestivirus*): Hyperemia of the brain blood vessels

• Porcine Cytomegalovirus (porcine herpesvirus 2): Hemorrhage and gliosis occur throughout the central nervous system, with a predilection for the choroid plexus, cerebellum, and olfactory lobes

• Hemagglutinating Encephalomyelitis Virus (family: *Coronaviridae*): nonsuppurative encephalomyelitis; perivascular cuffing; gliosis, neuronal degeneration, most pronounced in the gray matter of the pons Varolii, medulla oblongata, and dorsal horns of the upper spinal cord; neuritis of the peripheral sensory ganglia, particularly the trigeminal ganglia, also occurs

• Japanese Encephalitis and West Nile Viruses (family: *Flaviviridae*, genus: *Flavivirus*): Diffuse nonsuppurative encephalitis and spondylitis
• Porcine Reproductive and Respiratory Syndrome Virus (family: *Arteriviridae*): mild lymphohistiocytic leukoencephalitis or encephalitis involving the brainstem, cerebellum or cerebrum; multifocal gliosis; perivascular cuffing by lymphocytes and macrophages

• Eastern Equine Encephalomyelitis Virus (family: *Togaviridae*, genus: *Alphavirus*): encephalitis; perivascular cuffing by neutrophils early in disease followed by lymphocytes; neuronal necrosis; neuronophagia; glial nodules and malacia

• Rabies (family: *Rhabdoviridae*; genus: *Lyssavirus*): CNS changes range from mild vasculitis and focal gliosis in the brain to extensive meningoencephalitis and marked neuronal degeneration in the brain and spinal cord; Negri bodies are frequently absent in rabid swine

• Paramyxoviruses:
    - Blue eye paramyxovirus (BEP): reported only in Mexico; encephalitis and corneal opacity in piglets
    - Menangle virus: only one known outbreak; Australia 1997; mummified, autolyzed and fresh stillborn piglets with multiple congenital defects and extensive degeneration and necrosis of gray and white matter of the brain

and spinal cord, intranuclear and intracytoplasmic inclusion bodies in the neurons of the cerebrum and spinal cord; nonsuppurative meningitis
- Nipah virus: confined to Southeast Asia, zoonotic, epidemic in 1998-1999 in Malaysia resulting in significant human mortality; in cases with neurological disease there is nonsuppurative meningitis rather than encephalitis

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SLIDE 81
CONFERENCE 21 / CASE I – CPHU (AFIP 2988198)

Signalment: The presented sample is the lung of adult female rat (*Bandicota indica*) captured in the village at southern coast of Sri Lanka.

History: After the Sumatran earthquake on Dec. 26, 2004, the World Health Organization warned against a prevalence of zoonoses in tsumami-striken areas around the Indian Ocean. The Japanese government sent research teams of medical and veterinary scientists to these areas for the investigation of zoonotic diseases in humans and animals.

Gross Pathology: The lungs were diffusely consolidated. A cyst, 7 mm in diameter, containing larvae of *Taenia taeniaeformis* (*Cysticercus fasciolaris*) was found on the left lobe of the liver. Except for these visceral lesions, the rat appeared normal.

Histopathologic Description: Many eggs containing larvae are lodged in the thickened alveolar walls. Most of them seem to be located within alveolar capillaries and a few eggs within the arterioles. A few hatched larvae are phagocytized by multinucleated giant cells in the alveolar lumina. Many macrophages, epithelioid cells, multinucleated giant cells, eosinophils, lymphocytes and plasma cells infiltrate the alveolar walls and interstitium, and they frequently form granulomas around the eggs. Four sections of adult worms are in the small arteries showing severe proliferative endarteritis. Bronchiolar mucosa is thickened, and shows goblet cell hyperplasia and infiltrations of eosinophils.

In the cerebrum, not presented, mild meningitis was observed.

Contributor's Morphologic Diagnosis: Eosinophilic and granulomatous bronchopneumonia due to Angiostrongylus infestation (Pulmonary angiostrongyliasis of rodents)

Contributor's Comment: *Angiostrongylus (A.) cantonensis* lives in the pulmonary arteries and right ventricles of rodents. Copulation occurs in the pulmonary artery and females produce eggs which develop into first stage larvae. They penetrate the alveoli, migrate through the respiratory tract into the alimentary tract and pass out of the definitive host in the feces. They then infect a molluscan intermediate host, usually terrestrial snails such as *Achatina fulica* or aquatic snails such as *Pila* spp. Third-stage larvae emerge a little over 2 weeks later and infect the rodent definitive host, usually when it eats an infected mollusk. At least 24 species of rodents can act as definitive hosts for *A. cantonensis*, including *Rattus norvegicus* and *Rattus rattus*. A wide range of animals may act as paratenic hosts.[1] From the rat intestine, the third-stage larvae embark upon an obligatory migration through the CNS, to which they are carried by the bloodstream. In the brain or spinal cord, third-stage larvae leave the capillaries, wander through tissues apparently at random, moulting twice en route to the subarachnoid space. On reaching the brain or spinal cord surface, fifth-stage larvae attempt to re-enter the central circulation by penetrating veins, and are then carried to their ultimate destination, the pulmonary artery.[2]

A. cantonensis is found in Southeast Asia, the Pacific and Australia, including Polynesia, Indonesia and Papua New Guinea. Because of its lack of host specificity and the mobility of rats, *A. cantonensis* has become established throughout much of the tropical and sub-tropical parts of the world, such as Madagascar, Japan, Egypt, the Ivory Coast of Africa, India, Samoa and Fiji, Cuba, Puerto Rico and southeastern USA. Infection is transmitted to humans and animals by eating raw molluscs (slugs, snails and so on) or raw food that has been contaminated by them.

A. mackerrasae and *A. malaysiensis* are close relatives of *A. cantonensis* and they share virtually the same life cycle, but only *A. cantonensis* has been confirmed to be a human pathogen. We collected the adult worms from the lungs of rats and sent them to the parasitology department. Although the parasites presented here are most probably *A. cantonensis,* a final diagnosis of the subspecies has not been made yet.

AFIP Diagnosis: Lung: Pneumonia, granulomatous and eosinophilic, diffuse, severe, with numerous morulated and larvated eggs and intra-arterial adult metastrongyle nematodes, proliferative histiocytic and eosinophilic endarteritis, bronchiolar epithelial hyperplasia, and mild eosinophilic bronchiolitis, etiology consistent with *Angiostrongylus* sp., greater Bandicoot rat (*Bandicota indica*), rodent.

Conference Comment: The contributor provided an excellent review of *Angiostrongylus cantonensis* infection. Angiostrongylids belong to the Family Metastrongyloidea within the Order Strongylida. Unlike the other strongyle families i.e. the true strongyles and trichostrongyles, metastrongyles have coelomyarian musculature and accessory hypodermal chords. (3)

Within the pulmonary artery, adults measure 125-135 µm in diameter with a 3-5 µm thick smooth, eosinophilic, hyalinized, anisotropic cuticle, coelomyarian musculature that lines a pseudocoelom, accessory hypodermal chords, an intestine composed of a few, large multinucleated cells (known colloquially as a “strongyle gut”) and paired uteri with developing eggs.

Angiostrongylus cantonensis is the most common cause of human eosinophilic meningitis. Most cases of eosinophilic meningitis have been reported from Southeast Asia and the Pacific Basin, although the infection has spread to many other areas of the world, including Africa and the Caribbean. Humans can acquire the infection by eating raw or undercooked snails or slugs infected with the parasite or by eating raw produce that contains a small snail or slug, or part of one. There is some question whether or not larvae can exit the infected mollusks in slime (which may be infective to humans if ingested, for example, on produce). The disease can also be acquired by ingestion of contaminated or infected paratenic animals (crabs, freshwater shrimp). In humans, juvenile worms migrate to the brain, or rarely the lungs, where they eventually die. Clinical symptoms of eosinophilic meningitis are caused by the presence of larvae in the brain and the local host reaction. Symptoms include severe headaches, nausea, vomiting, stiff neck, seizures, and neurologic abnormalities. Occasionally, ocular invasion occurs. Eosinophilia is present in most cases. Most patients recover fully. (4)

Another angiostrongylid of veterinary importance is *Angiostrongylus vasorum*, a parasite of the right ventricle and pulmonary artery of dogs and foxes. The adult worms cause proliferative endarteritis; however, the most severe damage is caused by the eggs and larvae. Eggs lodge in arterioles and capillaries where emerging larvae erupt into alveolar spaces inciting marked fibrosis. Clinical disease is not usually noted until the

chronic stage, which can occur months to years after the initial infection. Fibrosis in the lung can lead to right-sided cardiac failure. It is rare for acute disease to result in death of the dog. (5)

This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP.

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SLIDE 82
CONFERENCE 21 / CASE II – 51307 (AFIP 2985121)

Signalment: Captive-born 8 day old male bottlenose dolphin (*Tursiops truncatus*)

History: Calf was born after prolonged labor and did not nurse until 11.5 hours of age. Animal did well nursing for next seven days. On the eighth day, animal did not nurse well and was repeatedly "disciplined" by dam. Briefly nursed only two more times before dying. Animal was submitted for necropsy examination.

Gross Pathology: Animal was presented in good post-mortem condition. Muscling was good, but subcutaneous and visceral fat stores were reduced. There were numerous recent and remote skin lacerations and abrasions, consistent with "disciplining" behavior. No teeth had yet erupted. The lungs were slightly rubbery and edematous, and there was a small amount of mucopurulent discharge in the trachea. The heart was grossly normal with a patent ductus arteriosus. The surface of the brain was grossly normal.

Laboratory Results: Cultures taken at necropsy produced heavy growths of *Streptococcus zooepidemicus* and *Escherichia coli*.

Histopathologic Description: Included are sections of cerebellum with or without brainstem. Prominence of the external granular layer of the cerebellum is consistent with animal's age. Expanding the meninges and Virchow-Robin spaces are large numbers of macrophages and viable and degenerate neutrophils, with frequent plasma cells and lymphocytes. There are large numbers of intra-lesional large bacterial cocci, appearing within the cytoplasm of macrophages as well as extra-cellularly in pairs and large colonies. Tissue Gram stains reveal large Gram positive cocci. Occasional vessels are thrombosed with fibrin and enmeshed erythrocytes and leukocytes. Inflammation was also present in the choroid plexus and the optic nerve meninges (not submitted).

Septic thrombi were present in the lungs, and bacteria were additionally observed in the adrenals, heart, several lymph nodes, and associated with inflammation in the soft tissue of the umbilicus. Aspirated squamous cells were observed in the lungs, consistent with fetal stress. There was extra-medullary hematopoiesis in the liver and spleen, and lipidosis in the liver and kidneys.

Contributor's Morphologic Diagnosis: Brain: Meningitis, suppurative, diffuse, acute, severe, with myriad intra-cytoplasmic and extra-cellular Gram positive bacterial cocci, consistent with *Streptococcus zooepidemicus*.

Contributor's Comment: Like cattle and horses, bottlenose dolphins have epitheliochorial placentation (http://medicine.ucsd.edu/cpa/index.html), although it is diffuse rather than cotyledonary or microcotyledonary. As such, there is no transfer of maternal antibodies in the dolphin fetus, and neonates are dependent on colostrum for passive transfer of pre-formed antibodies. In primates, rodents, and other animals with hemochorial placentation, there is accumulation of maternal IgG but not other classes of antibody in the fetus to levels approaching those of the dam[1]. In cats and dogs, where there is endotheliochorial placentation, transfer of IgG is less efficient, and antibody concentrations may only be 5-10% of those of the dam.

Colostrum contains primarily IgG (65-90%), with lesser amounts of IgA and some IgM and IgE. Unlike milk, the IgG in colostrum is derived predominately from serum, rather than local production by resident plasma cells in the mammary gland. Colostrum additionally contains trypsin inhibitors which, in combination with the immaturity of the digestive enzymes in the neonate, allow much of the antibody to bypass degradation. Specialized FcRn receptors, MHC class Ib heterodimers, on the apical surface of the enterocytes bind immunoglobulin, and it is taken into the cell by pinocytosis, later to enter lacteals or capillaries. In general, permeability is highest immediately after birth, and declines after about six hours to very low levels by 24 hours. Gut closure is generally considered complete by 48 hours.

This particular dolphin had clinical and pathological evidence of intra-uterine stress, and did not nurse for nearly twelve hours post-partum. Because the ability to passively absorb pre-formed antibodies declines rapidly from birth, the delay in nursing likely

contributed to at least partial failure of passive transfer (FPT). In domestic animals, septicemia as a sequela of FPT commonly occurs within the first 5-8 days post-partum, and may be manifest as polyarthritis, pneumonia, or meningitis [2]. The umbilicus is often the source for such infections. The bacteria cultured in these infections are often derived from the local environment or are commensals.

Strep. *zooepidemicus* (*Strep*. *equi subspecies zooepidemicus*, Lancefield group C) has been isolated from septicemic foals[2], and has been identified as a cause of mortality in captive *Tursiops* species[3]. In horses, *Strep*. *zooepidemicus* is a mucosal commensal and opportunistic pathogen, causing suppurative infections in joints, lymph nodes, nasal cavities and lungs in animals stressed by viral infection, heat or injury[4]. Additionally, *Strep*. *zooepidemicus* has been associated with meningitis in humans as a suspected zoonosis[5].

AFIP Diagnoses: Brainstem; cerebellum: Leptomeningitis, histiocytic and neutrophilic, acute, diffuse, severe, with myriad short bacilli, bottlenose dolphin (*Tursiops truncatus*), cetacean.

Conference Comment: The similarity of this case of apparent partial failure of passive transfer in a dolphin calf to cases in bovine calves is interesting in light of the close phylogenetic relationship between dolphins (and other cetaceans) and artiodactyls such as cattle.

Gram-stained sections examined at the conference demonstrated that the bacteria are short gram-negative bacilli compatible with *Escherichia coli,* which was cultured. No gram-positive bacteria were found. The case was further studied in consultation with AFIP's Department of Infectious and Parasitic Diseases. They found only gram-negative bacilli. The bacteria were compared to a case of known cultured *E. coli* in a human kidney and were found to be very similar. Their conclusion was that the bacteria are consistent with *E. coli.* They noted that the apparent absence of the cultured *Streptococcus* might be a result of rarity or sampling. *E. coli* is a common cause of neonatal septicemia in domestic animals and humans and is one of the first potentially pathogenic bacteria to which neonates are exposed following parturition.

The contributor noted an excellent web-page on comparative placentation provided by Dr. Kurt Benirschke and the University of California at San Diego. The web address is http://medicine.ucsd.edu/cpa/authorfs.html

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SLIDE 83
CONFERENCE 21 / CASE III – 399-04 (AFIP 3003942)

Signalment: 4 weeks old, female, Ayshire, bovine

History: The calf was reared in a calf-rearing farm where there had been problems with acute tympany. Calves were brought to this farm at around two weeks of age. A week after arrival several calves were anorexic, lethargic and bloated. The calf was found dead a few days later. The calves were fed milk replacer *ad libitum*. The milk replacer was mixed with 35 – 40°C water and added to large containers in which it gradually cooled down to environmental temperature.

Gross Pathology: The calf was in good nutritional condition but severely dehydrated. The abomasum was markedly dilated and contained gas and a moderate amount of blood stained liquid. The abomasal wall was edematous with multifocal hemorrhages and mucosal erosions. On the serosal surfaces, especially on the abomasum, there was fibrinous exudate. The rumen contained partly digested roughage and a large amount of oil. The small intestine was congested. No other specific lesions were observed.

Laboratory Results: Bacteriology and PCR: *Clostridium perfringens* was isolated from the abomasum and small intestine and PCR was used to specify it as type A.

Histopathologic Description: Abomasum: Within the mucosa there is marked diffuse congestion and moderate inflammatory infiltrate consisting mainly of neutrophils and lesser number of lymphocytes that multifocally expand and extend to the submucosa and through the muscle layer to the serosa. There is nearly diffuse superficial necrosis of the mucosa with multifocal hemorrhages that extend randomly deeper into the abomasal wall with disruption of the serosa and adhered fibrinous exudate admixed with neutrophils. Closely attached to the mucosal epithelium and within the intraluminal inflammatory exudate there are numerous rod-shaped bacteria (8-10 _m long).

Contributor's Morphologic Diagnosis: Abomasum: Abomasitis, hemorrhagic and suppurative, acute, diffuse, severe with necrosis and numerous rod-shaped bacteria.

Contributor's Comment: Abomasal tympany with inflammation is an emerging disease in calves. In Finland it is most prevalent in calves fed *ad libitum* milk replacer. Severe acute bloat and high mortality are typical for the disease. Typically *C. perfringens* type A or *Sarcina*-like bacteria alone or together are found in these cases. Many infectious agents are capable of causing abomasal lesions, however, only infection with *C. septicum-, C. perfringens* type A- and *Sarcina*- like bacteria are associated with moderate to severe tympany with necrotizing abomasitis in calves. Braxy, which is caused by *C. septicum,* is associated with frozen feed (1,2).

Clostridial organisms are part of the normal bacterial microflora of the gastrointestinal tract of ruminants and they are widely found in the environment. *C. perfringens* type A is the most common one of the five types of *C. perfringens* and the only one that is found both in the intestinal tract of ruminants and soil. The significance of this bacterium as a pathogenic organism in bovine enteric diseases is still not clear although it has been reported as the cause of enterotoxemia syndrome in Belgian Blue calves and clinical disease has been produced by intraruminal inoculation (3,4,5). *C. perfringens* strains are divided into five types on the basis of their production of four major exotoxins. All these five *C. perfringens* types can also produce enterotoxin during sporulation. This enterotoxin is the major factor in food poisonings in humans caused by *C. perfringens* type A containing the *cpe*- gene and it has also been isolated from foals and pigs with diarrhea. _-toxin is the major exotoxin produced by type A strains but some of these strains can also produce _2- toxin. This novel _2- toxigenic *C. perfringens* has been isolated from piglets and horses with enteric disease. In cattle it is still controversial how big a role these toxins have in the pathogenesis of abomasitis (3,6,7). *Sarcina*-like bacteria are part of the normal bacterial flora in the stomachs of monogastric animals and they are also very likely normal inhabitants of the rumen of cattle. In healthy lambs *Sarcina*-like bacteria have not been isolated from the abomasum. In lambs with abomasitis *Sarcina sp.* have been isolated with *C. fallax* and *C. sordellii.* (8,9,10).

Since a small amount of *C. perfringens* type A and *Sarcina*-like bacteria reside normally in the gastrointestinal tract of cattle, a change towards their excessive growth has to happen for them to cause clinical disease. Because bacterial growth in the rumen often precedes abomasitis, it is critical to prevent the milk replacer from entering the forestomachs during the first weeks of life. This can occur if there is insufficient closure of the reticular groove while feeding or if too much milk replacer is fed at a single time. Management factors are crucial in preventing the growth of other bacteria, for example *Lactobacillus*- or *Clostridium spp.* in the rumen and/or abomasum, that can contribute to the growth of *C. perfringens* type A and *Sarcina*-like bacteria in the gastrointestinal tract. The problems seem to be associated with poor hygiene, high storage temperature and inadequate cooling of the milk replacer, sudden change of feed and feeding of low quality milk replacer based on plant proteins (1,10,9,11).

AFIP Diagnosis: Abomasum: Abomasitis, necrotizing, fibrinosuppurative and hemorrhagic, acute, transmural, severe, with numerous mucosa-adherent bacilli, Ayshire, bovine.

Conference Comment: Of the five toxigenic types of *Clostridium perfringens*, designated types A-E based on their production of the four major exotoxins, *C. perfringens* type A is the most common. Enteric proliferation of this type is recognized as a cause of hemorrhagic enteritis in several species including black-footed ferrets, chickens, horses, pigs, lambs, goats, calves, and humans. Clinical signs and histologic lesions are caused by a lecithinase known as alpha toxin, the major toxin produced by *C. perfringens* type A. Alpha toxin is both hemolytic and cytotoxic by virtue of its effects on cell membranes. Enterotoxin, the principal toxin involved in foodborne illness, is released upon lysis of sporulating cells. (1,11,12)

Within the last few years another *Clostridia perfringens* toxin identified as ß2 has been described. The biologic activity of the ß2-toxin is similar to that of the ß1-toxin. The gene that codes for ß2-toxin is referred to as cpb2. All cpb2 positive strains of *C. perfringens* do not, however, produce ß2 toxin *in vitro*. Cpb2 positive *C. perfringens* has been found in various animal species with and without enteric disease. Some hypothesize that predisposing factors such as dietary change and antibiotic therapy can result in selection of ß2-toxigenic *C. perfringens* which may lead to enteritis or enterotoxemia. (13) Below is a simple chart of the five types of *C. perfringens*, the toxins they produce and diseases they cause.

Clostridium perfringens - Types, toxins and diseases

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

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

Braxy, caused by *Clostridium septicum*, is another disease of lambs and calves resulting in abdominal distention, abomasitis, and high mortality. The factors that initiate bacterial invasion are unknown but are thought to be secondary to injury by frozen, spiny or coarse ingesta. Microscopic findings characteristic of braxy include abomasal edema and emphysema, areas of coagulative necrosis bordered by neutrophils (occurring primarily in the submucosa but can be transmural),vasculitis, thrombosis, hemorrhage and the presence of Gram-positive bacilli.

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SLIDE 84
CONFERENCE 21 / CASE IV – Ex50c (AFIP 2987649)

Signalment: A 10 year-old male Pinscher, canine

History: A 10 year-old male Pinscher presented with a 4-month history of intermittent pain of the left forelimb. The dog occasionally lifted the affected limb and avoided using it, but was not consistently lame. No abnormalities were found on palpation, radiography and computerized tomography and a diagnosis was not made at the initial examination. The dog was re-examined a month later at which point a small mass was palpated in the region of the brachial plexus. Exploratory surgery revealed a localized fusiform swelling in the C7 spinal nerve at the level of the brachial plexus. The swelling was 6 cm long and 1.5 cm diameter at its widest point. Adjacent nerves adhered to the surface of the mass. The forelimb was amputated. At the time of case submission, 3 months following the operation, the dog is in good health.

Gross Pathology: On cut section the mass was pale and hard.

Histopathologic Description: Multiple sections of a mass surrounded by a fibrous capsule. Multifocally neoplastic cells extend through the capsule and form small nodules either in direct continuity with or adjacent to the main mass. Within the tumor, the cellularity and pattern of arrangement is variable. The most widespread element consists of oval to spindle cells in moderately to densely cellular sheets and fascicles with a small to moderate amount of collagenous stroma. Neoplastic cells have eosinophilic cytoplasm with indistinct margins and oval vesicular nuclei with one or more nucleoli. A trend to whorl formation is observed in a region of neoplastic proliferation immediately adjacent to an area with relative preservation of nerve fibers. Here, neoplastic cells multifocally form a few concentric laminae around a central axonal core. Small foci where the neoplastic cells assume an epithelioid appearance or are separated by large amounts of mucinous extracellular matrix are present.

There are several areas with smooth homogenous and eosinophilic stroma, suggestive of neoplastic osteoid with entrapped oval to polygonal neoplastic cells, either singly or in groups. Neoplastic cells in this area are spindled, irregular and polygonal with large vesicular nuclei and one or more prominent nucleoli. There is anisokaryosis and the morphology is reminiscent of osteosarcoma.

An anaplastic epithelial element is present and consists of groups of disorganized glandular and cystic structures lined by cuboidal, columnar, squamous, or attenuated cells with moderate to abundant occasionally vacuolated cytoplasm and large round to

oval vesicular to finely granular nuclei with one or more nucleoli. A few small clusters of squamous epithelial cells with localized keratinization in continuity with the neoplastic glandular structures are found (not present in all sections). Throughout the mass mitotic figures are uncommon and single necrotic cells are found in low number. There is multifocal mild lymphocytic and lesser plasmacytic infiltration of the tumor and the surrounding connective tissue.

Contributor's Morphologic Diagnosis: Malignant peripheral nerve sheath tumor with divergent differentiation

Contributor's Comment: Tumors of the peripheral nervous system (PNS) are uncommon in domestic animals, with the exception of multicentric schwannoma / neurofibroma, in cattle which is usually incidental (1, 2). Most cases are encountered in dogs, where the most common sites of origin are the nerve roots of the brachial plexus, often C6-C8. A typical history in these cases is of slowly progressive lameness, pain and muscle atrophy in the affected limb. Compression of the cranial thoracic spinal cord may lead to Horner's syndrome with mild upper motor neuron and general proprioceptive signs in the ipsilateral pelvic limb. Tumors of thoracic and lumbar spinal nerves tend to present with signs of acute spinal cord compression. The trigeminal nerve is the most commonly affected cranial nerve and a mass at this location may manifest as unilateral atrophy of the muscles of mastication and loss of facial sensation. If the tumor impinges on the CNS, brain stem signs may ensue (1).

The typical gross appearance of tumors arising in peripheral nerves is grayish fusiform thickening of several nerve trunks, sometimes with fusion into a common tumor mass (1, 2). Many PNS tumors in the dog are anaplastic and malignant as evaluated by histologic criteria and biologic behavior. Poorly differentiated PNS tumors are best designated malignant peripheral nerve sheath tumor (MPNST). Determination of the cell of origin – schwannian, perineurial, fibroblastic or other is often impossible in routine sections (1). Schwannoma may be used to describe well-differentiated variants (2). Both benign and malignant tumors may show divergent differentiation (2). In human MPNST, cytologic and histologic pattern variation is the rule. Distinctive features found in some cases include melanin pigmentation and divergent mesenchymal or epithelial differentiation (3). The most common secondary elements are cartilage and bone (4). In the dog differentiation to primitive cartilage, bone, skeletal muscle, keratinizing squamous epithelium, glandular epithelium and melanin production have been observed (1, 4).

The MPNST in this case has mesenchymal and epithelial components, both of which are morphologically malignant - osteosarcoma and adenocarcinoma respectively. A MPNST arising in LI and L2 with similar histologic features was reported in a dog (2). Immunohistochemical staining of that tumor showed that the mesenchymal cells stained diffusely for vimentin, S-100 and NSE while the epithelial cells were positive for pancytokeratin only (4). In general, immunohistochemistry is not considered reliable for the diagnosis of peripheral nerve sheath tumors (2).

The theory behind the divergent differentiation in MPNST is that migratory stem cells of the neural crest differentiate to neuroectodermal structures (which give rise to melanocytes, ganglion cells, Schwann cells etc.) and multipotential ectomesenchymal cells (which give rise to leptomeninges, bone, cartilage, and muscles of the head and neck). Studies have suggested that the epithelial component is not heterotopic but could arise from Schwann cells or mesoectodermal cells intrinsic to peripheral nerves. In humans, the presence of divergent differentiation warrants a poor prognosis (4).

AFIP Diagnosis: Malignant peripheral nerve sheath tumor with adenocarcinomatous divergent differentiation, Doberman Pincher, canine.

Conference Comment: The contributor has provided a rare case and a thorough review of malignant peripheral nerve sheath tumors with divergent differentiation. Attendees recognized the spindle shape and characteristic whorling pattern of a peripheral nerve sheath tumor but were surprised by the presence of the epithelial component. No unequivocal osteoid was identified in the sections examined at the conference.

Readers are encouraged to review conference 17, case 3, 2005-2006 for a case of a peripheral nerve sheath tumor of the skin and subcutis of dogs.

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SLIDE 85
CONFERENCE 22 / CASE I – EPL 3-25-05 (AFIP 2986960)

Signalment: One-year-old, golden retriever, canine (*Canis familiaris*), phenotypic female.

History: Canine presented for a routine ovariohysterectomy.

Gross Pathology: An enlarged clitoris was noted on physical exam. Two intraabdominal gonads were present and situated in the correct anatomical location expected for normal ovaries. One gonad appeared normal. The other gonad is smaller and has a firm cord of tissue on one edge resembling an epididymis. The uterine horn adjacent to the abnormal gonad exhibited segmental hypoplasia/aplasia. The remaining uterus appeared normal.

Histopathologic Description: Histologically the abnormal gonad contained both male and female characteristics. On one edge of the gonad, was a thick cord of fibromuscular tissue containing several tubular structures lined by cuboidal to low columnar epithelium with stereocilia. This structure appeared consistent with an epididymis. The majority of the gonad consisted of sheets or small aggregates of large polygonal cells containing eosinophilic vacuolated cytoplasm (consistent with interstitial cells) interrupted by occasional tubular structures that did not resemble normal ovarian follicles or seminiferous tubules. These tubules were lined by polygonal to spindle shaped cells containing vacuolated basophilic cytoplasm and exhibited a mild degree of anisokaryosis (atypical sex cord stromal cells). In several tubules, these cells appeared consistent with Sertoli cells looking similar to atrophied seminiferous tubules. A single layer of flattened cuboidal epithelium lined the external surface of this gonad (ovarian surface epithelium) with numerous tubular infoldings into the underlying superficial stroma (subsurface epithelial structures). The contralateral gonad was histologically unremarkable appearing consistent with a normal canine ovary (not present on slide).

Contributor's Morphologic Diagnosis: Ovotestis, unilateral

AFIP Diagnoses: 1. Gonad: Ovotestis, Golden Retriever, canine.
2. Uterus: Essentially normal tissue.

Conference Comment: The gonad contains both ovarian and testicular tissue. Ovarian features include the subsurface epithelial structures (SES) and surrounding ovarian bursa (not present in all slides). Testicular features include an epididymis, tubular structures resembling seminiferous tubules lined by Sertoli-like cells and abundant eosinophilic polygonal cells resembling interstitial or Leydig cells. In some sections of the gonad there is an expansile focus of sex cord germinal cells which may represent the early development of an interstitial cell tumor. Additionally, some slides contain a region of highly convoluted veins suggestive of pampiniform plexus, which is further evidence of testicular development.

Normal sexual differentiation in mammals requires successful completion of a series of consecutive steps under genetic control. These steps are marked by the establishment of chromosomal sex, gonadal sex, and phenotypic sex. Intersexuality is a general term currently used to describe ambiguity among any of these levels of development.

Chromosomal sex is normally determined at fertilization by the formation of either an XY or XX zygote. Studies in mice indicate that expression of the *Sry* (sex-determining region Y) gene located on the Y chromosome is responsible for initiating the cascade of events that are responsible for testicular differentiation. *Sry* also directs the differentiation of germ cells to form Sertoli cells in the developing testis. Sertoli cells are responsible for the secretion of antimüllerian hormone (AMH) which results in regression of the Müllerian duct, which normally develops into the oviduct, uterus and upper part of the vagina in female embryos. Cells in the interstitial space surrounding the developing testis cords differentiate into testosterone-producing Leydig cells. Testosterone, converted to the biologically active form dihydrotestosterone by the enzyme 5-alpha reductase, allows development of the vas deferens and epididymides from the Wolffian ducts and induces the development of the prostate gland and penis. (5) In the absence of the Y chromosome and *Sry* gene, the default pathway to female gonadal sexual differentiation is initiated. Clitorimegaly, as seen in this case, is a characteristic feature of hermaphroditism and develops under the influence of testosterone.

Hermaphrodites are classified according to the morphology of the gonads present. A true hermaphrodite has at least one gonad containing ovarian and testicular tissue (i.e. an ovotestis) or has one male and one female gonad. A pseudohermaphrodite has gonads of one sex and accessory reproductive organs of the opposite sex. A male pseudohermaphrodite has testes and female accessory sex organs; a female pseudohermaphrodite has ovaries and male accessory reproductive organs.

Another well-described anomaly of development is freemartinism. Freemartinism is an abnormality of chromosomal sex, in contrast to true hermaphrodism and pseudohermaphrodism which are abnormalities of gonadal sex and phenotypic sex, respectively. (3)

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 AMH from the male fetus to influence the female fetus. This suppresses female genital development and allows male structures 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. (1,2)

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SLIDE 86
CONFERENCE 22 / CASE II – 05-1467 (AFIP 2984137)

Signalment: 16 months old, intact female, Golden Retriever, canine (*Canis familiaris*)

History: During routine spaying, the submitting veterinarian noticed a mass in one uterine horn (side not specified). This was sent to the surgical biopsy service of the University of Tennessee for histological evaluation.

Gross Pathology: The submission consisted of a segment of uterine horn which was focally thickened by a firm swelling measuring 2.5cm long and 2cm in diameter. This was flanked by grossly normal uterine horn. On cut section through the swelling, the uterine lumen was much narrower than normal (ranged from 2-5mm) and the surrounding uterine wall was circumferentially and uniformly expanded by an inner pale tan/white layer which was 2mm thick and an outer homogeneous, tan layer up to 6mm thick (Fig 1. Bar = 1cm)

Histopathologic Description: The slide contains a transverse section of uterus (taken through the uterine horn) in which there is circumferential expansion of the endometrium by two distinct layers. The layer adjacent to the myometrium consists of fairly uniformly dilated glandular structures, corresponding to cystic hyperplasia of the basal zone of the endometrium. These glands are lined by a single layer of cuboidal to attenuated epithelial cells. Most are empty but some contain sparse eosinophilic, proteinaceous material, erythrocytes and necrotic leukocytes. This glandular layer surrounds a much thicker layer composed of thin, anastomosing trabeculae and papillary projections of fibrovascular connective tissue covered by a single layer of cuboidal to columnar epithelial cells. The trabeculae form a meshwork which narrows the uterine horn lumen and corresponds to hyperplasia of the crypt (glandular) epithelium. The epithelial cells have moderate amounts of sometimes highly vacuolated, bright pink cytoplasm with prominent apical blebbing and basally located nuclei. The crypts contain basophilic,

mucinous material (mucus). The meshwork immediately surrounding the lumen is largely necrotic, with multifocal mineralization, correlating with lesion regression.

Contributor's Morphologic Diagnoses: Uterine horn: Pseudocyesis (pseudopregnancy) site

Contributor's Comment: Pseudocyesis (pseudopregnancy; false pregnancy) is still a fairly poorly understood physiological condition. At least historically the term pseudopregnancy is confusing, especially in the bitch. This is because, in other species, "pseudopregnancy" has been traditionally used to describe lengthening of a normally short luteal phase following a sterile mating; the corpora lutea (CL) then persist for a length of time similar to pregnancy. The bitch is different because the luteal phase is the same length whether pregnant or not. The confusion is also partially due to some authors specifically using the term to describe the normal luteal phase which occurs in the bitch (1). It is now generally agreed that it should be reserved for those times when signs of late pregnancy/early lactation occur in the absence of a conceptus (2).

In this case, the uterine lesion was grossly and microscopically characteristic of that described in the literature for pseudopregnancy (3). Similar histological features were described in a subgroup of 24 bitches with a history of false pregnancy (2). This subgroup was composed of animals at the peak of their clinical signs. All had large CLs. We had no specific description of the ovaries (ovarian tissue was not submitted) and no pertinent clinical signs were mentioned by the referring veterinarian. However, it is recognized that the intensity of signs can be variable. While signs may be conspicuous in some bitches, others may have no symptoms at all, the latter known as covert pseudopregnancy (4). The histological features of pseudopregnancy recapitulate the placental zone in the canine gravid uterus but there are some important differences. In the pseudopregnant state the fetal membranes are absent and, since there is no chorion, the endometrium remains intact. The crypt zone visible in this case would have been completely lost in the truly gravid state since the canine placenta is endotheliochorial (2). Moreover, this particular animal had never been bred.
The original hypothesis proposed that pseudopregnancy was caused by overproduction of progesterone or abnormal persistence of the CL. This premise was challenged by one study which found no difference between circulating progesterone levels in non-pregnant bitches, regardless of whether or not they had symptoms consistent with pseudopregnancy (1). The advent of a canine prolactin assay in the 1970s confirmed that, during late pregnancy and in lactation, prolactin levels rose in conjunction with a sharp drop in progesterone levels. Since inhibitors of prolactin are therapeutically successful, it is generally now accepted that increased prolactin levels are key to the development of pseudopregnancy (3). Sex steroids (estrogens, androgens and progestins), which inhibit pituitary release of prolactin, also suppress the condition (1), indicating pituitary dysfunction. However, some propose other additional underlying factors to explain why the condition only occurs in some bitches, despite fairly similar hormonal patterns. For instance, it seems likely there are genuinely predisposed breeds, although this is poorly documented in the literature (4). Furthermore, susceptible bitches tend to have a high recurrence rate in succeeding estrus cycles

which may involve increased peripheral sensitivity to prolactin (4). It is also notable that, in humans, there is variation in the molecular composition of prolactin, with different forms having different degrees of hormonal potency. Since this heterogeneity has also now been demonstrated in the dog, it may ultimately help to clarify the exact role of prolactin in canine pseudopregnancy.

AFIP Diagnosis: Uterus, endometrium: Hyperplasia, labyrinthine, segmental, marked, with mucometra, periluminal necrosis and mineralization, Golden Retriever, canine.

Conference Comment: The contributor provides an excellent review of false pregnancy in the bitch. Conference attendees discussed the presence of the uterine gland cystic hyperplasia within the lamina-propria-submucosa and the marked, labyrinthine hyperplasia of the endometrial mucosa as the physiologic response of the uterus to pregnancy. In this case; however, there is no placental chorion and; therefore, no loss of the placenta-like zone of the endometrium, resulting in accumulation and persistence of the hyperplastic mucosa. Coagulative necrosis of the tips of the endometrial fold is a consistent feature of segmental endometrial hyperplasia.

During false pregnancy, bitches may exhibit behavioral changes such as restlessness, anorexia, decreased activity, aggression, licking of the abdomen and maternal behavior (nesting, mothering inanimate objects, adopting other bitches' puppies). Physical signs include weight gain, mammary enlargement, lactation and even abdominal contractions. (2,4)

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http://www.vet.utk.edu/departments/path/

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SLIDE 87
CONFERENCE 22 / CASE III – Case 1 (AFIP 2957438)

Signalment: Female, cynomolgus monkey (*Macaca fascicularis*), approximately 1 year 5 months of age.

History: An abdominal mass was found during a routine physical exam. Exploratory abdominal surgery was performed to determine the location of the mass and attempt resection. The mass was large and involved the uterus and left ovary. After excision of the mass the tissues were submitted for microscopic examination. The monkey was not pregnant and to our best knowledge had never been mated.

Laboratory Results: Immunohistochemistry findings:
AE1/AE3 – Strong positivity in all tumor cells
Wide spectrum cytokeratin antibodies – all tumor cells positive
Inhibin – large pleomorphic tumor cells were strongly positive, smaller tumor cells negative
Placental alkaline phosphatase – all tumor cells weakly positive
Human chorionic gonadotropin (hCG) – Very rare large multinucleated tumor cells were positive
Human placental lactogen – Diffusely positive, stronger signal in larger, more pleomorphic tumor cells.
Vimentin, CAE, AFP, EMA – tumor cells are negative

Histopathologic Description: [Submitted tissues: ovary and uterus] The mass consisted primarily of infiltrative sheets and cohesive clusters of neoplastic cells distorting and replacing uterine and ovarian tissue. Eosinophilic extracellular hyaline material surrounded some individual tumor cells and tumor cell aggregates in H&E slides. In PAS-stained slides (slides not provided) many aggregates of neoplastic cells and many individual cells were separated by eosinophilic, hyaline extracellular material. In the uterus the mass was located primarily in the muscle wall. The ovary was nearly effaced. Most tumor cells were round to polygonal with moderate amounts of eosinophilic cytoplasm, round to oval to irregularly-shaped coarsely stippled nuclei and single to multiple nucleoli. Lower numbers of neoplastic cells were of increased size, had marked amounts of cytoplasm, and had increased pleomorphism, karyomegaly and bizarre nuclear shapes. The degree of pleomorphism was moderate to marked. The neoplastic cells were morphologically consistent with neoplastic intermediate trophoblasts. Rare cells had multinucleation and were consistent with neoplastic syncytiotrophoblasts (hCG +). The morphology of the cells in the ovary and the uterus locations was similar; however, in the ovary most cells were of the larger more pleomorphic morphology. There were 2-10 mitoses per high power field. There were large areas of necrosis with hemorrhage, variable amounts of fibrosis, and minimal to mild multifocal lymphoplasmacytic infiltrates in both the uterus and the ovary.

Contributor's Morphologic Diagnosis: Uterus and ovary: Malignant trophoblastic neoplasia consistent with malignant placental site trophoblastic tumor.

Contributor's Comment: The monkey recovered uneventfully from the surgical procedure; however, it was later euthanized due to a poor prognosis. A limited necropsy was performed after euthanasia. Metastatic foci were in the lungs. No other

lesions were noted at the time. The microscopic appearance of the lung metastases was similar to that of the ovarian/uterine tumor.

Trophoblastic diseases in humans include hydatiform moles, invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), exaggerated placental site, and placental site nodules.[1] The current case was most consistent with trophoblastic neoplasia, specifically PSTT, based on the histomorphologic features and immunohistochemical pattern of immunoreactivity. The primary differential diagnosis was ETT. Other differential diagnoses included choriocarcinoma and ovarian or uterine carcinoma. PSTT and ETT are both tumors of the intermediate trophoblast. The intermediate trophoblast is one of 3 types of trophoblastic cells found in the placenta: cytotrophoblast, syncytiotrophoblast, and the intermediate trophoblast. The cell morphology and immunohistochemical pattern of staining of PSTT are consistent with intermediate trophoblasts of the implantation site while the cells of ETT are consistent with intermediate trophoblasts of the chorion laeve.[2-3] PSTT and ETT can be differentiated based on morphologic features, immunohistochemical expression patterns, and gene expression profiles.[4] The cells of PSTT are typically larger and more pleomorphic than those in ETT. PSTT grows in a more infiltrative pattern frequently invading the myometrium. ETT has a nodular expansile growth pattern. Deposition of eosinophilic extracellular material is a common finding in PSTT and ETT; however, in human PSTT the material is more homogeneous and in ETT it is reportedly more fibrillar and may resemble keratin.[5] Although PSTT and ETT share immunohistochemical features, there are differences that can be used to differentiate between the two tumors. For example, cells of PSTT have greater numbers of cells positive for MelCAM (CD146) consistent with their reported origin from the implantation site of the placenta.[3]

ETT and PSTT have been rarely reported to occur in non-human primates.[6-7] Interestingly, both reported cases and the current case occurred in non-gravid, female cynomolgus monkeys.[6-8] The reported case of ETT originated in the left ovary and metastasized to the lungs, and the reported case of PSTT originated in the left ovary but did not metastasize. The exact origin of the tumor in this case (ovary vs. uterus) could not be determined; however, the tumor involved the left ovary and the uterus. It is unknown if the involvement of the left ovary in all three cases is a coincidental finding. Similar to the reported cases of non-human primate PSTT and ETT, the monkey in the present report was not pregnant, was born in captivity, and to the best of our knowledge had never been mated. In humans PSTT and ETT are reportedly rare, typically occur after gestation, and may occur in sites other than the uterus.[4,6]

AFIP Diagnosis: Uterus; ovary, left (per contributor): Placental site trophoblastic tumor, Cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: Trophoblasts are the ectodermal cells that cover the blastocyst, erode the uterine mucosa and transfer nutrients from the mother. There are

three types: cytotrophoblasts, intermediate trophoblasts and syncytiotrophoblasts. Cytotrophoblasts are small, undifferentiated stem cells with a high nuclear:cytoplasmic ratio. Syncytiotrophoblasts are the terminally differentiated cells that produce the majority of the placental hormones, and regulate gas and nutrient exchange. Syncytiotrophoblasts are multinucleated, with abundant cytoplasm. Intermediate trophoblasts share some of the morphological and functional features of the other two types. They are usually mononucleate, and larger and more polygonal than cytotrophoblasts, but smaller than the multinucleated syncytiotrophoblast. Cytotrophoblasts are immunopositive for cytokeratin, and are negative for inhibin. Since they do not produce hormones, they are also negative for hPL and hCG. Syncytiotrophoblasts can produce both hCG and hPL, depending on the stage of gestation and trophoblastic maturation. Therefore, staining for hPL and hCG is variable, although staining for hCG usually predominates. Syncytiotrophoblasts also typically express cytokeratin and inhibin. Intermediate trophoblasts are positive primarily for cytokeratin, hPL and inhibin. As trophoblasts mature, the focus of their hormone synthesis changes from hCG to hPL. This change is reflected in the hormone profile seen during a normal gestation. (9,10)

PSTT is a neoplastic proliferation of intermediate trophoblasts that resembles the non-neoplastic infiltration of intermediate trophoblasts that occurs at the endometrial implantation site during pregnancy. It is a rare neoplasm that typically occurs in the postpartum uterus. There are rare reports of extrauterine locations, including the ovary, oviduct and testicle. (11,12) Like normal intermediate trophoblasts of the implantation site, neoplastic cells characteristically invade blood vessels and myometrium. Clinical signs include amenorrhea or abnormal vaginal bleeding. Grossly, the tumors are usually well demarcated and tan, with areas of hemorrhage and necrosis. Histologically, PSTT are primarily composed of intermediate trophoblasts, with rare cytotrophoblasts and syncytiotrophoblasts. Extensive deposition of fibrinoid matrix and vascular invasion is common. By immunohistochemistry, neoplastic cells are positive for cytokeratin, inhibin and hPL, with few cells positive for hCG. PSTT's are usually benign. However, extensive necrosis, large clusters of neoplastic cells, and a mitotic rate >5/10 HPF, as in this case, are suggestive of malignancy. (10) Rare cases of metastasis to the lungs, liver, abdominal cavity and brain have occurred in humans. (10)

The tumor cells were diffusely and strongly positive for keratin and inhibin. Approximately 50% of the cells were positive for hPL, and less than 2% of the cells were positive for hCG. PSTT's consist primarily of intermediate trophoblasts with a few widely scattered syncytiotrophoblasts. By immunohistochemistry, intermediate trophoblasts and syncytiotrophoblasts are positive for hPL and hCG, respectively. Therefore, PSTT's have predominant staining for hPL, with rare cells positive for hCG, as in this case. (10)

The differential diagnosis included epithelioid trophoblastic tumor, choriocarcinoma, anaplastic carcinoma, juvenile granulosa cell tumor and yolk sac tumor.

Epithelioid trophoblastic tumor consists of a monomorphic population of intermediate trophoblasts resembling those lining the chorionic laeve (chorionic-type intermediate trophoblasts), whereas cells of PSTT resemble the intermediate trophoblasts of the placental implantation site (implantation site intermediate trophoblasts). Neoplastic cells in epithelioid trophoblastic tumors are smaller with less nuclear atypia than PSTT. By immunohistochemistry, neoplastic cells are positive for cytokeratin and inhibin, and are focally positive for hCG and hPL. Epithelioid trophoblastic tumors are nodular and expansile and do not typically invade blood vessels, as opposed to the more infiltrative PSTT. (5)

Choriocarcinomas consist of a biphasic population of cytotrophoblasts and syncytiotrophoblasts, as opposed to the largely monomorphic population of intermediate trophoblasts in a PSTT. By immunohistochemistry, choriocarcinomas stain predominantly for hCG, with fewer cells positive for hPL. In contrast to PSTT, choriocarcinomas are often malignant and a fibrinoid matrix is not present. (9,10)

Anaplastic carcinoma was ruled out based on the positive immunostaining for hPL and hCG. (9)

Juvenile granulosa cell tumors are typically solidly cellular, with multifocal follicle formation, and a variable amount of thecomatous differentiation. Cells have more abundant eosinophilic to vacuolated cytoplasm than those of the adult granulosa cell tumor. Although juvenile granulosa cell tumors are more pleomorphic than their adult counterparts, the degree of atypia in this case would be unexpected. Granulosa cell tumors are typically immunoreactive for inhibin, and negative for hPL and hCG. (13)

Yolk sac tumors are malignant germ cell tumors that typically occur in the ovary and testicle, and recapitulate the different developmental stages of the normal yolk sac. The neoplastic polygonal cells form a variety of microscopic patterns, ranging from nests, cords, and papillary structures, to a reticular or microcystic pattern. Cells typically have clear cytoplasm, which contains lipid or glycogen. Other features may include enteric or hepatic differentiation, deposition of an eosinophilic extracellular basement membrane material, and eosinophilic PAS-positive intracellular eosinophilic inclusions. Neoplastic cells are positive for cytokeratin, and are negative for hPL and hCG. (13)

Contributor: Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN 46140.

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SLIDE 88
CONFERENCE 22 / CASE IV – 5 4984 (AFIP 2985454)

Signalment: Adult, female, Thoroughbred, *Equus caballus*, Equine.

History: The mare delivered the fetus and placenta 1 month prematurely. The mare had been receiving multiple antibiotics and other medications for a month prior to the abortion. The fetus and placenta were submitted for necropsy.

Gross Pathology: The allantochorion in the region of the cervical star and adjacent body was multifocally discolored light brown and thickened; the chorionic surface was covered by thick, tenacious, brown exudate. No gross lesions were observed in the remainder of the placenta or fetus.

Laboratory Results: Cultures of the placenta yielded numerous *Aspergillus* sp. *Pantoea (Enterobacter) agglomerans* was isolated from the fetal stomach contents. Florescent antibody test for *Leptospira* spp. on placenta and kidney was negative. Polymerase chain reaction tests for *Crossiella equi* and *Amycolatopsis* spp. were negative.

Histopathologic Description: Sections of the allantochorion are characterized by diffuse amorphous eosinophilic material on the chorionic surface containing degenerated cells and fungal hyphae. There is necrosis of the underlying chorionic villi. Additional alterations include a moderate inflammatory infiltrate consisting of degenerate and intact polymorphonuclear cells and macrophages in the chorionic villi and underlying chorionic stroma. The mycelia within the exudate contain dichotomous branching and septate hyphae, often with vesicular swellings.

Contributor's Morphologic Diagnosis: Equine, placenta, mycotic placentitis, focally extensive, chronic, severe.
Etiology: *Aspergillus* sp.

Contributor's Comment: Aspergillosis of the placenta was diagnosed based on the morphological characteristics of the hyphae and the results of the culture. Aspergilli are omnipresent in the soil and derive nutrients from decaying organic material. *Aspergillus* spp. have been found in poor quality hay and compost heaps (1). Aspergilli readily produce numerous spores that are dispersed by wind and dust. *Aspergillus* spp. have been isolated from the nasal passages of normal horses (2). Clinical cases of aspergillosis in horses are relatively uncommon and have been reported to cause nasal granulomas, guttural pouch mycosis, intestinal aspergillosis, keratitis, lower respiratory tract infections, and mycotic placentitis (1,2).

Mycotic placentitis in the horse primarily occurs in late gestation. Fungal infections may reach the placenta via a hematogenous route, but most ascend through a relaxed cervix (3). *Aspergillus* spp. is the most commonly isolated fungus in mycotic placentitis cases (3,4,5). Abortion may result from placental insufficiency due to the infection involving substantial portions of the chorionic surface (6). Also, the infection and resultant inflammation may stimulate cytokine elaboration leading to abortion (7). The route of infection in this mare was not determined, but it is probable that it was through the urogenital tract.

AFIP Diagnosis: Allantochorion: Placentitis, necrohemorrhagic, chronic-active, diffuse, severe, with pseudomembrane, congestion and edema, multifocal trophoblast squamous metaplasia, and many fungal hyphae, etiology consistent with *Aspergillus* sp., Thoroughbred, equine.

Conference Comment: Common causes of equine placentitis and abortion include bacterial, fungal and viral etiologies. Bacterial pathogens include *Streptococcus zooepidemicus*, *E. coli*, *Leptospira* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, and nocardioform placentitis caused by *Crossiella equi* and similar unclassified bacteria. *Aspergillus fumigatus* is the most common fungal cause of equine abortion. Other fungal causes include the phycomycetes (*Absidia* spp., *Mucor* spp., *Rhizopus* spp.), *Candida* spp. and *Histoplasma* spp. Viral causes include equine

herpesvirus 1 and equine arteritis virus. Poor perineal conformation of the mare may contribute to an ascending infection of bacterial or fungal placentitis. As mentioned by the contributor, ascending infections generally occur through a relaxed cervix. The cervical star or the portion of the placenta overlaying the internal cervical os is generally thickened and covered in plaque-like foci of inflammation in cases of both mycotic and bacterial infection.

The moderator stressed that in cases of mycotic abortion there are five key histologic features which can usually be identified in addition to the fungi: evidence of placental separation and an accumulation of debris around the chorioallantoic membranes; squamous metaplasia of trophoblastic cells; edema; congestion; and adenomatous dysplasia of the equine allantois. In this case, all five features are identifiable; however, adenomatous dysplasia of the equine allantois is not present in all tissue sections. Adenomatous dysplasia of the equine allantois has been associated with fetal disease, can occur anywhere within the allantois, and is characterized by nodules which may be solid or cystic. The affected allantois is thickened by variably sized glandular structures which are lined by cells that are occasionally continuous with the epithelial cells of the allantoic surface. (3) In this case there are multiple small cysts within the allantois which are lined by attenuated cuboidal to polygonal cells and which are either empty or contain sloughed cells, cellular debris, and/or homogenous, eosinophilic, proteinaceous material.

Aspergillus spp. fungi are an important cause of disease and serve as opportunistic pathogens in immunosuppressed domestic animals and humans. Typical gross findings include multifocal to coalescing pale nodules. Histologically, aspergillosis is characterized by fungal granulomas or pyogranulomas composed of a central area of necrosis containing hyphae that are 3-5 _m wide, with regularly septate parallel walls, and dichotomous acute angle branching, surrounded by variable numbers of neutrophils, lymphocytes, epithelioid macrophages, and fibroblasts. Many cases also demonstrate vasculitis (8).

Readers are encouraged to review WSC cases 3 and 4, conference 15, 2002 for examples of *Candida* sp. and nocardioform placentitis.

Contributor: University of Kentucky, Department of Veterinary Science, Livestock Disease Diagnostic Center
http://fp1.ca.uky.edu/lddc

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SLIDE 89
CONFERENCE 23 / CASE I – 2 or 3 (AFIP 2983562)

Signalment: 11 year old, wild-caught, female cynomolgus macaque

History: This macaque was imported from the Philippines in 1990 to augment the breeding colony at the California Regional Primate Research Center. Four years later she was brought to the attention of the veterinary service because of an equivocal tuberculin reaction and the presence of a skin rash. A follow up skin test using mammalian tuberculin, avian tuberculin and a sterile saline control produced a small induration at 24 and 48 hours to only the avian tuberculin inoculation. The result was considered equivocal for avian and negative for mammalian tuberculosis. She had previously been noted to have intermittent epistaxis, crusting of the nares, ulcerated nasal philtrum and nasal discharge. Thoracic radiographs revealed no significant findings.

Gross Pathology: On physical exam there was extensive necroulceration of the nasal philtrum, depigmentation of hands and feet, and multiple ulcerated 2-3 mm diameter nodules present symmetrically on the skin of the hands, wrists, ankles, feet, and tail base. Left axillary and bilateral inguinal lymphadenopathy were noted.

Laboratory Results: The animal was negative for type D retrovirus by ELISA. Tissue homogenates prepared from a skin biopsy specimen demonstrated acid fast bacteria (AFB) and positive reactivity for PCR amplified *Mycobacterium leprae.* Serum drawn at the time of the animal's arrival at the Primate Center was strongly positive by ELISA for the IgG anti-PGL-I (phenolic glycolipid-I), which is specific for *M. leprae*. Retrospective analysis of stored sera demonstrated a significant rise in IgM anti-PGL-I and IgM LAM (mycobacterium common lipoarabinomannan) as early as 6 months prior to the detection of cutaneous lesions.

Histopathologic Description: Two tissue samples have been submitted; ulcerated lesions from the foot and from the nares. Much of the dermis is effaced by multifocal to coalescing granulomas comprised of a central area of necrosis with few degenerate neutrophils surrounded by a well demarcated band of epithelioid macrophages admixed with multinucleated giant cells which is in turn is surrounded by a band of lymphocytes, plasma cells and reactive fibroblasts. The superficial dermis is diffusely infiltrated by large numbers of macrophages, neutrophils, lymphocytes and plasma cells that are associated with a focal ulceration of the overlying epidermis. The inflammatory infiltrate also extends into the deep dermis and in many sections is identified surrounding or infiltrating nerve bundles. In sections from the nares there is necrosis of cartilage. Acid fast stains revealed small numbers of AFB in the surface exudates, in the cutaneous histiocytic infiltrate, and within dermal nerve bundles. Immunohistochemical staining with Bacillus Calmette-Guérin (BCG) antibody was also positive.

Contributor's Morphologic Diagnosis: Skin, chronic multifocal to coalescing granulomatous and necrotizing dermatitis with cutaneous neuritis and epidermal ulceration (consistent with infection with *Mycobacterium leprae*)

Contributor's Comment: Submitted tissues are from archived samples of the first reported case of spontaneous leprosy in a cynomolgus macaque (1). Naturally occurring leprosy has been previously documented in only two species of nonhuman primates from West Africa – the chimpanzee and the sooty mangabey (2,3,4). Host immune response to *Mycobacterium leprae* is critical for control of the infection and is also responsible for the damage to skin and nerves, which is the basis for the clinical spectrum of disease. Different categories of leprosy exist as classified by Ridley and Jopling (5). Tuberculoid leprosy is characterized by a strong lymphocytic infiltrate and an effective histiocytic response to *M. leprae*, resulting in well-localized granulomas and few surviving organisms. The lesions are typical of the chronic delayed-type (type IV) hypersensitivity response. Lepromatous leprosy is characterized by a selective unresponsiveness to *M. leprae* antigens and an ineffective, diffuse histiocytic reaction with numerous intralesional organisms. Between these two groups are borderline forms of leprosy characterized by progressive reduction in the cellular-immune response and an increasing number of skin and nerve lesions, greater bacteria load and increasing antibody levels. The combination of the variably organized granulomatous inflammation, the paucibacillary nature of the lesions, the antibody responses to LAM and PGL-I, and the immunohistochemical data, place this animal in the paucibacillary mid-borderline category of leprosy.

Invasion of nerves by AFB and granulomatous inflammation in the submitted slides was variable but distinct and constitutes a defining characteristic of *M. leprae* lesions.

AFIP Diagnosis: Skin and subcutis, foot (per contributor): Dermatitis and panniculitis, pyogranulomatous, multifocal and coalescing, marked, with granulomatous neuritis, and focal ulceration, cynomolgus macaque (*Macaca fascicularis*), primate.

Conference Comment: There is some variation among slides with some of the contributors receiving a section of nares with cartilage. *Mycobacterium leprae* is an acid-fast obligate intracellular organism and the etiologic agent of leprosy, also known as Hansen's disease in humans. *M. leprae* causes a chronic granulomatous disease that primarily affects the skin and peripheral nerves. It is a zoonotic and is most common in tropical climates. Naturally acquired infections have been reported in wild nine-banded armadillos, sooty mangabey monkeys, and a cynomolgus macaque.

As mentioned by the contributor, there are two forms of the disease: the lepromatous form and the tuberculoid form, with a spectrum of intermediate stages between the two. The T-helper lymphocyte response to *M. leprae* determines whether an individual develops tuberculoid or lepromatous leprosy. (6)

Lepromatous leprosy (malignant disease) results from a lack of T cell immunity (anergy - unresponsiveness of host immune system) and is characterized by numerous macrophages filled with many mycobacteria (multibacillary). Lepromatous leprosy lesions lack effective CD4+ type 1 helper T cells but contain many CD8+ suppressor T helper 2 cells which secrete IL10 (inhibits helper T cells; mediates anergy), IL4 (induces antibody production by B cells), and IL-5 which may suppress macrophage activation. Antigen-antibody complexes may be produced resulting in vasculitis, glomerulonephritis and erythema nodosum. IL-12, produced by antigen presenting cells is important in the generation of T helper 1 cells, and low levels or unresponsiveness of T cells to this cytokine may reduce the T helper 1 response, leading to lepromatous leprosy. (6)

Tuberculoid leprosy (benign disease) is characterized by granuloma formation with few mycobacteria (paucibacillary). Numerous CD4+ type 1 helper T cells are located at the periphery of lesions where they secrete IL-2 and interferon gamma. Interferon gamma is critical to mobilizing an effective host macrophage response. (6) Few CD8+ lymphocytes are located in the center of the granuloma.

M. leprae grows more slowly than other mycobacteria and grows best at 32° to 34° C, the temperature of human skin and core temperature of armadillos. (6) Because of this affinity for cooler temperatures, human lesions are more common on the distal extremities, scrotum and upper respiratory tract. Typical clinical findings include macular, nodular and papular lesions with hypoesthetic or anesthetic skin lesions and paralytic deformities in the extremities. The ulnar and perineal nerves are most often affected resulting in loss of sensation to the hands and feet. Skin lesions may become ulcerated and are most common in the subperiorbital area, lips, chins, ears, and scrotum.

Mycobacteria lepraemurium or murine leprosy affects rats and mice and has also been reported in cats, but is not considered zoonotic. *M. lepraemurium* primarily affects the skin and viscera; but very rarely affects peripheral nerves. (7)

Contributor: Veterinary Medical Teaching Hospital (VMTH)
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SLIDE 90
CONFERENCE 23 / CASE II – N05-436 (AFIP 2994519)

Signalment: 12 yr old female Olive Baboon (*Papio cynocephalus anubis*)

History: This wild-caught African baboon presented with multiple subcutaneous nodules (fig 1). Hematologic and serologic testing of the animal demonstrated a moderate monocytosis, slight eosinophilia and hyperglobulinemia. Surgical biopsy of one of the nodules revealed several parasitic granulomas containing cestode larvae. Attempts to treat the animal with an antiparasitic drug proved unsuccessful. The animal was euthanized for humane reasons.

Gross Pathology: Multiple (too numerous to count) nodular tapeworm cysts ranging in size from 0.1 to 1.5 cm and containing viable cestode larvae were observed in the skin and subcutis. Infestation was particularly heavy in the lower right quadrant of the animal. Larvae were also observed grossly associated with lymphatic tissue, mesentery, skeletal muscle, and in pericardial, diaphragmatic and peripancreatic connective tissue (fig 2).

Contributor's Morphologic Diagnosis: Haired skin and subcutis: Granulomas, eosinophilic, multifocal, with epithelial hyperplasia, necrosis, hemorrhage and intralesional cestodes, etiology consistent with Spirometra sp.

Contributor's Comment: Multiple viable and degenerating cestode larvae are in the dermis and subcutis, forming eosinophilic granulomas. The parasites are morphologically consistent with plerocercoid larvae, or spargana (fig 3), of the pseudophyllidean cestode *Spirometra* spp. Histologic features that help identify the parasites as cestodes include an outer tegument surrounding a solid but loose mesenchymatous stroma containing calcareous corpuscles, excretory ducts, skeletal muscle bundles, and the absence of a digestive tract. Pseudophyllideans like *Spirometra* also lack anterior suckers that are present in Cyclophyllidean cestodes. Examples of cyclophyllidean larval stages include cysticercus, coenurus, hydatid cyst and the tetrathyridium of *Mesocestoides* spp.

The normal life cycle of *Spirometra* involves two intermediate hosts. The adult worms live in the definitive host, typically wild canids and felids. Water contamination with the eggs of *Spirometra* leads to infection of the primary intermediate host, a copepod (water flea), where it develops into the first larval stage (procercoid). The migrating second larval stage (plerocercoid or sparganum) of the cestode develops in the second intermediate host following ingestion of infected copepod. Infection with the plerocercoid stage, referred to as sparganosis, is known to occur in many different tissues and a wide variety of vertebrate hosts throughout the world, including small mammals, amphibians, reptiles, birds, and occasionally humans. It is also true that any number of mammal or avian predators can serve as paratenic hosts (transport hosts) by consuming infected tissues of second intermediate hosts, resulting in serial transmission of the plerocercoid stage. The plerocercoid eventually develops fully into the adult tapeworm in the intestine of the definitive host.

Sparganosis has previously been reported in non-human primates including baboons, vervets and a highland Syke. The disease is also well documented in feral pigs, where it poses a particular hazard for transmission to people. Although people typically acquire the parasite by eating raw or undercooked meat of plerocercoid-infected animals, other possible routes of infection include direct ingestion of procercoid-infected copepods, and also direct contact in cultures that use animal tissues as poultices. In humans, spargana larvae are most commonly found in the subcutis, but have been reported to migrate into the CNS, breast, eye, urinary bladder, and lung.

AFIP Diagnosis: Haired skin and subcutis: Granulomas, eosinophilic, multifocal, with necrosis, hemorrhage, and numerous larval cestodes**,** olive baboon (*Papio cynocephalus anubis*), primate.

Conference Comment: The contributor provides an excellent example and review of sparganosis in a nonhuman primate. As noted by the contributor, sparganosis may be acquired by humans through the ingestion of undercooked meat, in which the plerocercoid larvae remain alive, and through the application of infected fresh animal tissue poultices, as is practiced in some cultures. Frogs are

most commonly used in this practice. The sparganum invades the human tissue where the poultice is applied, most often the eye. Additionally, humans may contract the disease through drinking water contaminated with infected copepods.

A rare form of human sparganosis has been described in which the infective plerocercoids proliferate and invade every tissue except bone. The underlying pathogenesis of this variant of sparganosis is unknown. In general, sparganosis is a relatively benign human and animal disease; it may be more prevalent than reported due to this benign nature.

The main groups of cestodes encountered in histological section are the cyclophyllideans and the pseudophyllideans. Histologically it is almost impossible to determine the exact species of the parasite in question. However, two differences between members can be used to narrow the differential. Both the adult and larval forms of cyclophyllideans have four suckers on their anterior end. Pseudophyllideans lack these suckers. Cyclophyllideans also have muscles (within the parenchyma) that separate the medullary and cortical regions. Pseudophyllideans lack these muscles. (4)

For a simple algorithm to help identify parasites in tissue sections, readers are encouraged to review WSC 15, case 3, 2005-2006. Additionally, readers are encouraged to review Wednesday Slide Conference 5, case 4, 1998 for a case of sparganosis in a pig.

This case was reviewed by Dr. C.H. Gardiner, parasitology consultant for the Department of Veterinary Pathology, AFIP.

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SLIDE 91
CONFERENCE 23 / CASE III – PA-4195 (AFIP 2986824)

Signalment: Adult, male baboon (Papio sp.)

History: This adult male animal had a multiple month history of malaise with mild-moderate anorexia and some weight loss. The animal had peri-orbital hyperemia that was treated empirically with antihistamines. General physical exam findings and blood work at that time were inconclusive. The anorexia and malaise subsequently progressed significantly and the baboon experienced more marked body weight loss. It was also noted to be tachypneic, with increased respiratory efforts. On more recent physical exam a markedly enlarged spleen was palpated. A patchy, macular rash was present in the groin area. Thoracic radiographs showed severe infiltrates, especially in the left hemithorax (see Figures 1 & 2). Hematologic assessment revealed a lymphocytosis. The animal was euthanized. Prior to sacrifice, a bronchioalveolar lavage was performed under general anesthesia (see Figure 3).

Gross Pathology: The body condition was considered thin but not cachetic. The spleen was markedly enlarged but of regular general contour. A fine, diffuse nodular granularity was noted on the capsular surface, although the organ had normal architecture on cut surfaces (see Figure 6). The lungs were totally adherent to the adjacent parietal pleura with disseminated fine adhesions. The visceral pleura was markedly thickened and opaque. Lung parenchyma in the entire left lung and the right diaphragmatic and middle lobes was firm, tan and of “meaty” consistency and did not float in formalin (see Figures 4 and 5). Some remaining aeration was noted in the right upper lobe. Thoracic para-spinal and hilar lymph nodes were markedly enlarged.

Contributor’s Morphologic Diagnosis: 1) Diffuse, severe mixed cellular infiltration including prominent interstitial lymphoid aggregates with a significant blastic component and mixed epithelioid macrophage and granulocytic alveolar infiltrates, with numerous syncitialized giant cells (most prominently noted in slides labeled 1 & 1A) (see Figures 7 and 8)
2) Alveolar edema, patchy to diffuse, marked
3) Pleural thickening and fibrosis, focally extensive, marked

Contributor’s Comment: The microscopic findings are consistent with STLV-1 associated lymphoma. This entity in baboons has been well described in the literature and is often associated with severe pulmonary tumor involvement. This animal was serologically positive for STLV-1, as well as for a variety of other retroviruses. The lung findings in this case included a significant associated pulmonary inflammatory process and this is also a well-documented finding in animals with advanced disease. Numerous syncytia (multinucleated giant cells) are seen (again, as previously described in this disease entity). Their pathogenesis is not immediately apparent, however, HTLV-1 (the closely related human counterpart) does induce syncytia formation in susceptible cell lines upon contact with virus-producing cells – in fact syncytial assay is a tool used in the detection of neutralizing antibody and cellular receptors. The extra-pulmonary disease distribution and pattern in this case (spleen, skin & lymph nodes) are also typical. It is not clear whether the late-course hematologic findings reported as

peripheral lymphocytosis may have represented a leukemic component of the process. Peripheral smears are not available for review. STLV-1 seroprevalence is at high levels (>40%) in some colonies and this agent is closely related to HTLV-1, a type C retrovirus that has been linked to both adult T-cell leukemia and neurological disorders in humans. Other nonhuman primates, including rhesus macaques are susceptible to infection with STLV-1, and intraspecies transmission (macaques to baboons) has been linked to large outbreaks at some centers.

AFIP Diagnosis: Lung: Lymphoma, with marked alveolar edema, histiocytosis, and numerous multinucleate giant cells**,** baboon (Papio sp.), primate.

Conference Comment: Simian T-cell lymphotropic viruses (STLV) are currently listed within the genus Deltaretrovirus, family Retroviridae. Other viruses belonging to the genus Deltaretrovirus include the type species Bovine Leukemia Virus, as well as the human T-cell lymphotropic viruses (HTLV). (5) As mentioned by the contributor, HTLV and STLV share many similar characteristics and, in some primate colonies, lymphomas are recognized that have all the hallmarks of adult T-cell leukemia/lymphoma (ATLL). (2)

Transmission of HTLV is dependant on the transfer of infected lymphocytes. In humans, the known routes of infection include the transfer of blood, milk and semen, with breast milk considered to be the most common mode of transmission. Clinically, most baboons with non-Hodgkin lymphoma have been diagnosed because of severe dyspnea followed by radiographic findings of diffuse interstitial pneumonitis and multifocal, circumscribed lesions consistent with neoplasia. Generalized lymphadenopathy and obliteration of nodal and pulmonary architecture by pleomorphic lymphocytes with hyperlobulated nuclei is another prominent finding. Additional clinical findings include: lymphocytosis or leukemia with significant numbers of atypical lymphocytes and dermatologic lesions with lymphocyte infiltration. (2)

Like HTLV, the life cycle of STLV includes the random integration of proviral DNA into the host genome. In most cases of HTLV, leukemic cells display an activated CD4+ T-cell phenotype. The viral transcriptional regulatory gene *tax* is directly or indirectly responsible for the expression of interleukin-2 receptor alpha-chain (IL-2R alpha), interleukin-2 (IL-2), and other cell surface markers that may lead, in some cases, to overt leukemias and lymphomas. Increased expression of IL-2R alpha coinciding with the CD4+ T-cell population is considered the hallmark of HTLV/STLV infection. In both humans and nonhuman primates, the *tax* gene presumably plays a pivotal role in the induction and immortalization of peripheral blood lymphocytes leading to lymphoid cancers. By up-regulating IL-2R alpha, IL-2, and other cytokines, an autocrine loop for T-cell activation and proliferation is established. (2)

Conference attendees considered Simian Immunodeficiency Virus (SIV) in the differential diagnosis. Like STLV, SIV is a retrovirus; however, SIV belongs to the genus Lentivirinae. SIV targets lymphoid tissues, specifically cells expressing the CD4

molecule on their surface (helper/inducer T lymphocytes and antigen-presenting cells of monocyte-macrophage origin). Experimentally, SIV is readily transmissible via infected blood or serum, and through the genital mucosa. Pulmonary lesions associated with SIV infection include: interstitial pneumonia with lymphocytic infiltration of the interalveolar septa and peribronchial lymphoid hyperplasia (early infection); a characteristic giant cell pneumonia with multinucleated giant cells (syncytial cells) in alveoli and interalveolar septa; and, in some cases, diffuse alveolar fibrosis and bronchiolar epithelial metaplasia, especially in cases complicated by the atypical fungus, *Pneumocystis carinii*.

For a more complete list of retroviruses important in veterinary medicine, readers are encouraged to review Wednesday Slide Conference case 1, conference 15, 2005-2006.

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SLIDE 92
CONFERENCE 23 / CASE IV – 05-1294 (AFIP 3007305)

Signalment: 2 yr old, male, Sprague Dawley rat, Rattus norvegicus
History: This rat was found moribund and died less than one hour later. No experimental manipulations had been performed.

Gross Pathology: There is little fat throughout the body. A small amount of ingesta is present in the stomach and intestines. The pleural and pericardial cavities are filled with blood. Both auricular walls and the left ventricular wall are expanded by a thin, tan infiltrative mass. (Fig 1)

Histopathologic Description: Heart: Expanding the endocardium and infiltrating the myocardium is an unencapsulated neoplasm that consists of spindle cells arranged in short interlacing streams and occasional whorls. Neoplastic cells have indistinct cell borders and are surrounded by a moderate amount of eosinophilic, fibrillar matrix. Nuclei are oval with finely stippled chromatin or are elongate and hyperchromatic. Mitoses are not observed. Rarely, there are spindle cells with markedly elongate, wavy nuclei (Anitschkow cells). Occasionally, neoplastic cells infiltrate and surround myocardial cells that are swollen, vacuolated, with loss of cross-striations (degeneration). Multifocally, along the endocardial surface, neoplastic cells are more polygonal with round nuclei that contain a single, prominent nucleolus.

Contributor's Morphologic Diagnosis: Heart: Endocardial Schwannoma

Contributor's Comment: Endocardial proliferative lesions are rare in rats. One study of over 19,000 rats found 44 cases of endocardial hyperplasia (0.2% prevalence) and 16 cases of neoplasia (0.085% prevalence).[1] The nomenclature for proliferative endocardial lesions in rats has been confusing due to the controversy over the cell of origin. Common diagnoses include endocardial schwannoma, neurofibroma, subendocardial fibrosis, Anitschkow cell sarcoma, and endocardial mesenchymal tumor.[1-5]

Grossly, there is a variably sized, firm, gray to white mass that lines the luminal wall, most often of the left ventricle.[2,3] Histologically, endocardial schwannomas often consist of two distinct cell types. The superficial layer contains mostly round to polygonal cells, with ovoid nuclei, prominent nucleoli, and finely vacuolated cytoplasm. The deeper and thicker layer, adjacent to the myocardium, is composed of spindle cells with elongated, hyperchromatic nuclei interspersed between collagenous fibers.[3] In larger neoplasms, spindle cells sometimes exhibit nuclear palisading and form Verocay bodies (Antoni type A).[3,4] Anitschkow cells are typically present. These cells have elongated, ribbon-like heterochromatin and are thought to be macrophages or regenerative myofibers.[6]

Ultrastructurally, there are again two types of cells. The spindle cells contain mitochondria, rough endoplasmic reticulum, and occasional filaments. They are surrounded by a convoluted basement membrane with few desmosomes. These features are typical of neoplastic Schwann cells. The second cell type is pleomorphic with vesicular nuclei and short, densely packed cell processes and most likely is of mesenchymal origin.[2] Immunohistochemically, neoplastic cells are often positive for S100 and neuron-specific enolase.[4]

Endocardial schwannomas must be differentiated from endocardial hyperplasia. The latter consists of sharply demarcated subendocardial proliferation usually less than 20 cell layers thick. Neoplasia, however, exhibits active invasion with finger-like projections into the myocardium and increased mitotic activity. Hyperplasia and neoplasia can occur simultaneously. Metastasis is rare.[1,2,4]

Endocardial schwannomas appear to be unique to the rat. Heart tumors morphologically similar to endocardial schwannomas in the rat have been reported in hamsters, dogs, and man but they lack the subendocardial appearance of the rat tumor.[2]

AFIP Diagnosis: Heart, endocardium: Schwannoma, Sprague Dawley rat, rodent.

Conference Comment: The contributor provides an excellent review of endocardial schwannomas in rats. Schwannomas that arise within the hearts of rats can be either endocardial or intramural. Although both apparently arise from Schwann cells and form patterns that are typical of nerve sheath neoplasms, they have certain differences. Endocardial schwannomas always involve the left ventricle but can extend into the subendocardium and any of the adjacent cardiac chambers. In the present case, neoplastic cells are predominantly located within the left ventricle; however, some sections also have neoplastic cells within the right ventricular endocardium. The smallest endocardial schwannomas are little more than a subendocardial layer of spindle cells. As they enlarge, they infiltrate the adjacent myocardium, sometimes nearly replacing the intraventricular septum. They may also extend into the pericardial connective tissue or metastasize to other organs. In comparison, intramural schwannomas tend to form more discrete nodular masses and usually arise within either the left ventricular wall or intraventricular septum. They grow by expansion with minimal infiltration and do not metastasize. (7)

There are rare Anitschkow cells within the neoplasm. The nuclei of Anitschkow cells are ellipsoidal, have distinct nuclear membranes, and are pale except for an intensely stained central bar of chromatin along the long axis. As mentioned by the contributor, Anitschkow cells have been thought to represent either a cell of muscle origin or a cardiac histiocyte. In a study of fetal and neonatal hearts from humans, cells displaying the Anitschkow chromatin pattern were the predominant cell type in the myocardium and it was concluded that the Anitschkow pattern probably indicates cellular immaturity rather than a specific cell type. (8)

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SLIDE 93
CONFERENCE 24 / CASE I – 03F034 (AFIP 2890942)

Signalment: Channel catfish (*Ictalurus punctatus*) from recently constructed and filled farm pond.

History: Outbreak occurred in spring of 2003 and mortality was approximately 35%. Affected fish were observed swimming close to surface of pond.

Gross Pathology: Gill lamellae were hyperemic, blunted and thickened. All other internal organs and tissues were grossly normal.

Laboratory Results: Water quality data was not available

Contributor's Morphologic Diagnosis: Gill Lamellae – inflammation, granulomatous, chronic, diffuse, severe with epithelial hyperplasia, cartilage necrosis and intralamellar myxozoan-like parasites

Contributor's Comment: The lesions are those of proliferative gill disease (PGD) of channel catfish. This is an important disease of cultured channel catfish causing high mortality and significant economic loss. The disease is caused by a myxozoan parasite that has life stages in different hosts. A worm (*Dero digitata*) commonly found in catfish ponds can become infected with the myxozoan parasite *Aurantiactinomyxon* sp. (actinosporean stage). Another stage of this myxozoan parasite named *Henneguya ictaluri* (myxosporean stage) also infects catfish gills. Catfish can be experimentally infected 5 to 6 days after exposure to worms infected with *Aurantiactinomyxon* sp (1). Experimental infection is characterized by inflammation, epithelial cell hyperplasia, gill necrosis and the presence of cysts containing spores. The sequences of the SSU rRNA genes of the actinospore and myxospore life stages have been recently demonstrated to be identical (2).

In tissue section, *H. ictaluri* cysts are located within central regions of gill lamellae and are surrounded by heavy infiltrates of macrophages. Individual cysts appear to lack a capsule and consist of aggregates of small basophilic spores. Cartilage is either conspicuously absent where inflammation and parasites are located or is undergoing degenerative changes.

Please note: some sections of gill lamellae contain low numbers of monogenetic trematodes, *Chilodonella* sp protozoa and *Henneguya exilis* cysts. These are producing minimal tissue reaction and are not part of PGD.

AFIP Diagnosis: Gill: Branchitis, histiocytic and proliferative, diffuse, moderate to severe, chronic, with lamellar fusion, blunting, and loss and protozoa, etiology consistent with myxozoan parasites, channel catfish (*Ictalurus punctatus*), piscine.

Conference Comment: There is some variability among slides concerning the number and types of parasites present as well as the presence of cartilage necrosis. Proliferative gill disease (PGD) (a.k.a. hamburger gill disease) of channel catfish occurs throughout commercial catfish-producing regions of the United States and has caused severe economic losses to individual producers since 1981. The disease is characterized by a rapid onset with 10% to 95% mortality. Respiratory efficiency is reduced and fish may gasp for air at dissolved oxygen concentrations that are normally adequate. Fish present in severe respiratory distress with swollen, friable gills that may bleed when touched. Microscopically, there is intense granulomatous inflammation, swelling and epithelial hyperplasia of the gills with necrosis of the primary lamellar cartilage and distortion of lamellae. Cysts have also been observed in the brain, spleen, liver, and kidney. Water temperatures between 16 and 20^{o}C favor optimal growth of the organism.

PGD must be differentiated from disease caused by microsporidian parasites such as *Pleistophora* sp., *Glugea* sp., *Loma* sp. and *Noserag* sp. which are capable of infecting skeletal muscle, gills, epithelial and connective tissue resulting in high mortality. Microsporidian infection results in the formation of large cysts (up to several millimeters) called xenomas that are filled with numerous refractile spores. Xenomas induce proliferative inflammation leading to granuloma formation. Additional differentials for gill lesions include the gram-negative bacterium *Flavobacterium columnare* (formerly *Flexibacter columnaris*) causing **"**columnaris disease or saddleback disease"*;* and non-inflammatory hyperplasia of gill epithelium associated with poor water quality and overcrowding.

Other myxosporidian parasites of fish include:

- *Myxosoma cerebralis*: "Whirling disease" in trout; cartilage necrosis of head and spine

- *Henneguya* sp.: “Blister disease” in freshwater fish and catfish; gill epithelial and epidermal hyperplasia with pseudocysts
- *Hofferellus carassii*: “Kidney bloater disease” in goldfish in North America and Asia; marked dilation of some, but not all, renal tubules
- *Ceratomyxa shasta*: Ceratomyxosis; granulomatous peritonitis in salmonids
- *Sphaerospora* sp.: Proposed cause of Proliferative Kidney Disease (PKD, PKX, X Disease); myxozoan parasite; granulomatous interstitial nephritis in salmonids

Readers are encouraged to review Wednesday Slide Conference case 2, conference 22, 1996 for another case of PGD.

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SLIDE 94
CONFERENCE 24 / CASE II – L03 1060 (AFIP 2893497)

Signalment: Grouped samples of hepatopancreas from numerous freshwater crayfish (*Cherax quadricarinatus)* are submitted for histopathology.

History: Submitted for health evaluation during state inspection
Histopathologic Description: Hepatopancreas: Few to moderate numbers of epithelial cells lining hepatopancreatic tubules have enlarged nuclei with marginated chromatin and contain homogeneous, eosinophilic to basophilic, round to oblong inclusion bodies. In some areas, supporting interstitium has multifocal infiltrates of mononuclear and granular leukocytes. Leukocytes center about squamous epithelial lined ducts, which contain large numbers of rod shaped bacteria.

Contributor's Morphologic Diagnoses: 1. Intranuclear inclusion bodies in hepatopancreatic epithelial cells
2. Hepatopancreatitis, mononuclear and granulated leukocytes

Contributor's Comment: The presence of baculovirus virions were demonstrated in tissue sections by electron microscopy.

Rod shaped nuclear viruses are the most common viral agents detected in cultured crustaceans and all infect the epithelial cells of the digestive tubules and hepatopancreas (13). Baculoviruses of penaeid shrimps have been demonstrated to be serious pathogens, however the virulence and clinical significance of others (crayfish) remains unclear (13).

Baculovirus was discovered in crayfish in 1996 as the result of an inspection of an aquaculture facility in Northern Utah (14). Samples of crayfish were taken from the facility and tested at the Ross Smart Veterinary Diagnostic Laboratory at Utah State University. This virus was confirmed using electron microscopy. The baculovirus is known to infect crayfish in Australia and California, but had not been seen in Utah until its presence was confirmed in 1997 (14). The baculovirus is often found in the crayfish's internal organ called the hepatopancreas. The virus is not known to cause extensive mortality, but is suspect of stunting their growth and affecting their immuno-competency (14). The disease is not a threat to humans.

AFIP Diagnoses: 1. Hepatopancreas, tubular epithelial cells: Amphophilic intranuclear inclusion bodies, multifocal, moderate numbers, with karyomegaly, freshwater crayfish (*Cherax quadricarinatus*)**,** crustacean.
2. Hepatopancreas: Granulomas, multifocal, few, with mixed bacteria.

Conference Comment: There is some variability among slides. Some sections contain granulomas with a central core of mixed bacteria. As mentioned by the contributor, baculoviruses are known to cause disease in shrimp and prawns and have recently been recognized as pathogens of several species of crayfish.

Baculoviruses are a family of large, rod-shaped viruses which contain a circular, double-stranded genome ranging from 80-180 kbp. Baculoviruses have very species-specific tropisms among invertebrates with over 600 host species having been described. They are not known to replicate in mammalian or other vertebrate animal cells. (16)

Diseases of crayfish caused by baculoviruses include White Spot Virus (WSV) infection and *Cherax quadricarinatus* bacilliform virus (CqBV) infection. WSV has been associated with high mortality (90%) in crayfish fed frozen imported prawns. CqBV infections in *Cherax quadricarinatus* can result in lethargy, weakness, a failed-tail-flick response and an inability to right themselves when placed on their back. CqBV infections are associated with hepatopancreatic tubule epithelial cell necrosis and

atrophy. Nuclear changes include hypertrophy, margination of chromatin and the formation of a single, large, granular eosinophilic inclusion body. (17)

The following is an excerpt of supplemental information on penaeid (shrimp) baculovirus provided by the Office International des Epizooties (OIE manual):

Infections of the hepatopancreas by the nuclear polyhedrosis viruses *Baculovirus penaei* (BP) and *Penaeus monodon*-type baculovirus (MBV) are among the most readily diagnosable diseases of the penaeid shrimps and prawns. The inclusion bodies formed by the viruses are very conspicuous and easily demonstrated by direct light microscopy with fresh specimens or by routine histological methods with fixed specimens. Direct microscopic methods are most suitable for the post-larvae stages, which are commonly moved in regional and international commerce (1, 2, 7, 8). Highly sensitive molecular methods for BP and MBV are also available and provide alternative methods for surveillance applications, especially for nonlethal testing of broodstock (8, 9).

BP and MBV are considered to be potentially serious pathogens in the larval, postlarval, and early juvenile stages of host shrimps. These viruses possess a wide geographical distribution and diverse host range, and multiple strains of both viruses have been documented. Infections by both viruses are characterized by the presence of prominent, intranuclear occlusion bodies, which are referred to as polyhedral occlusion bodies or polyhedral inclusion bodies, in affected epithelial cells of the hepatopancreas and midgut, or free within lysed cell debris in the faeces (8, 9). Crowding, chemical stress, or environmental stress may enhance the pathogenicity and increase the prevalence of MBV or BP in their hosts. Infection by BP and MBV are exclusively by the oral route in which cannibalism and faecal-oral contamination are the principal mechanisms of transmission (6, 8, 11, 12).

The geographical distribution of BP is limited to the Western Hemisphere. However, within the Americas and Hawaii, multiple geographical strains of BP exist, and some of these may be distinguished by the size of the virion or by molecular methods. BP has a widespread distribution in cultured and wild penaeid shrimps in North and South America (3, 4, 5, 8, 9, 10).

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SLIDE 95
CONFERENCE 24 / CASE III – ZH S05-0926 (AFIP 2987670)

Signalment: 2-year-old, female, Bearded Dragon (*Pogona vitticeps*).

History: In a group of 4 animals, one died unexpected without any symptoms. The other remained asymptomatic and are still alive.

Gross Pathology: At necropsy, no gross abnormalities were observed.

Laboratory Results: *Salmonella* spp. were isolated from the intestinal tract after enrichment.

Histopathologic Description: The normal lobular architecture of the liver section is disturbed by multifocal to confluent necrosis. Many hepatocytes are karyomegalic as the result of large smudgy basophilic adenoviral inclusions. Anisokaryosis of hepatocytes and a marked increase of sinusoidal inflammatory cells, which are predominantly lymphocytes, are present. In the necrotic areas there are some fibrin clots. Intranuclear inclusion bodies were also found in the intestinal epithelium but pancreas and lungs were not affected.
Electron microscopy showed intranuclear inclusions consisting of crystalline aggregates of non-enveloped viral particles, some of them with electrodense centers.

Contributor's Morphologic Diagnosis: Acute severe multifocal to coalescing necrotising hepatitis, with basophilic intranuclear inclusions in hepatocytes and Kupffer cells.

Contributor's Comment: Adenoviruses (AdVs) are categorized into four genera: *Mastadenovirus* (from mammals), *Aviadenovirus* (from birds), and two recently accepted genera, *Atadenovirus* and *Siadenovirus*. Phylogenetic analysis indicated that Adenoviruses from reptiles belong to the genus *Atadenovirus* (1).

AdV-like particles have been identified in many reptile species, including 10 snake species, 4 lizard species, and 1 crocodilian species. Lesions in reptiles associated with AdV-like agents include hepatitis, enteritis, esophagitis, splenitis, and encephalopathy (1).

The virus appears to be established in certain breeding groups of the lizard genus Pogona in the United States, and although transmission studies have not been conducted, vertical transmission through the egg *in utero* or at the time of oviposition seems a likely route. Adults may survive as inapperent carriers (2).

AFIP Diagnoses: 1. Liver: Hepatocellular degeneration, necrosis, and loss, multifocally-extensive, marked to severe, with basophilic intranuclear inclusion bodies, bearded dragon (*Pogona vitticeps*), reptile.
2. Liver: Hepatocellular lipidosis, diffuse, moderate.

Conference Comment: Adenoviruses are double-stranded DNA virus that have been reported in numerous reptiles including Nile crocodiles, boa constrictors, rosy boas, rat snakes, Gaboon vipers, Savannah monitor lizards, Jackson's chameleon, and Rankin's dragon. All of these reptiles had intranuclear viral inclusion bodies in the gastrointestinal tract and/or liver; most also had lesions of hepatic necrosis. Adenovirus is established in some breeding groups of bearded dragons in the United States.

Adenoviral inclusions are intranuclear and typically appear basophilic to amphophilic with hematoxylin and eosin. Adenoviral inclusions are often referred to as large and "smudgy" in appearance.

Ultrastructural characteristics of reptilian adenoviruses include the formation of paracrystalline arrays in the nucleus of infected cells. The viral particles are 60-66 nm in diameter, non-enveloped, and have electron-dense or electron-lucent cores.

As mentioned by the contributor, adenoviruses are currently classified into four genera. Below is a comparative list of adenoviruses and some of the diseases they cause (3):
Genus Aviadenovirus (Group 1 avian adenoviruses)**:**

- Fowl, goose, duck, pigeon, turkey adenovirus: Inclusion Body Hepatitis (IBH); hydropericardium syndrome; respiratory disease; necrotizing pancreatitis and gizzard erosions
- Quail Bronchitis (avian adenovirus Type 1): Infects *Colinus virginianus*

Genus Siadenovirus:

- Marble spleen disease (MSD) (Adenovirus Type 2): Pheasants; splenic necrosis, respiratory edema, congestion and asphyxia
- Hemorrhagic enteritis (HE) (Adenovirus Type 2): Young turkeys; bloody droppings, death
- Avian adenovirus splenomegaly virus (AASV)
- Frog Adenovirus

Genus Atadenovirus:

- Egg drop syndrome (subgroup 3 avian adenovirus): Laying hens, viral replication in pouch shell gland epithelium; intranuclear inclusion bodies
- Ovine, bovine, duck, possum adenoviruses
- Reptilian Adenoviruses
    - Bearded dragon, snake, chameleon, gecko

Genus Mastadenovirus:

- Human adenovirus: respiratory disease, enteritis; keratoconjunctivitis
- Simian adenovirus (27 different viruses): mostly subclinical; some secondary to immunosuppression, mild to moderately severe respiratory and enteric disease, keratitis/conjunctivitis
- Canine adenovirus 1: Infectious canine hepatitis
- Canine adenovirus 2: most cases secondary to immunosuppression; produces necrotizing bronchiolitis and alveolar epithelialization

- Equine adenovirus: mild respiratory disease except in CID Arabian foals where adenoviral infection leads to severe bronchiolitis and atelectasis
- Bovine adenovirus: respiratory tract disease, pyrexia, KCS, colic, associated with respiratory and enteric disease in calves but not considered the primary pathogen in either syndrome
- Ovine adenovirus: respiratory tract disease, conjunctivitis, enteritis
- Porcine adenovirus: widespread, mostly subclinical, pneumonia, enteritis associated with encephalitis and diarrhea
- Murine adenovirus: oncogenic in newborns, experimentally induce CNS lesions
- Guinea Pig Adenovirus: pneumonitis
- Adenovirus can experimentally cause tumors in hamsters
- Wildlife: Adenovirus Hemorrhagic Disease in California mule deer produces similar lesions to Bluetongue virus and Epizootic Hemorrhagic Disease (EHD) (orbiviruses). Black-tailed deer adenovirus: hemorrhagic vasculitis, pulmonary edema. Brown bear, coyotes, wolves and raccoons also susceptible to CAV-1

Note, several viral isolates share the same name with isolates from other genera and are only differentiated by letter designators. For a complete table of the adenoviruses, readers are encouraged to visit the ICTV website listed in reference 3.

Readers are encouraged to review Wednesday Slide Conference case 2, conference 29, 1995 for another case of adenovirus in the intestine of a reptile.

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SLIDE 96
CONFERENCE 24 / CASE IV – 366724 (AFIP 2888664)

Signalment: Adult female green tree frog (*Rana* sp.)

History: Owner presented the frog to Angell Memorial Animal Hospital for a one-week history of decreased appetite, lethargy, and progressive erythema along the ventral abdomen and hind legs. The frog shared a peat moss enclosure with another green tree frog, which showed similar clinical signs. Both affected frogs exhibited accelerated skin shedding.

Gross Pathology: On gross examination, the body exhibited dark red, pinpoint mottling of the skin of the abdomen and pelvic extremities. The dorsal skin showed fewer pinpoint lesions but exhibited irregular shedding with numerous flakes of keratin that were easily removed with minimal digital pressure.

Laboratory Results: Routine fungal cultures were negative. Aerobic/anaerobic bacterial cultures exhibited mild to moderate growth of *Sphingobacterium multivorum* (incidental environmental flora).

Contributor's Morphologic Diagnosis: Skin (ventral abdomen): Moderate to severe diffuse orthokeratotic hyperkeratosis and acanthosis with intralesional chytrid organisms and mild superficial perivascular dermatitis

Etiologic diagnosis: Chytridiomycosis

Etiology: *Batrachochytrium dendrobatidis*

Contributor's Comment: Chytridiomycosis, recently identified as an emerging infectious disease of post-metamorphic amphibians, has been implicated in episodes of mass mortality with subsequent population declines in Australia, the Americas, Africa, Europe, Asia and India [1,2,3,4]. The etiologic agent, *Batrachochytrium dendrobatidis*, was first identified from carcasses collected at sites of mass mortalities in Australia, Costa Rica and Panama [1,4]. The gross lesions vary greatly in severity. They consist of epidermal hyperkeratosis with petechia, sloughing, ulceration and hemorrhage and usually involve the toes, pelvic extremities and ventral abdominal skin [1,2,3]. The organisms were identified as a non-hyphal chytrid fungus based on histologic examination of hematoxylin and eosin-stained tissue sections, transmission electron microscopy and 18s rDNA sequence data [1,4].

The organism has a wide host range that includes urodeles, aquatic amphibians, terrestrial and semi-terrestrial frogs and toads. It has a predilection for lower environmental temperatures (montane forests) [2]. Although it has been seen mainly in adult amphibians, tadpoles, which have normally non-keratinized skin, can serve as carriers and will on occasion exhibit lesions around the keratinized mouth parts [1,3].

The organisms infect only the *stratum corneum* and *stratum granulosum*, and develop into a 6-15 micron diameter spherical thallus (zoosporangia) with a discharge papilla that projects to the surface. The zoosporangia appear to be intracellular and display a relatively uniform, 1-2 micron thick, smooth wall. Within the zoosporangia there are variable numbers of any of four developmental stages of the chytrid (zoospores). On

routine hematoxylin and eosin-stained tissue sections, zoospores appear as round to oval basophilic bodies with poorly defined margins. The zoosporangia can retain their shape for some time after the zoospores have been released [1,3].

AFIP Diagnosis: Skin: Epidermal hyperplasia and hyperkeratosis, multifocal, moderate, with minimal subacute dermatitis, and numerous intracorneal fungi, etiology consistent with *Batrachochytrium dendrobatidis*, green tree frog, amphibian.

Conference Comment: The contributor provides an excellent review of an emerging aquatic fungal pathogen of captive and wild frogs and toads.

Chytridiomycosis is now believed to have originated in Africa with the earliest case found in an African Clawed Frog (*Xenopus laevis*) in 1938. In the wild, *X. laevis* does not show clinical signs and the species has not experienced any sudden die-offs, suggesting it is the natural host. Interestingly, *X. laevis* was also geographically disseminated around the world in massive numbers for over 3 decades following the discovery of the pregnancy assay for humans in 1934 (the assay is based on the principle that ovulation in *X. laevis* is induced by injection with urine from pregnant women because of high levels of gonadotropic hormones in the urine). Other factors contributing to the spread of the disease include the establishment of feral populations of *X. laevis* in importing countries (e.g. U.K., U.S.A., and Chile) and transmission to other amphibian species (e.g. the American bullfrog) which subsequently served as vectors through international trade as food items.(5) Recent *in vitro* studies have shown that *B. dendrobatidis* can survive in sterile river soil and sand for up to three months, can attach and grow on feathers and survive drying for up to three hours, suggesting that birds may be disseminating the disease between frog habitats.(6)

The pathogenesis and cause of mortality is unknown but three theories have been proposed: 1) epidermal hyperplasia and orthokeratotic hyperkeratosis incited by chytridiomycosis infection may interfere with important functions of amphibian skin (such as water absorption, osmoregulation and respiration) by creating an impervious barrier; 2) systemic absorption of a fungal toxin; and 3) a combination of the two.

Recent work shows that although there is little genetic variation among isolates of *B. dendrobatidis*, different strains exist with significantly different virulence and behavior (7). As mentioned by the contributor, changes such as hyperkeratosis and congestion occur more frequently on the ventrum. Additionally, the stratum corneum sloughs following a high intensity build-up of sporangia rather than being shed continuously (8).

Readers are encouraged to review Wednesday Slide Conference case 3, conference 23, 2002 and case 4, conference 29, 1999 for additional cases of chytridiomycosis in frogs.

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SLIDE 97
CONFERENCE 25 / CASE I – N85-2570 (AFIP 2994518)

Signalment: A geriatric female rhesus macaque, *Macaca mulatta*, nonhuman primate.

History: This rhesus monkey had a history of chronic weight loss and was being fed and medicated via nasogastric tube. The monkey was found dead during a routine morning health check.

Gross Pathology: Emaciated
Diffusely pale liver with accentuated centrilobular pattern (not submitted)
Cervical mass (not submitted)
No gross lesions were observed in the heart

Histopathologic Description: Multifocally expanding the epicardium, myocardium, and endocardium, and surrounding and replacing degenerate and necrotic myocardiocytes, are variable numbers of lymphocytes, plasma cells, macrophages, and fewer neutrophils. Degenerate myocardiocytes are characterized by fragmentation, pale to hypereosinophilic vacuolated sarcoplasm, loss of cross striations, and

occasional mineralization. Multifocal myocardiocytes contain variably sized oval, intrasarcoplasmic pseudocysts that contain numerous amastigotes. The amastigotes are 2-4 um in diameter, round to oval, and contain a nucleus and a variably distinct rod shaped kinetoplast oriented parallel to the nucleus.

Contributor's Morphologic Diagnoses: 1. Heart: Myocarditis, necrotizing, subacute, multifocal, moderate, with intrasarcoplasmic amastigotes, etiology consistent with *Trypanosoma cruzi*, rhesus macaque, *Macaca mulatta*, nonhuman primate.
2. Liver: Vacuolar change, lipid-type, diffuse, severe. (not submitted)
3. Cervix: Leiomyoma. (not submitted)

Contributor's Comment: *Trypanosoma cruzi* is a hemoflagellate protozoal organism that is the cause of American trypanosomiasis, or Chagas' disease. Unlike many other protozoal parasites it has little host specificity and has been isolated from over 100 mammalian species and dozens of insect vectors. Two forms of trypanosomiasis exist, American trypanosomiasis and African trypanosomiasis. In the United States, the American form is most prevalent in the southern states and has been reported in Maryland. Important reservoir host include dogs, raccoons, opossums, armadillos, and rodents.

Trypanosoma cruzi is passed between animals, and to humans, by reduviid bugs (family Reduviidae), also called cone-nose bugs, assassin bugs, or kissing bugs. The life cycle involves three morphologic forms. Two of the forms, trypomastigotes (blood form) and amastigotes (leishmanial or intracellular form), are found in susceptible animals. The other form, the epimastigote, is found in the digestive tract of the reduviid bug. The reduviid bug is infected when it ingests trypomastigotes in a blood meal from an infected host. Ingested trypomastigotes transform into epimastigotes and undergo asexual reproduction in the vector's gut. The epimastigotes move to the hind gut and transform back into the trypomastigote form. When the reduviid bug feeds it also defecates. Infection occurs when trypomastigote containing feces comes in contact with the broken skin at the site of the bite. Other means of transmission include ingestion of an infected bug, infected feces coming in contact the mucous membranes (eyes or mouth), ingestion of food contaminated with infective feces, and blood transfusions. Once the trypomastigote gains access to the host's blood stream it can enter smooth, skeletal, and heart muscle cells, and cells of the reticuloendothelial system. Transformation into the amastigote stage requires brief exposure to an acidic phagolysosome. The trypomastigote stimulates an increase in intracellular calcium promoting fusion of the phagosome with the lysosome. This results in an acidic environment and also actives hemolysins which disrupt lysosomal membranes and release the trypomastigote into the cell's cytoplasm. Within the cytoplasm trypomastigotes form pseudocysts, and reproduce as amastigotes. Amastigotes develop flagella; rupture the host cell eliciting an inflammatory response, and gain access to the blood stream. Upon entering the blood stream trypomastigotes penetrate smooth, skeletal, and heart muscles, or infect reduviid bugs when the insect takes a blood meal. *T. cruzi* also has the ability to disable the host's alternative pathway of the complement system. The parasite contains a surface homologue of the human

complement regulatory protein decay-accelerating factor (DAF). DAF binds C3b, inhibiting C3 convertase formation and alternative pathway complement activation.

Natural infection with *T. cruzi* has been reported in numerous New World primates from South America and Panama. Old World Monkeys, including macaques and lemurs, become susceptible when located in a geographic area containing reduviid bugs or when experimentally infected. Infection is often subclinical or can result in non-specific clinical signs including generalized edema, anorexia, fever, dyspnea, anemia, leukocytosis, hepatosplenomegaly, lymphadenopathy, and myocarditis. The most frequently occurring lesion in nonhuman primates is myocarditis. The degenerating pseudocysts and destruction of myocardial fibers elicits a mononuclear cell inflammatory response. In conjunction with myocardial cell damage, damage to the conductance pathways in the heart eventually result in dilated cardiomyopathy and cardiac arrhythmias. A similar pathogenesis can occur in the digestive tract resulting in damage to smooth muscle and the myenteric plexus, causing megacolon and megaesophagus.

A histopathologic differential diagnosis for *T. cruzi* in a rhesus monkey would include *Toxoplasma gondii*. Tissue cysts of *T. gondii* can be found in cardiac and skeletal muscle, and contain small (1-3um) oval, PAS-positive bradyzoites that lack kinetoplast. Other non-protozoal causes of myocarditis in some species of nonhuman primates include encephalomyocarditis virus and coxsackie B virus, both members of the family Picornaviridae.

AFIP Diagnosis: Heart: Myocarditis, necrotizing, subacute, multifocal, moderate, with rare intrasarcoplasmic amastigotes, etiology consistent with *Trypanosoma cruzi*, rhesus macaque (*Macaca mulatta*), primate.

Conference Comment: The contributor provides a thorough review of American trypanosomiasis, or Chagas' disease. Conference attendees discussed the infectious causes of myocardial necrosis and developed a differential diagnosis list for "dit-dot", intracellular microorganisms.

Differential Diagnosis list for intracellular "dit-dot" microorganisms:

- *Toxoplasma/Neospora*: No kinetoplast
- *Leishmania*: Larger kinetoplast perpendicular to the nucleus (parallel in *T. cruzi)*; restricted to macrophages (not myocytes); no trypomastigote form
- *Sarcocystis* spp: No kinetoplast
- *Histoplasma capsulatum*: Intracellular yeast; no kinetoplast
- Other *Trypanosoma* spp: No tissue amastigote form

Other causes of myocarditis that attendees considered include malaria as well as encephalomyocarditis virus (EMCV), coxsackie B virus, bacterial infections and vitamin E deficiency.

In addition to *T. cruzi*, several other species of trypanosomes have long been associated with both human and animal disease. Below is a simple chart listing those diseases and the species that cause them:

Species	Disease	Definitive Host	Intermediate Host	Geographic Distribution
T. cruzi	Chagas' disease; American Trypanosomiasis	Man, dog, cat, monkey, opossum, armadillo	Kissing bug	Central and South America and southern U.S.
T. evansi	Surra	Equidae, ruminants, dogs, elephants	Horse flies	Africa, Asia, S. America, Far East
T. equiperdum	Dourine	Equidae	Transmitted by coitus	Cosmopolitan; rare in the United States
T. vivax	Souma	Cattle, sheep, horse, goat, camel	Tsetse fly	Central and South America
T. brucei	Nagana	Man, domestic and wild mammals (not goats)	Tsetse fly	Tropical Africa
T. congolense	Trypanosomiasis	Equidae, ruminants, pig, dog, camel	Tsetse fly	Tropical Africa
T. rhodesiense, T. gambiense	African Sleeping Sickness	Man, antelope	Tsetse fly	East and Tropical Africa
T. equinum	Mal de Caderas	Equidae		Tropical and Subtropical South America
T. hippicum	Murrina de Caderas	Horses and mules		Central America

Chagas' disease in humans can be divided into acute and chronic forms. In the acute form, myocarditis and focal myocardial cell necrosis predominates, often with generalized lymphadenopathy or splenomegaly. The chronic form occurs in 20% of infected patients 5 to 15 years after initial infection and is characterized by intense lymphoplasmacytic and histiocytic interstitial and perivascular inflammation with scattered myocardial cell necrosis and fibrosis. Lesions are believed to be the result of either an immune response directed against the remaining parasites or the production of T-cells and antibodies which cross react with host myocardial and nerve cells. Damage to myocardial and myenteric plexus nerve cells results in dilated cardiomyopathy, megaesophagus and megacolon. (5)

As mentioned by the contributor, infection occurs when feces containing trypomastigotes comes in contact with broken skin at the site of the reduviid bug's bite. Interestingly, in contrast to the vectors found in South America, the vectors identified in the United States exhibit delayed defecation following ingestion of a blood meal, resulting in decreased chance of transmission.

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SLIDE 98
CONFERENCE 25 / CASE II – 5-2556-6-2 (AFIP 2991407)

Signalment: Adult female beluga whale (*Delphinapterus leucas*) with no evidence of reproductive activity.

History: One of 11 hunter harvested animals sampled from the Mackenzie Delta, NWT, Canada, July 1-13, 2004

Gross Pathology: There was diffuse moderate enlargement of the thyroid gland and on sectioned surface, immediately below the capsule and extending randomly within the parenchyma, there were numerous 0.2-1.0 cm, well circumscribed, firm white nodules. There were no other significant gross internal or external lesions.

Laboratory Results: PCR negative for *Brucella* spp. and dolphin morbillivirus. Liver mercury =8.8 ppm, selenium=32.44 ppm. Bacteriology yielded light growth of *Staphylococcus epidermidis* and *Corynebacterium* spp. from the liver and lymph nodes and there was no significant growth from the brain, small intestine, or lung.

Histopathologic Description: Randomly throughout the parenchyma, there are multiple variably sized, well delineated, moderately cellular, to multifocally hypercellular and cystic, partially encapsulated nodules which compress and distort adjoining thyroid follicles. The cells are arranged as sheets, bi- to multilayered cords and entrapped follicles that are occasionally cystic. There is a moderate amount of fibrovascular stroma. Throughout the intervening parenchyma, the follicles are variably sized, occasionally distended by colloid and multifocally bound by micropapillary proliferations.

In smaller follicles, there is multifocal follicular hyperplasia with apical cytoplasmic vesiculation.

Contributor's Morphologic Diagnosis: Thyroid gland: Hyperplasia, adenomatous, multifocal, moderate with follicular cyst formation and micropapillary proliferations

Contributor's Comment: Beluga whales (*Delphinapterus leucas*) are an integral part of the folklore and natural history of the Inuvialuit and federal legislation in Canada affords an inalienable right to these people to hunt and harvest marine mammals for consumption. Marine mammals are exempt from inspection by the Canadian Food Inspection Agency and monitoring and reporting of the health status and public health implications of disease processes in wild beluga stocks has been informally under the purview of the Department of Fisheries and Oceans. Due to ongoing concerns about human consumption and the potential impact of oil exploration and development in the Mackenzie delta, a baseline health assessment was initiated. Between 1 July to 13, July, 2004, 13 beluga whales were harvested by Inuvialuit in the vicinity of Hendrikson and Kendall Islands, North West Territories (NT). Nine animals were females and 4 were males. Two animals were severely emaciated. In 4 whales, there was moderate verminous pneumonia and 2 additional animals had diffuse interstitial pneumonia. Two animals were not available for diagnostic evaluation.

One of the most significant and consistent microscopic findings were within the thyroid gland (in 9 of 11 animals). These nodular proliferations have previously been reported in beach cast and hunter harvested beluga whales in the St Lawrence estuary and Hudson Bay, Quebec. The pathogenesis of these proliferations remains unknown. The nodules are expansile and encapsulated suggestive of adenomas; however, adenomatous hyperplasia may be differentiated from this neoplasm by multifocal glandular involvement, varying degrees of encapsulation, more cellular pleomorphism and similarity to follicular epithelia within the adjoining parenchyma. In humans, hyperplastic nodules are polyclonal; whereas, adenomas are monoclonal. Endocrine disruption associated with contaminant exposure has been proposed; however, tissue loads do not correspond to the degree of thyroid follicular involvement and there is considerable variation in contaminant exposure between the sampled regions. Representative tissues, including thyroid gland, heart, lung, kidney, adrenal gland, spleen, skin and small intestine were collected at the time of harvest and characterization and quantification of thyroid hormone receptors is currently underway.

AFIP Diagnoses: Thyroid gland: Hyperplasia, adenomatous, multifocal, moderate, with follicular cysts and micropapillary proliferations, beluga whale (*Delphinapterus leucas*), cetacean.

Conference Comment: An interesting case of a common lesion in an uncommon species. Conference attendees returned to the age-old debate of adenoma versus hyperplasia. In general, thyroid adenomas are focal, compressive and encapsulated

neoplasms; whereas, thyroid adenomatous hyperplasia is multinodular, non-compressive and unencapsulated. Attendees were divided over whether to assign one morphologic diagnosis of adenomatous hyperplasia or two morphologic diagnoses addressing both adenomas and hyperplasia. Some stressed that the likelihood of multiple neoplasms arising in the same tissue simultaneously is slight; however, others argued that if the same tissue is subjected to oncogenic promoters over a given period of time then the likelihood of multiple neoplasms increases.

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SLIDE 99
CONFERENCE 25 / CASE III– W6922-3 (AFIP 2987457)

Signalment: Two five-month-old pigeons, one female and one male (columbiformes; *Columba livia*)

History: Several pigeons became ill. Clinical signs: lethargy, severe weight loss and acute mortality. Examination of two animals revealed severe anorexia. They were euthanized to do a post mortem examination.

Gross Pathology: Anorexia, content of intestines: mucinous, faces in the rectum were normal. Congested lungs. Liver, spleen, kidney, brains: normal. Heart: dilated. Atrophic m. pectoralis. Bursa of Fabricius: small, dilated

Laboratory Results: Parasitologic examination faces: normal.
Microbiologic examination content of intestine: negative for Salmonella
Microbiologic examination liver sample: Escherichia coli

Histopathologic Description: Bursa of Fabricius: lymphocyte depletion, multifocal aggregates of intracytoplasmic, basophilic globular and botryoid inclusions in epithelial cells and macrophages.

Contributor's Morphologic Diagnosis: Pigeon circovirus infection

Contributor's Comment: Circovirus infection should be suspected as a possible primary agent in young pigeons with various clinical problems. This is due to a circovirus-induced immunosuppression with subsequent concurrent secondary infections. The clinical signs are associated with the secondary infections, which are often the cause of death.

Definitive diagnosis can be made by histopathology or electron microscopy[1,2]. On electron microscopy, the inclusions are nonenveloped and 14-17nm in diameter, arranged in a paracrystalline array.

AFIP Diagnosis: Bursa of Fabricius, follicles: Lymphoid depletion, diffuse, severe, with multifocal histiocytosis and intrahistiocytic basophilic and botryoid cytoplasmic inclusion bodies, pigeon, avian.

Conference Comment: Pigeons with Pigeon circovirus (PiCV) infections develop lesions in the spleen, bursa of Fabricius, gut-associated lymphoid tissue, and bronchus-associated lymphoid tissue. Changes in the spleen can range from lymphofollicular hyperplasia with scattered lymphocyte necrosis to lymphoid depletion and histiocytosis. Bursal changes can also range from scattered lymphocyte necrosis to severe cystic bursal atrophy. Basophilic, botryoid, intracytoplasmic inclusion bodies can be seen in macrophages in splenic, bursal, gut-associated, and bronchus-associated lymphoid tissue and in bursal epithelial cells.

Other avian circoviruses include:
- Psittacine Beak and Feather Disease Virus (PBFDV): feather loss, abnormal feathers, and beak abnormalities in psittacines; basophilic intracytoplasmic inclusions in follicular epithelium
- Chick Infectious Anemia Virus (CIAV): acute, immunosuppressive disease of young chickens characterized by anorexia, depression, anemia and bone marrow hypoplasia with thymic and bursal atrophy in young chicks
- Canary circovirus: "black spot"; abdominal enlargement and gall bladder congestion; feather dystrophy and circoviral inclusions
- Gulls and geese: "runting syndrome"; circovirus-associated disease

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ELECTRON MICROGRAPH
CONFERENCE 25 / CASE IV – EM (AFIP 2995867)

Signalment: 11 month old, female, DBA, mouse.

History: 2 cage mates had a 2 day history of conjunctivitis and were found dead in the cage. This animal was sacrificed and investigated for suspected ectromelia virus infection.

Gross Pathology: Lesions in various cage mates ranged from conjunctivitis (Fig 1) to ulcerative dermatitis (Fig 2).

Laboratory Results: PCR was positive for mouse pox virus.

Histopathologic Description: Histologically, there was multifocal proliferative and ulcerative dermatitis with large, eosinophilic, intracytoplasmic inclusions (Fig 3F). Skin lesions were immunohistochemically positive for vaccinia virus. (Fig 3)

TEM description: Conjunctiva: There are multiple epithelial cells that lie on a basement membrane and are covered by lamellations of keratin. The cytoplasm of most cells contains a large, generally round, moderately electron dense, amorphous inclusion that occasionally displaces the nucleus. All cells contain numerous, small, round, electron dense particles. On inset, these particles are round, measure 200-250 nm in diameter, and contain an elliptical or peanut-shaped, electron dense core (pox virus).

Contributor's Morphologic Diagnosis: Conjunctiva: Degeneration, with numerous intracytoplasmic pox viruses and few electron dense inclusions.

Contributor's Comment: Ectromelia virus is an orthopoxvirus closely related to Variola virus and Monkeypox virus. It is a useful model for the study of poxviral pathogenesis and both antiviral and vaccine testing.[1] There are generally three clinical forms of the disease. Susceptible mice, such as C3H, DBA, and BALB/c develop acute, fatal disease with coagulative necrosis of the liver, spleen, and other organs. Moderately susceptible mice develop chronic skin lesions with erosive and ulcerative dermatitis that can lead to amputation of the distal limbs and tail. Resistant mice, such as B6, are asymptomatic.[2,3] Resistant mice often exhibit a stronger and more rapid immune response when compared to susceptible strains.[1]

Grossly, livers can be swollen and friable with pinpoint, white foci of necrosis. There is conjunctivitis, cutaneous erythema, crusting, alopecia, and dry gangrene of extremities. Microscopically, there is multifocal and random coagulative necrosis of hepatocytes. Adjacent hepatocytes undergo degenerative changes and often contain basophilic, to

amphophilic, intracytoplasmic inclusions (type B). Within the skin there is epidermal hyperplasia, ballooning degeneration, and prominent eosinophilic, intracytoplasmic inclusions (type A). Similar lesions can be found in the conjunctiva, mucous membranes, and intestinal epithelium.

Ultrastructurally, infected cells exhibit typical degenerative changes. Within hepatocytes there is loss of glycogen, vesicular swelling of the endoplasmic reticulum, mitochondria, and Golgi, and sparse lipid droplets.[2] The cytoplasm is often displaced by low electron-dense matrices thought to be the ultrastructural correlate of type B inclusions.[2] Immature viral forms emerge that have a dense, eccentric body and a less dense matrix of viroplasm enclosed by a membrane with surface projections.[4] Mature virions are often brick-shaped and are 300 to 450 nm in diameter. They consist of an electron-dense, often dumbbell-shaped core, which contains viral DNA, and is surrounded by an intermediate coat and a lipoprotein envelope.[4]

Pox viruses encode several proteins that promote infection and survival. They often encode growth factors that mimic epidermal growth factor and stimulate keratinocytes. The stimulated keratinocytes have an increased susceptibility to pox viral infection.[4] In addition, ectromelia virus further promotes infection by encoding a protein that inhibits UV-induced apoptosis.[4]

AFIP Diagnosis: Conjunctiva (per contributor): Epithelial cell degeneration, with numerous intracytoplasmic pox virions, and few electron dense inclusion bodies, DBA mouse, murine.

Conference Comment: The contributor provides an excellent review of the ultrastructural characteristics of pox viral infections. Attendees described the normal ultrastructural anatomy of epithelial cells and then discussed the key features supportive of poxviral infection i.e. lamellations of keratin indicative of hyperkeratotic orthokeratosis; electron dense matrices interpreted as inclusion bodies; increased lucency within the cytoplasm supportive of intracellular edema (ballooning degeneration) and the characteristic 300-400 nm in diameter “brick-shaped” virions with a “dumbbell-shaped” DNA core.

Ectromelia virus was discovered in England in 1930 and has been extensively studied as a model of the pathogenesis of exanthematous diseases. The study of ectromelia virus led to the concept of a primary and secondary viremia as well as the role of cell mediated immunity, particularly T-lymphocytes and macrophages, in the recovery from infection. (5) Mousepox has a restricted host range and causes severe disease with a high mortality rate (50-100%). Voles are believed to be the natural host.

There are two recognized forms of mousepox: the rapidly fatal form with few, if any, cutaneous lesions and the chronic form with ulceration of the skin, particularly on the snout, feet and tail often resulting in loss of limbs or tail.

The typical gross findings in mice include conjunctivitis, alopecia, crusting and erythema of the skin and dry gangrene of the extremities and tail. The liver and spleen swell and develop multifocal hemorrhages and pinpoint white foci. Lymph nodes and Peyer's patches may be enlarged and hemorrhagic.

Typical light microscopic findings include intracytoplasmic eosinophilic A-type (Marchal) inclusion bodies within the epidermis, pancreas and intestine. Early in the disease there is epidermal hyperplasia, hypertrophy, spongiosis and ballooning degeneration with inclusion bodies. Later, the epidermis becomes necrotic and ulcerated. The liver develops multiple foci of coagulative necrosis, hepatocellular syncytia and vacuolar degeneration with minimal inflammation. There is splenic necrosis involving both lymphoid follicles and red pulp. Additionally, there may be intestinal mucosal erosions and bone marrow degeneration. The combination of hepatic and splenic necrosis along with cutaneous lesions and characteristic eosinophilic intracytoplasmic inclusion bodies is pathognomonic. (3)

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