Conference Note: In conjunction with the AFIP Department of Telemedicine, the AFIP Department of Veterinary Pathology held its first virtual slide conference. The four glass slides selected for this conference were digitally scanned, and the “virtual slides” were made available to department staff and residents a week prior to the conference. Residents used Aperio ImageScope® software on their PCs to evaluate the virtual slides, develop morphologic descriptions, and make their diagnoses. During the conference residents used the same software to navigate the slide and present the case to attendees. Residents and staff received the glass slides after the conference. Following the conference, this virtual microscopy experiment received mixed reviews from the participants. Most residents found the software easy to learn and user-friendly. The resolution and refresh rate of the projected image in the conference room was superior to that of our usual digital camera-projected glass slide image. Some individuals were frustrated during their conference preparation by the slow refresh rate on their individual PCs (varied widely based on computer speed and network/internet connection). The major concerns for most residents were their inability to use fine focus or easily mark key diagnostic fields on the slides.

CASE I – NOVARTIS CASE 1 (AFIP 2985458).

Signalment: 2-year-old, male, rat, IGS Wistar Hannover Rat, Crl: WI (G1x/BRL/Han) IGS BR (*Rattus norvegicus*).

History: This rat was part of a standard 2 year carcinogenicity study.

Gross Pathology: At scheduled sacrifice, there was a firm mass at the proximal tail that measured 30x20x20mm.
Histopathologic Description: Histologically, the tumor is composed of an expansile, unencapsulated mass of physalipherous (foamy, ‘bubble cells’) cells with clear, vacuolated cytoplasm, distinct plasma membranes, round to oval euchromatic nuclei and central nucleoli, growing in nests or cords and divided into lobules by a thin fibrous stroma. Mitotic figures are rare or not present. The tumor cells have locally invaded and replaced adjacent bone, muscle and neurovascular bundles. At multiple sites, the tumor has small foci of necrosis, mixed cell inflammation and islands of bone that are entrapped by the tumor cells. This bone consists of well-differentiated osteocytes with variable numbers of osteoclasts, some of which have assumed giant, bizarre morphology. No metastases were noted.

Contributor’s Morphologic Diagnosis: Tail: Chordoma, IGS Wistar Hannover, rat.

Contributor’s Comment: Chordomas are believed to arise from residual notochordal tissue in the axial skeleton, and have a predilection form the proximal and distal extremities. They are most common in the lumbosacral spinal cord of Fischer 344 rats. Chordomas have been reported in humans, rats, mice, dogs, cats, ferrets and mink.

Chordoma can be confirmed with histochemistry (fat negative, PAS positive) and immunohistochemistry (S-100, keratin, and neuron-specific enolase positive), which differentiate it from liposarcoma and chondrosarcoma.

Tissue sections and records of 56 rats with chordoma were identified in the National Toxicology Program’s (NTP) data base of approximately 115,000 Fischer 344 rats. Tumors were examined to determine morphological characteristics, incidence, and aspects of biological behavior. Chordomas occurred in aged rats, originated predominantly in lumbosacral vertebrae, were highly malignant, occurred three times more often in male versus female rats, and commonly produced bilateral posterior paresis, paralysis, and/or distention of the colon and rectum.

AFIP Diagnosis: Tail: Chordoma, IGS Wistar Hannover rat (*Rattus norvegicus*), rodent.

Conference Comment: Chordomas are usually composed of three concentrically arranged components in domestic animals: lobules of closely packed vacuolated (physalipherous) cells at the periphery, cartilage in between, and well-differentiated trabecular bone in the center. Physalipherous cells are pathognomonic for chordomas and may be surrounded by a mucinous matrix. The mitotic rate, as in this case, is low.
As pointed out by the contributor, immunohistochemical stains such as S-100, keratin, and neuron-specific enolase help to differentiate chordoma from liposarcoma and chondrosarcoma. Additionally, neoplastic cells express vimentin.3,4,7,8

Chordomas are the most frequently reported musculoskeletal neoplasm of ferrets and are rarely reported in other species. Chordomas can arise anywhere along the axial skeleton; however, predilection sites differ among species. In the ferret, chordomas are typically located distal to the last caudal vertebra expanding the tip of the tail forming a multilobulated club-shaped mass. Chordomas located on the tail of ferrets are slow-growing and rarely, if ever, metastasize. Cervical chordomas, on the other hand, can induce osteolysis as well as compression of the spinal cord and adjacent tissues. Rarely, chordomas occur in the thoracic vertebra of ferrets. Cutaneous metastasis and neurological signs may also occur in cervical chordomas. In other species, chordomas are more commonly located in the sacrococcygeal region.3,4,5,6,7

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References:
CASE II – LMU Neuropath 214/04 (AFIP 3031290).

**Signalment:** German Fleckvieh (*Bos taurus taurus*), 7-month-old, female, bovine.

**History:** Sudden onset of ataxia and paraparesis, progressing to paraplegia within hours. Further signs included unilateral hypopyon and fever. The animal was euthanized after unsuccessful symptomatic treatment.

**Gross Pathology:** Brain (not provided): On cut surface there were multiple, well circumscribed brownish-red foci of a few millimeters diameter confined to the watershed area of the telencephalon.

Spinal cord: On transverse slices, bilaterally asymmetric, non-contiguous brownish-red discoloration of the thoracolumbar gray matter with occasional softening and cavitation could be seen at multiple segments.

**Histopathologic Description:** Slices of the brain, spinal cord and spinal nerve roots reveal a severe multifocal thrombotic necrotizing vasculitis of small parenchymal blood vessels with associated edema, microinfarcts and rarefaction of the adjacent neuroparenchyma. Vasculitis is characterized by fibrinoid necrosis, perivascular fibrin deposition and massive transmural, mixed-cellular inflammatory infiltrations, comprising mainly polymorphonuclear leukocytes and macrophages as well as many eosinophils. These lesions are far most severe in the ventral column of the spinal cord, the adjacent ventral funiculus and some ventral nerve roots. Within the affected spinal gray matter, there are numerous eosinophilic nerve cell necroses, with or without neuronophagia, multiple axonal spheroids, and activated microglial cells. Affected parts of the spinal white matter tracts display signs of bystander demyelination and clusters of multiple eosinophilic spheroids. Large thrombi can also be found in branches of the ventral spinal artery and in the main trunk of the ventral spinal vein. Some of these thrombi present with an endothelial cell lining at the surface and already have been incorporated into the vessel wall. Others undergo puriform lysis. Some sections contain intralesional gram-negative coccoid bacteria.

**Contributor’s Morphologic Diagnosis:** Spinal cord, vasculitic radiculomyelitis, necrotizing, thrombotic, subacute, severe, multifocal, with gram-negative coccoid bacteria

**Contributor’s Comment:** Histopathologic patterns of this case strongly resemble those of thrombotic meningoencephalitis (TME) caused by *Histophilus somni* (former *Haemophilus somnus*) infection. *Histophilus somni* is a Gram-negative
inhabitant of the bovine nasopharynx and urogenital tract. Under circumstances that compromise local resistance and the immune system in general, \( H. \textit{somni} \) acts as an opportunistic pathogen causing severe localized or systemic infections. Thereby, infection occurs mostly around yearling age and in winter time, or is associated with transportation and/or crowding stress.

Septicemic courses present with meningoencephalitis, pneumonia, pleuritis, myocarditis, infertility, abortion, and arthritis. The initial lesion is a widespread necrotizing vasculitis with extensive thrombus formation. Although incompletely understood, \( H. \textit{somni} \)-mediated vascular damage induces endothelial cell apoptosis and immunopathic mechanisms.\(^3\)

Therefore, \( H. \textit{somni} \) is equipped with virulence factors that mediate adherence to endothelial cells, interference with phagocytosis, and resistance to killing by complement. Evasion from host’s immune defense is achieved by lipo-oligosaccharide (LOS) phase variation and sialylation, and employment of immunoglobulin binding proteins.\(^2\) Just recently a group of high molecular weight binding proteins (HMWBP), encoded by one single open reading frame, have been identified that are capable of binding bovine IgG2 Fc-segments. They are antigenic and have been protective to calves in an immunization /challenge experiment.\(^4\) Once uptaken by neutrophils and macrophages, \( H. \textit{somni} \) survives innate bactericidal immune responses by inhibition of \( \text{O}_2^- \) production and scavenging \( \text{H}_2\text{O}_2 \) in presence of carbohydrate energy sources.\(^2\)

HMWBP also mediate adhesion to bovine endothelial cells.\(^4\) These tackled cells undergo LOS-induced activation of caspases 1, 3 and 8 and upregulate IL-1 transcription.\(^3\) Unfortunately, recruitment of immune cells, complement and coagulation cascades through endothelial signals result in fulminant destruction of the blood vessels rather than elimination of these bacteria.

In the CNS, the vasculitis predominantly is seen in watershed areas. In early stages (vasculitic encephalomyelopathy), brain and spinal cord homeostasis is affected by reduced perfusion and blood-brain-barrier break down. With influx of polymorphonuclear inflammatory cells and bleeding, release of enzymes, cytokines/chemokines and reactive oxygen species aggravate cell and tissue destruction (bystander effect) as a vicious cycle.

Other specific agents causing similar pictures are \( \text{Salmonella} \) spp. and Zygomycetes.\(^1\) The latter could be excluded by absence of fungal hyphae in additional stains (periodic acid Schiff).
AFIP Diagnosis: Spinal cord: Vasculitis, leucocytoclastic, multifocal, with thrombosis, axonal degeneration, and Gram negative bacteria, German Fleckvieh (*Bos taurus taurus*), bovine.

Conference Comment: The contributor provides a concise overview of the pathogenesis and histologic lesions associated with *Histophilus somni*, the cause of thrombotic meningoencephalitis (TME). Grossly, random red-brown necrohemorrhagic foci (infarcts) are visible most frequently in the cerebrum at the cortical gray matter-white matter interface. The brain may be swollen with flattened gyri due to edema. Fibrinopurulent meningitis is common with cloudiness of the CSF. The lesions are visible externally as well as on cut surfaces and may be seen in the spinal cord as in this case.\(^5,6\) As pointed out by the contributor, the lesions in this case are most severe in the gray matter, but do extend into the adjacent white matter.

In addition to TME, pneumonia, pleuritis, myocarditis, infertility, abortion, and arthritis, *H. somnus* has also been implicated as a cause of necrotizing laryngitis (calf diphtheria) in conjunction with *Fusobacterium necrophorum*.\(^7\)

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References:
1. AFIP WSC 20/2005-2006, Case III – 37236 (AFIP 2996898)
CASE III – 2006904019 (AFIP 3031788).

Signalment: 5-year-old, neutered male, domestic ferret, *Mustela putorius furo*.

History: The animal had diarrhea for a long time and visited a veterinary hospital due to persistent diarrhea lasting more than 10 days before death. This animal received antibiotics and prednisolone but did not respond to therapy. It went into a coma and died.

Gross Pathology: The animal was necropsied by a veterinary practitioner. Two small whitish nodules were detected on each cut surface of the spleen and liver. The right adrenal was markedly enlarged (11x7x7 mm) and adhered to the liver.

Histopathologic Description: The structure of the cortical and medullary tissue of the right adrenal gland was within almost normal range except for slight proliferation of spindle cells in the medulla. No hyperplastic focus or neoplastic changes were seen in the submitted sample. A multilocular cyst lined by flattened or cuboidal monolayered epithelium occupied more than half of right adrenal gland (Fig. 1). These cysts were filled with eosinophilic mucoproteinaceous fluid containing cell debris and calcified granules. There were also many tubular structures lined by cuboidal epithelial cells adjacent to these cysts. These tubular structures were similar in appearance to the bile ducts and some of the tubules contained small amounts of eosinophilic fluid and connected to the cysts. The cysts and tubules were located in the cortex and medulla. In some areas, cysts and tubular structures were surrounded by small amounts of connective tissue and metaplastic bone. Clusters of hepatocytes were scattered near the cysts (Fig. 2). Although the morphological characteristics of the hepatocytes were very similar to those of cortical cells, the hepatocytes had darker cytoplasm and wider intercellular spaces in contrast to the cortical cells. Adjacent to the right adrenal, similar tubular structures and multiloculated cyst filled with eosinophilic mucoproteinaceous material were also observed in the liver. This area was well demarcated from surrounding liver tissue and contained cluster of adrenocortical and medullary cells (Fig. 3).

Immunohistochemically, epithelial cells lined the tubular structures and cysts were strongly positive for cytokeratin 7 (DAKO), weakly positive for cytokeratin AE1/AE3 (DAKO) and negative for cytokeratin 14 (Biomeda).

Contributor’s Morphologic Diagnoses: 1. Adrenal gland: Ectopic hepatic tissue with cystic dilatation of bile ducts filled with eosinophilic mucoproteinaceous fluid
2. Liver: Ectopic adrenal tissue
3. Small intestine and visceral lymph nodes: Malignant lymphoma (not submitted)

**Contributor’s Comment:** Solitary or multiple cysts and tubular structures lined by monolayered epithelial cells are rarely seen in the surgically resected adrenal gland tissue from pet ferrets. We have examined a total of 792 surgically resected adrenal glands and epithelial-lined cysts or tubular structures were detected in 20 cases (2.5%). They consisted of 16 out of 108 right adrenals (14.8%) and 11 out of 684 left ones (1.6%). Seven cases had bilateral lesions. All ferrets were gonadectomized at an early age.

Almost all adrenal glands were surgically resected, because proliferative changes were suspected in the adrenal cortices by the onset of clinical signs such as alopecia, enlargement of prostatic gland or swelling of vulva. Nodular or diffuse hyperplasia, adenoma or carcinoma of cortical cells was detected in the majority of the affected adrenal glands. In many cases, moderate to severe enlargement of the spleen with enhanced extramedullary hematopoiesis was also recognized at laparotomy. Clinical signs and enhanced extramedullary hematopoiesis of the spleen were attributable to the imbalance of steroid hormones.

Remnant of the mesonephric duct or ovarian tissue was considered for the genesis of cysts and tubular structures, although the pathologic significance was not clear. Ectopic bile cyst was also considered, because small cluster of hepatic cells was observed near the cysts in two cases. However, hepatocytes and adrenocortical cells took very similar morphologic characteristics and immunohistochemical staining for hepatic mitochondria (hepatocyte paraffin-1, DAKO) to differentiate the hepatic cells from cortical cells was not available for the ferret tissue.

From the changes of this case, it is confirmed that multilocular cysts or tubular structures lined by monolayered epithelial cells are derived from bile cysts from ectopic hepatic tissue. Results of immunohistochemical staining of epithelial cells for various cytokeratins are also identical to those of bile duct epithelium.

**AFIP Diagnosis:** Adrenal gland: Cysts, multiple, ferret (*Mustela putorius furo*), mustelid.

**Conference Comment:** Biliary cysts commonly occur in ferret adrenal glands and are considered an incidental finding. They are most often encountered in the right adrenal gland which may share a common capsule with the caudate lobe of the liver. Cysts are filled with a hard translucent, waxy material, which appears eosinophilic on H&E. In some cases, this material may be the only submission in surgical biopsies of ferret adrenal glands.
A recent study in Japan identified epithelial-lined cysts as incidental findings in 0.03% of adrenal glands of ferrets (11/440).\textsuperscript{1} In this study, the right adrenal gland was twice as likely to contain these structures as the left. Positive immunohistochemical staining for cytokeratin 7, 14 and 19, AE1/AE2 in the epithelial cells lining these cysts strongly suggest that the cysts are biliary in origin.

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**References:**

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**CASE IV – MK062234 (AFIP 3026799).**

**Signalment:** Five-year-old male rhesus macaque.

**History:** An approximately five-year old male rhesus macaque was inoculated intravenously with 5 X10\textsuperscript{3} TCID\textsubscript{50} SHIV DH12R obtained by mixing virus obtained from five previously SHIV infected rhesus macaques. Plasma viral loads decreased and remained at low levels until euthanasia. CD4 and CD8 levels remained between 500-1500 cells/ul over the course of 180 weeks. The animal was euthanized on 12/15/2005 due to scrotal edema and overall decline in condition.

**Gross Pathology:** At necropsy the macaque had moderate subcutaneous edema and moderate ascites with approximately 150 ml of clear fluid (total protein of 1.4 g/dl) in the abdominal cavity. The urinary bladder contained a moderate amount of urine (SG 1.010, 2+ protein). Both kidneys were severely enlarged, pale and tan. The right kidney weighed 64.9 g and measured 6.8 X 4.5 X 3.0 cm and the left weighed 67.4 g and measured 7.0 X4.0 X 2.8 cm. The average kidney weight for a normal adult male rhesus is 13.7 g for the left kidney and 12.9 g for the right with each measuring approximately 3.5 X 2.5 X 1.5 cm.\textsuperscript{1} The ascites and subcutaneous edema are consistent with hypoproteinemia and hypoalbuminemia likely secondary to renal disease.

**Laboratory Results:** 12/7/2005:

- **BUN** 67 mg/dl (9-23)
- **CREAT** 1.5 mg/ml (0.7-1.3)
- **PHOS** 3.5 mg/dl (0.9-8.0)
ALB 0.9 g/dl (3.3-4.7)
TP 3.1 g/dl (6.0-7.8)

**Histopathologic Description:** Sections of kidney revealed bilateral severe glomerulonephritis characterized by the following glomerular changes: enlarged glomeruli with thickened capillary basement membranes (wire loops), increased numbers of parietal and visceral epithelial cells, increased mesangial matrix and adhesions to Bowman’s capsule. Within glomeruli there were small numbers of necrotic cells and associated neutrophils. Prominent dilated tubules were present containing proteinaceous fluid. There was a moderate lymphoplasmacytic interstitial nephritis and multifocal lymphoid aggregates with associated tubular loss and tubular regeneration.

Other findings in this case included mild plasmacytic colitis, mild nonsuppurative pulmonary perivasculitis, mild lymphoid hyperplasia of lymph nodes and spleen, and mild hemosiderosis of the spleen and liver.

Electron microscopy (EM) of the kidney revealed, dense deposits primarily in the basement membrane, with lesser amounts on the epithelial side of the basement membrane. These dense deposits formed humps and the basement membrane was markedly thickened and wrinkled.

Frozen kidney sections were not available and immunohistochemistry performed on paraffin sections were negative for IgA, IgM and IgG.

**Contributor’s Morphologic Diagnoses:**
1. Glomerulonephritis, membranoproliferative, diffuse, severe, bilateral.
2. Interstitial nephritis, lymphoplasmacytic, multifocal, moderate, bilateral.

**Contributor’s Comment:** Morphological findings associated with membranoproliferative glomerulonephritis include proliferation of parietal epithelial cells with possible crescent formation, mesangial cell proliferation, leukocyte infiltration, thickening of basement membranes and adhesions to Bowman’s capsule. The thickened basement membrane occurs when the immune system, with an adequate number of T-cells, is overstimulated, and antibodies react with antigens located in and around the basement membrane. Additional pathological processes affecting the glomerulus include necrosis, apoptosis and activation of glomerular endothelial cells. Complement activation and has been experimentally shown to cause apoptosis. Silver stains can distinguish the splitting of the basement membrane due to the mesangium expanding into the capillary loops. Definitive diagnosis of glomerulonephritis is by renal biopsy and electron microscopy to localize the immune complexes in and around the basement.
membrane. Immunofluorescent labeling is needed to further characterize the immune complexes as IgG, IgM or C3 which typically has a linear pattern.

Based on ultrastructure and immunofluorescence membranoproliferative glomerulonephritis is classified into three morphologic types. In type I, immune deposits are primarily subendothelial with granular deposition of C3 and IgG. In type II, immune deposits are primarily within the glomerular basement membrane with granular deposition of C3 and IgG usually absent. In type III, the immune deposits are both subepithelial and subendothelial. In all three types there is often a double contour of the basement membrane caused by duplication of the basement membrane with interposition of mesangial cell processes giving a “tram-track” appearance.3

Glomerulonephritis has been reported in humans, monkeys, dogs, cats, mice, rats and white perch.4,5,6,7,8,9,10 Membranoproliferative glomerulonephritis has been reported in approximately 10% of HIV infected individuals.11 Immunofluorescence of the glomeruli may be positive for IgA, IgG, IgM, C3 and C1q. IgM deposits were most commonly seen in patients with HIV who had clinical signs of nephrotic syndrome and renal insufficiency. EM in these cases showed deposits in the subendothelial, subepithelial side of the basement membrane and within the mesangium.12 SIV has a similar genetic structure as HIV-1 and infects macaques causing similar adverse health effects including the loss of CD4+ T cells, thus making an excellent model for studying AIDS.13 Immune mediated glomerulonephritis is an infrequently reported finding in macaques.5 It is unclear in the present case if the finding of glomerulonephritis is incidental to the experimental infection with SHIV. Only one case has been reported in the literature of an SIV infected macaque which developed immune mediated glomerulonephritis.14 The possibility that long term antigenic stimulation from chronic low level virus infection may have predisposed this animal to immune mediated disease can not be ruled out.

An important differential diagnosis for glomerular disease associated with infection with SIV or HIV is focal segmental glomerulosclerosis (FSGS).15 FSGS is characterized by proteinuria, microhematuria, and mesangial expansion. By EM there is podocyte damage, foot process effacement, immune deposits in the filter slits and endothelial tubuloreticular inclusions. These immune deposits are a passive response to accumulation of clumps in the bloodstream that are trapped. By immunofluorescence there is IgM and C3 in the mesangium and/or sclerotic areas. With disease progression the sclerosis increases along with the amount of mesangial matrix.3 The pathology of SIV associated FSGS is similar to that seen with HIV with increased mesangial matrix, collapsing glomeruli capillaries, immunoglobulin deposits, increased macrophages and tubuloreticular inclusions.16 FSGS has been reported in approximately 60% of people infected with HIV that
have had renal insufficiency and occasionally in monkeys infected with SIV. The pathogenesis of FSGS is poorly understood. Additional differential diagnoses include minimal change disease (lipoid nephrosis), diabetic nephropathy, renal amyloidosis, lupus nephropathy and poststreptococcal glomerulonephritis. Congo red staining in this case was negative of amyloid deposition.

**AFIP Diagnosis:** Kidney: Glomerulonephritis, membranoproliferative, global, diffuse, with tubular degeneration, regeneration, protein casts, and multifocal, moderate lymphoplasmacytic interstitial nephritis.

**Conference Comment:** The contributor provides a complete and thorough overview of glomerulonephritis to include different types of glomerulonephritis, histopathologic and ultrastructural characteristics of each, as well as differential diagnoses. Glomerulonephritis, usually of immune origin, is a common cause of renal disease in domestic animals, and frequently precedes end-stage kidneys and renal failure, especially in dogs and cats. A list of causes of immune-mediated glomerulonephritis in domestic animals is summarized below.

### Diseases with Immune-Complex Glomerulonephritis

#### HORSES
- Equine infectious anemia
- *Streptococcus* sp.

#### CATTLE
- Bovine viral diarrhea
- Trypanosomiasis

#### SHEEP
- Hereditary hypocomplementemia in Finnish Landrace lambs

#### PIGS
- Hog cholera
- African swine fever

#### DOGS
- Infectious canine hepatitis
- Chronic hepatitis
- Chronic bacterial diseases
- Endometritis (pyometra)
- Pyoderma
- Prostatitis
- Dirofilariasis
- Borreliosis (Lyme disease)
- Systemic lupus erythematosus
- Polyarteritis
- Autoimmune hemolytic anemia
- Immune-mediated polyarthritis
- Neoplasia – mastocytoma
Hereditary C3 deficiency
Leishmaniasis

CATS
Feline leukemia virus infection
Feline infectious peritonitis
Feline immunodeficiency virus
Progressive polyarthritis
Neoplasia
Progressive membranous glomerulonephritis

MINK
Aleutian disease

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References:


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