



WEDNESDAY SLIDE CONFERENCE 2013-2014

Conference 5

16 October 2013

CASE I: N12-191 (JPC 4019350)

Signalment: 5-week-old male Yorkshire cross, (*Sus scrofa domesticus*).

History: Three days before submission, the piglet became listless, developed a head tilt,

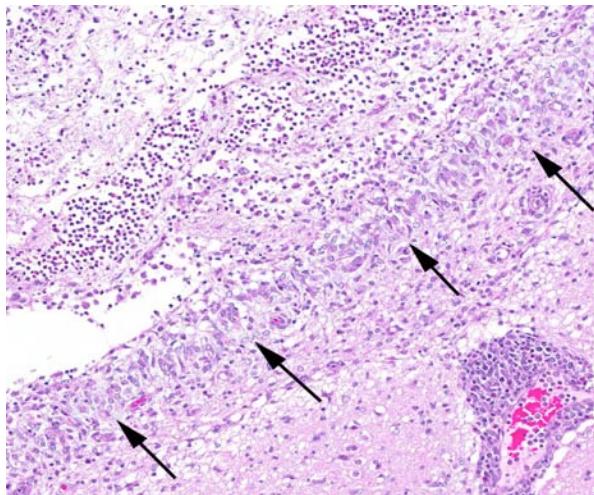
ataxia, hind limb paresis and was circling. The piglet was treated with oral meloxicam and subcutaneous ceftiofur. On the day of submission, the piglet was recumbent with generalized muscle tremors. Additional piglets in this litter were less severely affected; however, one additional piglet from this litter was submitted for necropsy 2 days later with a similar clinical history.



1-1. Cerebrum at level of lateral ventricle, pig: At subgross inspection, the lateral ventricle is markedly expanded, contains an exudate, and there is rarefaction in the periventricular white matter. (HE 0.63X)

Gross Pathology: There are small, multifocal, perivascular white to tan plaques on the surface of the cerebral cortex. The lateral ventricles are moderately dilated and contain light yellow, clear fluid.

Histopathologic Description: Brain: Within the parenchyma and surrounding vessels around the ventricle, there are low to moderate numbers of lymphocytes, macrophages, plasma cells, neutrophils and few eosinophils. Low numbers of neutrophils, lymphocytes, plasma cells and macrophages infiltrate the ependymal layer,



1-2. Cerebrum at level of lateral ventricle, pig: The ependyma is hypercellular with transmigrating inflammatory cells. At upper left, the ventricular exudate is composed of abundant fibrin and a mixed inflammatory population of numerous neutrophils, and fewer macrophages, eosinophils, lymphocytes, and plasma cells. At lower left, periventricular vessels are cuffed by moderate numbers of histiocytes, lymphocytes, and plasma cells. (HE 40X)



1-3. Lateral ventricle, pig: 1-2 μm Gram-positive paired cocci, consistent with *Streptococcus suis*, are present within phagocytes and adhered to necrotic debris. (Brown and Brenn 1000X)

which is disorganized, as well as the underlying parenchyma. The parenchyma surrounding the ventricle is edematous, and blood vessels with perivascular cuffs are lined by plump endothelial cells. The leptomeninges multifocally contain few to low numbers of lymphocytes, fewer macrophages and rare eosinophils and neutrophils. The ventricular lumen contains aggregates of fibrin, moderate numbers of neutrophils and macrophages, few multinucleated giant cells and rare eosinophils. Occasionally admixed with inflammatory cells and fibrin are Gram-positive cocci that are multifocally arranged in short chains.

Contributor's Morphologic Diagnosis: Brain: Meningoencephalitis and ventriculitis, neutrophilic, lymphohistiocytic and eosinophilic to pyogranulomatous, subacute, multifocally extensive, moderate, with intralesional gram-positive cocci.

Contributor's Comment: The cause for the neurologic signs was a meningoencephalitis that was localized primarily around and within the ventricles and choroid plexus. Multifocally within the ventricles, there were occasional clusters of intralesional gram-positive cocci arranged in short chains. Aerobic bacterial culture of the brain yielded *Streptococcus suis*, confirming suspicion of a streptococcal infection. The distribution of the inflammation was similar

to what is described in association with this bacterium,¹³ although the presence of multinucleated giant cells and eosinophils has not been reported within cases of streptococcal meningoencephalitis. This may speculatively have been due to an unusual serotype of the bacterium or an unidentified co-infection. The lesions in the other piglet submitted from this group the following week were similar.

Streptococcus suis is a gram-positive, facultative anaerobic, α -hemolytic streptococcus belonging to Lancefield group D.^{3,11} More than 30 serotypes have been identified, and most infections in pigs in most countries are caused by serotype 2.³ Disease is mainly seen in weanlings and growing pigs, with incidence peaking at weaning, and may include septicemia, serositis, meningitis, polyarthritis, pneumonia, abortions, abscesses and endocarditis.^{3,11}

Outbreaks of *S. suis* generally have low morbidity and mortality ranging from 0-20%, depending on treatment.¹¹ Carriers are significant factors in disease transmission, and outbreaks may occur in closed herds.¹¹ Stress can predispose to infection, and concurrent infections increase morbidity.¹¹

JPC Diagnosis: Cerebrum, lateral ventricle: Ventriculitis and paraventriculitis, fibrinosuppurative, granulomatous and eosinophilic, with mild to moderate meningitis

and intra-ventricular and intra-neutrophilic gram-positive cocci.

Conference Comment: *Streptococcus* species are catalase-negative, opportunistic pathogens affecting multiple organ systems in various species. They are generally categorized on the basis of their hemolytic pattern on blood agar as α, β or γ (non)-hemolytic. α- and γ-hemolytic streptococci are often normal inhabitants of the upper respiratory and lower urinary tracts, as well as the skin and gastrointestinal tract, while pathogenic species are usually β-hemolytic. *Streptococcus* species can be further designated into Lancefield groups A-V (excluding I and J) based on their cell wall polysaccharides.⁵ *S. suis*, considered one of the most important bacterial pathogens of swine, has several important virulence factors, including its capsular polysaccharide and virulence-related proteins such as muramidase-released protein, extracellular protein factor and hemolysin. Hemolysin (or suilysin), is thought to enhance bacterial invasion and lysis of host cells. Suilysin is expressed by many strains of *S. suis* and has been associated with high virulence.¹⁴

There are several *Streptococcus* species of veterinary importance in addition to *S. suis*. *S. canis* infection in neonatal and adult dogs (and less commonly cats) has been associated with pneumonia, abortion, septicemia, endocarditis, necrotizing fasciitis, keratitis, lower urinary tract infections, cholangiohepatitis, prostatic abscesses, mastitis, arthritis and meningoencephalitis.⁵ *S. equi* subsp. *equi* causes equine strangles, a contagious infection of the upper respiratory tract and local lymph nodes;¹² it has also been linked with immune mediated vasculitis and purpura hemorrhagica.⁷ *S. equi* subs. *zooepidemicus* and *S. equisimilis* are associated with equine reproductive disease, but have also been isolated from the lung, liver, brain, kidney and joints.^{8,12} *S. equi* subs. *zooepidemicus* also causes bursitis or fistulous withers in horses,⁷ and was implicated in an outbreak of acute hemorrhagic pneumonia in more than 1,000 shelter dogs in California.² *S. agalactiae* (and less commonly *S. dysgalactiae* and *S. uberis*) are important causes of bovine mastitis.⁹ *S. iniae* is a significant aquatic pathogen of tilapia and other reef fish, which causes necrosis, inflammation and vasculitis.⁴ Furthermore, several species of *Streptococcus* are

zoonotic, including *S. canis*,⁵ *S. equi* sub. *zooepidemicus*,¹² *S. iniae*⁴ and *S. suis*.^{11,14}

Conference participants outlined several potential causes for the gross and histologic lesions associated with *S. suis* in swine. The fibrinous polyserositis often noted grossly at necropsy¹¹ could also occur secondary to *Hemophilus parasuis* or *Mycoplasma hyorhinis* infection.¹ Ruleouts for the microscopic lesions of meningoencephalitis and ventriculitis include salt toxicity, edema disease and postweaning multisystemic wasting syndrome (PMWS). Salt toxicity is characterized by cortical laminar necrosis/malacia with eosinophilic meningoencephalitis.⁷ Shiga-toxin producing *E. coli* (STEC), the etiologic agent of porcine edema disease, induces fibrinoid vascular change and necrotizing vasculitis with subsequent edema in various tissues, including the brain.⁶ Cerebellar spongiosis, necrotizing vasculitis, edema and hemorrhage are occasionally described in conjunction with porcine circovirus type 2 and PMWS.¹⁰ However, as noted by the contributor, the ventricular localization and fibrinosuppurative character of the lesions in this case are fairly specific for *S. suis*.¹¹

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CASE II: D120073 (JPC 4032261).

Signalment: Wild-caught juvenile male raccoon, (*Procyon lotor*).

History: A juvenile male raccoon exhibiting neurological deficits was found by a member of the public and submitted to a wildlife rehabilitation facility in northern California in January 2012. The raccoon had wounds on the tail, pale mucous membranes, ataxia, head tremors, mild inappetence and an initial exacerbated startle response. The animal could never right himself, rolled over and was uncoordinated. Palliative treatment with meloxicam (Metacam®, 0.2 mg/kg subcutaneously q24h), procaine G penicillin (20,000 Units/kg subcutaneously q24h), iron dextran (10 mg/kg intramuscularly once) and vitamin B complex (30 mg/kg subcutaneously once), did not ameliorate the clinical signs. The raccoon was humanely destroyed and submitted to the California Animal Health and Food Safety Laboratory, Davis, California, for necropsy examination.

Gross Pathology: The raccoon had adequate fat stores. The liver was diffusely and markedly enlarged and pale with irregular, undulating surfaces. The spleen was also markedly enlarged and meaty. All lymph nodes noted were very pale and enlarged. The lungs were collapsed and there were occasional pinpoint pale subpleural foci. The gastrointestinal tract contained scant contents with a small amount of dry fecal matter in the distal large intestine.

Histopathologic Description: In the section of cerebellum the neuronal as well as glial cell cytoplasm was markedly distended (up to 3 times normal size) by aggregates of delicate clear round vacuoles (approximately 1µm in diameter). These aggregates occasionally displaced the nucleus to the periphery of the cell. Multifocally swollen eosinophilic axons were observed in the granular layer.

In the section of the spleen, foamy macrophages expanded the germinal centers and formed extensive sheets that replaced and effaced the red pulp.

Laboratory Results:

Test	Result
Aerobic culture- lung, liver, mesenteric lymph node	Mixed growth
Fecal PCR for <i>Salmonella</i> sp.	<i>Salmonella arizona</i>
Fecal flotation	Negative
Serology for <i>Toxoplasma</i> sp.	Negative
Heavy/trace mineral analysis (lead, manganese, iron, zinc, arsenic, cadmium, molybdenum, copper, mercury, selenium)	Within normal limits
Rabies testing via fluorescent antibody testing on brain tissue	Negative
Lysosomal enzyme analysis	See Table 1
Special stains: Oil red O (on formalin fixed frozen sections), PAS, Sudan Black, Luxol fast blue and acid fast	- Multifocal endothelial cell intracytoplasmic accumulation of Oil red O-positive material - Affected neurons and macrophages were Oil red O, PAS, Sudan Black, Luxol fast blue and acid fast negative
Autofluorescence (via UV scope)	Negative

Contributor's Morphologic Diagnosis: Cerebellum: Severe diffuse neuronal and glial cell vacuolation and swelling with occasional multifocal spheroids (suspect storage disease).

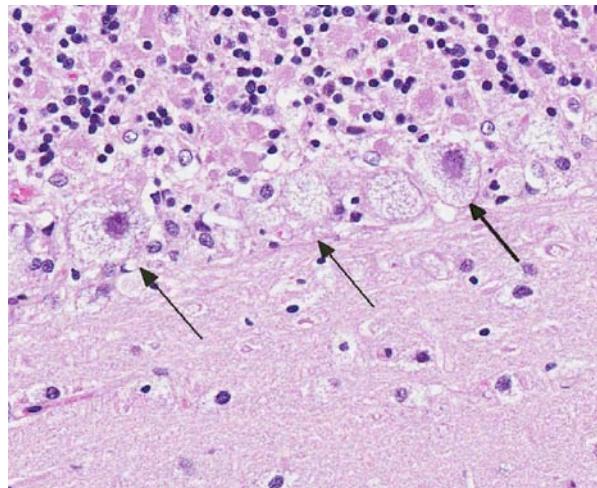
Spleen: Germinal centers and red pulp severe diffuse histiocytosis (suspect storage disease).

Contributor's Comment: In addition to the cerebellum and spleen, multiple tissues were infiltrated and expanded by aggregates or sheets of previously described foamy macrophages. These include multiple lymph nodes; lamina propria of the tongue, intestine, colon; portal areas of the liver; and around the pulmonary vessels, in the alveolar spaces and subpleurally in the lung. Additionally, cerebral neurons and glial cells as well and peripheral ganglia neurons were similarly affected. Various degrees of cytoplasmic foaminess were also observed in the epithelial cells of the renal tubules, parietal glomerular cells and hepatocytes.

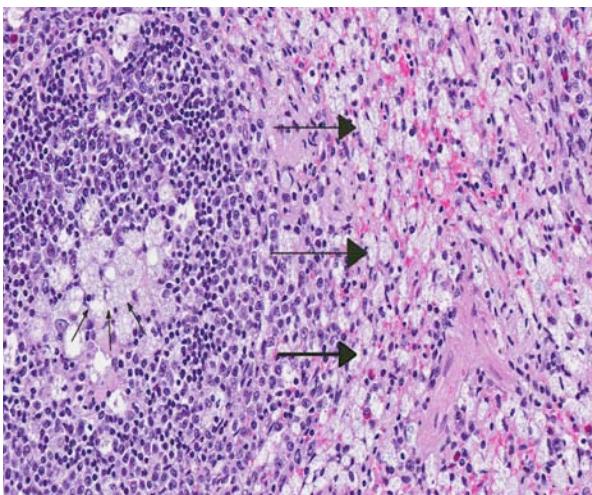
Transmission electron microscopy revealed lysosomal accumulations of floccular variably electron dense and frequently concentrically arranged lamellar material consistent with lysosomal storage disease. However, ultrastructural analysis is relatively non-specific regarding type of storage.



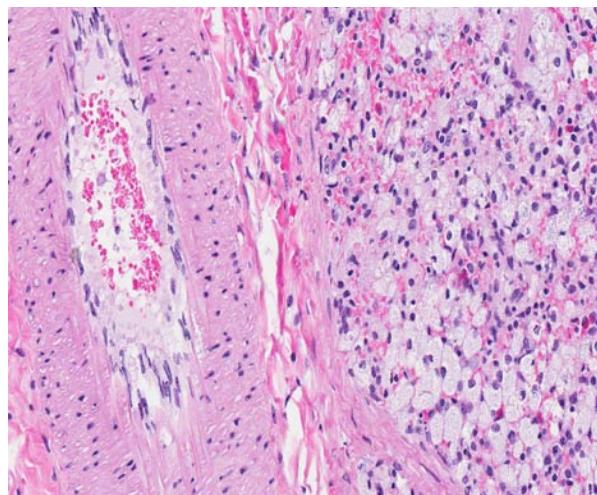
2-1. Cerebellum with medulla and spleen, raccoon: The granular layer of the cerebellum appears attenuated and the red pulp of the spleen is markedly expanded. The splenic white pulp appears hyperplastic. (HE 0.63X)



2-2. Cerebellum with medulla, raccoon: Purkinje cells (arrows) and smaller neurons within the granular cell layer are markedly swollen and contain large numbers of discrete clear vacuoles within their cytoplasm. (HE 250X)



2-3. Spleen, raccoon: Macrophages with abundant foamy cytoplasm expand the germinal centers of splenic white pulp (small arrows) and greatly expand the red pulp (large arrows). (HE 250X)

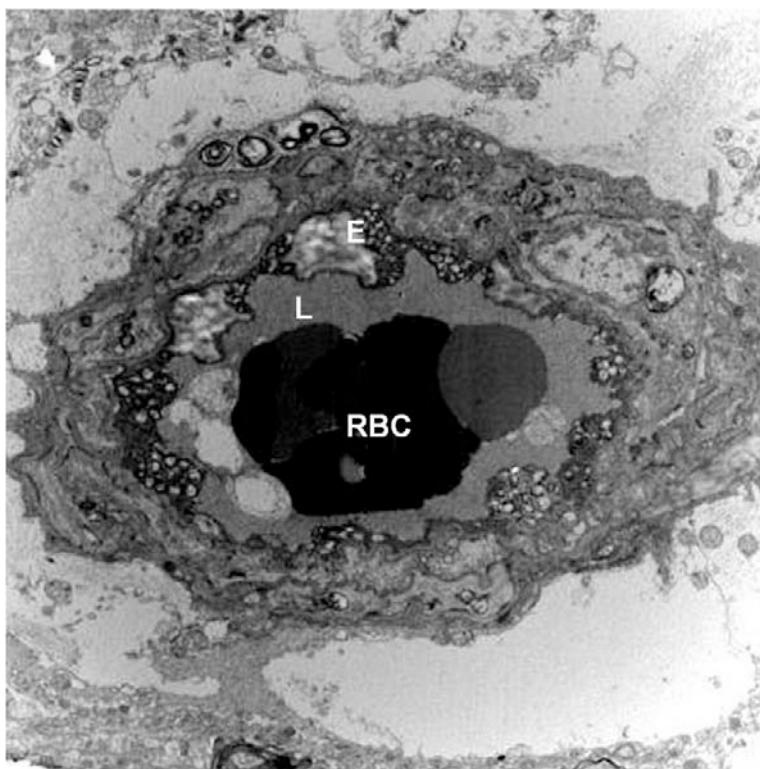


2-4. Spleen, raccoon: Endothelial cells also contain numerous discrete vacuoles within their cytoplasm. (HE 400X)

Measurement of lysosomal enzyme activity including sphingomyelinase, β -galactosidase, β -hexosaminidase and β -hexosaminidase A and B was performed in water homogenates of the brain samples from affected and age-matched non-affected raccoon⁸ (Table 1). This assay revealed complete absence of sphingomyelinase activity. The absence of sphingomyelinase activity is a criterion for the diagnosis of sphingomyelin lipidosis (also known as Niemann – Pick disease (NPD)).⁷

Sphingomyelin lipidosis belongs to a large group of sphingolipidoses lysosomal storage diseases

that also includes GM1 and GM2 gangliosidosis and globoid cell leukodystrophy, to name a few. In sphingolipidoses the spectrum of affected organs is wide and often includes viscera and macrophages because the substrate is derived from all cell membranes.³ The involvement of neurons in most LSDs is due to both the high metabolic activity of these cells and their long life span, which allows the gradual accumulation of undegraded substrate.³ Ultrastructural pathology offers useful information in the diagnosis of LSDs and helps categorize the type of LSD. In diseases accumulating sphingolipids, storage bodies are characterized by membranous material arranged



2-5. Spleen, raccoon: Ultrastructural analysis of endothelial cells of a capillary reveals the presence of numerous lysosomes filled with lamellar material (E). Unfortunately, electron microscopy is useful only for identify lysosomal storage diseases, but poor in determining which type.

concentrically (membranous cytoplasmic bodies). None of these forms is specific for a given disease, but concentric lamellae are most common in GM1 and GM2 gangliosidoses. In the present case the storage bodies were poorly defined, concentrically arranged lamellar whorls. Histochemistry, immunohistochemistry and fluorescence microscopy may also be of use in identifying storage material, but definitive and gold standard for diagnosis is by means of biochemical analysis.³

There is no single presentation common to all lysosomal storage diseases; the clinical and gross pathological manifestations are dependent on the deficient enzyme and the outcome of the deficiency in the organs that utilize the enzyme. Microscopically, LSDs are characterized by accumulation of enlarged lysosomes containing uncatabolized substrate in solution or complexed with related chemical species, which will be partially or wholly removed during fixation and preparation of the paraffin wax-embedded sections. If the substrate is soluble in water or lipid solvents, there will be a vacuolated appearance of the affected cells.³

Sphingolipids are an important group of structural lipids in which the unifying compound, ceramide, is esterified to sialyloligosaccharides to form gangliosides, to other saccharides to form neutral glycolipids such as globoside, or to phosphocholine to form sphingomyelin.³

The primary metabolic defect in Niemann–Pick disease (NPD) types A and B in man is the lack of sphingomyelinase enzyme that catalyzes the hydrolytic cleavage of sphingomyelin to ceramide and phosphocholine.³ The reduced or absent enzyme activity results in accumulation of sphingomyelin in lysosomes.⁷ Similarly to humans, an autosomal recessive mode of inheritance has been demonstrated in a cat and a dog.^{2,8}

Similar histological and ultrastructural features to those in this case were reported affecting neurons, oligodendroglial cells, macrophages, renal epithelial cells, endothelium and pericytes in the CNS, PNS, spleen, liver, lung and kidney in a cat,¹ a dog² and a Hereford calf.⁶ Consistent with the present case, these animals presented as juveniles, had neurological signs and virtually no sphingomyelinase activity was detected in the brain and liver compared with normal controls. Autofluorescence by UV light was reported in a dog with NPD,² but was not found in the present case.

JPC Diagnosis: Cerebellum: Neuronal, glial cell and endothelial cytoplasmic vacuolation, diffuse, marked, with gliosis.
Spleen: Histiocytosis, diffuse, marked with cytoplasmic vacuolation.

Conference Comment: Conference participants briefly reviewed select inherited (see Table 2) and acquired lysosomal storage diseases of veterinary importance. Sphingolipidoses result from defective catabolism of normal cell membrane constituents known as glycosphingolipids, and exhibit autosomal recessive inheritance. This case is an excellent example of sphingomyelinosis, or Niemann-Pick disease, the

Table 1: Lysosomal enzyme activities in brain homogenates from the affected and a normal raccoon

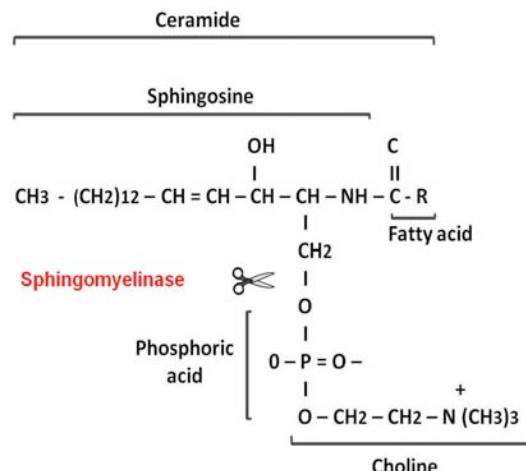
Enzyme activity (nmol/h/mg protein)	Affected Raccoon	Normal Raccoon
Beta-galactosidase ^a	64.0	22.6
Beta-hexosaminidase A ^b	81.4	28.9
Beta-hexosaminidase A and B ^c	611.1	118.0
Sphingomyelinase ^d	0.13 (3X)	5.2

a- measured using 4MU- β -galactoside

b- measured using 4MU- β -N-acetylglucosaminideSO4

c- measured using 4MU- β -N-acetylglucosaminide

d- measured using 14C-sphingomyelin; 3x means measured three times



2-6. *Derivation of sphingolipids (see contributor's comments for discussion.)*

pathogenesis of which is thoroughly described in the contributor's comment. GM₁ gangliosidosis has been described in dogs, cats, Friesian cattle and sheep. Accumulation of lysosomal GM₁ ganglioside occurs due to a deficiency in β -galactosidase, though GM₁ gangliosidosis in Suffolk sheep is actually due to deficiencies in both β 1-galactosidase and α -neuraminidase. GM₂ gangliosidosis results from insufficient activity of hexosaminidase (which exists as an $\alpha\beta$ - or $\beta\beta$ -dimer) or its activator protein. This condition is reported in domestic shorthair and Korat cats, German shorthaired pointers and golden retrievers due to a β -subunit deficiency, while in Japanese spaniel dogs and Yorkshire pigs there is an activator protein deficiency. Tay-Sachs and Sandhoff diseases are examples of GM₂ gangliosidosis in humans. As in sphingomyelinosis, neurons in GM₁/GM₂ gangliosidosis are expanded by abundant, foamy, PAS-positive cytoplasm, with faint granules; ultrastructurally lysosomal granules are composed of concentric membranous whorls. Glial cells and macrophages are also affected. Glucocerebrosidosis, which is similar to Gaucher disease in humans, has been reported in Sydney Silky Terriers, and results from deficient glucocerebrosidase, the catalyst for conversion of glucocerebroside to ceramide. Microscopically, glucocerebrosidosis manifests in hepatic and lymph node sinusoidal macrophages, as well as some neurons, but not in Purkinje cells or the spinal cord. Ultrastructurally, storage material appears twisted or branching.⁵

Globoid cell leukodystrophy, also known as galactocerebrosidosis, is an autosomal recessive disorder reported in dogs, cats and polled Dorset sheep. It is classified within the sphingolipidoses group of storage diseases and results from deficient activity lysosomal galactocerebrosidase, an enzyme which normally catalyzes the breakdown of galactocerebrosides. Galactocerebrosides are important components of myelin, but at high concentrations are cytotoxic; excessive accumulation in oligodendrocytes and Schwann cells causes extensive cellular degeneration and necrosis, halting active myelination. This combined with the degeneration of existing myelin, results in demyelination and axonal loss. Phagocytic macrophages, however, are unable to degrade galactocerebroside, and thus appear microscopically as characteristic swollen, PAS-positive "globoid cells," which exhibit perivascular cuffing within the white matter. In contrast to many of the other lysosomal storage diseases, neurons are not typically involved in the accumulation of excess storage material in galactocerebrosidosis.⁵

Glycoproteinoses, such as α - and β -mannosidosis, or α -L-fucosidosis, are characterized by defective degradation of the carbohydrate component of N-linked glycoproteins. In α -mannosidosis, a historically important entity in Angus cattle, a defective enzyme leads to decreased lysosomal α -mannosidase activity in all cells except hepatocytes, which leads to widespread mannose/

Table 2: Select inherited lysosomal storage diseases⁵

Condition	Enzyme Defect	Storage Material	Inheritance/species
GM ₁ gangliosidosis	β-galactosidase	GM ₁ ganglioside in lysosomes of neurons, glial cells, macrophages	- autosomal recessive - dogs, cats, Friesian cattle; - suffolk sheep- deficiencies in β1-galactosidase AND α-neuraminidase.
GM ₂ gangliosidosis (Tay-Sachs and Sandhoff diseases)	-hexosaminidase (αβ- or ββ-dimer) -activator protein	GM ₂ ganglioside in lysosomes of neurons, glial cells, macrophages	- autosomal recessive 1. domestic and Korat cats, German shorthaired pointers, golden retrievers: β-subunit deficiency 2. Japanese spaniel dogs, Yorkshire pigs: activator protein deficiency
Sphingomyelinosis (Niemann-Pick disease)	sphingomyelinase	sphingomyelin in lysosomes of neurons and macrophages	- autosomal recessive in cat and dog
Globoid cell leukodystrophy (glactocerebrosidosis)	galactocerebrosidase	galactocerebrosides in oligodendrocytes/Schwann cells, globoid cell macrophages (NOT in neurons)> demyelination, axonal loss	- autosomal recessive - dogs, cats and polled Dorset sheep
Glucocerebrosidosis (Gaucher disease)	glucocerebrosidase	glucocerebroside in lysosomes of hepatic/lymph node sinusoidal macrophages, some neurons (NOT in Purkinje cells or the spinal cord)	- Sydney Silky Terriers
α-Mannosidosis	α-mannosidase	mannose/N-acetylglucosamine oligosaccharide in lysosomes of neurons, macrophages, secretory epithelial cells	- Angus cattle
β-Mannosidosis	β-mannosidase	oligosaccharides in lysosomes of neurons, macrophages, secretory epithelial cells	- Salers cattle and Nubian goats
MPS I	α-L-iduronidase	mucopolysaccharide storage in mesoderm derived cells	- domestic shorthair cats and Plott hounds
MPS III	N-acetylglucosamine-6-sulfatase	heparan sulfate in mesoderm-derived cells; neurons contain gangliosides	- Nubian goats
MPS VI	arylsulfatase-B	mucopolysaccharide storage in mesoderm derived cells; neuronal storage does not occur	- Siamese and domestic shorthair cats
MPS VII	β-glucuronidase	widespread neurovisceral storage	- dogs and cats
Glycogenosis (type II in humans)	α-1,4-glucosidase	widespread glycogen storage within lysosomes and intracytoplasmically; including neurons	- autosomal recessive in shorthorn and Brahman beef cattle

N-acetylglucosamine oligosaccharide deposition. β-mannosidosis due to β-mannosidase deficiency is reported in Salers cattle and Nubian goats. In both α- and β-mannosidoses, neurons, macrophages and secretory epithelial cells are most severely affected, although storage material is typically lost during tissue processing so vacuoles appear empty on standard H&E slides. In α-L-fucosidosis deficient activity of α-L-fucosidase leads to a similar histological appearance; this condition is autosomal recessive in English springer spaniels.⁵

Mucopolysaccharidoses are distinguished by defective catabolism of glycosaminoglycans, so skeletal and connective tissue abnormalities such

as deformities, degenerative joint disease, and thickening of the heart valves or leptomeninges are often observed; neurons can be involved as well. Deficiency in α-L-iduronidase, known as mucopolysaccharidosis type I (MPS I) in humans, is reported in domestic shorthair cats and Plott hounds. Storage primarily occurs in mesoderm-derived cells. Deficiency in N-acetylglucosamine-6-sulfatase leads to the veterinary counterpart of human MPS III, which has been described in Nubian goats. Mesoderm-derived cells are packed with heparan sulfate, while neurons contain gangliosides, which accumulate due to interference with neuraminidase activity. Siamese and domestic shorthair cats occasionally have a deficiency in

arylsulfatase-B, a disorder known as MPS VI in humans. Mucopolysaccharidosis VI differs from MPS I and II in that neuronal storage does not occur. Finally, the counterpart to human MPS VII is reported in dogs and cats secondary to a lack of β -glucuronidase. Again, microscopic findings are similar to those described above; however, there is also widespread neurovisceral storage.⁵

Glycogenoses result from defective glycogen catabolism. α -1,4-glucosidase deficiency, an autosomal recessive condition documented in shorthorn and Brahman beef cattle, leads to widespread glycogen storage, both within lysosomes and intracytoplasmically. In contrast, other types of glycogen storage diseases are concentrated primarily within the liver and muscle. Since the excess storage material is composed of glycogen, it is PAS-positive and diastase-sensitive. Neurons are severely affected in this disease.⁵

Acquired lysosomal storage diseases often follow the ingestion of toxic plants, or, less commonly, drug administration. Swainsonine is an indolizidine alkaloid found in several plant species, such as locoweed (*Astragalus*, *Oxytropis* sp.). Ingestion of swainsonine by grazing livestock causes inhibition of α -mannosidase, inducing a form of α -mannosidosis. Ingestion by pregnant sheep can also result in abortion and fetal malformation. Ingestion of *Trachyandra divaricata* or *T. laxa* causes excessive storage of lipofuscin in central and peripheral neurons of South African/Australian livestock.⁵ Aminoglycosides, such as gentamicin, accumulate within lysosomes of renal proximal tubular cells where they inhibit lysosomal phospholipases, producing aggregates of phospholipid-containing myeloid bodies. As lysosomes become progressively distended with myeloid bodies, they rupture, releasing acid hydrolases as well as high concentrations of aminoglycosides into the cytoplasm, further damaging the cell.⁴

Regardless of the underlying genetic or acquired cause, most lysosomal storage diseases in veterinary species are characterized clinically by an early onset of neurologic impairment. Considering the similarity of clinical signs as well as the frequent overlap of the gross and microscopic lesions in many types of lysosomal storage diseases, electron microscopy and

especially measurement of lysosomal enzyme activity are often necessary to elucidate a specific etiology.

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CASE III: 11-342 (JPC 4032320)

Signalment: 11-year-old female spayed Maine coon cat, (*Felis catus*).

History: The cat was presented to a local emergency clinic for acute onset of tetraplegia. Neurologic examination revealed normal cranial nerve function, deep pain sensation in all limbs, hindlimb hyperreflexia, and forelimb hyporeflexia, consistent with a C6-T2 spinal cord lesion. The neck was non-painful with normal range of motion. Cervical and thoracic radiographs were unremarkable. The cat's neurologic status deteriorated overnight, with worsening forelimb hyporeflexia and loss of deep pain sensation in the left forelimb. A guarded prognosis was given, and the owners elected euthanasia and subsequent necropsy.

Gross Pathology: Transverse sectioning of the spinal cord revealed multiple asymmetric brown-grey foci of malacia and hemorrhage, extending from C4-T2. The vertebrae and intervertebral discs were grossly normal.

Laboratory Results: Brain slices tested negative for rabies virus antigen via immunofluorescence.

Histopathologic Description: Spinal cord, caudal cervical to cranial thoracic: Affecting up to 75% of the grey and white matter, and most severely affecting the ventral horns and ventral funiculi, are multiple asymmetric foci of malacia characterized by parenchymal vacuolation,

hemorrhage, and neuronal necrosis and loss (infarcts). The ventral spinal artery and numerous intramedullary arteries and veins contain luminal amorphous blue-grey material (fibrocartilaginous emboli). Vessel walls are rarely disrupted by brightly eosinophilic fibrillar material and cellular debris (fibrinoid vascular necrosis). Within malacic areas, blood vessels are surrounded by low to moderate numbers of neutrophils that multifocally extend into the surrounding meninges and neuroparenchyma (neutrophilic meningomyelitis) and glial cells are mildly increased in number. Adjacent white matter tracts contain large regions of myelin sheath dilation with numerous swollen axons (spheroids) and rare myelomacrophages (Wallerian degeneration). Within the ventral dura mater are multifocal basophilic concretions (dural mineralization).

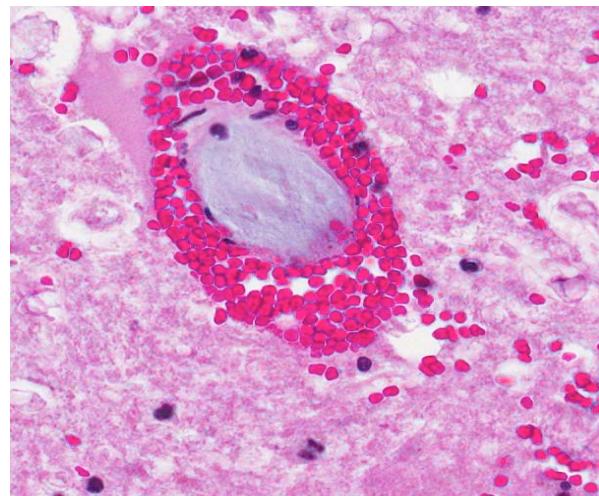
Contributor's Morphologic Diagnosis: Spinal cord (caudal cervical to cranial thoracic): Severe multifocal acute myelomalacia with intravascular fibrocartilaginous emboli, secondary

neutrophilic meningomyelitis, gliosis, and Wallerian degeneration.

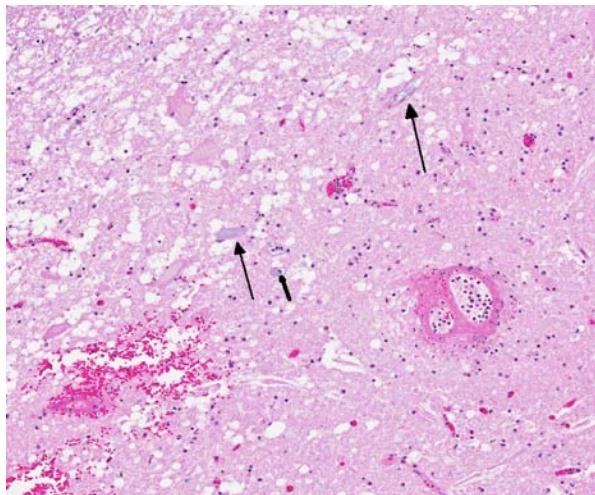
Contributor's Comment: Fibrocartilaginous embolism (FCE), though well-documented in dogs, is considered an uncommon cause of spinal cord disease in cats⁴ and has been reported in numerous other species including sheep, pigs, horses, turkeys, mustelids, and humans.² Affected cats are non-painful and develop peracute to acute, usually asymmetric, spinal cord-related



3-1. Cervical spinal cord, cat: Subgross inspection reveals asymmetric pallor (malacia) affecting the grey matter as well as all funiculi within the spinal white matter. (HE 0.63X)



3-2. Cervical spinal cord, cat: Multiple veins within the grey matter and white matter contain emboli of fibrocartilage. (HE 200X)

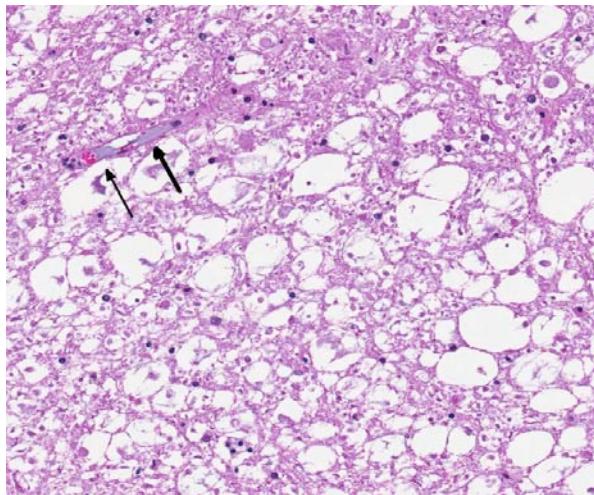


3-3. Cervical spinal cord, cat: Areas of infarction include white matter (upper left), multiple vessels containing fibrocartilaginous emboli (arrows), hemorrhage, and vessels with necrotic walls (lower right). (HE 220X)

signs that are non-progressive beyond the first 24-48 hours.^{2,5,7} While definitive diagnosis requires histopathologic examination, a presumptive diagnosis may be made antemortem based on history, clinical examination and MRI findings, and exclusion of other causes of myelopathy. Resolution of clinical signs, in the absence of specific therapy, is also highly suggestive of FCE.⁴

The myelomalacia seen in cases of FCE results from occlusion of the blood supply to the spinal cord by fibrocartilaginous emboli, which are thought to originate from the nucleus pulposus of the intervertebral disk.^{4,5,7} It is uncertain how the disk material enters the vasculature. Several theories have been proposed, including: 1) penetration of disk material into spinal vessels (e.g. due to trauma), 2) entry into a common blood supply of the intervertebral disk and spinal cord (e.g. remnant embryonic vessels or neovascularization), 3) entry into an anomalous arteriovenous communication, or 4) herniation of disk material into an adjacent vertebral body, allowing entry into the vertebral venous sinus.^{1,2,4,5,7}

The distribution and extent of the myelomalacia depends on the size, location, and number of affected blood vessels. Spinal arteries have extensive anastomoses, thus the presence of spinal cord infarcts is consistent with simultaneous occlusion of multiple vessels.⁶ Typical histopathologic findings, as seen in this case, include myelomalacia and fibrocartilaginous



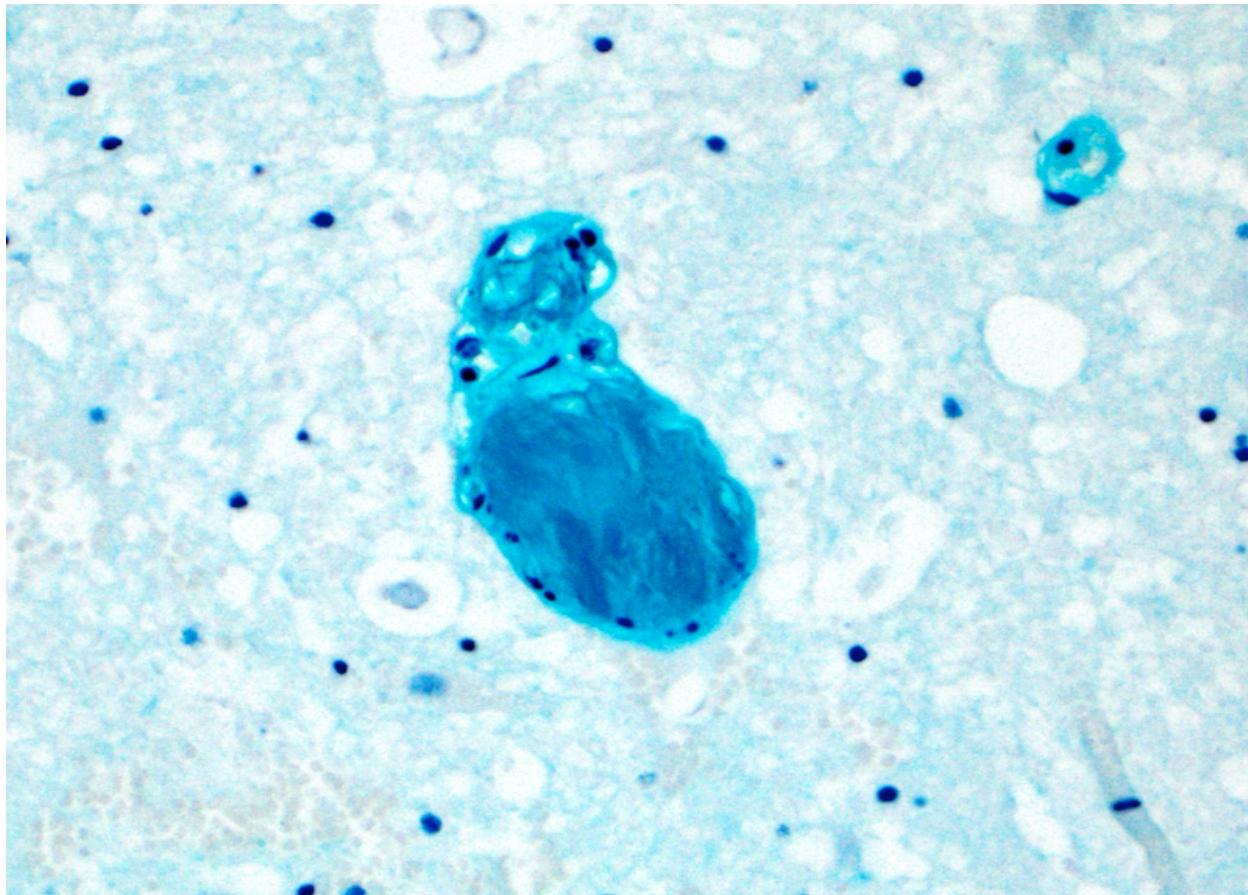
3-4. Cervical spinal cord, cat: Within the dorsal funiculus, axons in proximity of vessels with fibrocartilaginous emboli (arrows) exhibit degenerative changes such as dilated myelin sheaths, swollen axons, and axonophagia by Gitter cells. (HE 320X)

emboli within leptomeningeal and/or intramedullary blood vessels. Fibrocartilaginous emboli have been described in both arteries and veins, though often the type of vessel affected cannot be definitively identified.⁶ While emboli may be evident on routine histologic examination, their identification can be enhanced with Giemsa, toluidine blue, or alcian blue histochemical stains.⁷

JPC Diagnosis: Spinal cord: Infarcts, multifocal to coalescing, extensive, with wallerian degeneration, hemorrhage and numerous fibrocartilagenous emboli.

Conference Comment: The contributor provides a concise, thorough summary of the clinical presentation and pathogenesis of fibrocartilagenous embolism. Although FCE is definitively diagnosed via histopathology, a presumptive diagnosis can sometimes be made on the basis of history, clinical signs, a thorough physical and neurologic examination and imaging such as MRI. Differential diagnoses for FCE include causes of acute, asymmetrical paresis such as trauma, spinal cord or vertebral neoplasia, diskospondylitis, intervertebral disk disease, aortic thromboembolism and bacterial, viral or parasitic infections.^{1,2,8} In contrast to most of these conditions, however, animals with FCE are typically non-painful on spinal palpation and clinical signs are non-progressive after the first 24-48 hours.^{1,2,4}

Metabolic and degenerative disorders as well as toxicities that affect the brain and spinal cord and



3-5. Cervical spinal cord, cat: Fibrocartilaginous matrix appears bright blue-green when stained with Alcian Blue. (Alcian Blue 2.0, 400X) (Photo courtesy of: Laboratory of Pathology and Toxicology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA USA. <http://www.vet.upenn.edu/>)

may result in a similar clinical presentation, but unlike FCE, these conditions generally exhibit a symmetrical distribution. For example, bilaterally symmetrical polioencephalomalacia is observed secondary to dietary thiamine deficiency in carnivores, while lead toxicity can occasionally cause symmetrical laminar cortical necrosis in cattle. A focal, symmetrical poliomyelomalacia of unknown etiology is reported in sheep, goats, pigs and Ayrshire calves, while polioencephalomalacia (PEM) is well described in ruminants; salt toxicity in swine also causes similar lesions. In horses the ingestion of neurotoxin repin, from the yellow star thistle (*Centaurea solstitialis*) or Russian knapweed (*Centaurea repens*), induces symmetrical malacia of the pallidus and substantia nigra, while thiaminase from bracken fern (*Pteridium* sp.) and horsetail (*Equisetum arvense*) results in bilaterally symmetrical necrosis of the periventricular gray matter.³

Contributing Institution: Laboratory of Pathology and Toxicology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA. <http://www.vet.upenn.edu/>

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CASE IV: L11-8963 (JPC 4019357)

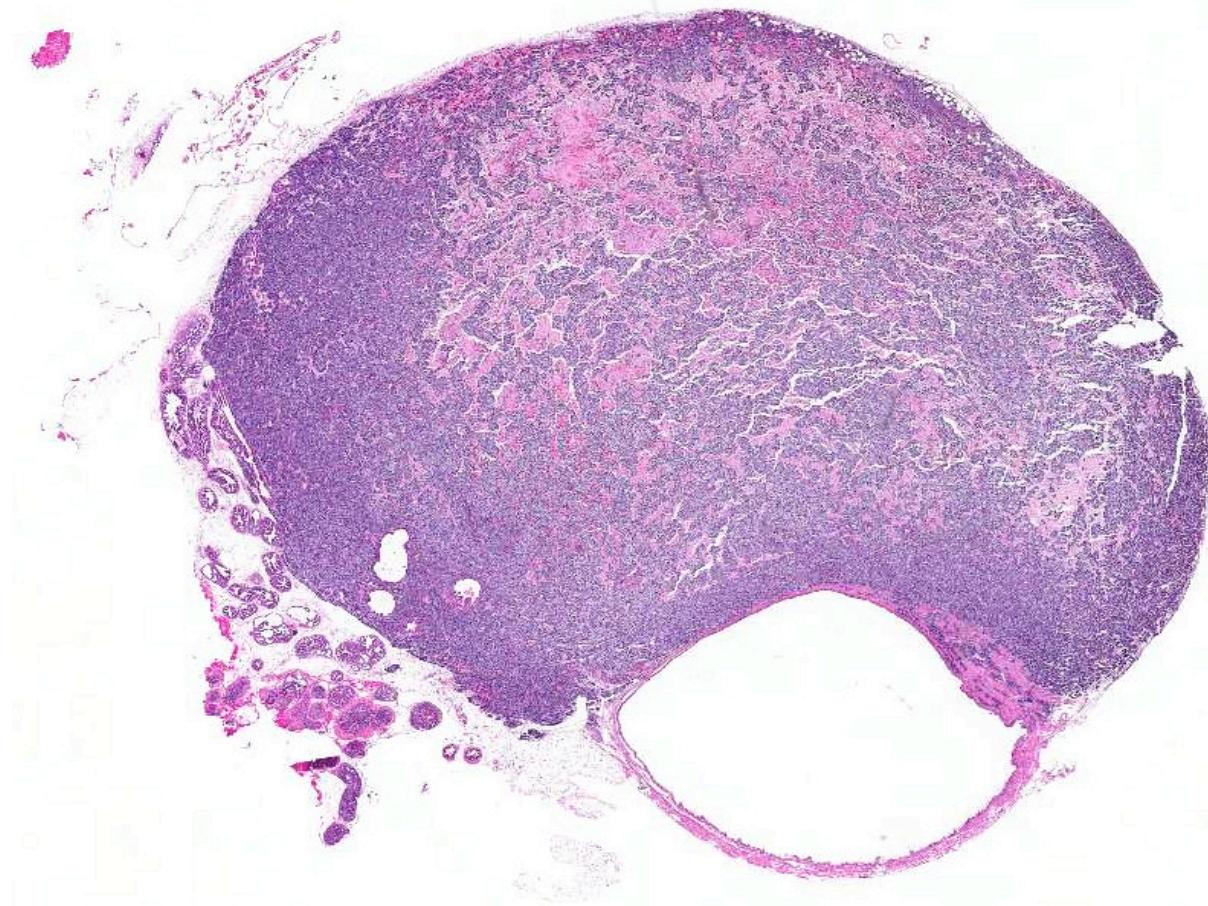
Signalment: 9-month-old male C57BL/6 TRAMP mouse, (*Mus musculus*).

History: This mouse was a control animal that was part of a novel imaging modality study. No clinical signs reported.

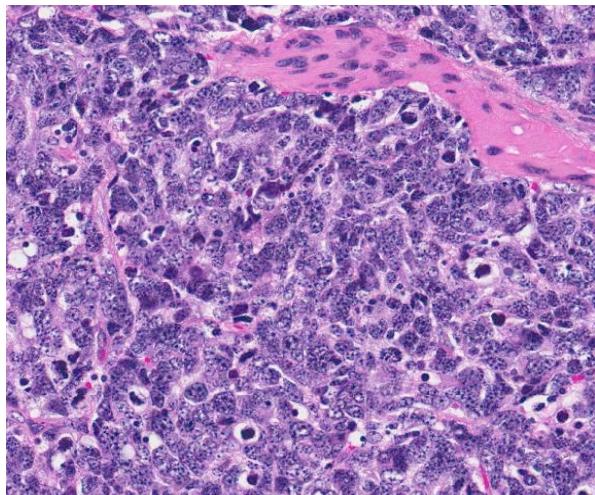
Gross Pathology: This mouse was presented alive in good body condition. There is a focally extensive, 1.2 x 1.0 x 1.0 cm, mottled pale tan to red, firm, somewhat circumscribed mass around the neck of the urinary bladder. All other organs and tissues are within normal gross limits.

Histopathologic Description: Prostate gland: There is a regionally extensive, invasive, unencapsulated mass arising from and replacing most of the anterior prostate lobe, with infiltration and effacement of other prostate lobes and the

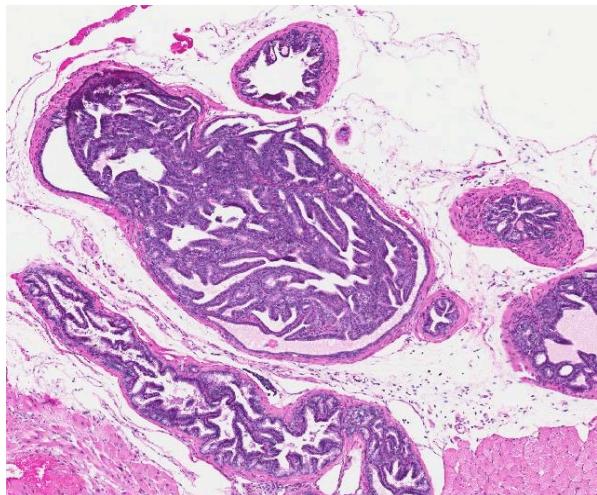
urinary bladder. The mass is comprised of sheets and anastomosing lobules of cells with scant fibrovascular stroma. The cells are pleomorphic (polygonal, elongated, round) with poorly defined cell borders enclosing small amounts of eosinophilic cytoplasm. The nuclei are round to ovoid with coarsely clumped chromatin and single inconspicuous nucleoli. A range of 15 to 25 and an average of 20 mitoses per 400X field is noted. Anisocytosis and anisokaryosis is marked (>3 fold). Apoptosis/single cell necrosis is noted throughout the mass, and there is multifocal to coalescing central lytic necrosis with hemorrhage throughout the mass. Within most remaining glands of the anterior lobe and the other lobes of the prostate gland, there are changes consistent with prostatic intraepithelial neoplasia (PIN), including areas of epithelial stratification, micropapillary formation, and cribriform structure formation.



4-1. Prostate, TRAMP mouse: The anterior lobe of the prostate is enlarged up to 1.3 cm and effaced by an infiltrative neoplasm that compresses the adjacent urinary bladder. (HE 0.63X)



4-2. Prostate, TRAMP mouse: Neoplastic cells are polygonal and pleiomorphic, forming sheets rather than acinar structures. There are large numbers of mitotic figures as well as apoptotic neoplastic cells. (HE 300X)



4-3. Prostate, TRAMP mouse: Adjacent glands show a disorderly array of columnar cells in contiguous acini, rather than the typical single layer. This preneoplastic change is referred to as prostatic intraepithelial neoplasia (PIN). (HE 35X)

Contributor's Morphologic Diagnosis:

1. Prostate gland (anterior lobe), poorly-differentiated prostatic carcinoma.
2. Prostate gland (all lobes), high-grade prostatic intraepithelial neoplasia (PIN).

Contributor's Comment: Transgenic adenocarcinoma of mouse prostate (TRAMP) is a transgenic mouse engineered to express the SV40 virus large T and small t oncogenes in the secretory epithelial cells of the prostate under the control of the androgen-responsive minimal rat probasin promoter.^{1,3,4} Expression of these transgenes results in inhibition of p53 and Rb tumor suppressor function.¹

The TRAMP model has been based on either C57BL/6 or C57BL/6 TRAMP x FVB hybrid mouse strains.^{1,3,4} The prostate tumors observed in male TRAMP mice progress in a stepwise fashion through different preneoplastic and neoplastic lesions, which is a feature of prostate tumors in humans.^{1,3} In TRAMP mice, commonly observed lesions include:¹

1. *6- to 12-week old mice:* hyperplastic epithelial lesions or prostatic intraepithelial neoplasia (PIN).
2. *12- to 24-week old mice:* well-differentiated prostatic adenocarcinoma.
3. *>24-week old mice:* poorly-differentiated prostatic adenocarcinoma, with development of metastases (commonly iliac lymph nodes and lungs).
4. *33- to 52-week old mice:* death.

TRAMP prostate tumors share other similarities with human prostate tumors, including metastases to distant sites, development of androgen independence, and neuroendocrine differentiations.^{1,3} As such, TRAMP mice have been extensively used to study the molecular events important in prostate cancer progression in humans.

The classification and grading of lesions in transgenic mouse models of prostatic tumors (including and mainly those observed in TRAMP mice) can be confusing and controversial. The most widely used scheme for classifying such tumors originates from the 2004 Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee.⁴ This classification scheme refined and stressed the importance of PIN lesions based on a wide body of published work. Recently, a grading scheme was proposed that incorporates some data and concepts developed and published since the 2004 Bar Harbor classification scheme, which in summary includes:¹

- *Grade 0: Normal.* Prostate glands lined by a monolayer of cuboidal to columnar epithelium with basally-oriented nuclei.
- *Grade 1: Low-grade PIN.* Crowding and occasional stratification of prostate epithelial cells, with increased nuclear to cytoplasmic ratio.

- *Grade 2: Moderate-grade PIN.* Similar to low-grade PIN, but there is more frequent stratification.
- *Grade 3: High-grade PIN.* Similar to moderate-grade PIN, but hyperplastic epithelial cells can form papillary projections and/or cribriform patterns.
- *Grade 4: Phyllodes-like tumor.* Consists of papillary projections of loose stroma with loosely-arranged stellate mesenchymal cells.
- *Grade 5: Well-differentiated adenocarcinoma.* Invasive tumor consisting primarily of well-differentiated tubules or acini. There is marked cellular and nuclear atypia, and high mitotic indices.
- *Grade 6: Moderately-differentiated adenocarcinoma.* Invasive tumor that consists of a mass of epithelial cells, some of which form recognizable acini or tubules. There is marked cellular and nuclear atypia, and high mitotic indices.
- *Grade 7: Poorly-differentiated carcinoma (neuroendocrine type).* Invasive tumors composed of solid sheets of polygonal to elongated cells. There is marked cellular and nuclear atypia, and high mitotic indices.

JPC Diagnosis: Prostate gland: Prostatic carcinoma, high grade, with adjacent prostatic intraepithelial neoplasia (PIN).

Conference Comment: The contributor has given a comprehensive overview of this important mouse model and given a clear summary of the different classification schemes. We concur with the contributor's use of the 2012 proposed grading scheme, which classifies the intraepithelial proliferative lesions as PIN rather than atypical hyperplasia for this model because, as the authors note, PIN lesions in TRAMP mice are clearly precursors of more invasive and aggressive carcinomas.¹

Physicians from the Joint Pathology Center (JPC) Genitourinary subspecialty were consulted on this case. These pathologists were impressed by the striking lack of differentiation within this aggressive tumor and noted that the histological features closely approximate the neuroendocrine phenotype described as a "grade 7" in the paper

previously referenced. Apparently this microscopic appearance is rare in human prostatic carcinomas, which are generally diagnosed and treated well before reaching this stage. JPC pathologists also expressed agreement that the changes in the remaining glands of the prostate gland are consistent with high-grade prostatic intraepithelial neoplasia, which represents an intermediate stage between normal epithelium and invasive malignant carcinoma. In human medicine, PIN is clinically significant in that it provides relatively early identification of patients at risk for malignancy.²

Contributing Institution: Veterinary Services Center

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Stanford School of Medicine

<http://med.stanford.edu/compmed/>

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