

The Armed Forces Institute of Pathology
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CASE I – 062418 (AFIP 3031120).

Signalment: Term fetus, male, Angus, bovid, *Bos Taurus*.

History: Abortion. No information regarding vaccination of the dam or other abortions in the group available.

Gross Pathology: Field necropsy performed by submitting veterinarian. No gross pathologic lesions described.

Laboratory Results:

Bacterial culture - stomach content, lung, liver, placenta: No significant bacteria isolated.

Serology, dam – negative for *Leptospira* (*L. pomona*, *L. grippotyphosa*, *L. hardjo*, *L. icthohemorrhagica*, *L. canicola*), negative for *Neospora caninum*. IBR positive at 1:128, BVD-1 positive at 1:64, BVD-2 positive at 1:256

Fetal fluid immunoglobulin: 23 mg/dl

PCR: Positive for IBR, negative for BVD

Fetal liver selenium: 1.58 µg/g weight (normal 1.5-3.5)

Viral isolation: Lung, kidney, thymus: No viruses isolated

Histopathologic Description: Fixed tissues submitted and examined were cerebrum, small intestine, skeletal muscle, heart, spleen, kidney, liver, lung, thymus, eyelid, colon, and placenta. Tissues were moderately autolyzed.

Cerebral sections contain numerous small perivascular cuffs of lymphocytes, with scattered lymphocytes within the parenchyma. Inflammation is associated with areas of parenchymal rarefaction and prominent neuronal necrosis, with suspect areas of gliosis. Neuronal nuclei are often markedly enlarged with marginated chromatin, and often contain large round to oval amphophilic inclusions. Mild lymphocytic infiltration is present within the meninges.

Other lesions detected are thymic and splenic lymphoid depletion, placental vasculitis and vascular necrosis, multifocal renal necrosis, and a focal crypt abscess in the small intestine. Bile is prominent within canaliculi, but no convincing necrosis is detected in the liver.

Contributor's Morphologic Diagnosis: Cerebrum: Necrotizing nonsuppurative encephalitis with intranuclear inclusions (herpesvirus infection).

Contributor's Comment: Findings are indicative of abortion and encephalitis due to herpesvirus. Two alphaherpesviruses, BHV-1 and BHV-5, have been associated with encephalitis and meningoencephalitis in calves and adult cattle.¹⁻⁶ Both are neurotropic but, although BHV-1 can be isolated from brain tissue of cattle with the respiratory form of disease, encephalitis due to BHV-1 is uncommon.^{1,2,5} Encephalitis due to herpesvirus infection in cattle is most often due to BHV-5.¹⁻⁷ To our knowledge, encephalitis in an aborted fetus infected with BHV has not been reported.

BHV-1 subtypes include subtype 1 (BHV-1.1, which is primarily associated with respiratory disease) and subtypes 2 (BHV-1.2a and BHV-1.2b), which are associated with genital disease.⁷ The virus previously classified as BHV-1 subtype 3 (BHV-1.3) is now classified as BHV-5. Experimental infection of calves with neurovirulent BHV-5 resulted in neuronal infection and encephalitis.³ BHV-5 meningoencephalitis is endemic in South America but occurs only sporadically elsewhere.²⁻⁴

Both BHV-1.1 and BHV-1.2a are capable of causing abortion.⁷ Foci of necrosis and leukocytic infiltration of multiple organs, but especially of liver, are characteristic of BHV abortion.⁷ In this case, characteristic hepatic necrosis was not detected. Intranuclear inclusions within neurons infected with either BHV-1 or BHV-5 encephalitis have been reported, but are uncommon.^{2,3,4,7} Viral inclusions are reported to be transient, appearing for approximately 2-3 days after infection, which likely explains their absence in most field cases.⁷

There is evidence that central nervous system infection occurs due to spread of virus from nasal mucosa via the trigeminal nerve to the trigeminal ganglion or via the olfactory nerves to the olfactory cortex.^{4,6} Central nervous system infection due to viremia has also been suspected.⁶ Viral transport by circulating leukocytes leads to placental and fetal infection.⁶

Differentiation of BHV-1 and BHV-5 can be difficult.⁴ Although the PCR primers utilized in this case are thought to be specific for BHV-1, viral isolation was attempted in order to further characterize the virus. Unfortunately, no unfixed brain tissue was available for viral studies, and no virus was isolated from other tissues. Immunohistochemistry for BHV was also positive in this case (courtesy of Dr. Fabio Del Piero, New Bolton Center, University of Pennsylvania), but further studies are needed to characterize the virus in this case.

AFIP Diagnoses: Brain, cerebrum: Encephalitis, lymphoplasmacytic and necrotizing, multifocal, moderate, with glial and neuronal intranuclear inclusion bodies, Angus (*Bos Taurus*), bovine.

Conference Comment: The contributor provides a concise summary of bovine herpesvirus 1 (BHV-1) and bovine herpesvirus 5 (BHV-5). Other important bovine herpesviruses include bovine herpesvirus 2 (pseudo-lumpy skin disease, bovine herpes mammillitis) and bovine herpesvirus 4 (bovine herpes mammary pustular dermatitis). Bovine herpesvirus 2 is dermatotropic and can cause generalized disease (pseudo-lumpy skin disease) or a localized infection of the teat. Localized infection occurs most commonly in dairy cattle secondary to trauma. Decreased milk production and secondary bacterial mastitis are common sequelae. Lesions develop on the teats and skin of the udder. Suckling calves develop lesions on the muzzle. Bovine herpesvirus 4 cause similar but milder disease than the localized form of bovine herpesvirus 2.⁸

Conference participants briefly reviewed other encephalitic herpes viruses that occur in various species to include malignant catarrhal fever (ovine herpesvirus 2, alcelaphine herpesvirus 1), equine herpesvirus 1, and pseudorabies (porcine herpesvirus 1). Encephalitic herpesviruses cause cell injury by inducing 1) neuronal and glial necrosis, 2) endothelial cell necrosis, and 3) secondary effects of inflammation, cytokines, and chemokines.⁹

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References:

1. Roels S, Charlier G, Letellier C, Meyer G, Schynts F, Kerkhofs P, Thiry E, Vanopdenbosch E: Natural case of bovine herpesvirus 1 meningoencephalitis in an adult cow. *Vet Rec* 146:586-588, 2000
 2. Ely RW, d'Offay JM, Ruefer AH, Cash CY: Bovine herpesviral encephalitis: a retrospective study on archived formalin-fixed, paraffin-embedded brain tissue. *J Vet Diagn Invest* 8:487-492, 1996
 3. Belknap EB, Collins JK, Ayers VK, Schultheiss PC: Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. *Vet Pathol* 31:358-365, 1994
 4. Perez SE, Bretschneider G, Leunda MR, Osorio, FA, Flores, EF, Odeón AC: Primary infection, latency, and reactivation of bovine herpesvirus type 5 in the bovine nervous system. *Vet Pathol* 39:437-444, 2002
 5. Penny CD, Howie F, Nettleton PF, Sargison ND, Schock A: Upper respiratory disease and encephalitis in neonatal beef calves caused by bovine herpesvirus type 1. *Vet Rec* 151:89-91, 2002
 6. Radostits OM, Gay CC, Blood DC, Hinchcliff KW: *Veterinary Medicine A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 9th ed., pp. 1173-1184. WB Saunders, Philadelphia, Pennsylvania, 2000
 7. Dungworth DL: The respiratory system. In: *Pathology of Domestic Animals*, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 556-558. Academic Press, San Diego, California, 1993
 8. Hargis AM, Ginn PE: The integument. In: *Pathologic Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 1176-1177. Mosby Elsevier, St. Louis, Missouri, 2007
 9. Zachary JF: Nervous system. In: *Pathologic Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 885-887. Mosby Elsevier, St. Louis, Missouri, 2007
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CASE II – 1051/05 (AFIP 3026718).

Signalment: 3 year-old neutered-female domestic short hair cat, feline.

History: The cat showed altered behaviour for one week with apathy and anorexia. The clinical examination revealed anisocoria whereas the pupillary reflex was normal. Radiographs were also unremarkable. Soon after the clinical examination the cat died. The carcass was submitted for necropsy by the clinical veterinarian in a frozen condition. The cat lived with two dogs and a rabbit in a household and

had free outdoor access. More than one year prior to death the cat had a bite wound in an unknown body region.

Gross Pathology: On gross examination of the brain, there was a round dark grey mass of about 3 cm in diameter without a distinct border in the caudal part of the right cerebral hemisphere. The meninges adhered on this spot. A brain tumor or encephalitis was suspected initially. The bones of the skull, the internal and middle ear as well as the nasal cavity showed no alteration.

Laboratory Results: Detection of FeLV p27-antigen (Witness FeLV[®]-Test, Synbiotocs Corp., Lyon, France) and evidence of antibodies against FIV gp40-antigen (Witness FIV[®]-Test, Synbiotocs Corp., Lyon, France) in samples of full blood were negative.

Histopathologic Description: Brain, cerebrum (right hemisphere): Unfortunately the carcass was frozen, so freezing and thawing artefacts are obvious. Throughout the whole slide there are parallel clefts dissecting the neuropil. They are free of any material and show no accompanying cellular reaction.

Within the cerebral cortex there is a focus of marked cellular degradation (necrosis) and debris. There are abundant intralesional septate fungal hyphae with yellow-brown coloured walls. The dichotomous branching hyphae have a diameter of about 5-7 μm and show occasional vesicular swellings. There is a dominant infiltration of macrophages and neutrophilic granulocytes, a moderate amount of plasma cells and lymphocytes and some giant cells which sometimes contain intracytoplasmatic melanized fungi.

There are also some slides from the periphery which do not show the central necrotic area. In some slides submeningeal clotted vessels with intraluminal hyphae can be seen. Vessels with a marked lymphocytic perivascular infiltration are surrounding the necrotic lesion.

The meninges overlying the necrosis revealed a moderate to marked infiltration with lymphocytes and macrophages.

Contributor's Morphologic Diagnoses:

1. Cerebrum: Encephalitis, pyogranulomatous and necrotising, subacute, focally extensive, severe with intralesional brown-pigmented fungal hyphae (cerebral phaeohyphomycosis).
2. Cerebrum: Meningitis, granulomatous, subacute, focal, moderate to severe.

Contributor's Comment: Phaeohyphomycosis, an uncommon opportunistic infection of human beings and a wide variety of animals (e.g. amphibians, birds,

and mammals), is caused by dematiaceous (naturally pigmented) fungi.¹⁻⁴ In cats about 15 genera of pigmented fungi have been recognized as agents of feline phaeohyphomycosis, including the species of *Cladophialophora bantiana*, *Exophiala jeanselmei*, and *Fonsecaea pedrosoi*.⁵⁻⁸ Most of them are known as plant pathogens, soil saprophytes or laboratory and environment contaminants.^{3,9} The infection occurs mainly after inhalation of spores or through traumatic fungal implantation of fungi from contaminated soil, thorns or wood splinters as well as the introduction through bite wounds.⁶ In domestic animals, cerebral phaeohyphomycosis is rarely seen and has been reported in humans, dogs and cats.¹⁰

The brown or black pigmentation of these fungi can always be seen in cultured fungal cells but may not be visible or only faintly in tissue sections. The hyphae have a width of about 2-6 μm , vary in length in tissue sections and are septated, branched or unbranched. There are different histomorphologic shapes from vesicular-like swellings, bizarre shapes to fragmented hyphae. Fungal cells can be found in the pus of abscesses or in granulomas as well as intracellularly.³

Dematiaceous fungi may cause phaeohyphomycosis, chromoblastomycosis or eumycotic mycetomas.^{3,11,12} These forms have to be differentiated by their tissue appearance. Eumycotic mycetomas are characterized by black grains or granules of pigmented fungi, whereas chromoblastomycosis reveals spherical fungal cells (sclerotic bodies) in tissue sections.

Frequently phaeohyphomycosis occurs in cutaneous or subcutaneous locations after the infection with for example *Phialophora verrucosa*, *Fonsecaea pedrosoi* or *Exophiala jeanselmei*. *Cladophialophora bantiana* seems to be a neurotropic agent causing cerebral phaeohyphomycosis predominantly after inhalation.^{5,7,8,13}

Unfortunately, a mycological determination of the fungal species was not carried out in this presented case. So it is not possible to confirm *Cladophialophora bantiana* as etiologic agent although some hints point it out. Although considered an infectious disease of immunocompromised patients, phaeohyphomycosis is often found in 'healthy' individuals with no obvious immunosuppression.^{5,6,8,14}

AFIP Diagnosis: Brain, cerebrum: Meningoencephalitis, necrotizing, pyogranulomatous, focally extensive, severe, with fibrinoid vasculitis, and numerous dematiaceous hyphae, domestic short hair, feline.

Conference Comment: The contributor provides a thorough concise summary of phaeohyphomycosis. *Ochroconis gallopavum* (formerly *Dactylaria constricta* var. *gallopava*) causes cerebral phaeohyphomycosis in various avian species and was recently reported to have caused fatal systemic phaeohyphomycosis in a dog.^{2,15} With sparsely pigmented fungi, special stains such as Fontana-Masson may be helpful in identifying melanin pigment; however, culture is required for definitive diagnosis.⁴ There is variation between slides with fibrinoid vasculitis and fibrin thrombi present in some sections.

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References:

1. Bube A, Burkhardt E, Weiß E: Spontaneous chromomycosis in the marine toad (*Bufo marinus*). J Comp Path 106:73-77, 1992
2. Salkin IF, Dixon DM, Kemna ME, Danneman PJ, Griffith JW: Fatal encephalitis caused by *Dactylaria constricta* var. *gallopava* in a snowy owl chick (*Nyctea scandiaca*). J Clin Microbiol 28(12): 2845-2847, 1992
3. Salfelder K, de Liscano TR, Sauerteig E: Phaeohyphomycosis. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 56-59. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990
4. Foil CS: Phaeohyphomycosis. In: Infectious Diseases of the Dog and Cat, ed. Greene CE, 2nd edition, pp. 426-428. W.B. Saunders Company, Philadelphia, Pennsylvania, 1998
5. Beccati M, Vercelli A, Peano A, Gallo MG. Phaeohyphomycosis by *Phialophora verrucosa*: first European case in a cat. Vet Rec 157:93-94, 2005
6. Abramo F, Bastelli F, Nardoni S, Mancianti F: Feline cutaneous phaeohyphomycosis due to *Cladophialophora bantiana*. J Fel Med Surg 4:157-163, 2002
7. Helms SR, McLeod CG: Systemic *Exophiala jeanselmei* infection in a cat. JAVMA 217(12):1858-1861, 2000
8. Fondati A, Gallo MG, Romano E, Fondevila D: A case of feline phaeohyphomycosis due to *Fonsecaea pedrosoi*. Vet Dermatol 12:297-301, 2001
9. Dixon DM, Shadomy HJ, Shadomy S: Dematiaceous fungal pathogens isolated from nature. Mycopathologia 70(3):153-161, 1980
10. Boulijhad M, Lindeman CJ, Hayden DW: Pyogranulomatous meningoencephalitis associated with dematiaceous fungal (*Cladophialophora bantiana*) infection in a domestic cat. J Vet Diagn Invest 14:70-72, 2002
11. Salfelder K, de Liscano TR, Sauerteig E: Chromoblastomycosis. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 145-150. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990

12. Salfelder K, de Liscano TR, Sauerteig E: Mycetomas. In: Atlas of Fungal Pathology, eds. Salfelder K, Gresham GA, pp. 151-159. Kluwer Academic Publishers BV, Dordrecht, The Netherlands, 1990
 13. Dixon DM, Merz WG, Elliott HL, Macleay S: Experimental central nervous system phaeohyphomycosis following intranasal inoculation of *Xylohypha bantiana* in cortisone-treated mice. Mycopathologia 100:145-153, 1987
 14. Mariani CL, Platt SR, Scase TJ, Howerth EW, Chrisman CL, Clemmons RM: Cerebral phaeohyphomycosis caused by *Cladosporium spp.* in two domestic shorthair cats. J Am Anim Hosp Assoc 38:225-230, 2002
 15. Singh K, Flood J, Welsh RD, Wyckoff JH, Snider TA, Sutton DA: Fatal systemic phaeohyphomycosis caused by *Ochroconis gallopavum* in a dog (*Canis familiaris*). Vet Pathol 43:988-992, 2006
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CASE III – 05-575 (AFIP 3026925).

Signalment: 5-month-old, intact male, Greater Swiss Mountain Dog.

History: This dog had a 2 week history of progressive hindlimb paraparesis consistent with a T3-L3 spinal cord lesion.

Gross Pathology: There was a focal yellow-tan subdural mass (1.7 x 1.2 x 0.5cm) in the spinal cord at L2 with minimal adjacent hemorrhage and spinal cord compression (Figure 1, Figure 2). All other organs appeared grossly normal.

Histopathologic Description: Within the dura and severely compressing and replacing the spinal cord is an expansile, well-demarcated neoplastic cellular proliferation arranged in bundles and small lobules separated by variably dense fibrous connective tissue. There are two distinct neoplastic cell populations. One population consists of interlacing fascicles of spindleoid cells with indistinct cell borders. Nuclei are elongate-ovoid, with finely stippled chromatin, and 1-2 nucleoli. The second population of cells forms tubules and acini, and occasionally form structures resembling glomeruli. Cells are columnar with ovoid-polygonal nuclei and finely stippled chromatin. Mitoses are variable and range from 0-2 per 5 high-power fields. Few dilated myelin sheaths with swollen axons (spheroids) are present in the adjacent white matter.

Contributor's Morphologic Diagnosis: Thoracolumbar spinal cord tumor of young, large breed dogs (Nephroblastoma)

Contributor's Comment: Thoracolumbar spinal cord tumor of young dogs (spinal cord nephroblastoma) occurs most often in dogs between six months and three years of age with clinical signs of pelvic limb paresis and ataxia, consistent with a compressive lesion between T10-L2 spinal cord segments. This intradural, extramedullary mass is thought to arise from residual embryonic tissue within the dura from fetal development.¹ The histologic appearance is variable, but contains distinct cell populations including a spindleoid cell component and an epithelial component forming tubules and glomeruloid-like structures.^{2,3,4} Differential diagnoses for thoracolumbar spinal cord tumors include primitive neuroectodermal tumors, poorly differentiated astrocytomas, and ependymomas.^{5,9}

Immunohistochemical staining properties of these tumors support a renal origin. The neural markers GFAP and NSE are negative, but tumor cells demonstrate positive immunoreactivity for both cytokeratin and vimentin.^{5,6} This immunoreactivity pattern is similar to renal nephroblastomas. Glomeruloid structures within the tumor are strongly labeled with the Wilms' tumor protein 1 (WT1) antibody.⁷ WT1 is inactivated in childhood Wilms' tumors (nephroblastoma), and the properties of WT1 in humans are identified as a transcriptional target implicated in renal differentiation. About 10% of sporadic Wilms' tumors have inactivating mutations in WT1, and many of these same tumors often contain B-catenin mutations as well.⁸

There is only one case report documenting a spinal cord nephroblastoma metastasis.⁹ A 2-year-old intact female Basset Hound was found to have two spinal cord masses at T11-T12 and L4-L6. The mass at T11-T12 was composed of embryonic blastemal cells, and an epithelial component of tubules and glomeruloid structures. The L4-L6 mass was composed of sheets of undifferentiated blastemal cells with no glomerular component and a higher mitotic index. Because the more distal tumor was much less differentiated, it was thought to represent a metastasis of the T11-T12 mass. It was hypothesized that the spread occurred through the subarachnoid space, or, less likely, occurred within the parenchymal blood vessels. A second case report details an extradural spinal cord metastasis of a renal nephroblastoma, and other sites of metastases included adrenal glands, hepatic and mediastinal lymph nodes, and bone marrow.¹⁰

In the case submitted, no other lesions were observed grossly or histologically, so the diagnosis of primary thoracolumbar spinal cord tumor was given.

AFIP Diagnosis: Spinal cord and dura mater: Thoracolumbar spinal cord tumor of young dogs (nephroblastoma), Greater Swiss Mountain Dog, canine.

Conference Comment: The contributor provides a thorough overview of spinal nephroblastomas in the dog. Nephroblastoma is the most common primary renal tumor of swine and chickens. Nephroblastomas occur less frequently in calves and dogs, and are very uncommon in other species. They are most often seen in young animals and sometimes in fetuses. Nephroblastomas are true embryonal tumors that arise from metanephric blastema.^{11,12}

Grossly, nephroblastomas in the kidney are typically encapsulated white to tan lobulated, meaty to firm with spongy and cystic areas, often with foci of hemorrhage and necrosis. They are usually located in the cortex and extend through the capsule. They are usually unilateral and located at one pole of the kidney, although they can be bilateral and extrarenal. They can be very large (up to 34 kg in swine) resulting in abdominal enlargement. In contrast to spinal cord nephroblastomas in the dog in which metastasis is rare, the tumors in the kidney have widespread metastasis to the lung and liver in over half the canine cases. Metastasis is rare in pigs and calves.^{11,13}

The characteristic histologic features include (1) an epithelial component that varies from glandular structures to normal tubules to glomeruloid structures that lack capillaries to islands and serpentine patterns (2) a mesenchymal component that may be arranged in undifferentiated lobules or streams of mesenchymal cells or differentiated into fibrous, mucoid or adipose tissue, or smooth/skeletal muscle, cartilage or bone and (3) blastemal cells found in clumps or dispersed between the epithelial and mesenchymal tissues. All three components are found in varying amounts and stages of differentiation resulting in tremendous variation in appearance. Regardless of the predominant element, the tumor has an embryonic appearance. The epithelial component can occasionally become squamous and keratinize. The proliferation rate and malignant potential of each element may vary even within a single tumor. Tubular and glomerular differentiation is associated with a good prognosis while an anaplastic sarcomatous appearance is associated with a greater likelihood of metastasis and a poor prognosis.^{11,13}

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References:

1. Macri NP, Alstine WN, Coolman RA: Canine spinal nephroblastoma. J Am Anim Hosp Assoc 33:302-306, 1997

2. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA, Van Winkle TJ: Histological Classification of Tumors of the Nervous System of Domestic Animals, Second Series, vol. V, pp. 26-27. The Armed Forces Institute of Pathology, Washington, D.C., 1999
 3. Koestner A, Higgins RJ: Tumors of the nervous system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 716-717. Iowa State Press, Ames, Iowa, 2002
 4. Summers BA, Cummings JF, de Lahunta A: Veterinary Neuropathology, pp. 386-390. Mosby-Year Book, Inc., St. Louis, Missouri, 1995
 5. Baumgärtner W, Peixoto PV: Immunohistochemical demonstration of keratin in canine neuroepithelioma. Vet Pathol 24:500-503, 1987
 6. Summers BA, deLahunta A, McEntee M, Kuhajda FP: A novel intradural extramedullary spinal cord tumor in young dogs. Acta Neuropathol 75:402-410, 1988
 7. Pearson GR, Gregory SP, Charles AK: Immunohistochemical demonstration of Wilms tumour gene product WT1 in a canine "neuroepithelioma" providing evidence for its classification as an extrarenal nephroblastoma. J Comp Path 116:321-327, 1997
 8. Rivera MN, Haber DA: Wilms' tumour: connecting tumorigenesis and organ development in the kidney. Nat Rev Cancer 5:699-712, 2005
 9. Terrell SP, Platt SR, Chrisman CL, Homer BL, de Lahunta A, Summers BA: Possible intraspinal metastasis of a canine spinal cord nephroblastoma. Vet Pathol 37:94-97, 2000
 10. Gasser AM, Bush WW, Smith S, Walton R: Extradural spinal, bone marrow, and renal nephroblastoma. J Am Anim Hosp Assoc 39:80-85, 2003
 11. Maxie MG: The urinary system. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 2, pp. 520-521. Academic Press, Inc., San Diego, California, 1993
 12. Newman SJ, Confer AW, Panciera RJ: Urinary system. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., p. 680. Mosby Elsevier, St. Louis, Missouri, 2007
 13. Meuten DJ: Tumors of the urinary system. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 518-519. Iowa State Press, Ames, Iowa, 2002
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CASE IV – Massey IVABS 30060-99 HE (AFIP 2788405).

Signalment: 18-month-old male New Zealand Huntaway dog.

History: The dog exhibited progressively worsening ataxia for a month. The owner had noted a high stepping, prancing gait affecting the forelegs and that the dog had difficulty jumping into a utility vehicle. In addition, the dog had started defecating in its kennel.

Upon clinical examination ataxia and hypermetria were noted in all 4 limbs, but there were no tremors or cranial nerve deficits. No abnormalities were observed on fundic examination. Postural reactions (conscious proprioception, wheelbarrowing, hemistand and hemiwalk) were also normal as were spinal reflexes in the forelimbs. A crossed extensor response was seen when the hindlimb withdrawal reflexes were being assessed. The patella, cranial tibial and gastrocnemius reflexes were exaggerated in both hindlimbs and clonus was observed on the right side.

Gross Pathology: The only abnormality noted at necropsy was an enlarged and congested liver.

Laboratory Results: Lysosomal hydrolase activities: A deficiency of sulphamidase activity was identified in the affected liver. Alpha-mannosidase is significantly elevated in the liver from the affected dog compared with the control.

Glycosaminoglycan analysis: GAGs from the affected and control dog livers were compared with each other and with those from two human patients and a mouse with mucopolysaccharidosis-III A. Complex banding patterns were different between samples from controls (human and dog) and affected subjects. A similar pattern of banding was evident between human and mouse mucopolysaccharidosis-III A GAGs and those of the affected dog, particularly at the low molecular weight oligosaccharide region of the gel.

Histopathologic Description: In the section provided, many neurons are distended with fine granular material which varies from eosinophilic to slightly basophilic. This storage material was variably PAS-positive, stained moderately with Luxol fast blue, lightly with Sudan black and slightly with alcian blue in some severely affected neurons. It gave a yellow autofluorescence on fluorescence microscopy. In some areas axonal spheroids are prominent. A moderate number of highly vacuolated macrophages are present in the meninges and perivascular spaces. There is a variable local loss of Purkinje cells in some cerebellar folia of the cerebellum, accompanied by thinning of the molecular layer in the same areas.

Electron microscopy: The storage material in neurons of the cerebral cortex was in the form of membrane-bound accumulations of membranous whorls. In occasional neurons there were also membranous stacks of the type known as 'zebra bodies' and these predominated in some cells. The fine vacuoles noted at the light-microscopic level in hepatocytes were seen as empty membrane-bound vesicles.

Contributor's Morphologic Diagnosis: Brain, cerebellum and medulla oblongata: Neuronal cytoplasmic accumulation of granular storage product, diffuse moderate. Histiocyte cytoplasmic vacuolation of meninges and perivascular spaces. Huntaway. Canine.

Aetiology: Mucopolysaccharidosis IIIA (Sanfilippo syndrome)

Contributor's Comment: A lysosomal storage disease was suspected and confirmed by histopathology and electron microscopy, which revealed widespread neuronal lesions of a lysosomal storage disease that were interpreted as indicative of one of the forms of mucopolysaccharidosis-III. This was on the basis of apparent ganglioside accumulation (indicated by membranous whorls/stacks as seen in electron micrographs) and vacuolated macrophages in perivascular spaces and leptomeninges. In other tissues examined from this dog (notably liver) there was also cytoplasmic vacuolation of fibroblasts and hepatocytes. The absence of notable skeletal or soft-connective-tissue lesions supported this diagnosis, rather than one of the other forms of mucopolysaccharidosis.

Most of the non-degraded heparan sulphate is excreted in urine and primary stigmata associated with its accumulation in tissues are limited to the macrophages in perivascular spaces and meninges, and vesicles in hepatocytes and connective tissue cells. Paradoxically, the major accumulated material in neurons is comprised of gangliosides, which as polar lipids, help form the membranous whorls and stacks noted on electron microscopy.

There are 4 phenotypically similar forms of MPS-III (**A-D**) resulting from deficiencies of 1 or the other of 4 enzymes particular to the sequential catabolism of heparan sulphate. Enzyme analyses undertaken were for potential diagnosis of MPS-III A,B,C and multiple sulphatase deficiency. The lack of sulphamidase in the affected liver indicated a probable diagnosis of MPS-IIIA. The presence of the same amount of total sulphatase in the control and the affected dog liver indicated that the sulphamidase-deficiency was not the result of a multiple sulphatase deficiency. The elevated α -mannosidase activity is consistent with a lysosomal storage disorder.

The mucopolysaccharidosis-IIIA (Sanfilippo syndrome) is an autosomal recessive inherited lysosomal storage disease of humans that has also been diagnosed in wire-haired Dachshund dogs and mice. A similar genetic cause is assumed for the disease in this Huntaway dog.

AFIP Diagnosis: Brain, cerebellum and brain stem: Neuronal and axonal degeneration, multifocal, moderate, with spongiosis, spheroids and abundant neuronal cytoplasmic eosinophilic granular material, New Zealand Huntaway dog, canine.

Conference Comment: Lysosomal storage diseases occur secondary to dysfunction of lysosome-mediated degradation of products (substrates) of normal cellular metabolism. These substrates cannot be degraded by lysosomes, accumulate, and eventually result in the death of affected cells. Lysosomal storage diseases that affect the central nervous system result in the accumulation of substrate in neurons and myelinating cells. When these cells die, the accumulated substrate is released into adjacent tissue. Macrophages phagocytose the unprocessed substrate; however, macrophages have the same genetic defect and accumulate the substrate in their lysosomes leading to their eventual death. With few exceptions, these are inherited autosomal recessive diseases.⁴

Originally, lysosomal storage diseases were thought to develop exclusively due to mutations resulting in a reduction in lysosomal enzyme synthesis; however, it is now clear that there are other defects including the following:

1. Synthesis of catalytically inactive enzymes that resemble normal active enzymes
2. Defects in posttranslational processing of the enzyme resulting in its being misdirected to sites other than lysosomes
3. Lack of enzyme activator (enzyme that normally increases the rate of an enzyme-catalyzed reaction) or protector protein (facilitate repair and refolding of stress-damaged proteins)
4. Lack of substrate activator protein required to assist with hydrolysis of substrate
5. Lack of transport protein required for elimination of digested material from lysosomes

Therefore, lysosomal storage diseases can result due to defects in any protein that is essential for normal lysosome function. Cell swelling and vacuolation occur due to accumulation of unprocessed substrate in lysosomes. Differences in size and appearance of cells (neurons versus hepatocytes) are therefore dependent on the availability of the substrate (carbohydrate or lipid) in a particular organ system. For example, many lipids and glycolipids are unique to the central nervous system (CNS). Therefore, when there is a lysosomal defect in the CNS, neural cells often accumulate lipids and glycolipids.⁴

The following chart summarizing inherited metabolic diseases affecting the nervous system of domestic animals was kindly provided by the moderator.⁴⁻⁷

Table Inherited metabolic diseases affecting the nervous system of domestic animals

Disease name	Stored material	Defective enzyme/gene	Morphology Histochemistry*	Neurons	Myelin	Other tissues/cells	Species/Breed
Ceroid-lipofuscinosis	Ceroid/lipofuscin	Numerous	Yellow-brown cytoplasmic granules (F,L,P,S)	X		axonal spheroids, neuronal necrosis, Wallerian degeneration, gliosis, macrophages	canine, feline, ovine, bovine
Fucosidosis	Fucose containing glycolipids, glycoproteins, poly-/ oligo-saccharides	Alpha-L-fucosidase	Clear fine vacuoles (P)	X		glia, macrophages, lymphocytes, peripheral nerves, kidney, pancreas, lymph nodes, lung	canine (English Springer Spaniel)
Galactosylceramide lipidosis (Globoid-cell leukodystrophy, Krabbe's-like disease)	Galactosylceramide (galactocerebroside and galactosylsphingosine (psychosine))	Galactosylceramidase (galactocerebroside β -galactosidase)	Demyelination with accumulation of macrophages filled with PAS positive material		X		canine (West Highland Terrier, Cairn Terrier, min poodle, Basset Hound, blue tick hound, Beagle, Pomeranian, Irish Setter), feline (DSH, DLH), ovine (Dorset), rhesus, twitcher mouse
Galactosialidosis	Glycolipids	Beta-galactosidase	Clear vacuoles (F, L, P)	X		hepatocytes, macrophages	canine (Schipperke)
Glucocerebroside (Gaucher's disease)	Glucocerebroside	Acid beta-glucosidase (glucocerebroside)	Clear to weakly eosinophilic small vacuoles (P)	X	X	macrophages in liver and lymph nodes, axonal spheroids	canine (Silky terrier), ovine, porcine
Glycogen storage disease IA (Von Gierke)	Glycogen	Glucose-6-phosphatase	Swollen hepatocytes (described as vacuoles, but looks like glycogen?)			liver, neurologic signs are due to hypoglycemia	canine (Maltese)
Glycogen storage disease II (Pompe's)	Glycogen	Acid alpha-glucosidase	Swollen cells	X		muscle (skeletal, cardiac, and smooth)	canine (Lapland), feline (DSH), ovine (Corriedale) bovine, (Shorthorn, Brahman), quail (Japanese)
Glycogen storage disease III (Cori's)	Glycogen	Amylo-1,6-glucosidase	Enlarged foamy? (P)	X		hepatocytes, muscle, glia	canine (German Shepherd, Akita)
Glycogen storage disease Type IV	Abnormally branched glycogen (alpha-1,4-D-glucan)	Branching enzyme	Pale blue granules (I, P)	X		muscle	feline (Norwegian forest cat)
GM1 gangliosidosis	Gangliosides	Beta-galactosidase	Clear to pale pink granular material in vacuoles (L, P, S)	X	X	hepatocytes, macrophages, renal tubular cells, pancreatic exocrine cells	canine (Beagle, English Springer Spaniel, Portuguese Water Dog, Alaskan Huskies, Shiba dogs, mixed breed), feline (Siamese, Korat, DSH), bovine (Holstein Friesian), ovine (Suffolk.,

							Coopworth-Romney)
Mucopolipidosis (I-cell disease)	Mucopolysaccharides, lipids, glycoproteins	N-acetylglucosamine-1-phosphotransferase	Clear vacuoles	X (rare)		bones, cartilage, skin	feline (DSH)
α -mannosidosis	Glycoprotein-derived mannose-rich oligosaccharides	Alpha mannosidase	Clear vacuoles	X	loss	glia, fibroblasts, endothelial and glandular epithelial cells, hepatocytes, macrophages	bovine (Angus, Murray Grey, Galloway), feline (Persian, DSH, DLH)
β -mannosidosis	Glycoprotein-derived mannose-rich oligosaccharides	Beta mannosidase	Clear vacuoles	X	loss	glia, fibroblasts, endothelial and glandular epithelial cells, macrophages	caprine (Nubian), bovine (Salers)
MPS I (Hurler's, Scheie, and Hurler/Scheie disease)	Heparan and dermatan sulfate	Alpha-L-iduronidase	Clear vacuoles Skeletal abnormalities predominate in most MPS syndromes	X		hepatocytes, macrophages, glia, chondrocytes, smooth muscle, fibroblasts, leukocytes	feline (DSH), canine (Plott hound, Rottweiler)
MPS II (Hunter syndrome)	Heparan and dermatan sulfate	Iduronate-2-sulfatase	Clear vacuoles (P)	X		epithelial and endothelial cells, macrophages	canine (Labrador Retriever)
MPS IIIA (Sanfilippo A)	Heparin sulfate gangliosides	Heparan N-sulfatase	Clear vacuoles (F, L, P, S)	X		hepatocytes, macrophages	canine (Wirehaired Dachshund, New Zealand Huntaway)
MPS IIIB (Sanfilippo B)	Heparin sulfate	Alpha-N-acetylglucosaminidase	Clear vacuoles (P, T)			monocytes, hepatocytes	canine (Schipperke), emu
MPS IIID (Sanfilippo D)	Heparin sulfate gangliosides	N-acetylglucosamine 6-sulfatase	Clear vacuoles	X		muscle, fibroblasts, chondrocytes, others	caprine (Nubian)
MPS VI (Maroteaux-Lamy)	Dermatan sulfate	N-acetylglucosamine 4-sulfatase (arylsulfatase B)	Clear vacuoles	X		hepatocytes, macrophages, wbc	feline (Siamese, DSH), canine (Miniature Pinscher, Miniature Schnauzer, Welsh Corgi, Chesapeake Bay Retriever)
MPS VII (Sly)	Chondroitin and dermatan sulfate	beta-glucuronidase	Clear vacuoles	X		hepatocytes, fibroblasts, chondrocytes, RPE, keratocytes, synovial cells, macrophages	canine (German Shepherd, mixed breed), feline (DSH)
Sphingomyelinosis (Niemann-Pick A & B)	Unesterified cholesterol (ganglioside)	Acid sphingomyelinase	Clear vacuoles (P variable)	X		macrophages, spheroids	feline (Siamese) canine (Miniature poodle)
Sphingomyelinosis (Niemann-Pick C)	Unesterified cholesterol (ganglioside)	NPC1	Clear vacuoles (P variable)	X		macrophages, spheroids	Feline (DSH, Siamese), Canine (Miniature poodle, Boxer)

*Histochemistry refers to the histochemical stains that stain the stored material assuming ideal conditions of tissue preservation, fixation, and processing. Most are only reliably positive on frozen sections of unfixed tissues.

(F = autofluorescent, I = Lugol's iodine, L = luxol fast blue, P = PAS, S = Sudanophilic, T = toluidine blue)

Cerebellar atrophy of varying degrees was present in some slides.

Readers are encouraged to read reference 3 for a comprehensive overview of lysosomal storage diseases of animals.

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References:

1. Jolly RD, Allan FJ, Collett MG, Rozaklis T, Muller VJ, Hopwood JJ: Mucopolysaccharidosis IIIA (Sanfilippo syndrome) in a New Zealand Huntaway dog with ataxia. *N Z Vet J* 48:144-148, 2000
2. Neufeld EF, Muenzer J. The mucopolysaccharidoses. *In: The Metabolic Basis of Inherited Disease*, eds. Scriver R, Beudet AL, Sly WS, Valle D, 6th edition, vol. 2, pp. 1565-1587. McGraw-Hill, New York, New York, 1989
3. Jolly RD, Walkley SU: Lysosomal storage diseases of animals: an essay in comparative pathology. *Vet Pathol* 34:527-548, 1997
4. Zachary J: Nervous system. *In: Pathologic Basis of Veterinary Disease*, eds. McGavin MD, Zachary JF, 4th ed., pp. 928-930. Mosby Elsevier, St. Louis, Missouri, 2007
5. Haskins M, Giger U: Lysosomal storage diseases. *In: In Press*, 2007
6. Braund K: Clinical Neurology in Small Animals: Localization, Diagnosis, and Treatment, VIN
7. Summers BA, Cummings JF, de Lahunta A: Degenerative diseases of the central nervous system. *In: Veterinary Neuropathology*, pp. 214-236. Mosby-Year Book, Inc., St. Louis, Missouri, 1995

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