The Armed Forces Institute of Pathology
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CASE I - 355/01 (AFIP 2834372)

Signalment: 4-year-old, female, Friesian horse, *Equus caballus*, equine

History: A 4-year-old female Friesian horse was presented for clinical evaluation of severe movement disorders. The mare revealed intense atactic gait progressing over a period of two weeks, supposedly after having been sold. Finally, the horse was put down because of poor prognosis.

Gross Pathology: Macroscopic inspection of the brain revealed a marked asymmetrical enlargement of the cerebellum with intense convoluted and thickened folia. Normal configuration appears only in some minor parts of the lateral hemisphere.

Laboratory Results: Hematology, CSF cytology and clinical chemistry of CSF and serum were inconclusive of systemic disease.

Contributor's Morphologic Diagnosis: Dysplasia, cerebellar, severe, chronic, with calcareous deposits in small vessels and spongiosis.

Contributor's Comment: Neuropathological findings closely resemble lesions described in human cases of Lhermitte-Duclos Disease (LDD). This disease occurs in early adulthood, arises from the cerebellar cortex and is characterized by an asymmetric dysplastic enlargement of the cerebellum without clear demarcation of diseased and normal tissue and highly enlarged and distorted folia. The lesion causes progressive mass effects and is commonly associated with cerebellar dysfunction and signs of increased intracranial pressure.
        Histopathological examination of the widened folia exhibits a disorganized cerebellar architecture in which Purkinje cell bodies are rarely recognized and the border between the molecular and granular layers cannot be discerned. The thickened granular cell layer contains large ganglion cells with clear nuclei and prominent nucleoli.

The broadened molecular layer is thickened and irregularly myelinated tracts extend from the abnormal nerve cells in the granular layer.

Severity of the findings varies from a recognizable granular cell layer containing occasional large dysplastic neuronal cell bodies to an unrecognizable granular layer occupied by a population of large nerve cell bodies between the molecular layer and internal white matter. The myelinated axis of the cerebellum is atrophic and cystic. Like in our case, the lesion gradually blends into normal cerebellar tissue. Mitoses are absent. Some slides contain blood vessels with intense mineralization of the tunica intima and tunica media.

LDD was first described in 1920 and has also been mentioned in the literature under the names of Purkinjeoma, granular cell hypertrophy of the cerebellum, hamartoma of the cerebellum, dysplastic gangliocytoma, ganglioneuroma and gangliomatosis of the cerebellum.

It resembles an extremely rare disease with only a few more than 100 so far documented human cases and only one reported case in a horse. The pathogenesis of this disease is still poorly understood. Lhermitte and Duclos regarded the lesion as a combination of a ganglion cell neoplasm and a malformation originating from precursors of Purkinje cells. LDD in humans is 40% associated with Cowden's syndrome, a rare autosomal dominant multiple hamartoma syndrome. Linkage analysis has determined that a single locus within chromosome 10q23 is likely to be responsible for Cowden's disease and probably for LDD. Located in this region is PTEN (**P**hosphatase and **TEN**sin homolog), acting as a tumor suppressor by negatively regulating the phosphoinositide3-kinase (PI3K) signaling pathway. PTEN is frequently mutated somatically in various human cancers. Besides carcinogenesis, it may play important roles in brain development. Knockout mice show enlarged, histoarchitecturally abnormal brains and the layering defects might be due to uncontrolled progenitor proliferation and/or altered cell migration normally regulated by PTEN.

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AFIP Diagnosis: Cerebellum: Dysplastic gangliocytoma, Friesian, equine.

Conference Comment: This exceptionally rare case was reviewed in consultation with the staff of the Department of Neuropathology, AFIP, who concur with the contributor that the histomorphology of this lesion closely resembles Lhermitte-Duclos disease (LDD). Conference participants compared the profoundly abnormal portion of the cerebellar folia to the more normal remaining tissue, noting that both small and medium size neurons, which resemble granule cells and Purkinje cells, respectively, populate the granular layer, and there is moderate widening of the molecular layer. The pathogenesis of this lesion is uncertain; neoplasia, malformation, and hamartoma have all been proposed. Luxol fast blue and Bielschowsky stains demonstrated the subpial layer of myelinated axonal fibers (white matter) oriented parallel to the underlying gray matter of the dysplastic folia, which is anecdotally referred to as "inside out". We are grateful to the Department of Neuropathology for their assistance.

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CASE II - N482/02 (AFIP 2840181)

Signalment: 2-month-old, male, Brown Swiss (*Bos taurus*), bovine

History: The two months of age calf was admitted to the clinic with weakness of the forelimbs. Clinical examination revealed that the calf had moderate acute pneumonia and normal cranial nerve function. Sensation was normal. Muscles of the thoracic limb were atrophic. In a few days, there was observed weakness of the rear limbs too. The calf was euthanized one week later after the admission.

Gross Pathology: At post-mortem examination, mild atrophy of the thoracic limbs was noted. The calf had lobar, cranioventral, mild bronchopneumonia. No gross changes were present in the central nervous system.

Laboratory Results: Not available.

Contributor's Morphologic Diagnosis: Spinal cord: Chromatolysis, mild, multifocal; neuronophagia, mild, multifocal; gliosis, mild to moderate, diffuse; calf, Brown Swiss, bovine.

Contributor's Comment: Lesions were identified in the slides from the spinal cord corresponding to the cervical intumescences, and especially in the ventral horns of the spinal cord. The lesions consisted of somatic motor neuron chromatolysis and loss of neurons. Nuclei of chromatolytic neurons appeared pyknotic and karyolytic. Diffuse, mild to moderate gliosis was evident in affected grey matter. Glial nodules and neuronophagia were multifocally seen.

Spinal Muscular Atrophy (SMA) is a Lower Motor Neuron (LMN) disease, a group of lesions characterized by neurofibrillary accumulation, involving the neurons of the ventral horn of the spinal cord, and brain stem; and occasionally the large motor neurons of the motor cerebral cortex (1). Degenerative changes are characterized by loss of neurons, glial nodules, and neuronophagia. Surviving cell bodies are often enlarged and chromatolytic. The pattern of chromatolysis is described variously as peripheral, central, or complete (2).

LMN disease has been reported in Brown Swiss and it is clinically characterized by skeletal muscle atrophy, decreased spinal reflex and motor weakness (1): affected calves become paraparetic between two and six weeks of age, and after a period of recumbency die. Bronchopneumonia is a common event (1, 2). The majority of the affected calves are female and the pathology could be also congenital.

Many LMN diseases have often a familial basis, the most common are autosomal recessive disorders, caused by mutations in the survival motor neurone gene (SMN). A possible other pathogenetic mechanism of SMA, invokes an inappropriate persistence of normally occurring motor neuron apoptosis, via mutations in the gene for neuronal apoptosis inhibitory protein (NAIP) (3).

Through the use of human SMA synthetic probes, the genetic position of bovine SMA has been assigned and, in the 1998, the full length cDNA sequence of the bovine SMN gene was identified. Molecular analysis and characterization of the sequence documents 85% identity to its human counterpart and three evolutionarily conserved domains in different species. The bovine SMN is localized to the chromosome region 20q12->q13 (4).

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AFIP Diagnosis: Spinal cord: Ventral motor neuron degeneration, necrosis, and loss, multifocal, mild, with central chromatolysis, satellitosis, and multifocal glial nodules, Brown Swiss, bovine.

Conference Comment: In veterinary medicine, motor neuron diseases are usually of the lower motor neuron type. Within the axon, neurofilaments (intermediate filaments) are cytoskeletal components, which in concert with microtubules, maintain axonal diameter. Under normal conditions, neurofilaments are phosphorylated, assembled and transported from the perikaryon into the axon. Phosphorylation defects lead to abnormal neurofibrillary accumulation in the perikaryon, which is characteristic of some lower motor neuron disorders, including spinal muscular atrophy of Brown Swiss and Red Danish calves and hereditary canine spinal muscular atrophy in Brittany Spaniels.

In this case, chromatolysis (neuronal degeneration) progresses to neuronophagia (loss of motor neurons), Wallerian degeneration, and neurogenic muscular atrophy. There are rare accumulations of macrophages among the fibers of the ventral funiculi, which signifies axonal degeneration due to loss of the ventral motor neuron in the ventral horn.

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CASE III - 02N160 (AFIP 2840311)

Signalment: 14-year-old female (spayed) Beagle-Shepherd mix, *Canis familiaris*, canine.

History: This 14-year-old dog was found unable to stand up (4-26-02). She was healthy except for a short history of renal disease. Her body and head were turned to the right, and her right forelimb was held in extension. Spinal reflexes were intact but increases on the right side. Clinical presentation was that of an upper motor neuron lesion injury in the area of the cerebellum and or brainstem. She was treated with corticosteroids and IV fluids. An MRI scan was performed the next day with a diagnosis of cerebellar infarction. The dog did not improve over a four-day period and was subsequently euthanized.

Gross Pathology: The perihilar lymph nodes were white, firm and mineralized. The caudate lobe of the liver had rounded edges and a rubbery firm texture. The kidneys were grossly normal. The adrenals were nodular on cut surface. There was 1.0 cm x 1.0 cm soft tan area in the left cerebellum.

Laboratory Results:
Elevated serum chemistries on 4-26-02:
BUN:45 mg/dl; creatinine: 1.8 mg/dl; alkaline phosphatase: 157 U/L; AST: 140 U/L; CPK: 2194 U/L
Leukogram: WBC 17.1 THDS/CMM
Total protein 6.6 g/dl and albumin: 3.5 g/dl were within normal range.

Urinalysis: Clear yellow urine; SG 1.017; pH 5.0; 2+ protein (100 mg/dl); trace blood and granular casts

Contributor's Morphologic Diagnoses: 1. Subacute infarction, cerebellum/medulla.
2. Severe chronic glomerulonephritis, left and right kidneys.
3. Marked diffuse hepatocelluar swelling with mild cholestasis and hemosiderosis, liver.
4. Mild biliary hyperplasia and fibrosis, liver.
5. Mild hemosiderosis, hilar lymph node.

Contributor's Comment: The cerebellum has a large area of edema and vacuolar change affecting the folia with extension into the medulla. A central area near the medulla is necrotic and small arteries near this lesion are affected by endothelial hyperplasia. A few small hemorrhages are recognized. The cause of the cerebellar infarct is not evident, but is most likely due to a vascular lesion not identified in the sections.

Cerebral infarction has been characterized in dogs and aging swine, as a consequence of vascular stenosis induced by severe atherosclerotic lesions. Severe cerebral infarction has been described in a young mature dog following aberrant migration of mature *Dirofilaria immitis* within branches of the right middle cerebral artery. There was no evidence of either atherosclerosis or heartworm infection in this dog.

Protein-losing disease has been associated with producing a hypercoagulable state leading to thromboembolism in 135 of dogs in one review study. Dogs with serum protein of less than 2 g/dl were shown to be at risk of thromboembolism. While this dog had moderate proteinuria she did not have hypoproteinemia. However, it was noted in the study that serum albumin was initially normal in 29% of cases.

Antemortem demonstration of a cerebellar infarction in a dog has been rare, but may become more common with the commercial availability of MRI. The MRI provided a diagnosis that correlated 100% with the gross and histopathological findings.

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AFIP Diagnosis: Cerebellum, cortex, vermis and hemisphere: Necrosis, focally extensive (infarct), with spheroids, Beagle-Shepherd cross, canine.

Conference Comment: Conference participants suspected an underlying vascular event that resulted in ischemia and necrosis (infarction). The vulnerability of central nervous system (CNS) cells to hypoxia and ischemia is greatest in neurons of the cerebral cortex and cerebellar cortical Purkinje cells, less in oligodendroglia, astrocytes, and microglia, and least in endothelial cells lining the vasculature. Few predisposing factors leading to CNS infarction have been identified in dogs. These include hypertension secondary to renal disease, hypothyroidism producing atherosclerotic plaques, and amyloidosis. Periodic acid-Schiff is useful to demonstrate vascular lesions associated with hypertension.

Contributor: Georgetown University, Division of Comparative Medicine, Washington, DC, 20057-1435

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CASE IV - S79/01 (AFIP 2839909)

Signalment: 8-week-old, 25 kg, female, Pietrain crossbreed, porcine

History: This tissue is from one out of 210 five- to eight-week-old recently weaned pigs. Twenty-eight animals of this group showed ataxia, tremors, convulsions, and to a variable extent, progressive paralysis of the hind limbs over the last two weeks. Eighteen pigs died acutely or had to be euthanized. Three of them were submitted for necropsy.

Gross Pathology: No gross lesions noted.

Laboratory Results: Using porcine kidney cells (PK-15) and embryonic testis cells (EFH) a porcine teschovirus (PTV) differing from serotype 1 was isolated from the spinal cord, brain, and heart of this animal and from spinal cord, brain, lymphatic tissue or liver, lungs, heart, and kidneys, respectively, of two other necropsied piglets from the same group. Antibodies against PTV strain isolated were detectable in serum samples with low titers and not of all animals. Virus isolation and RT-PCR for classical swine fever virus, PCR for porcine circovirus type 2, and pseudorabies were all negative.

Contributor's Morphologic Diagnoses: 1. Spinal cord: Poliomyelitis, lymphocytic and histiocytic, multifocal, mild to moderate.
2. Paravertebral ganglia: Ganglioneuritis, lymphocytic, mild (not submitted)
3. Brain, cerebrum and cerebellum: Meningoencephalitis, lymphocytic and histiocytic, multifocal, moderate to extensive (not submitted)

Contributor's Comment: Porcine polioencephalomyelitis comprises a group of closely related diseases of pigs caused by different types of variably virulent porcine teschoviruses, formerly grouped to enteroviruses (PEV, *Picorniviridae*). The prototypic

"porcine enteroviral encephalomyelitis" was first recognized in 1929 by Trefny in Teschen, in what is now the Czech Republic, and described as a particularly virulent, highly fatal, nonsuppurative inflammation involving all parts of the central nervous system and the craniospinal ganglia. Since then "Teschen disease" has occurred sporadically in numerous countries, predominantly in Central Europe and Madagaskar, and was the cause for severe economical losses during outbreaks in the 1930s to 1950s. Though several local eponyms have been applied in the past for milder forms like Talfan disease in the United Kingdom and benign enzootic paresis in Denmark, the hallmark of all of these different diseases is the nonsuppurative character and distribution of the inflammation in the CNS. In the spinal cord, there is extensive lymphocytic perivascular cuffing coinciding with lymphocytic and histiocytic infiltration into the neuropil in a specific topic pattern. The lesions of the gray matter are far more pronounced than in the white matter, where only moderate activation of microglia around small foci of malacia may be present. Motor neurons of the ventral columns of the gray matter and, to a lesser extent of the dorsal horn, are consistently affected and undergo different stages of degeneration from slight swelling to progressive chromatolysis and finally microglial perineuronal satellitosis with neuronophagia. Ultrastructural studies indicate that vesiculation of the endoplasmic reticulum is the characteristic finding in teschovirus-induced neuronal degeneration. Moderate diffuse astrogliosis may also be present in the gray matter.

Occasionally, hemorrhage and diffuse lymphocytic infiltration can be observed also. The medulla oblongata, cerebellar cortex, cerebellar peduncles, pons, and thalamus may also be variably involved depending upon the strain of porcine teschovirus in question. In pigs recovering from acute disease showing residual paralysis, wasting due to neuronal atrophy of the musculature of the hind limbs is present.

It should be noted that a non-suppurative encephalomyelitis is not pathognomonic or unique to porcine teschovirus infection, though inflammatory character, location and distribution of lesions in this case represent an excellent example of this disease. Non-suppurative encephalomyelitis can be associated with a broad spectrum of causes like classical swine fever virus (CSFV, *Flaviviridae)*, African swine fever virus (ASFV, *Asfarviridae)*, porcine hemagglutinating encephalomyelitis virus (HEV, *Coronaviridae)* causing vomiting and wasting disease and Ontario encephalomyelitis, rabies virus (RABV, *Rhabdoviridae)*, pseudorabies virus *(Herpesviridae)* causing Aujeszky's disease, Coxsackie virus B5 *(Picornaviridae)* causing swine vesicular disease, porcine rubulavirus *(Paramyxoviridae)*, porcine reproductive and respiratory syndrome virus (PRRSV, *Arteriviridae)*, and encephalomyocarditis virus (EMCV, *Picornaviridae)*. Bacterial meningoencephalitis (*Streptococcus suis, Haemophilus parasuis, Salmonella cholerasuis*) or sodium chloride poisoning as non-infectious causes for CNS disease have to be ruled out as differentials due to the different type and location of inflammatory exudate (Summers et al., 1994).

It is now generally accepted that at least 11 PTV subtypes (PTV-1 to 7, 11 to 13; PTV-Dresden, ICTV classification) can be differentiated by virus-neutralization tests and genome sequencing (Zell et al., 2001). Infection by one serotype does not protect against infection by another. Based on parameters such as cytopathic effect (CPE), replication in cell culture, and serological assays, porcine enteroviruses were previously

divided into three CPE groups: I (serotypes 1-7 and 11-13), II (serotype 8), and III (serotypes 9 and 10). However, sequencing of almost the complete genome of the group I virus strains revealed a phylogenetically distinct virus group (Kaku et al. 2001). Therefore, the former PEV-1 was reclassified as porcine teschovirus 1 (PTV-1, species *Porcine teschovirus*, genus *Teschovirus)*, and the other serotypes of PEV group I were proposed as tentative members of the same species (King et al., 2000; Krumbholz et al., 2002).

Interestingly, the isolated PTV from the submitted case reacted by indirect immunofluorescence assay strongly with the PTV group-specific Mab 040/B1, but also with Mab 041/3C3, which specifically recognizes PTV strain Dresden as different from PTV-1. As shown by phylogenetic analysis of the P1 region, which encodes the capsid protein, and due to the distinct reaction pattern in serologic tests, it has been proposed that this Dresden-like PTVs should be considered as a distinct serotype (Zell et al., 2001).

AFIP Diagnosis: Spinal cord: Myelitis, nonsuppurative, multifocal, moderate, with neuronal necrosis and radiculitis, Pietrain crossbreed, porcine.

Conference Comment: The contributor has provided an excellent summary of porcine polioencephalomyelitis.

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