**Joint Pathology Center**
**Veterinary Pathology Services**

# WEDNESDAY SLIDE CONFERENCE 2021-2022

## C o n f e r e n c e 12

**15 December 2021**

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**CASE I:** N-697-15 (JPC 4085531)

**Signalment:**
10-year-old, female, Crioula, horse (*Equus caballus*)

**History:**
This is one horse out of a group of 15 horses of the same breed held together exclusively on a native pasture without feed supplementation. Five adult horses got sick with a common history of neurological signs and progressive loss of weight – three adult horses and one colt died after a clinical course of 90 days and the surviving horse was euthanized for humanitarian reasons. One of the horses was necropsied. Clinical signs were characterized by progressive weight loss, incoordination, stiff gait, aimless walking, abnormal behavior, and hyperesthesia with locomotion difficultly when provoked to move. According to the owner, affected horses were found trapped in fences or in dense bushy areas. A heavy infestation of broomweed (*Sida carpinifolia*) was observed in the pasture where the 15 horses were held, mainly in shady places close to fences and riversides. All horses were vaccinated for rabies.

**Gross Pathology**:
Necropsy findings in the horse of this report included bilateral extensive lacerations of the skin of distal portion of hind limbs and poor body condition. The large colon was moderately distended by fecal content and there was moderate urinary bladder repletion.

**Laboratory Results:**
Fresh samples of brain and spinal cord were submitted to direct FAT for rabies with negative results.

**Microscopic Description:**
Histologically, neurons and astrocytes from telencephalic cortex, diencephalon, striate body, rhombencephalon, cerebellum and spinal cord (cervical and lumbar intumescences, and thoracic and sacral segments) contain varying degrees of swollen and fine vacuolated cytoplasm giving the cells a foamy appearance. The lesions were specially marked in thalamus, hippocampus, cerebellum and in all examined levels of the spinal cord. Occasionally, there were neurons with shrinking and hypereosinophilic cytoplasm (neuronal necrosis), and multifocal axonal spheroids. There was also moderate astrogliosis of the Bergmann glia which was highlighted by the GFAP IHC. In several ganglia (mesentery celiac, trigeminal and paraspinal) there was mild neuronal cytoplasmic vacuolization.

Sections of cerebellum, pons, thalamus, and hippocampus were submitted for lectin

histochemistry technique with commercial lectins (Vector Laboratories, Burlingame, CA). Immunohistochemistry (IHC) to detect anti-glial fibrillary acidic protein (GFAP) was performed in adjacent sections using streptavidin-biotin-peroxidase complex, with anti-glial fibrillary acidic protein (GFAP) at 1:500 and anti-S100 at 1:200 as primary antibodies. Antigens were retrieved through heat and reaction was revealed with 3'3-diaminobenzindine (DAB) chromogen (DAKO). As negative control, a brain section from a normal age-matched horse was used.

**Contributor's Morphologic Diagnoses:**
Neuronal vacuolar degeneration, moderate to severe, diffuse, chronic.

**Contributor's Comment:**
The diagnosis of *Sida carpinifolia* poisoning in these cases was based on clinical, epidemiological, histopathological, and lectin histochemistry findings.

Normal cellular catabolism directs a steady stream of endogenous complex macro-molecules into vesicular compartments (lysosomes) for degradation to simple molecules that may be reused or excreted. These essentially autophagic pathways are the means through which a cell recycles its own constituents. Storage diseases occur when there is accumulation of macro-molecules in a cell which has compromised intracellular mechanisms for digestion, disposal, or transport. Most storage diseases involve neurons and translate as neurologic impairment[5] although other populations of stable or permanent cells may be affected. Lysosomes are the kingpins of the de-gradation mechanism for intracellular digestion of macromolecules. Lysosomal digestion can be impaired in several ways. The most relevant is the deficient activity of a specific lysosomal hydrolase due to a genetic defect or to an acquired condition which compromises one or more of the lysosomal enzymes.[5]

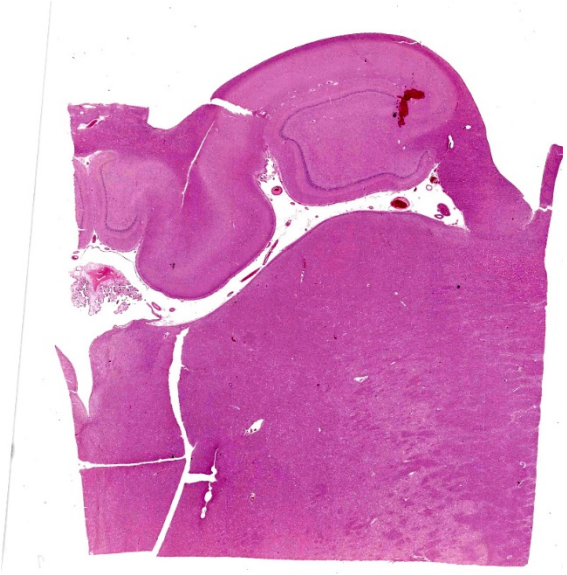

**Figure 1-1. Diencephalon, horse. A section of diencephalon with hippocampus is submitted for examination. (HE 6X)**

Acquired lysosomal diseases in domestic animals are usually, but not always, induced by the ingestion of plants containing swainsonine, including *Swainsona* spp. in Australia, *Astragalus* spp. and *Oxytropis* spp. in South and North America, China, and Africa,[9,14,17] *Ipomoea carnea* subsp. *fistulosa*,[2] *Ipomoea sericophylla* and *I. riedelii*,[3] *Ipomoea verbascoidea*,[16] *Turbina cordata*,[8] and *Sida carpinifolia*[10,11,15] in Brazil.

*S. carpinifolia* (Malvaceae) is a perennial shrub, frequently found in humid and shady areas of Brazil in the southern, southeast and midwestern regions. It is also known by the synonyms *S. acuta* Burm., *S. ulmifolia* Mill., *S. acuta* var.*carpinifolia* (L.f.) K. Schum, and *S. frutescens* Cav. The plant is an erected perennial shrub 30-70 cm high. The leaves are alternate and short-peciolate, with an upper glabrous surface and a lower surface with sparse hair over the veins. The flowers are distributed singly or in small clusters and

are yellow with seven or eight carpels. The ingestion of *S. carpinifolia* induces an intracellular storage of oligosaccharides caused by indolizidine alkaloid (swainsonine),[10] which causes inhibition of lysosomal α-mannosidase and α-mannosidase II from Golgi complex,[18] affecting typically neurons.[17] It is a chronic disease with clinical signs characterized by neurological disturbances. The toxicosis is also associated with abortions and stillbirths.[10]

Several species of grazing livestock and wildlife such as goats,[10] sheep, [22] cattle,[11] horses[15] and fallow deer[19] have been described to be affected by *S. carpinifolia* poisoning.

Lysosomal storage diseases, both genetic and acquired, are uncommon in horses, but there are reports of inherited neuronal ceroid lipofuscinosis[23] and acquired cases of poisoning by *Astralagus* spp. and *Oxytropis* spp. in North America and *Swainsona canascens* var *horniana* in Australia.[14]

The clinical findings in ponies naturally poisoned by *S. carpinifolia* were described[15] and include stiff gait, general muscular tremors, colic, rolling, moans, recumbence and death 15-20 days after introduction into an area with large amounts of *S. carpinifolia*. The affected horses of this report had abnormal behavior with exacerbated reactions and difficult locomotion after induced movement, incoordination, stiff gait and aimless walking. Affected horses were frequently found dead trapped in fences or in dense bush areas.

It is possible that signs of colic in these cases result from neurogenic motility dysfunction of the large intestine due to lesion in the celiac ganglia and submucosal and myenteric plexuses.

Abnormalities in coordination and behavior are caused, mainly, by chronic neurodegenerative lesions triggered by the toxic active principle of the plant, which leads to oligosaccharides storage in the cytoplasm of cells from multiple tissues.[18]

Pathogenesis involves a deficiency of the lysosomal enzyme α-mannosidase, which is responsible for the catabolism of multiple glycoprotein portions; thus, it inducing the lysosomal storage of a wide range of oligosaccharides.[13] The disease induced by the plant toxic principle differs from the inherited condition, since the former causes also the inhibition of mannosidase II produced in Golgi complex in addition to inhibition of α-mannosidase.[5] Experimentally it has been shown that the clinical evolution and the clinical signs are related with the duration of ingestion and with the amount of the plant that was consumed.[10,22] The clinical course of the horses of this report was 90 days, different from what was described in ponies, where the toxicosis showed a peracute to acute course.

The necropsy findings in cases of *S. carpinifolia* poisoning are not significant.[10,11,22] In the current case, there was only moderate distension of large colon by fecal content, which was also described in ponies poisoned by *S. carpinifolia*.[15] Microscopic lesions of brain and spinal cord were mainly characterized by marked cytoplasmic vacuolization of neurons and astrocytes, with few necrotic neurons and axonal spheroids; moderate proliferation of Bergmann astrocytes in cerebellum, and moderate vacuolization of thyroid follicular epithelial cells and proximal convoluted tubular cells, and mild vacuolization of hepatocytes. These were similar to what has already been described in affected cattle,[11] sheep,[22] goats,[10] and ponies.[15] Others have

also described cytoplasmic vacuolization of acinar pancreatic epithelial cells.[10,11,19,22]

Lectins are proteins or glycoproteins of non-immune origin that bind reversibly to specific residues of carbohydrates (glycoproteins, glycolipids and glycosaminoglycans).[10] The lectin histochemistry analysis may detect certain substances in a tissue where a lesion has occurred and helps to establish a correlation between its presence, quantity, severity and extension of the process. The efficiency of this analysis has been already demonstrated several studies through the detection of complex substances containing sugars, related or not with storage diseases, such as glycoproteinosis[10] and glicolipidoses.[1] The brain sections of the horse of this report submitted to this type of analyses revealed moderate to marked cytoplasmic stain in neurons when *Triticum vulgaris* (*WGA*) and *Succinyl Triticum vulgaris* (*sWGA*) were employed which indicates the expression of ß-D-N-acetylglucosamine and acetylneuraminic acid. Similar observations were made when *Concanavalia ensiformis* (*Con-A)* was employed, which is a specific lectin for α-D-mannose α-D-glucose. The mild to moderate stain for *UEA I*, *PNA* and *SJA* lectins observed in the horse of this report has been described in goats poisoned by the same *S. carpinifolia*, as well as in tissues without lesions.[10] IHC anti-S100 has highlighted the increasing number of Bergmann astrocytes in cerebellum, in addition to a higher expression of GFAP. The Bergmann's glia is a specialized population of astrocytes that occurs in the Purkinje cell layer of cerebellum.[4] The increased numbers of Bergmann astrocytes has been described in cases of primary loss of Purkinje cells with secondary depletion of the molecular and granular layers and in poisonings by plants containing swainsonine.

**Contributing Institution:**
Setor de Patologia Veterinária, UFRGS, http://www.ufrgs.br/patologia/

**JPC Diagnosis:**
Diencephalon with hippocampus and thalamus, neurons and glia: Cytoplasmic vacuolation, diffuse, marked with rare neuronal necrosis, satellitosis, and spheroids.

**JPC Comment:**
The contributor provides a thorough review of the pathogenesis of chronic swainsonine ingestion, as seen in this case associated with broomweed (*Sida carpinifolia*) ingestion.

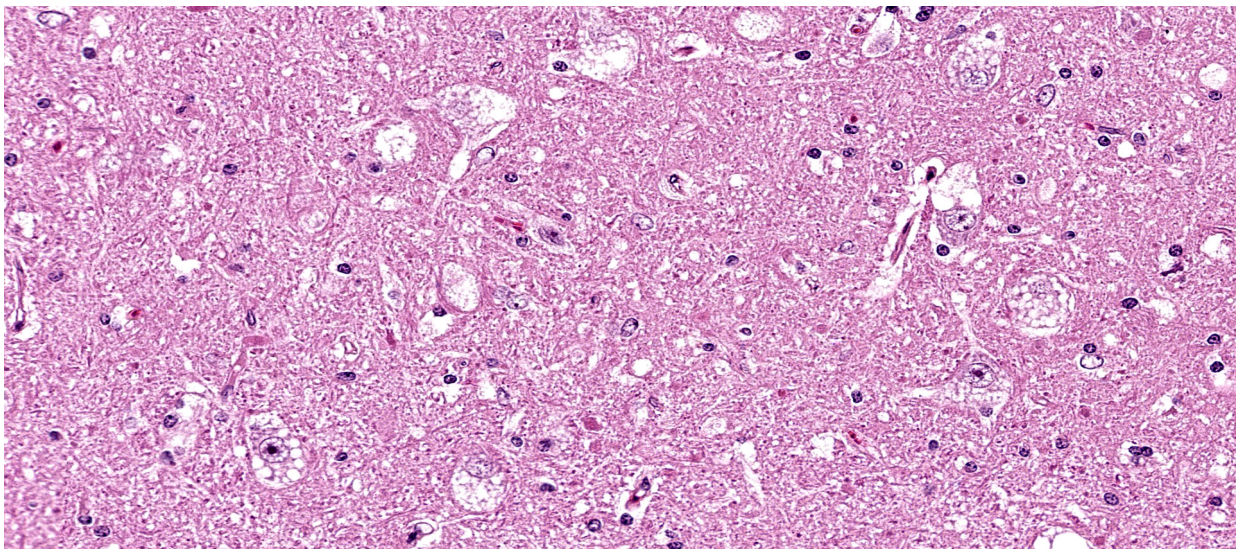

**Figure 1-2. Diencephalon, horse. Neurons are diffusely swollen with abundant vacuolated cytoplasm. (HE, 400X)**

Molecularly similar to the simple sugar mannose, swainsonine inhibits both lysosomal enzyme α-mannosidase and golgi mannosidase II, resulting in an inability of affected cells to process mannose rich oligosaccharides, which subsequently accumulate in lysosomes and cause cellular dysfunction.[6,20]

As noted by the contributor, swainsonine-containing plants naturally grow on several continents and poison significant numbers of livestock. In the United States, plant species in genera *Astralagus* and *Oxytropis* are colloquially known as locoweeds, with swainsonine commonly referred to as "locotoxin" and its associated neurologic clinical condition is known as "locoism". *Astralagus* and *Oxytropis* are both extensive genera, with 354 species and 198 varieties of *Astralagus* reported in the United States and Canada while there are 22 species and 35 varieties of *Oxytropis*.[20]

Identified as an issue by stockmen in the United States as early as 1873, locoism posed such a significant problem that a field station was established and dedicated to its study in Hugo, Colorado in 1905. Subsequent research lead to the publishing of a USDA bulletin titled "The locoweed disease of the plains" by C.D. Marsh in 1909. Interestingly, Marsh connected locoism with a similar condition in Australia known as "peastruck", which was associated with consumption of the Darling Pea (*Swainsonia* spp.). Seventy years later, Colgate *et. al.* isolated and described the toxin indolizidine alkaloid swainsonine from *Swainsonia canescens* in Australia. The toxin was subsequently isolated from *Astralagus* and *Oxytropis* in 1982.[20]

In Brazil, two recent studies have described antemortem techniques in regard to the presumptive diagnosis of swainsonine toxicity.[6,21] The first evaluated blood smears from guinea pigs fed 50 grams of *I. marcellia* per day. A repeatable finding after only five days of ingestion was the cytoplasmic vacuolation of lymphocytes in blood smears, predominantly at the periphery of the cell.[12] Another study evaluated the usefulness of percutaneous hepatic biopsies using Menghini needles in goats fed 4g/kg/day of dried *I. marcellia* (0.8mg/kg/day swainsonine). Hepatocellular vacuolization consistent with lysosomal storage disease was identified in all affected goats starting at day seven. Sections were subsequently labelled using lectin histochemistry staining with *Concanavalia ensiformis* (CON-A) and *Triticum vulgaris* (WGA).[21]

Swainsonine producing fungal symbionts, such as *Undifilum* spp., have been found to be associated with multiple plant species associated with swainsonidosis, including *Astragalus mollissimus, Oxytropis lambertii,* and *O. sericea* in North America[4], *Astragalus* and *Oxytropis* in China, and *S. canescens* in Australia.[5] Furthermore, swainsonine content has been found to correlate with the degree of endophyte infection, suggesting endophytes at least partially influence the degree of toxicity.[4]

**References:**


1. Alroy J., Ucci A.A., Goyal V. & Woods W. 1986. Lectin Histochemistry of Glycolipid Storage Diseases on Frozen and Paraffin-Embedded Tissue Sections. J. Histochem. Cytochem. 34(4):501-505.
2. Armién AG, Tokarnia C.H, Peixoto P.V et al. Spontaneous and experimental glycoprotein storage disease of goats induced by *Ipomoea carnea* subsp. fistulosa (Convolvulaceae). Vet. Pathol. 2007; 44:170-184.
3. Barbosa RC, Riet-Correa F, Medeiros RM, et al. Intoxication by *Ipomoea sericophylla* and *Ipomoea riedelii* in

goats in the state of Paraiba, northeastern Brazil. Toxicon 2006; 47: 371-379.

4. Braun K, Romero J, Liddell C, Creamer R. Production of swainsonine by fungal endophytes of locoweed. *Mycol Res*. 2003;107(Pt 8):980-988.
5. Cantile C, Youssef S. Storage diseases, In: *Jubb, Kennedy & Palmer Pathology of Domestic Animals,* ed. Maxie MD 6th ed., vol. 1. pp. St. Louis, MO: Elsevier; 2016: 284-293.
6. Cholich LA, Martinez A, Micheloud JF, et al. Alpha-mannosidosis caused by toxic plants in ruminants of Argentina. *An Acad Bras Cienc*. 2021;93(suppl 3):e20191496. Published 2021
7. Cook D, Gardner DR, Pfister JA. Swainsonine-containing plants and their relationship to endophytic fungi. *J Agric Food Chem*. 2014;62(30):7326-7334.377.
8. Dantas AF, Riet-Correa F, Gardner DR, et al.: 2007, Swainsonine-induced lysosomal storage disease in goats caused by the ingestion of *Turbina cordata* in Northeastern Brazil. Toxicon 49:111-116.
9. Dorling PR, Huxtable CR, Colegate SM, Inhibition of lysosomal alpha-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens.* Biochem J. 1980; 191:649–651.
10. Driemeier D, Colodel EM, Gimeno EJ, Barros SS. Lysosomal storage disease caused by *Sida carpinifolia* poisoning in goats. Vet Pathol. 2000; 37:153-159.
11. Furlan FH, Lucioli J, Veronezi LO et. al. Spontaneous lysosomal storage disease caused by *Sida carpinifolia* (Malvaceae) poisoning in cattle. Vet Pathol. 2009; 46: 343-347.
12. García EN, Aguirre MV, Gimeno EJ, Rios EE, Acosta OC, Cholich LA. Haematologic alterations caused by Ipomoea carnea in experimental poisoning of guinea pig. *Exp Toxicol Pathol*. 2015;67(10):483-490.
13. Jolly RD & Walkley SU. Lysosomal storage diseases of animals: an essay in comparative pathology. Vet Pathol. 1997; 34: 527-548.
14. Locker KB, McEwan DR, Hamdorf IJ.. Experimental poisoning of horses and cattle with *Swainsona canascens var horniana*. Aust Vet J. 1980; 56:379-383.
15. Loretti AP, Colodel EM, Gimeno EJ, Driemeier D. Lysosomal storage disease in *Sida carpinifolia* toxicosis: an induced mannosidosis in horses. Equine Vet J. 2003; 35:434-438.
16. Mendonça FS, Albuquerque RF, Evêncio-Neto J.et al. Alpha-mannosidosis in goats caused by the swainsonine-containing plant *Ipomoea verbascoidea*. J Vet Diagn Invest. 2012;24:90-95.
17. Molyneux RJ, James LF. Loco intoxication: Indolizidine alkaloids of spotted *locoweed* (*Astragalus lentiginosus*). Science. 1982; 216:190-191.
18. Moremen KW. Golgi α-manosidase II deficiency in vertebrate systems: implications for asparagines-linked oligosaccharide processing in mammals. Biochem Biophys Acta. 2002; 1573:225-235.
19. Pedroso PMO, Von Hohendorf R, Oliveira LGS, et al. *Sida carpinifolia* (Malvaceae) poisoning in fallow deer (*Dama dama*). J. Zoo Wildl. Med. 2009; 40:583-585.
20. Panter KE, Gardner DR, Lee ST, Pfister JA, Ralphs MH, Stegelmeizer BL, James LF. Important poisonous plants of the United States. In: Gupta RC, ed. *Veterinary Toxicology Basic and Clinical Principals.* New York, NY: Elsevier; 2007: 826-833
21. Rocha BP, Reis MO, Driemeier D, Cook D, Camargo LM, Rietcorrea F and

Mendonca FS. 2016. Biopsia hepatica como metodo diagnostico para intoxicacao por plantas que contem swainsonina. *Pesq Vet Bras*. 2016;36(1): 373-377.
22. Seitz AL, Colodel EM, Barros SS et. al. Intoxicação experimental por *Sida carpinifolia* (Malvaceae) em ovinos. [Experimental poisoning by *Sida carpinifolia* (Malvaceae) in sheep.] Pesq. Vet. Bras. 2005; 25:15-20.
23. Url A, Bauder B, Thalhammer J, et al. Equine neuronal ceroid lipofuscinosis. Acta Neuropathol. 2001;101:410-414.

**CASE II**: 21-031340 (JPC 4166761)

**Signalment:**
60-day-old, intact male, Katahdin, *Ovis aries*, ovine

**History:**
Day 1: Lamb started spinning in circles, ear down, and exhibited loss of balance. Day 2: Condition worsened to the point lamb could not get up. Day 3: Lamb was found dead.

**Gross Pathology**:
The lamb is in thin body condition based on thin skeletal musculature and fair subcutaneous and visceral adipose tissue stores. Postmortem autolysis is mild.

Two retropharyngeal lymph nodes at the level of the atlanto-occipital junction are symmetrically and diffusely enlarged, each measuring approximately 1.0 cm in diameter, as well as red-tinged with a dark red to black medulla.

**Laboratory Results:**
Culture of brainstem- moderate numbers of *Listeria monocytogenes.*

**Microscopic Description:**
Brainstem: Affecting gray and white matter, bilaterally and asymmetrically, with more pronounced involvement of the left half of the brainstem, are multifocal to coalescing aggregates of viable and degenerate neutrophils with variable numbers of histocytes and gitter cells (microabscesses), and occasional aggregates of glial cells (glial nodules). Within these areas, myelin sheaths are frequently dilated with swollen and hypereosinophilic axons (spheroids), and/or with an influx of neutrophils and necrotic cellular debris that effaces the normal parenchyma. Rarely, neuronal cell bodies are shrunken, rounded and hypereosinophilic with pyknotic nuclei (necrosis). The affected neuropil is extensively rarified (malacia) with variable edema. Blood vessels are often surrounded by small to moderate numbers of lymphocytes, plasma cells, macrophages, and neutrophils (perivascular cuffing) that fill Virchow-Robbins space. The meninges are segmentally infiltrated by moderate numbers of lymphocytes and plasma cells, with fewer macrophages and neutrophils.

**Contributor's Morphologic Diagnoses:**
Brainstem: Severe, subacute, multifocal to coalescing, necrosuppurative and lymphoplasmacytic meningoencephalitis, with malacia, Wallerian and myelin degeneration, microabscessation and perivascular cuffing.

**Contributor's Comment:**
Collectively, the clinical history, lack of gross findings, and histopathologic changes are classic for *Listeria monocytogenes* (LM) as the cause of clinical signs and death in this lamb. LM is a gram-positive, facultative anaerobic, coccobacillus to bacillus bacterium, that is ubiquitous and durable in the environment, virtually worldwide.[1,3-8,10,13] It is commonly isolated from healthy tissues and feces of ruminant animals. LM is an intracellular pathogen of leukocytes, partic-

ularly macrophages, and epithelial cells. Surface protein internalins (A and B) facilitate internalization of the bacterium, by interacting with E-cadherin to pass through the intestinal, placental and blood-brain barriers. The cholesterol-binding hemolysin, Listeriolysin-O, allows LM to escape phagosomes so they can then co-opt the host cell contractile actin to facilitate cell-to-cell transfer.[1,3-8,10,14]

There are 6 species of *Listeria*, 2 of which are considered pathogenic: LM and *L. ivanovii*. There are multiple serotypes, which fall under 3 lineages, the most pathogenic being 1/2a, 1/2b and 4b. Serovars 1 and 4b are most commonly isolated from cattle, while serovars 4b and 5 are most commonly identified in sheep. Serovar 5 is specific for *L. ivanovii*, which infrequently causes abortion, particularly in sheep.[1,3-8,10]

The most common syndrome of listeriosis is encephalitis. Colloquially known as "circling disease," it is almost solely observed in adult ruminants as a sporadic disease or, less commonly, in outbreak situations. Outbreaks are generally associated with feeding contaminated silage, typically during winter and spring.[1,8,10] While the exact pathogenesis has not been definitively described, the most widely accepted rationale, is that the bacterium enters oral mucosal epithelium through wounds, infects the trigeminal nerve branches or other regional cranial nerves, and centripetally travels via axons to the brain. This route explains why the medulla and pons are most severely affected, with less involvement of the thalamus, hippocampus, and cervical spinal cord. Clinical signs include confusion, depression, head pressing, and deviation of the head to one side or the other without rotation (causing the animal to walk in circles, hence the name "circling disease"), unilateral drooping of the ear, eyelid and lips due to paralysis of the 7th cranial nerve, and possible drooping of the masticatory muscles and pharynx. The clinical course spans from a few hours up to 2 days, and rare survivors have permanent neurological deficits. Associated lesions may include rhinitis and unilateral purulent endophthalmitis. At necropsy, there are typically no gross lesions, though in severe cases, the medullary meninges may be thickened with green-colored edema and multifocal brainstem malacia is possible. Histologically, the primary lesion is microabscessation of the brainstem with glial nodules, accompanied by perivascular cuffing, acute vasculitis, and fibrin. White matter is often edematous and rarefied. Meningitis, if present, is due to local spread of the infection.[1,3-8,10,13-14]

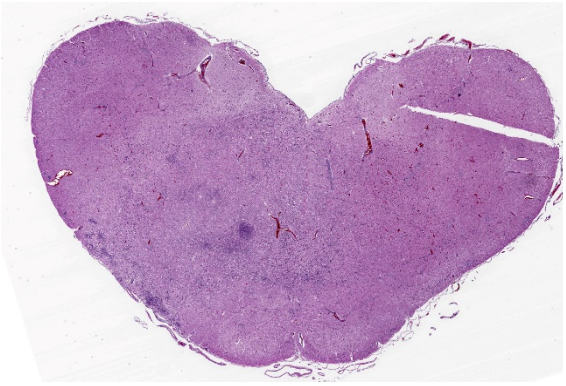

**Figure 2-1. Brainstem, sheep. There are multifocal foci of hypercellularity scattered throughout the section. (HE, 8X)**

Other syndromes include abortion, septicemia, conjunctivitis, endocarditis, and mastitis.[1,8] Infrequently, encephalitis may occur simultaneously with other syndromes, particularly abortion and septicemia, within a herd, but not within the same animal.[5,10] LM has an affinity for the gravid uterus, via suspected hematogenous spread from alimentary or intravenous inoculation of the dam, who may or may not show clinical signs of septicemia before abortion.[1,2,5,8-9] The incubation period is variable (days to weeks), typically causing late-term abortion.

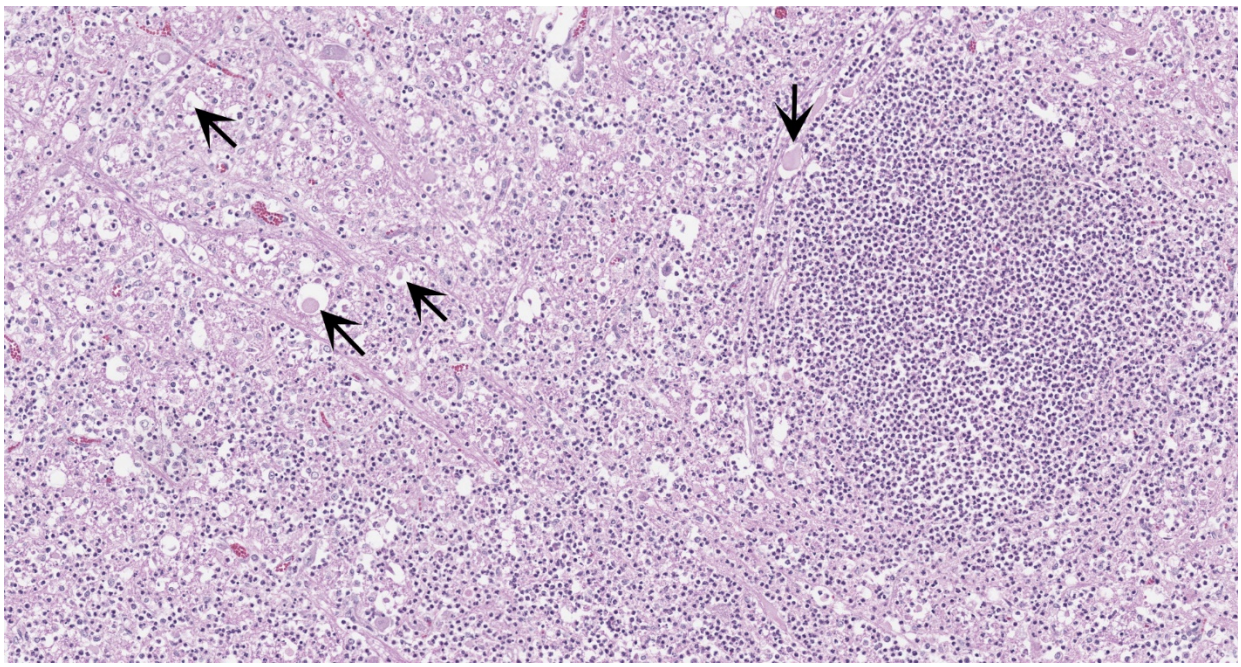

**Figure 2-2. Brainstem, sheep. Areas of rarefaction contain large numbers of viable and degenerate neutrophils, occasionally forming microabscesses (right). There are scattered dilated myelin sheaths containing axonal debris and spheroids (arrows) scattered through malacic areas. (HE, 141X)**

In the early part of the last trimester, LM infects the fetus, causing fetal septicemia and death. The dam typically aborts in 5-13 days.[2,7-9] This means the fetus is typically and markedly autolytic, which precludes evaluation of any gross lesions. The placenta is usually retained due to mild metritis, but LM is typically not cultured. If infected in the late part of the last trimester, the dam will undergo dystocia, often accompanied by severe metritis and septicemia. In near term infections, severe, diffuse, non-suppurative cerebrospinal meningitis is possible. Gross fetal lesions may include subcutaneous edema, hydrothorax, hydroperitoneum; enlarged, pale, bronze-red, friable liver with numerous, small necrotic foci; small abomasal erosions; increased amounts of yellow-orange, mucoid meconium; and enlarged mesenteric lymph nodes.[2] In bovine fetuses, there may also be severe necrotizing colitis, despite autolysis. Possible placental lesions include severe, necrosuppurative, cotyledonary placentitis; local to diffuse, yellow to red/brown exudative intercotyledonary placentitis with edema, and vasculitis.[2,8-9]

Septicemia syndrome is typically only observed in aborted fetuses and neonates, infected *in utero*. Multisystemic colonization is observed with coagulative necrosis or miliary microabscessation of the liver, as well as the heart and other viscera to a lesser degree.[2,8-9]

Conjunctivitis syndrome is suspected to be caused by contaminated dust in the eye.[8] Acute, suppurative myocarditis with abscessation and possible bacterial emboli, is the hallmark of the endocarditis syndrome.[8] Mastitis occurs most frequently in cattle, and ranges from subclinical to severely suppurative. Infection can be difficult to clear, resulting in intermittent shedding.[1]

Definitive diagnosis of listeriosis can be obtained via culture of fresh tissue (brain, aborted placenta, and fetus), fluids and swabs (cerebrospinal fluid, nasal discharge, urine,

feces, and milk). Cytology of cerebrospinal fluid from a lumbosacral tap will have increased protein (0.6-2.0 g/L) and mild pleocytosis. Immunofluorescence can be performed on smears from necropsy specimens, milk and meat, and may be helpful for fast diagnosis in public health situations. Serology is generally unhelpful, as many animals have high titers due to frequent environmental exposure.[11]

Listeriosis is an uncommon, but extremely important and well-documented food-borne illness of humans.[10,13] The encephalitis syndrome is suspected to be due to hematogenous spread rather than axonal migration.[3,7,10]

LM has been isolated from numerous animal species including birds, reptiles, and ticks.[14] Clinical disease has been described in horses, pigs, dogs and cats, though incidence is rare.[1,8,14]

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**JPC Diagnosis:**
Brainstem: Rhombencephalitis, necrosuppurative, multifocal to coalescing, severe, with numerous microabscesses and moderate lymphohistiocytic meningitis.

**JPC Comment:**
The contributor provides an excellent review of *Listeria monocytogenes* and its multiple associated syndromes. E.G.D Murray is credited with initially isolating this entity from the blood of laboratory animals in 1924. Unable to assign the organism to known genera at the time, he identified the new agent as *Bacterium monocytogenes*. The genus was later renamed *Listeria* by Pirie in 1940.

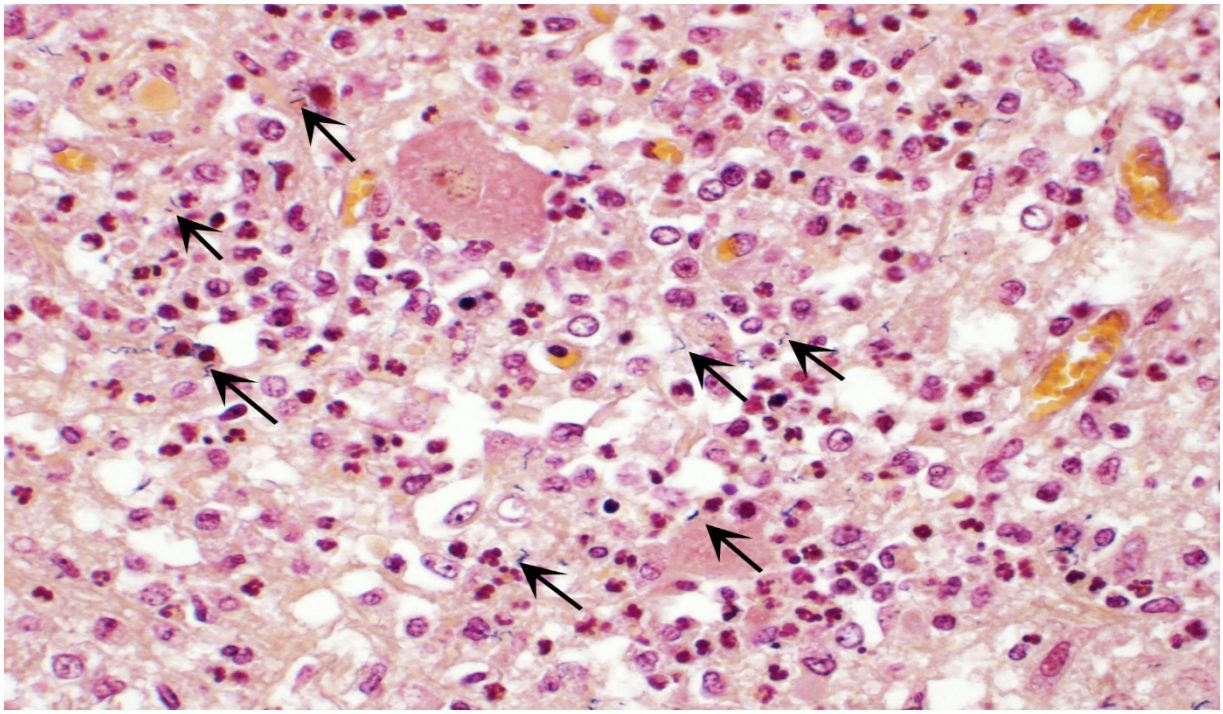

**Figure 2-3. Brainstem, sheep. There are small gram-positive rods (arrows) scattered throughout malacic areas. (Brown-Brenn, 600X)**

Although commonly isolated from humans, animals, food, and the environment, *L. monocytogenes* wasn't fully recognized as a pathogen until an epidemic of listeriosis in newborns (granulomatous infantiseptica), occurred in Germany in 1949. Histologic examination revealed granulomas within multiple tissues, including liver, spleen, brain and lung while bacteria were cultured from the meconium, blood, and other organs. Investigators initially identified *Corynebacterium infantisepticum* as the etiologic agent. However, H.P.R Seeliger shortly thereafter found the bacteria to be motile, inconsistent with bacteria of the *Corynebacterium* genus, and identified *Listeria* as cause of the outbreak.[6]

Capable of growth within both the environment and the extracellular space within the host, *L. monocytogenes* is also able to penetrate and replicate within virtually every nucleated cell. Following its release from the phagosome as the result of hemolysin and phospholipases as described the contributor, the organism utilizes the cell's cytoskeleton for intracellular movement via actin polymerization, primarily at the organism's apical aspect. This movement facilitates contact with the cell membrane, from which *L. monocytogenes* is then extruded as a membrane bound extracellular vesicle. These vesicles shelter the organism from the host's immune system and subsequently undergo endocytosis by the next target cell, completing its cell-to-cell transmission.[6]

Listeriosis in ruminants is classically associated the consumption of poor quality silage, a popular winter feed option utilized by producers formed under anaerobic conditions as the result of natural lactic acid fermentation. *L. monocytogenes* is often present, though in low numbers, in grass used for silage production. When properly performed,

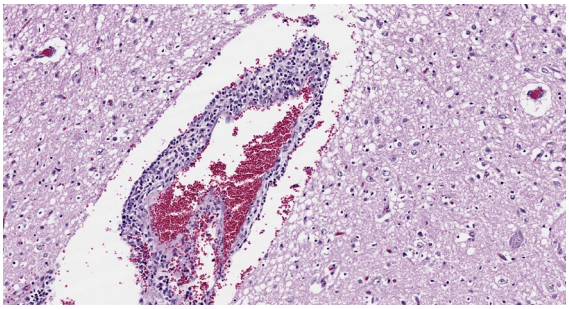

**Figure 2-4. Brainstem, sheep: Virchow-Robins spaces and the overlying meninges are expanded by a mixed inflammatory cell population. (HE, 198X)**

the silage fermentation process has an anti-listerial effect, largely as the result of organic acids. Both the initiation and maintenance of an anaerobic environment are essential for the production of suitable silage. This was demonstrated in a study that investigated the impact of both silage quality as well as a range of oxygen concentrations (0%, 0.1%, 0.5%, 1.0%, and 5%) on the inhibition of *L. monocytogenes* during the fermentation process.[4] With the exception of poor quality late season grasses, silage exposed to ≤ 5% oxygen underwent rapid acidification to a pH of <5. This drop in pH was maintained when oxygen concentrations were maintained between 0-0.1% whereas silage exposed to higher oxygen concentrations exhibited a subsequent increase in pH that corresponded with the recovery of *L. monocytogenes*, though at varying levels correlating with the increase in pH. With the exception of one sample of very poor quality late season grass, *L. monocytogenes* survival under strict anaerobic conditions was inhibited by a pH of <4.4. Prolonged survival was noted at 0.5% oxygen and growth readily occurred at higher oxygen tensions of 1-5%. Of note, the presence of moldy silage may be a useful indicator of *L. monocytogenes* contamination as mold growth in microaerobic silage corresponded well to the presence of *L. monocytogenes*.[4]

As noted by the contributor, listeriosis has been reported in other species, including rare

reports in companion animals. Sources of potential exposure that have become increasingly popular include raw meat-based diets. This was highlighted in 2018 study in Belgium that evaluated 35 commercial frozen raw-meat based diets from eight different brands. *Listeria monocytogenes* was isolated from 54% of products. Additional pathogens identified included *Escherichia coli* serotype O157:H7 in 23%, *Salmonella* species in 20%, and *Sarcocystis cruzi* in 11%.[12]

**References:**


1. Czuprynski CJ, Katharíou S, Poulsen K. Listeria. *Pathogenesis of Bacterial Infections in Animals*. 4th ed. Ames, IA: Wiley-Blackwell; 2010:167-187.
2. Dennis SM. Perinatal lamb mortality in Western Australia. *Australian Vet J*. 1975,51:75-79.
3. Disson O, Lecuit M. Targeting of the central nervous system by *Listeria monocytogenes*. *Virulence*. 2012;3(2):213-221.
4. Donald AS, Fenlon DR, Seddon B. The relationship between ecophysiology, indigenous microflora and growth of Listeria monocytogenes in grass silage. *J Appl Bacteriol*. 1995;79(2):141-148.
5. Dryer M, Thomann A, Bottcher S et al. Outbreak investigation identifies a single *Listeria monocytogenes* strain in sheep with different clinical manifestations, soil and water. *Vet Microbiol*. 2015;179:69-75.
6. Hof H. History and epidemiology of listeriosis. *FEMS Immunol Med Microbiol*. 2003;35(3):199-202.
7. Madarame H, Seuberlich T, Abril C et al. The distribution of E-cadherin expression in listeric rhombencephalitis of ruminants indicates its involvement in *Listeria monocytogenes* neuroinvasion. *Neuropath Appl Neurobiol.* 2011;37:753-767.
8. Maxie MG. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. St. Louis, MO: Elsevier; 2016.
9. Njaa BL. *Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals*. 4th ed. Ames, IA: Wiley-Blackwell; 2012:27-28, 71-72.
10. Oevermann A, Zurbriggen A, Vandevelde M. Rhombencephalitis Caused by *Listeria monocytogenes* in Humans and Ruminants: A Zoonosis on the Rise?. *Interdiscip Perspect Infect Dis*. 2010;2010:1-22.
11. Pritchard JC, Jacob ME, Ward TJ, Parsons CT, Katharíou S, Wood MW. Listeria monocytogenes septicemia in an immunocompromised dog. *Vet Clin Pathol*. 2016;45(2):254-259.
12. Scott PR. Overview of Listeriosis. Merck Veterinary Manual. https://www.merckvetmanual.com/generalized-conditions/listeriosis/overview-of-listeriosis. Updated March 2014. Accessed June 24, 2021.
13. van Bree FPJ, Bokken GCAM, Mineur R, et al. Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. *Vet Rec*. 2018;182(2):50.
14. Wagner W, Melzner D, Bago Z et al. Outbreak of Clinical Listeriosis in Sheep: Evaluation from possible Contamination Routes from Feed to Raw Produce and Humans. *J Vet Med B*. 2005;52:278-283.
15. Zachary JF, Huff TG, Britton R. *Pathologic Basis of Veterinary Disease*. 6th ed. St. Louis, MO: Elsevier; 2017:186-189.

**CASE III**: 265/0912 (JPC 4165419)

**Signalment:**
A two- month-old, male, Canary Black Pig (*Sus scrofa domesticus*).

**History:**
A litter of pigs exhibited weight loss and severe dyspnea. All animals died and one was submitted to our diagnostic laboratory for a complete necropsy. The animals belonged to a swine farm with no vaccination plan.

**Gross Pathology**:
The animal was in poor body condition. The cerebellum showed abundant petechial and ecchymotic hemorrhages in the meninges and parenchyma, affecting mainly the white matter.

The lungs were diffusely firm with moderate interlobular septal edema. Cranioventral portions were patchy dark red. There was generalized lymphoadenomegaly. Additionally, mild hydropericardium, ascites, fibrinous pleuritis and multifocal hepatic fibrosis (consistent with *Ascaris suum* larvae migration) were observed.

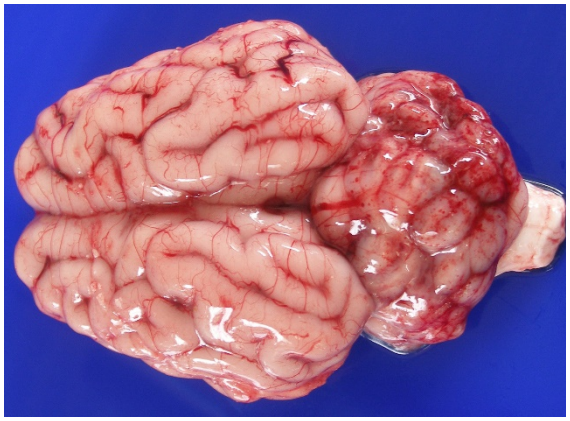

**Figure 3-1. Brain, pig. There are multifocal to coalescing hemorrhages covering the surface of the cerebellar folia and vermis. (*Photo courtesy of:* Unit of Veterinary Histology and Pathology. University Institute of Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria. http://iusa.ulpgc.es/)**

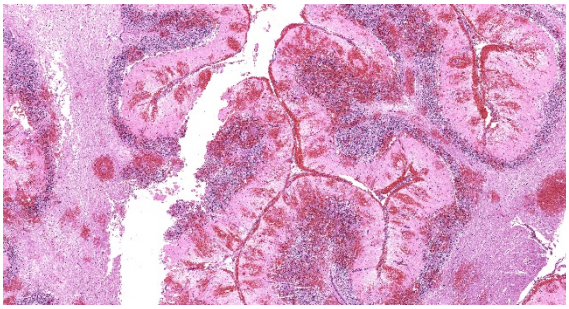

**Figure 3-2. Cerebellum, pig. There are multifocal to coalescing hemorrhages within all layers of the cerebellum and the overlying meninges. (HE, 33X)**

**Laboratory Results:**
Immunohistochemistry against PCV2 and PRRSV antigens was performed on brain, lung and lymph node tissue sections.

In the cerebellum, immunoreaction against PCV2 was observed in the cytoplasm and nuclei from intralesional perivascular macrophages and endothelial-like cells.

Immuno-labelling was also seen in alveolar macrophages, lymphocytes and syncytial cells in the lung, and in histiocytes, lymphocytes and occasional multinucleated giant cells in lymph nodes.

No PRRSV antigen was detected in any sample.

**Microscopic Description:**
Cerebellum: affecting the leptomeninges and both the grey and white matter there are numerous multifocal to coalescing and variably sized areas of hemorrhage, frequently centered on small caliber blood vessels. The wall of these vessels shows loss of cellular detail with karyorrhexis and karyolysis, and is replaced by eosinophilic amorphous material and cellular debris (fibrinoid necrosis). Occasionally, fibrin thrombi occlude the lumen. Around less affected blood vessels, the Virchow-Robin space is expanded by a mild infiltrate of lymphocytes, plasma cells, macrophages and rare eosinophils (perivascular cuffing), which

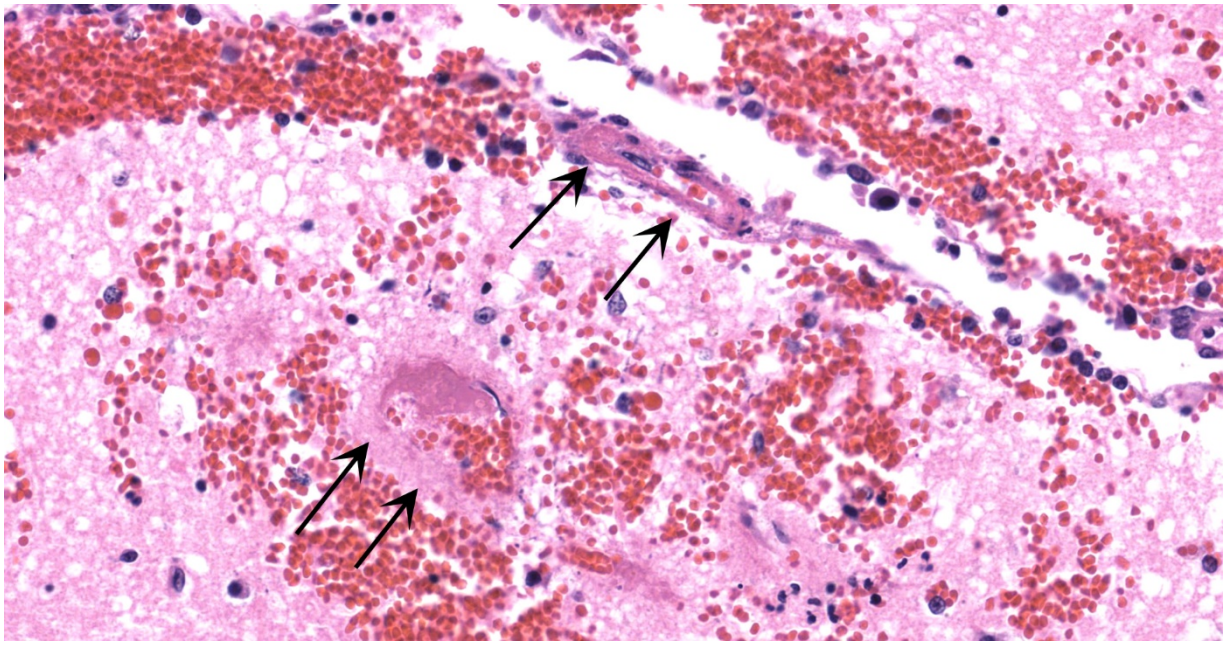

**Figure 3-3. Cerebellum, pig. Within the meninges and cerebellar parenchyma, there is vascular necrosis and thrombosis. The surrounding white matter of the granular cell layer is spongiotic. (HE, 454X)**

occasionally invade the wall (vasculitis) and extend into the adjacent neuroparenchyma. Endothelial cells are hypertrophied and have plump and vacuolated nuclei (reactive). Multifocally, the neuropil adjacent to affected areas shows moderate rarefaction. Diffusely, glial cells are mildly increased in number (gliosis).

**Contributor's Morphologic Diagnoses:**
Cerebellum and leptomeninges: Vasculitis, lymphohistiocytic, multifocal, moderate, with fibrinoid necrosis, perivascular cuffing, hemorrhage, thrombosis and mild gliosis.

**Contributor's Comment:**
In addition to the lesions observed in the cerebellum, other significant histological findings were severe lymphoid depletion with histiocytic infiltrate in lymph nodes, Peyer's patches, spleen and thymus, severe lymphohistiocytic interstitial pneumonia, and mild to moderate lymphohistiocytic interstitial nephritis. The postmortem and histological findings in combination with immunohistochemistry were consistent with PCV2-systemic disease.

Circoviruses (family Circoviridae) are small, nonenveloped, single-stranded DNA viruses, whose genome is circularly arranged (hence the name). They are worldwide distributed and have been identified in mammals, birds, fishes and even insects.[2, 29] Four porcine circoviruses (PCVs 1-4) have been recognized so far, named in the order of discovery. Only PCV1, a contaminant in PK-15 cells, is considered non-pathogenic.[27,28]

In the early 90's a new disease, named postweaning multisystemic wasting syndrome (PMWS), was described in pigs in Canada.[8] The disease was characterized by poor weight gain, wasting, dyspnea, pallor, diarrhea and jaundice. Few years later, a new porcine circovirus, different from the one of PK-15 cells (PCV1), was isolated from pigs with PMWS.[1,5,10,13] Since then, PCV2 has been associated with many other syndromes in pigs, and is now one of the most widespread viral infections in swine

husbandry, causing significant economic losses.

PCV3 and PCV4 have been discovered and isolated just recently, and the available information on their distribution, prevalence, and pathogenicity is still limited and sometimes controversial.[15,16,19,20,33]

PCV2 is characterized by a high nucleotide substitution rate, thus having the highest genetic variability among single-stranded-DNA viruses. At present, eight different PCV2 genotypes have been identified (PCV-2a to PCV-2h) and more are likely to appear in the future.[16] PCV2a, PCV2b and PCV2d are the most common ones. Recently, genotype prevalence has switched from PCV2b to PCV2d. [9]

All PCVs share two main open reading frames: ORF1 (rep gene) encoding proteins associated with replication, and ORF2 (cap gene) which encodes the capsid protein, the main antigenic determinant of the virus.[16] The ORF3 gene, present on PCV2 genome, encodes a protein which is thought to induce apoptosis of infected cells.[9]

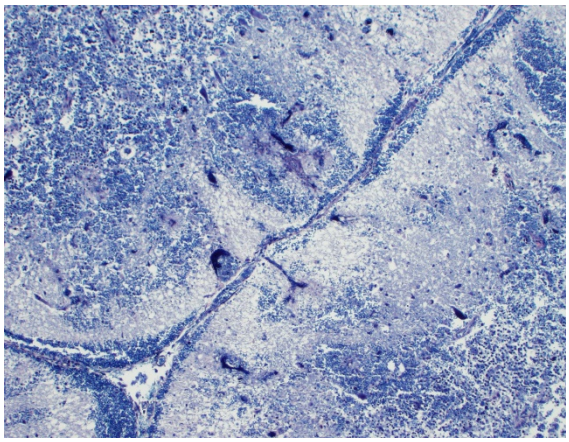

**Figure 3-4. Cerebellum, pig. A phosphotungstic acid hematin (PTAH) stain demonstrates the fibrin thrombi occluding vessels in areas of granular layer hemorrhage and necrosis. (PTAH, 200X)**

Infection with PCV2 occurs mainly in domestic pigs, but it also happens in feral pigs and wild boars.[4,6,11,30] The virus is transmitted both vertically and horizontally via inhalation/ingestion of fomites from oronasal-pharyngeal body fluids, feces, and urine.[14, 18] PCV2 uses a viral capsid attachment protein to bind to heparin sulfate and chondroitin sulfate B glycosamino-glycans on host cells. It enters the cell by several pathways including clathrin-mediated endocytosis, caveolin-mediated endocytosis, actin and Rho-GTPase [21, 32]. Viral DNA replication relies mainly on the DNA replication machinery of the host cells, since the small viral genome has a very limited coding capacity.[21] Cellular tropism seems to change depending on the age of the animal: in fetuses, PCV2 is found in cardiomyocytes, hepatocytes and cells of the monocytic lineage, whereas after birth it shows predilection for lymphoblasts and macrophages.[24] In vitro studies demonstrated that B lymphocytes are one of the most important cell population for viral replication.[31] PCV2 is able to persist but not replicate in dendritic cells, which are probably used as a vehicle for spreading.[7] As PCV2 directly targets the cells of the immune system, pigs develop severe immuno-suppression, which increases the risk of coinfections.

In most cases, infection is subclinical. Clinical forms manifest as a variety of syndromes, like systemic disease (which includes PMWS), respiratory disease, enteric disease, porcine dermatitis and nephropathy syndrome (PDNS), reproductive disease, encephalitis and congenital tremors.[3,17,18,26] Recently, severe granulomatous and necro-tizing myositis has been described for the first time in pigs with natural PCV2 infection.[12] All these syndromes have been grouped together under the name “Porcine Circovirus Associated Disease, PCVAD”.

In systemic forms, necrotizing arteritis, periarteritis, and fibrinoid vasculitis are seen in different organs, including the brain.[22] Neurological disease has rarely been reported with PCV2 systemic infection.[23,25] Symptoms include lethargy, ataxia, paddling, opisthotonos, nystagmus, and seizures. Gross lesions in the central nervous system consist mainly of hemorrhage of the leptomeninges and the grey and white matter of the cerebellum. Histologically, in addition to the fibrinoid degeneration and vasculitis, other reported lesions are apoptosis of endothelial cells, gliosis, lymphohistiocytic coroiditis and meningitis, spongiosis, and neuronal degeneration and necrosis.[23,25] Immunohistochemistry demonstrates cytoplasmic and nuclear labelling in intralesional perivascular macrophages and endothelial cells.

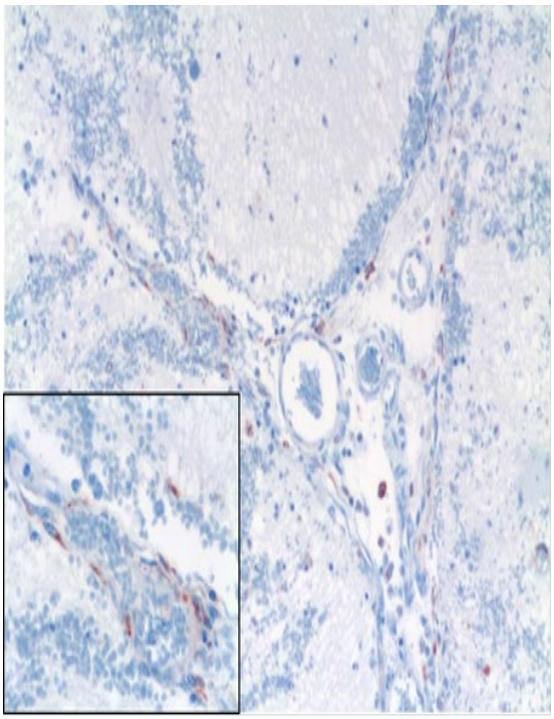

**Figure 3-5. Cerebellum, pig. PCV-2 antigen is present within macrophages within the cerebellar granular cell layer. (*Photo courtesy of:* Unit of Veterinary Histology and Pathology. University Institute of Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria. http://iusa.ulpgc.es/) (anti PCV-2, 400X)**

The presence of PCV2 within endothelial cells of the affected vessels suggests that the virus plays a role in the pathogenesis. However, as mentioned before, PCV2 is often associated with other agents, which could also contribute to the lesions. The presence of PCV2-labelled endothelial cells with increased activity of cleaved caspase-3 and apoptotic figures, suggests that a direct cytopathic effect of PCV2 may be possible.[22, 25] Vascular damage could also result from cytokine secretion by infected cells and recruited inflammatory cells or by deposition of immune complexes in the vessels wall.[22] In the study published by Seeliger and colleagues, no immune complexes were detected in the affected vessels.[25]

The reason why only a small percentage of animal develops disease, mechanisms beyond immunopathogenesis and the cause of persisting viremia are largely unknown. Both clinical and subclinical infections have an impact on porcine health and production. It is believed that factors related with the host (like age and genetics) as well as environmental factors play a role in the development of clinical forms.[18] The high viral load of infected pigs, the long-term shedding, and the resistance of the virus in the environment make PCV2 one of the most highly prevalent viruses in swine farms worldwide.

PCV vaccines are now the single most-selling prophylactic agents in porcine farming and appear to confer cross protection against genotypes 2a and 2b. Although efforts in vaccination have remarkably decreased the impact of clinical forms and improved production performances, the infection is still widespread among the vaccinated population. Furthermore, vaccines may not confer protection under conditions of repeated exposure and the

influence of other cofactors.[9] It has been shown that epitope variations, which have been identified between genotypes, can enable the virus to escape pre-existing immunity and have been implicated in apparent vaccine failure cases involving genotype 2d.[9]

**Contributing Institution:**
Unit of Veterinary Histology and Pathology. University Institute of Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria.
http://iusa.ulpgc.es/
https://hcv.ulpgc.es/web2/?page_id=3601

**JPC Diagnosis:**
Cerebellum: Vasculitis, fibrinonecrotizing, multifocal, severe, with multifocal hemorrhage, focally extensive necrosis, thrombosis, and mild lymphohistiocytic meningitis.

**JPC Comment:**
The contributor provides an outstanding review of PCV2. First identified in 1974 by Tischer et al., circoviruses are some of the smallest viruses known, measuring as little as 17 nm in diameter. Interestingly, evidence of PCV-2 has retrospectively been detected in archived tissues from as early as 1962 using in-situ hybridization and PCR.[16]

As previously mentioned by the contributor, PCV-2 specifically targets lymphoblasts and macrophages, with coinfection a common and expected feature as a result of immunosuppression. Furthermore, coinfection with porcine parvovirus, porcine reproductive and respiratory syndrome virus, and *Mycoplasma hyopneumonia* results in upregulation of PCV-2 replication, suggesting synergistic relationships between these infectious agents.[16]

Although circoviruses are typically regarded as host specific, PCV-2 and PCV-3 have both been detected in non-porcine hosts, indicating potential for cross-species infection. Natural infection with PCV-3 has been identified in domestic and wild pigs, as well as dogs, cattle, wild ungulates, and laboratory mice. Furthermore, PCV-3 was detected for prolonged periods of time in experimentally infected baboons.[16]

In addition to pigs, multiple avian species are affected by pathogenic members of genus *Circovirus* in the Circoviridae family, including psittacine beak and feather disease virus (BFDV), pigeon circovirus (PiCV), and goose circovirus (GoCV). Histopathologic features include intracytoplasmic botryoid inclusions of macrophages and depletion of both T and B lymphocytes. Chicken anemia virus, the only member of the closely related genus *Gyrovirus*, specifically targets hemocytoblasts and T lymphocytes, resulting in the T-lymphocytes depletion.[29] Clinical signs associated with infection bear resemblance to Postweaning Multisystemic Syndrome (PMWS) in pigs and include ill-thrift, lethargy, anorexia, poor performance, and increased mortality as the result of secondary infection.[29]

Although the majority of species affected by the *Circovirus* genera are avian, they do not include those of commercial significance. Particularly when compared to PCV-2, research interest toward these entities has been historically limited. However, additional discovery of disease mechanisms across these species may prove to be useful toward enhanced understanding and control of PCV-2.

**References:**
1. Allan GM, McNeilly F, Kennedy S, Daft B, Clarke EG, Ellis JA, Haines DM, Meehan BM, Adair BM. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and

Europe. J Vet Diagn Invest. 1998, 10, 3–10.
2. Blunt R, McOrist S, McKillen J, McNair I, Jiang T, Mellits K. House fly vector for porcine circovirus 2b on commercial pig farms. Vet Microbiol. 2011, 149, 452–455.
3. Choi J, Stevenson GW, Kiupel M, Harrach B, Anothayanontha L, Kanitz CL, Mittal SK. Sequence analysis of old and new strains of porcine circovirus associated with congenital tremors in pigs and their comparison with strains involved with postweaning multisystemic wasting syndrome. Can J Vet Res. 2002, 66, 217–224.
4. Dei Giudici S, Lo Presti A, Bonelli P, Angioi PP, Sanna G, Zinellu S, Balzano F, Salis F, Ciccozzi M, Oggiano A. Phylogenetic analysis of porcine circovirus type 2 in Sardinia, Italy, shows genotype 2d circulation among domestic pigs and wild boars. Infect Genet Evol. 2019, 71:189-196.
5. Ellis J, Hassard L, Clark E, Harding J, Allan G, Willson P, Strokappe J, Martin K, McNeilly F, Meehan B, Todd D, Haines D. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. The Canadian veterinary journal = La revue veterinaire canadienne. 1998, 39, 44–51.
6. Franzo G, Cortey M, de Castro AM, Piovezan U, Szabo MP, Drigo M, Segales J, Richtzenhain LJ. Genetic characterisation of Porcine circovirus type 2 (PCV2) strains from feral pigs in the Brazilian Pantanal: An opportunity to reconstruct the history of PCV2 evolution. Vet Microbiol. 2015, 178, 158–162.
7. Franzoni G, Graham S, Dei Giudici S, Oggiano A. Porcine Dendritic Cells and Viruses: An Update. Viruses. 2019, 11(5): 445.
8. Harding JC. The clinical expression and emergence of porcine circovirus 2. Vet Microbiol. 2004, 98, 131–135.
9. Karuppannan A and Opriessnig T. Porcine Circovirus Type 2 (PCV2) Vaccines in the Context of Current Molecular Epidemiology. Viruses. 2017, 9(5)
10. Kiupel M, Stevenson GW, Mittal SK, Clark EG, Haines DM. Circovirus-like viral associated disease in weaned pigs in Indiana. Vet Pathol. 1998, 35, 303–307.
11. Knell S, Willems H, Hertrampf B, Reiner G. Comparative genetic characterization of porcine circovirus type 2 samples from German wild boar populations. Vet. Microbiol. 2005,109, pp. 169-177.
12. Konradt G, Cruz RAS, Bassuino DM, et al.Granulomatous Necrotizing Myositis in Swine Affected by Porcine Circovirus Disease. Vet Pathol. 2018, 55(2):268-272.
13. Morozov I, Sirinarumitr T, Sorden SD, Halbur PG, Morgan MK, Yoon KJ, Paul PS. Detection of a novel train of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. J Clin Microbiol. 1998, 36, 2535–2541.
14. O'Connor B, Gauvreau H, West K. Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. Can Vet J. 2001, 42:551–553.
15. Oh T, Chae C. First isolation and genetic characterization of porcine circovirus type 3 using primary porcine kidney cells. Vet Microbiol. 2020, 241, 108576.
16. Opriessnig T, Karuppannanc A, Castrod A, Xiaoe C. Porcine circoviruses: current status, knowledge gaps and challenges. Virus Res. 2020, 286:198044.
17. Opriessnig T, Langohr I. Current state of knowledge on porcine circovirus type 2-associated lesions. Vet Pathol. 2013, 50, 23–38.

18. Opriessnig T, Meng XJ, Halbur PG. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest. 2007, 19, 591–615.
19. Palinski R, Pineyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM. A Novel Porcine Circovirus Distantly Related to Known Circoviruses Is Associated with Porcine Dermatitis and Nephropathy Syndrome and Reproductive Failure. J Virol. 2017, 91.
20. Phan TG, Giannitti F, Rossow S, Marthaler D, Knutson TP, Li L, Deng X, Resende T, Vannucci F, Delwart E. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. Virology journal. 2016, 13, 184.
21. Ren L, Chen X, Ouyang H. Interactions of porcine circovirus 2 with its hosts. Virus Genes. 2016, 52:437–444.
22. Resendes AR, and Segalés J. Characterization of Vascular Lesions in Pigs Affected by Porcine Circovirus Type 2–Systemic Disease. Vet Pathol. 2015, Vol. 52(3) 497-504.
23. Ribeiro Correa AM, Zlotowski P, Santos Neves de Barcellos DE, Farias da Cruz CE, Driemeier D. Brain lesions pigs affected with postweaing multisystemic wasting syndrome. J Vet Diagn Invest. 2007, 19:109-12.
24. Sanchez Jr RE, Meerts P, Nauwynck HJ, Pensaert MB. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life. Vet Microbiol. 2003, 95, 15–25.
25. Seeliger F, Brügmann M, Krüger L, Greiser-Wilke I, Verspohl J, Segalés J, Baumgärtner W. Porcine Circovirus Type 2-Associated Cerebellar Vasculitis in Postweaning Multisystemic Wasting Syndrome (PMWS)-Affected Pigs. Vet Pathol. 2007, 44:621–634.
26. Segales J. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res. 2012, 164, 10–19.
27. Tischer I, Rasch R, Tochtermann G. Characterization of papovavirus-and picornavirus-like particles in permanent pig kidney cell lines. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Erste Abteilung Originale. Reihe A: Medizinische Mikrobiologie und Parasitologie, 1974, 226, 153–167.
28. Tischer I, Mields W, Wolff D, Vagt M, Griem, W. Studies on epidemiology and pathogenicity of porcine circovirus. Arch Virol. 1986, 91, 271–276.
29. Todd D. Avian circovirus diseases: lessons for the study of PMWS. Vet Microbiol. 2004, 33:525–529.
30. Vicente J, Segales J, Hofle U, Balasch M, Plana-Duran J, Domingo M., Gortazar C. Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (sus scrofa). Vet. Res.2004, 35, pp. 243-253
31. Yu S, Opriessnig T, Kitikoon P, Nilubol D, Halbur PG, Thacker E. Porcine circovirus type 2 (PCV2) distribution and replication in tissues and immune cells in early infected pigs. Veterinary immunology and immunopathology. 2007,115, 261–272.
32. Zachary JF. Mechanisms of microbial infections. In: Pathologic Basis of Veterinary Disease, 6th ed. Zachary JF (ed) Elsevier, St. Louis, Missori, 2016, 219-220.
33. Zhang HH, Hu WQ, Li JY, Liu TN, Zhou JY, Opriessnig T, Xiao CT. Novel circovirus species identified in farmed pigs designated as Porcine circovirus 4, Hunan province. China. Transbound Emerg Dis. 2020, 67(3):1057-1061.

**CASE IV**: MW19-0525 (JPC 4152805)

**Signalment:**
6-year-old female zebra (*Equus zebra*)

**History:**
This zebra was born in Camp Verde, Arizona. It had a history of a dystocia in 2014 that appeared to cause neurologic problems including inability to stand and seizures that she appeared to have recovered from after 8 hours. However after the neurologic events, the zebra had some long term hind limb ataxia and seemed to have some pain after heat cycles. September 7 2017, the zebra showed signs of a head tilt, circling, pacing with front limb ataxia. The zebra was still eating and drinking; however, the front limb ataxia progressively worsened over 3 days. September 11, 2017 the zebra was sedated for laboratory work and physical examination. The physical exam was unremarkable with dirty ears with no ticks being the primary finding. Blood was collected (see below for ante mortem bloodwork results) and dexamethasone and IV fluids were administered. The zebra had a very prolonged recovery as she was unable to stand for 6 hours post sedation. The following day, the zebra showed mild improvement. However over the course of 7 days post-sedation, the zebra had worsening clinical signs of severe ataxia, circling, and head tilt that eventually progressed to leaning on the fence and walls. There was no vaccination history. The Zebra was euthanized on September 15, 2017 and was submitted for gross and histopathological examination.

**Gross Pathology**:
On external examination, there are locally extensive, erosive to ulcerated, flat, red to dark red areas on the skin of the supraorbital region, bilaterally. The skin of the right side is more affected than the left side. The underlying subcutis of the head has multiple large areas of subcutaneous hemorrhage and edema. The brain and the spinal cord are grossly unremarkable. Internal organs are within normal limits.

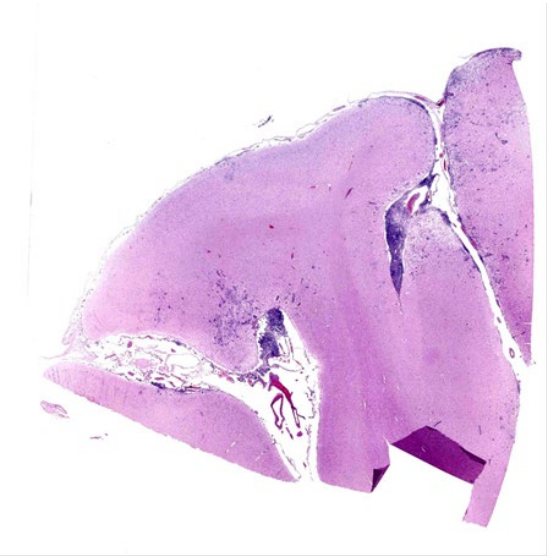

**Figure 4-1. Cerebrum, zebra. A section of cerebrum is submitted for examination. There is marked hypercellularity of the meninges and immediately subjacent, there is patchy hypercellularity and pallor of the neuropil. Virchow-Robins spaces are cuffed by multiple layers of inflammatory cells. (HE, 5X)**

**Laboratory Results:**
Complete blood count: Hct 38% (32.5-46.5%), WBC 10.3K/µL (4.3-11.4 K/µL), Neut 8.93 K/µL (2.46-7.23 K/µL), Lymph 1.082 K/µL (1.45- 5 K/µL), Monocyte 0.185 K/µL (0-0.6 K/µL), Eosinophil 0.093 K/µL (0-0.7 K/µL), Basophil 0.01 K/µL (0- 0.1 K/µL), Platelet 274 (70-250 K/µL)
Neutrophils appear slightly toxic per reviewer comments.
Fibrinogen: 400 mg/dL (100-400 mg/dL)

Chemistry: Glucose 55 mg/ dL (49-102 mg/dL),Creatinine 2.1 mg/dL (0.8-1.8 mg/dL), BUN 16mg/dL (11-25 mg/dL), Phosphorous 5.0 mg/ dL (2.0-4.8 mg/dL), Calcium 11.2 mg/ dL (10.2-12.8 mg/dL), Sodium 133 mmol/ L (132-141 mmol/L), Potassium 3.2 mmol/ L (2.5-5.2 mmol/L), Na:K ratio 42 (no range given), Chloride 95

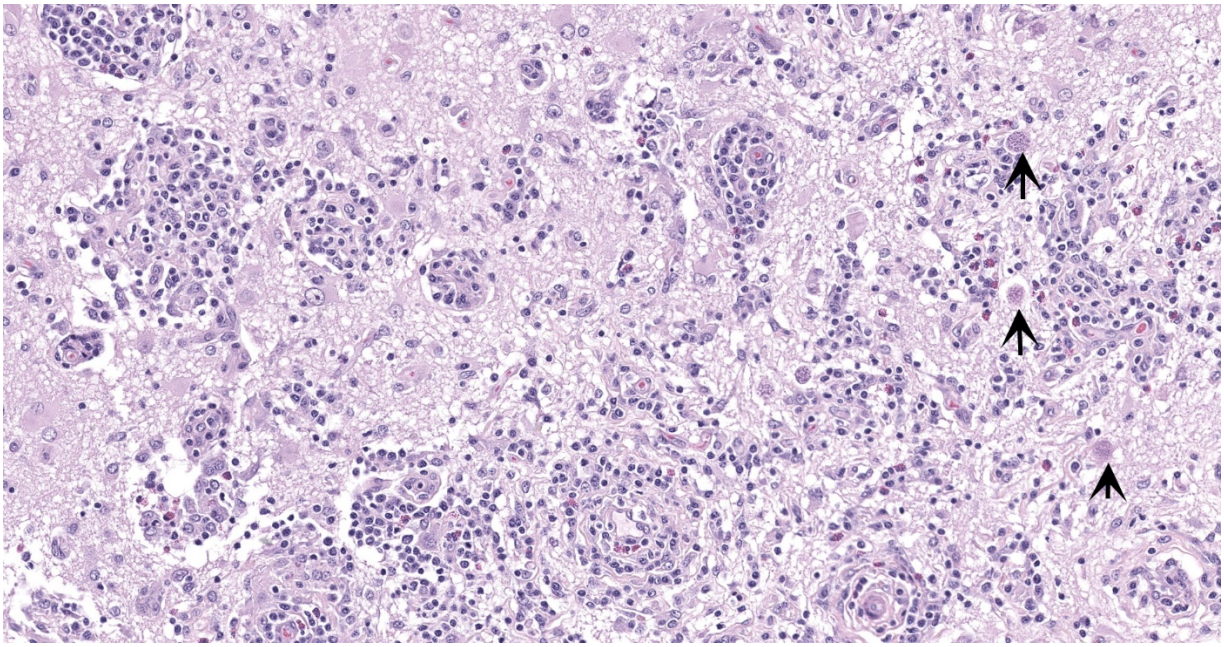

**Figure 4-2. Cerebrum, zebra. Higher magnification of the malacic neuropil with spongiosis, gliosis, marked lymphohistiocytic perivascular cuffing and numerous apicomplexan cyst. (HE, 223X)**

mmol/L (96-106 mmol/L), TC02 (bicarbonate) 18 mmol/L (21-31mmol/L) , Anion Gap 23 mmol/L (8-18 mmol/L), Total Protein 8.1 g/dL (5.7-7.5 mg/dL), Albumin 3.2 g/dL (3.0-3.9 mg/dL), Globulin 4.9 g/dL 2.3-4.1 g/dL), AST 1,377 U/L (194-431 U/L), ALP 127 U/L (76-262 U/L), Total bilirubin 0.5 mg/dL (0.4-2.8 mg/dL), Conjugated Bilirubin 0.1 mg/dL (0.2-0.6 mg/dL), Unconjugated Bilirubin 0.4 mg/dL (0.1-2.8 mg/dL), Cholesterol 124 mg/dL (49-150 mg/dL), Creatine Kinase 873 U/L (130-497 U/L)

EHV-1 Real PCR Serology:
Neuropathogenic: negative
Non-neuropathogenic: negative

Immunohistochemistry:
*Neospora caninum*: Protozoal cysts exhibit strong positive immunoreactivity.
*Sarcocystis neurona*: Protozoal cysts exhibit negative immunoreactivity.

PCR:
The DNA nucleotide sequence of Apicomplexan and *Neospora* spp PCR confirmed that the organism is most likely *Neospora caninum* (performed by Michigan State University).

**Microscopic Description:**
Brain: Multifocally, the neuroparenchyma is randomly disrupted with small to large numbers of inflammatory cells, large numbers of lymphocytes, small numbers of plasma cells, few macrophages, few eosinophils, rare multinucleated giant cells and malacia. Variable degrees of perivascular cuffing up to 5 cells thick with large numbers of lymphocytes, plasma cells, macrophages, few eosinophils, and reactive vascular endothelial cells expands the Virchow Robin spaces and into surrounding the neuro-parenchyma. Astrocytes are swollen (reactive) with large amounts of cytoplasm (gemistocytes) and swollen axons are occasionally identified. Multiple large areas of clear spaces, disruption of neuro-

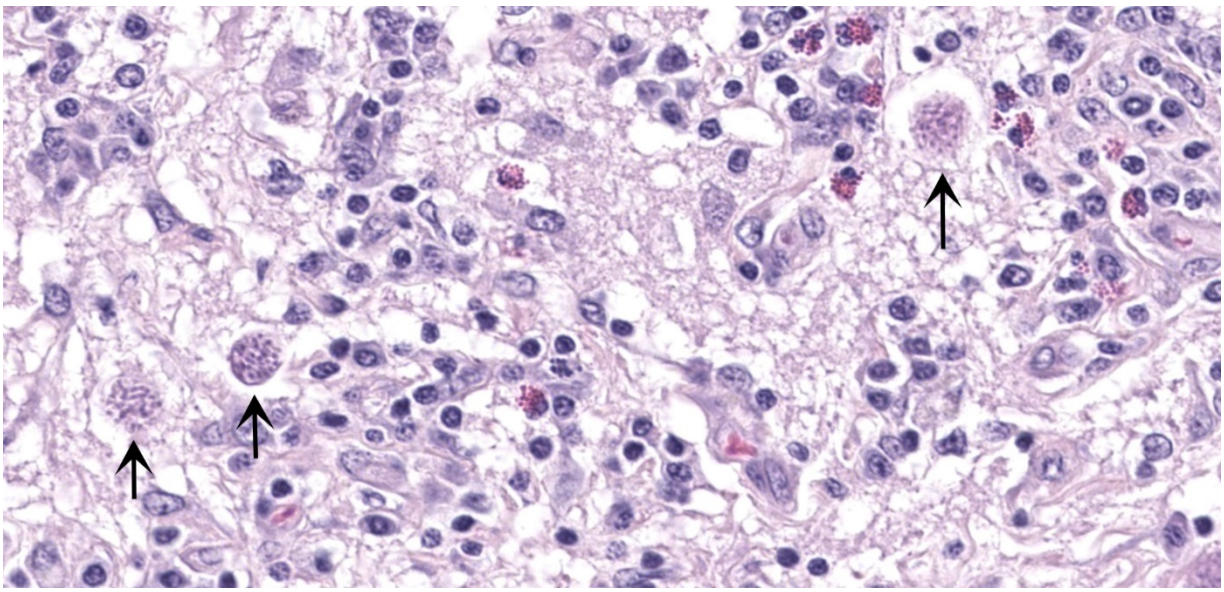

**Figure 4-3. Cerebrum, zebra. High magnification of apicomplexan cysts (arrows). Note the occasional eosinophils within the inflammatory infiltrate. (HE, 720X)**

parenchyma, and vacuoles (edema) are associated with these inflammatory cells. The leptomeninges are markedly expanded with similar inflammatory infiltrates, mild hemorrhage, fibrin, necrotic debris and edema. Within these inflammatory foci, there are multiple 20-25 µm in diameter protozoal cystic structures with a discernible outer wall containing numerous 2x4 µm oval to crescent-shaped tachyzoites and basophilic nucleus.

**Contributor's Morphologic Diagnoses:**
Meningoencephalitis, lymphoplasmacytic, histiocytic, multifocal, severe with peri-vascular cuffing, gliosis, free protozoal tachyzoites and numerous protozoal cysts.

**Contributor's Comment:**
The current name of equine protozoal myeloencephalitis (EPM) was given at American Association of Equine Practitioners (AAEP) meeting in 1977.[7] The causative agent of EPM is either *Sarcocystis neurona* or *Neospora* spp, although the majority of cases are caused by *S. neurona*.[3,5] Protozoa was first observed within the *Toxoplasma*-like encephalomyelitis in the horse in 1974 [1] but reviews of these cases were attributed to *S. neurona*. [3] *Neospora*-associated EPM is uncommon but many cases have been reported.[3-5,9] This is the first *Neospora*-associated EPM case reported in a zebra.

*Neospora caninum* was first described as a new genus and species in 1988 and caused meningoencephalomyelitis and myositis in dogs.[2] Bovine neosporosis causes abortion in both dairy and beef cattle worldwide and reproductive failure leads to major economic impact. Most of the cases are aborted at 5-6 months of gestation. *Neospora caninum* can be transmitted transplacentally which is the major mode of transmission in cattle.[2] The definitive host of *N. caninum* is domestic dogs which produce oocysts in feces.[2] In horses, *Neospora*-like organisms were identified in two aborted foals and adult horses.[2-4] In 1998, the new name of equine neosporosis, *Neospora hughesi*, is proposed.[3] *Neospora hughesi* has been isolated and has differentiated from *N. caninum* by molecular and biological techniques.[2,3,6] Neospora-

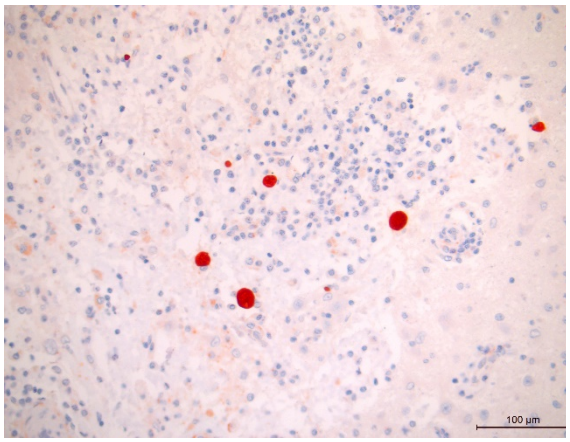


**Figure 4-4. Cerebrum, zebra. Apicomplexan cysts stain immunopositive for *Neospora caninum*. (anti-N. caninum, 200X). (*Photo courtesy of:* Diagnostic Pathology Center, Midwestern University College of Veterinary Medicine, 5725 West Utopia Rd., Glendale, AZ 85308, https://www.mwuanimalhealth.com/diagnostic-pathology-center)**

associated EPM (*N. hughesi* and possibly *N. caninum*) in horses is an uncommon infection in United States and only one case has been reported in Canada.[2,3,9]

In this zebra case, multifocal severe lymphoplasmacytic meningoencephalitis is identified in the cerebrum, brain stem and spinal cord. Within the inflammatory foci, numerous free protozoal tachyzoites (2-4 µm) and protozoal cysts (20-30 µm in diameter) are identified. The tissue cyst wall of *N. caninum* is 1-4 µm thick, whereas the tissue cysts of *T. gondii* is less than 1 µm.[3] The tissue cysts of *N. hughesi* can be compared with *N. caninum*. Tissue cysts of *N. hughesi* are smaller than *N. caninum* and the bradyzoites were smaller than those of *N. caninum*.[3] However, it is unclear whether *N. hughesi* is the only *Neospora* species for EPM in horses.[3] Immunohistochemistry (IHC) revealed a strong positive immuno-reactivity for the *N. caninum* antibody and negative immunoreactivity to *S. neurona* (performed in University of Minnesota). The DNA nucleotide sequence of Apicomplexan PCR (16-1 Cap 18S ribosomal RNA gene) and *N. caninum* PCR (NcCalr5 NC5 marker genomic sequence) revealed 100% similarity (187/187bp and 209/209bp) to *N. caninum*, respectively. However, *N. hughesi* is closely related to *N. caninum*. Therefore, performed IHC and DNA sequence of *N. caninum* may not be differentiated between *N. caninum* and *N. hughesi.* One paper differentiated *N. hughesi* from *N. caninum* based on their immunodominant surface antigen, SAG1 and SAG1-related sequence 2 (SRS2).[6] There was 6% difference in amino acid identity between NcSAG1 and NhSAG1, whereas there was a 9% difference when NcSRS2 and NhSRS2 were compared. These markers can be used to distinguish *N. caninum* from *N. hughesi*.[6] Wobeser et al 2009 targeted the first internal transcribed spacer (ITS-1) region to distinguish *N. hughesi* from *N. caninum*.[9]

EPM is one of commonly diagnosed infectious agents in adult horses in the United States, most commonly caused by *S. neurona* and uncommonly by *Neospora* spp.[2] Both *N. caninum* and *N. hughesi* have been reported and more cases of *N. hughesi* have been identified in recent cases.[3,7,9] The definitive host of *N. hughesi* has not been identified and has been speculated to be small rodents or canids.[7] Currently antemortem EPM is diagnosed with serum titer test from CSF and serum. All horses are believed to be susceptible to EPM, but not all infected horses with either *S. neurona* or *N. hughesi* will develop disease.[7] A recent study shows the seroprevalence of *N. hughesi* is low in horses (between 3-10%) depending on geographic differences.[7] EPM caused by *Neospora* spp. may be underestimated. Currently, SAG1, SRS2 and ITS-1 are pending to differentiate *N. caninum* and *N. hughesi* in this case.

**Contributing Institution:**
Diagnostic Pathology Center

Midwestern University College of Veterinary Medicine
5725 West Utopia Rd.
Glendale, AZ 85308
https://www.mwuanimalhealth.com/diagnostic-pathology-center

**JPC Diagnosis:**
Cerebrum: Meningoencephalitis, necrotizing, multifocal, severe, chronic with numerous apicomplexan cysts and extracellular zoites.

**JPC Comment:**
As mentioned by the contributor, additional molecular diagnostics were pending at the time of this case's submission to WSC. Subsequent molecular diagnostics utilizing the ITS-1 region revealed 100% genetic similarity to *N. caninum* while control *N. hughesi* DNA from the typed species was not detected, further supporting the diagnosis of *N. caninum*.[8] This case is significant in that it not only represents the first reported case of *N. caninum* associated EPM confirmed using molecular analysis but also the first case of *N. caninum* associated EPM reported in a zebra. Therefore techniques utilized with this case can be applied toward retrospective and future EPM studies to better understand the prevalence and geographic distribution of *N. caninum* compared to *N. hughesi* associated EPM in equids.[8]

Neosporosis was first identified as an entity by Bjerkås et al. in Norway during the 1980s. Distributed worldwide, reported seropositivity rates for *N. caninum* in dogs are as high as 38% in Argentina and 22% in New Zealand. Interestingly, multiple studies have demonstrated rural canines have significantly higher seropositivity rates of compared to their urban counterparts.[2]

As previously noted, canines are the definitive host of *N. caninum* but can also be intermediate hosts as well. Other domestic species serving as intermediate hosts include cattle, sheep, goats, and horses. The apicomplexan parasite's lifecycle is composed of three infectious stages, with intracellular tachyzoites and cysts found in the intermediate hosts and unsporulated oocysts in definitive hosts. Cysts are typically found in the CNS but can also be found in other tissues such as placenta and liver. They have a round to oval shape and can be up to slightly more than 100 µm in diameter with an approximately 4 µm thick wall surrounding bradyzoites. In the definitive canine host, unsporulated oocysts enter the gastrointestinal tract and are passed in the feces, contaminating the environment. The oocysts become infective following sporulation and are subsequently ingested by the intermediate host.

Although the source of intermediate host infection of is clear and multiple intermediate hosts have been identified, canine infection in regions without exposure to known intermediate hosts suggests the existence of additional intermediate host species, such as small animals that may be preyed upon by dogs in urban areas. Infected tissues from intermediate hosts in rural regions, such as aborted fetuses, fetal membranes, and dead calves likely serve as the major sources of infection and may explain the difference in seropositivity between rural and urban canines.[2]

In addition to *Neospora* spp., EPM is also caused by a closely related apicomplexan parasite, *Sarcocystis neurona.* In both cases, equids are believed to become infected following ingestion of infective oocysts passed in the feces of the definitive host. Whereas the definitive hosts of *N. caninum* include the domestic dog and the coyote

(*Canis latrans)*, *Sarcocystis neurona*'s definitive host is the opossum (*Didelphis* sp.). The definitive host for *N. hughesi* has not been identified.[9]

The moderator discussed the significance of multinucleated giant cells within the section, suggesting their presence in the CNS of any horse with a history of neurologic deficits should elevate the level of suspicion for a protozoal etiology.

**References:**


1. Beech J, Dodd D. *Toxoplasma*-like encephalomyelitis in the horse. *Vet Pathol*. 1974;11(1):87-96
2. Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol.* 2003;41:1–16.
3. Dubey JP, Lindsay DS, Saville WJ, et al. Granstrom DE, Speer CA. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). *Vet Parasitol*. 2001;95(2-4):89-131.
4. Hamir A, Tornquist, Gerros T, et al. *Neospora caninum*-associated equine protozoal myeloencephalitis, Vet parasitol. 1998:79:269-274
5. Lindsay DS, Steinberg H, Dubielzig R, et al. Central nervous system neosporosis in a foal. *J Vet Diagn Invest.* 1996;8:507–510.
6. Marsh AE, Howe DK, Wang G, et al. Differentiation of *Neospora hughesi* from *Neospora caninum* based on their immunodominant surface antigen, SAG1 and SRS2. *Int J Parasitol*. 1999;29(10):1575-1582.
7. Reed SM, Furr M, Howe DK, Johnson AL, MacKay RJ. Equine Protozoal Myeloencephalitis: An Updated Consensus Statement with a Focus on Parasite Biology, Diagnosis, Treatment, and Prevention. *J Vet Intern Med*. 2016;30(2):491-502.
8. Ruppert S, Lee JK, Marsh AE. Equine Protozoal Myeloencephalitis associated with Neospora caninum in a USA captive bred zebra (Equus zebra). *Vet Parasitol Reg Stud Reports*. 2021;26:100620.
9. Wobeser BK, Godson DL, Rejmanek D, Dowling P. Equine protozoal myeloencephalitis caused by *Neospora hughesi* in an adult horse in Saskatchewan. *Can Vet J*. 2009;50(8):851-853.