Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #1 16 August 2023

CASE I:

Signalment:
6-year-old, warmblood mare, equine (*Equus caballus*)

History:
The animal presented for treatment following a 24-hour history of lethargy and inappetence. The mare was diagnosed with strangles three weeks prior and was being medically managed with flunixin meglumine. At presentation, serum creatinine was 4.0 mg/dL, then rose to 10.2 mg/dL despite aggressive diuresis. The animal became anuric and was euthanized due to poor prognosis.

Gross Pathology:
The kidneys were mottled dark red and tan throughout, with dark red to black streaks extending from the cortex into the medulla. Multiple abdominal serosal surfaces contained petechiae and ecchymoses. There was serosanguinous thoracic and pericardial effusion and generalized intramuscular and ventral subcutaneous edema. The mandibular and retropharyngeal lymph nodes were swollen, edematous and reddened, and at least one lymph node exuded purulent material when incised. One guttural pouch contained a hard nodule of inspissated purulent material adhered to the ventral mucosal surface.

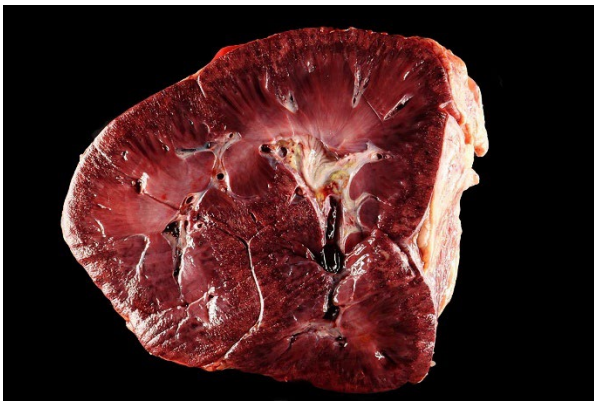

Figure 1-1. Kidney, horse. Dark red to black areas of hemorrhage extend from the cortex into the medulla. (Photo courtesy of: Colorado State University Veterinary Diagnostic Laboratory, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx)

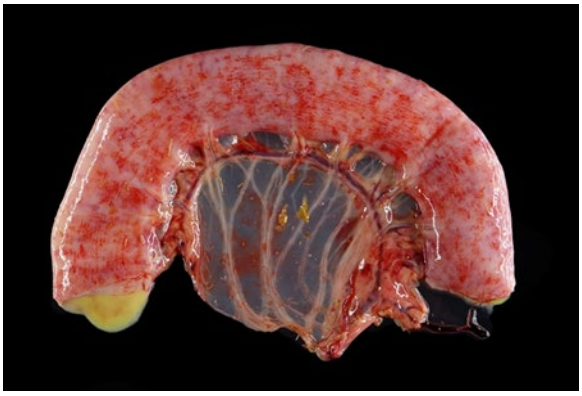

Figure 1-2. Intestine, horse. There are confluent areas of hemorrhage on the intestinal serosa and mesentery. (Photo courtesy of: Colorado State University Veterinary Diagnostic Laboratory, http://csu-cvmbs.colostate.edu/-vdl/Pages/default.aspx)

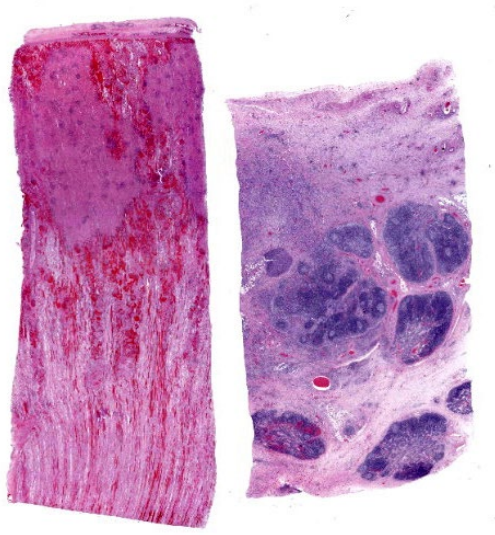

Figure 1-3. Kidney and lymph nodes, horse. There are multifocal to coalescing areas of necrosis within the renal cortex which extend into the medulla. Lymph nodes are separated and surrounded by fibrosis. (HE, 7X)

Laboratory Results:
Aerobic culture from a retropharyngeal lymph node swab yielded moderate mixed growth including *Streptococcus equi* subsp. *equi*, *Streptococcus equi* subsp. *zooepidemicus*, *Sphingobacterium* spp. and *Pseudomonas* spp.

Microscopic Description:
Kidney: Multiple irregular regions of the cortex are composed of coagulative necrosis characterized by partial loss of nuclear and cellular detail, hypereosinophilia, and karyorrhectic debris with maintenance of tissue architecture. Random scattered small to medium caliber blood vessels have segmental to circumferential transmural smudged eosinophilic walls infiltrated by intact and degenerate neutrophils, with some infrequent fragmentation of the tunica intima and media. Diffusely glomeruli in intact regions are segmentally to globally expanded by smudged homogenous eosinophilic matrix, intact and karyorrhectic neutrophils with fewer other mixed leukocytes (fibrinocellular crescents). Bowman's space is frequently filled with erythrocytes. Tubular epithelial cells are multifocally pyknotic and sloughed into the lumen. The interstitium is multifocally expanded by small aggregates of neutrophils. Tubules are multifocally filled with brightly eosinophilic proteinaceous fluid. Adipose tissue on the capsular surface is infiltrated by lymphocytes, plasma cells, and neutrophils within a thin layer of well-vascularized fibrous tissue.

Lymph node: The architecture of the node is nearly effaced by extensive fibrosis admixed with aggregates of neutrophils which are occasionally ringed by epithelioid macrophages. Occasionally small clusters of basophilic 1-3 micron cocci are present within inflammatory foci. Lymphoid follicles are multifocally distributed through the tissue. Occasionally, small caliber blood vessels along the periphery are surrounded or obscured by moderate numbers of lymphocytes, plasma cells, neutrophils, and macrophages.

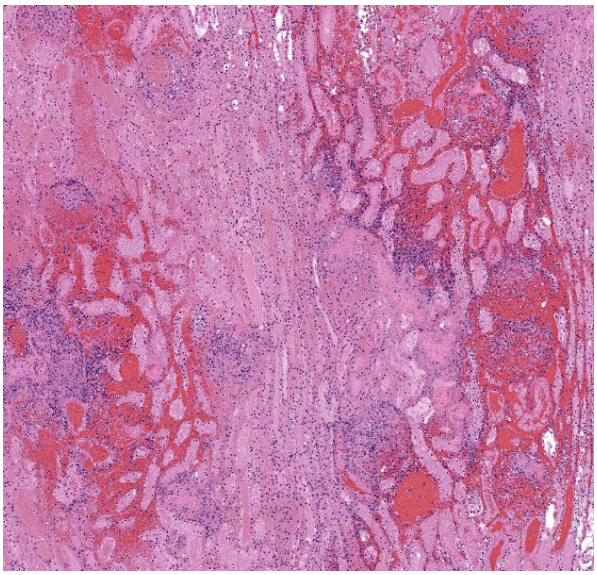

Figure 1-4. Kidney, horse. There is infarction and loss of glomerular and tubular detail within the renal cortex. (HE, 55X)

Contributor's Morphologic Diagnoses:
Kidney: Glomerulonephritis, severe, multifocal, segmental to global, with fibrinocellular crescents.

Kidney: Fibrinonecrotizing and suppurative vasculitis, severe, multifocal subacute-active, with coagulative necrosis (infarction), hemorrhage and edema.

Lymph node: Pyogranulomatous lymphadenitis, severe, multifocal to coalescing, chronic, with fibrosis and rare cocci.

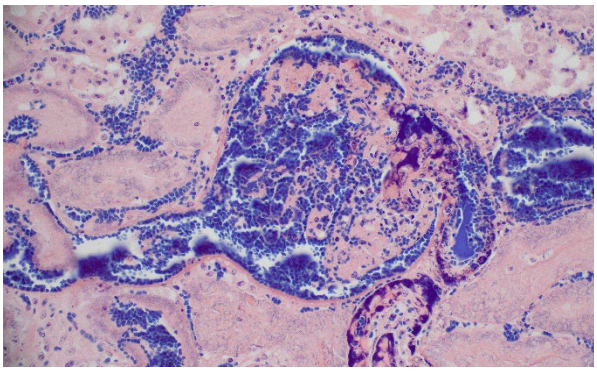

Figure 1-6. Kidney, horse. A PTAH stain demonstrates the presence of polymerized fibrin within glomeruli. Fibrin is a dark navy blue. (PTAH, 400X)

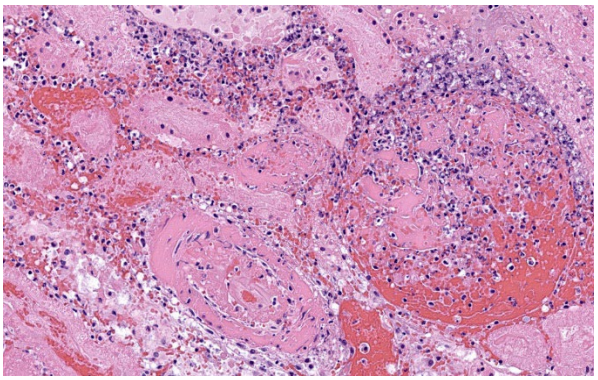

Figure 1-5. Kidney, horse. There is vasculitis and thrombosis of renal vessels and the adjacent glomeruli. There is coagulative necrosis of tubules in this area as well. (HE, 257X)

Contributor's Comment:
Streptococcus equi equi-associated purpura hemorrhagica (PH) is a well-characterized, though uncommon immune complex-driven vasculitis following prolonged infection with *S. equi equi*, otherwise known as strangles. In the case of *S. equi equi*-associated PH, complexes are formed between Streptococcal M protein (SeM) and IgA which deposit in vessel walls.[4] Immune complex formation and deposition in tissue depends on the amount of antigen, antigen:antibody ratio, and size of the complexes. Type III hypersensitivity reactions occur when there is slightly more antigen than antibody in circulation, forming complexes small enough to remain soluble but large enough to accumulate within tissue and initiate the complement cascade, resulting in the classic leukoclastic vasculitis.[7,9] Other individual immunologic factors may also play a role in defective clearance of immunocomplexes. Free SeM can also activate the NLRP3 inflammasome, inducing IL-1β production, promoting pyroptosis in macrophages, and worsening systemic inflammation.[8]

Immune complexes can be detected in the circulation of affected horses.[4] Horses with prior exposure or who are vaccinated are at a slightly higher risk of developing PH with subsequent *S. equi equi* infection, presumably due to preexisting antibody titers priming formation of immunocomplexes.[1] The percent of horses with Strangles that develop PH varies, with two studies of outbreaks reporting 6.5% and 5.4% respectively.[2] Other bacteria, including *Corynebacterium pseudotuberculosis,* and some viruses induce PH, also through a type III hypersensitivity mechanism.[9]

The typical clinical presentation of PH is variable but often includes well-demarcated, gravity-dependent edema with petechiae and/or ecchymoses on the mucous membranes and skin.[1,10] This case is unusual in that the primary clinical presentation was acute anuric renal failure, presumably secondary to the numerous and extensive renal infarctions. Infarctive PH resulting from vessel occlusion secondary to vasculitis is described most commonly with infarction of skeletal muscle, leading to stiffness and pain, or in the gastrointestinal tract, leading to colic. While the infarctions were responsible for acute severe renal failure, the non-infarcted glomerular tufts contained abundant inflammation, regions of necrosis, and fibrin thrombi (fibrinocellular crescents). Glomeruli are a well-known target for immunocomplex (IC) deposition in a variety of type III diseases such as systemic lupus erythematosus. Demonstration of glomerular IC deposition has not been confirmed in horses with *S. equi equi* associated PH, however glomerulonephritis with basement membrane IC deposition is reported as part of post-streptococcal infection in humans.[6] Henoch-Schonlein syndrome is a human form of IgA IC disease that causes purpura, arthritis, gastrointestinal symptoms, and glomerulonephritis and is thought to be caused by infections including streptococcal organisms, viruses, medication, insect bites and other causes.[5] It is suspected that the glomerulonephritis observed in this case may have been due to local IC deposition; however, the extensive tissue damage and inflammation precludes more careful evaluation of glomerular architecture and basement membrane thickness via electron microscopy. Special stains, including PTAH and PAS, confirmed fibrin deposition in glomeruli, though the mesangial basement membrane was predominantly obscured.

Contributing Institution:
Colorado State University
Veterinary Diagnostic Laboratory
https://vetmedbiosci.colostate.edu/vdl

JPC Diagnosis:
Kidney, vessels and glomeruli: Vasculitis, necrotizing, multifocal to coalescing, severe with thrombosis and extensive cortical and medullary infarction.

Lymph node: Lymphadenitis, suppurative, focally extensive, moderate, with reactive hyperplasia.

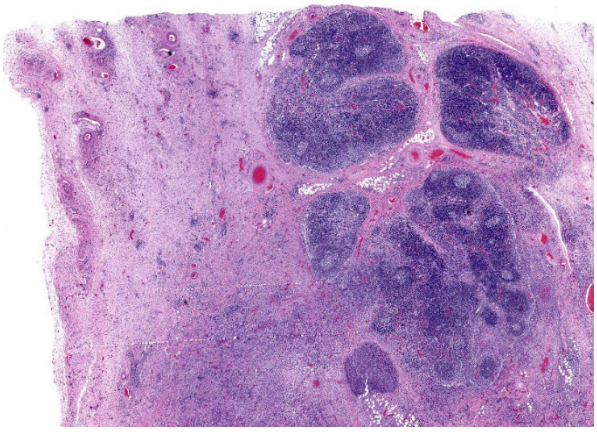

Figure 1-7. Lymph nodes, horse. Lymph nodes are hyperplastic separated and surrounded by fibrosis with a dicrete focus of suppurative necrosis at bottom center. (HE, 33X)

JPC Comment:
Purpura hemorrhagica is a prototypical Type III hypersensitivity-mediated disease. As the contributor notes, the classic histologic lesion is leukoclastic vasculitis, with an inflammatory infiltrate composed of neutrophils in which nuclei have disintegrated into fragments termed "nuclear dust" or, less poetically, "leukocytoclasia."[3] The contributor provides a nice discussion of the factors that lead to immune complex deposition within vascular walls and trigger the Type III hypersensitivity reaction.

Type III hypersensitivity reactions can be thought of as "innocent bystander" reactions because the injured tissue is not a direct target of the immune response; rather, once deposited within tissues, the immune complexes themselves activate multiple cellular processes and cascades that result in inflammation and tissue damage. [7] Most critically, immune complexes lead to complement activation when IgG and/or IgM are cross-linked with C1, leading to the formation of the C3 and C5 convertases of the classical complement pathway. Cleavage products of classical pathway, C3a and C5a, cause increased vascular permeability and vasodilation. C5a is also chemotactic for neutrophils and macrophages, luring them to sites of immune complex deposition where their released and elaborated proteolytic enzymes and free radicals damage surrounding tissues. Vascular damage can compromise the intima, leading to exposure of subintimal collagen, coagulation cascade and platelet activation, and the production of microthombi with subsequent infarction.

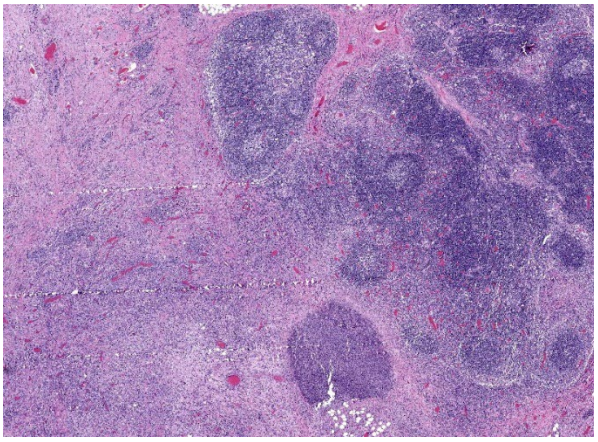

Figure 1-8. Lymph nodes, horse. Inflamed fibrous connective tissue surrounds and infiltrates a lymph node. Germinal centers are hypocellular due to lymphocyte loss. A suppurative focus is at bottom center.

This sequence of Type III hypersensitivity-mediated injury is not unique to Strangles-associated purpura hemorrhagica, but is part of a stereotyped pattern of injury in several immune-mediated diseases of veterinary importance. The contributor mentions systemic lupus erythematosus, where DNA and nuceloproteins serve as antigenic niduses for immune complex formation. Others include equine infectious anemia, equine recurrent infectious uveitis, "blue eye" secondary to canine adenovirus 1 infection, hypersensitivity pneumonitis, rheumatoid arthritis, and acute glomerulonephritis, among others.[7]

The conference moderator, COL Jeremy Bearss, outgoing JPC director, stressed the importance of examining vessels in all tissues. Though easily overlooked among more eye-catching histologic features, the vessels often contain a wealth of diagnostic information as in this case, where the leukoclastic vasculitis is a key histologic feature.

Careful consideration was given to the contributor's description of fibrinocellular crescents; however, no fibrinocellular crescents were noted in the histologic sections examined during the conference.

References:

1. Boyle AG, Timoney JF, Newton JR, Hines MT, Waller AS, Buchanan BR. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of Strangles—revised consensus statement. *J Vet Intern Med*. 2018;32(2):633-647.
2. Duffee LR, Stefanovski D, Boston RC, Boyle AG. Predictor variables for and complications associated with *Streptococcus equi* subsp *equi* infection in horses. *J Am Vet Med Assoc*. 2015;247 (10):1161-1168.
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4. Galan JE, Timoney JF. Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. *J Immunol*. 1985; 135(5):3134-3137.
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9. Valentine BA. Skeletal Muscle. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 6th ed. Elsevier; 2017:908-953.
10. Whelchel DD, Chaffin MK. Sequelae and complications of *Streptococcus equi* subspecies *equi* infections in the horse. *Equine Vet Educ*. 2009;21(3):135 -141.

CASE II:

Signalment:
1-month-old, male, Rasa Aragonesa sheep (*Ovis aries*)

History:
This animal was one of 400 lambs on a single farm. 250 lambs became lethargic and apathetic and 10 lambs died suddenly.

Gross Pathology:
Approximately 40% of the endocardium and myocardium of both ventricles had well-demarcated, multifocal foci of white, hard, crunchy tissue admixed with areas of mild hemorrhage.

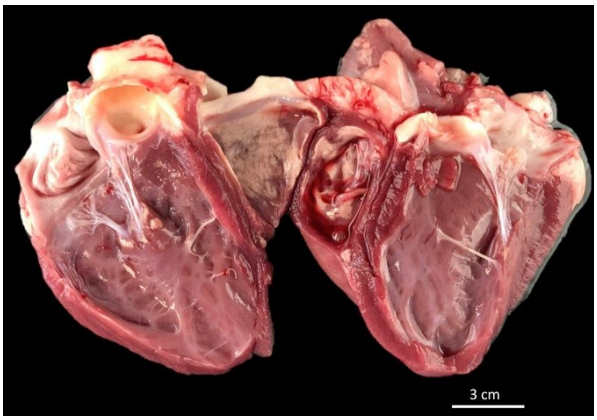

Figure 2-1. Heart, sheep. 40% of the ventricular myocardium, especially in subendocardial regions have foci of fibrosis, mineralization, and hemorrhage. (Photo courtesy of: Universidad de Zaragoza. Departamento de Patología Animal, https://patologiaanimal.unizar.es)

Microscopic Description:
Heart: Diffusely up to 40% of the tissue is affected by a degenerative and necrotizing process. On a focally extensive area of the myocardium close to endocardium, some cardiomyocytes are swollen with moderate amounts of vacuolation and pale eosinophilic cytoplasm (degeneration), others are shrunken and hypereosinophilic, with loss of cross striations and fragmentation of myofibrils, and occasional contraction bands, with pyknosis and karyorrhexis (necrosis). Many degenerate and necrotic myocytes contain abundant amounts of basophilic granular material (mineral). Multifocally, scattered among the tissue are few neutrophils and lymphocytes admixed with moderate edema and mild hemorrhage. Few satellite nuclei surrounding affected fibers are hyperplastic

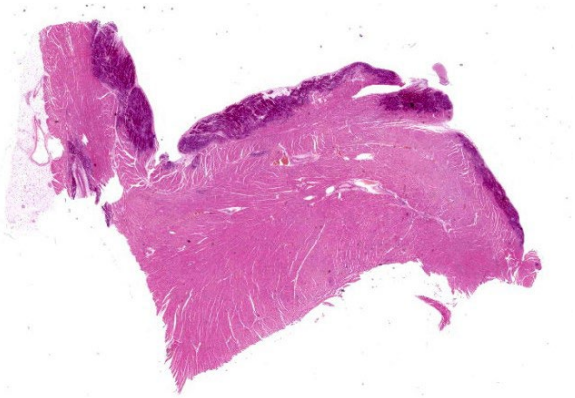

Figure 2-2. Heart, sheep. One section from the ventricular wall and septum is submitted for examination. There is well-demarcated dark discoloration of the subendocardial myocardium. (HE, 6X)

Contributor's Morphologic Diagnosis:
Cardiac muscle: Focally extensive necrosis and degeneration with mineralization, polyphasic, subacute, moderate.

Contributor's Comment:
The most common nutritional deficiency leading to nutritional myopathy is selenium deficiency. Nutritional myopathy resulting from vitamin E deficiency in the absence of selenium deficiency is uncommon in mammals but may be more common in birds and reptiles.[2] Selenium is primarily considered a powerful antioxidant because of its role in the glutathione peroxidase system.[4] Antioxidant systems are believed to have evolved as a means of surviving in an oxygenated atmosphere by dealing with free radicals and the toxic products of their metabolism. Animal antioxidant defense mechanisms are based on the synthesis of numerous biological antioxidants that include the antioxidant enzymes glutathione, thioredoxin, and coenzyme Q. There is also a range of dietary antioxidants which can be provided in feed, which include vitamin E, carotenoids, polyphenolics, and selenium (as a precursor to selenoproteins). Under stress conditions, the internal antioxidant system network alone cannot deal properly with excess reactive oxygen species formation and requires additional help from dietary antioxidant sources provided via feed/water. Vitamin E and selenium are major feed-derived antioxidants.[8]

Nutritional myopathy in sheep is probably more prevalent in more areas of the world than the disease in cattle. The names white muscle disease, rigid lamb disease, and stiff lamb disease were coined to describe the most frequently encountered clinical patterns in 2-4 week-old lambs, which very often are spring lambs, recently turned out onto the first green pasture.[2] Congenital nutritional myopathy does occur in lambs, but not often. The typical disease may occur as an outbreak among lambs from 1 day to 2 months of age or beyond. Mortality at this stage may be very low or may reach 50%. The next peak of incidence occurs at 4-8 months of age as weaned lambs are put onto lush pastures following mowing or into feedlots. Mortality is not usually very high, but the incidence of minimal clinical disease may be moderately high and that of subclinical disease may be higher still.[2]

Lesions and their corresponding clinical signs are as varied as the circumstances under which myopathy occurs. The lesions may be detectable in lamb fetuses at least 2 weeks before parturition. In the congenital disease, tongue and neck muscles used in suckling movements often contain the most severe lesions. When the lesions occur in lambs a few days older, they are likely to be much more extensive and involve primarily the major muscles of the shoulder and thigh but also back, neck, and respiratory (diaphragm and intercostal) muscles.[2] Severe, often fatal myocardial necrosis is typically part of the important vitamin E and selenium-responsive syndromes of nutritional myopathy of lambs, calves, swine, and horses,

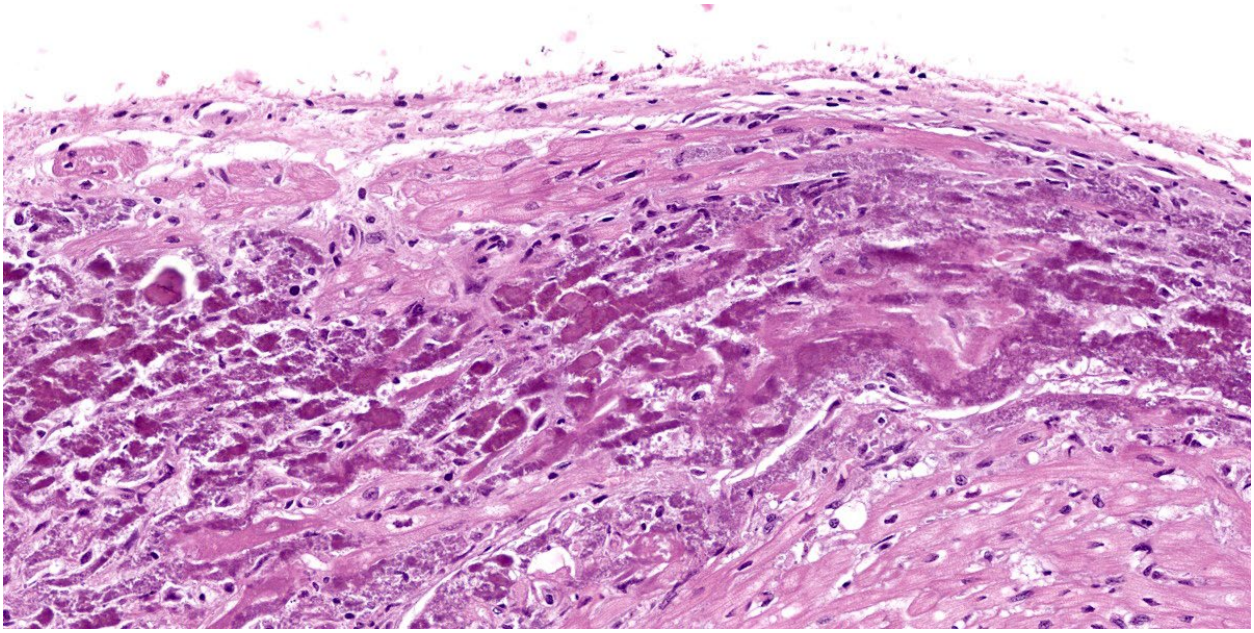

Figure 2-3. Heart, sheep. Higher magnification of an area of myofiber degeneration and necrosis with mineralization of deeper myofibers (HE, 270X)

and in mulberry heart disease of swine. It may also be seen as part of equine rhabdomyolysis and of capture myopathy and other exertional syndromes where vitamin E and selenium depletion produce cardiomyocyte alterations characterized by contraction band necrosis and myofibrillar lysis.[4,7]

Given the potential for a common mechanism of cell injury in both selenium deficiency and selenium toxicity, it is not surprising that the primary lesion in these two diseases is myocardial necrosis. Therefore, in cases of acute myocardial necrosis, it is imperative that tissue selenium levels are quantified before a diagnosis of selenium deficiency is rendered.[1] Polyunsaturated fatty acid levels must also be examined as supplementation with polyunsaturated fatty acids can greatly increase the severity of these changes.[4]

Several mineral deficiencies might be present at the same time in a sheep herd. Cases of poor growth performance in lambs should be investigated taking several mineral deficiencies, particularly cobalt, copper, and selenium into account. Clinical examination can often give only suspected diagnoses, so to access possible mineral deficiencies, a nutritional assessment should be performed sampling not only blood, but also liver tissue via biopsy or post-mortem samples.[3]

Contributing Institution:
University of Zaragoza
Departamento de Patología Animal (Área histología)
University of Zaragoza
Zaragoza, Spain

JPC Diagnosis:
Heart, myocardium: Mineralization and necrosis, subendocardial, diffuse, severe, with fibrosis.

JPC Comment:
The striking myocardial mineralization observed grossly and histologically in this case is an excellent example of pathologic calcification. Pathologic calcification is the abnormal deposition of calcium salts, along

with smaller amounts of iron, magnesium, and other mineral salts, within soft tissues.[6] Pathologic calcification comes in two forms: dystrophic calcification and metastatic calcification.

Metastatic calcification occurs primarily in normal tissues due to a calcium-phosphate imbalance ("metastatic is metabolic") and is more likely to occur when the serum product of the calcium-phosphorus concentration exceeds 70 mg/dL. Causes for calcium-phosphate imbalance are many and include chronic kidney disease, vitamin D toxicosis, inappropriately elevated parathyroid hormone, or humoral hypercalcemia of malignancy. Metastatic calcification tends to occur in tissues that exist in alkaline environments, primarily the stomach, kidneys, and lungs.

Dystrophic calcification, by contrast, occurs in areas of tissue necrosis ("dystrophic is dead") and typically occurs in patients with normal serum calcium levels. Intracellular calcium overload is an expected consequence of cell death as ischemia leads to the opening of membrane calcium channels and the flooding of the cytosol with calcium normally sequestered in the sarco- or endoplasmic reticulum and mitochondria.[5] Necrotic myofibers are particularly prone to dystrophic calcification due to the high levels of calcium ions stored in the sarcoplasmic reticulum; thus, selenium or vitamin E deficiency-driven necrosis may quickly lead to whole myocyte calcification and to the white, gritty lesion for which white muscle disease gets its common name.[5]

Nutritional myopathy, the preferred term for white muscle disease, may present differently in different species. The contributor provides a good summary of the typical disease presentation in young, suckling animals. In affected adult horses, the temporal and masseter muscles are often preferentially affected with swelling and stiffness leading to impaired mastication.[9] The disease in cattle, sheep, goats, and camelids more typically affects the postural muscles and the muscles of locomotion without the profound involvement of the temporalis and masseter muscles seen in horses.[9] Severe disease in all species can lead to necrosis and dystrophic calcification of the myocardium or endocardium, as illustrated in this case.

Lesions may also differ based on chronicity. In animals with severe, acute myopathy leading to death (typically young animals) lesions are characterized by widespread muscle necrosis and dystrophic mineralization with minimal inflammation. In subacute or chronic disease, lesions are polyphasic with active necrosis, macrophage infiltration, and regeneration all present simultaneously.[9]

There was dispirited discussion among conference participants about the phasic nature of the myocardial lesions in this case. Lesions resulting from nutritional myopathy, a chronic process, would be expected to be polyphasic; however, the slides reviewed in conference appeared to the moderator and conference participants to be largely monophasic and characterized solely by necrosis and mineralization. Participants discussed whether the young age of the animal at death precluded full lesion development or if the examined histologic section was unrepresentative. No satisfactory resolution was reached during conference discussion and the final JPC morphologic diagnosis slyly skirts the issue by omitting any reference to phase.

References:

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CASE III:

Signalment:
4-year-old, female, Southern Pre-alps sheep (*Ovis aries*).

History:
Several animals of a herd showed neurologic symptoms including amaurosis for several weeks. Some animals progressed to death, others were euthanized and submitted for necropsy. This case was referred (alive) to the veterinary hospital for pedagogic purposes. The animal presented depressed, with a left head tilt, circling to the left, and a hypermetric forelimb gait. MRI showed a 4 cm liquid mass in the left cerebral hemisphere compressing the lateral ventricle. The mass was surgically removed; 2 days later the animal was euthanized because of status epilepticus.

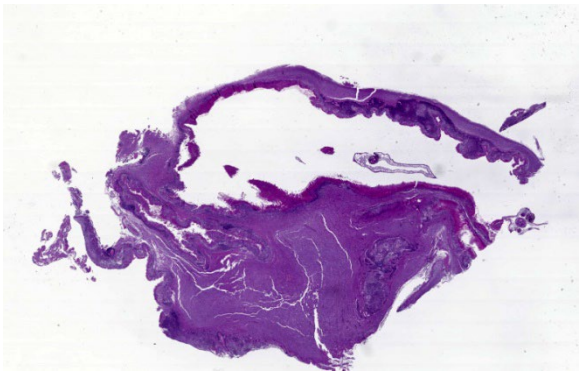

Figure 3-1. Cerebrum, sheep. Within the cerebrum, there is a cystic focus of inflammation containing a coenurus. At this magnification, cross sections of multiple scolices are visible. (6X)

Microscopic Description:
Cerebrum: The cerebral parenchyma is characterized by a compressive cystic nodular lesion lined by diffuse, moderate to severe inflammation composed of a large number of lymphocytes and plasma cells

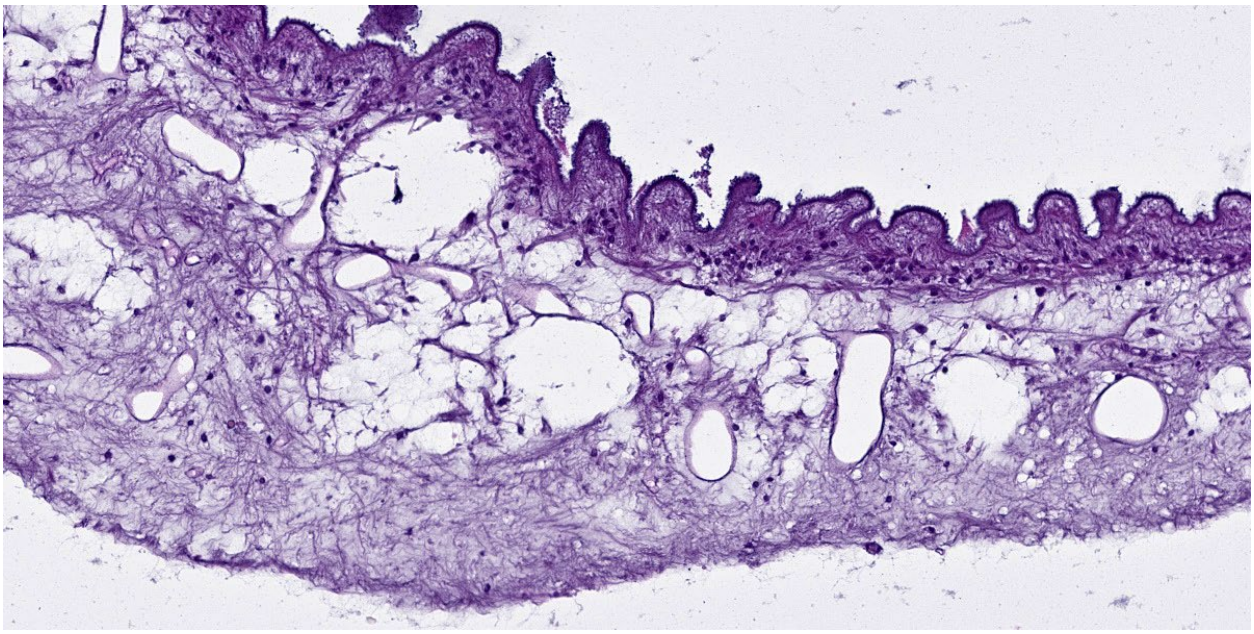

Figure 3-2. Cerebrum, sheep. A fragment of the bladder wall is free-floating within the cystic space. The bladder has a tegument with numerous ridges (top), a spongy parenchyma with numerous somatic cell nuclei subjacent to the tegument, and scattered 10-30um calcareous corpuscles within the spongy parenchyma. (316X)

surrounding several macrophages, epithelioid cells and multinucleated giant cells with up to 11 nuclei. Multifocally, between the granulomatous inflammation and the cyst wall there is a large amount of amorphous eosinophilic material and cellular debris (liquefactive necrosis). The cyst wall is composed of a thin outer undulating hyaline layer with microtriches (microvilli) and an inner layer of areolar tissue (germinative membrane). Arising from the germinative membrane, multiple larval metazoan parasites are visible (protoscolices). They are up to 1mm in diameter, lined by an eosinophilic smooth, ridged tegument, which is also visible inside the parasite (inverted tegument). They contain a solid parenchyma with many basophilic to clear oval, up to 15µm basophilic bodies (calcareous corpuscles), an invaginated scolex containing multiple anterior suckers composed of muscular rings comprising radial striations of muscle fibers, and a rostellum with multiple birefringent hooks. Digestive tract, reproductive tract, and pseudocoelom are absent. Lymphocytes and plasma cells are multifocally visible in perivascular areas (lymphocytic cuffs).

Contributor's Morphologic Diagnoses:
Cerebrum: Encephalitis, granulomatous, focally extensive, chronic, moderate with intralesional coenurus protoscolices.

Contributor's Comment:
Coenurus cerebralis is the intermediate stage of *Taenia multiceps*. *C. cerebralis* has a worldwide distribution, affecting primarily sheep and goats, though it has been described in other animals, including cattle, buffalos, yak, horse and pig.[4]

T. multiceps has an indirect life cycle. Canids, including domestic dogs, are definitive hosts. Adult worms, up to 100 cm in length, live in the small intestine of definitive hosts, who shed embryonated eggs and gravid proglottids in their faeces, contaminating pasture and water. When eaten by

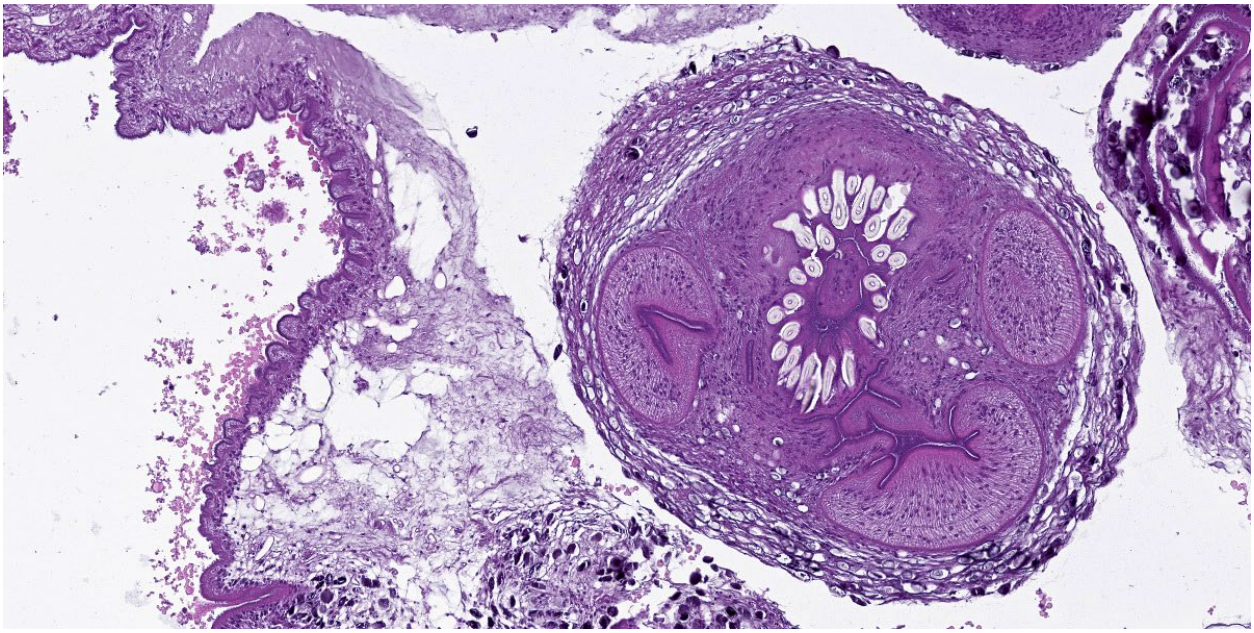

Figure 3-3. Cerebrum, sheep. A cross section of an inverted scolex contains an armed rostellum and several muscular suckers. (93X)

intermediate hosts, eggs release oncospheres (larvae) in the intestine. The oncospheres burrow through the intestinal wall, enter the circulation and migrate to the central nervous system, most often the cerebrum, where they form one or more unilocular bladder cysts.[4] Cerebral coenurosis is caused by *Coenurus cerebralis*, the larval stage of *T. multiceps*. The cysts grow over 6-8 months, eventually causing clinical signs.[4] *T. multiceps* protoscolices develop from the inner cyst wall and can reproduce asexually so that each coenurus may contain up to 400-500 scolices. The life cycle is completed when a definitive host eats the coenurus, releasing protoscolices, which attach to the intestinal wall and develop into adults in 42 to 60 days. This requires canids to have access to the brain of an infected animal.[4]

The cerebral clinical disease is commonly referred as "sturdy" or "gid." Acute and chronic forms exist. The acute disease is caused by larval migration through the brain. Its severity depends on the number of ingested eggs and migrating parasites. The chronic form is more commonly observed and it occurs in older animals.[4] It is due to cyst development in the brain, and less frequently in the spinal cord, causing parenchymal compression which leads to significant neurological signs and death.[4] Clinical signs of *C. cerebralis* include ataxia, blindness, head pressing and circling towards the affected side of the brain.[4,5,7] Signs progress to coma and death if untreated. Spinal cord lesions cause progressive hind limb paresis/paralysis.[4,5] Diagnosis is made using a combination of clinical signs, neurological examination, ultrasound and necropsy.[4]

Coenuri have been rarely reported in extracerebral locations. These reports are mostly from Asian countries. Non-cerebral coenurus has been described in the skeletal muscle, fascia, adipose tissue, lungs, peritoneum and pelvic cavity of sheep and goats.[1,4] Non-cerebral coenuri is often asymptomatic and only detected at slaughter, but may cause muscle pain.[4]

Grossly, *C. cerebralis* is composed of thin walled, fluid-filled, unilocular cysts up to 7cm in diameter. The cyst walls have multifocal white nodules comprising clusters of protoscolices.[6] Cysts more commonly occur in the cerebrum than in cerebellum or spinal cord. The cysts cause cerebral compression and can be responsible for hydrocephalus and cerebral or cerebellar herniation.[4,7]

Histologically, the cyst wall comprises an external eosinophilic layer with basophilic microtriches (microvilli) and an inner germinative layer made up of areolar tissue.[2] Multiple *T. multiceps* protoscolices arise from invaginations of the cyst wall. Protoscolices have four muscular anterior suckers, a rostellum with up to 34 hooks arranged in 2 rows, and an eosinophilic invaginated tegument. They also have standard features of cestodes including a parenchyma containing calcareous corpuscles, no pseudocoelom and no digestive or reproductive tract.[6]

Changes to the cerebral/cerebellar parenchyma range from mild to severe liquefactive necrosis and lymphocytic/ granulomatous inflammation with an infiltration of macrophages, lymphocytes, plasma cells and foreign-body multinucleated giant cells. Accompanying changes include neuronal degeneration and necrosis, satellitosis, neuronophagia, demyelination, gliosis and formation of microglial nodules, non-suppurative meningitis and meningeal hyperaemia and oedema.[2,6]

Humans are rarely infected. The main location of intermediate forms in humans is the subcutaneous tissue, but cerebral coenurosis is also described.[4]

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JPC Diagnosis:
Cerebrum: Encephalitis, granulomatous, focally extensive, severe, with coenurus.

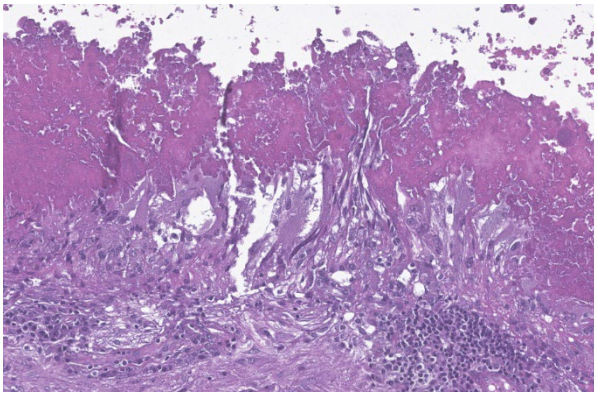

Figure 3-4. Cerebrum, sheep. The coenurus is surrounded by a thick layer of granulomatous inflammation with a peripheral layer of pleomorphic epithelioid macrophages. (306X)

JPC Comment:
Cerebral coenurosis is thought to have been reported for the first time by Hippocrates, who described a condition causing epilepsy in sheep and goats that was characterized by an excess of fluid in the brain.[8] More recently, studies from 1656 and 1724 reported the presence of water-filled sacs or bladders in sheep and cattle, remarking that these bladders were frequent causes of vertigo and death in affected animals.[8] It took until 1853, and feeding cysts taken from infected brains to unsuspecting dogs, to work out the life cycle that the contributor so nicely details.

Today, while coenurosis is reported sporadically in sheep and goats in many European countries (with a relatively high prevalence in Sardinia), the disease is a major endemic disease of small ruminants in the Middle East, particularly in Turkey, Egypt, Iraq, and Jordan.[8] In this group, prevalence rates

range from 2.9% of Jordanian sheep to 22.8-23.68% of Iraqi sheep and goats.[8] A wide range of prevalence is reported in Africa, from a low of 4-8% prevalence in Ethiopian sheep and goats to 42.1% in Tanzanian sheep and goats.[8] The disease burden borne by animals and farmers in these regions is significant, and transmission is difficult to control given that wild canids and herding dogs have the ability to travel and spread eggs and proglottids widely.

Infection prevention in endemic areas focuses heavily on educating farmers about proper sanitation and carcass disposal. In Sardinia, Italy, a recent novel preventive measure featured controlled feeding stations, accessible only to birds, where carrion and offal were fed to vultures.[8] Though not yet currently commercially available, vaccination against coenurosis is under development, with the first successful field test of a vaccine based on an oncosphere secretory antigen conducted in Sardinia in 2009.[9] Once neurologic signs of coenurosis are manifest, treatment options include antihelminthic treatments such as praziquantel, and surgical removal, which is frequently successful though uncommonly performed.

As the contributor notes, cerebral coenurosis is zoonotic and humans may become infected by ingesting eggs present in the feces of definitive hosts.[3] Once ingested, oncospheres hatch from the eggs, penetrate the intestinal wall, and travel via the circulation to target organs, including the brain, eyes, and muscle tissue.[3] Luckily, such transmission are rare, with only approximately 40 reported human cases; nevertheless, for those unlucky few, treatment requires invasive treatments with results as generally unsatisfactory as in other species.[3]

Conference discussion focused on the general approach to histologic evaluation of parasites. The moderator emphasized that the ability to recognize the broad category of parasite, such as cestode, trematode, or nematode, was often sufficient to get to a diagnosis when coupled with host species and anatomic location (this method is referred to, with disdain, by Dr. Christopher Gardiner, a PhD pathologist of great renown, as “playing the vet game”.

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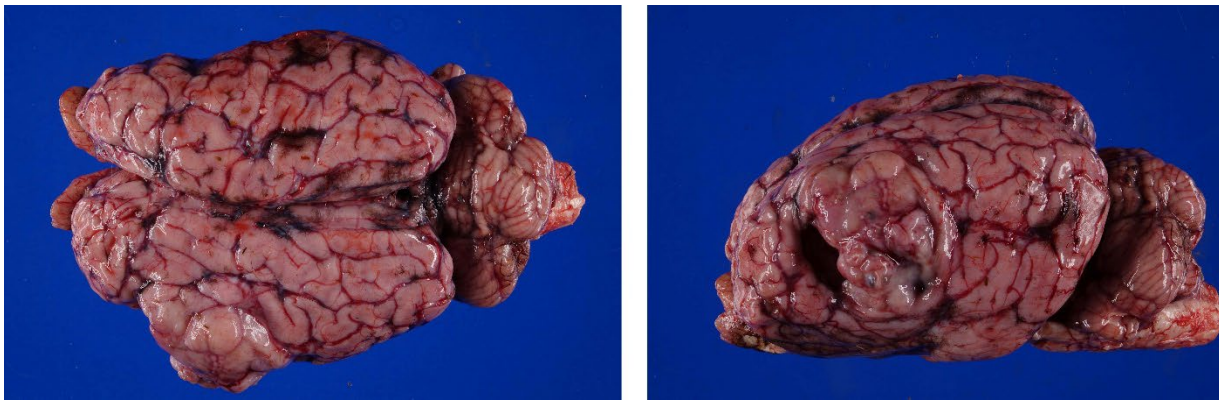

Figure 4-1. Cerebrum, alpaca. A 4.5cm mass is firmly adherent to the left dorsolateral hemisphere. (Photo courtesy of: Colorado State University College of Veterinary Medicine, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx)

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CASE IV:

Signalment:
8 year-old female alpaca (*Vicugna pacos*)

History:
The animal presented with a one-week history of lethargy, self-isolation from the herd, and progressive neurologic signs.

Gross Pathology:
Firmly adhered to the dura and compressing the left dorsolateral cerebrum, including the frontal, parietal and temporal lobes and causing rightward deviation of midline, is a 4.5 cm diameter, multilobulated, well demarcated, firm mass. On cut section the mass varies from pale to dark tan with multifocal regions of cavitation filled with clear, straw colored, viscous fluid. Additionally, there is moderate cerebellar herniation through the foramen magnum.

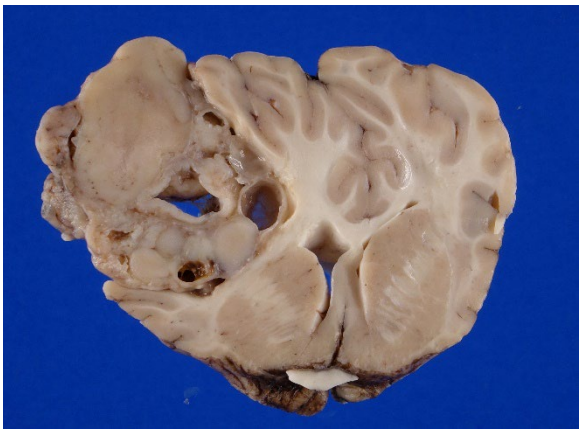

Figure 4-2. Cerebrum, alpaca. The mass in cut section infiltrates the underlying temporal lobe. (Photo courtesy of: Colorado State University College of Veterinary Medicine, http://csu-cvmbs.colostate.edu/vdl/Pages-/default.aspx)

Imaging Results:
On postmortem MRI, there is a large, broad-based mass in the mid aspect of the dorsal left calvarium causing marked rightward deviation and compression of the falx, left lateral ventricle, left frontal, parietal, and temporal lobes. The mass also causes mod-

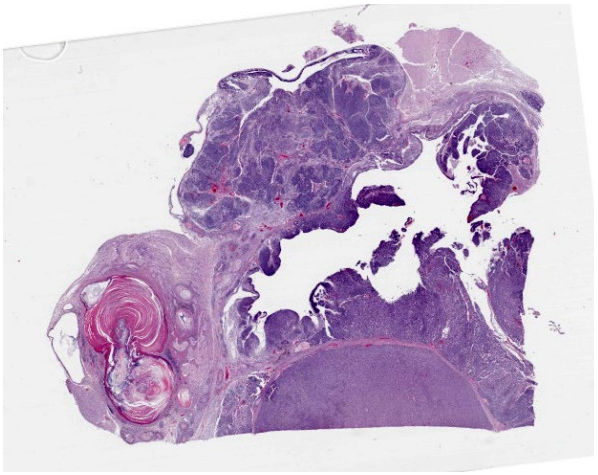

Figure 4-3. Cerebrum, alpaca. A heterogenous, multilobular, infiltrative and cystic mass effaces the neuroparenchyma. (HE, 5X)

erate dorsal compression of the left mesencephalon.

Microscopic Description:
Compressing the cerebrum is a well demarcated, densely cellular, multilobular neoplasm composed of several populations of primitive cells as well as tissue representing all three primordial germ cell lines. Primitive neuroectodermal cells are arranged in dense sheets, as well as pseudorosettes and occasionally rosettes. These primitive cells are often elongate with indistinct cell borders, a moderate amount of eosinophilic cytoplasm and ovoid to elongate nuclei. Anisocytosis and anisokaryosis in this population varies from mild to moderate and mitoses vary based of region. In more mitotically active regions, there are up to 15 mitoses in a single 400x field. Ectodermal components are comprised of well differentiated neurons associated with glial cells and neuropil. Additionally there are numerous cysts filled with lamellated keratin and keratin debris and lined by orderly stratified squamous epithelium. In a single keratin cyst, numerous cross sections of hair shafts are present (not available in all sections provided). Endodermal components are comprised of multifocal regions where neoplastic tissue forms variably sized cysts lined by a single layer of cuboidal to pseudostratified columnar epithelium. The apical surface of this epithelium occasionally has variably distinct, irregular cilia-like projections (respiratory epithelium). Mesodermal elements are comprised of nodules of mesenchymal cells (fibroblasts), as well as bands of smooth muscle adherent to the basal surface of both squamous and respiratory epithelial lined cysts. There is a single focus of well-differentiated cartilage (not present in all sections), as well as infrequent foci where there are individualized to small aggregates of adipocytes. The mitotic rate is low across all well-differentiated cell types. Adjacent normal neuropil is not present in all sections.

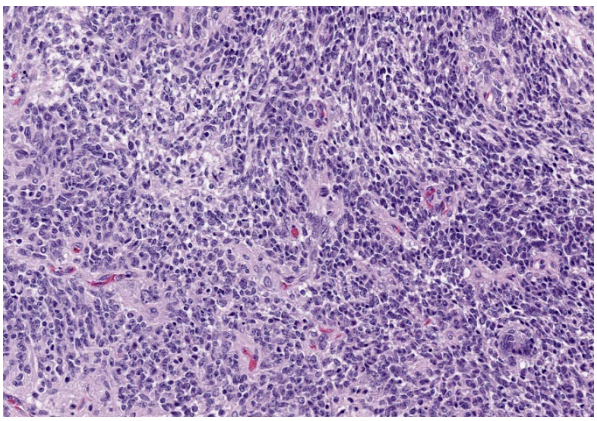

Figure 4-4. Cerebrum, alpaca. The predominant cell in the neoplasm is a poorly differentiated neuroectodermal cell which is arranged in nests and packets, sheets, streams, and occasional rosettes and pseudorosettes. (HE, 5X)

Immunohistochemistry for synaptophysin reveals primitive neuroectodermal cells that multifocally exhibit punctate to diffuse cytoplasmic immunoreactivity for synaptophysin. Neoplastic neurons and neuropil also exhibit strong cytoplasmic labeling for synaptophysin. Blood vessels, bands of smooth muscle dissecting between large nodules of

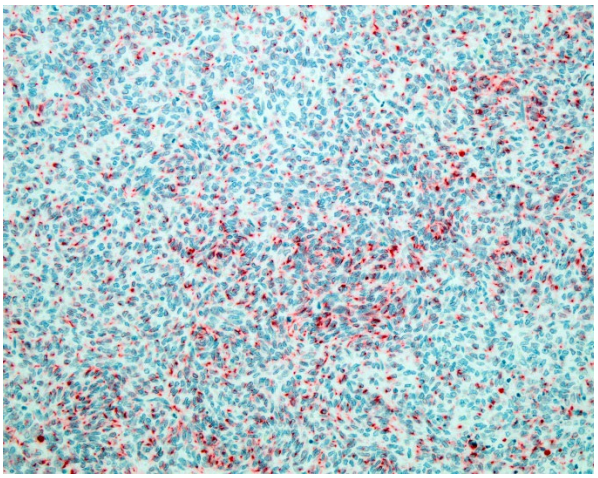

Figure 4-5. Cerebrum, alpaca: The predominant and primitive cell type is positive for cytokeratin. (anti-AE1/AE3, 200X) (Photo courtesy of: Colorado State University College of Veterinary Medicine, http://csu-cvmbs.colostate.edu/vdl/Pages/default.aspx)

primitive neuroectodermal cells, and smooth muscle adherent to the basal surface of epithelial lined cysts exhibits strong staining for smooth muscle actin. Several large nodules of primitive neuroectodermal neoplastic cells exhibit irregular, punctate cytoplasmic labeling with cytokeratin. Additionally, epithelium lining cysts exhibit strong cytoplasmic staining for cytokeratin. All neoplastic structures, except epithelial structures that are cytokeratin positive, have strong cytoplasmic staining for vimentin.

Contributor's Morphologic Diagnosis:
Cerebrum: Teratoma

Contributor's Comment:
In human literature, classification of teratomas histologically is divided into three categories: mature, immature, and malignant.[6] Mature teratomas are characterized by several types of mature, well-differentiated tissues, and immature teratomas are comprised of poorly differentiated, primitive tissues.[6] Both types are classically comprised of tissues from all three germ layers. Histopathology of teratomas is widely varied but distinct in complexity. Diagnosis is made when tissue types from two or more of the germinal layers are present. The three germinal layers and tissues representing each of them are as follows:[3,6]

- Mesoderm: notochord, musculoskeletal system (including bone and cartilage), muscular layer of stomach and intestine, circulatory and lymphatic systems, reproductive system (excluding germ cells), dermis of skin, adrenal cortex;

- Endoderm: epithelial linings (digestive tract, respiratory system, urinary system), liver, pancreas, epithelial component of the thymus, thyroid and parathyroid; and

- Ectoderm: epidermis of skin, sweat glands, hair follicles, epithelial lining of mouth and anus, cornea and lens, nervous system, adrenal medulla, tooth enamel, epithelium of pineal and pituitary glands.

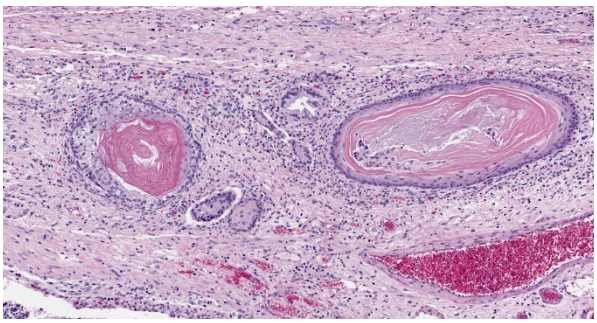

Figure 4-6. Cerebrum, alpaca. Ectodermal structures recapitulating hair follicles are scattered throughout the mass. (HE, 147X)

Teratocarcinomas (malignant variants) are, in both human and veterinary literature, very rare. These tumors contain somatic-type neoplastic components and, in people, the most commonly seen are rhabdomyosarcomas and

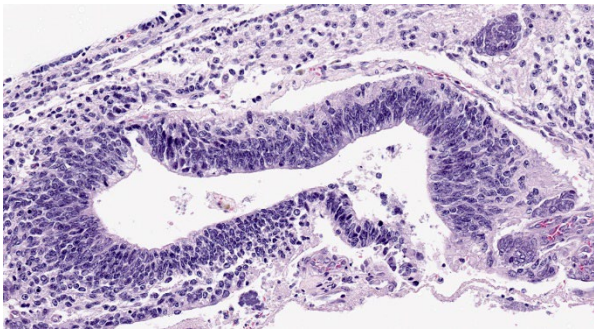

Figure 4-7. Cerebrum, alpaca. Pseudostratified epithelium recapitulates endodermal elements of gut or respiratory epithelium. (HE, 260X)

undifferentiated sarcomas.[6] Ultimately, a diagnosis of immature teratoma was given based on the high proportion of primitive neuroectodermal components.

In general, teratomas are benign tumors that most often arise in the gonads. In rare circumstances, however, they arise in extragonadal locations, including within the calvarium, and cause significant clinical disease secondary to local mass effect. A handful of reports exist documenting teratomas within the cranium in a variety of veterinary species including a rabbit, kitten, rat, dog, kestrel and an alpaca.[1,2,4,5,11] Other reports of nongonadal sites include adrenal teratomas in ferrets, a renal teratoma in a llama and retrobulbar teratomas in a cat, kestrel and great blue heron.[5,9,12,13,14]

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College of Veterinary Medicine
https://vetmedbiosci.colostate.edu/vdl/

JPC Diagnosis:
Cerebrum: Teratoma.

JPC Comment:
With a name derived from the Greek words "teras," meaning monster, and "onkoma" meaning swelling, teratomas have been objects of fascination and speculation for centuries, likely due to their startling propensity to produce a disorganized jumble of anatomic structures such as hair, teeth, and eyes.[10] Historically, teratomas have often been interpreted as evidence of Satanic possession or all manner of salacious activities, and all of this attention had an unexpected benefit: research surrounding this fascinating entity led fairly directly to the discovery of embryonic stem cells in the late twentieth century.[7]

As the contributor details, teratomas are distinguished from other tumors by the presence of tissue from multiple embryonic germ layers. This developmental plasticity is shared with embryonic stem cells which are defined by their pluripotency and capacity for self-renewal. In the last thirty years, tremendous strides have been made in developing pluripotent and induced pluripotent human stem cell lines which hold the tantalizing possibility of revolutionizing regenerative and transplant medicine.

A definitional concern when developing stem cell lines is how to test for pluripotency. This can be done by a variety of in vitro and in vivo measures; however, the gold standard method to confirm developmental pluripotency is the in vivo "teratoma assay."[8] The assay involves injecting presumptively pluripotent stem cells into immunodeficient mice, where, if truly pluripotent, they develop teratomas. These tumors are allowed to grow and are then removed and analyzed histologically to ensure that the tumor produced cell populations from all three developmental layers.[8] The teratoma assay also allows pathologists to assess whether the resulting mass contains malignant elements which may increase the incidence of stem cell-induced malignancies, a current roadblock to more widespread implementation of clinical trials for stem cell therapies.[8] While ethical and humane questions surround the assay and alternatives to

its use are being actively sought, the once-maligned teratoma has, for now, found new work as a cornerstone of the embryonic stem cell revolution.

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