Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2013-2014

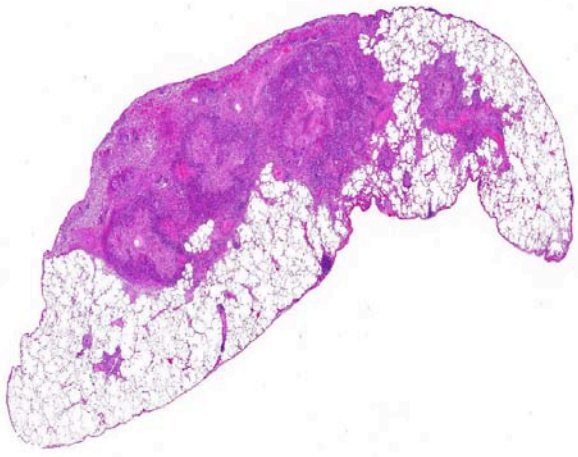
Conference 14

5 February 2014

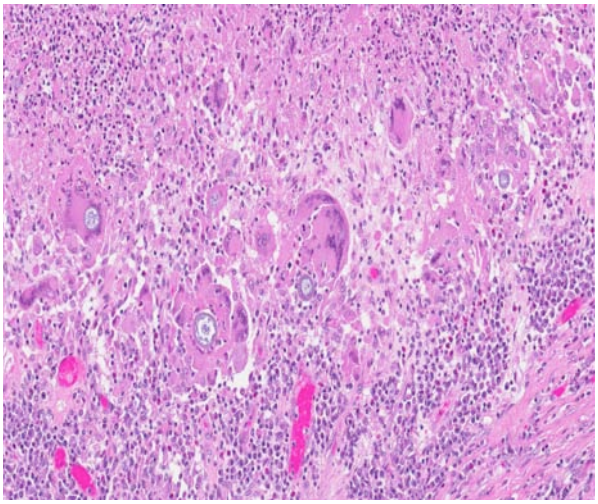
CASE I: 120657-05 (JPC 4032267).

Signalment: Adult male cynomolgus macaque (*Macaca fascicularis*).

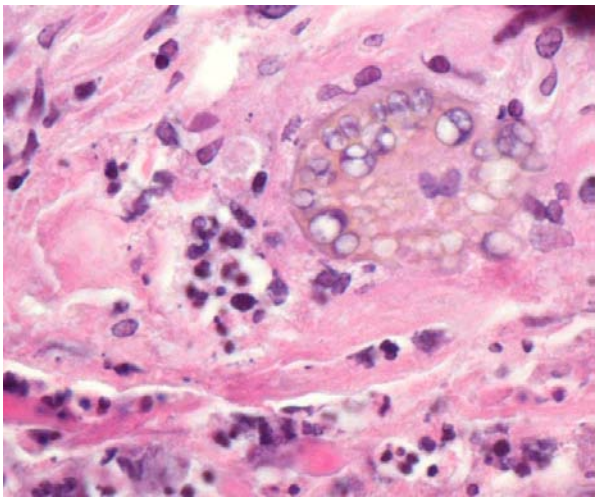
History: This monkey was procured from a national laboratory animal supplier and was part of a study to characterize infectivity and disease progression of monkeypox virus. Before virus challenge, there were no significant clinical signs noted. This monkey received an aerosolized dose of 1,608 plaque-forming units (PFU) of monkeypox virus, survived to the end of the study, and was euthanized at day 29 postinfection (PI). Clinical changes were evident by day 4. PI and key abnormalities included increases in body temperature, lymphadenopathy, and relatively mild cutaneous pox lesions. All noted abnormalities are attributable to the monkeypox infection and are typical of aerosol monkeypox



1-1. Lung, cynomolgus macaque: Approximately 50% of the section is effaced by multifocal to coalescing foci of pyogranulomatous inflammation. (HE 0.63X)



1-2. Lung, cynomolgus macaque: Inflammation is centered on numerous intra-and extracellular fungal sporangia ranging from 40-80 µm in diameter. Numerous foreign body and Langhans-type giant cell macrophages are present within the inflammatory foci. (HE 150X)



1-3. Lung, cynomolgus macaque: Mature sporangia rupture within the lesion, releasing endospores. (HE 400X) (Photo courtesy of: Pathology Division, USAMRIID, Building 1425, Fort Detrick, MD 21702 http://www.usamriid.army.mil/)

exposure.[11] No clinical respiratory signs were noted before or after viral challenge. This macaque was sourced from China and was quarantined in Texas before being transported to the contributing institute in Maryland.

Gross Pathology: Few faintly visible resolving pox lesions which consisted of 1 to 2 mm whitish/brown discolored spots were present on the skin of the axillary and inguinal areas. The lung lobes on the left side were diffusely very firm, while the right lobes contained few 5-10 mm pale firm foci affecting 10% of the apical and diaphragmatic lobes. All examined lymph nodes showed mild enlargement, and the spleen had marked enlargement with rounded edges and prominent white pulp on cut surface.

Laboratory Results: Hematology and clinical chemistry were performed before aerosol challenge and every 48 h after aerosol challenge; all results were unremarkable.

All tissues were evaluated with immunohistochemistry for orthropoxvirus antigen and all tissues were immunonegative suggesting either viral clearance (consistent with this animal's survival) or that the amount of antigen is too low for detection by immunohistochemistry.

Histopathologic Description: In 30% of the lung section are multifocal areas of granulomatous inflammation that obscure pulmonary architecture, fill the alveoli and bronchiolar lumina, and are composed of numerous multinucleate giant cells, admixed with lymphocytes, plasma cells, histiocytes, and viable and degenerate neutrophils and eosinophils. The bronchioles and alveoli are often dilated and filled with inflammatory cells, cellular debris, edema, and moderate numbers of fungal spherules that are 25-30 microns in diameter with a 2-3 micron wall. The spherules rarely undergo endosporulation and are filled with endospores that are 2-5 microns in diameter. The spherules are often contained within the cytoplasm of the multinucleate giant cells or are degenerate. There is hyperplasia of the epithelium lining the bronchioles with piling up and crowding of nuclei. Multifocally the alveoli are lined by plump cuboidal pneumocytes (type 2 pneumocyte hyperplasia). Multifocally there are peribronchiolar aggregates of histiocytes that contain a dark brown refractile material (anthracosilicosis).

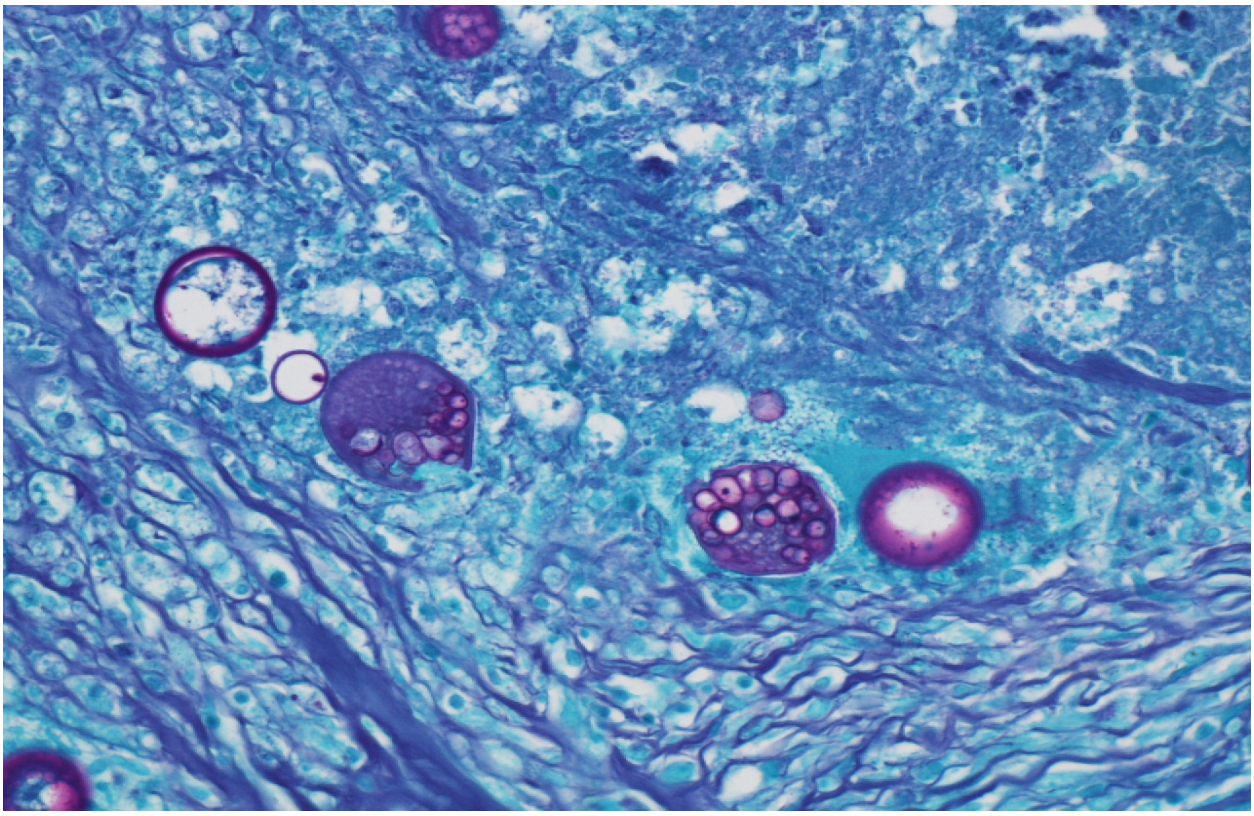
The periodic acid-Schiff (PAS) reaction revealed that each of the spherules had a PAS positive wall.

Similar granulomatous inflammation and fungal spherules are present within the tracheobronchial and mediastinal lymph nodes (not submitted).

Contributor's Morphologic Diagnosis: Lung: Pneumonia, pyogranulomatous, chronic-active, multifocal, marked with large numbers of multinucleate giant cells, epithelial hyperplasia, and moderate numbers of fungal spores consistent with *Coccidioides* sp.

Contributor's Comment: This is a case of coccidioidomycosis based on morphology of the spherules and character of the gross and histologic lesions. Electron microscopy was performed on the lung tissue for characterization of the fungal spores. Mature and immature spherules were identified. The spherules measured approximately 15-20 microns in diameter. Mature spherules contained endospores and immature spherules lacked endospores and contained a 1 micron-thick electron dense wall. These findings support the diagnosis of coccidioidomycosis.

Coccidioidomycosis is a fungal disease found only in the Western Hemisphere in semi-arid regions known as the Lower Sonoran Life Zone. This zone within the United States encompasses



1-4. Lung, cynomolgus macaque: Staining with periodic-acid Schiff stains highlight the positive cell-wall and endospores. (PAS 400X) (Photo courtesy of: Pathology Division, USAMRIID, Building 1425, Fort Detrick, MD 21702 http://www.usamriid.army.mil/)

the southern parts of Texas, Arizona, New Mexico and much of central and southern California.[12,15] Endemic regions outside of the United States include semiarid regions of Mexico, especially northern Mexico, as well as smaller endemic foci within Central and South America.[15] It is caused by a geophilic dimorphic fungus of which two nearly identical species are recognized, *Coccidioides immitis* and *Coccidioides posadasii*. *C. posadasii* was recently proposed as a new species based on genetic and phenotypic analysis,[12] and is generically known as the non-California species with a geographically unique distribution. *C. immitis* is restricted to the endemic areas of California while *C. posadasii* is found outside of California.[15] In this case, *Coccidioides posadasii* is the most likely etiologic agent based on the travel history of this primate.

Infection with *Coccidioides* sp. occurs primarily via inhalation of arthroconidia (also called arthrospores) that are aerosolized when the soil is disturbed by wind or human activities. After inhalation, the arthroconidia enlarge and become spherules that eventually undergo endosporulation. After endosporulation, the spherules rupture releasing hundreds of endospores into the surrounding tissue. These released endospores also mature into new spherules, and the cycle continues until host control is achieved.[14]

Coccidioides spp. appear capable of infecting all mammals and at least some reptiles, but it has not been reported in avian species. The disease has been reported in several species of primates including lemurs, chimpanzees, gorillas, macaques, and baboons,[1-4,16,19,21] and many of these reports describe disseminated disease. As a group, primates appear particularly susceptible.[21]

In dogs, the majority of infections are limited to the lungs and associated hilar lymph nodes. In 20% of recognized infections in dogs and 50% of infections in cats there is dissemination to other sites. In dogs, the most common sites of dissemination are the bones, joints, and lymph nodes, while in cats, the skin is the most common site of dissemination.[14] The sites of dissemination in non-human primates seems to follow a similar pattern as dogs and sites reported in the literature include the eye, bone, and esophagus.[2,4,5,16,18]

Pulmonary and disseminated histopathologic lesions consist of pyogranulomas or granulomas. There are frequently aggregates of intermixed neutrophils resulting from the initial reaction to the endospores released from matures spherules. The lesion forms a granuloma or pyogranuloma as it matures, and is composed of the common components of a granulomatous reaction including giant cells.[6]

In this case, there were no clinical signs that were attributed to coccidioidomycosis despite the extensive granulomatous inflammation in the examined sections. This finding of a subclinical infection is not surprising provided that serologic surveillance studies have shown that asymptomatic infections represent a high percentage of infections in humans and other animals. In a survey of dogs in Arizona, 70% of dogs with seropositivity were subclinically infected and exhibited no sign of disease.[20] Converse and Reed showed that 100% of naturally exposed monkeys developed subclinical infections after being contained in outdoor housing in a river basin for a year in an endemic region (Tucson, Arizona). None of these monkeys exhibited clinical signs of disease, but all were positive by a coccidiodin skin test and complement fixation test; while 40% of the exposed monkeys had histological lesions.[10] A survey of nonhuman primates housed outdoors at the California Primate Research Center in 1977-78 showed that four out of 119 (3%) primates were seropositive; the seropositive cases were attributed to a dust storm that affected the area because there were no positive cases when they were surveyed before the dust storm.[1] In humans, approximately 60 percent of infected persons are asymptomatic; the remainder can develop manifestations that range from mild to moderate influenza-like illness to pneumonia. Overall, less than 5 percent of infected persons have progressive pulmonary infection or extrapulmonary dissemination of the disease.[17]

Additionally in humans, coccidioidal pneumonia may be associated with erythema nodosum, especially in females.[17] Erythema nodosum is characterized by panniculitis of the lower extremities, especially over the shins, and is due to an immunologic response to a variety of causes. A similar condition has not been described in the reports of coccidioidomycosis in animals.

We can only speculate on the source of infection in this case, but this monkey most likely inhaled dust-borne arthrospores when it was in Texas; this would mean that the fungus is *C. posadasii*. Research by Converse and Reed showed that infection can be established after aerosol exposure of rhesus macaques to as few as 10 arthrospores.[10]

In the last decade, the number of reported cases in humans has increased, but the reason for this increase is uncertain. A multitude of contributing factors have been identified and include increased population in the endemic area, climactic change (drought and increasing temperatures in southwestern United States), dust storms, soil disturbance caused by increased construction activity, growing numbers of persons who are immunocompromised or have other risk factors for severe disease, and immigration of previously unexposed persons from areas where coccidioidomycosis is not endemic.[7,8]

Antemortem diagnosis of coccidiomycosis in animals is primarily done with serology and uses the same reagents and controls as for serodiagnosis of humans. There is overlap of seropositivity with clinical disease and subclinical disease in dogs, but seropositivity appears to correlate well with clinically important disease in cats.[14]

Morphology of the *Coccidioides* spherules in histological sections is fairly unique. Other fungal organisms with similar but distinguishable morphology include *Emmonsia* spp., *Blastomyces dermatitidis*, and *Rhinosporidium seeberi*. The major distinguishing factor of coccidial spherules is the formation of thick walled endospores within the coccidioidal spherule as it matures. *Blastomyces dermatitidis* does not form endospores but forms broad-based buds and is smaller than mature coccidial spherules. *Rhinosporidium seeberi* forms endospores that have thin walls. In addition, rhinosporidial sporangia are much larger than coccidioidal spherules. *Emmonsia* spp. have the closest morphology but do not form thick-walled endospores, but rather form fruiting bodies that line the interior of the wall and form a honeycomb pattern which are much less distinct than those of *Coccidioides* spp. Additionally, *Emmonsia* spp. adiaspore walls are thicker than *Coccidioides* sp.[6] Fungal culture, immunohistochemistry (IHC), polymerase chain reaction (PCR), and in situ

hybridization (ISH) are other methods to provide an unequivocal diagnosis. Fungal cultures must be treated with precaution because the infectious arthroconidia may develop after incubation at room temperature.[14]

Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

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Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

JPC Diagnosis: Lung: Pneumonia, pyogranulomatous, chronic-active, multifocal to coalescing, marked, with type II pneumocyte hyperplasia and intrahistiocytic endosporulating yeasts.

Conference Comment: The contributor provides an excellent review of coccidiomycosis in domestic and non-domestic species. Conference participants further explored the pathogenesis of this condition with a brief discussion of TH1 type cell mediated immunity (see WSC 2013-2014 conference 2 case 2). In addition to abundant pyogranulomatous inflammation, generally centered upon airways, there are discrete foci of perivascular inflammation, which are attributed to the migration of inflammatory cells from the circulation into infected tissue that occurs as part of the leukocyte adhesion cascade (see WSC 2013-2014 conference 6, case 2). The multifocal, marked type II pneuomocyte hyperplasia noted by the contributor is confirmed by immunohistochemical staining with TTF1, which, besides staining thyroid follicular cells, also stains the nuclei of type II pneumocytes and Clara cells.[11] Electron microscopy can also assist in identifying type II pneumocyte hyperplasia, as these cells typically contain characteristic lamellar inclusions.[9]

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CASE II: SN 11-1511 (JPC 4007165).

Signalment: 2 to 3-year-old male cynomolgus macaque (*Macaca fascicularis).*

History: This monkey was part of a study investigating candidate renal biomarkers. It received seven daily IV doses of two potential renal toxicants, everninomicin (30 mg/kg) and gentamicin (10 mg/kg). The study included pre- and post-dose monitoring of serum and urine chemistry with necropsy and histologic examination. Examined tissues included adrenal glands, kidneys, testes, heart, liver, skeletal muscle and urinary bladder at the end of a one week dosing period. Gentamicin is an aminoglycoside antibiotic for the treatment of gram negative bacterial infection that can induce proximal tubular necrosis.[2,5] Everninomicin is an experimental oligosaccharide antibiotic that has a renal toxicity profile that is similar to gentamicin.[3]

Laboratory Results: Serum and urine chemistry were tested twice prior to dosing (6 and 1 day prior to dosing) and 3 times during the week of dosing (post-dose days 3, 6 and 8). Values for the five time points are below. Standard units of measure and abbreviations were used unless otherwise noted. NS = not significant.

Clinical Chemistry:

Parameter	Values
BUN	125, 118, 101, 123, 89
CREA	125, 118, 101, 123, 90
ALT	125, 118, 101, 123, 91
AST	125, 118, 101, 123, 92
AP	125, 118, 101, 123, 93
GGT	125, 118, 101, 123, 94
T BILI	125, 118, 101, 123, 95
TP	125, 118, 101, 123, 96
ALB	125, 118, 101, 123, 97
GLOB	125, 118, 101, 123, 98
A/G Ratio	125, 118, 101, 123, 99
CHOL	125, 118, 101, 123, 100
TRIG	41, 31, 41, 41, 50
Ca	9.7, 9.7, 10.1, 10.0, 9.2
PHOS	6.8, 6.2, 6.9, 4.3, 4.6
Na	141, 143, 142, 141, 137
K	4.4, 4.3, 4.5, 3.4, 3.1
Cl	107, 107, 106, 110, 111

Urinalysis:

Parameter	Values
pH	8.5, 8.5, 8., 8.5, 7.5
Protein	neg, neg, neg, trace, 3+
Glucose	neg, 1+, 2+, NS, NS
Ketones	1+, neg, neg, trace, 2+
Bilirubin	neg, neg, neg, neg, neg
Blood	neg, neg, 1+, neg, neg
Specific Gravity	1.019, 1.018, 1.018, 1.015, 1.017
CREA	103.6, 104.3, 87.6, 52.0, 67.1
N-acetyl glucosaminidase (NAG)	7.7, 7.2, 23.3, 28.0, 30.4
Microalbumin	NS, NS, 7.2, 103.6, 143.3
Total urine volume (ml/ day)	71.0, 65.0, 81.0, 127.0

Gross Pathology: None.

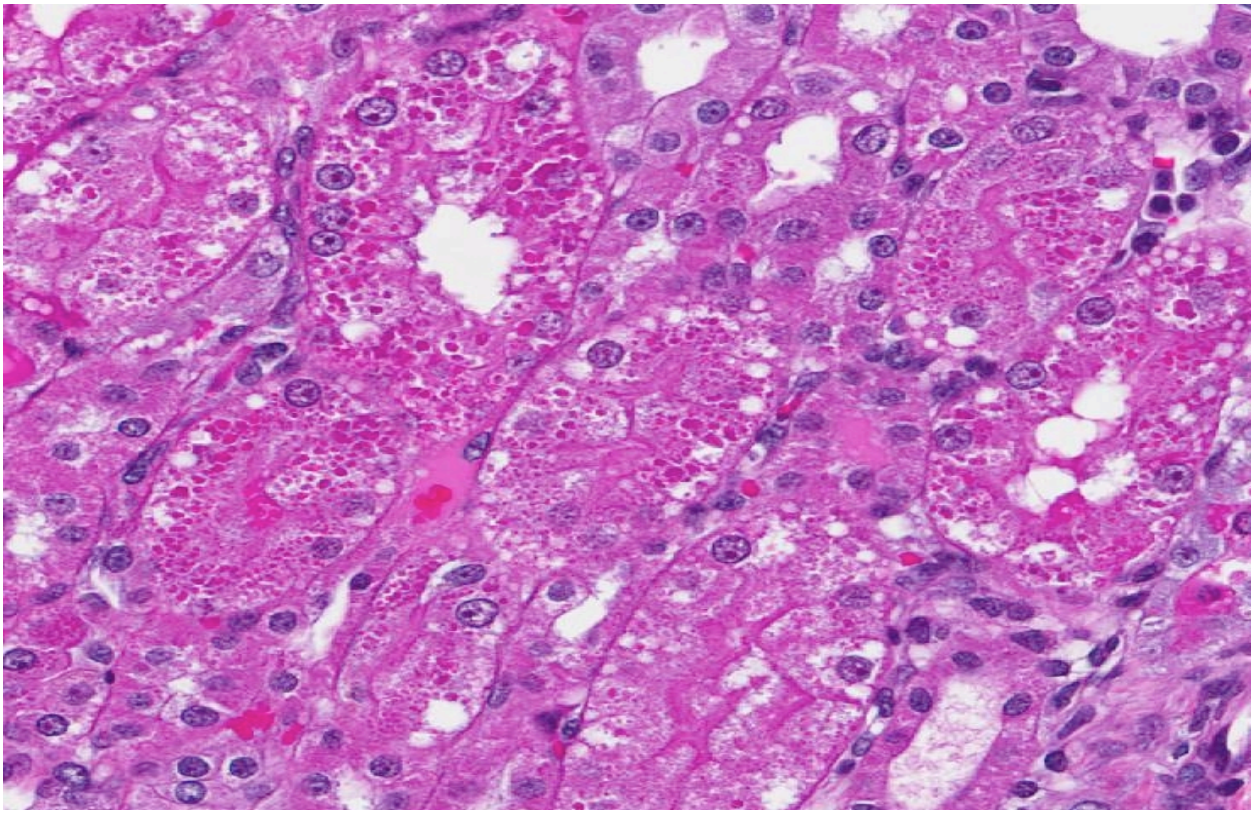
Histopathologic Description: There were everninomicin- and gentamicin-related kidney histologic changes that correlated with serum and urine chemistry changes. There were minimal casts, mild regeneration and marked degeneration. Degeneration was characterized by proximal tubule epithelial cell swelling, cytoplasmic hyaline droplets, epithelial sloughing and epithelial cell necrosis. Regeneration included tubule epithelial cell cytoplasmic basophilia, crowding and slight increases in mitotic figures. There was also tubular dilatation with occasional scattered mononuclear cell accumulations in interstitium adjacent to degeneration or regeneration in some but not all sections.

Contributor's Morphologic Diagnosis: Kidney: Subacute diffuse renal proximal tubular degeneration and necrosis with regeneration.

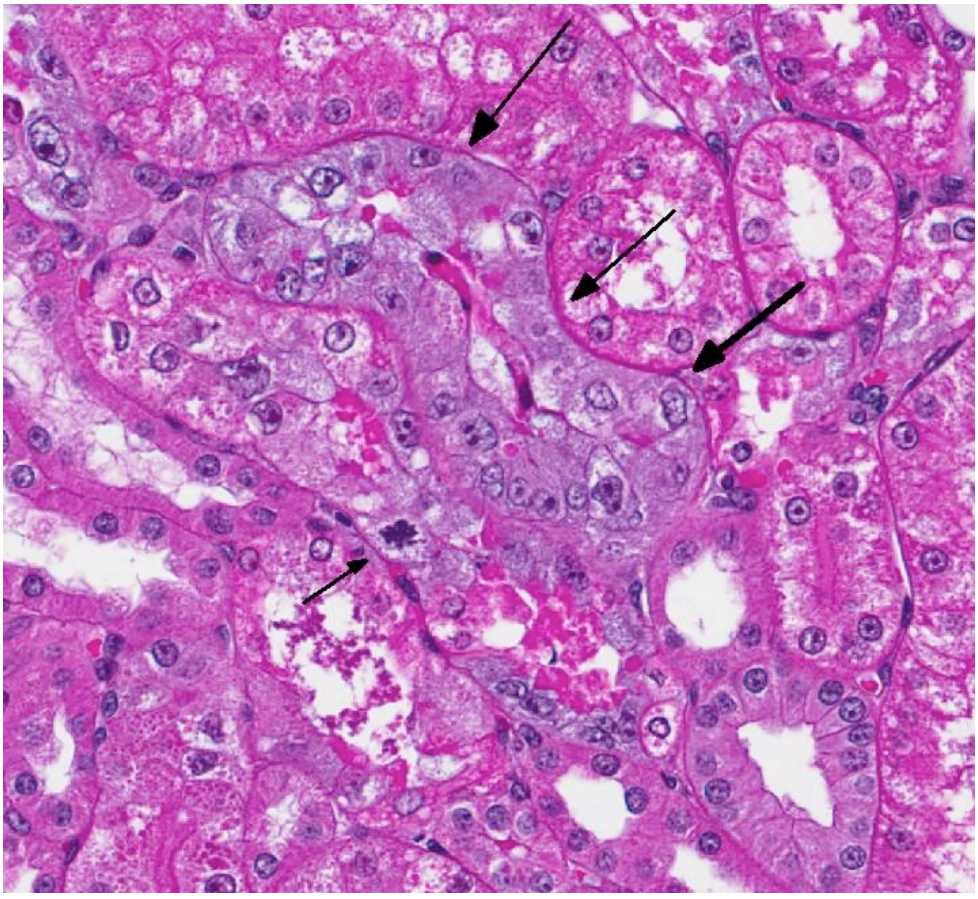
Contributor's Comment: Aminoglycosides cause acute renal failure by accumulating in proximal tubular epithelial cells where they damage mitochondria, ribosomes, and other intracellular components.[3] Proximal tubules resorb sodium, chloride, calcium, glucose, amino acids from glomerular ultrafiltrate.[3,8] Insufficient resorption following loss of proximal tubular epithelium can cause hyperkalemia, sodium alterations, hyperphosphatemia, azotemia, acid-base disturbances, enzymuria, cylinduria oliguria, isosthenuria and/or anuria.[3] A concise yet comprehensive review of renal physiology and antibiotic-induced renal failure can be found at http://www.vet.uga.edu/VPP/clerk/Matthews/index.php.[6]

JPC Diagnosis: Kidney, proximal convoluted tubules: Degeneration, necrosis and regeneration, diffuse, marked, with numerous cytoplasmic protein droplets.

Conference Comment: The primary function of the renal glomerulus is blood filtration, while the proximal convoluted tubules are predominantly involved with absorption; in fact, sixty percent of all reabsorption of the filtered solute occurs in the proximal tubules. They are responsible for



2-1. Kidney, cynomolgus macaque: A characteristic finding in cases of aminoglycoside toxicity is degeneration of tubular epithelium with formation of brightly eosinophilic hyaline droplets. The droplets represent concentric multilaminated phospholipid membrane whorls in the phagolysosome (myeloid bodies). (HE 288X)



2-2. Kidney, cynomolgus macaque: Regenerative changes within proximal tubular epithelium (large arrows) include cytoplasmic basophilia, vesicular nuclei with prominent nucleoli, and mitotic figures (arrow). (HE 288X)

reabsorbing water, glucose, sodium, chloride, amino acids and calcium from glomerular ultrafiltrate; thus damage can result in the clinicopathologic findings enumerated above.[3] There are two main types of acute tubular necrosis: nephrotoxic and ischemic. Antimicrobials such as aminoglycosides (considered obligate nephrotoxins), heavy metals, chemotherapeutic agents and other nephrotoxins damage the tubular epithelium while sparing the basement membrane.[6] In contrast, renal ischemia is usually a consequence of hypotension; it is characterized histologically by tubular epithelial necrosis or apoptosis with disruption of the basement membrane.[6] In this case, the presence of an intact tubular basement membrane may be demonstrated with the periodic acid-Schiff stain, supporting a toxic etiology rather than an ischemic insult.

Considerable discussion surrounded the pathogenesis of aminoglycoside nephrotoxicity in animals. Common aminoglycosides employed in veterinary medicine (in decreasing order of nephrotoxicity) include neomycin, kanamycin, gentamicin, streptomycin, tobramycin and amikacin. Foals are particularly susceptible to aminoglycoside-induced nephrotoxicosis, while in cats, this class of drugs has been associated with ototoxicity as well as nephrotoxicity. Aminoglycosides typically accumulate within proximal tubular epithelial cell lysosomes and damage is thought to be dose-dependent. They induce tubular damage via a number of ways: destruction of the sodium-potassium pump with subsequent oncotic necrosis; inhibition of phospholipase with accumulation of lysomosmes filled with cellular membranes ("myeloid bodies") impairment of mitochondrial respiration and cation transport; inhibition of protein synthesis; and loss of the epithelial brush border.[4,6,7]

In addition to the histological features described by the contributor, some conference participants noted that epithelial cells lining medullary collecting tubules are occasionally multinucleated, bulging into the tubular lumen. This is considered to be an incidental finding in cynomolgus macaques that is thought to be either a normal anatomic variation in this species or at best, a minor finding of little pathological significance.[1]

Contributing Institution: www.merck.com

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CASE III: PO-507/13 (JPC 4035680).

Signalment: 3-year-old female mixed breed pig (*Sus scrofa domesticus*).

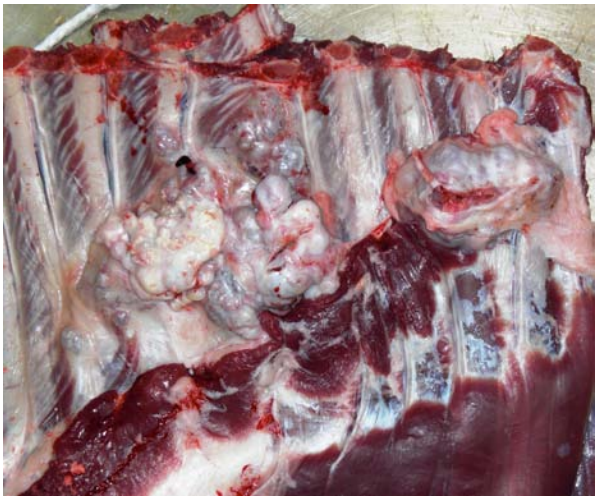
History: The animal was slaughtered in an officially inspected abattoir in Catalonia (Spain).

Gross Pathology: Two prominent exophytic, multinodular, cauliflower shaped masses of 4 and 5 cm in diameter were noticed in the inner side of the thoracic cavity arising from the rib surface. The masses had a smooth surface and were attached with a broad sessile base to the costal body of the ribs. The masses were solid, had a hard consistency and at cross section had bluish to white areas. During the inspection, no other abnormalities were observed in the carcass or in the internal organs of the animal.

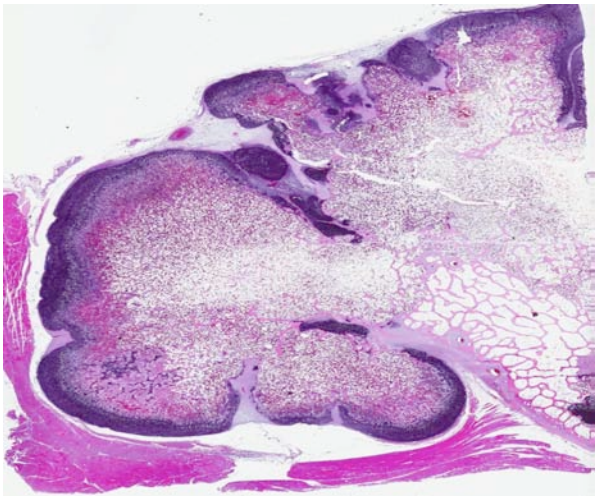
Histopathologic Description: Rib, transverse section: There is an exophytic, broad based, multinodular, well demarcated, expansive, and non-encapsulated neoplastic proliferation arising from the rib poriosteum. Every nodule is lined by fibrovascular stroma of variable thickness (perichondria). Neoplastic cells are well-differentiated chondrocytes enmeshed within an abundant hyalinized amphophilic extracellular matrix. They are arranged from the periphery to the center in well-defined layers mimicking growth plate endochondral ossification. The outer layer is made up of multiple small groups of chondrocytes with high nucleus/cytoplasm ratio (resting layer). Secondly, there is a thicker layer constituted by larger chondrocytes forming columns (proliferative layer), and a deeper layer where chondrocytes undergo hypertrophy (hypertrophic layer). Underneath the hypertrophic layer, the cartilage matrix is replaced by hyaline, eosinophilic osteoid matrix (calcification layer) and subsequently, there is loss and replacement of the chondrocytes by osteocytes. This is partially lined by osteoclasts and rows of plump osteoblasts. In the center of the nodule, several septa of mature trabecular bone are seen. Amongst the septa, bone marrow with a heterogeneous population of hematopoietic precursors and scattered adipocytes are present.

Contributor's Morphologic Diagnosis: Rib osteochondroma.

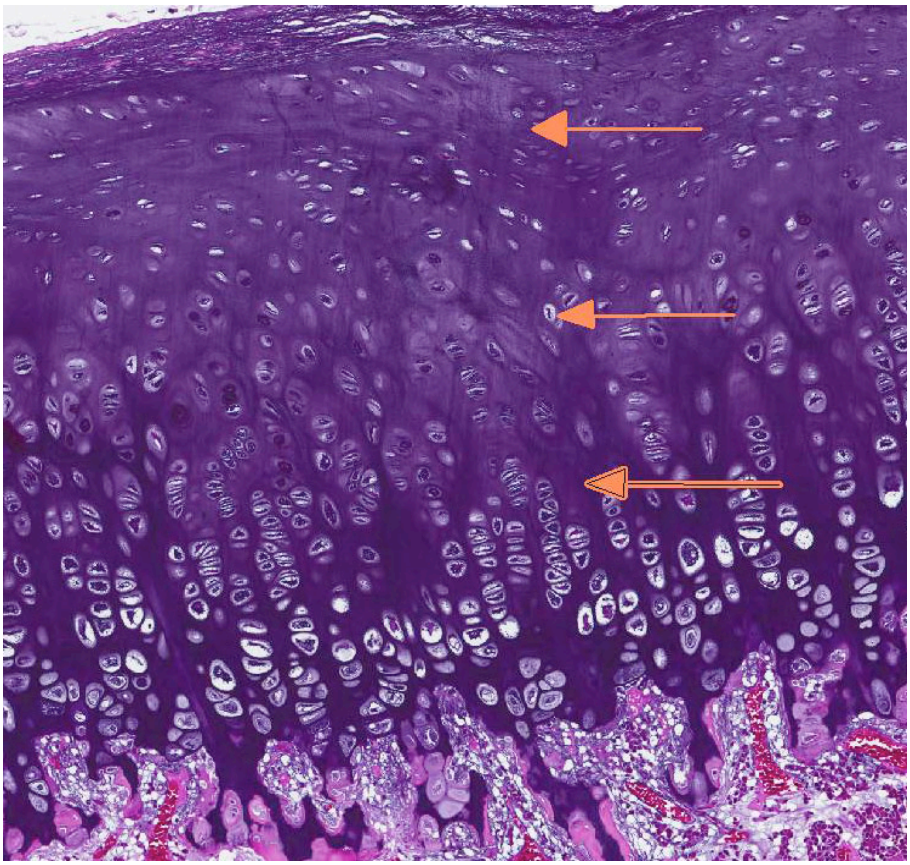
Contributor's Comment: An osteochondroma is a benign, cartilage-capped tumor arising from the surface of bones formed by endochondral ossification. Osteochondromas may occur in two forms: solitary or multiple.[2,5] This condition is usually recognized as an incidental finding during routine controls, radiographic examination or at necropsy. Occasionally, clinical signs might occur and are due to compression or distortion of adjacent structures. Osteochondromatosis is infrequently reported in humans, horses, dogs, a macaque and cats.[2,3,6] In the present case, considered differential diagnoses included chondroma and chondrosarcoma. Histologically,



3-1. Rib, pig: Two prominent exophytic, multinodular, cauliflower shaped masses of 4 and 5 cm in diameter were noticed in the inner side of the thoracic cavity arising from the rib surface. (Photo courtesy of: Servei de Diagnostic de Patologia Veterinaria, Facultat de Veterinaria, Bellaterra (Barcelona), 08193 SPAIN)

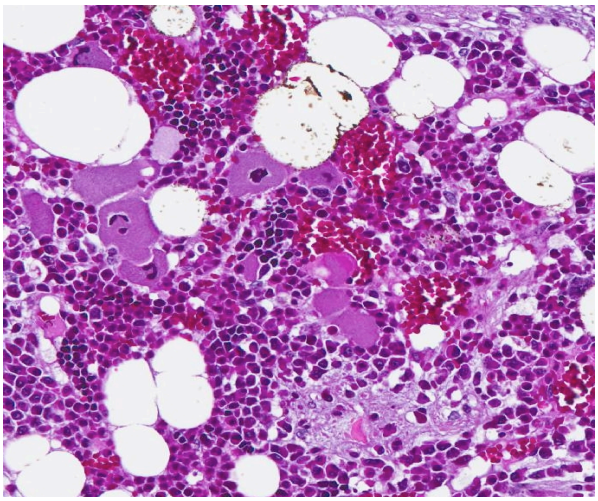


3-2. Rib, pig: A multilobular fungating bony mass covered by hyaline cartilage arises from the periosteum of the rib and communicates with the medullary cavity of the rib. (HE 0.63X)



3-3. Rib, pig: The hyaline cartilage is arranged in a zonal fashion similar to physeal cartilage – the resting zone (top arrow), zone of proliferation (middle arrow), and hypertrophic zone (bottom arrow). (HE 60X)

chondroma consists of irregular lobules of hyaline cartilage which may also show foci of endochondral ossification and mineralization.[7,8] However, chondroma misses the growth plate-like organization of the cartilaginous matrix that characterizes osteochondroma. Malignant transformation of osteochondroma to chondrosarcoma has occasionally been described in older dogs and humans. Histologically osteochondroma can be very difficult to differentiate from low grade chondrosarcoma, which may show few indications of malignancy and may closely resemble benign tumors of cartilage.



3-4. Rib, pig: Marrow spaces within the bony masses are filled with trilinear erythropoietic marrow. (HE 400X)

JPC Diagnosis: Rib: Osteochondromas (multiple cartilaginous exostoses).

Conference Comment: Osteochondroma are typically continuous with the marrow cavity of the underlying bone, a feature which is helpful in differentiation from other proliferative or neoplastic bone lesions.[8] This lesion is relatively uncommon in swine, however it occurs fairly frequently in dogs and horses, where it is inherited in an autosomal dominant pattern and typically arises from scapula, ribs, vertebrae, and pelvis of young animals.[1] Although the underlying genetic defect in these species is unknown, it likely involves the perichondrial ring. Since enlargement ceases at the time of physeal closure, and the cartilage cap is eventually replaced by bone in an orderly fashion, there is some debate regarding its classification; many do not consider osteochondroma to be a true neoplasm, but rather a skeletal dysplasia.[8] As a result, the terminology associated with osteochondromas can be somewhat confusing. Osteochondromatosis is also known as multiple osteochondromas, multiple hereditary exostoses, multiple cartilaginous exostoses, multiple osteochondromatosis, diaphyseal aclasis, and hereditary chondrodysplasia.[4]

In contrast, feline osteochondromatosis occurs in older animals and primarily affects

intramembranous flat bones, while long bones are seldom affected. The lesion does not generally communicate with the marrow cavity of the underlying bone. Cells may appear somewhat atypical and pleomorphic, and growth tends to be progressive, with the potential for malignant transformation and metastasis. Thus feline osteochondromatosis is more consistent with a true neoplasm and has a more guarded prognosis. Inheritance does not appear to play a role in the pathogenesis; instead, viral particles resembling feline leukemia virus (FeLV) have been demonstrated within the proliferating cells of the hyaline cartilage cap in these tumors.[1]

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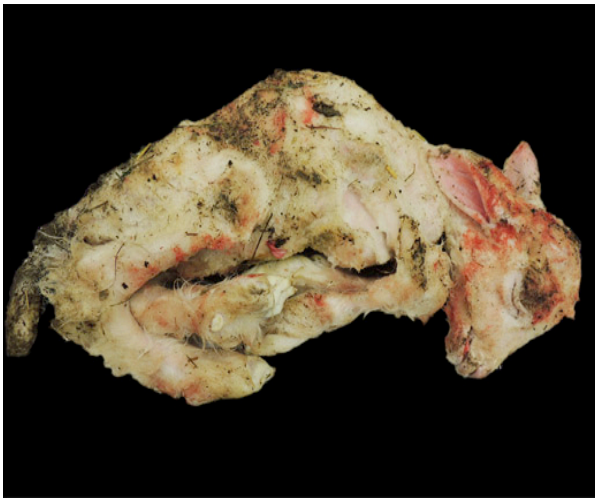
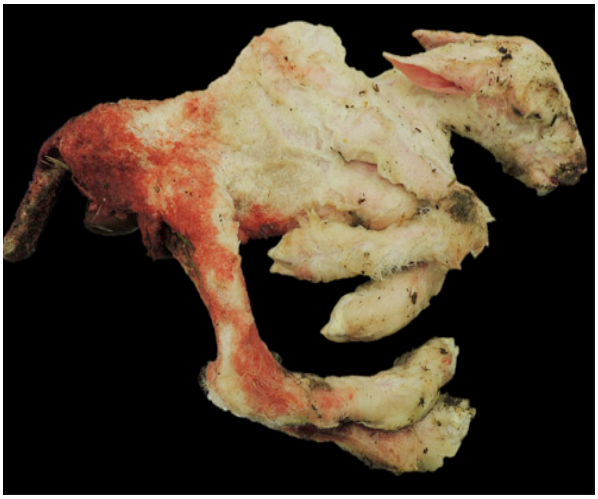
CASE IV: 13-6408 (JPC 4033119).

Signalment: One of twin male and female lambs stillborn at term from a white, mixed breed ewe (*Ovis aries*).

History: Twin stillborn lambs were received from a property in New York state housing 65 adult sheep purchased in November 2011. They began lambing in December 2012 and of the 22 lambs dropped by 14 ewes between December and early January, 12 were stillborn, some of which had deformities of the spine and limbs. Ten were born healthy and one was born deformed but alive. There were no clinical signs reported in the ewes.



4-1. Top, fetus A exhibits arthrogryposis, bottom, Fetus B demonstrates kyphosis as well as arthrogryposis. (Photo courtesy of: Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, S2-121 Schurman Hall, Ithaca, NY 14850 http://www.vet.cornell.edu/biosci/pathology/services.cfm)



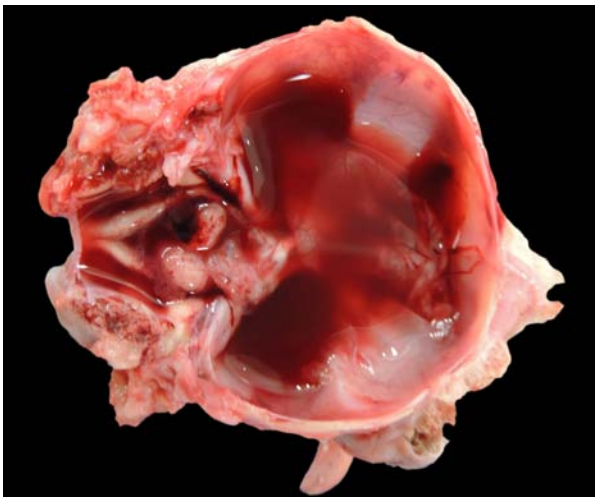
Gross Pathology: In one lamb, there was contraction of the elbow, stifle, carpal and tarsal joints (arthrogryposis). There was moderate lateral and dorsal deviation of the thoracic spine (scoliosis and kyphosis, respectively) and the sternum was curved dorsally. In the skull, the right orbit was located more rostroventally compared to the left orbit and there was deviation of the mandible to the right of the maxilla. The calvarium contained a thin membrane of cerebral cortex surrounding abundant clear, lightly red, thin fluid (hydranencephaly). The brainstem and hippocampi were small and the cerebellum absent. In the other lamb, there was arthrogryposis with contraction of the elbow and carpal joints and extension of the stifle and tarsal joints. There was severe scoliosis and kyphosis of the thoracic spine and the sternum was curved ventrally. The brain was fluctuant with marked dilation of the lateral ventricles (hydrocephalus) and cerebellar hypoplasia.

Laboratory Results: Virus neutralization testing (NVSL, Iowa) was performed in 7 ewes and 1 live affected lamb: 6 ewes, including the ewe of the fetuses examined, and the affected lamb were positive for Cache Valley virus and all were negative for Schmallenberg virus. Serum neutralization testing for Bovine Virus Diarrhea virus was negative. Polymerase chain reaction (PCR) testing for Bunyavirus and Schmallenberg virus and virus isolation in the two lambs were negative.

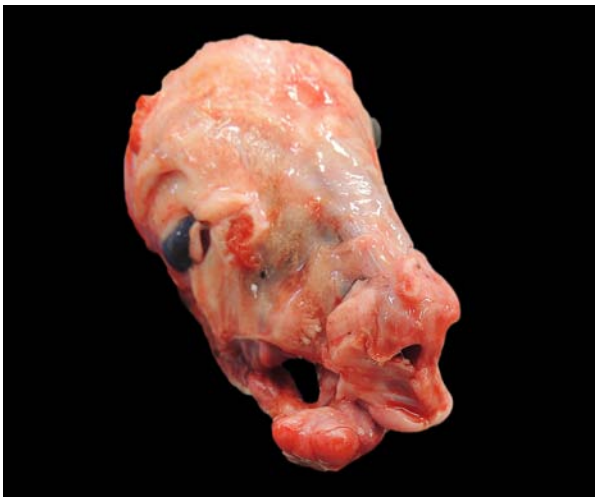
Histopathologic Description: Throughout the section, myocytes are small, thin, individualized and rounded. There is an absence of cross-striations and increased cytoplasmic basophilia. Diffusely, there is moderate expansion of the endomysium and perimysium with loosely arranged fibromyxomatous extracellular matrix material and edema. Scattered bundles of less affected muscle fibers composed of smaller myofibers with decreased sarcoplasm and peripheral and occasional central nuclei. Multifocally, there is parenchymal replacement by adipocytes and few small areas of hemorrhage.

Contributor's Morphologic Diagnosis: Skeletal muscle: Severe, diffuse myofiber hypoplasia and atrophy with fatty replacement.

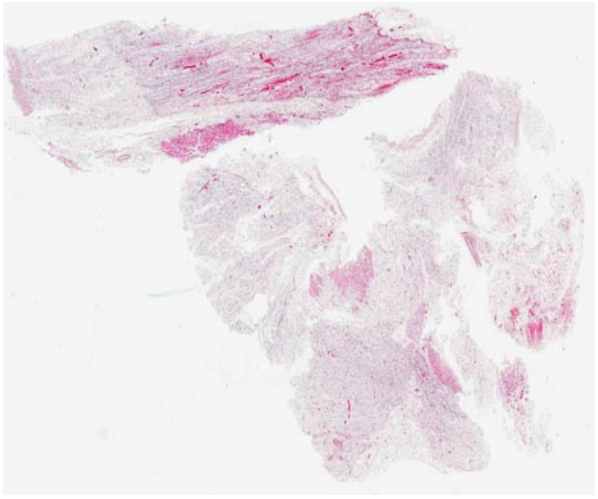
Contributor's Comment: Cache Valley Virus (CVV) belongs to the family Bunyaviridae, genus



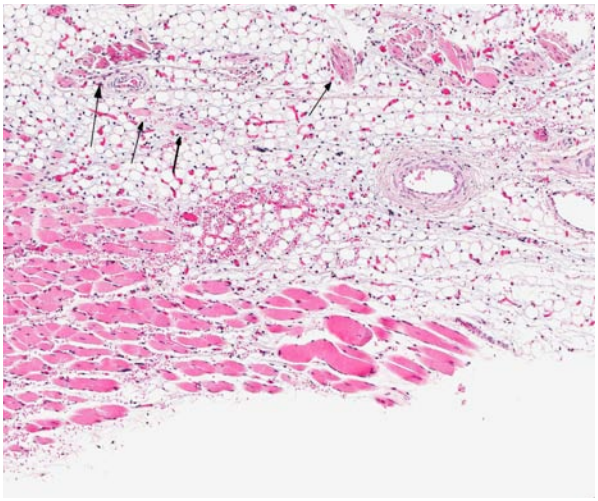
4-2. Fetus B demonstrates numerous neural defects within the cranium, with hydranencephaly being the most severe. (Photo courtesy of: Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, S2-121 Schurman Hall, Ithaca, NY 14850 http://www.vet.cornell.edu/biosci/pathology/services.cfm)



4-3. Fetus B exhibits severe craniofacial abnormalities. (Photo courtesy of: Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, S2-121 Schurman Hall, Ithaca, NY 14850 http://www.vet.cornell.edu/biosci/pathology/services.cfm)



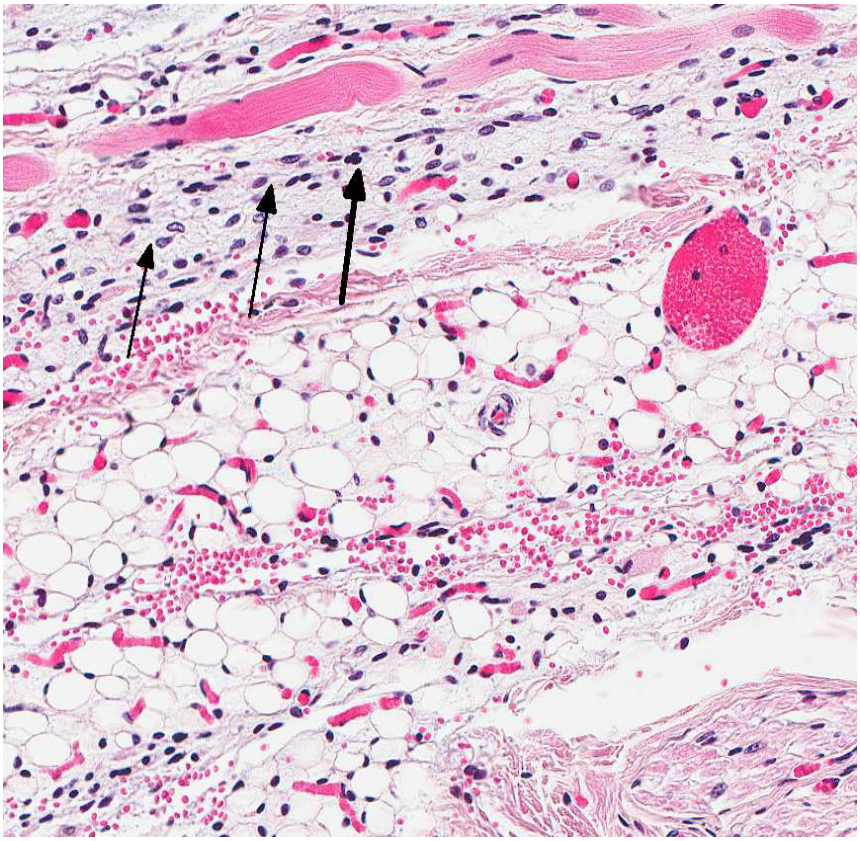
4-4. Stillborn lamb, skeletal muscle: Longitudinal (upper left) and transverse (lower right) sections of skeletal muscle show minimal development of skeletal muscle fibers, with marked infiltration by adipose tissue, as well as marked edema. (HE 0.63X)



4-5. Stillborn lamb, skeletal muscle: Longitudinal section demonstrates normal skeletal muscle at lower right, hypoplastic skeletal muscle (arrows) scattered amongst abundant fatty tissue. (HE 128X)

Orthobunyavirus, serogroup Bunyamwera, and is transmitted by arthropod vectors (arbovirus) to mammalian hosts. CVV is endemic to North America with a wide geographic distribution and was first isolated from *Culiseta inornata* mosquitoes collected in Cache Valley, Utah, in 1956. Other vectors for CVV include *Aedes* spp., *Anopheles* spp. and *Aedeomyia* spp. mosquitoes and *Culicoides* spp. biting midges. CVV is known to infect a wide range of mammals, including sheep, cattle, horses, white-tailed deer, cottontail rabbits and, rarely, humans. The lifecycle of CVV is poorly studied and the primary mammalian amplifying host is unknown. A potential primary host is the white-tailed deer, when experimentally infected develops a transient viremia lasting 1-3 days in naïve animals or less in previously exposed animals.[1] However, experimental infection of cottontail rabbits revealed a similar duration of viremia which was insufficient to infect mosquito vectors.[2] CVV is known to be present in the area of this farm[6] but malformed lambs are rare as local farmers breed at times which coincide with low numbers of flying insects.

The clinical signs of CVV infection in adults are generally subclinical causing only a transient



4-6. Stillborn lamb, skeletal muscle: In some areas, severely hypoplastic myofibers have a diameter equal or even less than a satellite cell nucleus. (HE 200X)

febrile response. In sheep, CVV is a well-recognized cause of congenital malformations and early fetal loss. Infection of ewes between 27 to 50 days of gestation results in congenital abnormalities including arthrogryposis, torticollis, hydranencephaly, hydrocephalus, porencephaly, microencephaly, cerebral and cerebellar hypoplasia, micromelia, anasarca and oligohydroamnios; mummification, reabsorption and dead embryos without deformities are also seen.[4] Infection at 28–36 days gives rise to central nervous system and musculoskeletal defects while infection at 37–42 days gives rise to musculoskeletal deformities only. Histological changes in addition to the muscular changes presented in this case include areas of necrosis and loss of paraventricular neuropil in the brain together with a reduction in the number of motor neurons.

CVV can be diagnosed on the basis of suggestive fetal malformations, histopathological changes, the demonstration maternal and neonatal antibodies and the presence of virus in pools of resident mosquitoes and viremic adults. At birth, CVV generally cannot be isolated aborted fetuses. Experimentally in sheep, CVV could be isolated from the allantoic fluid at less than 70 days of gestation but was not recovered from the allantoic fluid of fetuses after 76 days gestation.[4] Virus neutralization and ELISA testing are available for serological testing while PCR and virus isolation are available for virus detection.

JPC Diagnosis: Skeletal muscle and adipose tissue: Myofiber hypoplasia, diffuse, severe, with fatty infiltration.

Conference Comment: Histochemical staining with PTAH demonstrates the shrunken, irregular nature of fetal myocytes and highlights the multifocal loss of cross striations. The edema in the submitted tissue sections incited some debate among conference participants; several considered this a normal finding in fetal tissue and speculated that ongoing vasculogenesis may play a role, while others attributed the edema in the perimuscular connective tissue to a diminished intensity of skeletal muscle contraction secondary to myocyte hypoplasia. Since muscle contraction normally helps transport fluid from the interstitium into local lymphatics, skeletal muscle hypoplasia could theoretically contribute to widespread edema. Ultimately, participants concluded that both of these factors likely contributed to the edema.

Plant toxins and teratogenic viruses, including those belonging to the genera *Orthobunyavirus, Pestivirus* and *Orbivirus*, are often associated with congenital fetal malformations of the musculoskeletal and central nervous systems in ruminants. The most common cause of arthrogryposis, whether due to teratogenic plants or viruses, is denervation.[7] Ingestion of wild lupins (*Lupinus caudatus, sericeus*, or *formosus*) by pregnant cows may result in "crooked calf disease," which is characterized by fetal

musculoskeletal malformations such as arthrogryposis, torticollis, scoliosis, kyphosis, brachygnathia superior or palatoschisis. Similarly, maternal ingestion of *Veratrum californicum* can cause arthrogryposis or cyclopia in neonatal ruminants, and *Nicotiana* spp. (tobacco), jimsonweed and wild black cherry have also been associated with arthrogryposis; however, these plant toxins are not generally linked with hydranencephaly or cerebellar hypoplasia.[7]

Cache Valley, Akbane, Schmallenberg and Aino viruses belong to the genus *Orthobunyavirus* and are known for causing outbreaks of arthrogryposis, hydranencephaly and occasionally cerebellar hypoplasia in calves and lambs (see WSC 2012-2013, conference 22, case 3, table 1). Cache Valley virus is more common in sheep, while Akbane and Aino viruses primarily affect cattle. Fetal infection of calves and lambs with bovine virus diarrhea virus or border disease virus (pestiviruses) often results in cerebellar hypoplasia, or, less commonly, hydranencephaly, porencephaly, hydrocephalus or ocular abnormalities. Likewise, classical swine fever virus (also a pestivirus) infection can cause mummification, arthrogryposis and cerebellar hypoplasia in piglets. Infection with bluetongue virus (orbivirus) causes hydranencephaly and porencephaly in lambs and occasionally calves; however, arthrogryposis is not a characteristic lesion. Wesselsbron virus (flavivirus) is associated with mummification, hydranencephaly, arthrogryposis, porencephaly and cerebellar hypoplasia in lambs and calves.[3,7]

Virus-induced teratogenic effects are far less common in small domestic animals and laboratory species than in livestock. One exception is feline parvovirus which causes cerebellar hypoplasia in kittens due to selective necrosis of the external granular cell layer. Less commonly, canine parvovirus and Kilham rat virus (also a parvovirus) can also result in cerebellar hypoplasia in neonatal dogs and rats, respectively.[3,5]

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