CASE I: WSVL 08B4372 (JPC 3187132).

Signalment: Stillborn, late-term, male Red Angus calf (*Bos taurus*).

History: A registered Red Angus seedstock and cow-calf operation in northern Wyoming recognized a distinctive syndrome of late-term abortions/stillbirths in 2008. It occurred after genetic disease was recognized in progeny of one bull’s full brother on a property in Kansas. The Wyoming rancher was concerned the bull he owned had the same trait, and bred one bull, BUF CRK Romeo L081, to 25 of his daughters. The goal was to establish that this valuable bull was free of the trait. Unfortunately, of 25 calves born to Romeo and his daughters in 2008, 5 were identical to malformed calves born to the bull’s full brother in Kansas. All five were stillborn or premature (7 months – term).

Gross Pathologic Findings: This calf had a flat skull, impacted molars, short mandible (brachygnathia inferior), and a protuberant tongue. Gestational age was approximately 8 months.

Laboratory Results: Negative for bovine viral diarrhea virus by attempted virus isolation.

Histopathologic Description: Rib; persistent primary spongiosa, with empty resorption lacunae and scant osteoclasts.

Contributor’s Morphologic Diagnosis: Osteopetrosis.

Contributor’s Comment: Osteopetrosis has been recognized in the Angus breed since the 1960s, when a series of studies by Dr. Horst Leipold and colleagues documented the condition following an initial case report from New York. The gross appearance of calves is distinctive. It is one of the few abortifacient inherited diseases in cattle that can be diagnosed with confidence over the telephone when concerned ranchers phone veterinarians or their diagnostic laboratory.
Gross diagnosis is established by splitting long bones and demonstrating persisting primary spongiosa in the medullary cavity. Histologically, osteoclasts are markedly reduced in number, in spite of the presence of resorption lacunae. In this section of rib there are scant osteoclasts. Where present, they are lightly stained with washed out vesicular nuclei.

The basis for the defect is a mutation in the anion exchanger protein coded for by SLC4A2 on chromosome 4. A similar mutation causes osteopetrosis in mice. To date, no comparable mutation has been recognized in severe forms of osteopetrosis in children. Human osteopetrosis is classified morphologically into forms with reduced, normal or excessive numbers of osteoclasts. In Angus cattle it is likely that absence of the anion exchanger results in alkalinized cytoplasm, resulting in death of osteoclasts. Death in utero is due to compression of the cerebral cortex with posterior herniation of cerebellum as a result of osseous crowding in the cranial vault. An important diagnostic rule out is osteopetrosis due to in utero infection with bovine viral diarrhea virus. In this instance this and other calves sired by the bull were negative for BVDV.

**JPC Diagnosis:** Bone, rib: Persistent primary cartilaginous spongiosa with osteoclastopenia.

**Conference Comment:** The contributors published an excellent report of the calves in this case, with gross and histopathologic descriptions as well as comparisons with osteopetrosis conditions in humans. Although, as the contributor explains, osteopetrosis can be caused by several genetic mutations, all affected individuals share similar clinical characteristics (increased skeletal mass, increased likelihood of fractures and neurological complications).

The distinctive gross appearance of affected calves identified by the contributor are:
- compression of cerebral hemispheres with herniation of the cerebellar vermis into the foramen magnum
- dorsoventrally compressed brain with a concave depression in the parietal cortex
- thickened parietal bone
- mildly proptosis
- protruding tongue
- brachygnathia inferior
- restricted movement of temporomandibular joints
- loose, misaligned, not fully erupted incisors
- misaligned and unerupted molars
- abnormally smooth cranial calvarium surface with prominent bilaterally symmetrical bony ridges at the frontal–parietal synchondrosis and a corresponding depression in the temporoparietal cortex
- 1-2 cm fontanelle between frontal and parietal bones
- bilaterally symmetrical transverse ledge of bone at the frontal–sphenoid synchondrosis that involved the orbital wing of the sphenoid bone

Distinctive histologic findings are:
- trabecular bone with cores of hyaline cartilage
- rare osteoclasts in long bones, with numerous osteoclasts in the skull
• significant reduction or absence of retinal ganglion cells
• axonal loss and gliosis in the optic nerves and optic chiasm
• chromatolysis and neuronal atrophy in nuclei of the medulla oblongata (facial, vestibulocochlear, and hypoglossal nuclei, and red nuclei)
• atrophy of muscles of the tongue and larynx
• axonal swelling and Wallerian degeneration in pyramidal tracts and medial longitudinal fasciculus perivascular paraventricular corpora amylacea in the thalamus, basal nuclei, and midbrain with similar but larger structures in the choroid plexus of the third ventricle
• calcified vessel walls and mineralization of neuropil associated with the corpora amylacea
• reduced Purkinje cell numbers (but no other histologically identifiable cortical atrophy in compressed parietal cortex or in herniated cerebellum)
• disorganized mixture of cementum, dentin, enamel and trabecular bone in teeth (particularly around apical foramina within the intra-alveolar portion of premolars)
• mineralized and ossified connective stroma in the tooth chamber
• periodontal hemorrhage
• increased ratio of acellular to cellular cementum

Interestingly, 4 of the 10 affected calves in this report were heterozygous for the SLC4A2 mutation. The heterozygous calves were born alive, survived 1-7 days post birth and had milder brain lesions than the homozygous calves.

Conference participants noted that the cortex in the examined sections appears thin. Since we are unclear as to the level at which these sections were taken, we cannot fully evaluate this cortical thinning. It was speculated that the sections may be through the cutback zone, in which case the cortex would be expected to be discontinuous and thin.

Contributing Institution: Wyoming State Veterinary Laboratory
1174 Snowy Range Road
Laramie, WY 82072
http://wyovet.uwyo.edu/

References:
CASE II: A11-650 (JPC 4017812).

Signalment: 4-year-old, male intact, rhesus macaque (Macaca mulatta).

History: This animal was born and maintained as a specific pathogen free (SPF) animal within the NEPRC colony. Over a one-week period, a hard swelling, approximately 40 cm in diameter, developed over the left shoulder and biceps. Radiographs revealed a lytic bone mass within the left humerus with 1-2 areas of metastasis to the lungs; bone biopsy of the left humerus was non-diagnostic and revealed only reactive bone. Based on the rapid growth of the bony mass and poor prognosis based on the presence of pulmonary metastasis, humane euthanasia was elected.

Gross Pathologic Findings: The proximal and middle left humerus is swollen to five times normal size and on cross section has been replaced by a large 12 x 8 cm multilobular, fleshy, white to red friable mass that is variably gritty to hard on cross section. The neoplasm also infiltrates and surrounds the shoulder joint and the articulation of the humerus and radius. Scattered throughout the lungs, most numerous in the caudal right lung lobe, are approximately two dozen 1 to 4 cm diameter firm, pale nodules.

Laboratory Results: Chemistry panel revealed elevated ALP and LDH, hypoalbuminemia and hypoproteinemia.

Histopathologic Description: Lung: Arising within the pulmonary parenchyma are multiple well-demarcated nodules of neoplastic spindle-shaped cells arranged in sheets and cords that surround and are producing abundant osteoid. The nodules are variable in appearance with some having more osteoid than others. The neoplastic cells have abundant eosinophilic cytoplasm and large, round to ovoid nuclei with finely stippled chromatin and often prominent nucleoli. There is marked cellular and nuclear atypia with numerous mitotic figures (>5/hpf), many of which are bizarre. The nodules of neoplastic tissue are rimmed by a thick layer of fibrous connective tissue and are often traversed by numerous small blood vessels.

Contributor’s Morphologic Diagnosis: Lung: Metastatic osteoblastic osteosarcoma.

Contributor’s Comment: Histologically, this tumor is consistent with an osteosarcoma (OSA) arising from the left humerus, with extensive metastases to the lungs. Although this tumor can arise from any bone (and rarely extrasosseous sites), the proximal humerus is one of the most common sites for this neoplasm to occur, along with the distal femur and proximal tibia.² The histomorphology of this tumor (marked cellular atypia and extraordinarily high mitotic rate) is consistent with the extraordinarily rapid growth of a severely dysplastic and invasive metastatic neoplasm with numerous areas of lymphatic invasion and metastasis. Based on the amount of osteoid, this is considered a productive osteoblastic osteosarcoma.

Different subsets of OSA include those that produce osteoid (bone) (productive osteoblastic type), bone and cartilage (compound or combined type), are anaplastic with little extracellular
osteoid production (poorly differentiated type), or rarely may form blood filled cysts lined by malignant osteoblasts (telangiectatic type). Of these, osteoblastic (progressing to anaplastic) and combined type OSAs have been previously reported in rhesus macaques. Based on the paucity of reported cases, little is known about the prognosis of these bony tumors in non-human primates.

Differential diagnoses for the grossly lytic lesion included bacterial or fungal osteomyelitis, which was deemed less likely given the radiographic evidence of pulmonary nodules. Specific infectious agents reported to cause osteomyelitis in non-human primates include *Salmonella enteritidis*, *Mycobacterium tuberculosis*, *Coccidiodes immitis*, and *Histoplasma duboisi*. In addition, other neoplasms such as multiple myeloma, chondroblastoma, or other metastatic tumors could be considered. In this case, the bone biopsy revealed only reactive bone and was uninformative in distinguishing between neoplastic and inflammatory causes of bony lysis; this is a common issue with non-diagnostic specimens that miss the primary lesion.

Specific predisposing genetic mutations for the development of OSAs have not been reported in non-human primates, but several have been identified in dogs and humans. These include hereditary mutation in retinoblastoma (Rb) as well as sporadic mutations in p53, both common tumor suppressor genes. Mutations leading to overexpression of the p53 ubiquitin ligase MDM2 or decreased expression of the p14 gene product lead to increased p53 degradation and have been associated with OSA development. In addition, alterations in other genes include HER2/neu overexpression correlated with decreased survival and RECQL4 in the human Rothmund-Thomson Syndrome.

**JPC Diagnosis:** Lung: Osteosarcoma, metastatic.

**Conference Comment:** As the contributor notes in the excellent discussion, OSA occurs in dogs and humans, as well as non-human primates. Additionally, it is fairly common in cats, but rare in other domestic species.

Conference participants compared and contrasted OSA in primates and dogs, with the following table provided by the conference moderator:

<table>
<thead>
<tr>
<th></th>
<th>Primates</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Age</td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Sites</td>
<td>Away from the elbow and towards the knee</td>
<td>Away from the elbow and towards the knee, except distal tibia &gt; proximal*</td>
</tr>
<tr>
<td>Prognosis</td>
<td>87% survival</td>
<td>Dismal</td>
</tr>
</tbody>
</table>

*The moderator stressed an important caveat to the popular memory device “away from the elbow and towards the knee” (i.e., proximal humerus, distal radius, distal femur, proximal tibia) for common sites for canine OSA, noting that the distal tibia is actually more commonly affected than the proximal tibia.*
Discussing hereditary predispositions for developing osteosarcoma, the contributor brings up the interesting topic of tumor suppressor genes. Tumor suppressors such as RB and p53 are part of an intricate regulatory system in which they act to halt cellular proliferation in response to genetic damage. Active (i.e., hypophosphorylated) RB controls the cell cycle at the gap between mitosis and DNA synthesis (i.e., G1-S transition) by complexing with E2F transcription factors and recruiting chromatin-remodeling factors (e.g., histone deacetylases and histone methyltransferases), thereby inhibiting transcription of genes whose products are necessary for DNA synthesis in the S phase. When RB is inactivated via phosphorylation by the cyclin D-cyclin-dependent kinase (CDK) 4, cyclin D-CDK6 and cyclin E-CDK2 complexes, it releases E2F, which then activates the transcription of genes required for the S phase, and the cell cycle continues. Further regulation of this checkpoint occurs when growth inhibitors stimulate CDK inhibitors such as p16 (p16/INK4A) and p21 to inactivate the cyclin D-CDK complexes, leading to activated RB and subsequent cell cycle arrest. Thus, the G1-S checkpoint can be dysregulated by mutations in the genes that control the phosphorylation of RB: RB1, CDK4, and genes that encode cyclin D and p16.

Tumor suppressor p53 is known as the “guardian of the genome” due to its critical role in regulating the cell cycle both at the G1-S and G2-M checkpoints. In healthy cells, p53 is quickly degraded by the ubiquitin pathway via MDM2; however, when DNA damage is sensed by ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3 related (ATR) proteins, p53 undergoes post-transcriptional modifications causing it to be released from MDM2. Additionally, p14, another INK4A protein transcribed from the same gene as p16, has a protective effect on p53 by binding to and inhibiting MDM2. Once released from MDM2, p53 activates the transcription of numerous genes that cause cell cycle arrest and repair or apoptosis. Although p53 induces hundreds of genes, some of the key players include p21, which mediates cell cycle arrest by binding cyclin-CDK complexes and thus activating RB; GADD45, which aids in DNA repair; and BAX, which promotes apoptosis. If the DNA damage is successfully repaired, p53 up-regulates MDM2 transcription, which leads to its own destruction. If the DNA cannot be repaired, the cell becomes senescent (i.e., undergoes permanent, irreversible cell cycle arrest) or undergoes apoptosis. Similar to RB, mutations that cause loss of function in p53 or in the genes coding for proteins involved in the p53 pathway can disrupt this vital checkpoint and promote tumorigenesis.

**Contributing Institution:** New England Primate Research Center
http://www.hms.harvard.edu/nerprc/

**References:**
CASE III: 09-1057 (JPC 3134056).


History: The dog presented for a 2-month history of vomiting and diarrhea and later anorexia, which were partially responsive to antibiotics. She also had a history of left forelimb lameness. Ultrasound revealed a thickened gastric wall with loss of layering and enlarged gastric lymph nodes. Cytology on ultrasound-guided fine needle aspirate of the stomach revealed a gastric carcinoma. Radiographs of the left front limb revealed multiple radiolucent medullary lesions. On the day of euthanasia, the dog developed left hind limb lameness.

Gross Pathologic Findings: The dog weighed 23 kg, had a body condition score of 3/5 and was in fair postmortem condition. An area measuring 9cm x 3cm had been removed post mortem from the antrum, pylorus, and duodenum for purposes of tumor tissue banking. The tissue remaining at the pyloric-duodenal junction was 5 to 7 mm thick, firm and white to gray on cut surfaces. The pancreas was nodular, firm and up to 8 mm thick with multifocal areas of hemorrhage. There was a mild to moderate amount of petechiae and ecchymoses throughout the small and large intestinal mucosa. Three of the lymph nodes adjacent to the stomach were 1.3 to 1.5 cm in diameter, firm, dark red on the capsular surface and tan on section. Multiple round, white, soft to hard nodules (3 to 4 mm in diameter) were present throughout the medulla of the left humerus.

Histopathologic Description: Tissue is from the diaphysis of the left humerus. The lesions are most marked in the medullary cavity with marked infiltration with solid to occasionally acinar carcinoma associated with marked reactive fibrosis and marked reactive woven bone. Marrow cavity not involved with reactive woven bone or reactive fibrosis has mixed fatty and hematopoietic marrow. In some sections, there is tumor infiltrate into this mixed marrow without reaction. Not present on all slides is invasion of tumor into the cortex with variable cortical bone lysis, cortical bone necrosis and periosteal reactive woven bone formation. Neoplastic cells are not apparent in the periosteal lesion.

Contributor’s Morphologic Diagnosis: Left humerus: moderately differentiated, metastatic, solid carcinoma (presumed of gastric origin based on post mortem findings) with marked...
medullary reactive fibrosis and woven bone formation, variable cortical bone lysis and variable periosteal woven bone formation.

**Contributor’s Comment:** Carcinoma was confirmed in the stomach and based on pattern of infiltrate/absence of other primary tumors, metastatic gastric carcinoma was found in pancreas, gastric lymph nodes and left humerus. The clinical findings relevant to the left rear leg were unexplained (no lesions were apparent in the left rear leg grossly or microscopically).

Mammary gland and prostatic carcinoma are the major origin of metastatic carcinoma to bone in humans. Such metastatic pattern in spontaneous animal disease is rare. Most carcinoma metastatic to bone cause lysis; prostatic carcinoma in dog and man can be associated with osteoblastic metastases. The mechanisms by which prostatic tissue induces reactive woven bone is unclear but seems to have an endothelin-dependent mechanism. PtH-rp and prostaglandins are likely not involved. In the current case, the radiographic appearance of the lesions in the humerus were that of medullary lysis. It is difficult to explain the discrepancy between the microscopic appearance of a productive process in the medullary cavity and the radiographic interpretation of a lytic one. It is possible that because of reduced superimposition of bone, the cortical lysis (present in some sections) gave the appearance of medullary lysis radiographically. In a series of 19 dogs with skeletal metastatic carcinoma, most dogs were presented for veterinary care because of clinical signs relating to the skeletal metastasis and not the primary carcinoma. In 58% of the dogs, a primary site of the carcinoma was not determined. Most skeletal metastases are in axial skeleton and proximal long bones.

**JPC Diagnosis:** Long bone: Adenocarcinoma, metastatic.

**Conference Comment:** As the contributor states, metastasis of carcinoma to bone is rare in dogs relative to humans. Interestingly, malignant pilomatrixomas appear predisposed to metastasize to bone in dogs. During a review of 13 reported cases of canine malignant pilomatrixoma, Carroll et al. identified seven neoplasms which metastasized to or extended into bone (sites included vertebrae, ribs, mandible, maxilla and femur). In humans, tumor metastasis to bone is most common in well-vascularized sites (axial skeleton, proximal aspect of long bones and ribs), where the microenvironment provides “fertile soil” for solid carcinomas. For example, once human breast cancer metastasizes to bone, it produces colony stimulating factor 1, parathyroid hormone related protein, and tumor necrosis factor alpha. The resulting activation of nuclear factor kappa B ligand stimulates osteoclast formation and activation. Osteoclast degradation of bone matrix releases insulin-like growth factor 1, transforming growth factor beta and bone morphogenic proteins, which promotes survival of metastatic cells and stimulates parathyroid hormone related protein synthesis, thus resulting in a positive feedback loop of metastatic growth and osteolysis. Other factors that have been found to promote metastasis to bone in humans include chemokines in bone that attract circulating receptor-bearing tumor cells, cytokines that bind tumor cells bearing E-cadherin and laminin, and the presence of tumor associated macrophages. It is unclear if these factors play a role in the tendency of canine malignant pilomatrixoma to metastasize to bone.

**Contributing Institution:** Department of Veterinary Biosciences
The Ohio State University
1925 Coffey Road
CASE IV: 12-2197-6 (JPC 4019352).

Signalment: 4-year-old, castrated male, Bullmastiff dog (*Canis lupis familiaris*).

History: Dog had a lump on the lateral aspect of L elbow for an unknown period of time. Punch biopsy sent to a diagnostic laboratory resulted in a suspected diagnosis of chondroma. Dog continued to be lame and the owners elected amputation.

Gross Pathology: Received an elbow joint, which had an ulcerated firm mass, measuring approximately 5 x 5 cm, over the lateral aspect. The ulceration was from the site of the previous punch biopsy. On section, the mass was cavitaded; continuous with the elbow joint space; filled with variably-sized, smooth, white, hard masses. Sections of the mass were placed into decalcification to allow sectioning. Fluid injected into the joint space opposite the mass drained out through the section area of the mass suggesting the mass communicated with the joint space.

Histopathologic Description: The mass consists of numerous nodules consisting of well-differentiated hyaline cartilage arising from the synovial membrane and occasionally found within it. Residing within the lacunae are mature and well-differentiated chondrocytes. The nuclei are round and eccentric and consist of homogeneous dark purple chromatin. There is a moderate amount of eosinophilic cytoplasm, often containing small basophilic granules. The cells have irregular, almost spiculated cellular margins. Often these nodules show evidence of endochondral ossification. The synovium is markedly thickened and infiltrated with lymphocytes and plasma cells. The overlying fibrous capsule is also thickened by numerous reactive fibroblasts.


Contributor’s Comment: Synovial osteochondromatosis (SOC) is a condition described infrequently in dogs, and rarely in cats, horses, pigs, great horned owls and a red tailed hawk. It is characterized by the formation of small intra- or periarticular cartilaginous or
osseous nodules. Histologically, the cartilaginous lobules are hypercellular and can show features of nuclear and cellular atypia which can complicate the diagnosis, particularly if only small samples are taken. Compared to the condition in humans, relatively little is known about the pathogenesis of the disease in dogs. The majority of the descriptions in veterinary medicine are based on case reports of individual dogs.\textsuperscript{1,2,3,4,5,9}

In humans, SOC is considered to be a metaplastic condition rather than a neoplastic disease.\textsuperscript{11} There are however, at least two case reports of malignant transformation of synovial osteochondromatosis to chondrosarcoma in dogs.\textsuperscript{1,2}

Based on the description of the condition in humans numerous subcategories have been described. These include chondromatosis where the nodules are strictly cartilaginous in nature and osteochondromatosis where some of the nodules have undergone endochondral ossification as in this case. They are further subdivided based upon the suspected origin as either primary or secondary. SOC is defined as being secondary when there is a preexisting history of joint trauma or chronic mechanical irritation, while primary SOC is an idiopathic condition.\textsuperscript{12} In this case, there was no previous history of joint injury or disease and careful examination of the articular surfaces of the joints did not reveal any sign of disease suggesting that this is a primary SOC. There are limited numbers of cases describing the treatment of this condition; those that do exist suggest that removal of the nodules and partial synovectomy may improve the clinical signs, although radiographic reoccurrence of nodules was noted in some of these cases.\textsuperscript{4}

**JPC Diagnosis:** Synovium: Cartilaginous nodules, multiple, with marked proliferative and lymphocytic synovitis.

**Conference Comment:** The contributor provides an excellent summary of this uncommon condition. Conference participants considered both primary and secondary synovial chondromatosis as differential diagnosis for this case, and discussed differences between the two. The moderator provides the following table for comparison:

<table>
<thead>
<tr>
<th></th>
<th>Primary synovial chondromatosis</th>
<th>Secondary synovial chondromatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>Rare</td>
<td>Fairly common</td>
</tr>
<tr>
<td>Joints affected</td>
<td>Single</td>
<td>Multiple</td>
</tr>
<tr>
<td>Degenerative joint disease</td>
<td>Mild</td>
<td>Severe</td>
</tr>
<tr>
<td>Number of nodules</td>
<td>More</td>
<td>Fewer</td>
</tr>
</tbody>
</table>

Participants also considered chondroma and chondrosarcoma as potential diagnoses; however, most agreed that a chondroma would more likely present as a single, discrete mass rather than as multiple nodules, and chondrosarcoma would likely not appear so well-demarcated or demonstrate such a repeatable and orderly appearance within each nodule.

The classification of primary synovial chondromatosis is controversial, as there is evidence of both a reactive process and a metaplastic/proliferative process.\textsuperscript{1} Recent cytogenic studies demonstrate that in human primary osteochondromatosis, a subpopulation of mesenchymal stem cells undergo clonal proliferation, suggesting a benign neoplastic process.\textsuperscript{1}
Contributing Institution: Department of Veterinary Pathology
Western College of Veterinary Medicine and Prairie Diagnostic Services
52 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 5B4, Canada
www.usask.ca/wcvm/vetpath

References: