



## WEDNESDAY SLIDE CONFERENCE 2014-2015

### Conference 8

25 March 2015

**Guest Moderator:**

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**CASE I:** 65647 (JPC 4048575).

**Signalment:** 9-year-old spayed female DSH feline, *Felis catus*.

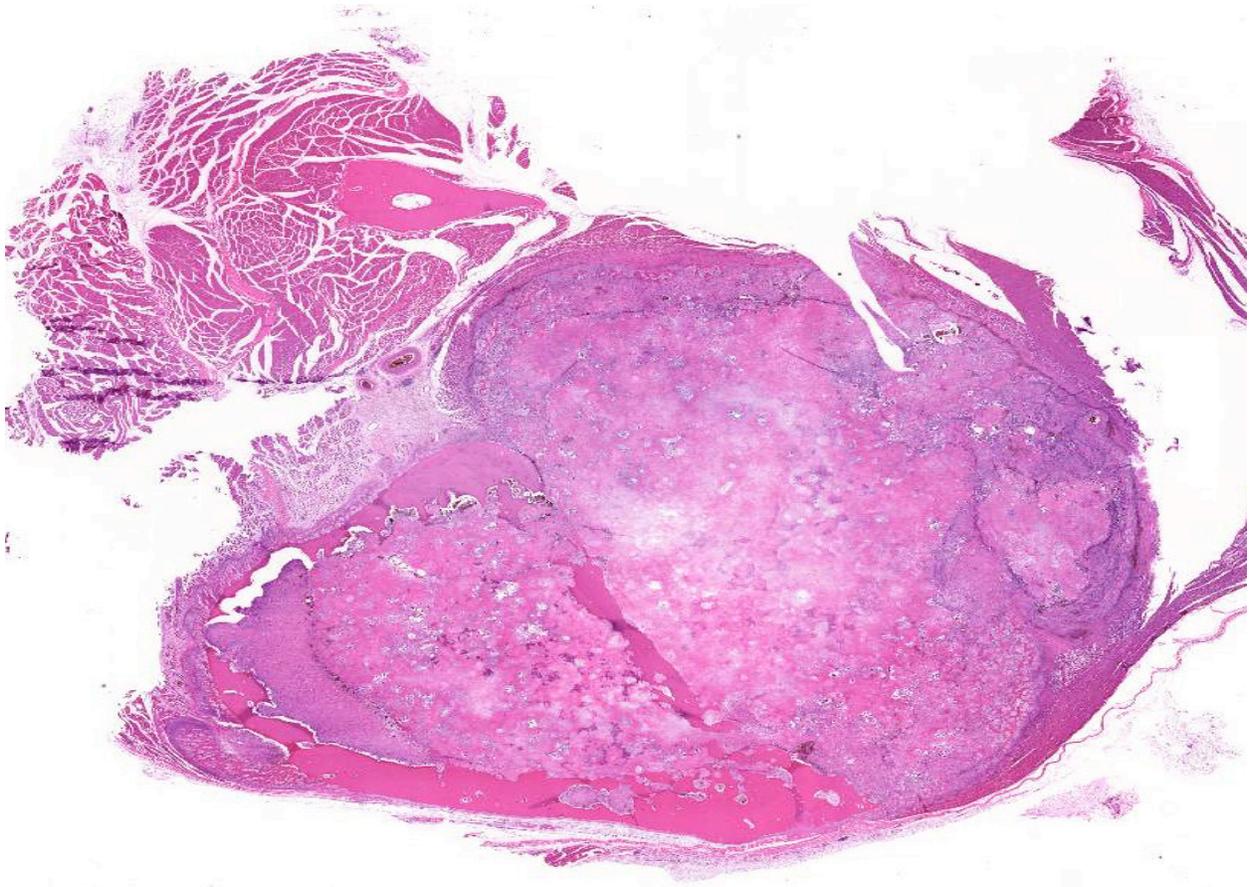
**History:** Three-month history of progressively worsening left hind limb lameness. On external examination, a large, firm mass was noted on the left proximal tibia. Radiographs of the limb revealed a focally extensive area of osteolysis with periosteal elevation along the proximal tibia. The left rear leg was amputated mid-femur and the entire limb was submitted for histopathological analysis.

**Gross Pathology:** Submitted for histopathology was the entire left hind limb that had been amputated at the level of the middle femur. Dissection revealed a pronounced thickening of the proximal tibia with irregular and lytic areas of periosteum and cortical bone.

**Laboratory Results:** N/A

**Histopathologic Description:** Examined is a section of bone and surrounding soft tissue (tendon, muscle) where the bone is markedly expanded and focally replaced by a poorly demarcated, non-encapsulated, densely cellular mass. Neoplastic cells fill greater than 50% of the

marrow spaces, surrounding and replacing trabeculae, multifocally replacing the cortex and extending into the periosteum. Neoplastic cells are arranged in sheets and streams, supported by a fine fibrovascular stroma. Cells are pleomorphic; most cells are polygonal to stellate with poorly defined cell borders, moderate eosinophilic fibrillar cytoplasm, eccentric ovoid nuclei, finely stippled chromatin, and a single amphophilic prominent nucleolus. There is moderate anisocytosis and anisokaryosis. There are numerous scattered binucleate and multinucleate giant cells, sometimes containing >20 nuclei, often adjacent to osteoid matrix. Mitotic figures are rare with 1 or fewer per 10 hpf. Neoplastic cells appear to produce a dense fibrillar to homogenous eosinophilic matrix (osteoid). There are extensive multifocal to coalescing regions of cartilaginous differentiation. Scattered throughout and adjacent to the neoplasm there is bone lysis, necrotic bone, and mild multifocal to coalescing areas of hemorrhage. The cortical bone is discontinuous, interrupted by clusters of neoplastic cells surrounded by abundant fibrous connective tissue (scirrhous reaction). Multifocally, there is periosteal proliferation of reactive bone (exostosis) and few perivascular infiltrates of lymphocytes, plasma cells, and fewer



1-1. Tibia, cat: The tibial diaphysis is markedly expanded and replaced by an infiltrative neoplasm producing extensive osteoid. The tibial cortex is largely replaced, except at the lower left. The fibula is present at upper left. (HE 6.3X)

macrophages that extend into the adjacent soft tissue.

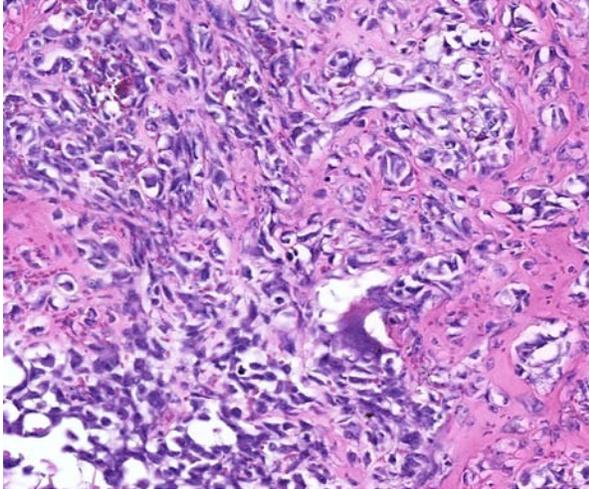
**Contributor's Morphologic Diagnosis:** Bone, proximal tibia: Osteosarcoma.

**Contributor's Comment:** Primary bone tumors are an uncommon finding in the feline patient with reported incidence of 3.1 to 3.9 per 100,000 cases.<sup>5</sup> Of the primary bone tumors, osteosarcomas (OS) account for 70 to 80 percent of findings.<sup>3</sup> Differing from canine OS, which exhibits a biphasic impact distribution, feline OS tends to impact middle age to older cats (average age 8-10 years).<sup>3</sup> Tumor locations can be divided into axial, appendicular, and extraskeletal. Extraskeletal sites have been noted to occur sporadically in multiple tissues and anatomical locations with a propensity for occurring in locations commonly associated with vaccine administration. The most common skeletal locations for feline OS include distal femur and proximal humerus and tibia and overall hind

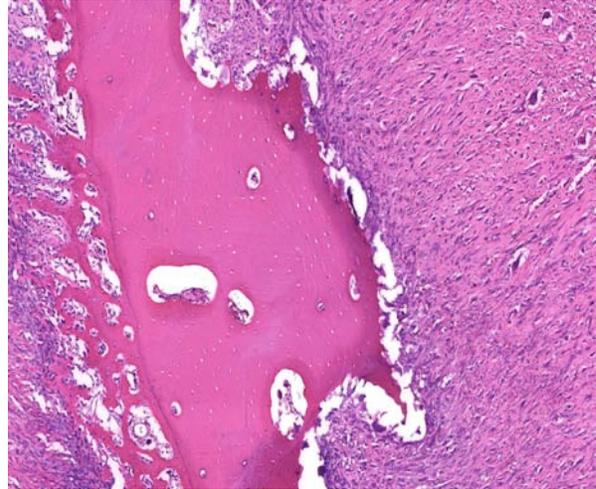
limbs are more commonly impacted. Further differing from canine OS, feline OS exhibits a low rate of (5-10%) of pulmonary metastasis.<sup>5</sup> Radiographic features of osteosarcoma in cats are variable with the aggressive periosteal proliferation often noted in canine OS being less prevalent.<sup>4</sup>

Cats with appendicular or extra skeletal forms of OS tend to survive longer when compared to those with axial forms. In a study published by Dimopoulou, survival prognosis for cats with osteosarcoma was related to histologic grade and mitotic index.

Spugnini, reported similar findings with median survival of 49 months for cats following amputation alone with appendicular OS, compared with a median survival time of only 5.5 months for cats with axial skeletal OS. If the tumor permits removal, surgery alone may be curative with extended survival time for those undergoing advanced adjunctive therapies. The



1-2. Tibia, cat: Neoplastic cells are spindle to stellate, and produce (and eventually incorporated within) abundant osteoid. Rare giant cells (center) are scattered throughout the neoplasm. (HE 220X)



1-3. Tibia, cat: The advancing front of the neoplasm results in resorption of overlying lamellar bone. There is periosteal new bone growth at the outer edge (at left), trying in vain to reinforce the rapidly disappearing cortex. (HE 61X)

histologic grade in this case was relatively low (rare mitotic figures, low to moderate tumor cell density, abundant tumor matrix, minimal to mild necrosis, moderate pleomorphism) and is suggestive of a fair to guarded prognosis. Although amputation is often curative, neoplastic cells were noted rarely in blood vessels within the section in this case. Interestingly, tumor invasion into vessels was not found to be a significant prognosticator in one retrospective study of feline osteosarcoma.<sup>3</sup> No long-term follow up is available for this patient.

This tumor was made up of predominantly osteoblast-like neoplastic cells with numerous clusters of multinucleate giant cells scattered amongst a prominent matrix of osteoid, mature bone, and cartilage. Scattered multinucleate giant cells are not uncommon in feline osteosarcoma, though a giant cell variant osteosarcoma such as this one, with numerous giant cells, is unusual. The origin of multinucleated giant cells in OS is poorly understood and both osteoclast and osteoblast origins have been postulated. In a report by Negrin<sup>8</sup>, both osteoclast and osteoblast immune staining features were noted in a feline OS of the calvarium. Osteoclast like features included TRAP, vimentin, and S-100 positive staining with cytokeratin-negative staining. An osteoblast like feature includes MHC II-negative reaction.<sup>8</sup> No prognostic significance has been related to the giant cell variant osteosarcoma seen in cats.

**JPC Diagnosis:** Bone, tibia: Osteosarcoma.

**Conference Comment:** Osteosarcomas (OSA) are commonly described as malignant long bone tumors of large breed dogs, with the distal radius, distal tibia, and proximal humerus being the most common sites of occurrence<sup>1</sup> and are much more common than their feline counterpart. The differences between OSA of dogs and cats have been highlighted by the contributor. Conference participants discussed the particulars of obtaining a definitive diagnosis of OSA. In many cases, diagnosis is often complicated by a small sample submission and the fact that these neoplasms are often heterogenous and admixed with reactive bone which may result from a proliferative response due to nonneoplastic mechanisms. Bone reacts to local and systemic stimuli through systematic and regimented processes, regardless of etiologic stimulus (e.g. fracture, benign or malignant neoplasm or infectious disease). This case nicely illustrates this point, as in some areas, woven bone is overtly neoplastic and characterized by large, atypical, irregularly clustered osteoblasts haphazardly oriented to disorganized bone trabeculae, or embedded within lacy deposits of osteoid. The disorganized areas of bone are intermixed with “reactive” or reparative woven bone characterized by plump osteoblasts coalescing around, and oriented perpendicular to the longitudinal axis of woven bone trabeculae, thus demonstrating a structural uniformity distinguishable from neoplastic bone islands. However, in small samples without clinical or

radiographic context, distinguishing osteosarcoma from reactive bone can prove problematic. As stated in the WHO tumor fascicles "practice and experience cannot be supplanted," in the case of interpreting proliferative bony and reactive periosteal lesions.<sup>9</sup>

The variety of histologic presentations of OSA has led to the description of nine separate subclassifications in a recent paper.<sup>7</sup> Subclassifying these tumors is also hindered by their heterogeneous nature as often several histologic subtypes are evident within a single neoplasm; however, their subclassification, with the exception of the aggressive telangiectatic variant, does not appear prognostically significant.<sup>7</sup> Histologic grade, on the other hand, is often cited as offering considerable prognostic value, though only mitotic index was identified specifically in cats.<sup>3</sup> Evidence has accumulated in the literature supporting the hypothesis that cyclooxygenase-2 (COX-2) is involved in the pathogenesis of osteosarcoma, and recently prostaglandin E<sub>2</sub> was pinpointed as the downstream culprit of interest, lending credence to the use of COX-2 inhibitors in chemotherapy regimens and suggesting selective inhibition of PGE<sub>2</sub> may be equally effective.<sup>6</sup>

**Contributing Institution:** <http://www.hopkinsmedicine.org/mcp/index.html>

#### References:

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**CASE II: RE-13-121 (JPC 4048075).**

**Signalment:** 4-month-old male Newfoundland dog, *Canis lupus familiaris*.

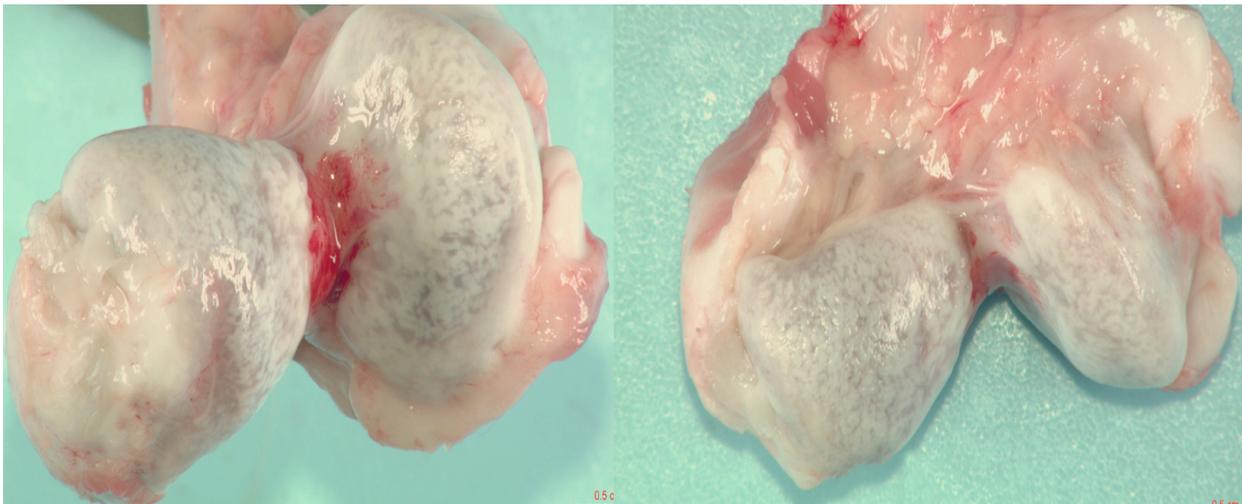
**History:** This dog had a history of being slow to get up and having trouble with the rear legs. The onset and duration of these clinical signs was not reported and could not be obtained. The referring veterinarian (rDVM) reported that the dog had swollen and thickened elbows and that the right elbow was worse than the left. The right elbow was possibly luxated. The dog also had severe pain in the hips and at the base of the tail. Additional clinical findings were not reported. Radiographs were reported to show white stippling due to decreased endochondral bone formation in all epiphyses of the limbs. The vertebrae were also affected. The owner elected euthanasia and declined a full necropsy. The rDVM removed the right front leg at the level of the scapula and the right rear leg at the level of the proximal femur and submitted them for examination. No other information could be obtained regarding status of littermates, diet, age of onset, etc.

**Gross Pathology:** The right front limb, including the scapula, and the entire right rear limb were submitted fresh. The soft tissues were removed so that the bones could be examined and sectioned. The articular surfaces of all bones were smooth with a characteristic mottled white appearance. The apophyses were the most affected. The trochlea of the humerus appeared thickened and

flared. On cut section, the epiphyses contained increased amounts of cartilaginous tissue which was most evident and extensive in the humeral condyles and the apophyses of other bones; these regions also had much smaller ossification centers than less affected bones. Occasionally, the metaphyseal physes were irregular and there were tongues of cartilage that extended from the physis into the metaphysis. Small islands of cartilage were also present in the metaphysis.

**Laboratory Results:** None.

**Histopathologic Description:** Multiple longitudinal sections of bone, including the proximal and distal femur, the proximal and distal tibia, the proximal and distal humerus, and the proximal radius were decalcified and examined microscopically. The slide submitted contains a section of the distal femur. The articular-epiphyseal (AE) complex and areas of persistent epiphyseal cartilage are highly irregular with various staining patterns. There are multifocal to coalescing areas of eosinophilic cartilage alternating with increased amounts of basophilic hyaline cartilage creating a mosaic type appearance. The eosinophilic regions of cartilage are indicative of loss of proteoglycans suggesting degeneration. There are two adjacent irregular spaces within the hyaline cartilage that contain fragments of basophilic debris. The cartilage is irregularly organized and there are separate multifocal islands of cartilage within the epiphysis that are undergoing ossification. Some of these islands extend to, and merge with, the



2-1. Humerus, cranial and caudal views of the condyle, dog: The trochlea are thickened and widely flared. The articular surface has the same mottled white appearance as shown for the femur. (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))



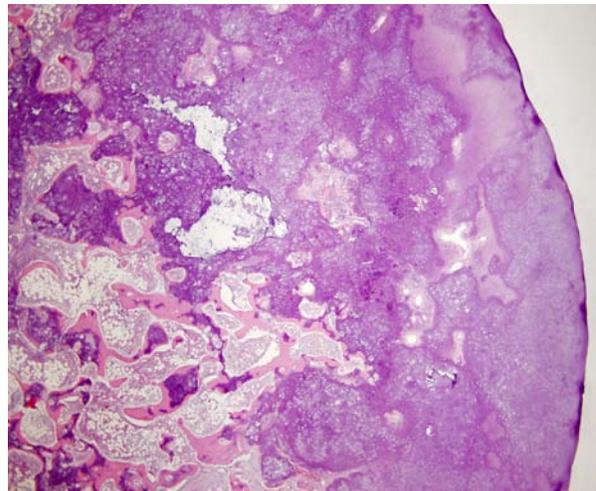
2-2. Humerus, trochlea (sagittal section), dog: There is a marked increase in cartilaginous tissue in the epiphysis with a smaller than normal ossification center (\*). The metaphyseal physis is irregular with a tongue of cartilage extending into the metaphysis. There is also a separate island of cartilage present in the metaphysis (arrow). (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))



2-3. Femur, trochlea, dog: The articular surface has a mottled white appearance. (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))



2-4. Femur, medial condyle (sagittal section), dog: There is increased epiphyseal cartilage (arrow) and a mildly irregular metaphyseal physis. (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))

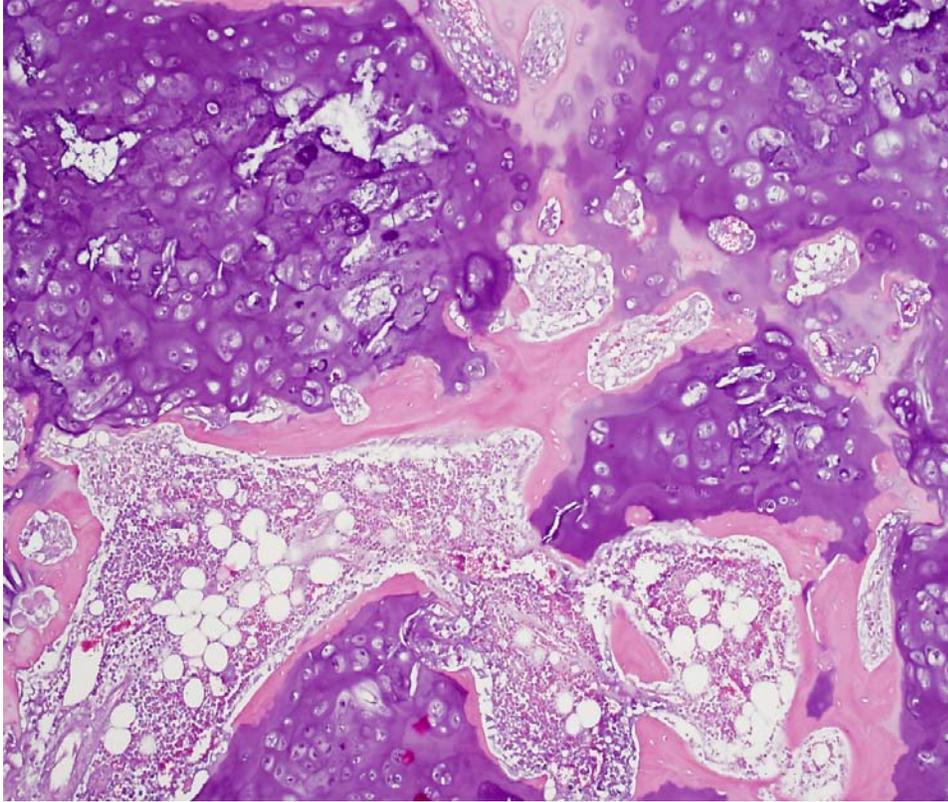


2-5. Femur, medial condyle (sagittal section), dog: The articular-epiphyseal (AE) complex and areas of persistent epiphyseal cartilage are highly irregular with various staining patterns. There are multifocal to coalescing areas of eosinophilic cartilage alternating with increased amounts of basophilic hyaline cartilage creating a mosaic type appearance. (HE 2X) (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))

metaphyseal growth plate. There appears to be reduced ingrowth of vascular channels along the edges of the reduced ossification centers. The metaphyseal growth plate (not present in all submitted sections) varies from normal regions to multifocal irregular areas characterized by disorganization of the three cartilage zones (resting, proliferating and hypertrophic) as well as multifocal extensions of cartilage tongues into the metaphysis. In some of these irregular regions,

islands of cartilage within the epiphysis merge with the metaphyseal growth plate causing marked disorganization.

Similar lesions of varying degrees were noted in all of the other bones examined histologically. In some bones the metaphyseal physes were more affected than in others, especially in the proximal radius and trochlea of the distal humerus.



2-6. Femur, medial condyle (sagittal section), dog: There is reduced ingrowth of vascular channels along the edges of the reduced ossification centers in the epiphysis. (HE 10X) (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))

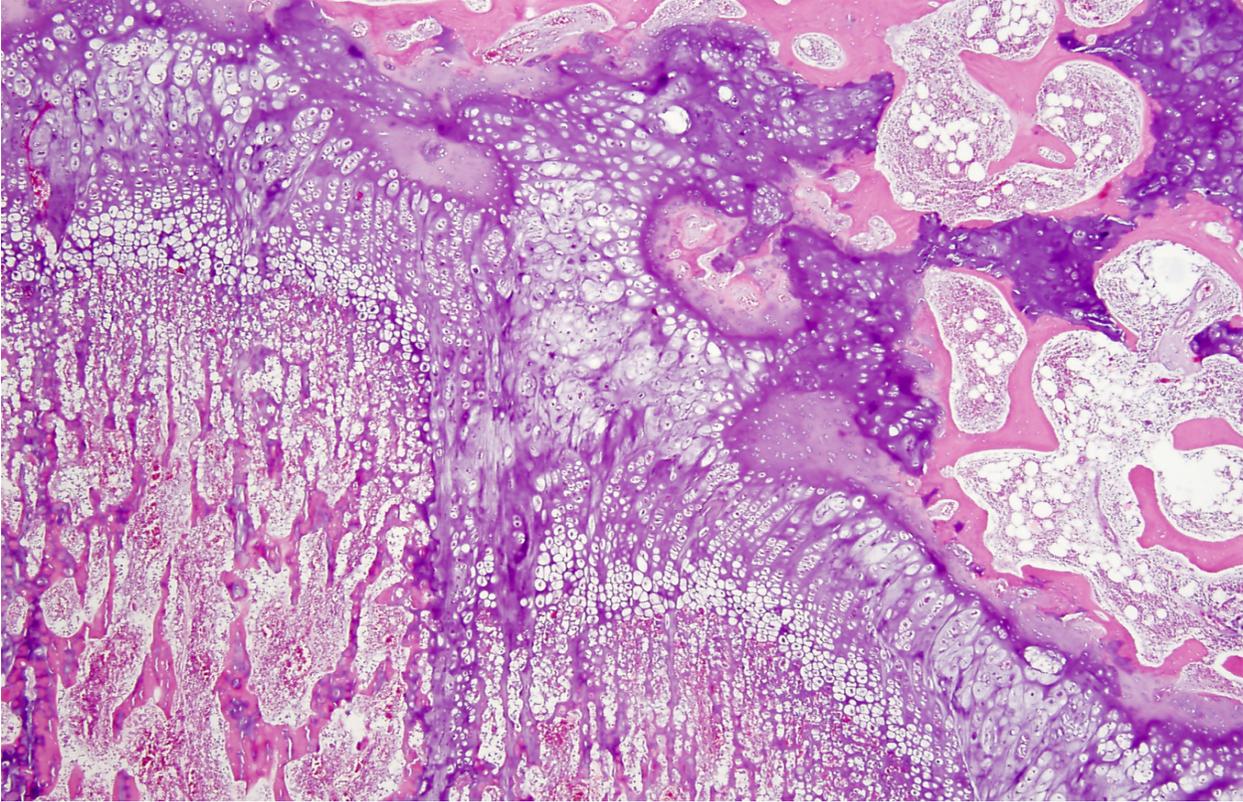
**Contributor's Morphologic Diagnosis:** Distal femur: Severe epiphyseal dysplasia and mild metaphyseal dysplasia.

**Contributor's Comment:** A variety of inherited osteochondrodysplasias have been described in dogs and some are considered normal characteristics of certain breeds. Those that cause disease may be present at birth or develop later in life, especially as weight-bearing increases.<sup>5</sup> Many bone dysplasias are breed specific, such as chondrodysplasias in the Alaskan Malamute, the Norwegian Elkhound, the English Pointer, and in the Great Pyrenees.<sup>5</sup> In these types of chondrodysplasias, the main lesions are in the metaphyseal growth plates and are generally associated with dwarfism or short stature.<sup>5</sup> The growth plates are disorganized with tongues of cartilage often extending into the metaphysis.<sup>5</sup> There are specific differences among breeds in terms of which zones of cartilage, and which bones, are most affected. Pseudoachondroplastic dysplasia of miniature poodles is another type of dysplasia that was originally termed epiphyseal dysplasia. Affected dogs are smaller than their

littermates.<sup>5</sup> Enlarged costochondral junctions, long costal cartilages, shortened vertebrae, and abnormal formation of the trachea and nasal septum have been described.<sup>1</sup> The limb bones often have enlarged epiphyses that are sometimes flared over the metaphyses.<sup>1</sup> The cartilage matrix is described as sparse with a lack of basophilia.<sup>1</sup> Chondrocytes have variable size and sometimes are clumped in large lacunae.<sup>1</sup> Radiographic findings show irregular multifocal development of ossification centers that create a stippled

appearance.<sup>1</sup> Decreased sulfation of glycosaminoglycans despite normal collagen synthesis is proposed as a cause of these lesions.<sup>1</sup> Osteochondrodysplasia in the Scottish Deerhound is characterized by growth plates that are irregular in width and physal-metaphyseal junctions that are uneven.<sup>1</sup> There is also a syndrome in Labrador Retrievers and Samoyeds that involves both ocular and skeletal dysplasia.<sup>1</sup> An autosomal recessive inheritance has been suggested for most of these breed specific conditions.<sup>1</sup>

Multiple epiphyseal dysplasia (MED) has been reported rarely in single dog case reports and in two case series. One case series included a litter of Beagle puppies<sup>5</sup> and the other included 19 dogs of various breeds.<sup>6</sup> MED has been described in dogs as a rare condition that involves a deficiency in ossification of the epiphyses of the long bones and vertebrae, the cuboidal bones, and the apophyses.<sup>6</sup> An autosomal recessive mode of inheritance has been suggested.<sup>6</sup> This is in contrast to the disease in humans which most commonly has a dominant pattern of inheritance. However, recessive forms of the disease in



2-7. Femur, medial condyle (sagittal section), dog: An island of epiphyseal cartilage merges with the metaphyseal growth plate causing marked disorganization in this region. There is also extension of a cartilage tongue into the metaphysis. (HE 4X) (Photo courtesy of: Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu))

humans have been reported and have been linked to a mutation in the gene encoding the oligomeric cartilage matrix protein on chromosome 19 as well as mutations in genes encoding type IX collagen (COL9A2) and matrilin-3.<sup>1</sup> These mutations lead to an anomaly in the matrix of hyaline, articular, and physeal cartilage.<sup>1</sup> In dogs, specific causative mutations have not yet been identified and it has been suggested that environmental factors such as toxins, drugs, and nutritional deficiencies may aid penetrance.<sup>6</sup>

In the case series of 19 affected dogs, dogs were normal at birth, lesions were noted on radiographs as early as 8-weeks-old, mild clinical signs were reported at 2 to 3 months of age, and most dogs had severe lameness by 5 to 8 months of age.<sup>6</sup> Radiographic findings in that case series were reportedly similar to those described in dogs with congenital hypothyroidism.<sup>6</sup> Thyroid testing could not be performed in the present case. Radiographic findings include: a delay in ossification of the epiphyses, apophyses, and cuboidal bones of the appendicular skeleton, the patella, the fabellae, and the epiphyses of the

vertebrae; normal appearing metaphyses and diaphyses of the long bones and vertebrae which also seemed normal in length; continued abnormal appearing epiphyses throughout the growth phase but bone formation proceeded from the normal ossification centers and the size of ossification centers increased with age; and a stippled appearance to the distal epiphysis of the tibia.<sup>1</sup>

The shoulder, stifle, and hip joints have been reported to be the most severely affected in dogs, likely due to greater weight bearing on these joints.<sup>6</sup> Reported gross lesions in dogs with MED include: a whitish appearance to all epiphyses, including those in the vertebrate, and in the apophyses and cuboidal bones; occasional loose fragments of cartilage in some joints; and smaller ossification centers noted in sagittal sections.<sup>6</sup> Histologic findings described in affected dogs include: smaller than normal ossification centers with poorly developed bone tissue and an irregular poorly developed hypertrophic zone in the epiphyses; decreased ingrowth of vascular channels at the periphery of ossification centers

and few vacuoles in the adjacent chondroid tissue; cartilaginous tissue in the epiphyses with uneven staining, pale areas, and many lacunae with large, often vacuolated chondrocytes with round dark nuclei; and few or no blood vessels within the epiphyseal cartilage.<sup>6</sup> Flocculent accumulation of chondroitin sulfate and glycoprotein in chondrocyte lacunae is described as the initial lesion.<sup>1</sup> Adjacent lacunae then coalesce and liquefy to form cysts and their contents mineralize.<sup>1</sup>

As the epiphyses of multiple long bones were the most severely affected in the current case, a diagnosis of MED was made. The gross and histologic findings were similar to those described in other reports of MED.<sup>1,5,6</sup> Other differentials considered for this case included pseudoachondroplasia and spondyloepiphyseal dysplasia. The former condition has been described in humans and Miniature Poodles and the latter has been described in humans. These dysplasias are characterized by lesions in both the physes and metaphyses, thus resulting in severe dwarfism.<sup>5,1</sup> In this case, the metaphyseal lesions were generally mild to moderate in most bones and dwarfism was not evident. In the 19 dog case series, some dogs had moderate lesions in the metaphyses, and the authors stated that MED should not be excluded in dogs with these lesions.<sup>5</sup> Nonetheless, another form, or multiple forms, of dysplasia cannot be entirely ruled out in the current case. As there is currently no treatment that can relieve the pain in affected dogs, euthanasia is recommended.<sup>6</sup>

**JPC Diagnosis:** Bone, distal femur: Epiphyseal/metaphyseal dysplasia (epiphyseal/metaphyseal (osteo) chondrodysplasia).

**Conference Comment:** This is an interesting and complex entity in which pinpointing causation to a specific genetic anomaly has not been successful. The clinical, radiographic and morphologic abnormalities associated with skeletal dysplasias are heterogeneous with over 200 described disorders, and can be broadly divided into connective tissue disorders that disrupt formation of bone (osteodysplasia) or cartilage and endochondral ossification (chondrodysplasia).<sup>3</sup> The nomenclature is confusing and often terms such as dysplasia (processes involving generalized defects caused by intrinsic alterations), dysostosis (processes

limited to a specific bone or bone segment), and dystrophy (defects caused by an extrinsic process) are often used interchangeably.<sup>1</sup> Often the term osteochondrodysplasia is applied when morphologically both cartilage and endochondral bone is altered; however, given the complexity of bone development, in some disorders, tissues other than bone are affected, as this case in chondrodysplasia of the Alaskan Malamute. Several animal models of chondrodysplasia have been established.<sup>8</sup> In humans, several causative genes have been identified, which include cartilage matrix proteins (e.g. *COL2A1*, *COMP*), transcription factors (e.g. *Hox* and *Pax* genes) growth factor receptors (e.g. *I*), and chondrocyte maturation/hypertrophy factors (e.g. *PTH/PTHrP*, *CBFA1*).<sup>3</sup> Recently, single gene mutations associated with specific disorders have been identified, such as the chondrodysplastic “breed-standard” phenotypes of nineteen breeds of domestic dogs.<sup>4</sup> The underlying genetic mechanism presented in this case has not been determined.

Relevant to the discussion is the acquired atypical expression of *FGF4* which manifests as the characteristic skeletal breed traits among chondrodysplastic breeds such as dachshunds and basset hounds.<sup>4</sup> *FGF4* induces the expression of sprouty genes which interfere with ubiquitin mediated degradation of *FGF* receptors, causing their overactivation.<sup>4</sup> The receptor *FGFR3* is a negative regulator of bone growth, thus chondrocyte proliferation is downregulated in these breeds leading to their short stature.<sup>2</sup> In contrast, spider lamb chondrodysplasia is caused by inhibition of *FGFR3* leading to uncontrolled chondrocyte proliferation, resulting in the long, splayed legs of black-faced lambs.<sup>2</sup>

For this case conference participants preferred a morphologic diagnosis of epiphyseal and metaphyseal chondrodysplasia to identify the histologic abnormalities of chondrocytes/cartilage matrix and disruption of normal ossification within both the epiphysis and metaphysis visible in most sections. However, the more general term “osteochondrodysplasia” would be appropriate as well. However, conference participants recognize the difficulty involved in characterizing these complex abnormalities, and note the contributor’s diagnosis of MED correlates with previous case reports in dogs and people with the distinctive

stippling of the epiphyses evident grossly, radiographically and histologically.<sup>6</sup>

The contributor provides an in-depth discussion on MED while contrasting other breed-specific osteochondrodysplasias. The diversity in causes and presentations of both osseous and chondrous dysplasias reveal the intricate and complex nature of osteogenesis both during development and in repair from injurious stimuli.

**Contributing Institution:** Michigan State University, Diagnostic Center for Population and Animal Health, [www.animalhealth.msu.edu](http://www.animalhealth.msu.edu)

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**CASE III: EHO-5 ZNFE960 (JPC 4050817).**

**Signalment:** 12-week-old male Sprague-Dawley rat, *Rattus norvegicus*.

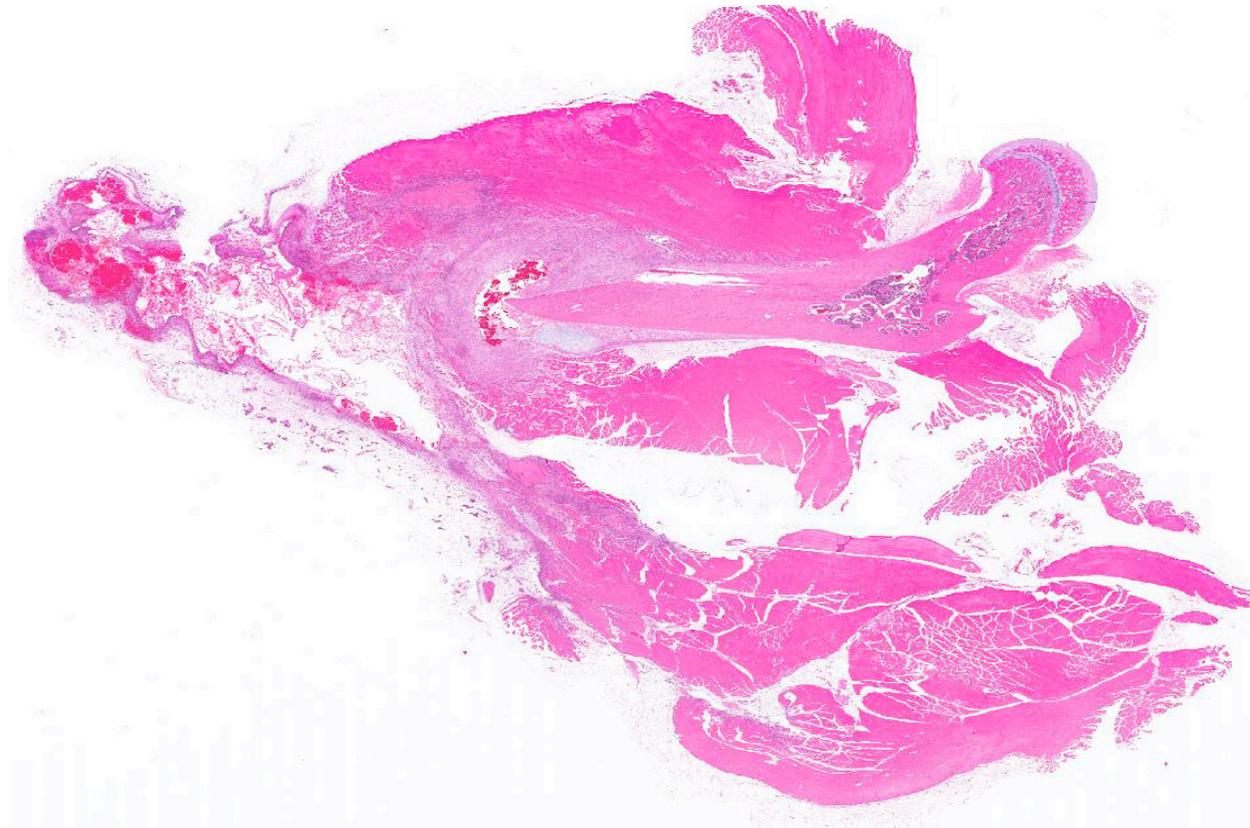
**History:** While under anesthesia, this animal underwent blast over-pressure at 120 kPa, traumatic fracture of the right rear limb with a drop weight apparatus, soft tissue crush injury at 20 psi for 1 minute and a trans-femoral amputation. The animal was maintained on an appropriate sustained-release pain control regimen and humane euthanasia was performed 7 days post injury. The right rear limb was disarticulated from the hip joint and submitted for routine processing. All animal care was done in accordance with the WRAIR/NMRC institutional animal care and use committee's guidelines.

**Gross Pathology:** Not available.

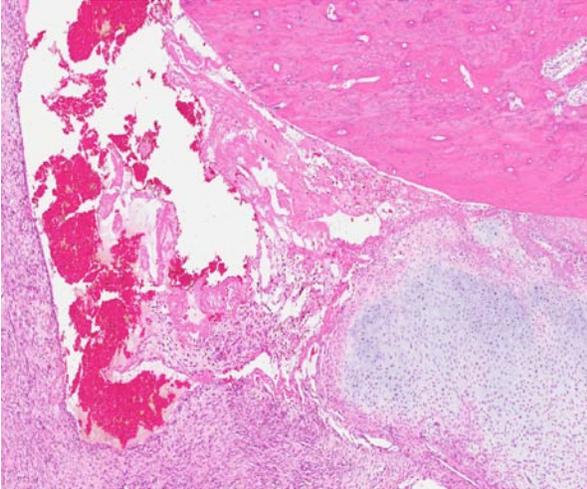
**Laboratory Results:** N/A

**Histopathologic Description:** Femur and associated soft tissues: There is a mid-diaphyseal, blunt, angled, traumatic fracture of the femur with

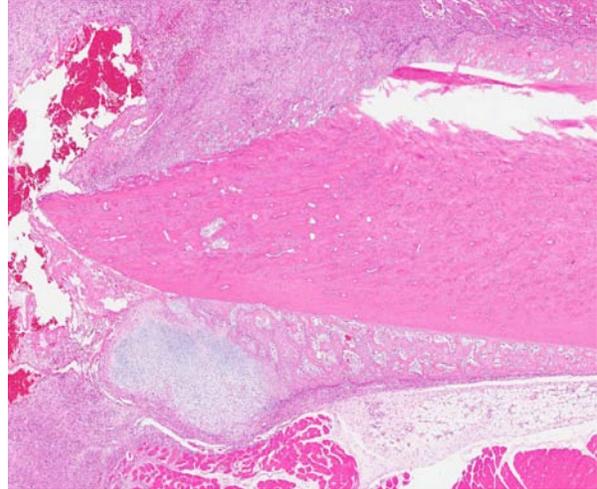
loss of the distal bone fragment. At the distal end of the bone fragment, there is a small hematoma composed of erythrocytes admixed with aggregates of disorganized fibrin. The hematoma is surrounded by densely packed proliferating mesenchymal cells (callus) which contain numerous perpendicularly oriented arterioles, fragments of woven bone, collagen, and few scattered multinucleate cells (osteoclasts). There is subperiosteal new woven bone composed of irregular and random to densely organized collagen fibers which widens from proximal to distal as it extends towards the fracture site and forms a small region of hyaline cartilage adjacent to the fracture. Woven bone is hypercellular with increased numbers of osteoblasts, enlarged osteocytes, and fewer osteoclasts. Within the callus, there is a small osteophyte embedded within dense connective tissue. Multifocally, the surrounding myofibers are variably characterized by: pallor, swelling, and vacuolization (degeneration); sarcoplasmic hypereosinophilia, loss of cross-striations, fragmentation, and pyknosis (necrosis); or sarcoplasmic basophilia with nuclear internalization with rowing of nuclei,



3-1. Femur, rat: There is a mid-shaft, angled femoral fracture with removal of the distal fragment. (HE 6.3X)



3-2. Femur, rat: At the distal end of the fracture site, there is a hematoma with hemorrhage and loosely polymerized fibrin strands. (34X)



3-3. Femur, rat: The distal end of the fragment is surrounded by a thick band of proliferating mesenchymal cells (callus). The periosteum (right) is elevated by proliferating trabeculae of woven bone, and a focal area of cartilage is present at the distal end. (HE 45X)

and large nuclei with large nucleoli (regeneration). Multifocally, myofibers are separated, surrounded and replaced by loose connective tissue, fibrin, edema, and hemorrhage. Distal to the fracture, there is a focally extensive area of inflammation forming a pseudocyst around a pocket of fibrin, hemorrhage, edema, and eosinophilic cellular and karyorrhectic debris (necrosis). Multifocally, there are clusters of histiocytes, neutrophils, lymphocytes and plasma cells at the periphery. Multifocally there is scattered golden yellow pigment (hemosiderin) within the callus and within surrounding connective tissues.

**Contributor's Morphologic Diagnosis:** Femur and associated soft tissues: Fracture, mid-diaphyseal, with subperiosteal new bone growth, cartilage growth, and callus formation, with adjacent myofiber necrosis, degeneration, and regeneration.

**Contributor's Comment:** Unlike most other tissues, bone is capable of repair by regeneration rather than scar formation.<sup>3</sup> The first stage of fracture repair is formation of a hematoma.<sup>3</sup> The hematoma is rapidly replaced by mesenchymal cells from the medullary cavity, endosteum, and periosteum to form a callus which is initially composed of loose connective tissue.<sup>3</sup> Next, primitive mesenchymal cells in the fracture gap differentiate into chondroblasts and replace the loose connective tissue with chondroid matrix.<sup>3</sup> The fracture site is revascularized and cartilage is

replaced by trabeculae of woven bone.<sup>3</sup> The final phase, which may take months or years, involves the replacement of woven bone in the callus with mature lamellar bone.<sup>3</sup> This coincides with modeling of the callus to restore the bone to its original shape and strength.<sup>3</sup> In adults, persistence of medullary trabeculae and thickening of the periosteal bone surface are likely to persist at the healed fracture site, whereas in younger animals, the fracture may completely resolve.<sup>3</sup>

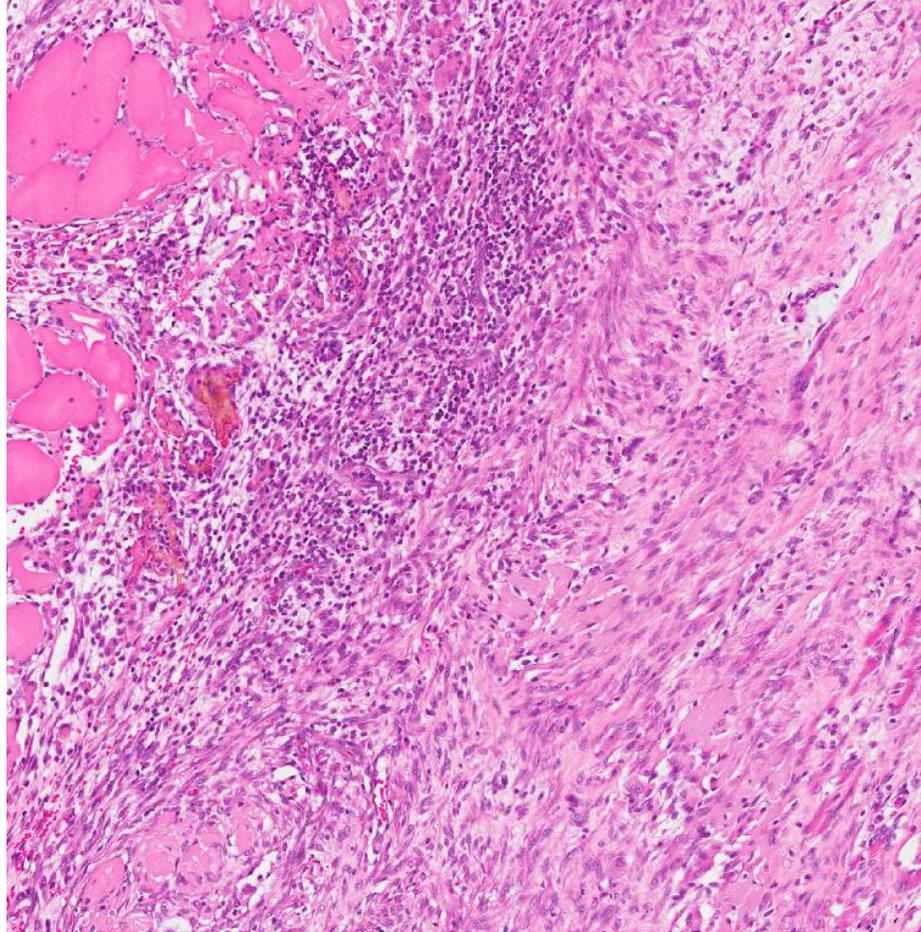
Three critical constituents are required for formation of bone: (1) the presence of collagen, (2) the availability of phosphate (and calcium), and (3) removal or absence of inhibitors of mineralization, such as pyrophosphate.<sup>1</sup> The bony biochemical milieu is complex and involves the interplay between soluble factors, extracellular matrix proteins and enzymes, and biomechanical forces that influence the modeling of bone.<sup>1-3</sup> This discussion will focus on the signaling molecules involved in bone homeostasis.

**Signaling Molecules.** Signaling molecules include hormones, cytokines and growth factors.<sup>1,3</sup>

**Hormones.** Parathyroid hormone and calcitonin are hormones that function to maintain a stable serum calcium concentration.<sup>1,3</sup> Estrogens and androgens are important regulators of skeletal growth and maturation.<sup>3</sup> The anabolic effect of estrogen is quite complex, but it appears to act indirectly by modulating other factors such as interleukin 6 (IL-6) and transforming growth factor beta (TGF-).<sup>1</sup> A significant effect of

estrogens appears to be inhibiting bone resorption.<sup>3</sup> Estrogen depletion induced by ovariectomy in a rat model markedly increases the synthesis of IL-6 by osteoblasts or their precursors in the bone marrow stroma.<sup>3</sup> Androgens also exert anabolic effects on bone either directly or after aromatization to estrogen.<sup>1</sup> Androgens tend to increase periosteal bone formation and radial growth, whereas estrogens decrease it, thus accounting for some of the differences noted between the sexes.<sup>1</sup> Thyroxine is essential for skeletal tissue formation, and if deficient leads to cretinism characterized by short stature and developmental abnormalities.<sup>1,3</sup> Growth hormone (GH) is secreted throughout life, but is highest in childhood and peaks during puberty.<sup>1</sup> While several hormones influence longitudinal bone growth, GH is generally regarded as the most important.<sup>1</sup>

**Cytokines.** Cytokines include interleukins, interferons, lymphokines, prostaglandins, and other polypeptides involved in host defense and homeostasis.<sup>1</sup> Interleukins are pro-inflammatory cytokines that are involved in bone resorption and remodeling.<sup>1</sup> Cytokines such as IL-6 and IL-11 appear to play a crucial role in the recruitment, proliferation, and differentiation of osteoclast progenitors that eventually lead to reduced bone mass in estrogen deficiency.<sup>2</sup> Prostaglandins are a subclass of eicosanoids that are enzymatically derived from fatty acids and act as potent messengers in regulation of vascular tone, inflammation, and cell growth.<sup>1</sup> However, they also have been shown to be an important mediator of local bone resorption.<sup>1,3</sup>



3-4. Femur, rat: The callus transitions into abundant granulation tissue which infiltrates the adjacent atrophic skeletal muscle. (HE 218X)

**Growth Factors.** The principal function of growth factors is regulation of cellular growth and function.<sup>1</sup> The actions of the TGF- superfamily, the bone morphogenetic protein (BMP) family, insulin-like growth factor (IGF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) have all been shown to influence bone metabolism.<sup>1</sup>

**Transforming Growth Factor- (TGF-).** TGF- has powerful effects on both osteoclasts and osteoblasts, and probably plays a key role in bone remodeling.<sup>3</sup> Expression of TGF- is especially high during active matrix secretion.<sup>1</sup> In vivo rat models have shown that osteoblast and osteoclast TGF-s (especially TGF-1) increase during fracture healing.<sup>1</sup> Functionally, TGF-1 has several effects that are synergistically conducive to matrix production and ossification.<sup>1</sup> First, TGF-1 recruits the appropriate cells for bone

formation and remodeling such as osteoprogenitor cells and fibroblasts.<sup>1</sup> During bone healing, TGF- $\beta$ 1 initially inhibits activation of osteoclasts, which is permissive for net bone formation.<sup>1,3</sup> Later, TGF- $\beta$ 1 is an indirect promoter of osteoclast activation.<sup>1,3</sup> This is crucial when osteoclasts are required not only to remodel bone, but to liberate protein-bound enzymes within the extracellular matrix.<sup>1</sup> In addition to recruiting cells, TGF- $\beta$ s are a potent promoter of collagen production without which ossification cannot occur.<sup>1,3</sup>

**Bone Morphogenic Proteins.** BMPs are a family of growth factors which belong to the TGF- $\beta$ -superfamily.<sup>1,3</sup> Like their parent TGF- $\beta$ s, the BMP signaling molecules regulate myriad cellular processes, including proliferation, differentiation, and growth.<sup>1,3</sup> In the context of bone formation, at low concentrations they promote chemotaxis and cellular proliferation.<sup>1</sup> At high concentrations, they favor cellular differentiation and bone formation.<sup>1</sup> BMPs are believed to stimulate production of osteoprotegerin (OPG) which is an osteoblast-secreted decoy receptor that specifically binds to osteoclast differentiation factor and inhibits osteoclast maturation.<sup>1</sup> BMPs are the most osteoinductive growth factors described.<sup>1</sup> BMP-specific antagonists, such as noggin and chordin, have also been identified.<sup>3</sup>

**Insulin-like Growth Factor-1, Fibroblast Growth Factor-2, and Epidermal Growth Factor.** In vivo and in vitro data suggest that insulin-like growth factor-1 (IGF-1) stimulates osteoprogenitor cell mitosis and differentiation, thereby increasing the number of functionally mature osteoblasts.<sup>1</sup> FGFs act in concert with heparin sulfate-containing proteoglycans to modulate cell migration, angiogenesis, bone development and repair, and epithelial-mesenchymal interactions.<sup>1</sup> FGF-2 is the most abundant ligand and has been shown to stimulate osteoblast proliferation and enhance bone formation.<sup>1</sup> FGF-2 expression is elevated during fracture healing.<sup>1</sup> Exogenously applied FGF-2 accelerates osteogenesis in critical-size bone defects and fracture sites.<sup>1</sup> EGF has been shown to stimulate bone resorption and upregulate production of matrix metalloproteinases, thus promoting remodeling.<sup>1</sup>

**Platelet-Derived Growth Factor and Vascular Endothelial Growth Factor.** PDGF is best known for its role in angiogenesis, but also is

instrumental in embryological development and postnatal cellular migration and proliferation.<sup>1</sup> As its name suggests, PDGF is secreted systemically by platelets.<sup>1</sup> In bone, its cellular origin is unknown.<sup>1</sup> Functionally, PDGF exerts one of the strongest chemotactic effects on osteoblasts and stem cell precursors.<sup>1</sup> Furthermore, PDGF is a potent activator of osteoclasts, fibroblasts, and endothelial cells.<sup>1</sup> PDGF is a potent stimulator of new bone formation, but also promotes bone resorption.<sup>3</sup> The VEGFs are involved in both angiogenesis and vasculogenesis.<sup>1</sup> VEGF increases endothelial cell and endothelial progenitor cell chemotaxis and mitogenesis, promoting new vessel formation.<sup>1</sup> In bone, osteoblasts secrete VEGF and express VEGF receptors.<sup>1</sup> Further, osteoclasts also express VEGF receptors, and VEGF chemotactically recruits osteoclasts to remodeling zones.<sup>1</sup> Following fracture or osteotomy, vascular disruption of nutrient arteries, release of lysosomal enzymes from necrotic bone edges and soft tissues, and vasoconstriction of periosteal and medullary arteries result in the formation of a hypoxic interfragmental zone of injury.<sup>1</sup> These stimuli serve collectively as a catalyst for new blood vessel formation.<sup>1</sup> VEGF-mediated angiogenesis has been demonstrated to be an absolute requirement for successful bone induction in fracture zones.<sup>1</sup>

**JPC Diagnosis:** Bone, femur: Severely displaced (traumatic amputation), simple, mid-diaphyseal femoral fracture with organizing hematoma, subacute subperiosteal woven bone and cartilaginous callus, adjacent myofiber degeneration, necrosis, and regeneration.

**Conference Comment:** Appropriate fracture healing requires alteration in expression of several thousand genes.<sup>2</sup> The contributor provided a comprehensive overview of the most well known signaling molecules which play a prominent role, and this case is an opportunity to observe the process histologically.

Indirect, or secondary, fracture healing occurs most commonly, and consists of endochondral and intramembranous bone healing in situations of weight-bearing fractures with a small degree of motion. Excessive motion or load results in delayed healing or non-union. No anatomical reduction is required with these types of fractures;

however, some fixation techniques may initiate it if they induce subtle motion at the fracture site.<sup>2</sup>

Direct fracture healing does not occur as a natural process, but rather following anatomical reduction and stable fixation. It is the desired end state of surgical fracture repair, as the direct remodeling of lamellar bone, Haversian canals and blood vessels may lead to complete healing within months, while indirect healing often occurs for years before the bone is completely remodeled from a fracture callus to lamellar bone.<sup>2</sup> This process also alters the electrical potential of bone, and recent studies have demonstrated that measured electrical potentials may correlate with prognosis of adequate fracture repair.<sup>4</sup>

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**CASE IV: HB6280 (JPC 4033562).**

**Signalment:** 8-month-old female thoroughbred horse, *Equus caballus*.

**History:** The mare showed sudden renal failure and no response to treatment. Anorexia and severe uremia were continuing and the animal was euthanized.

**Gross Pathology:** After formalin fixation, the surfaces of the both kidneys were slightly irregular and the cortex showed whitish to tan on the cut sections. No significant gross lesion was observed in other organs.

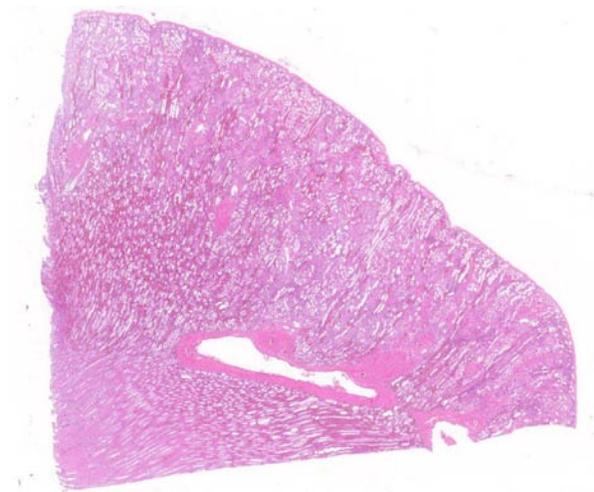
**Laboratory Results:** Blood test revealed high BUN 206.7 mg/dl (normal: 9.0-20.0) and CRE: 9.69 mg/dl (normal: 0.9-1.8). Serology (antibody titer) and PCR showed no evidence of *Leptospira sp.* infection.

**Histopathologic Description:** Diffuse and global, partially segmental glomerular sclerosis and enlarged glomeruli with mild to moderate proliferation of mesangial cells are remarkable. Irregularly dilated tubules are predominant in renal cortex, and papillary projections of tubular epithelium into the lumens are occasional. Immature small tubules with indistinct luminal structures and dysplastic tubules with enlarged clear nuclei are frequent. Protein cast formation, deposition of oxalate crystal and hyaline droplet degeneration are rarely in tubules/tubular

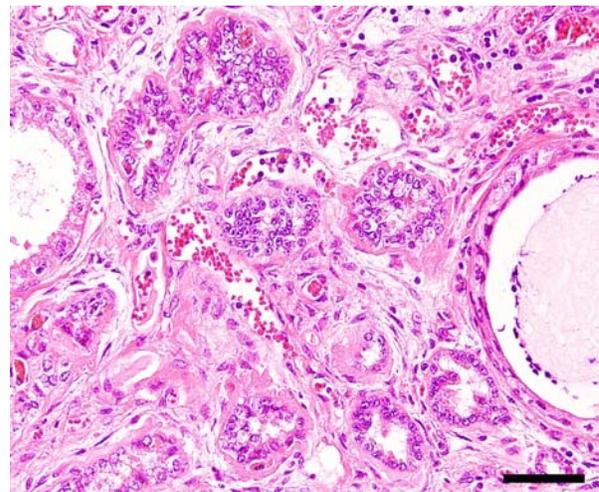
epithelium. Diffuse and mild fibrosis is in interstitial tissues with mild lymphoplasmacytic infiltration, sometimes along with concentric fibrosis around renal tubules and/or Bowman's capsules and deposition of eosinophilic homogenous material. Small to mid-sized arterioles are increasing in the cortex. Rarely, abnormal large muscular arteries are in subcapsular cortex (not in all slides). Pale basophilic myxoid material depositions are rare in the interstitium.

**Contributor's Morphologic Diagnosis:** Kidney: Equine renal dysplasia.

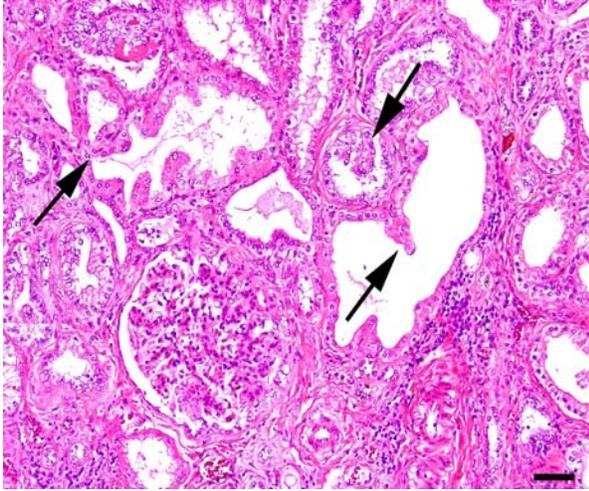
**Contributor's Comment:** Based on the histopathological findings, renal dysplasia was considered most likely in the present case. Abnormal proliferation of arterioles in the renal cortex also supports a developmental anomaly of the kidneys in this mare. Various affected glomeruli with hypercellularity and sclerosing changes suggest that the primary lesions are not located in glomeruli. However, regenerative tubules with juvenile epithelial cells, interstitial fibrosis and mild to moderate mesangial proliferation suggest a differential diagnosis of tubular nephritis following renal injury, such as toxicosis or leptospire infection. The present case was negative for leptospire. Further, plant toxicosis is unlikely because no other case of toxicosis was found in the ranch.



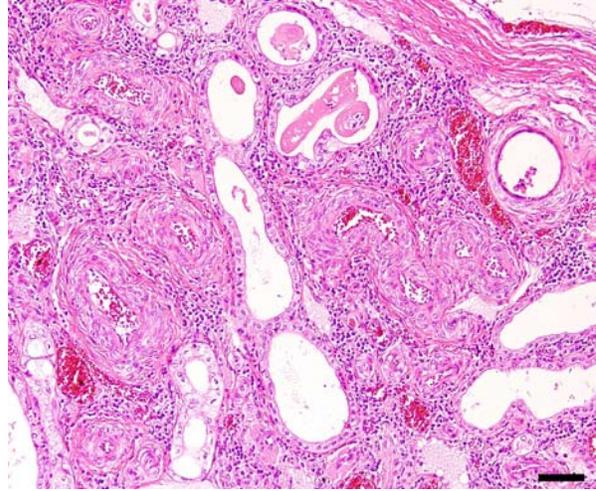
4-1. Kidney, 8-month-old thoroughbred foal: There is diffuse distortion of renal architecture with dilatation of tubules and markedly enlarged and tortuous renal vasculature.



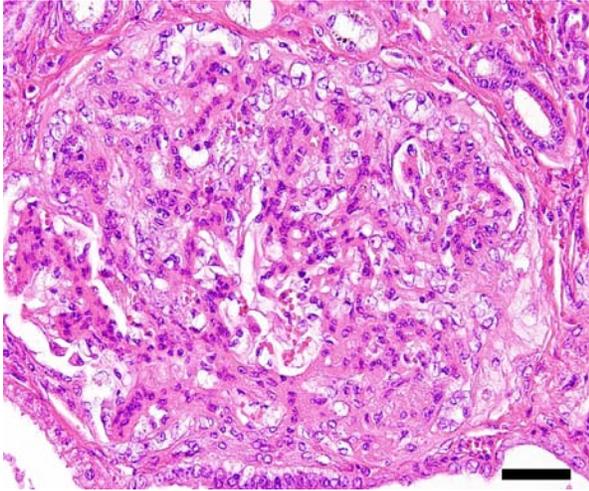
4-2. Kidney, foal: Immature renal tubules with atypical epithelial cells and incomplete luminal structures. HE. Bar=50µm. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, JAPAN <http://www.hokudai.ac.jp/veteri>)



4-3. Kidney, foal: Papillary projections (arrows) into the lumen of tubules. HE. Bar=50µm (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, JAPAN <http://www.hokudai.ac.jp/veteri>))



4-4. Kidney, foal: Many arterioles are present in renal cortex. Dilation of tubules is also noted. HE. Bar=50µm. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, JAPAN <http://www.hokudai.ac.jp/veteri>))



4-5. Kidney, foal: Enlarged glomerulus with proliferation of mesangial cells and adhesion with Bowman's capsule. (Photo courtesy of: Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, JAPAN <http://www.hokudai.ac.jp/veteri>))

Renal dysplasia is a rare congenital disease of developmental uni- or bilateral kidney anomaly reported in various animals.<sup>4</sup> Renal dysplasia in dogs is well documented; however, equine renal dysplasia is also reported. The size of the affected kidney shows small and irregular surface with demonstration of immature histological structures like undifferentiated stromal tissues, immature renal tubules and glomeruli. Primitive ductal structures and cartilage and/or bone formation are sometimes found.

The major histological findings reported in equine renal dysplasia are papillary proliferation of

tubular epithelium, immature tubules and islands of interstitial fibrosis.<sup>1,2,7</sup> Renal artery dysplasia is also described in one adult horse with chronic renal failure<sup>1</sup> and also in humans. The histopathological changes in the present case are very complicated; however, some of them, including oxalate deposition, appear to be secondary changes following renal failures. The various proposed pathogeneses of renal dysplasia, like abnormal metanephrons or vascular malformation, may be putative but clear mechanisms are still unknown.

**JPC Diagnosis:** Kidney: Dysplasia characterized by hypercellular glomeruli, glomerulosclerosis, tubular epithelial hyperplasia, hypertrophic arterioles and arteries, and interstitial myxoid matrix.

**Conference Comment:** Equine renal dysplasia is rarely reported in the horse and often has a variable histopathologic presentation. Many of the well-defined, characteristic histologic features observed in dogs, including persistent metanephric ducts, primitive mesenchyme and cartilaginous or osseous tissue, are not present in this case. Yet the clinical history and glomerular changes are consistent with the diagnosis. Some reports of equine renal dysplasia describe renal cysts, fetal glomeruli, and tubules lined by cuboidal epithelium, while others have identified normal kidney size with normal appearance and number of glomeruli but with hypoplastic nephron tubules.<sup>5,9</sup> Conference participants were

intrigued with the aberrant arterioles and large muscular arteries present throughout the renal cortex, and speculated whether these may have played a primary role in the development of other pathologic changes in this case. Interestingly, these aberrant vascular features have been reported in human cases of segmental or complete renal dysplasia.<sup>8</sup> Segmental hypoplasia with renal vascular anomalies (Ask-Upmark kidney) has also been described in young Boxer dogs.<sup>3</sup>

A related, possibly equivalent, condition to renal dysplasia is referred to as progressive juvenile nephropathy and is associated with specific dog breeds including Lhasa Apso, Shih Tzu, and the golden retriever. These conditions share several features with dysplasia though typical presentation depends on the breed. Often a glomerulopathy resembling membranoproliferative glomerulonephritis is present and may progress to glomerulosclerosis. Overall gross and microscopic features are comparable to those of chronic renal disease with renal fibrosis in aging dogs, but this condition affects dogs under 2 years of age.<sup>6</sup>

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