



WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 24

09 May 2012

CASE I: 0S10400 (JPC 4002913).

Signalment: 12 week-old male (castrated) and female commercial cross (for meat production) pigs, *Sus scrofa domestica*.

History: Porcine reproductive and respiratory syndrome virus (PRRSv) negative herd. Historical mortality; 2% in the nursery phase of production (3-13 weeks of age). 40% mortality from weeks 9-12 in the nursery with no response to antimicrobial therapy.

Clinical signs included sudden death, seizure like activity, lameness with joint enlargement, and weakness with muscle fasciculations.

Gross Pathology: Swollen joints with increased synovial fluid, swollen chondro-costal junctions, multiple fracture calluses on ribs, rib bones were soft and rubbery and bent 20-30 degrees before breaking.

Laboratory Results: Serum Chemistry

Serum calcium	Result	Units
Pig 1	4.1	mg/dl
Pig 2	4.8	mg/dl
Pig 3	4.9	mg/dl
Pig 4	5.3	mg/dl
Pig 5	4.6	mg/dl
Pig 6	5.9	mg/dl
Pig 7	4.7	mg/dl
Pig 8	5.1	mg/dl
Pig 9	5.9	mg/dl
Pig 10	4.7	mg/dl

Serum Vitamin D

Vitamin D analysis in serum from 10 pigs reveals no detectable (<2.0 ng/ml) amount of 25-hydroxy vitamin D3.

Normal reference range for serum vitamin D	
Age of animal	25-OH-D3 (ng/ml)
Neonate	5-15
10 days	8-23
Finishing pigs	30-35
Mature	35-70
At Parturition	35-100

Bone analysis

Identification	Bone Ash	Bone density	Bone Calcium	Bone Phosphorus
Pig 1	0.26	1.29 g/ml	32%	16%
Pig 2	0.33	1.2 g/ml	36%	18%
Pig 3	0.37	1.27 g/ml	38%	18%
Pig 4	0.29	1.08 g/ml	34%	18%
Pig 5	0.33	1.09 g/ml	40%	18%

Swine; rib bone, normal references	
Bone ash	58-62 %
Bone density	1.4-1.5 g/ml.
Bone ash Calcium	32-39%
Bone ash Phosphorus	13-22%

PCRs

Negative for SIV, *Mycoplasma hyopneumoniae*, and PRRS (lung)

Negative for *Erysipelothrix rhusiopathiae*, *Mycoplasma hyorhinis* (joint fluid)

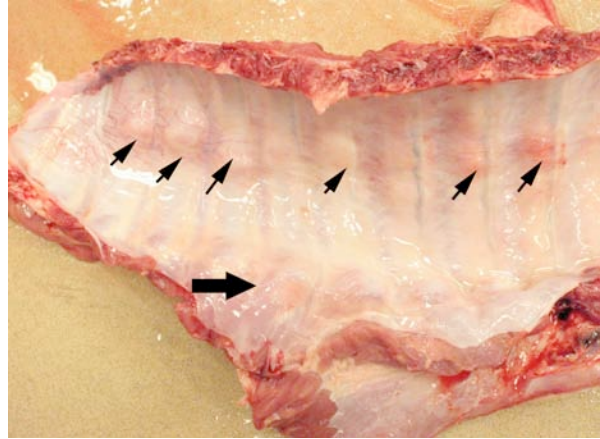
Bacteriology

Lung, liver, spleen, and joint fluid; No significant growth

Contributor’s Histopathologic Description: Rib bone, costochondral junction (slide Bd): Physal cartilage is diffusely thickened and irregular resulting in flaring of the distal metaphysis. Hypertrophic chondrocytes are arranged in poorly organized columns and there are multiple islands of retained cartilage within the metaphysis. Long tongues of cartilage remain within the primary spongiosa that are often surrounded by variably thick seams of unmineralized osteoid which are lined by cuboidal osteoblasts. Chondrocytes within retained cores are variably degenerate and occasionally lost. There is disorganization of primary spongiosa with multifocal fractures and areas of hemorrhage and fibrin accumulation. Osteoclasts are often associated with secondary spongiosa, many of which are present within Howship’s lacunae, and marrow spaces are often devoid of marrow elements and contain moderate numbers of mesenchymal cells and variable amounts of fibrous connective tissue. There is multifocal thinning of cortical bone with increased numbers of associated osteoclasts, and the periosteum is variably thickened.

Contributor’s Morphologic Diagnosis: Rib bone, costochondral junction: Failure of endochondral ossification with hyperplasia of physal cartilage and retention of cartilage cores (nutritional osteodystrophy; Rickets).

Contributor’s Comment: Metabolic bone disease broadly categorizes disturbances related to bone formation and remodeling. Rickets and osteomalacia are metabolic bone diseases associated with flawed bone mineralization in growing and adult animals, respectively.⁵ The pathogenesis is similar for these diseases and is linked to imbalances of phosphorus or vitamin D.³ In some instances, there are inherited forms of rickets associated with vitamin D metabolism or renal function. However, most reported cases of



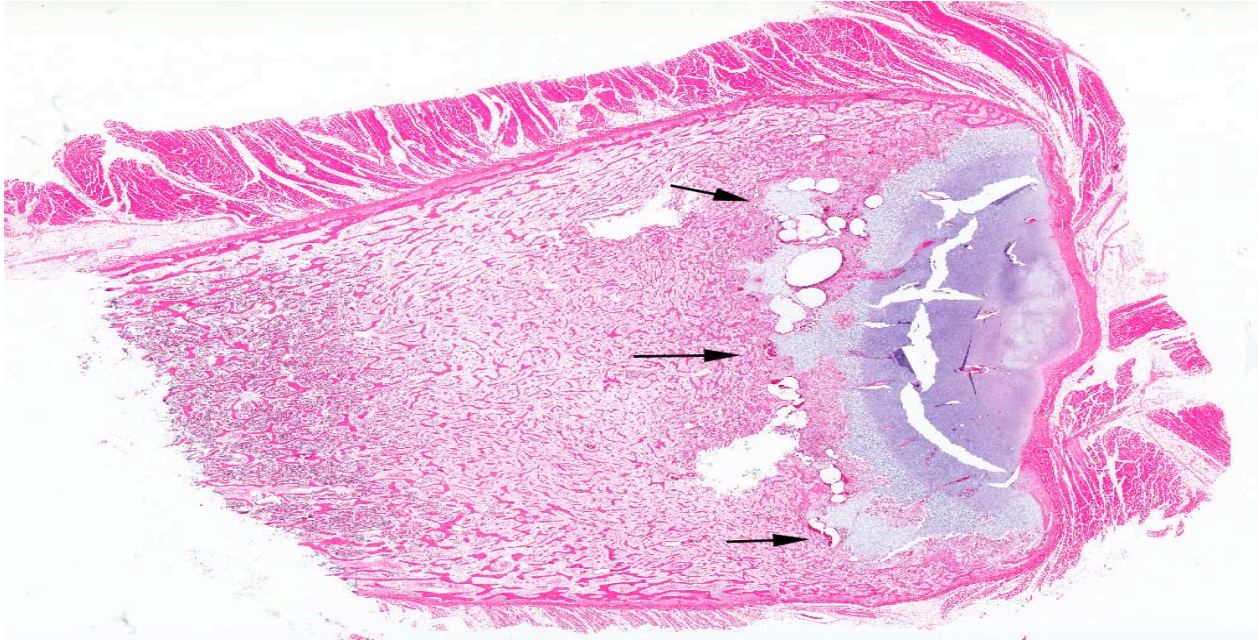
1-1. Pig, ribs: Multiple rib fractures (arrows) as well as an enlarged costochondral junction (large arrow) is present in this 12-week-old pig. Photo courtesy of: The Department of Veterinary Pathology, 2764 Veterinary Medicine, Iowa State University, Ames, Iowa 50011. <http://vetmed.iastate.edu/vpath> and <http://vetmed.iastate.edu/vdpam/>



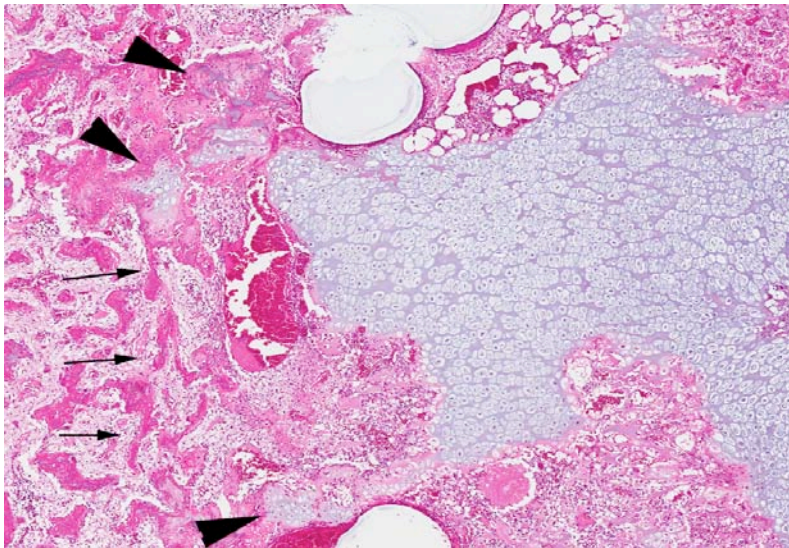
1-2. Pig, rib: Tangential section of the costochondral junction in a 12-week-old pig. The growth plate is expanded and scalloped in an irregular fashion. Photo courtesy of: The Department of Veterinary Pathology, 2764 Veterinary Medicine, Iowa State University, Ames, Iowa 50011. <http://vetmed.iastate.edu/vpath> and <http://vetmed.iastate.edu/vdpam/>

rickets in production animal medicine are outright diet mixing errors. Swine are particularly sensitive to rickets development because of rapid growth and confined facilities.⁵

Swine rickets has not garnished much attention in the last few decades as most nutritional programs provide adequate phosphorus and vitamin D required for normal bone growth and homeostasis. However, current industry practice for diet composition in market swine is tailored for lean muscle mass growth, not bone formation. Variations in quality or quantity of feed ingredients can cause clinical signs and lesions compatible with rickets. This old and well characterized disease process can be easily overlooked and forgotten. Investigation of recent cases of metabolic bone disease/acute deaths with high



1-3. Pig, rib: Subgross image of the growth plate from the rib in Fig. 1-2. The irregular tongues of cartilage (arrows) are present. The metaphysis is flared, marrow spaces within the metaphysis are filled with collagen rather than marrow, and the cortex is markedly thinned. Artfactual dilation of subphyseal presence is marked. (HE 5X)



1-4. Pig, rib: Areas of unmineralized cartilage are present within the primary trabeculae (arrowheads). Secondary trabeculae are often oriented parallel to the growth plate (arrows). (HE 34X)

morbidity and mortality presented to the Iowa State University Veterinary Diagnostic Laboratory (VDL) ultimately led to the discovery of a feed with a base mix product inadequate in vitamin D₃ and recall of that product.

Vitamin D can be synthesized in the skin from 7-dehydrocholesterol following ultraviolet light exposure

or be supplied in the diet. Dietary supplementation of vitamin D is considered a necessary practice for swine. Following skin conversion or small intestinal absorption, vitamin D is hydroxylated to 25(OH)D in the liver with biologically active vitamin D, 1,25(OH)₂D, produced thereafter in the kidney. The main activity of 1,25(OH)₂D is to regulate small intestinal calcium absorption along with lesser phosphorus absorption and inhibit parathyroid hormone.⁴

Lack of calcium absorption results in calcium mobilization from bone to maintain the finely regulated physiologic homeostasis of blood calcium concentration. The result of inadequate deposition or excess mobilization is metabolic bone disease.³ If bone reserves of calcium are exhausted or delayed in release, hypocalcaemia can result in death.

Growing pigs with classic rickets will have weak bones that bend before they break. Enlarged growth plates can give the appearance of swollen joints.¹ Osteomalacia is usually seen in late finishing or adult swine since this is a condition of increased absorption of previously formed bone. Fractured femurs,

vertebrae, or ribs at load out or at slaughter occur with increased frequency when there is osteomalacia. Lactational osteoporosis has similar features but occurs in lactating or newly-weaned sows. These aforementioned clinical signs are classical for metabolic bone disease. However, there are atypical presentations that can result in sudden death without premonitory signs.²

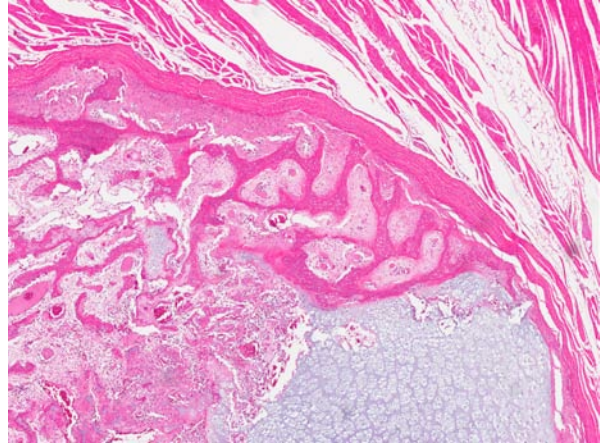
Chronic nutrient imbalances can have acute clinical manifestations other than pathologic fractures or rubbery or weak bones. Somewhat more unusual is the rather abrupt onset of clinical signs associated with acute hypocalcemia. In clinical observations from recent cases, growing pigs unexpectedly develop one or more of the following clinical signs: tremors, tetany, seizure-like muscle fasciculations, weakness, lameness, painful gait with reluctance to move, and bone fractures (macroscopic and/or microscopic). Often, the first clinical sign observed in affected animals in our cases was acute death. In a large population of pigs, many of these clinical signs may occur simultaneously.

Most common mechanisms of rickets in growing pigs are: inadequate dietary supplementation of vitamin D₃; inadequate absorption of phosphorus due to low phosphorus in diet, phosphorus unavailable as phytate, or inadequate phytase usage; imbalance of feed calcium to phosphorus ratio; and improper formulation of Ca:P ratio in diet (should be roughly 1.2:1).²

JPC Diagnosis: 1. Rib bone: Physeal chondrodystrophy with delayed endochondral ossification.
2. Rib bone: Fibrous osteodystrophy.
3. Rib bone: Cortical osteopenia.

Conference Comment: It is common for rickets to present with focally thickened physeal cartilage, as in this case, which represents pockets of disorganized retained hypertrophied chondrocytes within areas of normal endochondral ossification. This represents a timeline of variations of adequate and inadequate dietary vitamin D, and can look similar to osteochondrosis; however, rickets also presents with trabecular disruption, hemorrhage, and infractions.^{1,5}

The moderator discussed the fact that in rickets, the physeal cartilage is not hyperplastic, indicating proliferation of chondrocytes, but rather a failure of resorption of normal chondrocytes, and they are thus considered retained. For this reason conference participants preferred the term of physeal chondrodystrophy. This presents as a failure of orderly maturation of physeal chondrocytes, disorganization of columns of chondrocytes, irregular retention of hypertrophied chondrocytes, rare mineralized



1-5. Pig, rib: The metaphyseal cortex is very thin (osteopenic) and bolstered by trabeculae of woven bone. (HE 34X)

longitudinal septa formation, disorganized and thickened primary and secondary trabeculae without normal mineralized longitudinal septa, and secondary and tertiary trabeculae lined by hypertrophied osteoblasts and many osteoclasts forming Howships lacunae. In mammals, failure of endochondral ossification occurs because blood vessels can only penetrate into the physis when there is apoptosis of chondrocytes and mineralization of the longitudinal septa, which does not occur properly due to decreased available serum ionized calcium.¹

The presence of a flared metaphysis with coarse spicules of chondro-osseous tissue and fibrosis in the perichondrial groove and cutback zone is due to the presence of unmineralized bone matrix that cannot be bound by osteoclasts, which are only able to bind and resorb mineralized bone. This is the reason for the presence of prominent thickening along costal-chondral junctions, colloquially known as rachitic rosary.^{1,5}

The cortex is composed mostly of woven bone with increased cortical porosity, and the cortical osteopenia is due to increased cortical lysis as a result of the attempt at calcium resorption. In the diaphysis, the marrow fibrosis and increased osteoclasts is mostly intracortical and endocortical, but there is some classic peritrabecular fibrous connective tissue. Conference participants interpreted this as fibrous osteodystrophy (FOD). FOD occurs along with rickets due to hypocalcemia from decreased resorption from bone and absorption from the intestines and resultant secondary hyperparathyroidism. In the early stages of FOD, fibrous proliferation can be subtle and the fibrous connective tissue begins and is oriented around bone spicules. This is in contrast to myelofibrosis, where fibrous connective tissue is oriented toward the middle of the marrow cavities.^{1,5}

In addition to decreased dietary phosphorus or vitamin D, rickets in young animals or osteomalacia in adults can be the result of chronic fluorosis. It is also theoretically possible to be caused by low dietary calcium with normal dietary vitamin D.

Contributor: Iowa State University
Department of Veterinary Pathology
2764 Veterinary Medicine
Ames, Iowa 50011
<http://vetmed.iastate.edu/vpath>
<http://vetmed.iastate.edu/vdpam/>

References:

1. Carlson CS, Weisbrode SE. Bones, joints, tendons, and ligaments. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2011:926-7, 948-50.
2. Crenshaw TD. Calcium, phosphorus, vitamin D and vitamin K. In: Lewis AJ, Southern LL, eds. *Swine Nutrition*. 2nd ed. Boca Raton, FL: CRC Press LLC; 2001:187-212.
3. Dittmer KE, Thompson KG. Vitamin D metabolism and rickets in domestic animals: a review. *Vet Pathol*. 2011;48:389-407.
4. Moe SM. Disorders involving calcium, phosphorus, and magnesium. *Prim Care*. 2008;35:215-2vi.
5. Thompson K. Bones and joints. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 1. New York, NY: Elsevier Saunders; 2007:75-80.

CASE II: 10-1124 (JPC 4006473).

Signalment: 6-month-old intact female Yorkshire sow (*Sus scrofa domesticus*).

History: This case was part of a traumatic bone injury study involving a comminuted fracture of the femoral shaft with stabilization via surgical pinning. This particular animal developed a suppurative infection along the pins, but was maintained until the end of the study.

Gross Pathology: The mid femur was markedly swollen with a dense fibrous capsule surrounding the fractured bone, sequestrate and woven bone. Multifocal abscesses were noted tracking along the surgical pins.

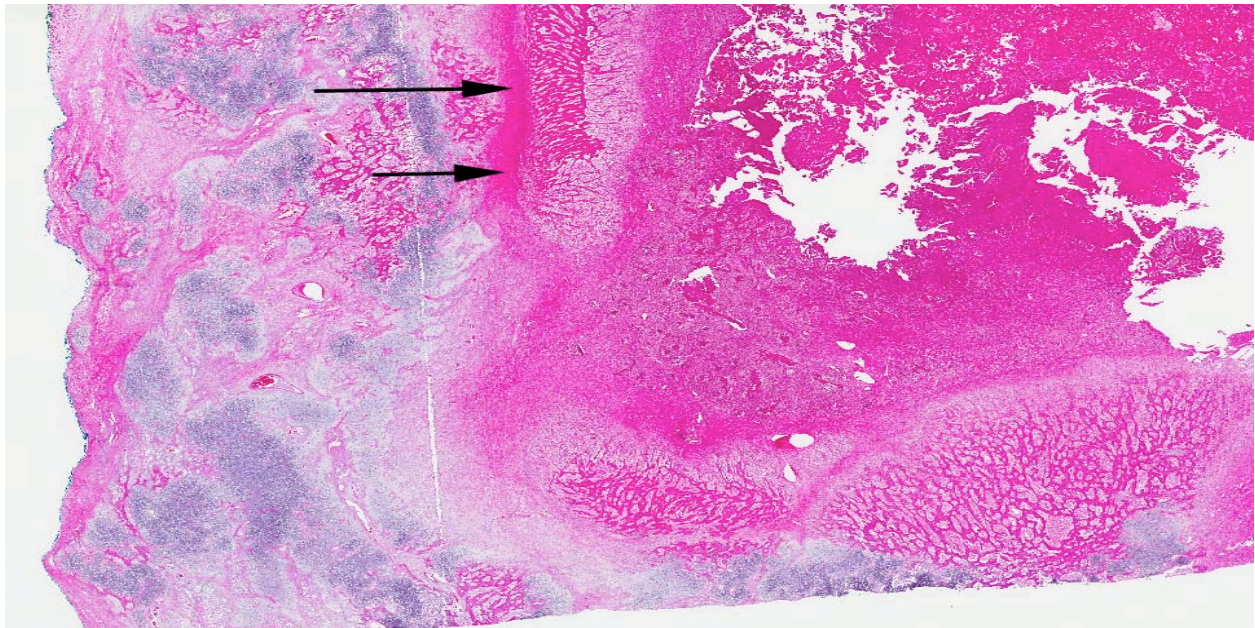
Laboratory Results: *Staphylococcus aureus* was cultured from the open pin lesions; however, no bacteria was visualized in the submitted tissue samples.

Contributor's Histopathologic Description: Bone, femur (per contributor): Focally extensive, pre-existing cortical bone is discontinuous (fractured) and thinned with scalloped edges, and replaced by irregular trabeculae of woven bone in varying degrees of maturation. Multifocally, woven bone is contiguous with the remodeled cortical bone and intertwined trabeculae are perpendicular to the cortical bone (reactive bone). Both cortical bone and woven bone are frequently surrounded and bounded by disorganized islands of cartilage undergoing endochondral ossification (callus) composed of tightly

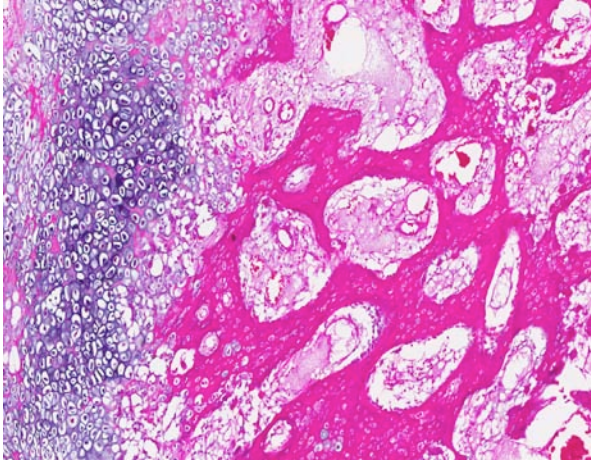
packed chondrocytes in a basophilic matrix as well as densely packed fibroblasts and collagen fibers which diffusely fill and obscure the marrow cavities (myelophthisis). Diffusely, bone trabeculae are lined by plump reactive osteoblasts which are frequently in close apposition to the bone or in regions which are scalloped and discontinuous, and frequent multinucleated osteoclasts rest in resorption bays (Howship's lacunae). Multifocally deep to the reactive bone is a swath of loose fibrosis admixed with myriad neutrophils, fewer macrophages and lymphocytes, high numbers of plump reactive fibroblasts, eosinophilic cellular and karyorrhectic debris (necrosis), eosinophilic proteinaceous material (edema), fibrin, hemorrhage and moderate numbers of small caliber vessels with reactive endothelium (granulation tissue). Multifocally within this area there are frequent multinucleated osteoclasts which are either free or surround fragments of osteolysis. These are also often centered on sequestrae characterized by sharp angulated fragments of necrotic bone with empty lacunae. The larger sequestrae are surrounded by the previously described granulation tissue (involucrum).

Contributor's Morphologic Diagnosis: Bone, femur (per contributor): Osteomyelitis, necrotizing, neutrophilic, focally extensive, marked with cortical fracture, reactive bone, callus formation, myelophthisis and sequestrae, Yorkshire sow, *Sus scrofa domesticus*.

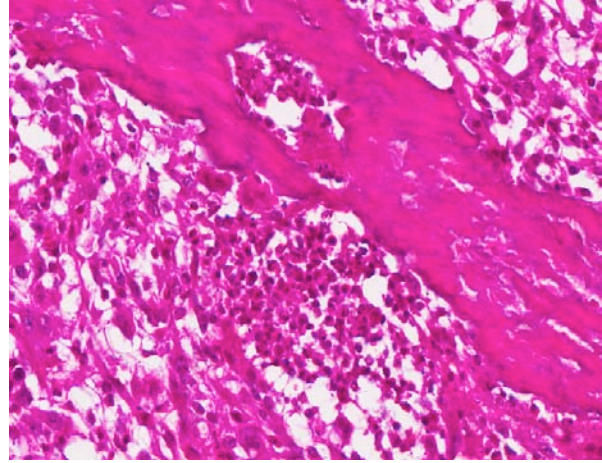
Contributor's Comment: Cases of acute osteomyelitis are most frequently associated with aerobic bacteria such as *Staphylococcus* sp. and *Streptococcus* sp. resulting from hematogenous spread



2-1. Pig, long bone: Subgross of callus formation following fracture. Numerous islands of chondroid metaplasia are present in the section. A fragment of osteopenic cortex is present (arrows) with marked periosteal and endosteal new bone growth. (HE 4X)



2-2. Pig, long bone: Scattered throughout the section, contiguous with trabeculae of woven bone at right (note the numerous haphazardly arranged lacunae characteristic of woven bone), are foci of chondroid metaplasia (left). Chondrocytes in these foci are also very numerous and haphazardly arranged. (HE 150X)



2-3. Pig, long bone: Fragments of necrotic, pre-existent lamellar trabecular bone are surrounded by numerous viable and degenerate neutrophils. (HE 340X)

in younger animals in which the physis is still open, or resulting from sepsis secondary to omphalitis. The veterinary literature describes less pathogenic agents such as *Fusobacterium necrophorum* or coliforms which can be opportunistic infections causing acute osteomyelitis.

In pigs, *Actinomyces pyogenes* is frequently isolated from osteomyelitic lesions as well as in cattle and sheep, while *Salmonella* sp. are commonly isolated from foals.

Additionally, anaerobic bacteria, such as *Peptostreptococcus anaerobicus* or *Bacteroides asaccharolyticus*, as well as mixed aerobic and anaerobic infections are also frequent causes of acute osteomyelitis.¹

Bacteria such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* contain fibronectin-binding surface proteins, which have the ability to interact with collagen, or the surface proteins contain binding sites that attach to sialoprotein, a noncollagenous bone matrix protein.⁵

Posttraumatic osteomyelitis usually represents a form of exogenous infection in which the bacteria are introduced through a traumatic wound as in this case, or through a surgical wound. Entrance of infection can occur following simple wounding in which the formation of hematomas under the skin may allow bacterial contamination and infection to develop. Such an infectious process may proceed without obviously apparent clinical signs. Posttraumatic osteomyelitis is frequently avascular as in this case, resulting from a nidus of necrotic bone and the probable inoculation of environmental or skin bacteria secondary to

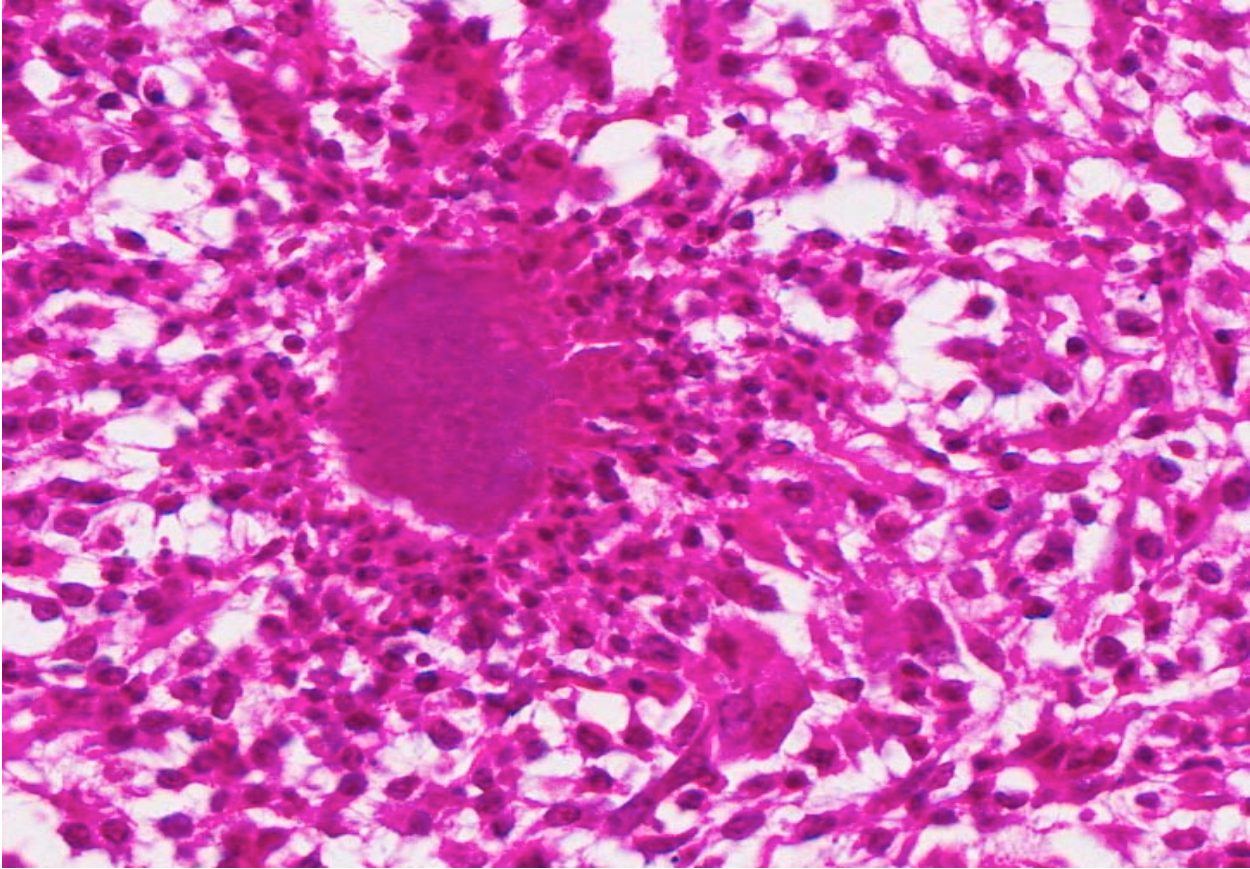
penetrating wounds, such as those caused by bullet, tooth or other foreign bodies.

As the fractured femur in this case was stabilized through open treatment of closed fractures, the posttraumatic or iatrogenic infection may have resulted from the placement of the pins or may have contaminated a sterile wound through that portal of entry. This pathogenesis represents the most common forms of osteomyelitis reported in small animals. When the skin is devitalized to the point that bacterial contamination is possible, infection of bone is an ever present danger.

Subsequent to inadequate treatment of acute osteomyelitis, the condition can obviously progress to become chronic and therefore represents an infection that is well established in bone and has been present for several weeks, months, or even years. Chronic infection can occur after bony union and structural stabilization has occurred or prior to union of the fracture. If the infection occurs before bone union, the treatment of the condition is made more difficult by the presence of the non-union or delayed union. An infected non-union is the worst possible treatment scenario in which the clinician is first required to allow for healing of the fracture and then to deal with the infection.³

JPC Diagnosis: Long bone: Osteomyelitis, suppurative, chronic, with medullary and periosteal new bone growth.

Conference Comment: There is marked variations in the slides submitted for this case. Lesions identified within the conference which were not present on all slides include callus formation, (either from the



2-4. Fig, long bone: Scattered throughout the medullary cavity, colonies of bacteria enmeshed in protein are surrounded by large numbers of viable and degenerate neutrophils as well as fewer macrophages.

fracture repair or a pathologic fracture from the osteomyelitis), reactive periosteal changes without antecedent callus formation, extensive chondrous metaplasia, medullary osteosclerosis, and cortical osteopenia. Additionally, although the contributor did not find bacteria present histologically in the lesion, conference participants were able to find cocci bacteria within the area of suppuration.

It was difficult to determine the presence of a fracture from review of the submitted slides alone. Some slides had areas of very regular periosteal new bone growth arranged perpendicularly and radiating outwards from the periosteum with endosteal perpendicular reactive woven bone formation, with no callus formation. Other slides had very haphazard spicules of reactive woven bone and periosteum with extensive areas of disorganized cartilage, and this is likely the callus formed from the original fracture. Conference participants interpreted the islands of cartilage as chondrous metaplasia in reaction to decreased oxygen tension rather than endochondral ossification, which in the moderator's experience is generally minimal in a callus and occurs late in the process.

Ideal fracture repair begins with the formation of a hematoma from locally disrupted periosteum, blood vessels, and tissue. Macrophages, platelets, and the dead bone release various growth factors, including bone morphogenic proteins (BMPs), transforming growth factor-beta, platelet derived growth factors, which stimulate the proliferation of woven bone and is the initial scaffolding laid down to stabilize the area. Proliferating undifferentiated mesenchymal cells invade the area, which eventually undergo osseous or chondrous metaplasia to form the haphazard reactive woven bone and hyaline cartilage, and is termed the primary callus. The primary callus is eventually replaced by lamellar bone and may be remodeled by osteoclasts over a long period of time. If the fracture is unstable and there is excessive movement, the area instead forms mature fibrous tissue, which does not serve as a substrate for bone formation and becomes a nonunion. With time pseudoarthrosis can occur.²

In this case, we prefer the use of the term medullary osteosclerosis (a condition in which increased fibrous connective tissue fills spaces between spicules of woven bone) rather than myelophthisis (which is usually reserved to describe bone marrow suppression

secondary to marrow infiltration by malignant cells or local production of myelosuppressive cytokines).

The contributor mentioned the presence of sequestrum formation within the lesion. Conference participants interpreted the small fragments of necrotic bone as the result of surgical trauma from the initial surgery, not as an involucrum (a dense layer of fibrous connective tissue or reactive bone which walls off a focus of necrotic bone). An involucrum separates the sequestrum from its vascular supply and thus prohibits its resorption; however, in this case, the small fragments of dead bone appear to have access to a vascular supply and conference participants predicted that they should eventually be resorbed.²

In regards to the observed osteopenia, cortical bone normally compacts from the endocortical surface and progresses outward, (a finding not observed in this case). Conference participants discussed the potential reasons for this increased cortical porosity, such as osteoclastic resorption due to the elucidation of cytokines such as tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-16, prostaglandin E₂ from the suppurative inflammation and triggering the expression of receptor activator for nuclear factor kappa B ligand (RANKL), also known as osteoclast differentiation factor (ODF), which stimulates osteoclasts upon binding their RANK receptors; or periosteal new bone growth in response to disuse osteopenia from the surgical implant, called stress shielding. In accordance with Wolff's Law, the reduction of stresses relative to normal would cause bone to adapt by reducing its mass, either by becoming more porous, called internal remodeling, or by getting thinner, called external remodeling.^{2,4} Lesions of skeletal muscle, which were not present in all slides, include myofiber atrophy, edema, reactive fibrosis, or foci of coagulation necrosis and regeneration of skeletal muscle, and multifocal areas minimal lymphocytic inflammation.

Contributor: Armed Forces Radiobiology Research Institute
Veterinary Services Department
4301 Jones Bridge Road
Bethesda, MD 20814-4712
<http://www.usuhs.mil/afrr/>

References:

1. <http://vetpath.wordpress.com/systemic-pathology/bone-pathology-steve-weisbrode-2/>
2. Carlson CS, Weisbrode SE. Bones, joints, tendons, and ligaments. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2011:921-3, 932, 951-2, 961-2.
3. Newton C, Nunamaker. Textbook of small animal orthopaedics. In: Nunamaker D, ed. *Osteomyelitis*. J.B. Lippincott Company. Online: http://cal.vet.upenn.edu/projects/saortho/chapter_37/37mast.htm

[projects/saortho/chapter_37/37mast.htm](http://cal.vet.upenn.edu/projects/saortho/chapter_37/37mast.htm)

4. Thompson K. Bones and joints. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 1. New York, NY: Elsevier Saunders; 2007:92-8.

5. Woodard JC. Outline of Veterinary Skeletal Pathology, Online: http://www.cldavis.org/woodard_bone/contents.htm

CASE III: 07/11 (JPC 4003387).

Signalment: 4-year-old female Leister cross, *Ovis aries*, ovine.

History: The ewe was presented to the clinic with severe dyspnea and weight loss. Respiratory signs of variable degree had been present during the last month, no fever was recorded. At clinical examination there was marked inspiratory and expiratory dyspnea with respiratory noise from the upper airways. The sheep was depressed and the temperature 39.1° C. A trial medical therapy with antibiotics and corticosteroids was unsuccessful in relieving clinical signs and the ewe was euthanized.

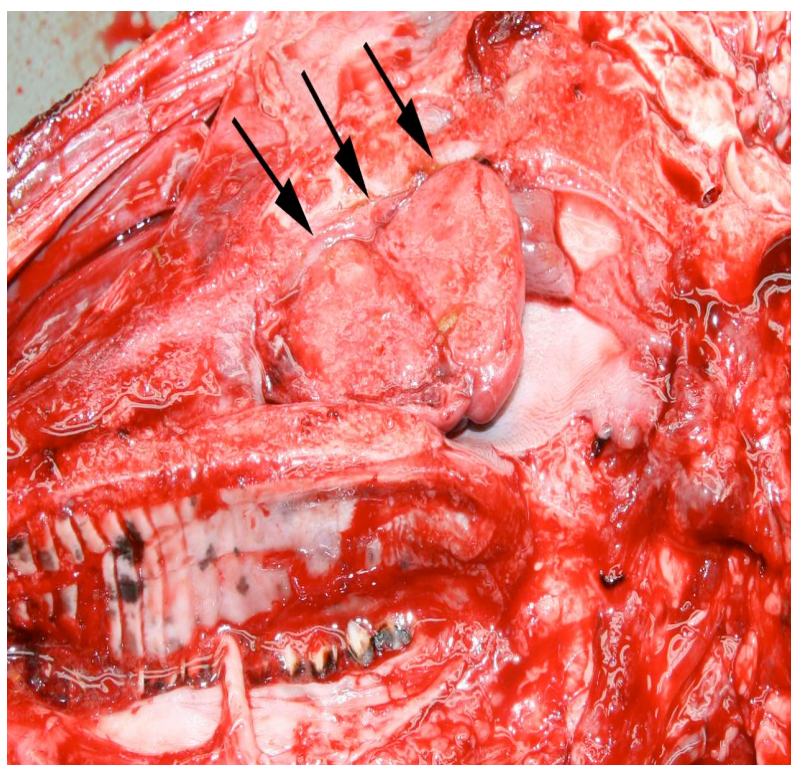
Gross Pathology: A space occupying mass was found in the caudal part of the nasal cavity, filling a large proportion of both the left and right side of the caudal nasal cavity. It markedly compressed the ethmoid bone and middle nasal concha on the left side, and was attached to the caudal region of the ethmoid bone. The mass was firm, pale and fibrous. When cutting through the mass there was a gritty sensation and marked resistance.

Laboratory Results: Aerobic bacterial culture was performed and yielded no specific growth.

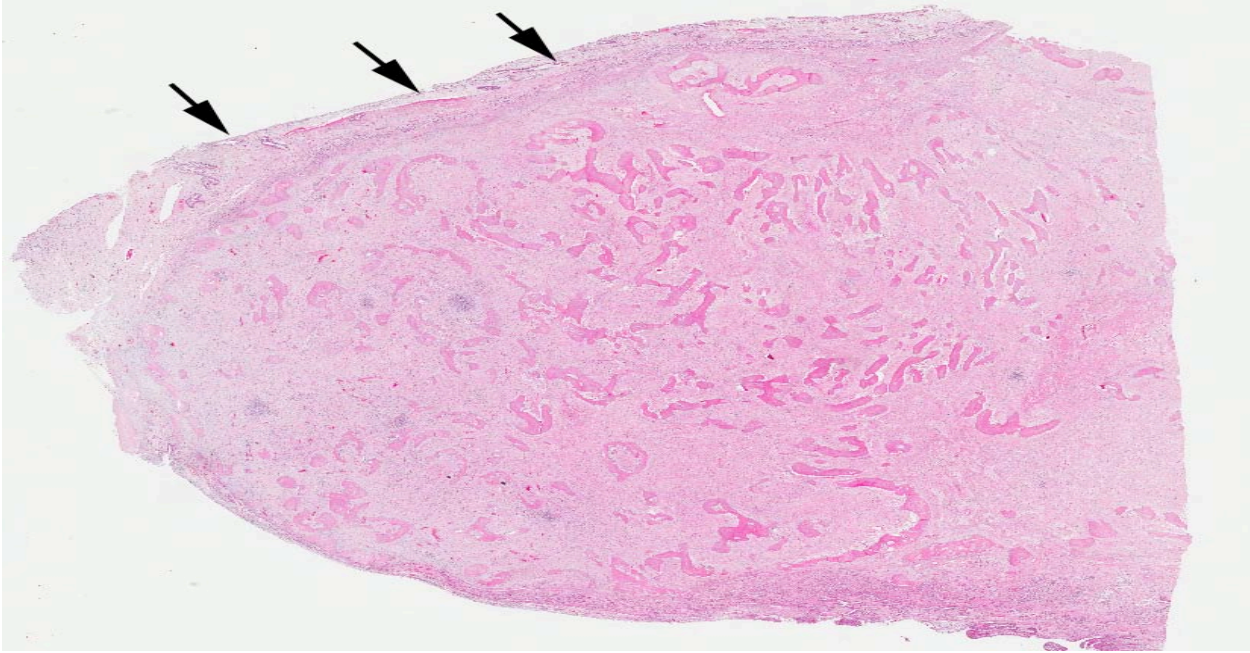
Contributor's Histopathologic Description: Nasal mucosa: In the lamina propria is a large expansile, partially encapsulated and demarcated mass composed of numerous spicules of woven bone separated by an abundant fibrous stroma. Osteoblasts outline bone spicules, although a transition from collagenous fibrous matrix to bone matrix is occasionally seen. In several places along bone margins osteoclasts are present, as well as adipose tissue. Within the stroma streams of plump and slender spindle shaped fibroblast-like cells and abundant collagenous extracellular matrix is seen. Dense collagen tissue is mixed with areas of myxoid matrix tissue. No mitotic figures were seen in examined sections. Moderate numbers of scattered as well as smaller multifocal accumulations inflammatory cells are present throughout the mass. Inflammatory cells consist of large numbers of plasma cells and smaller numbers of lymphocytes and neutrophils, and occasional eosinophils. Multifocally there are dense accumulations of inflammatory cells with a high proportion of neutrophils and these are sometimes centered on bone margins. Rarely fusiform microorganisms are found (not present in all slides). In the respiratory mucosa there is also subepithelial and periglandular diffuse moderate to marked infiltration of plasma cells and lymphocytes with smaller numbers of neutrophils. Extravasated erythrocytes are multifocally present.

Contributor's Morphologic Diagnosis: 1. Nasal cavity: Ossifying fibroma with plasmacytic and neutrophilic inflammation.
2. Nasal cavity: Rhinitis, lymphoplasmacytic and neutrophilic, chronic, moderate, diffuse.

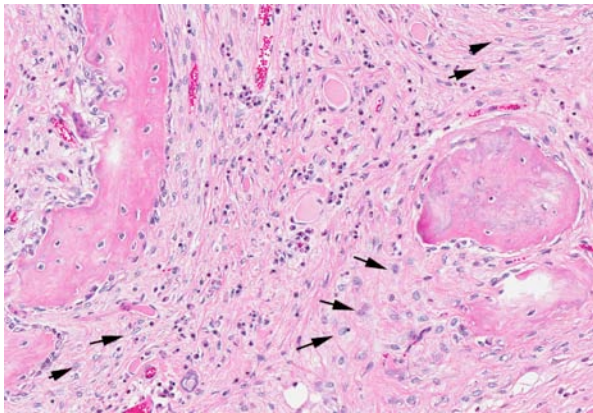
Contributor's Comment: Benign lesions arising from membranous bone include ossifying fibroma, osteoma and fibrous dysplasia.¹⁰ The morphologic features of the present case are most consistent with an ossifying fibroma. Ossifying fibromas show an intermediate morphologic architecture between osseous metaplasia of fibrous connective tissue, with predominate fibrovascular stroma separating poorly differentiated bone (as seen in fibrous dysplasia), and dense accumulations of well differentiated cancellous or compact bone with delicate intervening



3-1. A space occupying mass was found in the caudal part of the nasal cavity, filling a large proportion of both the left and right side of the caudal nasal cavity. It markedly compressed the ethmoid bone and middle nasal concha on the left side, and was attached to the caudal region of the ethmoid bone. Photo courtesy of: Department of BVF, Division of Pathology, Pharmacology & Toxicology, SLU (Swedish University of Agricultural Sciences), Box 7028, SE-750 07 Uppsala, Sweden. <http://www.bvf.slu.se/>



3-2. Nasal cavity, sheep: An expansile mass composed of spindle cells on a collagenous matrix with interspersed bony spicules expands the nasal mucosa. (HE 5X)



3-3. Nasal cavity, sheep. Within the mass, neoplastic spindle cells (arrows) on a loosely arranged fibrous stroma separate spicules of woven bone lined by a single layer of osteoblasts. (HE 150X)

fibrovascular tissue (as seen in osteoma).¹⁰ However, osteomas may show features of both ossifying fibromas and fibrous dysplasia, and it has been suggested that osteomas could represent the end stage lesion of other processes.¹⁰ Ossifying fibromas are rare in all species.¹⁰ In sheep, ossifying fibroma has previously been reported in the mandible of a young adult.⁸ Also involving the mandible, ossifying fibroma has been described in several cases of young horses.⁴ Furthermore, osteoma of the nasal cavity and frontal bone has been described in a sheep, and recently myxomatous fibroma with presence of bone spicules rimmed by osteoblasts and fewer osteoclasts was described in bighorn sheep.^{3,6} In addition, mucinous osteoma with features of ossifying fibroma has been

reported in the nasal cavity of a horse.⁷ In the present case, there was a marked inflammatory reaction within the mass and in the respiratory mucosa. The neoplasia may be related to chronic inflammation, although it is also possible that inflammation could arise secondary to disturbed airway function due to the presence of neoplasia. Chronic rhinitis is known to induce proliferative lesions including polypoid thickening of nasal mucosa, which may cause obstruction of the nasal passages.² Metaplastic ossification of connective tissue components incepted in inflamed nasal mucosa was discussed in the pathogenesis in an atypical osteoma in a bull.⁹ Chronic rhinitis was also found in the ovine case of skull osteoma described by Pérez *et al.*, who also described presence of inflammatory cells in the fibrous stroma in part of the neoplasm.⁶

JPC Diagnosis: Nasal cavity: Ossifying fibroma.

Conference Comment: Ossifying fibroma is an intraosseous lytic mass that destroys bone, and in early lesions is often intramedullary and does not produce a mass effect. Although considered benign, they are expansile and destroy adjacent normal bone. If this lesion was present in soft tissue it would be termed a fibroma with osseous metaplasia. This neoplasm differs from osteoma or exostoses, which arise from the periosteum and are proliferations of bone rather than fibrous tissue.^{1,11}

Fibrous dysplasia looks and behaves in a very similar manner to ossifying fibroma, Fibrous dysplasia is also more common in young animals and is an important

differential diagnosis for this case. However, fibrous dysplasia does not have spicules and trabeculae of woven to lamellar bone lined by osteoblasts, which is a defining feature of ossifying fibroma, as in this case. Another distinguishing feature is that fibrous dysplasia typically has lamellar bony trabeculae concentrated centrally in the mass, with predominantly woven bone at the periphery.^{1,5,11}

Fibrous osteodystrophy (FOD) was also discussed as a possibility, but in this case there is lamellar bone overlying woven bone in the spicules and trabeculae, and lamellar bone is usually not seen in FOD. Developing cortical bone occurs with woven bone laid down first which becomes compacted with overlying lamellar bone, and the lamellar bone present in this case likely represent preexisting bone. FOD also presents as a more fibroblast-rich lesion with less collagenous matrix as compared to ossifying fibroma, and is bilateral.^{1,11}

Because of the areas of inflammation, osteoclasts, and fibrosis, conference participants discussed chronic osteomyelitis as a possibility in this case; however, in osteomyelitis there is a concentric orientation of the inflammation to the fibrous connective tissue or the reactive bone, and in this case there is relatively little inflammation compared to the amount of fibrous connective tissue.¹

Contributor: SLU (Swedish University of Agricultural Sciences)
Department of BVF
Division of Pathology, Pharmacology & Toxicology
Box 7028, SE-750 07 Uppsala, Sweden
<http://www.bvf.slu.se/>

References:

1. Carlson CS, Weisbrode SE. Bones, joints, tendons, and ligaments. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 5th ed. St. Louis, MO: Mosby; 2011:951-2, 956-8.
2. Caswell JF, Williams KJ. Respiratory system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Philadelphia, PA: Saunders Elsevier; 2007:533-534.
3. Fox KA, Wootton SK, Quackenbush SL, et al. Paranasal sinus masses of Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). *Vet Pathol*. 2011;48:706-712.
4. Morse CC, Saik JE, Richardson DW, et al. Equine juvenile mandibular ossifying fibroma. *Vet Pathol*. 1988;24:415-421.
5. Nelson AM, Baker DC. Pedal osteosarcoma in a donkey. *Vet Pathol*. 1998;35(5):407-9.
6. Pérez V, Rúa P, Benavides J, et al. Osteoma in the skull of a sheep. *J Comp Pathol*. 2004;130:319-322.

7. Puff C, Ohnesorge B, Wagels R, et al. An unusual mucinous osteoma with features of an ossifying fibroma in the nasal cavity of a horse. *J Comp Pathol*. 2006;135:52-55.
8. Rogers AB, Gould DH. Ossifying fibroma in a sheep. *Small Ruminant Res*. 1998;28:193-197.
9. Rumbaugh GE, Pool RR, Wheat JD. Atypical osteoma of the nasal passage and paranasal sinus in a bull. *Cornell Veterinarian*. 1978;68:544-554.
10. Thompson KG, Pool RR. Tumors of bones. In: Meuten DJ, ed. *Tumors in Domestic Animals*. 4th ed. Ames, Iowa: Iowa State Press; 2002:248-255.
11. Thompson K. Bones and joints. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 5th ed. Vol 1. New York, NY: Elsevier Saunders; 2007:111-2.

CASE IV: 11/247 (JPC 4004158).

Signalment: 5 male and 1 female 22 day-old Ross 308 broiler chickens, *Gallus gallus domesticus*.

History: Six chickens were received for necropsy from a flock of 14000 individuals. The chickens showed signs of poor growth during the first 2 weeks, and on day 11 a new batch of feed was introduced. In the 3rd week the chickens weighed 40-50 grams less than normal. The feed intake was slightly increased. From day 18 signs of leg problems were observed in the flock, and tibiotarsal fractures were detected. On day 20, 40 chickens had fractures and 235 had been euthanized. From day 22, another new batch of feed was introduced. On day 27, an improvement in flock health had occurred and the remaining flock was slaughtered at normal time.

Gross Pathology: The six chickens had been euthanized and they weighed 482-654 grams. Four chickens had a unilateral fracture in the distal tibiotarsus, one chicken had a unilateral fracture in a femur, and in one chicken no fractures were detected. Surrounding the fractures there were moderate to large hemorrhages. Mineralization of bones was severely reduced in all six chickens. The hypertrophic zone of the growth plate in the proximal tibiotarsus was moderate to severely widened towards the metaphysis in all six chickens. Enlarged parathyroid gland was not detected macroscopically.

Contributor's Histopathologic Description: In the proximal tibiotarsus there is severe widening of the hypertrophic zone of the growth plate. The hypertrophic zone is dominated by wide cartilage

columns consisting of large hypertrophic chondrocytes in an orderly fashion. These cartilage columns are either naked or lined by a very narrow zone of osteoid. Multifocally, the lining of the columns are ragged and scalloped, and in some areas with clusters of osteoclasts.

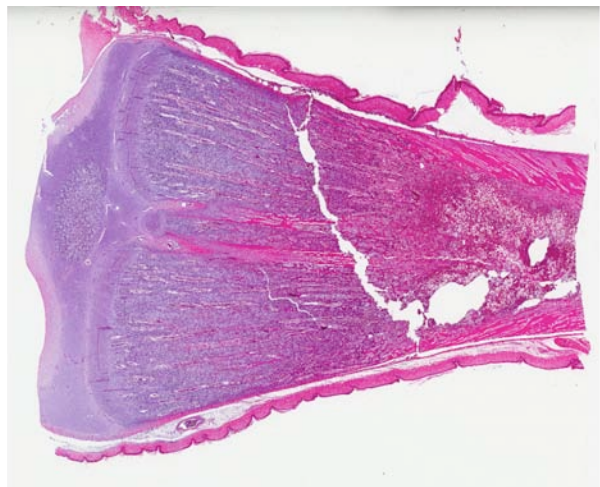
The thickness of the proliferating zone of the growth plate was considered normal. In the metaphysis, the cortical bone is severely thinned, and there is mild multifocal proliferation of fibroblasts subperiosteally. In some sections bacterial emboli are seen in medullary vessels.

Contributor's Morphologic Diagnosis: Proximal tibiotarsus: Failure of endochondral ossification, with retention of hypertrophic chondrocytes, severe, consistent with rickets.

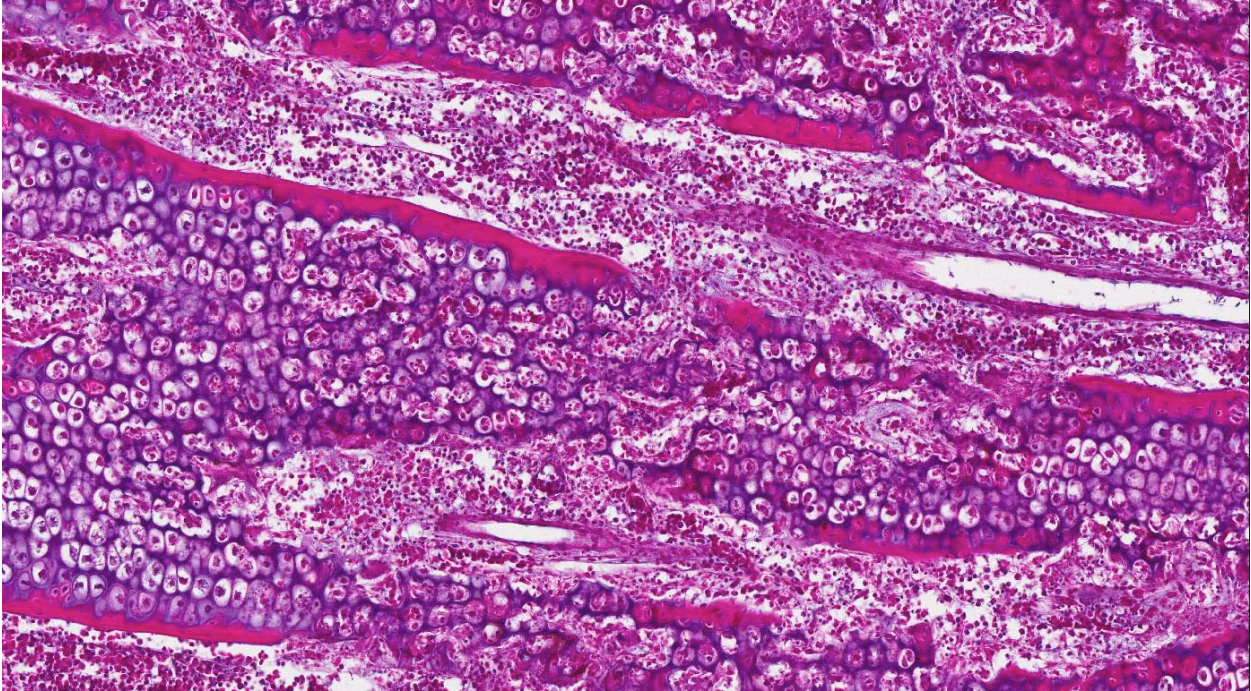
Contributor's Comment: Rickets in chickens is characterized by severe fragility of long bones and bending of long bones due to poor mineralization, and it may be caused by an imbalance between calcium and phosphorus or vitamin D deficiency.³ The histopathology of rickets differs depending on the cause of the disease.^{3,9} A hypocalcemic form is characterized by accumulation of proliferating chondrocytes;² and chickens fed a diet low in calcium showed, by 2 weeks of age, a variably lengthened and disorganized proliferating zone and shortened cartilage columns of the hypertrophied zone.⁷ A hypophosphatemic form is characterized by accumulation of hypertrophic chondrocytes in the metaphyseal zone.⁴ Chickens fed a diet low in phosphorus showed, by 2 weeks of age, lengthening of the hypertrophic zone,⁶ and a diet with excess calcium



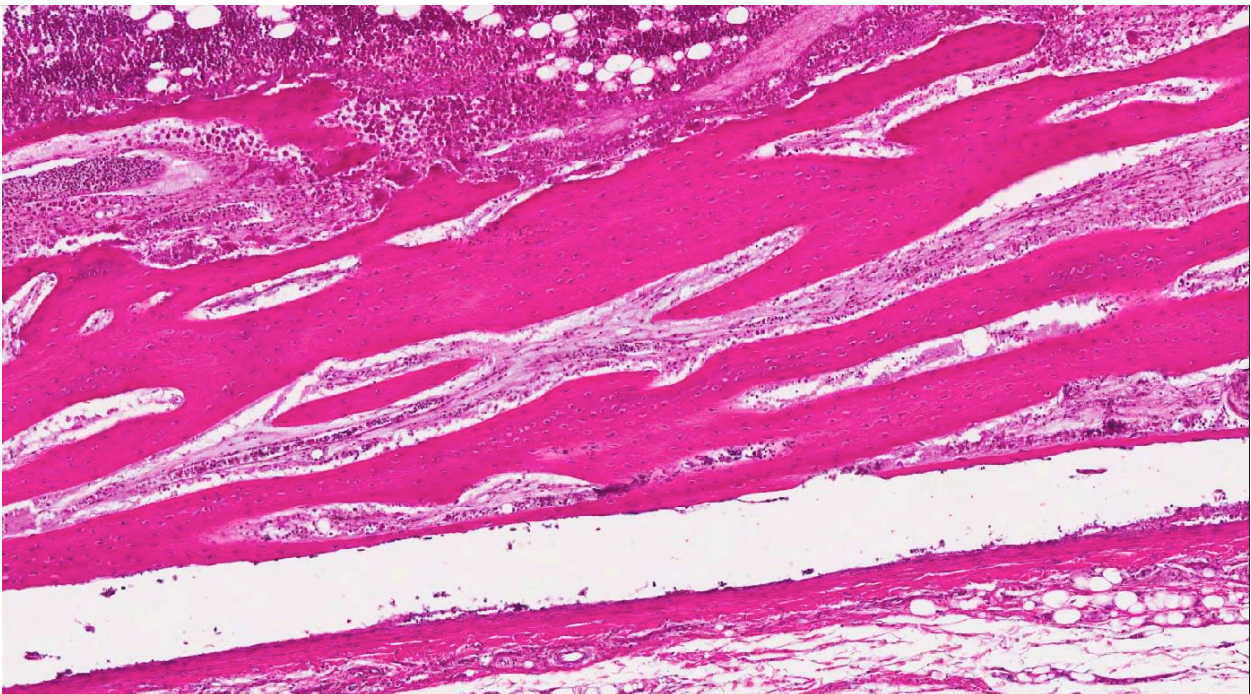
4-1. Tibia, 22-day-old chicken. The proximal tibial metaphysis is filled by a large core of unmineralized cartilage. The hypertrophic zone of the growth plate in the proximal tibiotarsus was moderate to severely widened towards the metaphysis. Photo courtesy of The Norwegian School of Veterinary Science, PO box 8146 Dep., 0033 Oslo, Norway. www.vetinst.no



4-2. Tibia, 22-day-old chicken. Subgross histologic image of the tibia in image 4-1. (HE 4X)



4-3. Tibia, 22-day-old chicken: Long trabeculae composed of unmineralized cartilage from the hypertrophic zone are lined by a wide osteoid seam. (HE 140X)



4-4. Tibia, 22-day-old chicken. The cortex is markedly thin and composed of woven bone. There are numerous osteoclasts within Howship's lacunae on the endosteal surface, and abundant loose fibrous connective tissue within endocortical vascular spaces. (HE 200X)

caused similar lesions.⁶ These two forms differ in histopathology from the rickets of vitamin D deficiency.³ In vitamin D deficiency, the enlargement of the epiphyseal plate initially is due to widening of the proliferating and hypertrophic zones; as the

deficiency progresses it may be primarily the former.³ The extensive lengthening of the hypertrophic zone found in these chickens may indicate a hypophosphatemic rickets; however, preliminary analyses of the feed used for this flock showed

decreased levels of vitamin D₃, while calcium and phosphorus were within normal range.

JPC Diagnosis: 1. Proximal tibiotarsus: Physeal chondrodystrophy with marked elongation of the zone of hypertrophy, delayed endochondral ossification, and cortical osteopenia.
2. Proximal tibiotarsus: Fibrous osteodystrophy.

Conference Comment: Although the lesions may be most similar to hypophosphatemic rickets, the history and dietary analysis are convincing for hypovitaminosis D. Conference participants discussed the importance of relying on dietary analysis to determine the type of deficiency rather than fine variations in bone lesions.

Some slides have areas of poor tissue preservation or autolysis with colonies of coccoid bacteria and homogeneous, eosinophilic, acellular material filling medullary spaces. PTAH and Masson's trichrome histochemical stains were performed to determine if the acellular material is widespread fibrin exudation; however, PTAH did not stain fibrin, and conference participants considered serous atrophy of fat affecting the metaphysis and epiphysis as the more likely cause for the acellular material. The Masson's trichrome revealed perivascular fibrosis of vessels penetrating the growth plate, mild peritrabecular fibrosis in the metaphysis, endocortical fibrosis, fibrosis in the vascular spaces of the cortex, and minimal fibrosis in the epiphysis interpreted as fibrous osteodystrophy secondary to hypovitaminosis D, similar to case 1.³

Also similar to case 1 are the findings of cortical osteopenia with woven bone in the cortex and increased cortical porosity due either to delayed osteonization or increased cortical lysis. Rickets in avian species differs from that in mammals in that there is delayed onset of osteoid deposition, atrophy of osteoblasts on cartilage cores, and retention and minimal disorganization of hypertrophied chondrocytes in wide regular columns which often extend deep into the metaphysis. As mentioned by the contributor, the multifocal marked osteoclast hyperplasia of metaphyseal trabeculae and endocortical surfaces at the diaphysis was interpreted by conference participants as normal endocortical osteoclasts, or modeling of the endocortical surface of a rapidly growing bird.^{1,3}

The primary differential diagnosis in this case is tibial dyschondroplasia (TD), a spontaneous lesion of rapidly growing birds which is characterized by a large mass of avascular cartilage in the tibiotarsal metaphysis with necrosis of the prehypertrophic zone of chondrocytes, and is similar to osteochondrosis in mammals. The lesions in TD are very similar to those of rickets, and

are the result of failure of chondrocytes to fully differentiate and allow vascularization, mineralization, and resorption of cartilage matrix.^{3,5,8}

Contributor: The Norwegian School of Veterinary Science
PO box 8146 Dep.
0033 Oslo
Norway

References:

1. Hedstrom OR, Cheville NF, Horst RL. Pathology of vitamin D deficiency in growing turkeys. *Vet Pathol.* 1986;23(4):485-98.
2. Jande SS, Dickson IR. Comparative histological study of the effects of high calcium diet and vitamin D supplements on epiphyseal plates of vitamin-D-deficient chicks. *Acta Anat* (Basel). 1980;108:463-468.
3. Klasing KC. Nutritional diseases. In: Saif YM, ed. *Diseases of Poultry*. 12th ed. Oxford, England: Blackwell Publishing; 2008:1121-1148.
4. Lacey DL, Huffer WE. Studies on the pathogenesis of avian rickets. I. Changes in epiphyseal and metaphyseal vessels in hypocalcemic and hypophosphatemic rickets. *Am J Pathol.* 1982;109:288-301.
5. Leach RM jr, Gay CV. Role of epiphyseal cartilage in endochondral bone formation. *J Nutr.* 1987;117(4): 784-90.
6. Long PH, Lee SR, Rowland GN, et al. Experimental rickets in broilers: gross, microscopic, and radiographic lesions. I. Phosphorus deficiency and calcium excess. *Avian Dis.* 1984;28:460-474.
7. Long PH, Lee SR, Rowland GN, et al. Experimental rickets in broilers: gross, microscopic, and radiographic lesions. II. Calcium deficiency. *Avian Dis.* 1984;28:921-932.
8. Orth MW, Cook ME. Avian tibial dyschondroplasia: a morphological and biochemical review of the growth plate lesion and its causes. *Vet Pathol.* 1994;31(4): 403-4.
9. Randall CJ. Nutritional deficiencies and metabolic disorders. In: Randall CJ, ed. *A Colour Atlas of Diseases and Disorders of the Domestic Fowl and Turkey*. 2nd ed. London, UK: Wolfe Publishing Ltd; 1991:109-124.