Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

C o n f e r e n c e 23

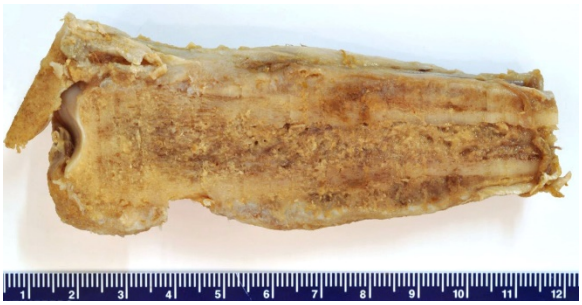
25 April 2018

Julie Engiles VMD, DACVP
Associate Professor of Pathology
Department of Pathobiology- New Bolton Center Murphy Laboratory
382 West Street Road
Kennett Square, PA 19348

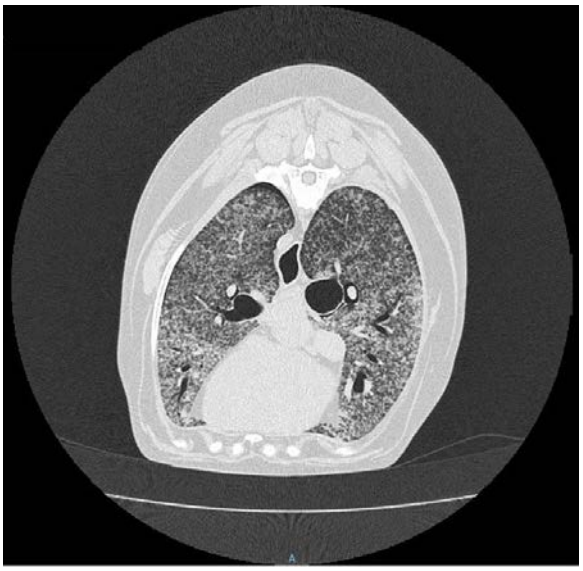
CASE I: 164361-16 (JPC 4100935).

Signalment: 3-year-old, male, castrated, Doberman pinscher (*Canis familiaris*), canine.

History: The patient presented to Cornell University Hospital for Animals (CUHA) Emergency Service for respiratory distress. Nine months prior, he had been evaluated for bilateral swollen carpi and radiographs revealed an aggressive bone lesion; the carpi became progressively worse leading to forelimb lameness, coughing, sneezing and epistaxis. The patient was administered diphenhydramine, 1.25 mg/kg (0.56 mg/lb), PO, q 12 h, as needed and amoxicillin, 25 mg/kg (11.36 mg/lb), PO, q 12 h, for 30 days. Cytological examination of a needle aspirate from an enlarged popliteal lymph node revealed histiocytic inflammation. The patient was administered prednisone, 0.5 mg/kg (0.22 mg/lb), PO, q 12 h for 7 days, then 0.5 mg/kg (0.22 mg/lb), PO, q 24 h for 14 days and doxycycline, 5 mg/kg (0.22 mg/lb), PO, q 12 h for 30 days. Because the carpal swelling continued to increase, the dose of prednisone was increased to 1 mg/kg (0.45 mg/lb), PO, q 12 h, at which point the respiratory signs worsened. Bronchoscopy showed inflamed, hemorrhagic bronchi, and radiographs showed progressive bilateral periosteal reaction and lytic lesions of the right and left distal radius and ulna, and a severe generalized miliary nodular lung pattern.



Radius, dog. The distal radius was expanded up to 5cm with loss of cortical bone.(HE, 4X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)



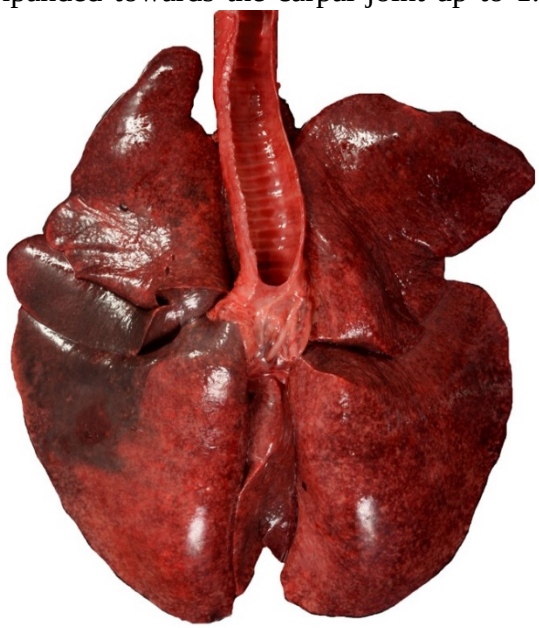
Computed tomography, lungs, dog. A CT scan of the lungs demonstrates a generalized bilateral military nodular pattern. (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

Gross Pathology: Prior to post-mortem examination, educational computed tomography (CT) scans were recorded of the head, neck and thorax and confirmed the bilateral, generalized miliary nodular pattern within the lungs. The CTs also revealed periosteal new bone formation on the calvaria.

At necropsy, the dog was in good body condition (4 out of 9, Purina scale) with mild postmortem autolysis. Bilaterally, the carpal joints were severely enlarged, with the left being worse than the right. Both carpal joints were hard with focal, soft, depressed areas. On the medial aspect of the right carpus, there was a 0.6 cm diameter full-thickness defect containing 0.1 mL of creamy, soft material (draining tract). Bilaterally, the skeletal musculature of the brachium, predominantly the triceps brachii, was less prominent (atrophy). The left popliteal lymph node was firm.

The lungs were diffusely mottled dark red and light pink, failed to collapse and mildly firm (interstitial pneumonia). Diffusely throughout the lungs were dozens of miliary, white-tan foci that extended into the parenchyma. The trachea contained approximately 2 mL of frothy, red fluid. On cut section, the large airways oozed a small amount of cloudy red to gray mucoid material (exudate).

Bilaterally, the distal one-third of the radius and ulna were expanded approximately 7 cm in diameter on the left and 5 cm in diameter on the right. On cut section, the right and left carpal joints exuded approximately 1 mL of viscous, brown, cloudy fluid (suppurative synovitis and periarthritis). The periarticular fascia on the medial aspect of the right and left carpi had cystic spaces filled with similar brown, cloudy fluid. On sagittal section, the periosteum of the distal one-third of the radius, ulna and carpal bones was gradually expanded towards the carpal joint up to 1.7

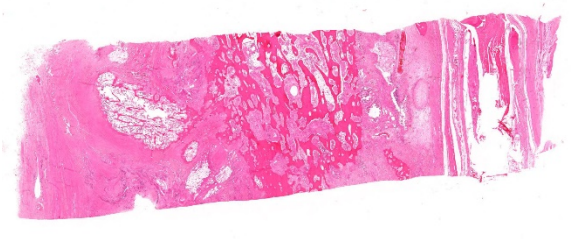


Lung, dog. The lungs were mottled, failed to collapse and contain numerous granulomas throughout the parenchyma. (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

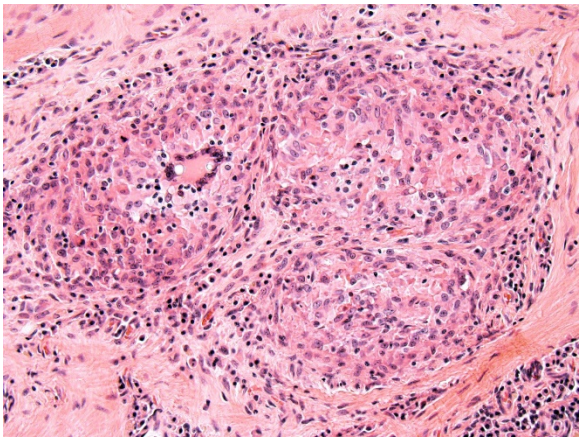
cm thick. The outer cortical surface was rough and uneven.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):
Blastomyces dermatitidis was partially cultured from lung tissue (culture was not finalized due to human health risks). *Blastomyces dermatitidis* infection was confirmed by PCR of the lung tissue.

Microscopic Description:
The cortical bone is expanded by numerous cavities filled with numerous macrophages together with fewer multinucleated giant cells, and rare lymphocytes (osteomyelitis). The inflammatory infiltrate varies, with multiple clusters of neutrophils and degenerated neutrophils in random areas. In multiple areas, the inflammatory cells are centered upon variable size islands of an intensely eosinophilic to magenta, acellular material, with indistinct margins (bone necrosis). The cortical bone is covered by an expanded layer of woven bone that is incompletely mineralized (periosteal reaction) and contains numerous osteocytes. The immature woven bone is lined by a single layer of plump osteoblasts (reactive). Frequently, the edges of the woven bone are scalloped, irregular with adjacent osteoclasts (Howship's lacunae). Randomly scattered in the inflammatory infiltrate are numerous intrahistiocytic and extracellular, 8-20 µm in diameter, round yeast-like organisms with a 2-3 µm thick, clear to lightly basophilic, refractile capsule. The yeast-like organisms frequently exhibit broad-based budding consistent with *Blastomyces spp*. The inflammatory infiltrate extends to the bone marrow spaces and dissects through the periosteal reaction and adjacent skeletal muscles (myositis).



Radius, dog. There is diffuse loss of cortical bone with marked proliferation of anastomosing trabeculae of woven bone and extensive inflammation that extends into the surrounding soft tissue. (HE, 6X)



Radius, dog. Numerous poorly formed granulomas populate the bone and adjacent soft tissue. Rare multinucleated giant cell macrophages contain yeasts (arrows). (HE, 200X)

Contributor's Morphologic Diagnosis:
Pyogranulomatous osteomyelitis and myositis, with bone necrosis, woven bone deposition and yeasts consistent with *Blastomyces spp*.

Contributor's Comment: This is a classic case of disseminated blastomycosis caused by infection with *Blastomyces dermatitidis* with severe lung and skeletal involvement. Additionally, histopathology examination of the heart revealed severe, multifocal, chronic degeneration and necrosis of cardiomyocytes with dystrophic mineralization, suggestive of hypoxia attributable to the severe pneumonia. Histopathology examination of decalcified sections of distal radius/ulna and calvaria revealed a severe, chronic osteomyelitis with large numbers of yeast organisms, consistent

with disseminated blastomycosis. Histochemical staining of lung sections using periodic acid-Schiff and Grocott-Gomori's methenamine silver further highlighted the yeast organisms.

Although the classic presentation for fungal pneumonia on radiographs is a generalized, random, miliary nodular pattern, blasto-mycosis can have various presentations ranging from multiple pulmonary nodules, patchy or lobar lung consolidation (alveolar pattern), to a solitary pulmonary mass.[3] Histopathology ruled out other potential causes of pulmonary nodules, such as neoplasia and infection with other mycoses including *Coccidioides immitis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*.

Blastomycosis is one of the most common systemic mycotic infection of dogs that live in endemic areas including the Ohio and Missouri river valleys, the southern Great Lakes, and southern mid-Atlantic states.[4] In the state of New York, the incidence of blastomycosis in dogs has increased over the past 20 years and is endemic in the Adirondacks where this dog lived.[5] Blastomycosis occurs in dogs and humans and is rare in other animals. Young, male, intact dogs living in endemic areas are at an increased risk of becoming infected.[5] Sporting dogs such as Labrador and Golden retrievers and Doberman Pinschers are more frequently affected.[6,7]

Blastomyces dermatitidis is a saprophytic fungus found in moist acidic or sandy soil, with high organic content.[6] It is a thermally dimorphic fungus, that forms septated mycelia in the environment, but becomes yeast in infected tissues. In its mycelial form, it produces conidia (spores) that are inhaled by the host and then phagocytized by alveolar macrophages.[7] In the presence of higher body temperature, spores become yeast with thick double-contoured outer walls and characteristic broad-based budding.[6,7] The yeast causes local suppurative to pyogranulomatous inflammatory response, which may be self-limiting.[8] However, phagocytized yeasts can be transported into the pulmonary interstitium where they can disseminate hematogenously or through the lymphatic system to other organs.[8] Inoculation directly through a skin wound is also possible, but rare.[8] By contrast with other mycotic diseases such as *Coccidioides immitis* and *Aspergillus sp.* where infectious spores can be easily aerosolized from infected tissues and transmit the disease, the yeasts of *B. dermatitidis* are not infective, and thus, do not require special biosafety precautions during necropsy.

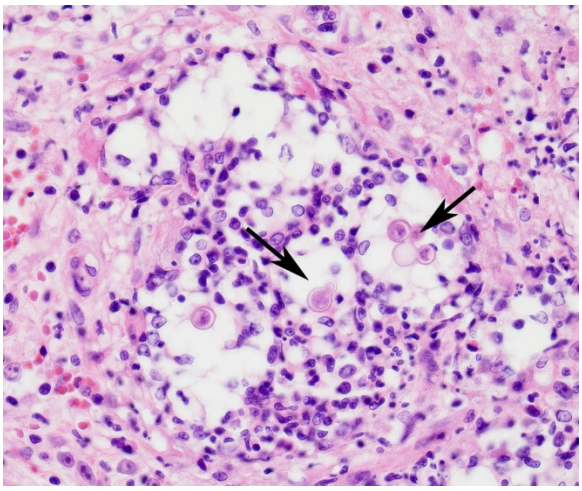
Clinical signs with blastomycosis depend on the organ system most severely affected; however, respiratory signs is the most common clinical presentation.[7,8] In dogs, *B. dermatitidis* often disseminates to the lungs, lymph nodes, skin, eyes, bones, reproductive system, and nervous system.[8] Lameness caused by osteomyelitis or paronychia is reported in 25% of dogs with blastomycosis, and fungal osteomyelitis in 10-15% of cases.

Itraconazole is the treatment of choice at a dosage of 5 mg/kg (2.27 mg/lb) every 24 hours for 60 days. Treatment should be continued for 30-60 days after resolution of clinical signs. Clinical signs often become more severe in the early phase of treatment, as the yeast organisms die off and elicit severe inflammation.[8] Prognosis for blastomycosis is good if severe pulmonary or CNS involvement is not present. Relapse is possible in dogs with initially severe clinical disease or in dogs treated for an insufficient length of time. Prolonged administration of prednisone might have contributed to the severe disseminated disease seen in this case.

JPC Diagnosis: Bone: Osteomyelitis, pyogranulomatous, diffuse, severe with marked cortical and trabecular osteolysis and numerous yeasts, Doberman pinscher (*Canis familiaris*), canine.

Conference Comment: Generally, bacterial osteomyelitis is more common than fungal, but there are three dimorphic fungi that can affect bone: (1) *Blastomyces dermatitidis*, (2) *Coccidioides immitis*, and (3) *Cryptococcus neoformans*. These three are dimorphic fungi, meaning they are in the filamentous form in the environment and yeast form in tissues. *Blastomyces dermatitidis* is fully described by the contributor above.[1,2]

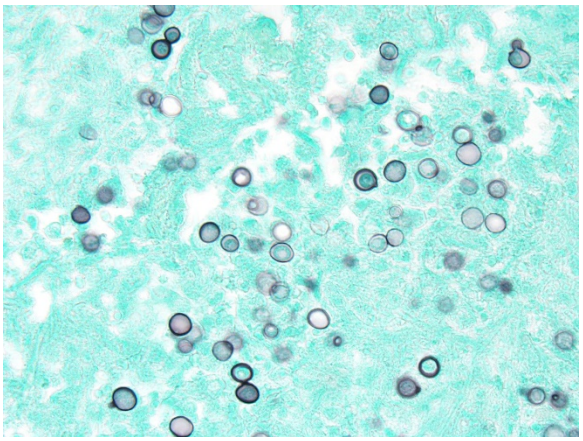
Coccidioides immitis, an endosporulator, is a common inhabitant of the desert climates in the southwestern United States, areas of Mexico, Central, and South America and is transmitted primarily by inhalation of arthroconidia (spores). Once at body temperature, the spores transition into spherules (yeast form) which can migrate along the pleura creating damage by inducing production of arginase I and coccidioidal urease by host tissues. Infection can be subclinical and manifestation in bone often takes years to develop, generally long after respiratory lesions have healed. Dissemination to bone is most common in dogs with the distal diaphysis of long bones affected by isolated nodules. Spherules have an outer wall glycoprotein which acts to modulate host immune responses by compromising cell-mediated immunity and preventing phagocytosis. Grossly, coccidiomycosis forms firm, fibrous nodules often with caseating centers which microscopically are composed of granulomatous to pyogranulomatous inflammation surrounded by a thick fibrous connective tissue capsule. The fungus is often present at various stages of maturation, from the immature spherule (10-20um in diameter)



Radius, dog. Areas of pyogranulomatous inflammation within the bone also contain numerous 8-14um yeasts with a 2um hyaline wall and narrow based budding. (arrows). (HE, 400X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

to the mature spherule (up to 200um in diameter) which typically has a double contoured wall and contains numerous endospores (2-5um in diameter). Other organisms that reproduce by endosporulation include: *Rhinosporidium seeberi*, *Prototheca* sp., *Chlorella* sp., and *Batrachochytrium dendrobatidis*.[1,2]

Cryptococcus neoformans is a common inhabitant of pigeon feces and soil which typically affects immunocompetent cats and dogs (cats more so) with transmission via inhalation and primary lesions in the lungs and nasal cavity. In cats, osteolytic lesions occur in the bones of the maxilla with soft tissue swellings over the bridge of the nose. Grossly, lesions appear gelatinous or cyst-like. Microscopically, there are numerous yeast which exhibit narrow based budding, with thick heteropolysaccharide capsules which form a clear halo and characteristic "soap bubble" appearance. Their mucopolysaccharide capsules stain positive with Mayer's mucicarmine and Alcian blue. Inflammation is scarce in these lesions due to



Radius, dog. A silver stain easily discloses large numbers of budding yeasts within the section. (Gomori methenamine silver, 400X)

the action of multiple virulence factors. The polysaccharide (glucuronoxylomannin) capsule prevents phagocytosis as well as inhibiting migration and recruitment of inflammatory cells. Additionally, an enzyme called laccase is produced which forms a melanin-like pigment that acts as an antioxidant. Two other enzymes, serine protease and urease aid in tissue invasion and promote sequestration in microcapillaries, respectively.[1,2]

During the conference, the moderator pointed out the marked loss of cortical bone which had been remodeled by osteoclasts, resulting in a trabecular appearance. Osteoclasts are of monocyte-macrophage lineage and many granulomatous reactions affecting bone result in marked bone loss and remodeling through osteoclastic activation.

Radiographically, osteosarcoma and granulomatous inflammation may appear very similar. A key difference identified by the moderator is that infectious organisms usually cross the joint with abrupt transition from lesion to normal bone, whereas most neoplastic processes generally do not cross joints and have a longer zone of transition from lesion to normal. Additionally, clinically neoplasia is generally one large mass, whereas, with infectious organisms several joints may be infected. Radiographic interpretation and clinical history is essential for accurate histologic diagnosis.

Contributing Institution:
http://vet.cornell.edu/biosci/pathology/

References:

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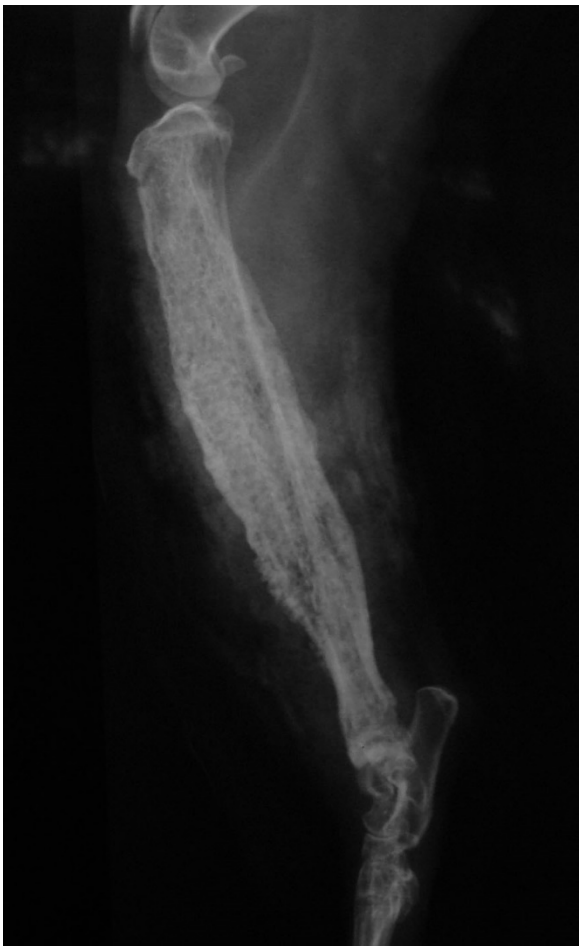
and Cat. 3rd ed. St Louis, MO: Elsevier Inc.; 2006:569-576.

CASE II: UFMG 247/12 (JPC 4018791).

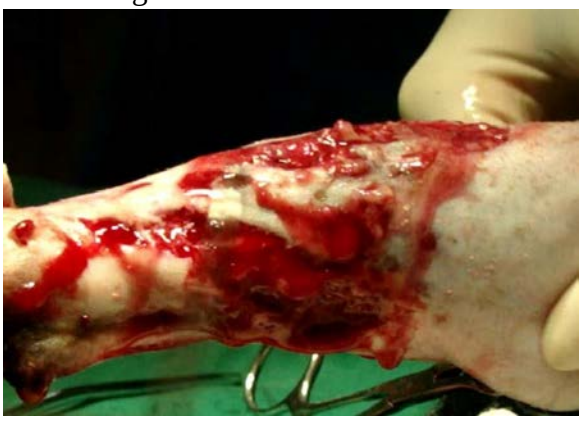
Signalment: 9-month-old, male, mixed breed (*Felis catus*), feline.

History: Initially this cat had a history of lameness of the right hind limb. The owner reported that the lameness started after a surgical procedure (orchiectomy) five months ago. Some days later, the cat returned to the same clinic where the orchiectomy had been performed. The right hid limb of this animal was subjected to radiography (craniocaudal and mediocaudal), and periosteal thickening of the metaphysis of the tibia was observed. Prednisone and enrofloxacin were prescribed, but improvement was not observed. After 50 days, two draining fistulae were observed on the medial tibia and topic rifamycin was prescribed. Fifteen days later, more draining fistulae were detected, and treatment with prednisone and enrofloxacin was reinitiated. Due to poor response to therapy and progressive weight loss, the cat was submitted to the veterinary hospital at the Universidade Federal de Minas Gerais (UFMG). On the clinical examination, the cat had temperature of 39ºC, regular body condition and the right tibia was diffusely thickned. Radiography was performed, and blood was collected for hemogram and biochemical analysis. Radiography revealed marked changes in the metaphysis and diaphysis of the right tibia. Changes were characterized by extensive loss of cortical bone and replacement by irregular and disorganized bone. There was no cortex-medullar definition and irregular radiodensity (osteoproliferation) was observed within marrow bone. In addition, marked periosteal reaction and thickening of the soft tissues lateral to the tibia were observed (Figure 1). Considering the presence of deep dermatitis, myositis, and involvement of the marrow bone (probably osteomyelitis), another therapy was attempted. A treatment with cefazolin, meloxicam, cefovecin sodium, tramadol, and fluid therapy was prescribed. After four days, a new blood test was performed, exudate was collected for bacteriological examination, and a surgical curettage was performed. The cat responded poorly to treatment, and a new blood test did not indicate any recovery. Due



Tibia, cat. A prominent proliferative and lytic lesion extends along the length of the tibia with loss of cortical outline, as well as cortical and medullary differentiation. (Photo courtesy of Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Clínica e Cirurgia Veterinárias, Av. Antônio Carlos, 6627; 31270-901. Belo Horizonte, MG, Brazil – www.vet.ufmg.br)

to unfavorable prognosis, amputation of the referred right hid limb was performed. One week after amputation, the clinical condition improved notably and no sign of disease was detected again.



Tibia, cat. The skin along right tibia is multifocally to ulcerated and a red exudate is observed in the underlying subcutis and underlying muscle. (Photo courtesy of Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Clínica e Cirurgia Veterinárias, Av. Antônio Carlos, 6627; 31270-901. Belo Horizonte, MG, Brazil – www.vet.ufmg.br)

Gross Pathology: Grossly, the skin along right tibia was intensely and multifocally to coalescing ulcerated. A reddish-yellow viscous exudate was observed in the subcutis and muscles (Figure 2). The right tibia was enlarged with moderate new bone formation along the metaphysis and diaphysis. Longitudinal and transverse sections showed loss of the medullary cavity and replacement with white and firm woven bone with scattered yellowish-white and granular material. There was no evidence of differentiation between medullary cavity and cortical bone.

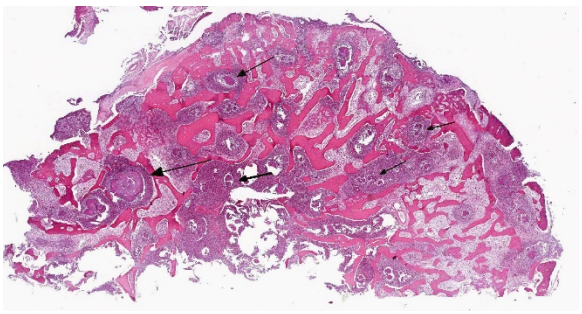
Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):

Clinical pathology:

- CBC two weeks prior amputation: 4,6 Hgb, 17,0 Hct, 4,0 RBC, 86/34.744 Seg, 08/ 3232 Lym, 03/1212 Mno.
- CBC one day prior amputation: 6,7 Hgb, 24,0 Hct, 6,0 RBC, 90/28.630 Seg, 07/ 1.449 Lym, 01/207 Mno.
- No changes were detected in the biochemical profile.

Microbiology: *Pseudomonas aeruginosa* was isolated from draining purulent exudate.

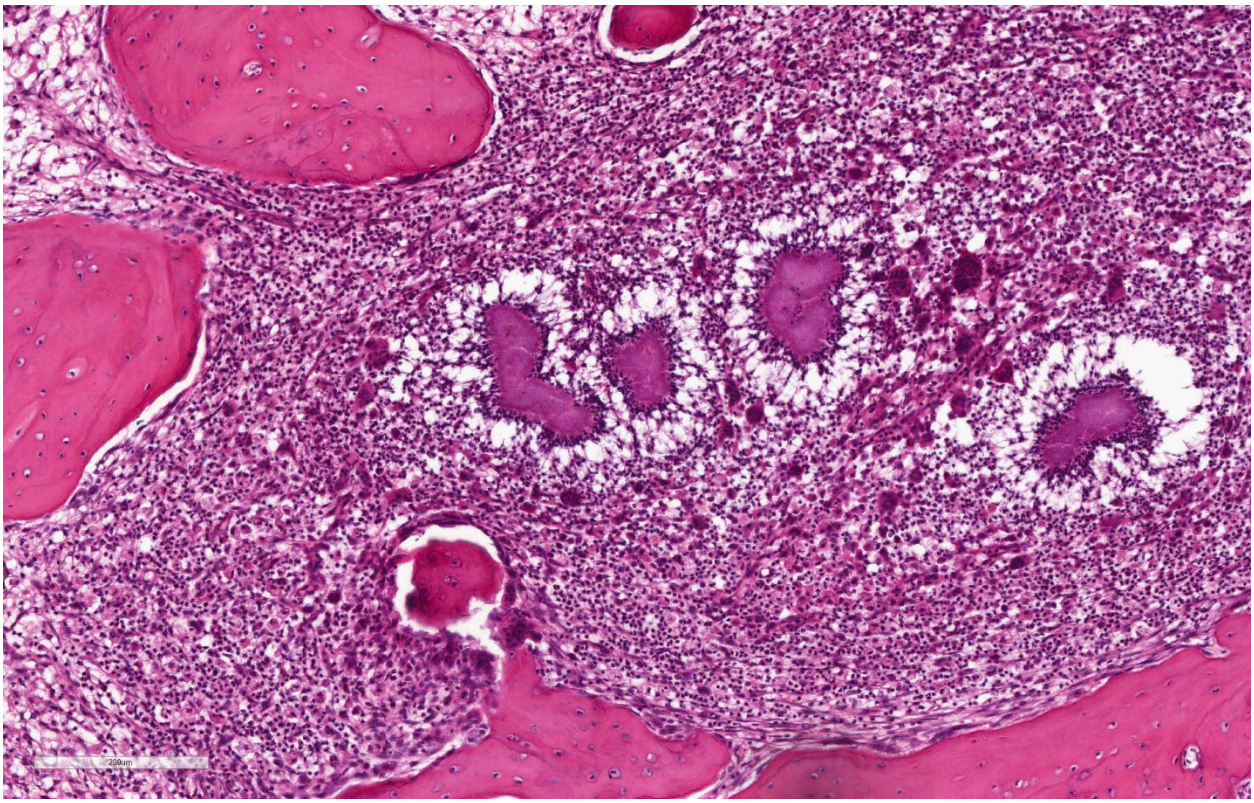
Microscopic Description: Histologically, the skin was extensively ulcerated and replaced by many neutrophils and cellular debris. In the subjacent dermis, there was multifocal mature granulation tissue and numerous neutrophils and macrophages. Deep in the dermis, multifocal variably-sized areas with colonies of bacteria surrounding by neutrophils, macrophages and giant multinucleated cells are found and irregular format with basophilic centers and eosinophilic filamentous radiating aggregates in the periphery (Splendore-Hoeppli phenomenon). Adjacent to the pyogranulomatous reaction, connective fibrous tissue and neovascularization were observed. The reaction invaded the hypodermis and muscular fascia. Where the infection reaches the muscles, marked necrosis and loss of muscular fibers could be seen. The periosteum of the tibia was thickened due to marked fibrous tissue



Tibia, cat: A cross section of the tibia is present with areas of trabecular lysis, formation of anastomosing woven bone, and an almost total loss of lamellar cortical bone. An inflammatory infiltrated is present between trabeculae and is centered on variably-sized bacterial colonies (arrows). (HE, 144X)

proliferation and inflammation. The compact bone of cortical was multifocally replaced by an inflammatory reaction and trabecular bone. The Harvey's canals were expanded and there was bone necrosis (loss of osteocytes) and reabsorption. The marrow bone was replaced by multiple Splendore-Hoppli reactions surrounding by neutrophils and giant cells (several containing 20 to 60 nuclei). Many foamy macrophages were infiltrating interconnected trabecular bone containing few osteoblast cells and no mineralized osteoid layer. Multifocal areas with new endosteal bone forming a network were found among remnants trabeculae. Thromboses were observed in the periosteal layer in some slides. Popliteal lymph node presented marked cortical lymphoid hyperplasia. Within lymphatic sinuses, numerous macrophages and giant cells with foamy cytoplasm were observed. Selected tissues from tibia, lymph node and adjacent muscles were subjected at Good Pasture, Giemsa, periodic acid – Schiff (PAS), Ziehl Neelsen and Grocott's methenamine silver (GMS) special stains. Good Pasture stained Gram-positive bacteria within colonies in the marrow bone, periosteum, muscles, fascia, skin and lymph node. A Goodpasture gram stain revealed many coccobacteria grouped in the central area or associated to the filamentous radially arranged material. In the periphery of reaction, cocci grouped in pairs or single chains could be seen. No organisms were identified in identical tissue sections stained by all others special stains aforementioned.

Contributor's Morphologic Diagnosis:



Tibia, cat: Bacterial colonies are surrounded by a layer of neutrophis, and the intertrabecular spaces are filled with numerous neutrophils, epithelioid macrophages, lymphocytes and few plasma cells. Osteoclasts are present within the exudate at a distance from trabeculae, which have numerous reversal lines. (HE, 144X)

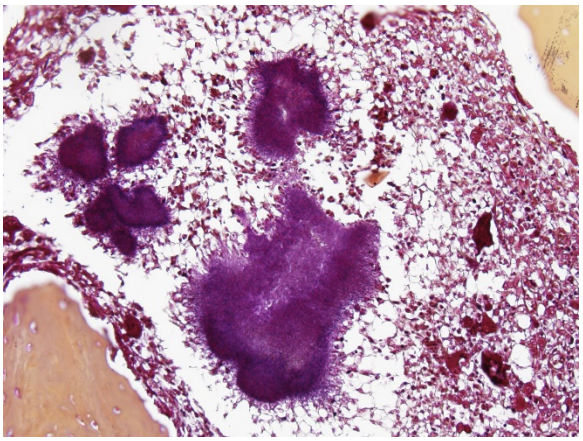
1. Bone (tibia): Marked diffuse pyogranulomatous periostitis, osteomyelitis, and marrow osteoproliferation associated with numerous granule formation (Splendore-Hoeppli phenomenon), and multifocal cortical loss, reabsorption, fibroplasia and new endosteal bone formation.
2. Skin and muscles (not included): Marked multifocal to coalescing pyogranulomatous and necrotic dermatitis and myositis associated with numerous granule formation (Splendore-Hoeppli phenomenon)

Contributor's Comment: Bacterial pseudomycetoma (botryomycosis) is a pyogranulomatous skin disease characterized by an unusual, presumed immunologic, reaction to nonfilamentous bacteria[4]. Some specific bacteria elicit Splendore-Hoeppli reaction (an antigen-antibody complex), which are characterized by the presence of radiating, club-shaped eosinophilic material around infectious and non-infectious agents[6]. Grossly, purulent material discharged from fistulae frequently contains white to yellow sand-like granules[3]. The antigen-antibody complex, morphologically unique reaction, was first described in sporotrichosis by Splendore and is schistosomiasis by Hoeppli[6]. Splendore-Hoeppli reaction can be caused by filamentous bacteria including *Actinomyces* spp. and *Nocardia* spp.[4]. Also, nonfilamentous bacteria including *Pseudomonas*, *Proteus*, *Eschericiha coli*[6], *Staphylococcus* and *Streptococcus*[4] are described and should be considered in the differential diagnosis. Gram's stain for bacteria can be used for differential diagnosis among Gram-positive (*Actinomyces, Nocardia, Staphylococcus, Streptococcus)* and Gram-negative (*Pseudomonas, Proteus* and *Eschericiha coli)* organisms[6]. Also, *Nocardia* spp. can be identified by Ziehl Neelsen[5], PAS and GMS stains.[3]
In cats, infectious with *Actinomyces viscosus*[8] and *Nocardia* spp.[5] were reported. However, report of nonfilamentous bacteria causing pyogranulomatous infection in the skin, muscles and bone in cats was not found.

The bacteria isolate in this case (*Pseudomonas aeruginosa*) could not be identified on special stains. Myriads of slightly elongate Gram–positive bacteria arranged in pairs or single chains were suggested of *Streptococcus* spp. infection. However, *Staphylococcus* spp. cannot be excluded. Gram-negative bacilli suggested of *Pseudomonas* spp. were not detected in all tissues evaluated.

Clinically, fungal organisms including *Microsporum canis* causing dermatophytic pseudomycetoma should be considered for differential diagnosis in cats. In fungal infection, the histopathology is very useful for differential diagnosis. Fungal hyphae are visible within granulomatous inflammation in tissue stained by hematoxilin and eosin and are strongly positive using PAS and GMS special stains.[4]

Skin and subcutaneous infections probably to develop as a result of wound contamination or trauma such as bites, lacerations, or puncture wounds with foreign bodies. Thus, infections localized in the skin and subcutis may extend deep to involve bone and muscle.[3] Bacterial infectious of bones usually originates in vascular areas of periosteum (determining periostitis) or medullary cavity (determining osteomyelitis). During bacteremia or septicemia, bacteria can become localized in many organs. In bones, there is a strong predilection for sites of active endochondral ossification within the metaphyses and epiphyses of long bones and vertebral bodies. The medullary sinusoidal



Tibia, cat: Filamentous bacilli are gram-positive. (Brown-Hopps, 400X)

capillaries are fenestrated, permitting ready escape of bacteria into the bone marrow. Thus, a combination of physeal, meta-physeal, or epiphyseal injury and concurrent bacteremia may be involved in the pathogenesis of hematogenous osteo-myelitis.[10] Probably, extension of infection to periosteum and marrow bone from adjacent tissue (skin and muscles) occurred in this animal. Indeed, evidence of other foci of infection was not detected in this cat.

Conversely, bacteria can be localized in the bone marrow and the infection can disseminate to adjacent tissues. Clinical manifestations of osteomyelitis may not develop until several months later when the bone lesion becomes extensive enough to cause pain, disfigurement of the bone, or perhaps result in pathological fracture.[10]

The recognition of reactive conditions associated with Splendore-Hoeppli reactions and differentiation among different agents (bacteria or fungi) are important to therapeutic measures. Also, aseptic collection of exudate and confidence culture following antibiogram is important to obtain success in the treatment.

JPC Diagnosis: Bone: Osteomyelitis, pyogranulomatous, chronic-active with diffuse, severe osteolysis and multifocal woven bone production with numerous colonies of filamentous bacilli, mixed breed (*Felis catus*), feline.

Conference Comment: Osteomyelitis, or inflammation of the medullary cavity and adjacent bone, is most commonly caused by bacteria (rather than fungi). Bacteria can spread to bone by three routes: (1) hematogenously, (2) local extension, or (3) implantation.[1]

Hematogenous spread is most common in young horses and ruminants with omphalophlebitis, however, these animals usually succumb to septicemia before boney lesions become evident. Bacteria seem to prefer areas of active endochondral ossification within metaphyses and epiphyses of long bones and vertebral bodies for two reasons, (1) capillaries there are fenestrated and allow bacteria to enter the bone marrow and (2) they make sharp turns within the growth plate allowing for accumulation of bacteria. Local extension is most common in older animals with severe periodontal disease and secondary bacterial spread into the maxilla and mandible, and implantation of bacteria usually results from adulteration of surgical fracture repair, bite wounds or gunshot injury, or open fractures.[1]

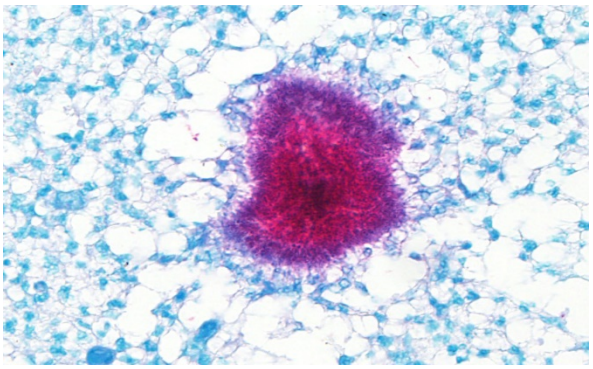
Certain bacteria are partial to bone, like *Staphylococcus aureus*, which invades osteoblasts, protecting it from host immune defense.[1] *Pasteurella multocida* wreaks havoc by retarding the growth and differentiation of osteoblasts and activating osteoblasts to resorption.[2] Moreover, at sites of infection, host defense mechanisms can be counter-productive, for example, cytokine production (IL-1, IL-6, TNF-α) by inflammatory cells induces osteoblasts to

activate osteoclasts to resorb bone.[1] A summary of the numerous manifestations of bacterial osteomyelitis is below for your viewing pleasure.

Table 1: Common manifestations of bacterial osteomyelitis[1]

Location	**Species affected**	**Predisposing factor**	**Route of dissemination**	**Common isolates**
Vertebral body	Horses, livestock	Inadequate passive immunity	Hematogenous (through umbilicus)	*Trueperella pyogenes* (common) Foals: *Escherichia coli, Salmonella enterica* serovar Typhimurium, staphylococci, streptococci, *Rhodococcus equi* Calves: *Fusobacterium necrophorum* Sheep: *Mannheimia haemolytica, F. necrophorum*, staphylococci Pigs: *Erysipelothrix rhusiopathiae*, staphylococci, streptococci
	Piglets, lambs	Inadequate passive immunity	Hematogenous (tail biting or docking)	
Mandible	Cattle "lumpy jaw"	Penetrating injury to oral mucosa	Implantation	*Actinomyces bovis* or *Trueperella pyogenes*
	Numerous	Periodontitis	Local extension	*Fusobacterium necrophorum*, other oral commensals
Numerous locations	Small animals	Open fractures, bite wounds, gunshot injury	Implantation	*Staphylococcus pseudointermedius*, streptococci
Nasal cavity	Pigs	Bacterial toxins	Local extension	*Pasteurella multocida* and *Bordetella bronchiseptica*

Pseudomonas aeruginosa, isolated from the purulent exudate in this case, is traditionally thought of as the causative agent of numerous dermatologic disorders, such as post-grooming furunculosis and otitis externa.[9] However, as a potent gram-negative bacterium, it can cause a myriad of clinical



Tibia, cat: Filamentous bacilli are strongly acid-fast (Fite-Furaco, 400X)

syndromes (endometritis (mares), mastitis (cattle), pneumonia (foals and mink), enteritis (primates), and abortion and abnormal fetal development (cattle and horses)) and, in these cases, is often part of a consortium of fellow gram-negative and gram-positive bacteria.[7]

Ruleouts discussed in this case included: *Nocardia* sp. and *Actinomyces* sp. The moderator and conference attendees noted that they didn't appreciate Splendore-Hoeppli material (radiating eosinophilic club-shaped antigen-antibody protein) surrounding bacterial colonies. Gram and acid-fast stains were run to further classify the bacteria which in the submitted unstained sections appear to be gram-positive and acid-fast. In the moderator's and attendee;s cumulative experience, these organisms are most indicative of *Nocardia* sp, which we favor as the causative agent in this case.

Actinomyces sp. and *Nocardia* sp., often lumped together in textbooks, come in cutaneous, subcutaneous, and visceral forms. They generally manifest as nodular ulcerated lesions on the extremities which may arise from underlying bone and are often secondary to wound contamination. Grossly, yellow to green sulfur granules are present corresponding with microscopic aggregates of bacteria with small amounts of adherent neutrophils awash in a sea of suppurative or pyogranulomatous inflammation. Attendees agreed that the aggregates of fulamentous bacilli in these sections are strongly reminiscent of sulfur granules.

Contributing Institution:

Universidade Federal de Minas Gerais
Escola de Veterinária
Departamento de Clínica e Cirurgia Veterinárias
Av. Antônio Carlos, 6627; 31270-901.
Belo Horizonte, MG, Brazil
www.vet.ufmg.br

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CASE III: 16-41394 (JPC 4100931).

Signalment: 14-week-old, male, intact, mixed breed (*Canis familiaris*), canine.

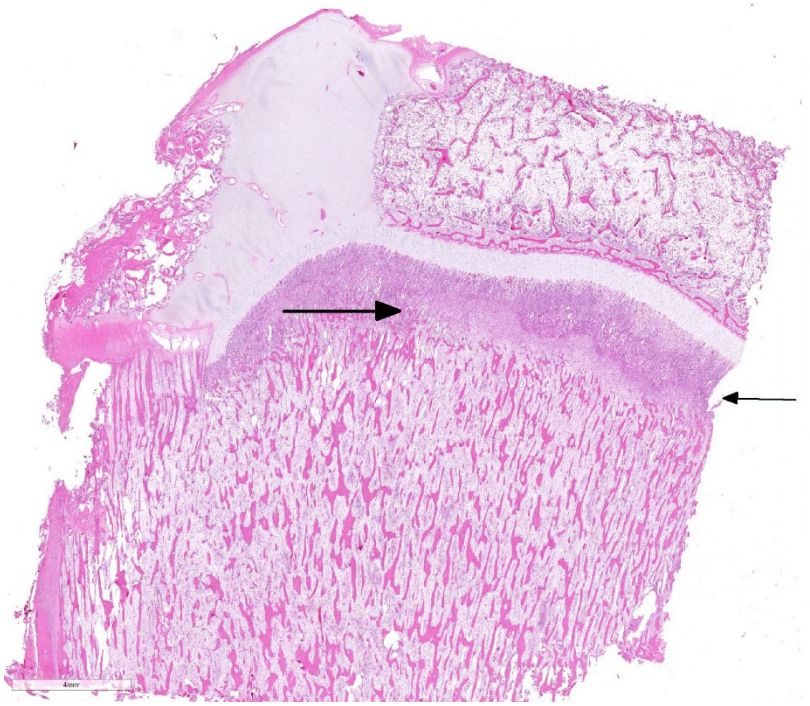
History: This animal had an approximately 4-week-long history of intermittent difficulty or inability to walk and/or stand, and vocalization due to pain upon light palpation. Clinical examination revealed positive deep pain on all four limbs and radiographs were unremarkable. Due to the poor quality of life, the animal was humanely euthanized.

Gross Pathology: The animal was in poor nutritional condition, and the costochondral joints from the seventh to thirteenth ribs were moderately enlarged and prominent. Upon longitudinal sectioning of long bones (femur, radius and ulna, tibia and fibula), no evident abnormalities were observed.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.): None provided.

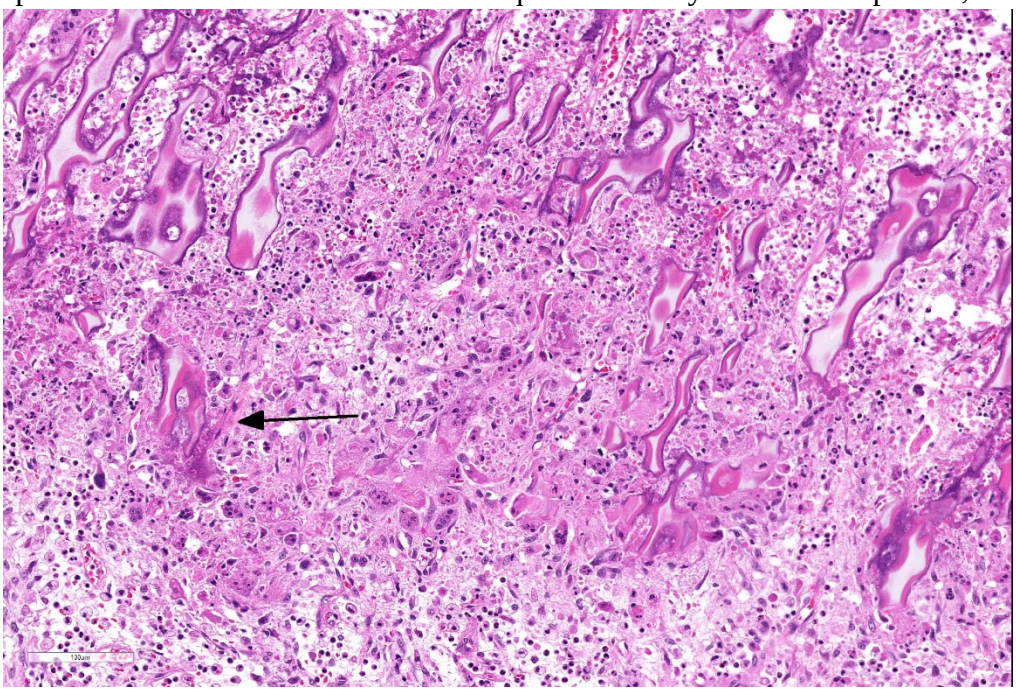
Microscopic Description:

Long bone (tibia): Diffusely, the metaphysis is severely affected by inflammation accompanied by degeneration and necrosis. The primary spongiosa (ossification zone) is diffusely distorted and infiltrated by a thick band of numerous viable and degenerate neutrophils and fewer macrophages, among abundant pyknotic and karyorrhectic cellular debris and eosinophilic, loose, fibrillar material (fibrin). Inflammatory cells frequently replace the primary spongiosa and expand intertrabecular spaces, separating fragmented remnants of thinned and tortuous trabeculae, composed of amphophilic to hyperbasophilic, finely stippled to amorphous material (mineralized cartilage



Long bone with growth plate, puppy: Epiphyseal primary spongiosa is basophilic as a result of a cellular infiltrate, and there is an irregular linear area of necrosis at the metaphyseal aspect (arrows). (HE, 6X)

spicules). There is marked paucity to absence of osteoblasts lining the fragmented and sparse trabeculae. There are frequent



Long bone with growth plate, puppy: Necrotic regions contain fractured trabeculae of the primary spongiosa (arrow) which are minimally lined by osteoid. There is abundant fibrin, edema, infiltrating neutrophils and cellular debris often replacing primary spongiosa and osteoclasts are free within the remaining space. (arrows). (HE, 150X)

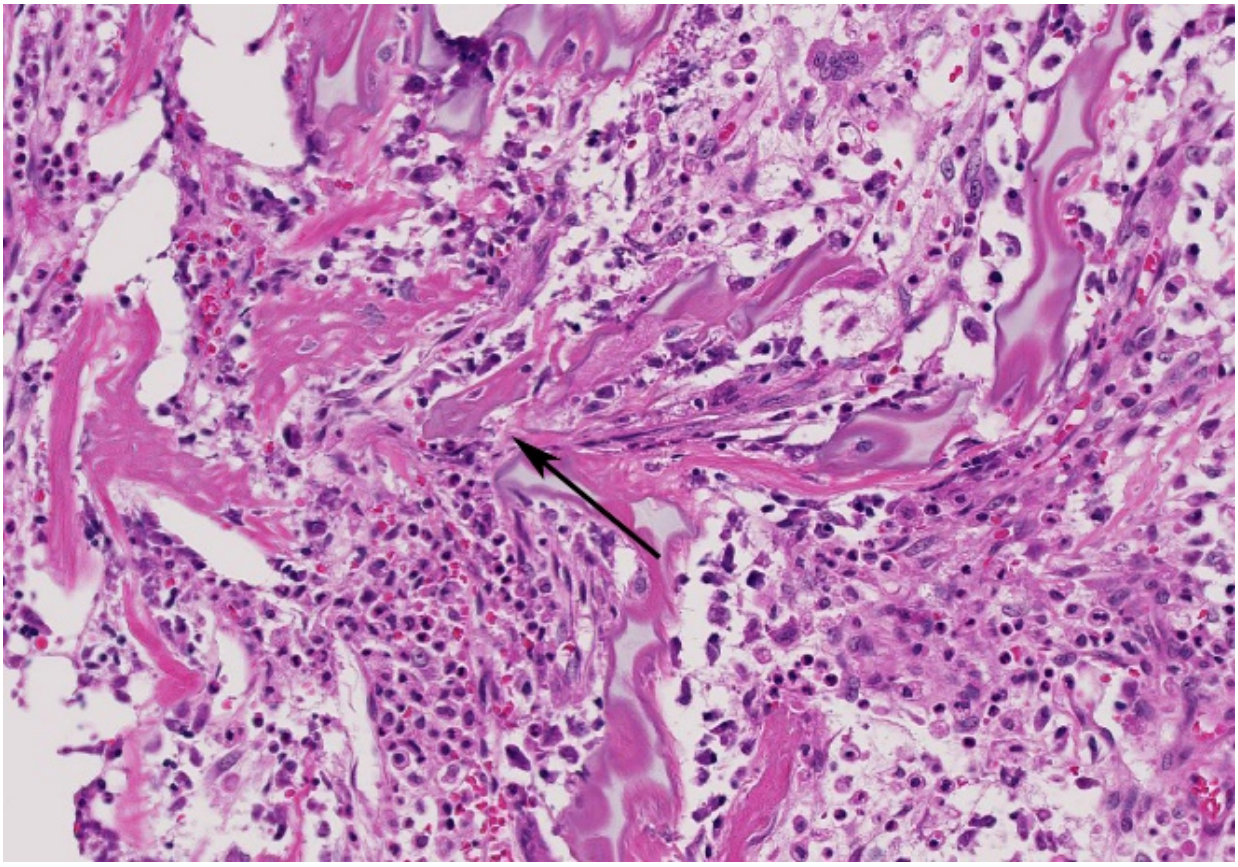
osteoclasts scattered among the inflammatory infiltrate, abutting scalloped metaphyseal spicules. The inflammatory cells loosely extend into the underlying secondary spongiosa and, in a lesser extent, into the proximal and mid-diaphyseal medullary cavity. Frequent small caliber and thin walled blood vessels within the medullary cavity present hyaline profile due to bright eosinophilic, fibrillar material concentrically replacing the vascular wall (vascular fibrinoid necrosis).

Contributor's Morphologic Diagnosis:
Metaphysis (tibia): Diffuse, severe, subacute, necrosuppurative osteomyelitis (metaphyseal osteopathy).

Contributor's Comment: Considering the signalment, clinical history and similar histopathology affecting the costochondral joints, femur and tibia in a bilateral and symmetrical pattern, a diagnosis of metaphyseal osteopathy was concluded in this case.

Metaphyseal osteopathy, also known as hypertrophic osteodystrophy (HOD), is a developmental bone disease involving necrosis and suppurative inflammation affecting the metaphyseal region of long bones of rapidly growing young dogs.[1,2] The disease has been reported in over 40 breeds, including mixed breed dogs. However, breed predisposition is commonly reported in Great Danes, Boxers, German Shepherds, Irish Setters, and Weimaraners.[1,4] The latter is reported as the only breed where entire litters and closely related animals have been affected by the disease, strongly supporting a heritable component.[4]

The etiopathogenesis of metaphyseal osteopathy is not fully understood, and proposed mechanisms involving nutritional, infectious or vaccine-related reactions lack supporting scientific evidence.[1] A recent study demonstrated overall overexpression of pro-inflammatory cytokines regulating innate immunity, such as Il-1b, IL-18, GM-CSF, CXCL10, TNF and IL-10, in affected dogs of two different breeds (Irish Setter and



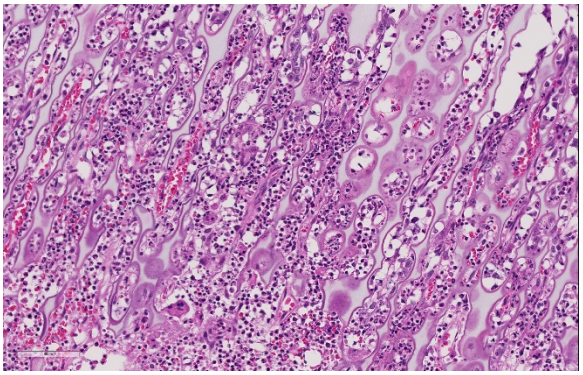
Long bone with growth plate, puppy: Horizontally-oriented trabeculae of primary spongiosa (arrow) are excellent evidence of microfracture. In this field, neutrophils are numerous and there is abundant polymerized fibrin. (HE, 200X)

Weimaraner) supporting the hypothesis of an autoinflammatory disease.[3]

The condition can range from self-limiting with management with corticosteroid therapy to multisystemic involvement, potentially reaching overall debilitation and eventual death or euthanasia[2], as ultimately happened in this case.

JPC Diagnosis: Long bone, metaphysis: Osteomyelitis, metaphyseal, necrotizing and neutrophilic, multifocal, moderate to severe with osteolysis and microfractures of the primary spongiosa, mixed breed (*Canis familiaris*), canine.

Conference Comment: Metaphyseal osteopathy is a condition with an unknown etiology that affects young, growing, large breed dogs (breed predispositions listed above) with relapsing lameness, joint swelling, and pain of the distal radius and ulna most commonly. Radiographically, these areas correspond to the metaphysis parallel to the physis and have alternating linear, parallel radiodense and radiolucent zones. Grossly, lesions are bilaterally symmetrical and characterized by a pale band in the primary spongiosa adjacent to the physis that can develop into small fractures within the boney spicules of the spongiosa. With chronicity, the periosteum thicken to support the dysfunctional ossification within the spongiosa and appears as bulbous dilations. The microscopic appearance is diagnostic for this condition with persistence of the mineralized cartilage lattice of the primary spongiosa, marked neutrophilic inflammation surrounding trabeculae,



Long bone with growth plate, puppy: Proximal to the area of necrosis, spaces between cartilage columns contain large numbers of neutrophils. In this field, neutrophils are numerous and there is abundant polymerized fibrin. (HE, 275X)

necrosis and loss of osteoblasts, and sometimes increased osteoclasts. Secondarily, as noted grossly, trabeculae frequently fracture and the periosteal bone is markedly thickened. The suppurative inflammation frequently extends into the marrow cavity of the metaphysis resulting in necrosis of marrow contents and fibrin thrombi.[1]

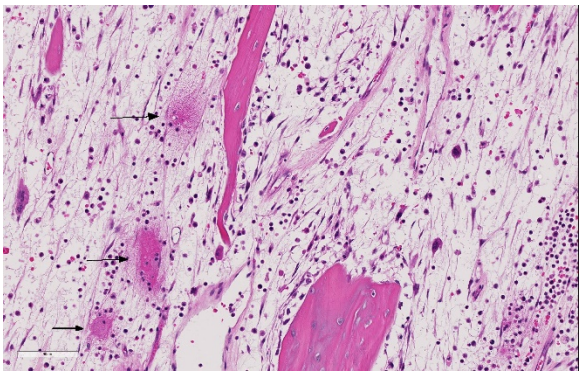
The main differentials for metaphyseal osteopathy include: bacterial osteomyelitis/septic metaphysitis/polyarthritis, hypovitaminosis C, and panosteitis.

All bacterial causes would have a different radiographic appearance and colonies of bacteria microscopically. Hypovitaminosis C, or scurvy, also exhibits decreased osteoid deposition on the cartilaginous trabeculae ("scorbutic lattice") of the primary spongiosa but lacks inflammation and necrosis. Ascorbic acid (vitamin C) is required for hydroxylation of proline and lysine during fibrillary collagen cross-linking. Cartilage that is not properly cross-linked is inherently weak leading to decreased osteoid, increased fragility of blood vessels (hemorrhages), and microfractures in bone.[1]

Finally, panosteitis does not occur within the metaphysis and often appears as cotton-like densities within the medullary space of long bones radiographically. Clinically, panosteitis affects similar ages and breeds as metaphyseal osteopathy, but manifests as "shifting leg lameness" which often resolves on its own as the animal grows. The radiodensities seen radiographically correspond to increasing amounts of fibrovascular tissue with are soon replaced by woven bone within the medullary cavity. There are several subsequent episodes of bone resorption and formation leading to characteristic resting and reversal lines microscopically. Additionally, there is rarely any inflammation present, despite the name "panosteitis".[1]

Contributing Institution:
University of Illinois at Urbana-Champaign
Veterinary Diagnostic Laboratory
http://vetmed.illinois.edu/vet-resources/veterinary-diagnostic-laboratory/

References:



Long bone with growth plate, puppy: Throughout the epiphysis and metaphysis, small vessels exhibit fibrinoid necrosis. (HE, 275X)

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CASE IV: 109234-16 (JPC 4100936).

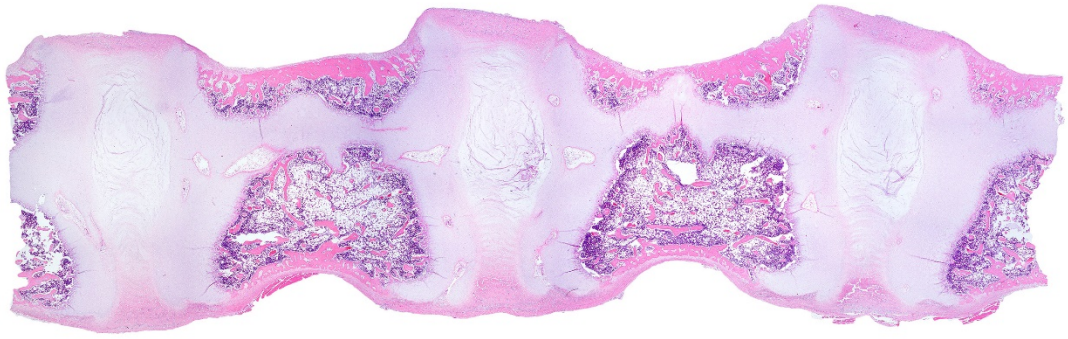
Signalment: 15-week-old female intact Boston terrier (*Canis familiaris*), canine.

History: A 15-week-old intact female Boston Terrier was presented to the Neurology Service of the Cornell University Hospital for Animals with a 7-week history of hyporexia, dysphagia, difficulty ambulating, failure to gain weight, and lethargy. The physical examination revealed dull mentation, poor body condition, non-ambulatory tetraparesis, severe joint laxity, and bilateral corneal opacities. MRI and CT imaging showed evidence of skull malformation, hydrocephalus, cerebral atrophy, severe vertebral and intervertebral disc malformations, and epiphyseal dysplasia. Cytology of the blood, cerebrospinal fluid, and joint fluid revealed cytoplasmic vacuolation and metachromatic cytoplasmic granules in neutrophils, lymphocytes, and macrophages. Based on these findings a presumptive diagnosis of mucopolysaccharidosis (MPS) was made and the owner elected euthanasia due to the poor prognosis.

Vertebrae, puppy: The vertebral bodies are shortened, the intervertebral disc is widened and rounded, and the disc material is loose. (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

Gross Pathology: Within both corneas were 0.4 cm in diameter, irregularly shaped areas of white-blue opacity. The left and right 10th, 11th, and 12th ribs were widened, flattened, and bulging laterally and ventrally. All four limbs had severe laxity of all of their joints and the left forelimb was externally rotated at the carpus. On the ventral midline at the level of the umbilicus was a small, 0.6 cm in diameter, focal, round, soft, reducible, protrusion (umbilical hernia).

Bilaterally, the proximal epiphyseal plates of the femur and humerus and distal epiphyseal plate of the radius and ulna were displaced and irregular (epiphyseal dysplasia). All vertebral bodies were shortened with thinning of the cortex and an approximate 1:1 ratio of the vertebra to intervertebral disc length. The intervertebral discs were rounded, widened, and hollow at the center and contained clear, loose, gelatinous material (dysplastic nucleus pulposus). The rostral mandible at the level of the symphysis was widened and thickened up to 3.0 x 3.0 x 1.0 cm. The xiphoid process extended 3.0 cm caudal to the last sternebra. On the dorsal



Vertebrae, puppy: Low magnification image of the vertebral bodies demonstrating the bridging trabecular of cartilage between the epiphyses. (HE, 6X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

surface of both carpi and arising between the carpal bones were five (right) and four (left) raised, up to 0.9 x 0.5 x 0.3 cm, well-demarcated, tan to pink, soft, fluctuant, cystic structures continuous with the joint capsules. These cystic structures contained <0.5 mL of slightly opaque, red-tinged fluid. The joints of all four limbs contained mildly increased amounts of cloudy, red-tinged fluid and the joint capsules had increased elasticity.

The heart weighed 26.0 g. The leaflet of the tricuspid valve along the interventricular septum was glistening, smooth, and focally thickened up to 1.0 x 0.6 x 0.2 cm. The mitral valve had seven similar, nodular thickenings up to 0.3 x 0.1 x 0.1 cm.

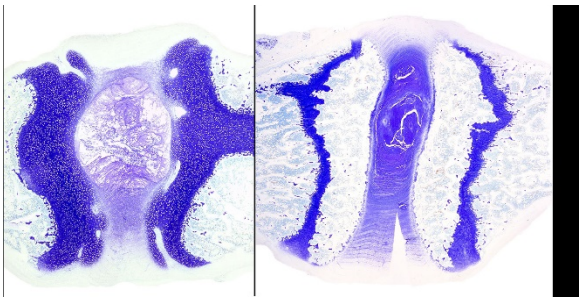
The meninges were diffusely opaque and moderately thickened. Sectioning of the brain revealed a mild dilation of the lateral ventricles (hydrocephalus).

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):

Berry urine MPS spot test: Positive

Microscopic Description:

Diffusely, the epiphyseal cartilage is severely thickened with an irregular contour and lacking secondary centers of ossification. Multifocally within, and occasionally crossing the epiphyseal cartilage, are broad tracts that lack cartilage with small blood vessels surrounded by abundant large polygonal cells with foamy cytoplasm and eccentric nuclei (transphyseal vessels). Consistently bridging the medullary space between epiphyseal cartilage are broad columns of cartilage with occasional extensions into the medullary space ventrally and dorsally. Chondrocytes are mildly enlarged, rounded, and have finely vacuolated cytoplasm and are occasionally binucleated. At the chondro-osseous junction is a narrow proliferative and hypertrophic zone with mineralized cartilage and a paucity of osteoclasts. Trabecular bone is sparse and discontinuous with retained cartilage cores. The nucleus pulposus is composed of abundant wispy to frothy basophilic matrix, small numbers of scattered 2 to 4 µm in diameter round, eosinophilic globules, and moderate numbers of large polygonal cells with vacuolated cytoplasm and eccentric nuclei (variable severity between sections). Expanding the collagen fiber matrix of the ventral and dorsal longitudinal ligaments are large numbers of individual and clustered large polygonal cells with foamy cytoplasm and eccentric nuclei.



Vertebrae puppy: The toluidine blue stain reveals the cartilage that otherwise stains very pale and highlights the lack of a site of secondary ossification in the epiphysis, the rounding of the epiphysis, the abnormal contents of the nucleus pulposus, and the bridging trabeculae of cartilage in the vertebral metaphysis. (Toluidine blue, 2X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

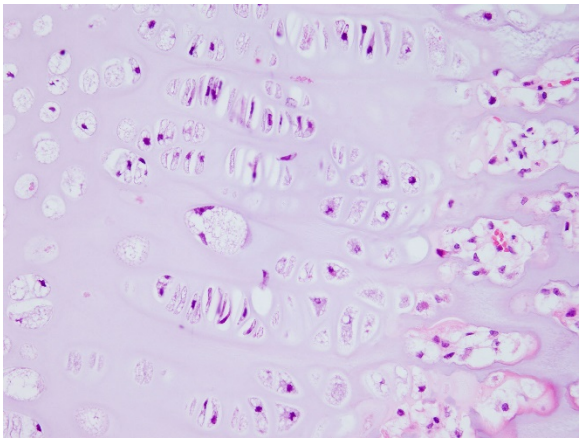
Contributor's Morphologic Diagnosis:
Lumbar vertebrae:

1. Chondrodysplasia
2. Epiphyseal dysplasia with loss of secondary site of ossification
3. Intervertebral disc dysplasia
4. Osteopenia

Contributor's Comment: The combined clinicopathologic, gross, and histologic findings of this puppy were strongly suggestive of mucopolysaccharidosis (MPS) with features that overlap with other reported cases of MPS in dogs. To confirm the diagnosis, urine was submitted to PennGenn Laboratories at the University of Pennsylvania for a urinary Berry MPS spot test[3]. In addition, a severe deficiency of β-glucuorindase was detected in the serum (personal communication, Dr. Urs Giger). These results were consistent with a Type VII MPS, also known as Sly syndrome in humans. Unfortunately, insufficient samples were available to determine the underlying genetic mutation in this case.

Mucopolysaccharidoses are a group of related lysosomal storage diseases that are the result of genetic deficiencies of key enzymes involved in the normal degradation of mucopolysaccharides. Muco-polysaccharides are glycosaminoglycans; long-chain carbohydrates attached to protein cores that are commonly found in the ground substance of connective tissues throughout the body. Examples of glycosaminoglycans include dermatan sulfate, heparin sulfate, keratin sulfate, and chondroitin sulfate. MPS disorders are broken down into types designated by the underlying enzyme deficiency. For example, Type I MPS is the result of a deficiency in the α-L-iduronidase enzyme and results in the accumulation of dermatan sulfate and heparin sulfate within lysosomes and in the urine. Thus far, only MPS types I, II, IIIA, IIIB, IIIC, IIID, VI, and VII have been identified in domestic animals.

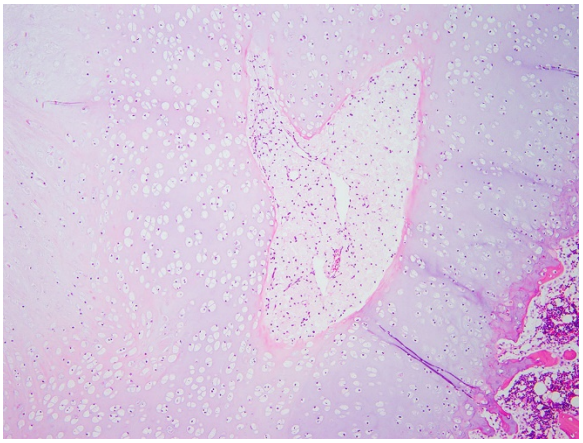
Lysosomes are small organelles within the cytoplasm that are responsible for the breakdown of endogenous and exogenous materials, including byproducts of normal cellular processes[9]. When deficiencies of specific lysosomal acid hydrolases or components of enzyme trafficking occur, substrates are not properly catabolized and



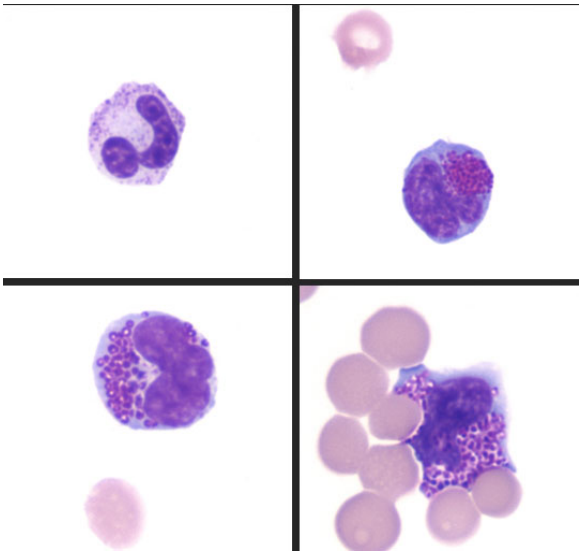
Vertebrae, puppy: Swelling, rounding, and vacuolation of chondrocytes in the growth plate cartilage with vacuolated cells the medullary space between spicules of primary spongiosa. (HE, 400X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

can accumulate within the lysosomes. This accumulation of the substrate is characteristic of lysosomal storage diseases, a heterogeneous group of disorders with a common feature of accumulating metabolites. The buildup of this material can cause lysosomes to become large and hinder normal cellular processes and may even lead to cell death. Errors in lysosomal processing can also result in the accumulation of autophagic substrate during normal cell turnover which can cause altered cellular metabolism and result in apoptosis. Long-lived postmitotic cells, fixed and mobile macrophages, and the cells most active in metabolizing these substrates are most susceptible to storage disease.

The lesions associated with MPS are related to the distribution and functions of the glycosaminoglycans affected by the enzyme deficiency. The key gross findings of this case that are consistent with MPS include skeletal deformities with shortened vertebral bodies, incomplete ossification of the vertebral end plates with subsequent widening of the intervertebral disc spaces, deformed epiphyses of the appendicular skeleton, facial dysmorphism, nodular thickening of the atrioventricular valves, meningeal thickening and opacity, and bilateral corneal opacities. The secondary ossification center of the distal epiphysis of the tibia, for example, is expected to be developed by 10-30 days of age but was completely lacking in this dog[5]. In addition to the vacuolation of chondrocytes, macrophages, and other stromal cells in the vertebral column, vacuolation in the Purkinje cells of the cerebellum, the stromal cells and macrophages of the aorta and trachea, proximal convoluted tubular epithelial cells, and synovium of appendicular joints were also seen in this case. The vacuolation of the epithelial cells of the kidneys is thought to reflect elevated levels of circulating glycosaminoglycans in the blood that are excreted through the glomerulus and reabsorbed in the renal tubular epithelium[11]. Surprisingly, vacuolation of Kupffer cells and hepatocytes



Vertebrae, puppy: A transphyseal vessel is highlighted by expansion of the perivascular space by vacuolated cells. (HE, 400X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)



CSF neutrophil/lymphocytes: Large numbers of metachromatic granules in the cytoplasm of a neutrophil and lymphocytes (Alder-Reilly bodies). (Wright-Giemsa, 1000X) (Photo courtesy of: Cornell University School of Veterinary Medicine, Department of Biosciences, 240 Farrier Road, Ithaca, NY 14853, http://www.vet.cornell.edu/biosci/pathology/)

was not a feature in this case despite being reported in another case of type VII MPS[6].

One of the early clues of MPS in this case was the presence of metachromatic intracytoplasmic granules (Alder-Reilly bodies) in neutrophils, lymphocytes, and macrophages in peripheral blood, joint fluid, and cerebrospinal fluid. This is a common finding in cases of MPS and is best observed with Leishman or Wright-Giemsa stains as Diff-Quick-stained smears do not reveal inclusions as readily in some reports[8].

Histochemical staining of the vacuoles was variable with Alcian blue and periodic acid-Schiff; however, it is suspected that the inclusion material can be lost during processing. In this case, the only cells highlighted with Alcian blue stain were within the wall of the aorta. Some reports have suggested that better success is obtained by using these stains on frozen tissues[12]. Electron microscopy could reveal dilated lysosomes that appear empty (electron-lucent), electron dense, or contain lamellar swirls (zebra bodies). Electron microscopy was not performed in this case.

JPC Diagnosis: Vertebral column: Chondrodysplasia, diffuse, severe with epiphyseal dysplasia, loss of secondary ossification sites, intervertebral disc dysplasia, and chondrocytic, fibroblastic and histiocytic vacuolation, Boston terrier (*Canis familiaris*), canine.

Conference Comment: Mucopolysaccharidosis is aptly described by the contributor above.

Table 1: Selected mucopolysaccharidoses in domestic animals [5,11,13,14]

Disease	Storage product(s)	Deficient enzyme(s)	Species	Breed(s)
MPS type I "Hurler's disease"	Different glycosaminoglycans (dermatan sulfate, heparan sulfate, keratin sulfate, chondroitin sulfate)	α-L-iduronidase	Dog, cat	Plott hound Domestic shorthair
MPS type II "Hunter syndrome"		Iduronate-2-sulfatase	Dog	Labrador retriever (1)
MPS type III A, B, D "Sanfilippo syndrome"		Heparan sulfate sulfamidase (SGSH; MPS type IIIA), a-N-acetylglucosaminidase (NAGLU; MPS type IIIB), and N-acetylglucosamine-6-sulfatase (GNS; MPS type IIID)	Dog, cattle, goat	IIIA – Dachshund, New Zealand Huntaway dogs IIIB – Schipperke dogs, cattle, emus IIID – goats
MPS type VI "Maroteaux-Lamy disease"		ARSB (arylsulfatase B)	Dog, cat	Miniature Pinscher, Miniature Schnauzer, Toy Poodle, Welsh Corgi, Chesapeake

				Bay Retriever, Great Dane Domestic shorthair, Siamese
MPS type VII "Sly disease"		B-glucuronidase	Dog, cat	German Shepherd Dog Domestic shorthair

During the conference the moderator noted that the cartilage cores are not running parallel to the axis of the bone but perpendicular, indicating infractions or microfractures in the bone. In general, the bone is weakly mineralized with few primary trabeculae being formed and a disorganized zone of hypertrophy, indicative of a developmental lesion.

Contributing Institution:
Cornell University
240 Farrier Road
Ithaca, NY 14853
http://www.vet.cornell.edu/biosci/pathology/

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