Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

C o n f e r e n c e 21

30 March 2022

CASE I: 1326 (JPC 4118329)

Signalment:
Adult, female, cynomolgus macaque (*Macaca fascicularis*)

History:
This macaque was part of the primate colony at USAMRIID and was awaiting assignment to a research study. Although it was at USAMRIID for several months and had no history of clinical disease during this time, it was suddenly noticed to have weakness of the left arm and left leg. Further physical examination revealed an approximately 3 cm X 2.5 cm open wound on the back of its neck with a purulent discharge. Routine aerobic bacterial cultures of the wound yielded *Staphylococcus aureus* and other unremarkable species of bacteria. There appeared to be a fistulous tract on the neck that extended into the cervical spine and radiographs of this area revealed that several cervical vertebrae had "mottled" areas of decreased bone density. Because of the overall poor prognosis for this animal and its unsuitability for use in a research protocol, euthanasia was performed.

This monkey was being kept in a primate research colony that is under the purview of an Institutional Animal Care and Use Committee (IACUC) and this research colony is maintained in compliance with the Animal Welfare Act, PHS Policy, and other federal statutes and regulations relating to animals and experiments involving animals. The facility where this primate colony is kept is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the 8th edition of the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Gross Pathology:
At necropsy, there was a tan purulent exudate that emanated from the wound on the right dorsal area of the neck. A fistulous tract extended from the open skin wound into the

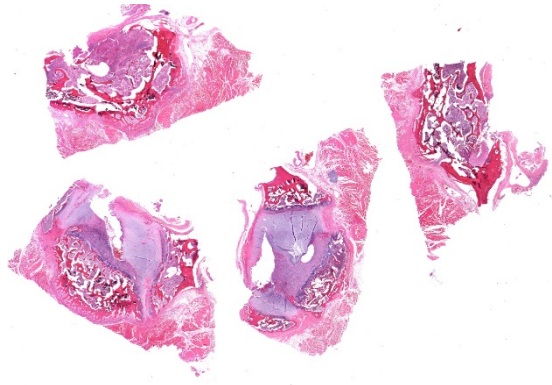

Figure 1-1. Cervical vertebra, cynomolgus macaque: Oblique sections of a cervical vertebrae are submitted for examination. (HE, 6X)

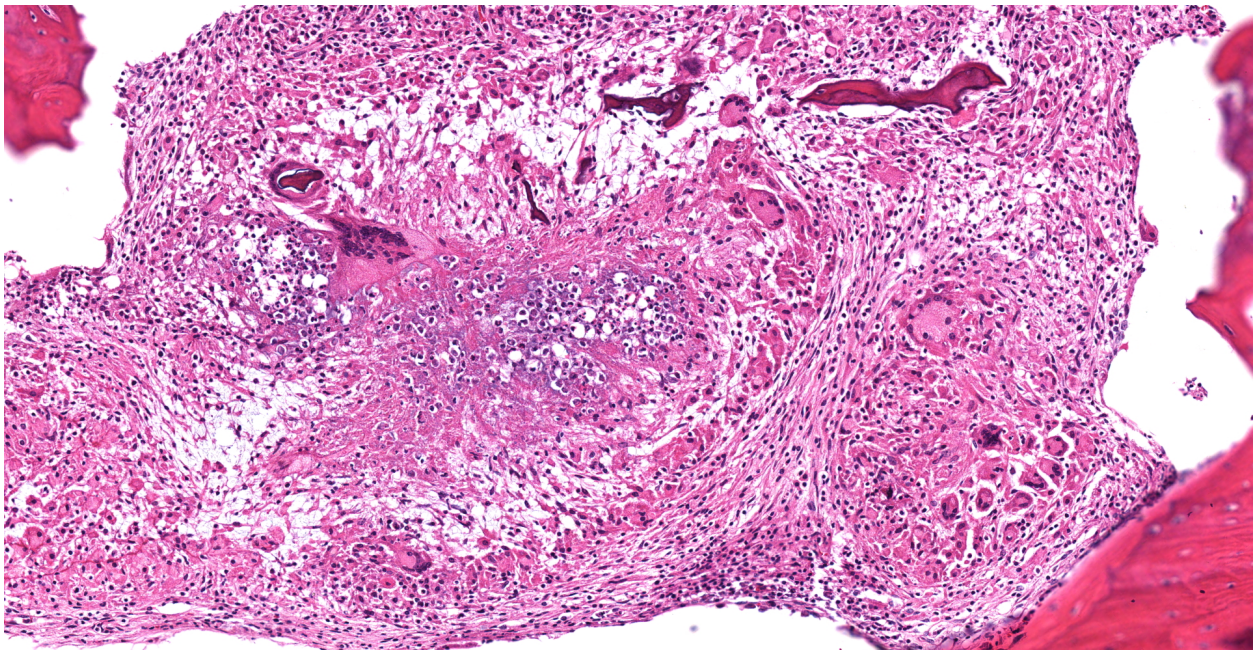

Figure 1-2. Cervical vertebra, cynomolgus macaque: Multifocally, the marrow is replaced by variably-sized, poorly formed granulomas which contain innumerable epithelioid macrophages, contain central areas of caseous necrosis and numerous Langhans and foreign body multinucleated giant cells (HE, 160X)

underlying muscles and cervical vertebrae and appeared to also involve the cervical spinal cord.

An approximately 1 cm diameter white nodule was present in the left superior lung lobe and multiple pinpoint white foci were noted throughout the spleen and liver.

Laboratory Results:
None submitted.

Microscopic Description:
Cervical vertebrae (4 sections): Within the bone marrow, there are multifocal and coalescing cellular infiltrates composed of neutrophils, macrophages (primarily epithelioid), and multinucleated giant cells. There is often caseous necrosis in the centers of these infiltrates and fibroplasia is present multifocally around the periphery of the infiltrates. There is multifocal necrosis and/or resorption of bone spicules within the marrow cavities, as well as multifocal periosteal new bone formation and fibrosis along the outer margins of the vertebral cortices.

Contributor's Morphologic Diagnoses:
Cervical vertebrae; multifocal and coalescing chronic pyogranulomatous osteomyelitis, moderate to marked, with bone resorption, bone remodeling, and periosteal new bone formation.

Contributor's Comment:
In addition to the lesions present in the cervical vertebrae, granulomatous and/or pyogranulomatous lesions were found in the adjacent soft tissues around these vertebrae and in the lungs, liver, spleen, kidneys, femoral bone marrow, and left eye. Acid-fast stains were then performed and revealed low numbers of intracellular and extracellular acid-fast bacteria within the lungs, liver, cervical bone marrow, and skin overlying the cervical vertebrae.

This macaque had repeatedly tested negative for tuberculosis using intradermal tuberculin. The most recent test had been administered

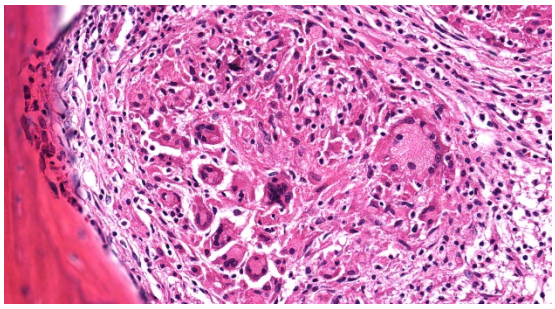

Figure 1-3. Cervical vertebra, cynomolgus macaque: Higher magnification of a granuloma, demonstrating both foreign body and Langhans-type giant cells. (HE, 402X)

approximately 3.5 months before the monkey was euthanized. However, based on the histologic findings, a mycobacterial infection was strongly suspected. Unfortunately, there were no fresh tissue samples available then for microbial culture. Formalin-fixed paraffin-embedded samples of lung were submitted to the National Veterinary Services Laboratory in Ames, Iowa for testing by PCR and these results were positive for organisms in the *Mycobacterium tuberculosis* complex but negative for organisms in the *Mycobacterium avium* complex (A. Lehmkuhl, pers. comm.).

The *Mycobacterium tuberculosis* complex consists of several species of closely related bacteria in the genus *Mycobacterium*, including *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, and *M. microti.* The *Mycobacterium avium* complex includes *M. avium, M. intracellulare*, and *M. chimera*. The causative agent of Johne's disease in ruminants is currently classified as a subspecies of *M. avium* (i.e. *Mycobacterium avium* subspecies *paratuberculosis).*

Immunofluorescence assays were then performed "in-house" at USAMRIID on formalin-fixed paraffin-embedded sections of lung using a commercially-available rabbit polyclonal antibody (Abcam ab43019) that specifically detects *Mycobacterium tuberculosis* antigen "Ag85B". Low numbers of immunofluorescent bacteria were detected in the lung granulomas.

Based on the test results, we concluded that this monkey was infected with *Mycobacterium tuberculosis* rather than another bacterial species in this "complex". Follow-up surveillance and testing of other macaques that had been exposed to this index case revealed additional affected animals and some of these have been culture-positive for *M. tuberculosis*.

Tuberculosis in humans (and macaques) is usually caused by *M. tuberculosis* although infection with *M. bovis* in humans was also common before pasteurization of milk as well as testing and culling of TB-positive dairy cows became standard practices.[4] Human tuberculosis is primarily a disease of the respiratory tract.[4] However, secondary infection of vertebrae can occur due to direct spread from the thorax or as a sequela to bacteremia.[3,4,6] The thoracic vertebrae are most commonly affected, with lumbar and/or cervical vertebrae less commonly affected.[3] Cases of spinal tuberculosis were described

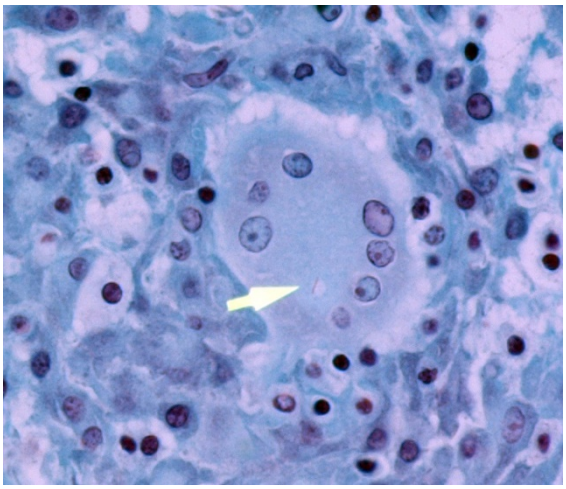

Figure 1-4. Cervical vertebrae, cynomolgus macaque. A single acid-fast bacillus is present within a cytoplasmic vacuole within a Langhans-type giant cell. (Ziehl-Neelsen, 600X) (*Photo courtesy of:* U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011, http://www.usamriid.army.mil/)

in detail in a 1779 publication by Sir Percival Pott.[1] Consequently, spinal tuberculosis in humans became known as "Pott Disease" or "Pott's Disease".[2-4,6] Another common term for this condition is tuberculous spondylitis.[3] The chronic osteomyelitis often results in destruction and collapse of vertebral bodies causing spinal cord damage and neurologic signs ("Pott's paraplegia").[2,3] It is interesting to note that the initial clinical signs that this cynomolgus macaque presented were weakness and movement difficulties involving its left arm and left leg.

Deformation of the spinal column in chronic cases of Pott's Disease often caused affected humans to have a "hunchback" appearance.[2,3] It is believed that observing victims of Pott's Disease inspired Victor Hugo to create the character of "Quasimodo" for his novel "*The Hunchback of Notre Dame*".[6]

Contributing Institution:
U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID)
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JPC Diagnosis:
Cervical vertebrae: Discospondylitis, pyogranulomatous, multifocal to coalescing, marked, with periosteal new bone formation.

JPC Comment:
The contributor provides a concise review of spinal tuberculosis, which represents 1-2% of all tuberculosis cases in humans and is the most common site of musculoskeletal infection with this entity. Although Pott described the manifestations of spinal tuberculosis in 1779, it has been identified in Egyptian mummies dated before 3300 BC, making it one of the oldest diseases known. Despite being an ancient disease, tuberculosis continues to be a significant

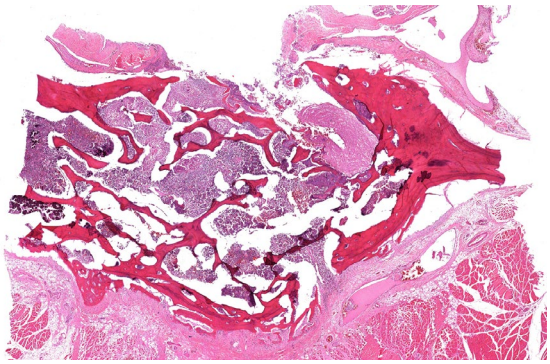

Figure 1-5. Cervical vertebra, cynomolgus macaque: The cortex of the ventral vertebral body is thickened by perpendicularly arrayed trabeculae of woven bone arising from the hypercellular periosteum. There is fibrosis which extends into surrounding adipose tissue and atrophic skeletal muscle.

public health issue with 10.4 million new cases reported worldwide in 2015. In regard to spinal tuberculosis specifically, the highest incidence is in impoverished populations, most commonly affecting young adults and children. Another significant risk factor for tuberculosis is HIV co-infection, which results in suppression of CD4+ lymphocytes and degraded cellular immunity. In addition, Chinese patients with the FokI polymorphism in the vitamin-D receptor gene are predisposed to spinal tuberculosis.[1]

M. tuberculosis is readily phagocytized by macrophages once it enters the body, where it is resistant to intracellular digestion by preventing phagosome-lysosome fusion. This feat is accomplished by recruiting a host protein known as coronin, which prevents fusion of these organelles by activating phosphatase calcineurin. The organism then replicates within macrophages until pattern recognition receptors, such as TLR2 and TLR9, bind substances such as mycobacterial lipoarabinomannan and unmethylated CpG nucleotides. This in turn initiates a Th1 inflammatory response, which most notably results in the production of IFN-γ by CD4+ lymphocytes. IFN-γ is essential for the containment of *M. tuberculosis* for multiple

reasons, including 1) stimulation of phagolysosome maturation in infected macrophages, 2) stimulation of inducible nitric oxide synthase, resulting in the creation of reactive nitrogen intermediates, 3) mobilization of antimicrobial peptides (defensins), and 4) stimulation of increased autophagy, which is essential for the destruction of intracellular bacteria.[4]

As noted by the contributor, the *M. tuberculosis* may disseminate to the vertebrae by either direct or hematogenous routes. Vertebrae are particularly vulnerable to the latter due to the presence of rich vascular plexuses formed in the subchondral regions supplied by the posterior and anterior spinal arteries. The osteomyelitis that occurs following colonization initially causes paradiscal destruction of the vertebral body; the disc typically remains intact until late in the disease process. Neurologic sequelae typically manifest late in the disease process, as the result of spinal compression secondary to kyphosis in addition to epidural pus and disc and osseous debris.[1]

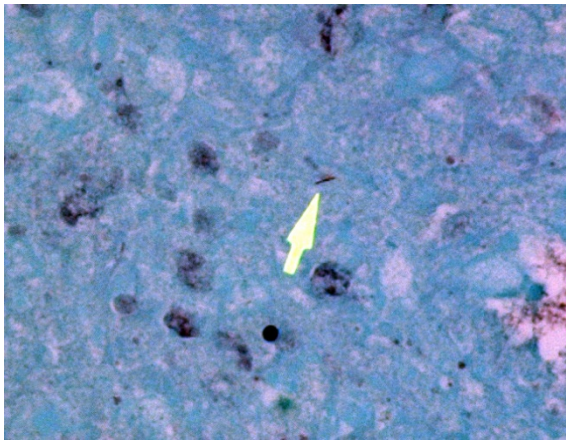

Figure 1-6. Lung, cynomolgus macaque. A single acid-fast bacillus is present within a cytoplasmic vacuole within a Langhans-type giant cell in a granuloma (Ziehl-Neelsen, 600X) (*Photo courtesy of:* U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Pathology Division, 1425 Porter Street, Fort Detrick, MD 21702-5011, http://www.usamriid.army.mil/)

Vertebral infections are typically insidious and progress over four to 11 months, with weight loss being the most common clinical sign, followed by fatigue, pyrexia, night sweats, and pain. Neurologic deficits are also common, particularly when cervical or thoracic vertebral regions are affected.[1]

In humans, spinal tuberculosis infections are typically paucibacillary, with acid fast bacilli observed in only 38% of biopsies in one report. Histologic features such as caseating granulomas and giant cells are often considered diagnostic in endemic regions, although similar lesions also occur with conditions such as sarcoidosis and cat scratch disease (*Bartonella henselae)*. *Mycobacterium tuberculosis* can be identified with a specificity of 80-90% and sensitivity of 95% using PCR technology. However, this technology is not readily accessible in socioeconomically deprived communities with poor access to healthcare.[1]

In regard to non-human primates (NHPs), tuberculosis is rare in wild populations living in isolation from human contact. However, it is also the most common reported infectious disease of captive NHPs. *Mycobacterium tuberculosis* is typically introduced to a naïve NHP population following an exposure to an infected human, most commonly via inhalation. Following its introduction to a previously naïve population, the pathogen undergoes monkey-to-monkey transmission, predominantly via aerosol and occasionally ingestion.[5]

Rhesus macaques are quite sensitive to *M. tuberculosis* and develop a variety of lesions similar to those reported in humans. Tuberculosis also tends to be rapidly progressive in this species, which can lead to widespread dissemination within a colony. In addition, surveillance testing utilizing the traditional intradermal tuberculin skin testing

method is associated with both false positives and negatives (such as in this case); therefore additional testing methods should be considered.[5]

According to the WHO, 80% of human tuberculosis cases occur in 22 countries. Two countries on this list include India and China, which both export large numbers of NHPs. Therefore, it is likely many NHPs become infected by caretakers or during the subsequent international trade process prior to their arrival at their final destination.[5]

Conference participants discussed use of the terms 'osteomyelitis' and 'discospondylitis' while determining how to best characterize the lesion in this case. Osteomyelitis is defined as inflammation of the bone and marrow while discospondylitis is defined as inflammation of the intervertebral disk as well as the adjacent vertebrae. Given the previously discussed pathogenesis of spinal tuberculosis, this lesion most likely originated as an osteomyelitis. However, due to involvement of the intervertebral disk, as evidenced by extruded basophilic material consistent with nucleus pulposus, the majority of participants favored discospondylitis.

References:

1. Dunn RN, Ben Husien M. Spinal tuberculosis: review of current management. *Bone Joint J*. 2018;100-B(4):425-431.
2. Garg RV, Somvanshi DS. Spinal tuberculosis: a review. *J Spinal Cord Med*. 2011; 34(5):440-454.
3. Hoch BL, Klein MJ, Schiller AL. Bones and Joints. In: Rubin R, Strayer DS, eds. *Rubin's Pathology: Clinicopathologigic Foundations of Medicine 5th Ed.* Baltimore, MD: Lippincott Williams & Wilkins; 2008:1083-1151.
4. Kumar V, Abbas AK, Aster JC. *Robbins and Cotran Pathologic Basis of Disease 9th Ed*. Philadelphia, PA. Elsevier Saunders; 2015.
5. Mätz-Rensing K, Hartmann T, Wendel GM, et al. Outbreak of Tuberculosis in a Colony of Rhesus Monkeys (Macaca mulatta) after Possible Indirect Contact with a Human TB Patient. *J Comp Pathol*. 2015;153(2-3):81-91.
6. Rosenberg AE. Bones, Joints, and Soft Tissue Tumors. In: Kumar V, Abbas AK, Aster JC eds. *Robbins Basic Pathology 9th Ed.* Philadelphia, PA. Elsevier Saunders; 2013:774.

CASE II: 14-699 (JPC 4074325)

Signalment:
3 y/o, Female Entire, German shepherd, *Canis lupus familiaris* (canine)

History:
This dog presented with a left hindlimb lameness, and a three week history of hesitancy to jump following a successful mating, and began a course of amoxicillin/clavulanic acid antibiotics and tramadol. Assessment at a referral practice the following day showed no evidence of hindlimb lameness, instead pain in the L2-L3 region, which was confirmed radiographically as a benign lucency, considered most likely an old discospondylitis lesion or Schmorl's node. *Aspergillus* antibody titers performed at the time were negative. In the subsequent two weeks the dog became anorexic, developed marked pain in both the lumbar and cervical spine which was refractory to multimodal analgesia, and was unable to bear weight on its forelimbs. The dog was euthanized and submitted for a complete post mortem examination.

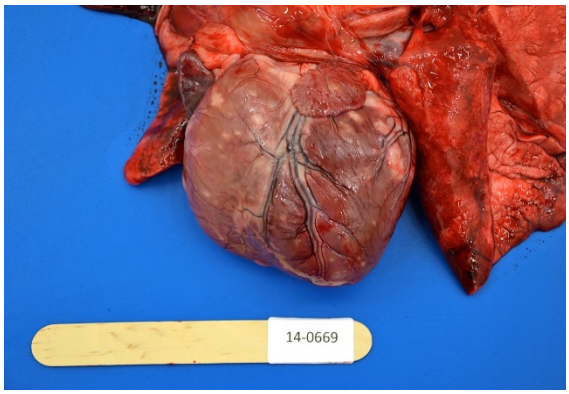

Figure 2-1. Heart, dog: Multiple foci of granulomatous inflammation are scattered throughout the myocardium. (*Photo courtesy of:* The University of Adelaide, Roseworthy, South Australia. https://www.adelaide.edu.au/vetsci/)

Gross Pathology:

A three-year-old female entire German shepherd dog in fair body condition was received for necropsy examination. The major finding at post mortem was pyogranulomatous discospondylitis and osteomyelitis at intervertebral space C5-C6 with bony destruction evident in the adjacent C5 and C6 vertebral bodies. Cytological examination of exudate from the C5-C6 disc space revealed neutrophilic and histiocytic inflammation admixed with numerous fungal hyphae. Disseminated granulomatous inflammatory foci were also identified in the myocardium and throughout the cortices of the left and right kidneys. There was multicentric subcutaneous, mediastinal, tracheobronchial and mesenteric lymphadenomegaly, and follicular lymphoid hyperplasia in the spleen. The uterus was gravid and four fetuses estimated at 5 weeks gestation age were present.

Laboratory Results:

Fungal organisms identified as *Paecilomyces variotii* were isolated from the vertebral disc lesion at C5-C6. Bacterial cultures from renal and vertebral lesions revealed no growth. Urinalysis (cystocentesis at necropsy) showed sub-optimal urine concentration (USG – 1.015), a pH of 6, and was otherwise unremarkable. There were no significant findings on sediment examination.

Histopathologic Description:

C5 and C6 vertebral bodies, intervertebral disc and spinal canal and cord (following decalcification): There is fragmentation and fibrillation of the intervertebral disc body, with expansion and effacement of the disc, marrow of adjacent vertebral bodies, and above subdural space by dense infiltrates of degenerate and non-degenerate neutrophils, macrophages, lesser lymphocytes and plasma cells, hemorrhage and fibrin. There is focally extensive loss of cellular detail and nuclear loss in trabecular bone (necrosis) and increased osteoclastic activity along the scalloped margins of thinned vertebral trabeculae in regions adjacent to inflammation (osteoclastic resorption). Refractile pale yellow-brown-walled fungal hyphae are present admixed with dense inflammatory infiltrates, and PAS stain highlights extracellular and phagocytosed fragments of fungal hyphae. Hyphae are septate with non-parallel walls and occasionally show dichotomous branching and terminal budding structures (chlamydospores).

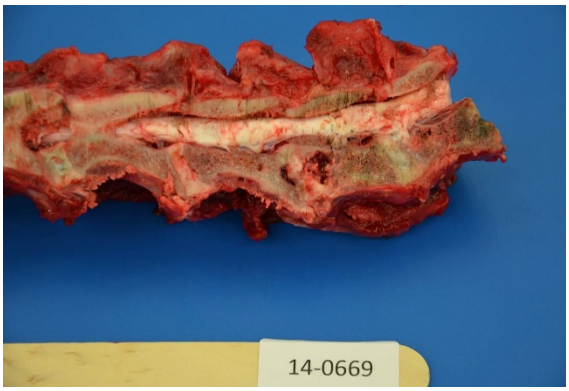

Figure 2-2. Cervical spinal column with cord: There is discospondylitis and osteomyelitis affecting C5 and C6 with compression of the overlying spinal cord. (*Photo courtesy of:* The University of Adelaide, Roseworthy, South Australia. https://www.adelaide.edu.au/vetsci/)

Microscopic examination of additional tissues: Granulomatous or pyogranulomatous inflammation with intralesional fungal organisms were present in the myocardium, kidneys, spleen, mediastinal, mesenteric and subcutaneous lymph nodes, myocardium, adrenal glands, pancreas and liver.

Contributor's Morphologic Diagnoses:
C5 and C6 vertebrae and intervertebral disc space: Pyogranulomatous and fibrinous discospondylitis, osteomyelitis, osteolysis and meningitis with intralesional fungal hyphae

Contributor's Comment:
Discospondylitis and osteomyelitis with concurrent disseminated systemic fungal infection was the cause of illness in this pregnant female dog, and organisms isolated from the C5-C6 intervertebral disc were identified as *Paecilomyces variotii*. Disseminated fungal disease is not an uncommon finding in German shepherd dogs (GSDs), although bacterial causes of discospondylitis are far more common across all breeds, particularly *Staphylococcus aureus* and *S. pseudintermedius*.[2] Paecilomycosis is also an uncommon cause of canine systemic

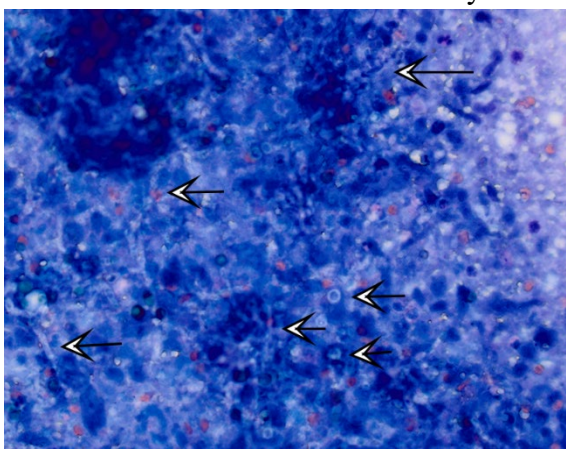

Figure 2-3. Cervical spinal column with cord: Cytological examination of exudate from the C5-C6 disc space revealed neutrophilic and histiocytic inflammation admixed with cross-sections of fungal hyphae (*Photo courtesy of:* The University of Adelaide, Roseworthy, South Australia. https://www.adelaide.edu.au/vetsci/)

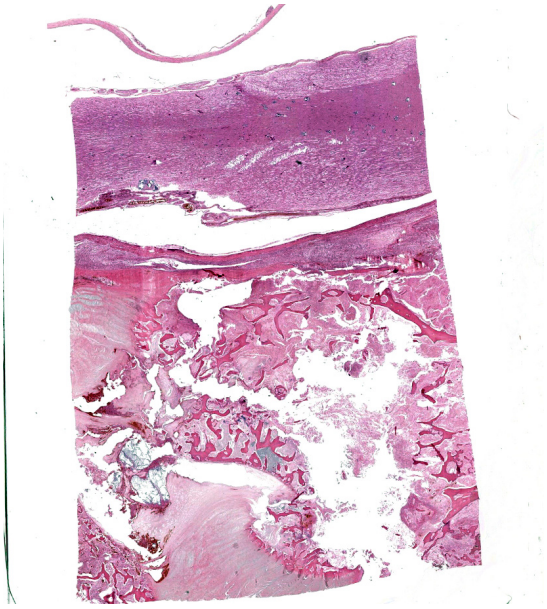

Figure 2-4. Cervical spinal column, C5-C6: One section at the junction of C5-C6 is submitted for examination. Spinal cord is at top. There is marked alteration of the vertebral endplates with loss of cortical bone of the ventral vertebral body, and there are numerous thin trabeculae of woven bone in the area of the cortex and medulla. The intervertebral disk is fragmented. The bone marrow is replaced by a dense cellular infiltrate. (HE, 5X))

mycosis, with the main fungal agent being *Aspergillus terreus*, and less commonly *A. deflectus, A. flavus, A. flavipes,* and other genera such as *Acremonium, Penicillium* and *Chryso-sporium*.[8] *Paecilomyces* is one of several causes of hyalohyphomycosis, a term referring to local or disseminated granulomatous disease caused by opportunistic, non-pigmented, hyphal fungal organisms.[9] Although rarely pathogenic, *Paecilomyces* spp. are an important albeit infrequent cause of morbidity in dogs, reptiles and humans, and have also been noted in cats, horses and rodents.[1,6,7,14,15] The species *P. variotii* has been isolated from canine, equine and rodent patients.[7,12] *Paecilomyces* spp. are common envi-ronmental filamentous saprophytic fungi found airborne, and in soil, vegetative material, dust and food products worldwide, as well as being part of the normal microflora of canine hair.[1,2,6,7,9,12,14,15] Consequently interpretation of positive cultures is

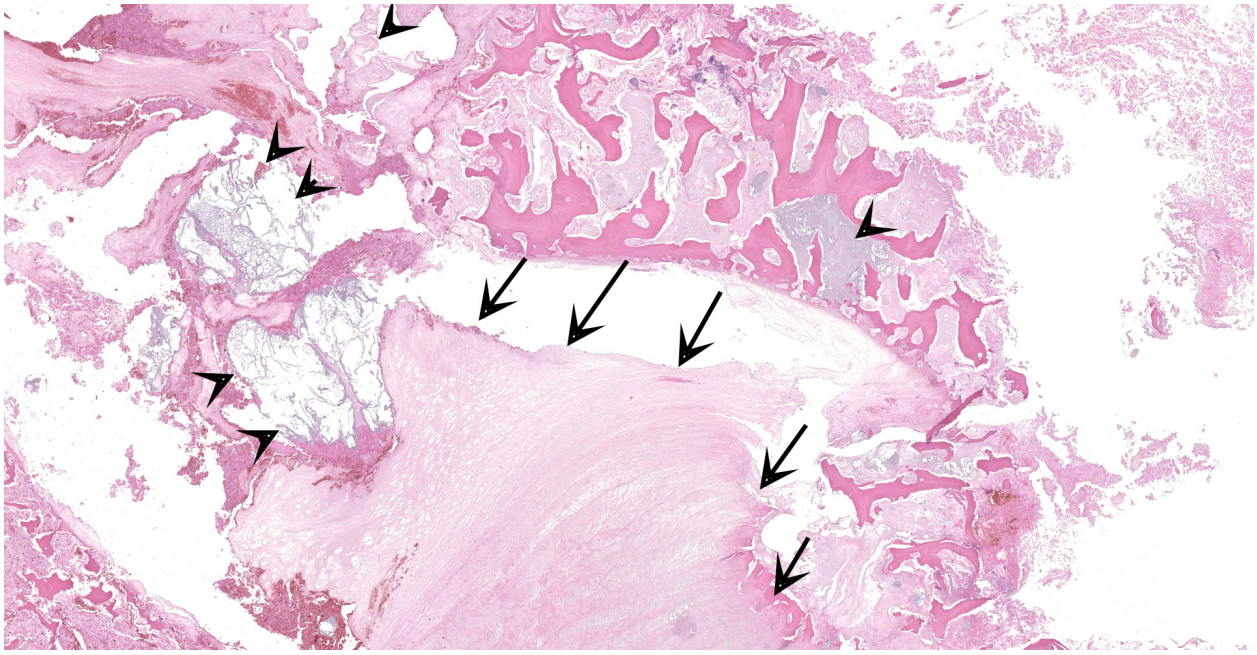

Figure 2-5. Cervical spinal column, C5-C6: There is fragmentation of the intervertebral disc with intermingled fragments of basophilic nucleus pulposus (arrows), eosinophilic annulus fibrosis, (arrowheads) and fragments of bone. (HE, 18X)

somewhat subjective given the possibility of contaminants, which may contribute to *Paecilomyces* being over looked as an aetiological agent.[2,6,7] In addition culture is typically poorly sensitive, possibly because of poor tissue concentrations of fungus in accessible areas.[7]

Paecilomyces spp. are anamorphic ascomycete molds, and are close relatives of *Penicillium* spp.[7] Their mycelia have yellow-brown, broad, branching, septate hyphae, leading into smooth walled conidiophores bearing wide-based, long necked phialides which often bend away from the main axis, and themselves bear ellipsoidal smooth-walled conidia.[6,11] Chlamydospores, the result of asexual or less commonly sexual reproduction, may also be present either singularly or in short chains, and are brown, roughened, globose and thick-walled.[11] Their morphology may be confused with *Aspergillus* spp. and *Candida* spp., hence fungal culture is always recommended.[14] The *Paecilomyces* genus is comprised of 15 different species including *P. variotii* (several spelling variations exist in literature), *viridis, tenuipes, sinensis, pericinus, marquandii, lilacinus, javanicus, fumosoroseus, flavinosus, carneus, canescens* and *aerugineus*. The majority of these species have been isolated from human and veterinary patients, most frequently *P. variotii* and *P. lilacinus.*[9] Speciation of *Paecilomyces* is important in clinical cases because it carries therapeutic implications, with *P. variotii* susceptible to most common antifungals, and *P. lilacinus* highly resistant.[2]

Clinical paecilomycosis ranges from mild to severe localized infections such as keratitis, endocarditis, sinusitis, nephritis and pneumonia, to disseminated infections with fungemia.[7] Granulomatous or pyogranulomatous nephritis, myocarditis, splenitis, lymphadenitis, pancreatitis, adrenalitis and hepatitis noted in this case is consistent with hematogenous seeding of a systemic fungal infection. The most common presentation of paecilomycosis in dogs is discospondylitis with or without dissemination.[6,7]

Foley *et al.* (2002) conducted a review of paecilomycosis in dogs at the University of California Davis Veterinary Medical Teaching Hospital between 1980 and 2000 (10 dogs) as well as case reports in veterinary medical literature (9 dogs): German shepherd dogs (GSDs) (8/19) and females (16/19) were over represented. The exact mechanisms for these gender and breed predispositions are unknown, although in general both female dogs and GSDs have increased rates of fungal diseases.[2,6,7,14,15] Proposed mechanisms in GSDs include depressed local cellular responses, and IgA dysregulation resulting in decreased mucosal immunity.[15] The role of immunomodulation due to pregnancy in this dog is uncertain; in humans, pregnancy is a reported risk factor for development of disseminated coccidiomycosis.[3,10,16]

Most canine patients in the 2002 review study had discospondylitis with (7/19) or without (6/19) disseminated fungal infection, with the remainder having disseminated or localized mycosis in other locations, including the liver, spleen, visceral or peripheral lymph nodes, and the kidneys.[6,7,9] The primary lesion of systemic paecilomycosis is usually not determined although authors in previous case reports have suspected cutaneous or mucosal wounds.[2,6,8] An unusual presentation was documented in a 2008 case report, in which a 4-year-old spayed female mixed-breed dog with disseminated *Paecilomyces variotii* developed generalized calcinosis cutis, a phenomenon only previously reported in three dogs with blastomycosis.[9]

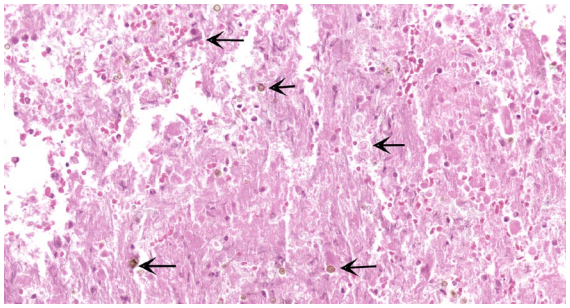

Figure 2-6. Cervical vertebra, C5-C6: Scattered throughout the eosinophilic (artifact of decalcification) inflammatory infiltrate, there are numerous cross sections of brown thick-walled fungal conidia (arrows).

Localized paecilomycosis in dogs has been reported in the skin, nasal cavity, inner ear, prostate and bladder.[7,9] A recent case report described an unusual presentation of localized paecilomycosis; a six-year-old entire female GSD with bilateral obstructive pyelonephritis caused by *Paecilomyces* bezoars, without concurrent disseminated disease.[15] In this case fungal infection was first identified using a pyelocentesis sample for urine sediment examination, and confirmed with urine culture. In a similar case of paecilomycosis in a 3-year-old castrated male GSD, also involving both kidneys, a cystocentesis sample was similarly use to reach a definitive diagnosis.[6] Interestingly in some cases, such as the case presented here, urine sediment and urine culture results are negative, despite the presence of fungal organisms in both kidneys.[2]

Patients with paecilomycosis sometimes have concurrent, possibly predisposing conditions, with examples in literature including immunosuppressive corticosteroid therapy, neoplasia, skin trauma, diarrhoea and otitis.[7,15] By contrast in man paecilomycosis is usually accompanied by an underlying immunosuppressive disorder, facilitating spread from localized areas of infections, which in humans are usually contaminated wounds or prosthesis implants.[1,9,14,15] Paecilomycosis associated with pneumonia, discospondylitis or disseminated infection carries a grave prognosis, with reported mortality rates ranging from 57-100%.[5,15]

Contributing Institution:
The University of Adelaide, Roseworthy, South Australia. https://www.adelaide.edu.au/vetsci/

JPC Diagnosis:
Cervical vertebral body and adjacent inter-vertebral disk: Discospondylitis, pyogranulomatous, diffuse, severe with numerous intra- and extracellular pigmented fungal conidia and hyphae.

JPC Comment:
The contributor provides an outstanding and thorough review of the host range, risk factors, pathogenesis, clinical signs, gross and histologic features, and comparative pathology associated with paecilomycosis.

Interestingly, the first published case of a disseminated canine mold infection (DCMI) was attributed to *Paecilomyces* spp. in a Weimaraner in 1971, despite the now known overrepresentation of German shepherd dogs (GSDs) and *Aspergillus* spp. The first DCMI case reported in a GSD was in 1978 and attributed to *Aspergillus terreus.*[5]

Similar to as reported in the contributor's comment, a 2018 review[5] of 112 cases of DCMI found GSDs to be predisposed, with the breed representing 67.8% of the cases. Furthermore, 89.7% of infections in this breed were attributed to *Aspergillus* section *Terrei*, which includes *A. terreus sensu stricto*, *A. carneus*, *A. niveus*, A. *alabamensis*, and A. *terreus* var. *aureus*. These species are morphologically identical and require molecular techniques for speciation. Across all breeds, females are at increased risk for DCMI, while sterilization is not associated with significantly increased risk in either sex. A wide age range (1-13 years) was affected, with the average age of infection reported to be 4.3 years.[5]

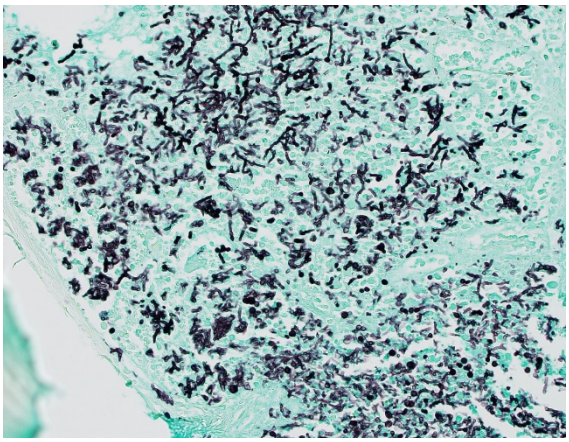

Figure 2-7. Cervical vertebrae, C5-C6: A Grocott's methenamine silver demonstrates the large numbers of hyphae within the inflammatory exudate. (GMS, 400X)

DCMI is thought to undergo dissemination primarily via the hematogeous route, which is facilitated by specialized structures produced by each organism. *Paecilomyces* spp. produce conidia in vivo, known as 'adventitious forms'. Similarly, *A. terreous,* as well as other fungi in sections *Flavipedes* and *Jani* produce structures known as 'accessory spores' or 'aleuriospores', which differ from phialidic conidia by growing directly on hyphae in both culture and infected tissue. It is believed these structures enter systemic circulation and subsequently arrest in capillary loops and other regions with reduced speed of blood flow, likely explaining why the kidneys and vertebrae are frequently affected in cases of DCMI.[5]

In addition to domestic and laboratory species, paecilomycosis has been identified in multiple wildlife species. Reptiles appear to be particularly susceptible, with confirmed infections reported in an American alligator (*Alligator mississipiensis*), an estuarine crocodile (*Crocodylus porosus*), a spectacled caiman (*Caiman crocodilus*), 2 Nile crocodiles (*Crocodylus niloticus*), 4 carpet chameleons (*Chameleo lateralis*), 2 Aldabra giant tortoises (*Geochelone gigantea*), and several green sea turtle (*Chelonia mydas*)

hatchlings. Proposed risk factors associated with infections in these species include stress of shipping with chameleons, skin trauma with crocodilians, and excessively cold water with green sea turtle infections. Reptiles are most commonly infected by *P. lilacinus.*[7]

Interestingly, *Paecilomyces* spp. have been identified as a source of numerous bioactive products, with 223 metabolites identified in one recent report[4], including highly toxic linear peptides known as leucinostatins, paeciloquinones (tyrosine kinase inhibitors), macrocyclics, and trichothecans. Therefore, there is tremendous potential associated with genus *Paecilomyces* in regard to the development of applications such as antimicrobials, antivirals, nematocidals, and free radical scavenging.[4] In addition to producing potentially useful compounds, some species having been found to have potentially useful applications in regard to agriculture, including both *P. farinosus* and *P. fumosoroseus.* Both of these species are known to infect insect and arachnid hosts, with the latter having been evaluated as a biological agent to suppress agricultural pests such as Russian wheat aphid and silver-leaf whiteflies.[7]

Although there is great potential in regard to the ongoing discovery of positive applications of *Paecilomyces,* the saprophytic fungus has also been implicated with food spoilage and other detrimental effects.[4] As an example, *P. varioti* thrives in substrates such as aviation fuel, while also producing corrosive acid metabolites and clogging filters with its mycelia. In addition, other species are known to contaminate laboratories, allegedly sterile solutions, and chlorinated drinking water.[7]

Participants did not observe an inflammatory process within the meninges within the section presented and therefore did not favor inclusion of 'meningitis' in the morphologic diagnosis. However, it is possible this lesion is represented in other sections due to slide variability.

Additional discussion centered on the use of the terms 'yeast', 'spore', and 'conidia' in regard to fungal propagules. Yeasts are a single celled form of fungi that produce by budding. Dimorphic fungi grow as yeast (or spherules) at 37 ºC and as molds (multicellular hyphae) at 25 ºC. Spores can be produced either asexually or sexually. Asexual spores are always formed in a sporangium and undergo cytoplasmic cleavage following mitoses. Sexual spores undergo meiosis and are associated with several unique structures such as basidiospores, zygospores, and ascospores. Conidia are similar to asexual spores in that they are always asexual in origin but never develop in a manner that involves cytoplasmic cleavage.[13]

Finally, several fungi (as in this case) have melanin in the cell wall of conidia, hyphae, or both. In such cases, the fungi are considered to be dermatiaceous.[13]

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CASE III: P963-20 (JPC 4168140)

Signalment:
4 ½ - 5 months old, female, Holstein veal calf (*Bos taurus*)

History:
Several milk-fed calves, in a herd of 800, developed multiple fractures at around 140 days of age. These calves were suddenly unable to get up or stand following a benign fall or after struggling to escape physical restraint for routine procedure. These calves had no prior locomotor problem and were in excellent body condition. They were fed a commercial feed supplemented in minerals and vitamins.

Gross Pathology:
The necropsy of two calves from the affected group revealed multiple fractures, varying from an individual to another, involving the appendicular long bones, ribs, vertebrae and/or pelvis. Some fractures appeared old (bone callus on the ribs) but others were fresh and associated with severe hemorrhage in the surrounding soft tissues. The bones were firm, not rubbery, but were easier to cut than normal. The growth plate of the long bones was abnormally thin and the cancellous bone appeared less dense than expected. Notably,

the decalcification process of long bones to make histological sections was significantly reduced and easier than normal. The kidneys of both animals were normal grossly and their parathyroids were not enlarged. Veal calf No 2 had soft feces.

Laboratory Results:
Bones from both calves were sent for analysis. Bone ash (mineral content of bone) and density were significantly decreased in Calf No 1 (bone ash 24% (normal 59-60%), density 1.13 g/ml (normal 1.29-1.35)). Bone ash was mildly decreased in Calf No 2 (53%) but density was normal. Calcium was mildly increased in both animals (39% and 39.4%; normal 37%) and phosphorus was minimally decreased (17.1% and 17%; normal 18.5%). Serum copper was evaluated in Calf No 2 and appeared decreased (10.0 µmol/L; normal 12-19 µmol/L). In the same animal, RT-PCR for Rotavirus type A was mildly positive (CT 30.07) and coprology revealed no intestinal parasites.

Microscopic Description:
The examination of long bones revealed a decrease in thickness of the growth plate with

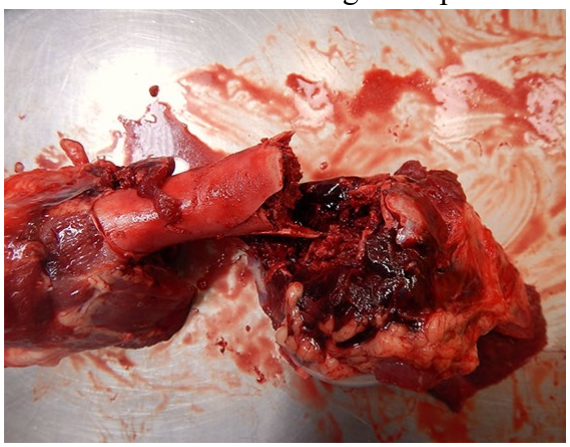

Figure 3-1. Humerus, veal calf 1: There is a transverse fracture of the right humerus. (*Photo courtesy of :* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/ centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

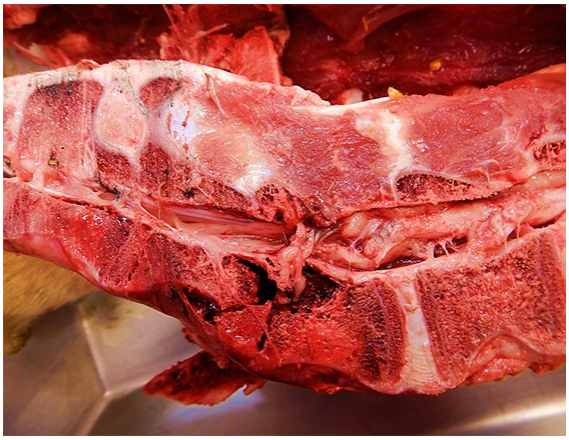

Figure 3-2. Sacrum, veal calf 1: There are multiple fractures in the vertebral body of S1. (*Photo courtesy of :* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/ centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

variable narrowing of the hypertrophic zone and a multifocal loss of the *secondary spongiosa* and sometimes even of the *primary spongiosa*. Numerous osteoclasts were located between the chondroid trabeculae. Osteoblasts were hypertrophied and hyperplastic and lined the osteoid trabeculae of the metaphysis. There was a marked reduction of bone trabeculae abundance in the epiphysis and metaphysis but those still present had a normal thickness and appeared normally ossified. A line of fibrosis, parallel to the growth plate, was observed in the metaphysis and was associated with an older incomplete fracture and bone remodeling.

Other tissues showed no significant lesions. The intestinal mucosa was not inflamed and crypts were not hypertrophied. However, the apex of the intestinal villi could not be evaluated due to post-mortem desquamation of epithelial cells.

Contributor's Morphologic Diagnoses:
Femur, Calf No 1: Osteoporosis with incomplete/transverse metaphyseal fracture with fibrosis

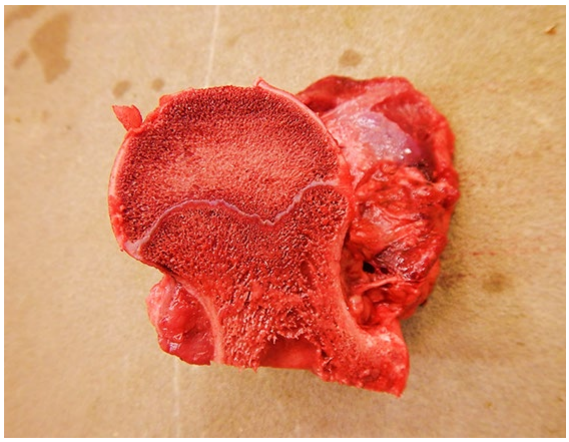

Figure 3-3. Femur, veal calf 2. There is a thin growth plate of the femoral head and reduced density of epiphyseal and metaphyseal cancellous bone. (*Photo courtesy of :* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

Contributor's Comment:

Osteoporosis is characterized by loss of bone density with normal mineralization: the number of bone trabeculae is reduced but those that remain have a normal structure. However, this reduction in density decreases bone strength and affected animals are prone to pathological fractures, as was the case with those veal calves that developed such lesions without any significant trauma or excessive strength used for restraining. Osteoporosis should be differentiated from rachitism and osteomalacia, in which the osseous matrix is normal but its mineralization is deficient, and from fibrous osteodystrophy, characterized by excessive bone resorption, proliferation of fibrous tissue and formation of immature and poorly mineralized bone.[6]

Observed in humans and domestic/farm animals, osteoporosis has also been described in wild ruminants such as moose (*Alces alces*) in Sweden, similar to the condition in cattle/sheep with a secondary copper (Cu) deficiency and/or molybdenosis, and in a Mountain Sheep (*Ovis canadensis*) and another moose both with osteoporotic skull anomalies.[3,6,10,11] Osteoporosis is also frequently observed in commercial high-producing laying hens.[20]

The major causes of osteoporosis are advanced age (senile osteoporosis), inactivity (disuse osteoporosis), corticosteroid treatment and nutrition. Other potential causes to consider include vitamin A toxicity, hyperthyroidism, chronic exposure to cadmium and the use of some immunosuppressive or anticonvulsant agents.[6] In this particular case, all causes were not considered relevant with the exception of a potential nutritional problem, which was deemed the most likely etiology. Such a nutritional problem could be primary (unbalanced diet, starvation) or secondary (intestinal malabsorption/inflammatory bowel disease). Although in humans chronic pancreatitis has been linked to osteoporosis and increased risk of bone fractures, no abnormalities were noted in bone pliability/fragility in an 8-y-old lactating Holstein cow in spite of pancreatolithiasis, pancreas atrophy/fibrosis and a fractured lumbar vertebrae.[14] In a case series of ten 2-y-old dairy heifers in first-lactation from New Zealand, humeral fractures were

Figure 3-4. Femur, veal calf 2. There are multiple fractures of the left femur. (*Photo courtesy of :* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

associated with osteoporosis, possibly linked to insufficient bone deposition during growth following protein-caloric malnutrition.[7] A short communication details osteoporosis in a group of 15 goats from Switzerland in which the cause was a combined calcium/ phosphorus and vitamin D deficiency as well as malnutrition attributed to gastrointestinal parasitism.[5]

Veal calves from our case were in excellent body condition so their diet seemed sufficient to fill their energy requirements. Even if the second calf had soft feces and a mildly positive RT-PCR for rotavirus type A, these two animals showed no significant inflammation and/or atrophy of the intestinal villi and coprology revealed no intestinal parasitism. All other tissues were unremarkable grossly and microscopically. As such, a dietary imbalance was considered the most likely hypothesis in the present case. Calcium, phosphorus and copper deficiency could cause osteoporosis. In ruminants, calcium deficiency is often associated with this type of lesion, even if in other species this imbalance mostly results in fibrous osteodystrophy.[6] Parathyroid activity and size increase when the serum calcium is low, leading to bone resorption; however, in the present case, parathyroids appeared normal

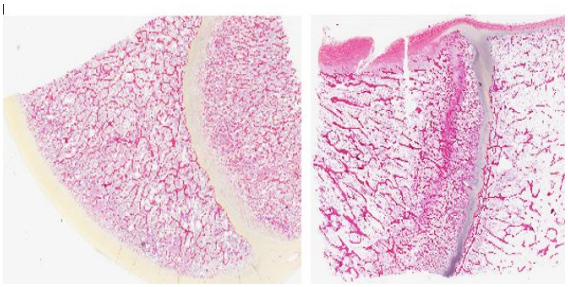

Figure 3-5. Femur, veal calf 1. There is reduced density of bony trabeculae, which appear otherwise morphologically normal with a narrowed growth plate. Age/breed matched control on left. (HE,1X) (*Photo courtesy of* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

grossly and calcium levels were mildly elevated in the bone ash of our two animals (39% and 39.4%, respectively).

Apart from this elevated calcium level and a reduced bone density, bone ash analysis demonstrated a reduction in phosphorus level in the mineral matrix (17% and 17.1% in animals Nos 1 and 2, respectively). According to Puls, phosphorus bone level below 17.6% could be considered critical for the health of an animal.[18] However, bone ash analysis must be interpreted with caution as the reference intervals for calcium and phosphorus are not necessarily reliable and because there would be no significant changes in most osteoporosis cases due to the stable calcium: phosphorus ratio in hydroxyapatite crystals that form the mineral matrix. But if we still consider this decreased phosphorus level as significant, it is noteworthy that chronic phosphorus deficiency usually induces rickets or osteomalacia rather than osteoporosis. Current scientific literature is not clear on the factors that could trigger bone to develop osteoporosis instead of rickets when phosphorus levels are low.[6] Interactions with other minerals could explain these differences. Copper, molybdenum and manganese have all been reported to be involved in some cases of osteoporosis. Bone copper was not assessed in the present cases but serum copper was evaluated in the second animal and was slightly decreased. Since we have very few data to determine the normal serum copper values in bovine and because this result reflects the copper level at one specific time point in a process that is most likely subacute to chronic, this mild serum copper decrease should be interpreted with caution. Unfortunately, we had no follow up from the referring veterinarian concerning this herd problem and its origin remains obscure.

Osteoporosis in humans is a chronic systemic bone disease of growing relevance due to the on-going demographic change in our aging societies. As defined by the world Health Organization (WHO), osteoporosis corresponds to a bone mineral density (BMD) T-score less than -2.5 SD as measured by dual-emission x-ray absorptiometry (DXA), commonly affecting 30% of women and 12% of men at some point during life.[1] The annual costs in the US of caring for osteoporotic-related fractures parallel or exceed the annual costs of caring for myocardial infarction, breast cancer and/or cerebrovascular accident.[15] In humans, risk factors associated with osteoporotic fractures include low peak bone mass, hormonal factors, use of certain drugs, cigarette smoking, low physical activity, low calcium/ vitamin D intake, race, small body size and a personal/family history of fractures.[5,8]

As in humans, glucocorticoid-induced postmenopausal/ovariectomy-related osteoporosis in lab animals is a significant form of osteoporosis, with higher risk of osteoporotic fractures.[21] Many different animal species have been used as models of that bone disorder for research. Although rodents are attractive to that effect because of the possibility of specifically modifying their genetic background, some aspect of that research can only be addressed with large animal models, such as metaphyseal fracture healing. Among large animal species, sheep have proven invaluable for osteoporosis research in this context.[17] Different ewe models for osteoporosis have been successfully established and are very important for orthopedic research, such as the lumbar spine vertebral compression fracture in a sheep osteoporosis model.[9,16] In the context of osteoimmunology, TNF-α and IL-6 are considered potential candidates for clinical application in spite of contradictory roles in immune responses versus bone metabolism.[19]

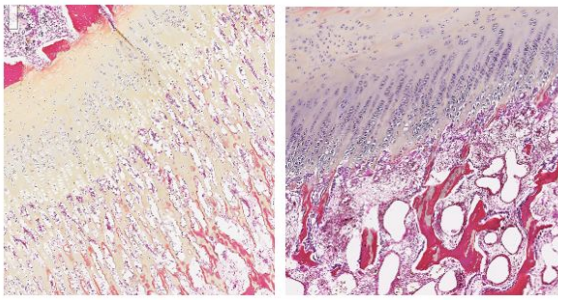

Figure 3-6. Femur, veal calf 1. There is multifocally reduced to absent primary spongiosa. Age/breed matched control on left. (HE, 5X)(*Photo courtesy of:* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/ centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

Another interesting research area involves an osteoporosis model using the red deer (*Cervus elaphus*) stag, in which physiological osteoporosis is a consequence of the annual antler cycle. This phenomenon could allow the identification of genes involved in the regulation of bone mineral density using comparative genomics between human and deer.[4]

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JPC Diagnosis:
Long bone with growth plate: Osteopenia, diffuse, marked with metaphyseal infraction and fibrotic endosteal callus.

JPC Comment:
The contributor provides an excellent review of multiple underlying pathogeneses associated with the development of

osteoporosis, a condition associated with increased bone fragility. Osteoporosis occurs as a result of excessive bone resorption in comparison to deposition, with a reduced quantity of bone while normal bone quality is retained.

As noted by the contributor, osteoporosis commonly affects postmenopausal women as well as older men, posing a significant risk factor for the development of fragility fractures, which most commonly involve the spine and metaphyseal bone in the hips and wrist. Furthermore, the features of osteoporosis may inherently complicate stabilization efforts (e.g. surgical fixation) necessary for optimal bone apposition, leading to unfavorable mechanical conditions and prolonged healing.[2]

Historically, rodent models have predominantly been used in ovariectomy-induced osteoporosis fracture studies, as well as to identify genes involved in fracture healing of osteoporotic bones. Although these species are ideal for use in these types of studies, their small size makes them less than ideal animal models for other related studies, such as those assessing orthopedic implants or in depth analysis of metaphyseal fracture healing. As noted by the contributor, sheep have been utilized as an animal model for these types of studies, with experimental induction traditionally via ovariectomy and/or glucocorticoid administration. Compared to ovariectomy, which only causes marginal osteoporosis in sheep, significant bone loss is induced by glucorticoid administration. Unfortunately, the dosage of glucocorticoids required to induce osteoporosis raises animal welfare concerns due to undesirable side effects. An alternative method, surgical disconnection of the pituitary gland from the hypothalamus, has been found to induce osteoporosis in sheep, with considerable bone loss after 8 months. Disruption of the hypothalamo-pituitary axis inhibits leptin signaling, which plays a key role in bone regulation, while also altering levels of other hormones that regulate bone homeostasis such as gonadotropins, thyroid hormones, insulin-like growth factor 1, and cortisol. The only clinically symptoms reported to be associated with hypothalamic-pituitary disconnection in sheep are polydipsia and polyuria as a result of iatrogenic diabetes insipidus.[2]

The most common pharmaceutical class used to treat for osteoporosis in humans are bisphosphonates, which have been in clinical use since 1968. These inorganic pyrophosphate analogs have a high affinity for calcium and accumulate in the skeleton, becoming embedded in newly formed bone during the anabolic phase of remodeling. Following their deposition, these substances remain inert until they're released in the acidic Howship's lacuna and absorbed by osteoclasts. Following their absorption, bisphosphonates induce osteoclast apoptosis,

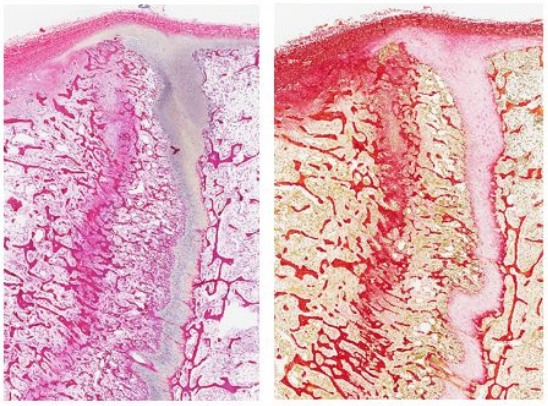

Figure 3-7. Femur, veal calf 1. There is a linear area of intramedullary fibrosis parallel to the physis and with haphazardly arranged bone trabeculae (remodeling). A picro Sirius red stain highlights the area of intramedullary fibrosis. (HE, PSR, 1X) (*Photo courtesy of :* Centre de Diagnostic Vétérinaire de l'Université de Montréal, Faculté de médecine vétérinaire, https://fmv.umontreal.ca/services/centre-de-diagnostic-veterinaire-de-luniversite-de-montreal/)

resulting in an overall suppression bone resorption.[12]

Bisphosphonates have a long half-life (up to 10 years) due to their incorporation into bone. This is significant in that bone turnover can continue to be suppressed long after administration is discontinued. While this feature is beneficial in regard the treatment of osteoporosis, it may also associated with impairment of fracture repair. Although these medications do not appear to be detrimental to initial callus formation following a fracture, studies in multiple species have found they hinder the callus' remodeling due to the same mechanisms as previously described, resulting in delayed the conversion of woven bone to mature lamellar bone. Therefore a history of bisphosphonate administration may be considered a risk factor for delayed union. However, human studies have also found de novo bisphosphonate administration prescribed as the result of an osteoporosis related fracture is not associated with delayed healing in human patients, likely due to insufficient accumulation of the bisphosphonates in the skeleton. Furthermore, other studies have paradoxically found bisphosphonates may also accelerate fracture healing, such in rabbits with mandibular fractures treated with zoledronic acid.[12]

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CASE IV: L20 163 (JPC 4152938)

Signalment:
7-year-old, spayed female, Labrador retriever, domestic dog (*Canis lupus familiaris*)

History:
The dog had a history of anorexia, progressive weakness, and lameness of undetermined duration. On presentation to the Louisiana State University Veterinary Teaching Hospital, the dog was depressed but responsive, with tachypnea and tachycardia. On thoracic radiographs, a lytic lesion at the dorsal border of the right scapula was noted as well as a right caudodorsal lung mass/consolidation. Abdominal ultrasound showed numerous targetoid lesions in the spleen as well as a right caudodorsal lung mass. Due to the poor prognosis, humane euthanasia was elected.

Gross Pathology:
The dorsocranial aspect of the right scapula was expanded by a 6.0 x 7.5 x 0.3 cm mass that extended to the dorsocaudal aspect of the bone. On the cut surface, the affected scapula was replaced by an infiltrative, ill-defined, soft and tan mass that filled the adjacent marrow spaces and was delimited by abundant, superficial cortical bone/ periosteum. The right femur had the proximal aspect of the metaphysis adjacent to the femoral neck expanded by a focal, ill-defined area of periosteal thickening measuring

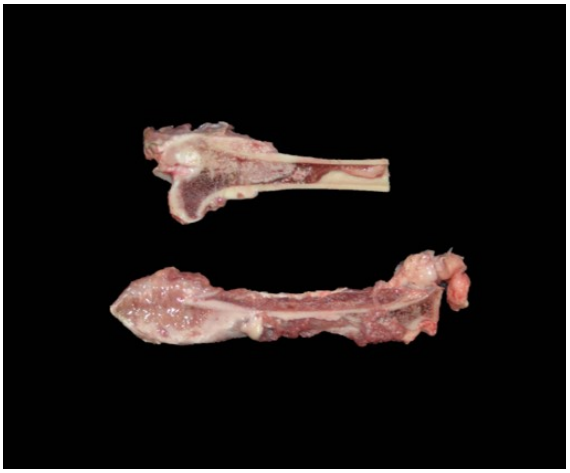

Figure 4-1. Scapula, dog. Scapular bone is replaced by an infiltrative neoplasm. There is marked periosteal bone proliferation. (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

approximately 2.0 x 1.0 x 0.4 cm; on the cut surface, the intralesional marrow spaces were filled with soft and tan tissue similar to that noted in the right scapula. The parenchyma of the right caudal lung lobe was extensively effaced by a well-demarcated, multilobular, firm and tan mass that measured 8.0 x 7.5 x 6.0 cm and slightly bulged on the cut surface. In the heart, the endocardial surface of the interventricular septum had a focal, white, and slightly firm nodule that measured 0.5 x 0.3 x 0.4 cm and protruded into the left ventricle. In the spleen, multiple round, soft, mottled tan to dark red and variably sized nodules (ranging in size from 0.2 x 0.2 x 0.1 cm to 1.5 x 1.5 x 1.5 cm) were noted throughout both the visceral and parietal surfaces.

Laboratory Results:

- Complete Blood Count:
  - Mean corpuscular value (MCV): 59.9 fL (62-77 fL)
  - Red cell distribution width (RDW): 17% (12-14%)
  - White blood cell count (WBC): 23.3 x 10^3/µl (8-14.5 x 10^3/µl)

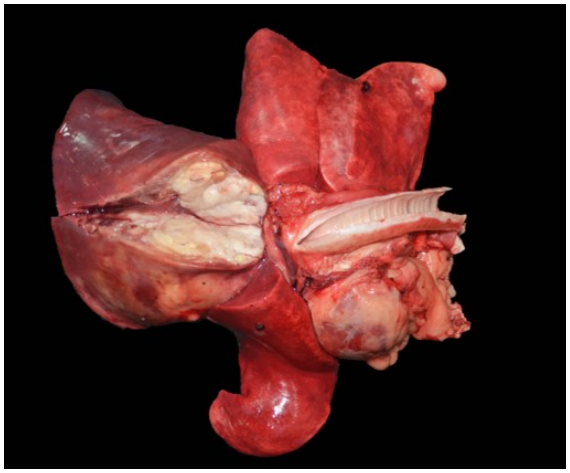

Figure 4-2. Lung, dog. A similar neoplasm is present in the femur. (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

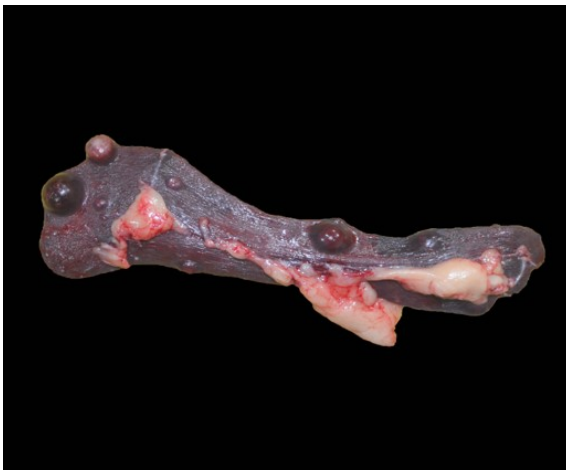

Figure 4-3. Spleen, dog. Multiple neoplastic nodules are present in the spleen and visible from the visceral and parietal surface. (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index .php)

  - Segmented neutrophils (absolute value): 18.8 x 10^3/µl (3-11.5 x 10^3/µl)
  - Lymphocytes (absolute value): 0.70 x 10^3/µl (1-4.8 x 10^3/µl)
  - Monocytes (absolute value): 3.7 x 10^3/µl (0.1-1.4 x 10^3/µl)

No significant abnormalities were identified in the biochemical profile.

Cytology:
Scapula: Four smears with overall low cellularity were examined. Rare individualized, rounded to elongated multinucleated cells with abundant basophilic cytoplasm containing few small, discrete vacuoles and round to ovoid nucleus (10-12 µm in diameter) with finely stippled chromatin and a single small, prominent nucleoli were noted. No infectious agents were seen.

Spleen and lung: Multiple smears from each location were evaluated. Round to stellate neoplastic cells with variably sized nuclei, small discrete intracytoplasmic vacuoles and occasional erythrophagocytosis were ob-

served along with numerous binucleated to multinucleated cells.

Microscopic Description:
Bone (right scapula): The normal architecture of the bone is extensively effaced by a non-encapsulated, infiltrative, and densely cellular neoplasm composed of sheets of neoplastic round to spindle cells supported by scant pre-existing stroma. Neoplastic cells are pleomorphic, are often large, and have distinct cell borders, with moderate to abundant eosinophilic cytoplasm, and a round to oval central nucleus with coarsely stippled chromatin and one to two prominent nucleoli. Anisocytosis and anisokaryosis are severe, with frequent giant and multi-nucleated neoplastic cells with bizarre nuclei. Nine atypical mitotic figures are counted in 10 high power fields. Occasionally, neoplastic cells contain intracytoplasmic erythrocytes (erythro-phagocytosis) and intracytoplasmic hemo-siderin pigment. At the periphery, the surface of the bone is irregularly expanded by proliferation of new trabecular bone, multifocal islands of partially ossified hyaline cartilage, marrow spaces filled with either neoplastic round cells or loose fibrous connective tissue, and markedly thickened periosteum composed of numerous proliferating osteoblasts and fibroblasts. The peripheral skeletal muscle is composed of multifocal shrunken myofibers (atrophy).

Histologic changes in other tissues (not submitted):
The splenic parenchyma is multifocally expanded by variably sized neoplastic nodules composed of similar neoplastic cells as described in the scapula, with frequent bizarre nuclei and occasional erythro-phagocytosis. Similarly affected tissues include the lungs, heart, adrenal glands, pancreas, and proximal right femur.

Immunohistochemistry:
Anti-Iba-1 and anti-CD11d-specific immunohistochemistry were performed on sections of spleen. Neoplastic cells had strong cytoplasmic immunostaining for Iba-1 but did not have any immunostaining for CD11d.

Contributor's Morphologic Diagnoses:
Bone (right scapula): Histiocytic sarcoma, disseminated (based on multiple tissues examined).

Contributor's Comment:
Canine proliferative histiocytic disorders include benign (histiocytoma) and malignant (e.g. histiocytic sarcoma [HS] complex) neoplastic as well as nonneoplastic diseases (e.g. cutaneous and systemic [reactive] histiocytosis). Among the entities grouped within the histiocytic sarcoma complex, the localized and disseminated forms are proliferative disorders originating from interstitial dendritic cells, while the

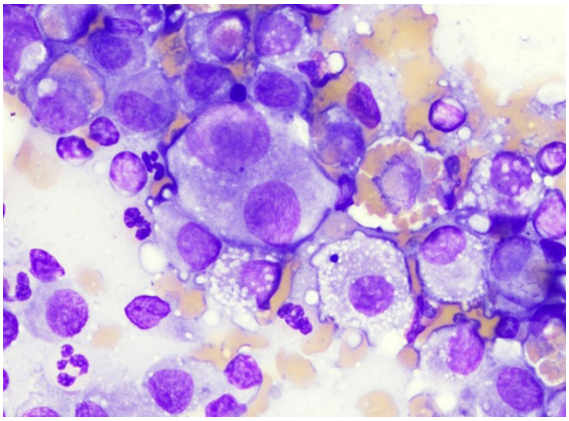

Figure 4-4. Spleen, dog. Round to stellate neoplastic cells with variably sized nuclei, small discrete intracytoplasmic vacuoles and occasional erythrophagocytosis were observed along with numerous binucleated to multinucleated cells. (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

Disease	Cell of Origin	Immunophenotype
Histiocytoma	Langerhans cell	CD1a+, CD11c/CD18+, E-cadherin+, CD204-, Iba1+
Cutaneous Langerhans cell histiocytosis	Langerhans cell	CD1a+, CD11c/CD18+, E-cadherin+, CD204-, Iba1+
Histiocytic sarcoma	Interstitial dendritic cell	CD1a+, CD11c/CD18+, CD204+/-, Iba1+
Histiocytic sarcoma, hemophagocytic	Macrophage	CD1a+/-, CD11d/CD18+, CD204+, Iba1+
Cutaneous histiocytosis	Activated interstitial dendritic cell	CD1a+, CD4+, CD11c/CD18+, CD90+, CD204-, Iba1+
Systemic histiocytosis	Activated interstitial dendritic cell	CD1a+, CD4+, CD11c/CD18+, CD90+, Iba1+

Table 1. Immunophenotypic differentiation of canine histiocytic diseases (From Tumors of Domestic Animals [2016], Chapter 8, Canine and Feline Histiocytic Diseases by Peter F. Moore).

hemophagocytic histiocytic sarcoma is a distinctive entity arising from bone marrow-derived macrophages, mostly within the splenic red pulp or bone marrow.[1-3,6,9-13,15] Canine HS may initially present either as a localized, solitary tumor or as a disseminated, multicentric disease. Metastatic disease is frequently observed as the disease progresses.[2,9,10,15]

The clinical presentation in dogs affected by HS is highly variable, depending on the organ systems affected.[10,15] Clinical signs can therefore include anorexia, weight loss, lethargy, dyspnea, lameness, neurological signs, among others.

HS most commonly affects the spleen, lungs, bone marrow, lymph nodes, skin and subcutis, joints, and central nervous system. The masses are often destructive and composed of large round to spindle neoplastic cells with ovoid to reniform nuclei and abundant eosinophilic cytoplasm with marked atypia and variably sized nuclei with multinucleation and frequent atypical mitosis.[3,10] In this particular case, neoplastic cells exhibiting erythrophagocytosis were observed both cytologically and histologically, resembling a hemophagocytic HS, which was initially considered as the presumptive diagnosis; however, immunohistochemical characterization of neoplastic cells revealed lack of expression of CD11d, an integrin that has been shown to distinctively characterize this hemophagocytic form.[15] Hemophagocytic HS is reported in similar canine breeds as histiocytic sarcoma, namely Bernese mountain dogs, golden retrievers, Labrador retrievers, flat-coated retrievers, and Rottweilers. Clinicopathological abnormalities are characterized by rapidly progressive, regenerative hemolytic anemia and thrombocytopenia with negative Coombs test, hypoalbuminemia, and hypocholesterolemia (not present in this particular case).[10,15] Macroscopically, hemophagocytic HS does not form mass lesions within primary sites (spleen and bone marrow) as opposed to histiocytic sarcoma, but causes a diffuse enlargement of the spleen instead.[10]

Disseminated HS is often difficult to grossly differentiate from systemic reactive histiocytosis,[10,11] a systemic disorder primarily affecting Bernese Mountain dogs, characterized by lesions in the skin and internal organs (such as lung, liver, spleen, kidneys, and bone marrow). Histologically, reactive histiocytosis is characterized by vasocentric and often vasodestructive proliferation of large, mildly atypical histiocytes. Cells with bizarre or multiple nuclei are lacking, however, in contrast to HS.

Differential diagnoses for tumors with high nuclear atypia include the following:

- Anaplastic lymphoma
- High grade/Grade III mast cell tumor
- Poorly differentiated carcinoma
- Amelanotic melanoma
- Osteosarcoma
- Undifferentiated sarcomas

Immunohistochemistry using specific antibodies for monocyte/macrophage/ dendritic cell lineage, B and T cell lineage, and other cells (e.g. epithelial cells or melanocytes) is often imperative to reach a definitive diagnosis (Table 1).[9,10,15]

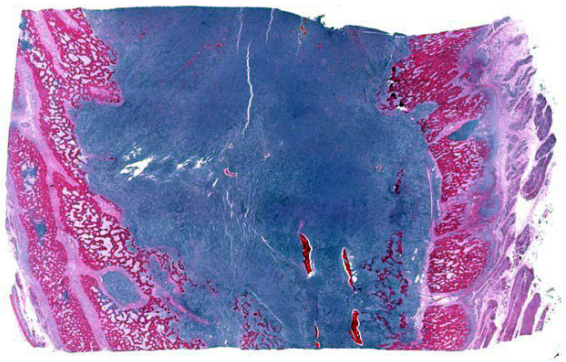

Figure 4-5. Scapula, dog. The normal architecture of the bone is extensively effaced by a non-encapsulated, infiltrative, and densely cellular neoplasm composed of sheets of neoplastic round to spindle cells supported by scant pre-existing stroma. (HE, 5X) (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

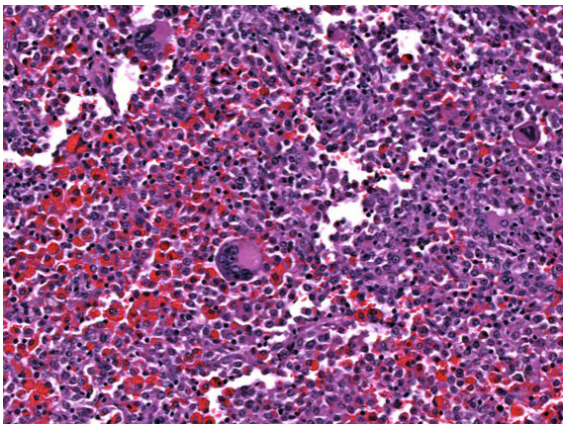

Figure 4-6. Scapula, dog. Scapula, dog. The normal architecture of the bone is extensively effaced by a non-encapsulated, infiltrative, and densely cellular neoplasm composed of sheets of neoplastic round to spindle cells supported by scant pre-existing stroma. (HE, 400X) (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

Although histiocytic sarcoma occurs mainly in dogs and cats, other animals such as cows, mice and some birds can develop this tumor as well; however, their occurrence is rare.[4,8,10,13]

Contributing Institution:
Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

JPC Diagnosis:
Bone: Histiocytic sarcoma.

JPC Comment:
The contributor provides an excellent overview of canine proliferative histiocytic disorders, a diverse complement of benign

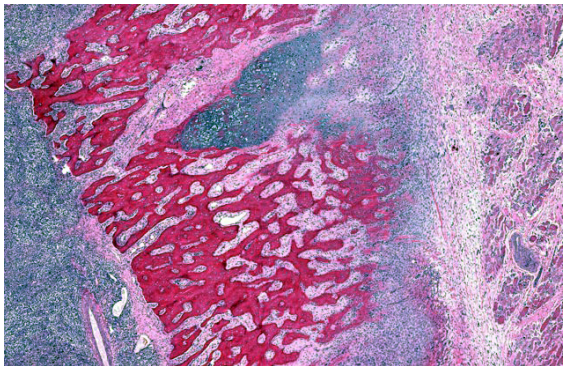

Figure 4-7. Scapula, dog. At the periphery, the surface of the bone is irregularly expanded by proliferation of new trabecular bone, multifocal islands of partially ossified hyaline cartilage, marrow spaces filled with either neoplastic round cells or loose fibrous connective tissue, and markedly thickened periosteum composed of numerous proliferating osteoblasts and fibroblasts (HE,100X) (*Photo courtesy of:* Louisiana Animal Disease Diagnostic Laboratory (LADDL) and Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University (http://www1.vetmed.lsu.edu/laddl/index.html and https://www.lsu.edu/vetmed/pbs/index.php)

and malignant entities originating from various histiocytic linages that may arise in and affect various organ systems.

Multiple dog breeds have demonstrated an increased incidence of histiocytic sarcoma (HS), including Bernese mountain dogs, flat coated retrievers, golden retrievers, and Rottweilers, while other breeds are also sporadically affected. Bernese mountain dogs are particularly predisposed and are estimated to be 225 times more likely to develop HS compared to other breeds, with up to 25% of individuals being affected in their lifetime.[14]

A recent report[5] describing a genome-wide association study (GWAS) using Bernese mountain dog DNA from 172 HS and 128 control cases confirmed previous reports that the main locus associated with HS development in this breed is the *MTAP-CDK2A* region on chromosome #11 (CFA11/HSA9q21). An additional single nucleotide variation associated with HS was identified on chromosome #20 at an intron of *FHIT* (a tumor suppressor gene), while a suspected locus was also identified on chromosome #5 in an intron of the *SPNS3* gene. *SPNS3*'s function is largely unknown, however, its paralogous gene (*SPNS2*) has a role in immunological development and has been associated with inflammatory and autoimmune disease in addition to defective macrophage phagocytosis. Interestingly, each of these three regions have previously been associated with other malignancies including osteosarcoma (*CDK2A*), lymphoma and hemangiosarcoma (*SPNS3*), and mast cell tumors (*FHIT*).[5]

In addition, multiple studies have found dogs with a history of orthopedic disease are at increased risk of developing periarticular HS, the most common synovial neoplasm in dogs. One study found Bernese mountain dogs are five times more likely to develop periarticular HS in a diseased joint compared to a healthy joint. Although this phenomenon occurs in breeds classically predisposed to HS, it does not appear to be restricted to only those breeds. One retrospective study[7] reviewing 28 canine patients from nine breeds with periarticular HS found those with a history of prior joint disease were over 13 times more likely to develop periarticular HS

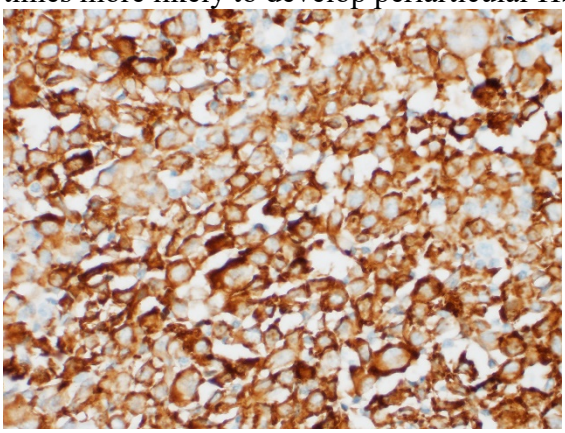

Figure 4-8. Scapula dog. Neoplastic cells demonstrate strong cytoplasmic immunoreactivity for IBA-1. (anta IBA-1, 400X)

compared to the control group. Even more striking, history of joint disease in the stifle joint was associated 64 times greater risk of developing periarticular HS when affected joints were compared independently. Rottweilers were found to be the most commonly affected when breeds were assessed independently.[7]

The pathogenesis involved in the development of HS as the result of prior joint disease is unknown. However, inflammation likely plays a role as increased numbers of antigen presenting cells are present in synovial membranes of dogs with cruciate disease, degenerative joint disease, and rheumatoid arthritis. In regard to cruciate disease specifically, synovitis has been identified as risk factor for future rupture of the cruciate ligament, potentially due to weakening as a result of degradation by inflammatory mediators. In addition, synovitis and osteoarthritis are known to persist following surgical repair of cruciate injuries. Therefore, breeds predisposed to cruciate injuries, such as Rottweilers, may inherently be predisposed to the development of periarticular HS.[7] Interestingly, administration of anti-inflammatory medications has been found to reduce the risk of HS in Bernese mountain dogs, further supporting the theory in regard to inflammation's role in the development of periarticular HS.[7,14]

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