The Armed Forces Institute of Pathology
Department of Veterinary Pathology
WEDNESDAY SLIDE CONFERENCE
2006-2007

CONFERENCE 10
13 December 2006

Conference Moderator: Dr. Steven Weisbrode, Diplomate ACVP
The Ohio State University
Dept of Veterinary Biosciences
Columbus, OH

CASE I – AVC F-4686-06 (AFIP 3031128).

Signalment: 12 year-old, spayed, female, domestic short-haired cat (*Felis domesticus*), feline.

History: Owners had noticed a hard mass in the dorsal sacral area which had been present for approximately 2 months.

Gross Pathology: Surgical excision was attempted. The mass peeled out of a hard capsule in 5 pieces, several of which were sent for histologic examination.

Laboratory Results: Radiographs revealed a multilobulated mass which invaded and replaced most of a coccygeal vertebra. Tail and anal tone were apparently normal.

Histopathologic Description: The submitted tissue consisted of a densely cellular tumor composed of short, interlacing bundles and clusters of plump, spindloid to polygonal neoplastic stromal cells. These cells have relatively uniform, oval nuclei with coarsely stippled chromatin, small nucleoli and small amounts of poorly-defined cytoplasm. Mitotic figures are infrequent (1 per 5 HPF). Interspersed uniformly throughout the tumor are many, large, multinucleated, neoplastic giant cells. These cells generally have 5-25 uniform nuclei and variable amounts of eosinophilic cytoplasm. These cellular infiltrates are supported by small to moderate amounts of uniformly distributed, dense, hyalinized, coarse collagenous stroma which contains frequent small foci of fine, mineral deposition. In some areas, small deposits of pale, acellular, pink matrix (interpreted as osteoid) are also scattered amongst stromal cells. A fibrous capsule (likely the periosteum) lines the periphery of the tissue. There are multifocal, small aggregates of mineralized bone (likely remnants of the coccygeal vertebrae) beneath this capsule.

Contributor's Morphologic Diagnosis: Giant cell tumor of bone, coccygeal vertebra.

Contributor's Comment: Giant cell tumors of bone are well-described but rare in domestic animals. Most reports involved sporadic tumors in dogs and cats. In humans, most giant cell tumors are regarded as low grade malignancies and local recurrence is not uncommon. Wide tissue resection tended to produce the lowest rate of recurrence. A small percentage of tumors may metastasize, typically to the lung. Interestingly in humans, most cases of metastasis of giant cell tumors occurred after attempts at surgical excision, likely due to local tissue damage promoting access to systemic circulation.[1]

Giant cell tumors are typically expansile, osteolytic lesions often involving long bones in humans and animals but which have also been reported in vertebral sites. Because few cases have been reported the biologic behavior of giant cell tumors of bone in animals has not been well-defined. However, those tumors described tended to be locally aggressive with rare incidences of distant metastasize. Retrovirus infection has been suggested as a primary etiology in cats in some cases.[2]

AFIP Diagnosis: Vertebra, coccygeal (per contributor): Giant cell tumor of bone, Domestic Shorthair, feline.

Conference Comment: This case sparked much discussion among conference participants and other consulted pathologists in reference to the differentiation of giant cell type osteosarcomas from giant cell tumors of bone since both contain multinucleate giant cells. Some participants favored a diagnosis of giant cell type osteosarcoma.

As stated by the contributor, giant cell tumor of bone is very rare and has been reported in dogs, cats, and cattle. The cases that have been described tend to behave like giant cell tumors in humans and occur most commonly in the epiphyses of long bones; however, involvement of the axial skeleton and metacarpal bones has been recorded in dogs and cats. The tumor tends to destroy cortical bone as it expands, but always tends to be at least partially encapsulated by a thin shell of bone (as in this case) which gives the tumor a characteristic "soap-bubble" appearance on radiographs. Giant cell tumors are locally aggressive, but usually do not metastasize.[3]

Two cell types are present in giant cell tumors. Mononuclear stromal cells are most numerous and have a histiocytic or fibroblastic appearance. Multinucleate giant cells compose up to 35% of the cell population and resemble osteoclasts.

There may be some collagen or osteoid in the tumor, but this is not a prominent feature in contrast to osteosarcomas in which the production of osteoid is characteristic and usually prominent. When present in giant cell tumor of bone, osteoid is very sparse. Hemorrhages and cavernous vascular spaces commonly occur.[2,3]

Giant cells occur in many bone lesions, but in giant cell tumor they are an integral part of the neoplasm. The giant cells are often very large, are scattered uniformly throughout the tumor and their nuclei resemble those of the mononuclear cells. The cytoplasmic borders of the giant cells are often indistinct. In contrast, in the giant cell variant of osteosarcoma, giant cells are increased in regions rather than distributed uniformly throughout the tumor. Additionally, the mononuclear cells in osteosarcoma have darker, angular nuclei or some other distinguishing shape clearly different from the multinucleate cells. Opinions differ as to whether the giant cells in giant cell tumor of bone are formed by coalescence of stromal cells or by amitotic division or nuclear segmentation without cytoplasmic separation. There is some controversy regarding the cell of origin. According to Jubb, Kennedy, and Palmer, the tumor giant cells have histochemical and ultrastructural characteristics of osteoclasts suggesting giant cell tumors probably arise from osteoclast stem cells of the bone marrow. Meuten's Tumors in Domestic Animals states that the histiogenesis of giant cell tumor is uncertain, but immunohistochemical staining suggests that the mononuclear cells are of histiocytic origin and that the giant cells arise from their fusion.[2,3,4]

In a study performed by Josten and Rudolph, multinucleate giant cells present in various neoplasms were classified as neoplastic or reactive (non-neoplastic). The nuclei of neoplastic giant cells were immunohistochemically positive for MIB1 (Ki67) while the cytoplasm was immunohistochemically negative for tartrate resistant acid phosphatase (TRAP). The nuclei were polymorphic and atypical mitoses were observed. In contrast to neoplastic giant cells, osteoclast-like giant cells were negative for MIB1 and positive for TRAP. Osteoclast-like giant cell nuclei were homogenous. Other non-neoplastic giant cells (e.g. foreign body cells, Langhans-giant cells) were negative for both MIB1 and TRAP.[5]

This case was reviewed in consultation with Dr. Roy Pool who preferred the diagnosis of atypical giant cell tumor of bone. In his opinion, this case represents one end of a spectrum between a low grade fibrosarcoma of bone with some tumor giant cells present and a giant cell tumor of bone with less than normal numbers of the multinucleate cells that characterize this tumor.

Contributor: Department of Pathology/Microbiology, Atlantic Veterinary College University of Prince Edward Island, Charlottetown, PEI, Canada, abourque@upei.ca

References:
1. Rosai J: Ackerman's Surgical Pathology, 8th ed., vol. 2, pp. 1957-1962. Mosby-Year Book, Inc., St. Louis, Missouri, 1996
2. Thompson KG, Pool RR: Tumors of bones. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 294-296. Iowa State Press, Ames, Iowa, 2002
3. Palmer N: Bones and joints. In: The Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, p. 135-136. Academic Press, San Diego, California, 1993
4. Slayter MV, Boosinger TR, Pool RR, Dämmrich, Misorp W, Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, Second Series, vol. I, p. 12, Armed Forces Institute of Pathology, Armed Registry of Pathology, The World Health Organization Collaborating Center for Comparative Oncology, Washington, D.C., 1994
5. Josten M, Rudolph R: Methods for differentiaton of giant cells in canine and feline neoplasia in paraffin sections. Zentralbl Veterinarmed A 44(3):159-166, 1997

CASE II – 06-29801 (AFIP 3031049).

Signalment: 8-week-old female wirehaired Dachshund; *Canis familiaris*; canine.

History: This pup was the first born and runt of a litter. Beginning at the age of 4 weeks, she developed motor problems and an intention tremor that progressed from walking only a few steps at a time to complete inability to walk by the time of euthanasia at 8 weeks of age. No information was supplied regarding the health status of this pup's littermates.

Gross Pathology: All long bones and ribs were fragile and easily fractured on manipulation. Fractures with callus formation were present on multiple ribs, thoracic vertebrae 5 and 6, mid-shaft right humerus, and left carpus.

Histopathologic Description: Histologic lesions were similar in all long bones and ribs examined but varied in severity among anatomic sites. Primary spongiosa was present in the epiphysis and metaphysis and was histologically normal, but secondary spongiosa was sparse or absent in both sites. Peri-trabecular fibroplasia was present in the epiphysis. There was a paucity of trabeculae in the metaphysis, and those present were thin and frequently fractured, with surfaces covered by a thin and often discontinuous layer of osteoid. The cortex was very thin and consisted mainly of woven bone with loose vascular stroma in wide spaces

between trabeculae. Moderate subperiosteal fibrosis was present. Few osteocytes and osteoclasts were present.

Contributor's Morphologic Diagnosis: Osteopenia with failure of development of secondary spongiosa, consistent with osteogenesis imperfecta.

Contributor's Comment: Osteogenesis imperfecta (OI) is a hereditary osteopenic condition described in humans, dogs, cats, cattle, and sheep that results from a qualitative and/or quantitative defect in collagen I production.[1,2,3,4,5] Although growth plate organization and mineralization of primary spongiosa occurs in normal sequence and pattern, osteoid deposition on primary spongiosa of long bones is impaired, leading to defective endochondral ossification through failure of development of secondary spongiosa.[1,2] In humans, the phenotypic expression of OI is quite variable and has been classified into multiple groups based on clinical and prognostic features.[2] In most severely affected cases, pathologic fractures with callus formation may develop *in utero*. Animals with OI typically appear normal at birth but have variably severe locomotor difficulty that progresses over the first few weeks of life. Contribution of collagen I to other tissues, such as teeth (dentin) and tendons, may also predispose to additional lesions, including abnormal tooth production (dentinogenesis imperfecta) and joint laxity.[1,2] Affected humans frequently have blue discoloration of sclera due to diminished collagen content.

Both autosomal dominant and recessive forms of OI have been identified in humans. Most human cases of OI result from mutations in either the COL1A1 or COL1A2 gene, resulting in glycine substitution in the procollagen molecule that leads to abnormal formation of the collagen I triple helix, or mutations resulting in a premature stop codon. Evidence of similar mutations in dogs with OI is limited, but this latter mutation has been identified in the COL1A2 gene of one Beagle pup with OI.[6] No consistent mutation was identified in several related Dachshund pups with clinical OI.[3]

AFIP Diagnoses: Long bone: Osteopenia, diffuse, marked, with failure to develop secondary spongiosa, Dachshund, canine.

Conference Comment: Osteogenesis imperfecta (OI) is an osteopenic disease that has been described in calves, lambs, puppies, domestic cats, mice, and tigers involving bone, dentin, tendons, and sclera which are composed primarily of type I collagen. Interestingly, other type I collagen-rich tissues, such as the skin, are rarely affected. Typical clinical findings include bone fractures, joint laxity (tendon hypoplasia), defective dentin (fragile/fractured teeth with translucent pink

discoloration), and scleral thinning (blue discoloration). Animals born alive often cannot stand.[1,3,7,8]

The pathogenesis involves a defect in osteoblastic/odontoblastic production of type I collagen. In some cases, decreased synthesis of noncollagenous proteins is involved (e.g. osteonectin). As pointed out by the contributor, in humans, and most likely in animals, OI is primarily due to mutations in one or both genes that code for type I collagen. Defects in COL1A1 or COL1A2 genes have been found in mice and Golden Retrievers. In cases in which mutations are not found in these genes, alterations in genes for enzymes responsible for posttranslational modification of collagen should be evaluated.[3,7]

The primary histomorphologic lesion in OI is osteopenia. Growth plates are not affected as cartilage is composed primarily of type II collagen. There is deficient deposition of osteoid on cartilage spicules and the chondro-osseous complex persists into the metaphysis. The metaphysis may have multiple growth arrest lines due to formation of transverse trabeculae. Trabeculae are not modeled (retention of cartilage cores), but are resorbed at the metaphyseal-diaphyseal junction. The marrow cavity contains loose mesenchymal tissue. In severe cases, there is much less trabecular bone than normal. In some cases, the amount and histologic appearance of bone is normal, but there is evidence of fracture disease. In these cases, bone fragility is most likely due to errors in helix-formation or cross-linking of tropocollagen molecules. Osteoblasts can appear normal or small. Additionally, there is a delay in compaction of cortical bone in which the cortices are composed of spicules of woven bone with large vascular spaces. Dentin is dysplastic and thin (dentinogenesis imperfecta).[1,7]
The moderator stressed that teeth should always be collected in suspected cases of OI as the qualitative and quantitative changes present in the dentin are specific for the disease; whereas the bony changes are secondary and can be non-specific lesions due to a variety of causes (e.g. osteopenia due to disuse, nutritional causes, etc.). Histologically, dental tubules are short, tortuous, and sometimes absent.

Differential diagnoses for multiple fractures in young dogs include trauma, nutritional or renal secondary hyperparathyroidism, and OI.

OI is best defined in cattle and has been reported in Charolais and Hostein-Friesians in Denmark as well as in Holsteins in the U.S. and Australia. Several chemical defects occur in the bone tissue of calves with OI including the following:

1. Decreased apatite crystal size (American, Australian)
2. Reduced amount of type I collagen
3. Levels of osteonectin decreased to less than 10% of normal (American)
4. Decreased bone acidic plasma proteins (American, Australian)
5. Decreased bone proteoglycans (American, Australian)

6. Decreased bone sialoprotein (American)
7. Decreased phosphophoryn – dentin-specific protein
8. Markedly deficient levels of an osteonectin-like protein of dentin

Calves with notable bone fragility can have bone that is histologically normal without obvious reduction in bone mass. These calves most likely become osteopenic secondary to disuse due to bone pain from fractures.[1,7,8,9]

OI in lambs is similar to calves in that joint laxity, reduced tendon size and increased fragility of long bones are present; however, teeth and collagen and osteonectin levels are normal.[1]

Fragilitas ossium (*fro*), an autosomal recessive mutation in the mouse, is similar to the severe form of OI.[1]

Contributor: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, http://ahl.uoguelph.ca

References:

1. Palmer N: Bones and joints. In: Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 42-44. Academic Press, Inc., San Diego, California, 1993
2. Rauch F, Glorieux FH: Osteogenesis imperfecta. Lancet 363:1377-1385, 2004
3. Seeliger F, Leeb T, Peters M, Brügmann M, Fehr M, Hewicker-Trautwein M: Osteogenesis imperfecta in two litters of Dachshunds. Vet Pathol 40:530-539, 2003
4. Campbell BG, Wootton JAM, MacLeod JN, Minor RR: Clinical signs and diagnosis of osteogenesis imperfecta in three dogs. J Vet Am Vet Med Assoc 211:183-187, 1997
5. Agerholm JS, Lund AM, Block B, Reibel J, Basse A, Arnbjerg J: Osteogenesis imperfecta in Holstein-Friesian calves. Zentralbl Veterinarmed A 41:128-138, 1994
6. Campbell BG, Wootton JAM, MacLeod MH, Minor RR: Canine COL1A2 mutation resulting in C-terminal truncation of pro-α2(I) and severe osteogenesis imperfecta. J Bone Miner Res 16:1147-1153, 2001
7. Weisbrode, SE: Bones and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1065-1066. Mosby Elsevier, St. Louis, Missouri, 2007
8. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp. 909-910. Williams & Wilkins, Baltimore, Maryland, 1997
9. Denholm LJ, Cole WG: Heritable bone fragility, bone laxity and dysplastic dentin in Friesan calves: a bovine syndrome of osteogenesis imperfecta. Aust Vet J 60(1):9-17

CASE III – N312/99 (AFIP 2810381).

Signalment: 10-year-old, male, Springer Spaniel, canine.

History: An immovable, smooth contoured, hard mass on the right dorsal cranium increased in size over a 6 month period with the growth rate accelerating over the final 6 weeks. The overlying skin was non-adherent. The animal was euthanized.

Gross Pathology: Carcass of moderate nutritional body condition. A large (~7x6 cm), multinodular, firm to hard mass extended from the dorsal cranium to the right of midline. On sectioning the mass contained multiple cavitations and gritty foci and extended into the caudal cranial cavity compressing the caudal cerebrum, the cerebellum, and brainstem. The mass extended caudally as far as the atlas. Multiple subpleural hard nodules, 1-5 mm in diameter were detected. Prostatic enlargement was apparent.

Histopathologic Description: Extending to the margins of the section are multiple irregular nodules containing varying amounts of intermixed cartilaginous and osseous tissue. Nodules are separated by and blend with surrounding dense fibrous stroma. Densely packed spindle-shaped cells at the periphery of the nodules contrast with plumper ovoid cells surrounded by basophilic cartilaginous or paler staining interconnecting spicules of osteoid matrix in more central locations. Atypical mitotic figures and nuclear hyperchromasia are noted. One to two mitotic figures per hpf are observed in peripheral areas. Mineralization of matrix extends out from the center of nodules. Necrosis of cells within lacunae and multinucleate giant cells (osteoclasts/chondroclasts) are present in some areas.

The lung lesions have a similar microscopic appearance (not included).

Contributor's Morphologic Diagnosis: Skull: Multilobular tumor of bone.

Contributor's Comment: Multilobular tumors of bone are infrequently reported, locally aggressive neoplasms usually involving the membranous bones of the canine skull. They are usually found as solitary masses in mature medium to large breed dogs. Synonyms of the lesion are chondroma rodens, calcifying aponeurotic fibroma, juvenile aponeurotic fibroma and cartilage analog fibromatosis. These tumors can produce clinical signs as a result of their compression of adjacent brain of lacrimal duct. They are most commonly found on the temporo-occiptal area and zygomatic process of the skull. Locally aggressive growth and recurrence following

surgery is the typical pattern of behavior with pulmonary metastases occurring late in the clinical course.

AFIP Diagnosis: Bone, skull (per contributor): Osteosarcoma, chondroblastic, Springer Spaniel, canine.

Conference Comment: Although conference participants carefully considered the contributor's diagnosis of multilobular tumor of bone, they felt that the histomorphology is consistent with a chondroblastic osteosarcoma. The characteristic pattern of multilobular tumor of bone is not present in this case, i.e., the repetitious tri-laminar appearance of multiple lobules of centrally located cartilage or bone surrounded by plump mesenchymal cells that are further bounded by interlobular fibrous septa.[2,3,4]

Osteosarcomas are the most common primary bone neoplasm in dogs comprising 80-85% of canine bone tumors and occur most commonly in older, male, large-breed dogs. Metastasis is common, primarily to the lung and lymph nodes. Osteosarcomas of the canine axial skeleton metastasize less readily. Common primary sites in the dog include the distal radius, proximal humerus, distal femur, and the distal tibia. The front limbs are affected twice as often as the hind limbs. Osteosarcomas can also occur in other bones such as the ribs, vertebrae, and skull, as in this case. Rarely, extraskeletal osteosarcomas may arise in soft tissue. Most osteosarcomas originate centrally from the medulla and display more malignant behavior than osteosarcomas of periosteal origin. Osteosarcomas of periosteal origin include periosteal osteosarcomas that behave like central osteosarcomas and parosteal (juxtacortical) osteosarcomas with a high degree of structural differentiation, slower growth, and a better prognosis than central osteosarcoma.[2,3,5,6]

No specific cause of osteosarcoma has been established; however, several associations have been made. Osteosarcomas have been associated with bone infarctions, previous fractures, and the use of metallic fixation devices in domestic animals. Osteosarcomas of viral origin have been reported in mice.[2,3,5]

Grossly, osteosarcomas have a gray-white appearance and lyse and replace normal bone extending into adjacent soft tissues, but do not penetrate articular cartilage and invade into joint spaces. Areas of infarction may be present characterized by large pale areas surrounded by a zone of hyperemia. Cortical bone is usually destroyed with varying amounts of reactive periosteal bone formation. Pathologic fractures are not uncommon.[2,3]

Osteosarcomas are malignant neoplasms in which neoplastic osteoblasts form osteoid, bone, or both and can be classified based on the matrix produced, the predominant cell type involved, radiographic appearance (lytic, sclerotic, or mixed) and origin (central, juxtacortical, periosteal).[3]

Classification based on matrix produced:[2,3]

1. Simple – produce osteoid and bone
2. Compound – produce osteoid, bone and cartilage
3. Pleomorphic – anaplastic with only small islands of osteoid

Classification based on cell type and activity:[2,3,5]

1. Osteoblastic – anaplastic osteoblasts and plump to spindle-shaped osteogenic precursor cells with angular borders; eccentrically located, hyperchromatic nuclei; dark staining cytoplasm
2. Chondroblastic – neoplastic bone and cartilage produced
3. Fibroblastic – spindle cell population in early lesions resembles fibrosarcoma, later tumor cells form tumor bone; better prognosis than other types
4. Poorly differentiated – malignant cells produce small amounts of osteoid and occasionally spicules of tumor bone; malignant mesenchymal cells vary from small, reticular-appearing cells to large, pleomorphic, sarcoma cells; highly aggressive; lytic lesions lead to pathologic fracture

Giant cell type osteosarcomas resemble osteoblastic osteosarcomas, but contain large areas in which giant cells predominate and must be differentiated from malignant giant cell tumor of bone.[5]

Telangiectatic osteosarcomas are uncommon and are composed of osteoblasts, osteoid, and large-cystic, blood-filled cavities lined by malignant osteoblasts rather than endothelium. The tumor metastasizes easily and is highly fatal.[2,3,4]

Osteosarcomas are rare in animals other than the dog and cat. In the cat, osteosarcomas account for 70% of feline bone tumors with the hind limbs most commonly affected. As in the dog, feline osteosarcomas are commonly of medullary origin. In contrast to dogs; however, metastasis occurs less frequently. Additionally, feline axial osteosarcomas are more likely to metastasize than are those arising in long bones. In horses, sheep and cattle, most osteosarcomas involve the head, especially the mandible. Osteosarcomas are rare in pigs, but have been occasionally seen in extremely young animals.[2,5,6]

Contributor: Department of Veterinary Pathology, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4, Ireland

References:
1. Weisbrode SE, Doige CE: Bone and joints. In: Thomson's Special Veterinary Pathology, eds. McGavin MD, Carlton WW, Zachary JF, 3rd ed., pp. 523-524. Mosby Inc., St. Louis, Missouri, 2001
2. Palmer N: Bones and joints. In: The Pathology of Domestic Animals, eds. Jubb KVF, Kennedy PC, Palmer N, 4th ed., vol. 1, pp. 130-132. Academic Press, San Diego, California, 1993
3. Weisbrode, SE: Bones and joints. In: Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1086-1091. Mosby Elsevier, St. Louis, Missouri, 2007
4. Slayter MV, Boosinger TR, Pool RR, Dämmrich, Misorp W, Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, Second Series, vol. I, pp. 13, 39-40, 9-11, 27-32, Armed Forces Institute of Pathology, Armed Registry of Pathology, The World Health Organization Collaborating Center for Comparative Oncology, Washington, D.C., 1994
5. Thompson KG, Pool RR: Tumors of bones. In: Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 263-283. Iowa State Press, Ames, Iowa, 2002
6. Jones TC, Hunt RD, King NW: Veterinary Pathology, 6th ed., pp.940-941. Williams & Wilkins, Baltimore, Maryland, 1997

CASE IV – 06-1264 (AFIP 3026963).

Signalment: Seven-year-old, intact, male, Bassett Hound.

History: The dog was reported to have a skin mass and protrusion of the penis for an "extended period of time". The dog was euthanatized by the local humane society because of alleged neglect by the owner.

Gross Pathology: Findings pertinent to the submitted tissue: Approximately four inches of the penis protruded from the prepuce and was firm and dark red with ulceration and congestion of the tip. The bulbus glandis was one and a half inches in diameter and congested. Maggots were present on the penis. A one inch diameter semi firm subcutaneous mass was present on the left shoulder which on cross section was tan to dark brown with gray layers of soft material. Within the caudal abdominal cavity, there was a 12cm, lobulated, rubbery to hard, round, irregular, white gritty mass involving the pelvis, all sacral vertebral bodies, the bodies of the proximal caudal vertebrae, and the bodies of the caudal lumbar vertebrae with sparing of the intervertebral discs. On cut section, the mass was hard and gritty. A 6cm mass was present in the right perianal area as was a 5cm

diameter mediastinal lymph node with both consisting of the same material as the larger mass. The mediastinal mass was obstructing lymphatic drainage. Approximately twenty-five percent of the lung tissue involving all lobes was replaced by multifocal firm to hard white nodules ranging in size from 3-5cm that were gritty on cut surface. The remaining lung was mottled dark red.

Histopathologic Description: Vertebrae: Portions of annulus fibrosis are present with most of the vertebral bodies on either side of the annulus replaced by a mass composed of anastamosing stratified squamous epithelial cells with large coalescing areas of necrotic (coagulation) epithelium ("ghost" cells) and extensive woven bone formation. The limited amount of pre-existing bone present has undergone marked osteolysis at the interface with the infiltrating mass.

Contributor's Morphologic Diagnoses: Vertebral bodies: Metastatic (malignant) pilomatricoma with bone lysis and reactive bone formation.

Contributor's Comment: The subcutaneous shoulder mass was a malignant pilomatricoma and was presumed the primary. The masses in the pelvis, vertebrae, lung and mediastinal lymph nodes were the same processes and presumed metastatic sites. This mass likely impinged on lumbar and sacral nerves causing damage and impaired neurologic function leading to the paraphimosis.

Pilomatricoma, also referred to as Malherbe's epithelioma or calcifying epthelioma, is a benign tumor of the hair follicle showing matrical differentiation.[1] Pilomatricomas are most frequently diagnosed in the dog, accounting for between 1 and 3 per cent of all dog skin tumors and are rare in other domestic animals.[2,3] According to Goldschmidt and Hendrick, these tumors typically arise on the neck, thorax, back, and tail, and take the form of a solitary, well-marginated, firm, and freely moveable mass affecting the dermis and hypodermis with alopecia and ulceration of the overlying skin.[1] On cut surface, the tumors consist of lobulated gray-white, chalky tissue with occasional areas of mineralization. Histologically, the lobules are characterized by zones of two different cells types: basophilic cells resembling hair matrix cells at the periphery and necrotic, keratinized 'ghost cells' centrally. Calcification and osseous metaplasia are frequently seen within the ghost cell region. Malignant pilomatricoma, or pilomatrix carcinoma, is extremely rare, and has been described only in dog and man. Histological features are the same as those of the benign variant, but the basal cells are invasive into the adjacent tissue and lymphatic invasion may be seen at the tumor's margins.[1] Published cases of malignant pilomatrixoma in dogs have described metastases to lymph nodes, lung, and, in two cases, bone.[2,4] Both cases of bone metastases involved the thoracic vertebrae and resulted in neurological deficits in the limbs. The primary tumors in these cases were likely cutaneous nodules removed approximately one year prior to presentation of metastases. However, only one of

these cutaneous tumors was examined histopathogically and diagnosed as pilomatricoma. No skin tumors were present in either dog at the time of death.

Skeletal metastasis in dog

Cooley and Waters conducted a study of 19 dogs that showed skeletal metastases as the initial clinical manifestation of metastatic carcinoma.[5] They found that the most common sites for metastasis to the skeleton were the axial skeleton and proximal long bones. Only 4 of the 36 skeletal carcinoma sites in these dogs occurred distal to the elbow. The primary sites most frequently identified in this study were mammary gland, prostate, and bladder.

Factors in bone metastasis

Blood flow is a major determinant of the site of skeletal metastasis.[6] In both humans and dogs, skeletal metastases show a predilection for the most heavily vascularized areas of the skeleton - the vertebral column, ribs, and proximal ends of long bones.[5,6] However, it is clear in human medicine that bone is a favored site of metastasis for certain solid tumors, including breast and prostate carcinoma, suggesting that there are more specialized processes at work than random hematogenous seeding.[6] Tumor cell metastasis is a complex, multi-step process involving interactions between the tumor cells and the microenvironment of the host tissue. There are several features of bone that make it a ready site for tumor metastases. Bone is a major storehouse for growth regulatory factors including transforming growth factor β, bone morphogenic proteins, platelet-derived growth factor, and many others that may enhance the survival rate of certain tumors and facilitate their aggressive behavior in this site. Local bone resorption spurred by skeletal mesatsasis may work to release these factors from the bone, resulting in a continuous cycle of tumor growth stimulation and osteoclastic bone destruction. In addition, a number of chemoattractant factors produced by bone marrow stromal cells and osteblasts, such as monocyte chemoattractant protein 1 and stromal cell derived factor-1, may play a key role in directing cancer cells to their metastatic destination. Recognizing the importance of these complex interactions has led to the concept of therapeutic interventions aimed at blocking the expression of growth factors that are critical to the metastatic process.[7]

Also critical to the migration of cancer cells from primary to metastatic sites are the interactions between tumor cells and vascular endothelia. Inflammatory cytokines, adhesion molecules, and chemotactic factors produced by endothelial cells may influence the passage of tumor cells through the vasculature as well as their eventual arrest at the metastatic site.[8] Cancer types showing a propensity for bone metastasis may express adhesion factors specific to bone marrow-derived endothelial cells, as was suggested by studies of prostatic carcinoma and bone-homing myeloma.

Mechanisms of heterotopic ossicifaction in pilomatricomas

Because the ghost cell regions of pilomatricomas are necrotic, the mineralization frequently associated with these tumors is presumed to be dystrophic. The mechanism of heterotopic bone formation within carcinomas is not understood. Studies have speculated that stimuli generated by malignant epithelial cells induce pluripotent mesenchymal cells to become osteoblasts, which go on to produce metaplastic bone.[9] A case study by Kypson et al. of osseus metaplasia in a rectal adenocarcinoma found overexpression of bone morphogenic protein 2 (BMP-2), a known inducer of osteoblastic differentiation, within the tumor cells.[9] BMP-2 secretion likely results from random activation of the BMP gene in certain cells. Although heterotopic ossification in rectal carcinoma is rare, this finding offers an intriguing glimpse into the complex interactions occurring between epithelial cancers and their surrounding stroma. Stromal changes, which were once believed to be largely reactive in nature, may play an important part in carcinoma progression.[10]

A 2006 study by Rifas proposed that T-cell cytokines released at local sites of inflammation may induce differentiation of local mesenchymal stromal cells into osteoblasts and thereby play a major role in heteoptropic ossification in chronic inflammatory diseases.[11] The study demonstrated that activated T-cell conditioned medium effectively induced BMP-2 and alkaline phosphatase production in human mesenchymal stromal cells in culture.

AFIP Diagnosis: Bone, vertebral body: Malignant pilomatricoma, metastatic, Bassett Hound, canine.

Conference Comment: The contributor provides an excellent and thorough overview of malignant pilomatricomas to include histomorphologic and gross appearance, skeletal metastasis in the dog, factors in bone metastasis, and mechanisms of heterotopic ossification in pilomatricomas.

The mechanisms of heterotopic ossification in pilomatricomas generated discussion in conference regarding epithelial to mesenchymal transitions. Some believe that members of the transforming growth factor (TGF-beta) family of growth factors can initiate and maintain epithelial to mesenchymal transitions while others believe that there is no convincing evidence that epithelial cells are able to convert to mesenchymal cells *in vivo*.[12,13]

Readers are encouraged to read references 12 and 13 for further information on this controversial topic.

Contributor: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Road, Columbus, Ohio 43210, http://vet.osu.edu/biosciences.htm

References:

1. Goldschmidt MH, Hendrick, MJ: Tumors of the skin and soft tissues. In: Tumors of Domestic Animals, ed. Meuten DJ, 4th ed., pp. 61-63. Iowa State Press, Ames, Iowa, 2002
2. Rodriguez F, Herraez E, Rodriguez JC, Gomez-Villamandos A, Espinosa de los Monteros A: Metastatic pilomatrixoma associated with neurological signs in a dog. Vet Rec 137:247-248, 1995
3. Madewell BR, Theilen GH: Tumors and tumor-like conditions of epithelial origin. In: Veterinary Cancer Medicine, eds. Thelien GH, Madewell BR, 2nd ed., p. 257. Lea & Febiger, Philadelphia, Pennsylvania, 1987
4. Van Ham L, van Bree T, Maenhout T, Tshamala M, Broekart D, Hoorens J, Mattheeuws D: Metastatic pilomatrixoma presenting as paraplegia in a dog. J Small Anim Pract 32:27-30, 1990
5. Cooley DM, Waters DJ: Skeletal metastasis as the initial clinical manifestation of metastatic carcinoma in 19 dogs. J Vet Intern Med 4:288-293, 1998
6. Mundy GR: Mechanisms of bone metastasis. Cancer Oct15;80(8 Suppl):1546-1556, 1997
7. Roodman GD: Role of stromal-derived cytokines and growth factors in bone metastasis. Cancer Feb 1;97(3 Suppl):733-738, 2003
8. Choong PFM: The molecular basis of skeletal metastasis. Clin Orthop Relat Res Oct.;(415 Suppl)S19-31, 2003
9. Kypson AP, Morphew E, Jones R, Gottrfried MR, Seigler HF: Heterotropic ossification in rectal cancer: rare finding with a novel proposed mechanism. J Surg Oncol 82:132-136, 2003
10. Magliocco AM: Commentary. J Surg Oncol 82:137, 2003
11. Rifas L: T-cell cytokine induction of BMP-2 regulates human mesenchymal stromal cell differentiation and mineralization. J Cell Biochem Jul1;98(4):706-14, 2006
12. Zavaldi J, Bottinger EP: TGF-beta and epithelial-to-mesenchymal transitions. Oncogene Aug 29;24(37):5764-5774, 2005
13. Tarin D: The fallacy of epithelial to mesenchymal transition in neoplasia. Cancer Res 65(14):5996-6001, 2005

Michelle E. Thompson, DVM
Captain, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists and the C. L. Davis Foundation.