The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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CONFERENCE 9
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Conference Moderator: Dr. Steven E. Weisbrode, VMD, PhD, Diplomate, ACVP
Professor, Department of Veterinary Biosciences
The Ohio State University, Columbus, OH 43210

CASE I – 00-3180 (AFIP 2841691)

Signalment: Neonate, female, llama

History: Multiple congenital defects and fractures.

Gross Pathology: Pertinent gross findings included brachygnathism inferior, pectus escavatum. Multiple rib fractures with variable to no callus formation, acute transverse fractures of left femur and tibia, transverse fracture of right femur with prominent callus formation and equivocally thin cortices of appendicular bones, pink teeth and blue sclera.

Laboratory Results: None given.

Contributor's Morphologic Diagnosis: Molar tooth: dysplasia of dentin compatible with dentinogenesis imperfecta.

Contributor's Comment: There is marked irregularity in the arrangement and marked reduction in the numbers of dentin tubules. The dentin matrix has numerous wavy parallel basophilic lines interpreted as mineralization fronts. The layer of predentin is irregular in thickness and stains only weakly with eosin. The odontoblasts are small, reduced in number and disorganized. These dental lesions are consistent with those reported in cattle and humans with osteogenesis imperfecta (OI) and the apparently pink teeth, blue sclera and multiple fractures in this neonatal cria support a diagnosis of OI. The metaphyseal trabeculae had less than expected bone matrix on cartilage cores. These changes also were like those reported in Friesian calves with OI.

Osteogenesis imperfecta in humans is caused by a variety of defects in the genes that code for the alpha one and two chains of type I collagen. In humans, over 150 mutations have been defined. Transgenic and knockout mice have been created as animal models. Dentinogenosis imperfecta (DI) is not a consistent finding in cases of OI in humans and when present can vary markedly in its severity. In addition, in humans, DI can exist as an independent disease not associated with skeletal lesions.

AFIP Diagnosis: Maxilla, tooth (per contributor): Dentin dysplasia (dentinogenesis imperfecta), llama, camelid.

Conference Comment: As the contributor noted, dentinogenesis imperfecta (DI) can be present in conjunction with osteogenesis imperfecta (OI), or as a separate disease entity. Collecting teeth at necropsy can verify the presence or absence of DI histologically when the characteristic opalescent dentin is not recognized grossly.

Osteogenesis imperfecta (brittle bone disease) is a rare, heritable disease of calves, lambs, and puppies. There is a single case report of OI in a kitten. The lesions in this disease include osteopenia, excessive bone fragility with increased susceptibility to fracture, skeletal deformity, joint laxity, translucent teeth, otosclerosis, and blue-tinged sclera. Conference participants discussed the degree and severity of osteopenia in this section, in addition to the lack of an age-matched control. Having a control animal could help to verify whether the reduced bone mass evident in this case is within normal limits for this age animal or is a true lesion, suggestive of osteogenesis imperfecta.

Contributor: Department of Veterinary Biosciences, The Ohio State University, 1925 Coffey Rd., Columbus, OH 43210

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CASE II - R125/02 (AFIP 2840180)

Signalment: 4-years-old, male, Green Iguana (*Iguana iguana)*

History: This animal was presented to the clinic showing a diffuse and severe swelling surrounding the wrist joint of the left forelimb. A private owner, keeping the subject in optimal alimentary and environmental conditions, described a progressive enlargement of the lesion in a month. No traumatic events were registered and apparently, the subject didn't show any pain or lameness. During the clinical examination, the animal was found particularly aggressive. Amputation of the forelimb at the elbow joint level was performed and histopathology of different transversal sections followed.

Gross Pathology: Presenting a compact texture undefined margins and complete integrity of the superficial cutaneous tissue. In association with the lesion, deformity of the bone segment profile was present. On the cut surface, a compact fibrous tissue was present associated to multifocal hyperemic-hemorrhagic areas and mixed with calcified deposits and bone residues.

Laboratory Results: RX examination: The lesion compared with the contralateral joint, shows diffuse and severe wrist joint bone lysis and rarefaction of distal parts of both radius and ulna, presenting diastases, and proximal metacarpal bones. In association, diffuse and severe involvement of the surrounding soft tissues is noticed.

Fine Needle Aspiration Cytology: A fine needle aspirate was sampled from the wrist joint region showing lysis at RX examination. The sample is characterized by many erythrocytes associated to an inflammatory component, mainly composed of heterophils, and occasional disseminated round cells showing an abundant, often vacuolated, cytoplasm and central vesicular nuclei. Mild anisocytosis and anisokaryosis are detectable. Binucleated and multinucleated elements are present.

Special stains:

1) PAS staining: negative for fungal structures
2) Gram staining: colonies of Gram-negative cocci, associated to multiple central necrotic areas.
3) Z.N. staining: negative for acid-alcohol fast staining microorganisms.

Contributor's Morphologic Diagnosis: Osteomyelitis, granulomatous, chronic-active, severe, diffuse; osteolysis, diffuse, severe; fibrous dysplasia diffuse, severe; osseous and chondroid metaplasia, multifocal, moderate; presence of microorganisms referable to bacteria.

Contributor's Comment: Different sections have been examined and similar histological aspects are detectable. The normal bone and muscular tissues have been replaced by compact, prominent and infiltrative fibrous connective tissue. Mixed to this tissue, multifocal necrotic areas are present, characterized by central amorphous bright eosinophilic material associated to moderate edema, macrophages and heterophils, and surrounded by numerous multinucleated giant cells (2-10 round regular nuclei and moderate granular cytoplasm, possibly referable to osteoclasts) and epithelioid cells. In association to some of the necrotic debris, multiple slightly basophilic colonies of cocci microorganisms are evident. Residual necrotic bone trabecules are also evident

associated to granulomatous inflammation. Inflammatory infiltrates, mainly composed by heterophils and lymphocytes, are multifocally distributed in perivascular areas. Moreover, large deposits of chondroid and osteoid extracellular matrix and specific cell differentiation (metaplasia) are present. Disseminated small blood vessels are also present mixed to the fibrous tissue that infiltrates the peripheral muscular fibers, showing moderate and diffuse degeneration. The superficial epithelium is characterized by severe and diffuse orthokeratotic hyperkeratosis, mixed with numerous colonies of cocci.

The histopathology allowed us to exclude a suspected neoplastic lesion and confirmed the diagnosis of a bacterial osteomyelitis associated to severe osteolysis and a prominent fibrous peripheral reaction. Bacterial isolation and identification was not performed. In this case, the absence of cutaneous wounds and traumatic events indicates a possible hematogenous infection.

These animals are often presented in the clinics for environmental infections, sometimes mild and superficial, but sometimes located in the viscera, which are severe and difficult to treat (1). Frequently, *Salmonella* species are implicated in reptilian pathology and in reptilian transmitted zoonoses (1). Few reports regarding traumatic osteomyelitis in iguanas, characterized by swelling of the lesions, are described in specific web sites (*i.d.* www.lbah.com/reptile/iginfection.htm), even if to our knowledge, there is no literature describing infective skeletal infections. Septic osteomyelitis with similar severe tissue reactions and histopathological aspects has been described in other reptiles (2). Within a four months follow up after the amputation of the forelimb, associated to an appropriate pharmacological treatment, the animal was recovering well and showed neither any signs of systemic involvement nor dramatic problems of locomotion.

The Department of Padova would like to thank J.G. Trupkiewicz, DVM of the Zoological Society of Philadelphia, Penrose Research Laboratory, Philadelphia, Pennsylvania, USA, 19104-1196, for his scientific contribution to this case.

AFIP Diagnosis: Bone, carpus and adjacent soft tissue (per contributor): Cellulitis, granulomatous and heterophilic, diffuse, severe, with fibroplasia, reactive bone formation, osteolysis, and luxation, Green Iguana (*Iguana iguana*), reptile.

Conference Comment: In reptiles, a wide range of pathogenic microorganisms may induce focal or diffuse cellulitis with a heterophilic or histiocytic inflammatory response. Focal infections may progress to an abscess or granuloma. Heterophilic inflammation is often induced by extracellular pathogens, while histiocytic inflammation is commonly associated with intracellular pathogens. In this case, it is not evident if the infection began in the soft tissues and extended locally to involve the bone, or if the bony tissues were affected first, with subsequent extension to the soft tissues. Infectious osteomyelitis can originate via hematogenous spread, extension from infection in an adjacent joint or soft tissue, or contamination of an open fracture or during orthopedic surgery. Osteomyelitis is most commonly caused by bacteria, but infection by fungal, viral, or protozoal agents can occur.

Contributor: Department of Public Health, Comparative Pathology and Veterinary Hygiene, Faculty of Veterinary Medicine, University of Padova, Agripolis, St. Romea 16, 35020 Legnaro, Padova, Italy

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CASE III – 02-25738 (AFIP 2839353)

Signalment: 6-month-old male Boxer, canine.

History: This dog has had a history of swollen joints, severe pain, lameness and recurrent fever for 2 months. The condition deteriorated until the dog would not get up and was euthanized.

Gross Pathology: Firm diffuse swellings of the right and left distal radioulnar metaphyses and left distal tibial metaphysis were noted. A fluctuant mass over the right olecranon process and similar lesion in the subcutis of the left temporal area were also found. The metaphysis and epiphysis of the radius, ulna and tibia all had extracortical thickening on cut section with calcification and the costochondral junctions were markedly enlarged.

Laboratory Results: Bacteriologic culture found no significant pathogens and virology including FA's for CPV, CAV, CDV and viral isolation, were all negative.

Contributor's Morphologic Diagnosis: Bone, physitis and metaphyseal osteomyelitis, necrosuppurative, hemorrhagic, severe with extraperiosteal fibro-osseous proliferation and retained cartilagenous cores

Contributor's Comment: This is a relatively classic case of canine hypertrophic osteodystrophy. Although a variety of causes have been implicated including

hypovitaminosis C and hypervitaminosis D, the condition remains idiopathic. Concurrent fever and frequent diarrhea, as well as suppurative osteomyelitis, suggest an infectious cause. Recently, a correlation was noted between differences in vaccine status and the occurrence of the disease among littermates. This dog was well vaccinated and no evidence of viral or bacterial disease could be identified.

AFIP Diagnosis: Long bone, ulna, metaphysis (per contributor): Osteomyelitis, necrosuppurative, chronic-active, diffuse, moderate, with hemorrhage, trabecular fractures, and fibrin deposition, Boxer, canine.

Conference Comment: Hypertrophic osteodystrophy (metaphyseal osteopathy) is a disease of primarily large or giant breed puppies that develop metaphyseal pain and lameness in multiple long bones, accompanied by depression, anorexia and variable pyrexia. It begins as a fibrinosuppurative osteomyelitis of the metaphysis. The clinical history of multiple, painful, metaphyseal swellings, fever, lameness and the breed and age of this dog correlate well with typical cases of hypertrophic osteodystrophy. Conference participants noted that both acute and chronic changes are present in this section.

Contributor: The University of Georgia, Veterinary Diagnostic & Investigational Laboratory, PO Box 1389, Tifton, GA 31793

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CASE IV - NIAH No2 (AFIP 2840313)

Signalment: Newborn, female, Holstein, calf.

History: A female Holstein calf was delivered alive but died after birth in April in a dairy farm where a hundred cows were kept. The farm had had an episode of abortion in December of the previous year and the calf was submitted under suspicion of Akabane disease.

Gross Pathology: The head of the calf was relatively flat, giving it a dog-like appearance with a short nose and sloping forehead. Limb bones were dumbbell-like with short diaphyses and irregularly widened metaphyses. Bone marrow was whitish and fatty. The other organs including central nervous system were grossly normal.

Laboratory Results: Laboratory results did not suggest any etiology of this pathology; no viral or bacterial agents were isolated, and antibodies to BVD/MD, Akabane, IBR or adenoviruses were negative and concentrations of vitamin A and calcium were in the normal range.

Contributor's Morphologic Diagnosis: Tibia: Chondrodysplasia, with dyshematopoiesis, Holstein, newborn calf.

Contributor's Comment: Histopathologically, the metaphyseal plates were mildly thickened and had many cartilage canals lined with flat shaped cells, in which capillary vessels were minimal. In proliferative to hypertrophic zones, accumulations of degenerative chondrocytes were irregularly arranged. Columns of calcified cartilage were poorly formed, losing comb-like structure. Most of the chondrocytes in the resting zone were pyknotic with swollen and ghost-like cytoplasm. Alcian blue (pH 2.5) staining showed that the matrix was not homogeneous but was fine granular or fibrous. The fine granular or fibrous matrix showed metachromasia when stained with toluidine blue, as well as degenerative chondrocytes and cartilage canals. Bone trabeculae are poorly formed and frequently contained cartilage matrix. Bone marrow was ischemic with poor hematopoiesis and was moderately replaced by adipose tissue. In cortical bone, the arrangement of outer basic lamellae and haversian lamellae were normal, while that of the inner basic lamellae was compressed vertically. In the epiphyses, most chondrocytes were also degenerated with ghost-like cytoplasm, and formation of the bone trabeculae was poor. Occasional bone marrow-like structures were formed in articular cartilage, as well as canals lined with flat shaped cells. Capillary vessels were found in some of these canals. There was no extramedullary hematopoiesis in the liver and spleen, while liver, lungs and spleen showed congestion. Mild fibrosis was present in the sinusoid, portal triad and around the central vein of the liver.

In the present case, degeneration of chondrocytes, malformation of the matrix and dyshematopoiesis were characteristic. These chondrocytes might not differentiate to fulfill normal endochondral ossification in osteogenesis. The lack of hematopoiesis in the bone marrow suggests insufficient interaction between growth factors and osteogenic tissues. Development of bone is regulated in the bone marrow mainly through interaction of bone marrow mesenchymal stem cells and many cytokines such as interleukin-1 beta, bone morphogenetic proteins or fibroblast growth factor. The dyshematopoiesis may be systemic, as suggested by the lack of extramedullary hematopoiesis.

Besides the present case, inherited chondrodysplasias have been known, but the causal gene loci are still unclarified. A Dexter-type calf has epiphyses that appeared normal but were enlarged. The gene is heterozygous for an incompletely dominant, but when homozygous it is lethal called a bulldog calf. Telemark and brachycephalic snorter types are also hereditary, but the histopathology is still unclear. A calf of the

Telemark-type shows a domed cranium, cleft palate and short, bulky and rotated limbs. Snorter dwarfs have a broad head and protruding mandible, and the ratio of metacarpal to diaphyseal diameters is less than that of normal animals. Congenital dwarfism of calves was reported with the possible nutritional etiology of manganese deficiency or dry and spoiled feed. An affected calf has a sheep-like appearance associated with joint laxity. Chondrodysplastic dwarfism of unknown etiology was reported in the Japanese brown breed. The characteristics were rhizomelia, with deformity of the end of the long bones and partial disappearance of the epiphyseal plate that resulted mainly from disturbance of chondrocytic differentiation and abnormal formation of the matrix.

AFIP Diagnosis: Long bone, tibia, epiphysis (per contributor): Chondrodysplasia, with osteopenia and dyshematopoiesis, Holstein, bovine.

Conference Comment: Chondrodysplasia refers to abnormalities in cartilage growth and matrix production, resulting in abnormal endochondral ossification. Cartilage grows by both interstitial proliferation, which increases growth plate thickness, and by superficial apposition, which increases primarily growth plate width. In many chondrodysplasias, interstitial growth is defective while appositional growth remains unaffected. Abnormal interstitial growth results in premature growth plate closure, resulting in short bones. The contributor did an excellent job of describing the histologic abnormalities. In addition, conference participants commented on the pallor of the cartilaginous matrix. The basophilia of cartilage on H&E sections is due to its high proteoglycan content, which is decreased in this case resulting in the pale matrix. Chondrodysplasia (dwarfism) is divided into proportionate and disproportionate forms. Most animal and human cases are of the disproportionate type. In the heterozygous form of dwarfism, described in Herefords, Aberdeen Angus, Holstein and Shorthorn breeds, the disproportionate dwarfs have normal longevity with discrepant development of the axial and appendicular skeleton. This results in a seemingly larger head and body than the markedly shortened extremities. In the homozygous form of dwarfism (congenital lethal chondrodysplasia), described in Jerseys, Guernseys, Holsteins, Dexters and Japanese Brown breeds, individuals are stillborn or die shortly after birth. These animals have a bulging forehead, depressed and shortened nose, protruding tongue, short and thick neck, and shortening of the limbs. Inherited chondrodysplasia has been described in cattle, dogs, rabbits, pigs, and sheep. Examples include Spider lamb chondrodysplasia affecting Suffolk and Hampshire sheep, ocular and skeletal dysplasia in Labrador Retrievers, and chondrodysplasia in Alaskan Malamutes.

Contributor: National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki, 305-0856, Japan

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Kathleen A. Ryan, DVM
Major, Veterinary Corps, U.S. Army
Wednesday Slide Conference Coordinator
Department of Veterinary Pathology
Armed Forces Institute of Pathology
Registry of Veterinary Pathology*

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