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Department of Veterinary Pathology
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Conference Moderator: Dr. Steven Weisbrode
Department of Veterinary Biosciences
The Ohio State University
Columbus, OH 43210

CASE I – A (AFIP 2735503)

Signalment: One-day-old, male, crossbred, porcine

History: Neonatal piglet born with thickened forelimbs. Cannot walk.

Gross Pathology: Front legs are markedly thickened distal to the elbow. On cross-section, the cortex of the radius is approximately twice normal thickness.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Congenital hyperostosis.

Contributor's Comment: Cross sections of the radius and ulna show marked periosteal edema, fibrosis with radiating trabeculae of periosteal new bone.

This is a congenital condition of pigs, thought to be inherited as an autosomal recessive trait. Affected piglets are often born dead at term; the rest usually die in one to two days, although the cause of death is not known, and has been attributed to starvation, trauma (rolled on by sow), or cardiac insufficiency. Skeletal lesions are restricted to the limbs, which are thickened, rigid, hard, the skin tense and firmly affixed to underlying tissue. One or both forelimbs are always affected, while hind limbs may or may not be involved.

While joints, epiphyses and metaphyses are histologically normal, excess periosteal intramembranous new bone is present in radiating trabeculae laid down on the original cortex of diaphyseal bone. Soft tissues of the affected limbs are infiltrated with edematous connective tissue, with reduced muscle mass.

The pathogenesis of this lesion is not known. It may be associated with disorganization of the perichondrial ossification groove of Ranvier, or possibly with

local circulatory abnormalities leading to prolonged edema and associated periosteal reaction. Localized arteriosclerosis in antebrachial arteries from affected piglets has been described.

AFIP Diagnoses: 1. Radius and ulna: Hyperostosis, periosteal, circumferential, moderate, crossbred pig, porcine.
2. Radius and ulna, adjacent soft tissue: Edema, focally extensive, severe.

Conference Comment: Fine radiating spicules of woven bone arranged perpendicular to the cortex is the characteristic histological appearance of congenital hyperostosis. In addition to the increased bone production enlarging the limb diameter, the layers of the periosteum are thickened as well. The inner (cambium) layer is proliferative while the outer (fibrous) layer is expanded by edema.

Histologically, bone is described as woven, lamellar, or a combination of the two. Woven bone is associated with repair, inflammation, or rapid deposition and is characterized by numerous large lacunae and the haphazard appearance of collagen fibers when viewed by polarized light. Lamellar bone is typical of more normal bone growth. It contains fewer small fusiform lacunae and collagen fibers are more orderly when viewed by polarized light.

Laminar bone, evident in the cortex of this specimen, is an arrangement of bone consisting of evenly spaced concentric laminae. The deposition of lamellar bone on the many endosteal surfaces eventually fills in the spaces between laminae. This arrangement and method of growth allows for rapid expansion of the diaphysis. Through primary remodeling, the laminar bone is eventually replaced by compact (osteonal) bone. Laminar bone is unique to farm animals and large dogs.

Contributor: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada N1H 6R8

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CASE II – 99-22936 (AFIP 2790125)

Signalment: 2-year-old, male castrated, Siamese cat (feline)

History: Unknown duration of hindlimb lameness without any history of trauma (indoor only cat). Radiographs revealed bilateral slipped capital femoral epiphysis with resorption of femoral neck.

Gross Pathology: None.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Physeal dysplasia with slipped capital femoral epiphysis.

Contributor's Comment: This is a newly described entity recognized with increasing frequency by radiologists and pathologists. The physeal lesion is characterized by irregular clusters of chondrocytes separated by an abundant matrix. The chondrocytes are large and most closely resemble cells from the reserve zone of a normal physis. The fracture is through the center of the physis with abnormal physeal cartilage on both the epiphyseal and metaphyseal side (metaphysis not provided). The epiphysis is viable, with no necrosis as would be seen in Legg-Calve-Perthes disease. This lesion differs from traumatic physeal fracture (Salter Harris type I) in that the chondrocytes have lost their linear orientation.

Recently two cats with this condition presented for complete necropsy (unpublished results). The cause of death was unrelated to the physeal dysplasia. The physeal dysplasia was present in other physes without evidence of slippage. Therefore, this condition represents a generalized physeal dysplasia with slippage of only the capital femoral epiphysis. The capital femoral physis presumably slips as a result of the shear forces on the femoral head. The forces are more compressive on the other physes.

The epidemiology, clinical history, and histopathology of this condition in cats is very similar to the same condition in humans. In cats the affected population is 85% male, 25% Siamese, and 15% Maine Coon cats. Both intact and neutered cats are affected. Most of the cats are of above average weight. This includes both obese cats and large cats in lean body condition. The average age of onset is 16 months with a range of 4 months to 4 years. The disease is frequently bilateral, but there may be months between the slippage of each side.

In humans, this condition primarily affects overweight and above average height adolescent boys. The onset is atraumatic and often insidious. Undiagnosed cases are thought to be a frequent cause of degenerative joint disease of the hip in later life. Blacks are more often affected than whites. Familial cases suggest an autosomal dominant inheritance with variable penetrance. This condition has also been described in two dogs, both overweight male Shetland Sheepdogs.

AFIP Diagnosis: Femoral capital epiphysis: Dysplasia, physeal, with degeneration, necrosis, and pathological fracture, Siamese, feline.

Conference Comment: The normal growth plate or physis contains three zones which blend from one to another as chondrocytes mature during the process of enchondral ossification; the reserve zone, proliferation zone, and hypertrophic zone. As chondrocytes pass through the proliferative zone they arrange in columns, accumulate glycogen, and enlarge as they enter the hypertrophic zone. Chondrocytes in the hypertrophic zone will lose their glycogen, accumulate calcium, and initiate matrix mineralization. In the distal hypertrophic zone, chondrocytes are surrounded by tubes of mineralized matrix (cartilage cores), and undergo apoptosis. Capillaries grow into the mineralized cartilage followed by osteoblasts, which deposit woven bone on the remaining mineralized septa (primary spongiosis). This area of primary spongiosis (the chondroosseous junction) is the most susceptible to traumatic physeal fracture; the differential diagnosis in this case.

Contributor: University of Pennsylvania School of Veterinary Medicine, Laboratory of Pathology, Philadelphia, PA 19104

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CASE III – 99-2804 (AFIP 2787840)

Signalment: Three and a half-year-old, female, paca (Cuniculus paca)

History: Two years history of intermittent lameness, lethargy, anorexia and weight loss. In attempts to stimulate appetite, the animal was offered sweet potatoes and avocados, and for the most of the two years period, the animal preferably ate them to the exclusion of other foods. The radiographs showed diffuse hypercalcification of the spine, which progressed to elbows and stifles. The lameness progressed to the level where the animal could not stand by its own.

Gross Pathology: The vertebrae had a markedly decreased range of motion and were diffusely and irregularly thickened. On the cross section, lumbar vertebral bodies had hard, up to 0.4 cm, white tissue proliferation on the periosteum and the hard tissue density within the vertebral body was greatly increased. Similar changes were present in other bones and there was widespread soft tissue mineralization.

Laboratory Results: Results of all laboratory tests were provided to us by the submitting veterinarian. The following were recorded over the two year history: BUN from 44 to 49 mg/dL (↑); Creatinine from 2.7 to 3.0 mg/dL (↑); Alkaline phosphatase from 500 to 1349 IU/L (↑); and Calcium from 11.0 to 13.9 (↑). Late in the clinical course serum 25-hydroxycholecalciferol (calcidiol) level was 105 nmol/L and considered within normal range for humans by the laboratory doing the test. Post mortem liver samples had retinol 1190 ug/g with expected normal values for humans being about 20 ug/g.

Contributor's Morphologic Diagnosis: Lumbar vertebra: osteosclerosis and exostosis.

Contributor's Comment: The osteosclerosis and periosteal bone formation were generalized throughout the skeletal system, including the skull, long bones, vertebrae and sternebrae. Other significant findings included widespread, metastatic mineralization of multiple organs (kidney, lung, diaphragm and heart). Due to the presence of osteosclerosis, hypercalcemia and soft tissue mineralization, hypervitaminosis D was suspected, but serum 25-hydroxycholecalciferol was considered within normal range. Hypervitaminosis A was suspected in this case because of the marked osteosclerosis, exostoses and dietary history. The liver retinol level was considered increased. Sweet potatoes are an excellent source of Vitamin A and possibly along with the avocados were likely the source of the hypervitaminosis A. The cause of the soft tissue mineralization in this case was

likely secondary to hypercalcemia and uremia. The kidneys had mineralization and reactive fibrosis; the cause was not apparent. The pathogenesis of the hypercalcemia is undetermined but hypercalcemia is reported due to hypervitaminosis A. In the adult, skeletal lesions due to hypervitaminosis A are osteosclerosis and exostosis. In the growing skeleton, bone growth is stunted due to inhibition of osteoblastic activity and degeneration of growth plates. The pathogenesis of the skeletal changes due to hypervitaminosis A are not well understood. The proliferative changes in bone in response to hypervitaminosis A might be mediated by the transcription factor ets1. Retinoic acids can increase expression of ets1 which has been associated with bone morphogenesis.

AFIP Diagnosis: Vertebra and rib: Hyperostosis and osteosclerosis, diffuse, severe, paca (Cuniculus paca), rodent.

Conference Comment: The contributor has provided a concise review of this condition. Conference participants favored hypervitaminosis A based upon the two primary processes evident in the section, hyperostosis and osteosclerosis. In addition to hypervitaminosis A as a cause of generalized hyperostosis, chronic fluoride ingestion was discussed as a differential diagnosis. Fluorosis, which occurs most frequently in herbivores, causes enamel hypoplasia in developing permanent teeth and generalized osteodystrophy. Teeth have a dark mottled or chalky appearance and wear unevenly. Osseous changes include exostosis of any bones, but most commonly the mandible and metatarsals. The effects are dose, rate, and species dependent.

Contributor: The Ohio State University, College of Veterinary Medicine, Department of Veterinary Biosciences, Columbus, OH 43210

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CASE IV – P20X2A (AFIP 2790119)

Signalment: 5-month-old, male, FGFR3G374Rneo-/+ heterozygous mouse

History: None.

Gross Pathology: Body as a whole: dwarf (body weight is approximately half of control), kyphosis. Skull: dome shaped with hypoplasia of the midface, inferior prognathism and protruding incisors.

Laboratory Results: None.

Contributor's Morphologic Diagnosis: Humerus: Chondrodysplasia.

Contributor's Comment: The humerus is misshapen - its length is markedly decreased (control: approximately 12 mm; affected: approximately 7 mm - see gross slide) and there is prominent flaring of the proximate metaphysis. The growth plate is somewhat narrower than the control and throughout most of it, orderly cartilage zones cannot be discerned. Chondrocytes are haphazardly distributed within eosinophilic extracellular matrix. Where residual cartilage zones are evident (e.g. in the cranial aspect of the plate in some specimen), proliferative cartilage columns are very short, separated by abundant extracellular matrix, and hypertrophic chondrocytes are not uncommon. Basophilia of the matrix is limited to this area. The cartilage matrix is multifocally mineralized along the epiphyseal and metaphyseal ends of the growth plate. Primary spongiosa is absent. The growth plate is sealed by horizontal bone struts deposited parallel to its metaphyseal aspect. Occasional foci of cartilage mineralization, degeneration, and loss with formation of irregular and optically empty spaces are present in the growth plate in some slides.

The growth plate in this five-month-old knockout mouse is sealed and shows early regressive changes. During active growth moderate shortening of the proliferative and hypertrophic zones was the main growth plate abnormality. The absence of qualitative changes in endochondral ossification in these mice is consistent with the histological findings in human achondroplasia. The squat shape of the bone is typical of achondroplasia and related disorders.

Dwarfism may be proportionate or disproportionate. Proportionate dwarfism is usually caused by endocrinological, nutritional, environmental, and chromosomal abnormalities. Bone involvement in these cases is secondary. Disproportionate dwarfism is due to disturbance of bone development. Achondroplasia, hypochondroplasia (a similar but milder disorder) and thanatophoric dysplasia (a severe form leading to early postnatal death) are among the more important disorders leading to disproportionate dwarfism in man. The common morphological finding in these diseases is retarded endochondral ossification in the presence of normal membranous ossification, leading to the formation of short bone with normal circumference.

These disorders have been linked to point mutations in the fibroblast growth factor receptor 3 (FGFR3) gene. Most, if not all, of the mutations lead to constitutive activation of FGFR3. FGFR3 is normally expressed by chondrocytes in the resting zone of the growth plate, where it is thought to be important in maintaining their quiescent and undifferentiated state. FGFR3 thus exerts a restraining effect on chondrocyte growth. In this knockout mouse, a point mutation (Gly380Arg), which is responsible for >97% of cases of human achondroplasia was introduced into one allele of the mouse FGFR3 gene. The mutation results in constitutive activation of FGFR3 and leads to excess inhibition of chondrocyte proliferation. The resultant gross and histological alterations closely resemble human achondroplasia. Conversely, mice with targeted FGFR3 inactivation have expansion of the zone of proliferating and hypertrophic chondrocytes and increased length of their vertebrae and long bones.

Achondroplasia is the most common of the cartilage dysplasias in man. Heterozygous and homozygous forms occur, but most cases occur without a family history. Persons affected with heterozygous achondroplasia have shortened proximal extremities, a relatively normal trunk, an enlarged head with bulging forehead, and depressed root of the nose (the last two are due to hypoplasia of the bones of the cranial base, ethmoids, and turbinates). Histologically, regular and well-organized endochondral ossification is seen, consistent with a quantitative disruption.

In domestic animals dwarfism is well described in cattle and in several breeds of dogs and sheep.

AFIP Diagnosis: Humerus: Chondrodysplasia, with medullary osteopenia and growth arrest, FGFR3G374Rneo-/+ heterozygous mouse, rodent.

Conference Comment: During conference, discussion centered on the appearance of the humeral growth plate. The consensus among participants was that there appeared to be a qualitative defect in growth plate chondrocytes. Chondrocytes displayed irregular maturation, varied in size, and lacked an orderly arrangement. Debate ensued as to whether these findings were consistent with achondroplasia in humans. The quantitative defect (decreased length of the femur) was difficult to assess without the benefit of the 2x2 normal control provided by the contributor. References provided by the contributor described and demonstrated lesions with this mouse model which are consistent with human achondroplasia (i.e. chondrocytes which exhibit normal columnization and maturation).

Additional features discussed included the paucity of trabeculae within the medullary canal (osteopenia) and the growth arrest line with transverse trabeculation.

Contributor: Weizmann Institute of Science, Rehobot, Israel 76100

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Brad A. Blankenship, DVM
Captain, 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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