COMPANION OR PET ANIMALS

Osteogenesis and dentinogenesis imperfecta in a four-month-old English mastiff

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SUMMARY
Osteogenesis imperfecta, also known as 'brittle bone disease', is an inherited connective tissue disorder caused by defects in type 1 collagen. The disease results in low bone mass and reduced bone strength, often manifesting as multiple intrauterine fractures, skeletal abnormalities and death before adulthood. A four-month-old, female entire, English mastiff was presented for multiple limb fractures. Due to a poor prognosis, euthanasia was elected. Gross examination revealed diffuse osteopenia with multiple chronic and acute skeletal fractures. All adult teeth were undersized and opalescent, and multiple deciduous incisors were retained. Histopathology of the long bones demonstrated severe, diffuse osteopenia with retention of non-osified cartilage spicules in the secondary spongiosa. The incisor teeth had multifocal disorganisation of odontoblasts and ameloblasts that exhibited piling (dysplasia) and hypoplasia of the dentin. Diagnoses of osteogenesis imperfecta and dentinogenesis imperfecta were made. Osteogenesis imperfecta should be considered as a cause of diffuse osteopenia in young dogs.

BACKGROUND
Osteogenesis imperfecta is a debilitating inherited and congenital disease that can vary from mild clinical signs to perinatal death. Most reports in domestic animals are in calves and lambs; however, rare cases are reported in various breeds of dogs and cats. To the authors’ knowledge, there are no reports in the scientific literature of osteogenesis imperfecta and dentinogenesis imperfecta in a mastiff dog.

CASE PRESENTATION
A four-month-old, entire female, English mastiff puppy was presented to the emergency service of the Texas A&M University Veterinary Medical Teaching Hospital (VMTH) for multiple proximal limb fractures of both acute and chronic durations. Two months earlier, the dog collapsed while on a walk. The referring veterinarian detected a palpable fracture of the right femur and applied a splint to provide stabilisation. Shortly thereafter the dog started to exhibit signs of pain in the left front limb. After confirming a fracture in the left front limb, the dog was referred to the VMTH. There was no history of trauma and the dog’s diet was appropriate (three to five cups of Science Diet Large Breed Puppy food and eight tablets of Pet-Form Vitamin/Mineral Supplement daily).

INVESTIGATIONS
Routine clinicopathological testing was performed. The only abnormalities on haematology and blood chemistry were a mild non-regenerative anaemia (4.91 M/µl; reference interval (RI): 5.63–8.87 M/µl) and a mild increase in gamma-glutamyl transferase activity (4 U/l; RI: 0–2 U/l). Radiographs showed diffuse osteopenia in the bones of the left forelimb and right femur and confirmed both long bone fractures (figure 1; referring veterinarian radiographs unavailable). The left humerus (radiograph taken at the VMTH) had a complete, caudally, laterally displaced, over-riding spiral fracture of the mid to proximal diaphysis. There was subtle, smoothly marginated periosteal proliferation along both the caudal proximal aspect of the distal fracture segment and the distal caudal aspect of the proximal fracture segment. There was mild rounding of the fracture ends. The cortices of the humerus and proximal radius and ulna were thin. The proximal humeral diaphysis was mildly sclerotic. There were striated areas of increased opacity throughout the medullary cavities of the humerus, radius and ulna. The imaged osseous structures were otherwise mildly and diffusely decreased in mineral opacity. The puppy was discharged but returned two weeks later for euthanasia after it sustained a third long bone fracture in the right humerus.

DIFFERENTIAL DIAGNOSIS
Differential diagnoses for osteopenia in a young dog with fractures include the following:
1. Metabolic bone disease (eg, secondary hyperparathyroidism).
2. Nutritional deficiency.
3. Osteogenesis imperfecta.

Figure 1 Orthogonal radiographs of the left humerus taken two weeks before euthanasia. There is a complete, subacute to chronic spiral fracture of the left mid to proximal humeral diaphysis. All bones are diffusely decreased in opacity.
Figure 2 Gross image of the chronic fracture sites. Left: acute, long-oblique to spiral, mildly comminuted, diaphyseal fracture of the right humerus. Middle: chronic, displaced fracture of the left humerus with callus formation. Right: chronic, displaced, over-riding fracture of the right femur with callus formation.

OUTCOME AND FOLLOW-UP
Gross postmortem examination findings demonstrated that all bones were diffusely brittle and had thin cortices that could be cut with a scalpel blade. Cortices of the long bones had a thickness measuring between 0.5 mm and 2 mm. Multiple ribs were easily broken with firm pressure. The most recent of the three long bone fractures involved the right humerus and had a recent spiral fracture. The initial fracture in the right femur and the subsequent fracture in the left humerus each had a callus over-lying a displaced, chronic fracture of the diaphysis (figure 2). Right ribs (8–10) had calluses, presumably from fractures occurring in utero during parturition. All erupted teeth were undersized, mildly translucent with a pink opalescent hue, and they were easily fragmented with moderate pressure (figure 3). Most deciduous incisors were retained.

Histological observations were recorded of the cortices of the diaphyses of the distal right tibia, left radius and ulna, and of the physis of the distal right tibia. The growth plate of the distal right tibia was of anticipated normal thickness when compared with that of another large breed puppy of similar age. Zonation with the appearance and numbers of chondrocytes in each zone was as anticipated for the physis in the affected puppy with the exception of the retention of central cartilage cores and lack of bony remodelling as you transition to the secondary spongiosa (figure 4). Multifocally there were trabecular microfractures and adjacent areas of haemorrhage. Separating trabeculae were abundant, loosely arranged mesenchymal and fibrous connective tissue (myelofibrosis). Haematopoietic tissue was hypocellular. Interspersed throughout the trabeculae were variable numbers of osteoclasts (resorption), which were also free within the fibrous stroma. Small numbers of attenuated osteoblasts line the trabeculae with decreased osteoid production. The periosteum was hypercellular and expanded by multiple rows of fibrous connective tissue (figure 4, inset). Within the diaphysis osteopenia was marked, characterised by a lack of cortical bone and markedly thin trabeculae of the woven bone.

Figure 3 Dentition. All adult teeth were undersized and mildly translucent with a pink, opalescent hue. Multiple deciduous incisors were retained.

Longitudinal sections of the undersized maxillary incisor teeth found that the dentin was thin, and while the superficial dental tubules were initially orderly, after a short distance (<0.5 mm) the tubules became irregular as they approached the surface of the pulp cavity. Instead of lining the pulp cavity surface of the dentin with an orderly, single-file alignment of columnar odontoblasts with cell processes protruding into the openings of dental tubules, the odontoblasts were a haphazard layer of disorganised and piled, polygonal cells that dispersed into the pulp cavity (figure 5). There was artefactual contraction and displacement of the lining of dysplastic odontoblast from the surface of the dentin. Multifocally ameloblasts were shrunken and disorganised with an increased cell layer and loss of their columnar appearance. The normal compact and trabecular alveolar bone was osteopenic with increased space between the trabeculae that was filled with a moderate amount of densely cellular fibrous connective tissue. Multifocally within the fibrous connective tissue were random bony trabeculae that were scalloped with basophilic cementing lines (remodelling). Vessels were often ectatic and congested. A diagnosis of osteogenesis and dentinogenesis imperfecta was made based on the diffuse osteopenia with retention of unossified cartilage spicules from the primary spongiosa, osteoblast atrophy, multifocal odontoblast and ameloblast disorganisation and piling (dysplasia), and dentin hypoplasia.
in dogs with OI include intrauterine fractures, skeletal deformities, postnatal fractures, bowing of the limbs, reduced growth and joint laxity. These abnormalities can result in stillbirth or prenatal death. In cases such as this one with concurrent dentinogenesis imperfecta, the dentin is thin and dysplastic with irregularly oriented tubules and irregular mineralisation. In all instances of OI, the physis remains unaffected since its fibrillar matrix is made up of type II collagen.

The primary differential diagnoses for OI are nutritional/metabolic bone disease, physical abuse and trauma, most of which carry a more favourable prognosis than OI. Of these, secondary hyperparathyroidism is most common. In this case, the puppy had been weaned onto a normal puppy diet and there was no evidence of hyperparathyroidism of renal origin as the parathyroid glands were of normal size and the kidneys were histologically normal. A blood chemistry profile revealed normal levels of phosphorus (6.7 mg/dl; RI: 5.1–10.4 mg/dl) and calcium (9.8 mg/dl; RI: 7.8–12.6 mg/dl). The dog was receiving two times the product’s recommended dosage of vitamin and mineral supplementation. However, percentage, international units (IU) and milligram dosages were still under the maximum dosage allowance for a dog of its size. Medical management of OI is notoriously unsuccessful. In human beings, physiotherapy, rehabilitation and orthopaedic surgery are the mainstay of treatment; however, these treatments are primarily palliative and do nothing to alter the extreme bone fragility.

**DISCUSSION**

Osteogenesis imperfecta in cattle and small ruminants has previously been documented in the Angus, Hereford, Charolais, Belgian blue and Holstein-Friesian cattle breeds and Romney sheep. To the authors’ knowledge, there are no reports in the scientific literature of OI in a mastiff dog. OI in dogs is inherited by autosomal dominant and/or recessive modes with gene mutations discovered in COL1A1 (golden retrievers), COL1A2 (beagles) and SERPINH1 (dachshunds). COL1A1 and COL1A2 code for the α1 and α2 collagen chains that are responsible for the production, folding, maintenance of structure and secretion of type 1 collagen. SERPINH1 encodes for an essential collagen chaperone protein, heat shock protein 47 (HSP47). This protein is involved in the correct folding of collagen and stabilises the collagen triple helix. For other breeds of dog, the underlying genetic defect has not been determined. Genetic testing for only the COL1A2 mutation was done in this case due to cost but was negative; thus, the affected gene in this case remains unknown.

OI is characterised by excessive bone fragility with a wide array of clinical signs and variations in severity. Many dogs initially present with lameness due to a fracture with no history of trauma, as in this case. The dog in this report had multiple long bone fractures in different limbs at different time intervals during its short lifetime of four months. The first two fractures involving the mid-diaphyses of the right femur and left humerus had, by the time of the terminal fracture in the right humerus, shared a similar ineffective and delayed healing effort with an external callus that failed to stabilise the fracture line. The dog’s bone marrow was extensively hypocellular and replaced with multifocal areas of myelofibrosis, likely contributing to the non-regenerative anaemia. Abnormal clinical findings in the skeleton that have been previously described

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