The pathology of vitamin D deficiency in domesticated animals: An evolutionary and comparative overview

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ABSTRACT

Although vitamin D is critical to calcium/phosphorus homeostasis, bone formation and remodeling, there is evolution-based variation between species in vitamin D metabolism and susceptibility to rickets and osteomalacia. Most herbivores produce vitamin D₃ in response to sunlight, but dogs and cats have generally lost the ability as carnivore diets are rich in vitamin D. Nutritional deficiencies and/or poor exposure to sunlight can induce rickets in birds, swine, cattle and sheep, but horses are less susceptible as they have evolved a calcium homeostasis that is quite different than other animals. Adaptations to specific environments also affect disease incidence: llamas/ alpacas out of their natural high altitude intense solar radiation environments are highly susceptible to vitamin D deficiency. The pathology of rickets/osteomalacia is similar across species, however fibrous osteodystrophy is more common and may also be present. Rickets/osteomalacia were likely more common in animals before the advent of commercial diets, but can be difficult to definitively diagnose especially in single archeological specimens. Consideration of species susceptibility, location – especially in terms of latitude, and any available information on diet, season of occurrence, husbandry practices or descriptions of affected animals can support the diagnosis of metabolic bone disease in animals.

1. Introduction: evolutionary origins of susceptibility to vitamin D deficiency disease

To understand susceptibility to vitamin D deficiency disease, it is useful to take an evolutionary perspective (Fig. 1). Of particular importance to understanding the pathogenesis of the bone lesions, is consideration of the role of vitamin D in the regulation of phosphorus as well as calcium, and the related roles of parathyroid hormone (PTH) and the phosphatonin fibroblast growth factor 23 (FGF23). Most of the mediators involved in calcium-phosphorus homeostasis evolved in fish, although their roles were repurposed during the adaptation to land environments (Bouillon and Suda, 2014; Doherty et al., 2015). Thus, while susceptibility to vitamin D deficiency in vertebrates goes back to fish, the specifics of how it is manifested as disease has both similarities and differences to what is observed in humans.

Because of their vital roles in numerous cell functions, calcium and phosphorus are very tightly regulated in all animals. During the colonization of land, vertebrates transitioned from a sea environment that was comparatively richer in calcium than phosphorus, to a land environment with a six fold higher gravity that was poor in calcium and generally richer in phosphorus (Bouillon and Suda, 2014; Doherty et al., 2015). This led to the evolution of major adaptations to bone structure and calcium/phosphorus regulation. These adaptations included a highly efficient system for intestinal calcium absorption and flexible bone remodeling, which allowed bone to be used as an internal reservoir of calcium to compensate for variations in dietary intake (Bouillon and Suda, 2014). Three main hormones facilitated these vertebrate adaptations and coordinate the overall homeostasis of calcium and phosphorus: PTH, the vitamin D endocrine system and FGF23 (Bouillon and Suda, 2014).

Vitamin D appears early in the evolution of life as an end product of a photochemical reaction of 7-dehydrocholesterol, which explains why sun exposure increases vitamin D levels in most species (Bouillon and Suda, 2014). However, because of subsequent adaptations, this capacity varies across species. For example, in the cat and to some extent the dog, dermal concentrations of 7-dehydrocholesterol are too low to produce vitamin D through UVB exposure, and they are more dependent on their carnivorous diet, which contains good sources of vitamin D (blood, fat), phosphorus (meat) and calcium (bones) (Bouillon and Suda, 2014). In contrast, llamas and alpacas adapted their dermal vitamin D production to the high actinic environments of South American mountain ranges and their susceptibility to vitamin D deficiency is increased when they are kept at lower altitudes in temperate regions, especially during the winter and early spring (Dittmer and...
teoblasts and osteocytes in response to 1,25(OH)2D3, PTH and long malacia being relatively rare in equids, although intestinal absorption of calcium, which may contribute to rickets/osteomalacia. Horses, in particular, are less dependent on vitamin D for calcium homeostasis in terrestrial vertebrates, as it acts to raise blood calcium through stimulation of bone breakdown by osteoclasts (Bouillon and Suda, 2014; Brenza et al., 1998; Hardcastle and Dittmer, 2015). PTH is a potent inducer of renal synthesis of 1,25(OH)2D3 (the hormonally active metabolite of vitamin D) which in turn stimulates active intestinal absorption of calcium (Bouillon and Suda, 2014; Brenza et al., 1998; Hardcastle and Dittmer, 2015). Species differences in the relative importance of vitamin D for the intestinal absorption of calcium also impact susceptibility to the effects of vitamin D deficiency (Wilkens et al., 2017). Horses, in particular, are less dependent on vitamin D for intestinal absorption of calcium, which may contribute to rickets/osteomalacia being relatively rare in equids, although fibrous osteodystrophy (see below) is comparatively common.

Because our understanding of calcium-phosphorus metabolism originated from early studies of rickets, the regulation of phosphorus in metabolic bone diseases has generally been considered of secondary importance to the control of calcium by PTH and vitamin D (Hardcastle and Dittmer, 2015). However, both calcium and phosphorus are critical elements of the hydroxyapatite crystals in the bone matrix, and recent discoveries of important regulators of phosphorus metabolism, in particular the hormone FGF23, have provided a more complete picture of the pathogenesis of bone diseases, especially those affecting bone mineralization (Hardcastle and Dittmer, 2015). FGF23 is secreted by osteoblasts and osteocytes in response to 1,25(OH)2D3, PTH and long term increases in serum calcium and phosphorus (Hardcastle and Dittmer, 2015). FGF23 inhibits PTH secretion, decreases serum phosphorus by inhibiting renal absorption and reduces activation of vitamin D3 (Hardcastle and Dittmer, 2015). Gene mutations that result in excessive FGF23 signaling have been found in several cases of congenital hypophosphatemic rickets, while a mirror image disease, tumoral calcinosis has been associated with a lack of FGF23, which is characterized by increased serum phosphorus but decreased renal phosphorus excretion, normal to increased vitamin D3 levels and generalized soft tissue mineralization (Georges et al., 2016; Hardcastle and Dittmer, 2015).

The emerging picture is that hormonal regulation of calcium and phosphorus by PTH, the vitamin D endocrine system and FGF23 occurred early in the evolution of vertebrates and was critical for their adaptation to land environments. Subsequent to the colonization of land, the relative roles and importance of its various components has changed as vertebrates adapted to different niches, which has affected species susceptibility to disease (Fig. 1). Overall, this system functions to maintain serum calcium and phosphorus homeostasis through the tightly coordinated regulation of bone mass. The disease tradeoff is that under some conditions calcium/phosphorus homeostasis will be maintained at the expense of the bones.

2. Metabolic bone disease: comparative considerations

In veterinary medicine, metabolic bone disease is a broad term for pathological conditions affecting multiple bones. They are most commonly caused by genetic, nutritional and/or hormonal abnormalities affecting bone growth and remodeling, usually through disruptions in calcium/phosphorus metabolism. Traditionally, they have been broken down into rickets/osteomalacia, fibrous osteodystrophy and osteoporosis; however, in many cases, especially in those induced by nutritional deficiencies, it is difficult to specifically classify an affected individual, as multiple conditions may be present. This means that cases reported in the literature should be carefully scrutinized, and those confirmed by histopathology considered more definitive (Dittmer and Thompson, 2011). Additionally, in some non-mammalian species, particularly reptiles, which appear to have a combination of fibrous osteodystrophy and osteomalacia in response to deficiencies, the more generic diagnosis of ‘metabolic bone disease’ has typically been used (Dittmer and Thompson, 2011). The pathogenesis and lesions of rickets/osteomalacia in modern domesticated species whose remains are likely to be found in archeological settings are considered in detail in the following sections. However, a brief overview of the pathological features of osteoporosis and fibrous osteodystrophy is useful, as lesions of these metabolic bone diseases can be present with those of rickets/osteomalacia.
Osteoporosis is the most common of the metabolic bone diseases in animals and humans (Craig et al., 2016) However, osteoporosis is a lesion, not a specific disease, and occurs when bone resorption chronically exceeds bone formation; it can be induced by a variety of conditions that either increase bone resorption and/or decrease bone formation. The result is bone loss with the remaining bone being of normal size and shape; if the condition is severe, bone mass is noticeably reduced and pathological fractures are often present (Fig. 2). Confirmation of mild to moderate cases of osteoporosis in animals is difficult, as parameters for objective measurements of bone loss, like those used in human cases, have not been determined for all species. In domestic animals kept in groups, a high incidence of fractures is suggestive, although severe collective trauma must also be considered (Craig et al., 2016). Gross lesions are most pronounced in areas of cancellous or trabecular bone, with vertebral bodies, fl at bones of the skull, scapula, ilium, and ribs being especially affected (Craig et al., 2016). In the long bones, loss of cancellous and cortical bone with the formation of prominent transverse trabeculi, can be observed (Craig et al., 2016) (Fig. 2). Causes of osteoporosis in modern animals are varied and include: starvation, age, disuse, lactation (under poor
nutritional conditions), uncomplicated calcium deficiency (dietary phosphorus and vitamin D levels are optimal—is rare outside of experimental conditions), severe gastrointestinal parasitism and corticosteroid therapy (Craig et al., 2016).

In contrast to osteoporosis, both fibrous osteodystrophy and rickets are characterized by distinctive pathological changes in addition to loss of bone. Fibrous osteodystrophy (osteodystrophia fibrosa, osteitis fibrosa cystica) is a relatively common metabolic bone disease in animals that can occur in combination with rickets. It is characterized by extensive osteoclastic bone resorption and fibrosis (Fig. 3). The proximate cause of fibrous osteodystrophy is prolonged elevation of PTH, which can result from a parathyroid tumor (primary hyperparathyroidism), dietary imbalance in calcium and phosphorus, and, in some species, chronic renal disease (secondary hyperparathyroidism) (Craig et al., 2016). Secondary hyperparathyroidism is much more common in animals than the primary variant. As is the case with rickets, animal species appear to vary in their susceptibility to fibrous osteodystrophy: horses, pigs, dogs, cats, New World primates and reptiles being more...
likely to be affected than sheep and cattle (Craig et al., 2016). Because of the close interactions between PTH, FGF23 and vitamin D in the regulation of calcium and phosphorus metabolism, all three mediators are often involved in the pathogenesis of fibrous osteodystrophy. Nutritional secondary hyperparathyroidism in animals can be induced by dietary deficiency of calcium, excess dietary phosphorus or in association with vitamin D deficiency (Craig et al., 2016). The distribution of the lesions of fibrous osteodystrophy also varies across species, but the lesions are typically characterized by bilateral enlargement of the bones of the skull, especially around the teeth in the maxilla and mandible. This enlargement is due to resorption of bone by osteoclasts and its replacement by fibrous tissue, which makes the bones porous and prone to fracture (Fig. 3). That fibrous osteodystrophy affects the bones of the skull as well as those of the body and limbs is useful to distinguish it pathologically from rickets: a disease of endochondral ossification with often pronounced effects on the growth plates of the long bones. Growth plates are normal in cases of fibrous osteodystrophy unless the animal also has rickets (Craig et al., 2016).

3. General pathology of rickets and osteomalacia in animals

Rickets and osteomalacia have similar causes and pathogenesis; pathologically, rickets is a disease of the developing skeleton characterized by abnormal endochondral ossification and defective bone
formation, while in adults with osteomalacia only bone formed during remodeling is affected. Both diseases occur in all domesticated species and in wildlife, but there are differences between species as to their specific causes and inciting environmental conditions (Craig et al., 2016). Defective mineralization is the primary feature in both rickets and osteomalacia, although the lesions vary depending on cause and duration, as well as age, growth rate and activity of the affected animal. In addition, dietary deficiencies are often multiple, resulting in lesions overlapped with those of other metabolic bone diseases, which can complicate interpretation (Craig et al., 2016).

Although any interference with maturation and mineralization of cartilage and bone matrix can induce characteristic lesions, the most common causes of rickets and osteomalacia in animals are dietary deficiencies of vitamin D or phosphorus. While calcium is required for mineralization, it is unclear that an uncomplicated calcium deficiency will induce rickets. Calcium is unlikely to be a limiting factor at sites of mineralization as any decrease in extracellular concentration of calcium is rapidly corrected by the action of PTH and vitamin D (Craig et al., 2016). Persistent dietary deficiency of calcium is therefore more likely to lead to fibrous osteodystrophy or osteoporosis due to hyperparathyroidism and excessive bone resorption. However, vitamin D deficiency will induce hypocalcemia, which in turn will stimulate the release of PTH, thus concurrent lesions of both rickets and fibrous osteodystrophy can be present, especially in vitamin D deficiency rickets (Craig et al., 2016). Hereditary cases of rickets resulting from genetic defects have been described in several species including humans, sheep, pigs and domestic cats (Dittmer and Thompson, 2011; Papadopoulou et al., 2013). These genetic mutations have primarily resulted in defects in vitamin D synthesis or abnormalities in phosphorus metabolism, which supports the central role of both vitamin D and phosphorus in the pathogenesis of rickets (Papadopoulou et al., 2013).

Rickets is induced by defective endochondral ossification and thus can only occur if the overall dietary conditions are adequate enough to allow the animal to grow. A suggestive history is that young animals in a period of rapid growth are most affected and that their legs start to bow as they grow, although similar limb deformities are also observed in other conditions, in particular chondrodysplasia, which has been selected for in some breeds and avian tibial dyschondroplasia (Leach and Monsonego-Ornan, 2007; Parker et al., 2009). The lesions of rickets are similar across species, are found in multiple bones, and are most prominent at sites of rapid growth, which include the metaphyseal and epiphyseal regions of the long bones and the costochondral junctions (note: the costochondral junctions are normally prominent in many fast growing animals and any enlargement should be interpreted carefully). In animals with rickets, the growth plate is irregularly thickened, the cortex is thinned and the bones are poorly mineralized. Limb deformities and/or pathological fractures, as well as areas of articular cartilage damage secondary to collapse of the subchondral bone may also be present. Histologically two major features characterize rickets: expansion of the hypertrophic chondrocytes in the growth plate and accumulation of unmineralized bone matrix (osteoid) (Fig. 4). At the tissue level, the effects of hypophosphatemia best explains these features, as low phosphorus both blocks apoptosis in the hypertrophic chondrocytes and inhibits mineralization of osteoid (Tiosano and Hochberg, 2009).

Accumulation of unmineralized osteoid also characterizes osteomalacia in adult animals. Osteomalacia affects bone remodeling resulting in the excessive deposition of unmineralized osteoid where mechanical forces are the strongest (i.e.: entheses and stress oriented trabeculae). The result is that affected bones have reduced resistance to mechanical stress and are prone to fracture in severe cases (Craig et al., 2016). However, because full examination of the skeleton at necropsy is not routinely performed, osteomalacia in animals is likely under diagnosed.

Although essential for bone development and formation, vitamin D is highly toxic and chronic excess can induce bone lesions as well as chronic deficiency. Vitamin D poisonings in animals can result from ingestion of plants (i.e.: Solanum spp., Cestrum diurnum, and Trisetum flavescens) containing various forms of vitamin D including vitamin D$_2$ and 1,25-dihydroxycholecalciferol-glycoside, excess dietary supplementation, or ingestion of rodenticides containing cholecalciferol (vitamin D$_3$) (Craig et al., 2016; Crossley et al., 2017). In vitamin D toxicity, intestinal calcium absorption is increased, as is mobilization of calcium from the bone, while excretion from the kidney is reduced (Selby et al., 1995; Vieth, 1990). The result is hypercalcemia and hyperphosphatemia, which, if chronic, results in soft tissue mineralization (Craig et al., 2016; Lopez et al., 2016). Death from renal failure usually occurs before there are noticeable changes in the skeleton; however, bone lesions may be found, especially if the toxic doses of vitamin D are intermittent (Craig et al., 2016; Lopez et al., 2016). An early response to high levels of vitamin D is an increase in osteoclastic activity, which can lead to bone resorption, while over time a disorganized matrix produced by osteoblasts accumulates resulting in sclerotic lesions (Craig et al., 2016). All bones are affected but the lesions are most prominent in the long bones at the sites of rapid growth.

4. Poultry

Vitamin D and its metabolites have been well studied in chickens; however, rickets is still an economically important problem in the modern poultry industry, especially in fast growing birds raised for meat (Dinev, 2012; Edwards, 2000). Rickets in modern birds usually occurs between 2 and 4 weeks of age and can be induced by disturbances in calcium, vitamin D or phosphorus metabolism secondary to dietary deficiencies (Dinev, 2012). Clinically, it is characterized by poor growth, weakness, lameness, inability to stand, and prominent valgus or varus deformations of the femur and/or tibiotarsus (Dinev, 2012). Deformation of the sternum and vertebrae may also be present. As is typical of rickets in other species, the bones are soft, the growth plates are enlarged and pathological fractures may be present. In birds, rickets induced by calcium or vitamin D deficiency is characterized by abnormal ossification with irregularity of the cartilaginous zone of proliferation, while in cases of hypophosphatemic rickets expansion of the hypertrophic cartilage is observed (Dinev, 2012). Rickets has also been diagnosed in waterfowl living in public parks and fed inappropriate foods by the public (Ewbank et al., 2013). Familiarity with species differences in normal morphology is especially important in interpreting growth plates in birds, as the zone of hypertrophied cartilage is larger than in mammals.

Poultry are also susceptible to tibial dyschondroplasia, which is a disease of complex etiology induced by rapid growth rates and clinically characterized by lameness and leg deformities (Leach and Monsonego-Ornan, 2007). Lesions of tibial dyschondroplasia are similar to that of physeal osteochondrosis in pigs and foals and consist of accumulations of poorly vascularized cartilage extending into the proximal metaphysis of the tibiotarsus and tarsalmetatarsus (Leach and Monsonego-Ornan, 2007). The retained growth plate chondrocytes have gene expression profiles compatible with endoplasmic reticulum stress responses that are similar to those observed in forms of chondrodysplasia (Cameron et al., 2011; Leach and Monsonego-Ornan, 2007). Environmental as well as genetic factors are important in disease pathogenesis, as, although tibial dyschondroplasia is one of the most common problems in modern poultry, it has not been observed in chickens living in Tibet (Huang et al., 2017a, 2017b). This is most likely due to metaphyseal angiogenesis being stimulated by living in a hypoxic high altitude environment (Huang et al., 2017b). Tibial dyschondroplasia has been described in 19th century turkey tibiotarsi found at the Royal London Hospital (Fothergill et al., 2012). Although bowing of the tibiotarsus was present, rickets was considered less likely because the distal metaphysis and distal articular surface were not affected (Fothergill et al., 2012).
5. Cattle

There are few published reports of rickets or osteomalacia in cattle, which was described by Sir Arnold Thieler in South Africa in the early 20th century (Dittmer and Thompson, 2011; Prozesky et al., 2016; Thieler, 1931). The reported clinical signs included: osteophagia, stiff or lame gait, swollen joints and spontaneous fractures; interestingly some affected animals died of botulism from ingestion of contaminated bone (Thieler et al., 1927). Lesions of rickets and osteomalacia in cattle are similar to those reported in other species: soft bones, irregular enlargement of the growth plates due to expansion of the hypertrophic cartilage with tongues of cartilage extending into the metaphysis (Dittmer and Thompson, 2011; Thompson and Cook, 1987). Posterior paralysis from vertebral fractures has also been reported (Thompson and Cook, 1987). Drought conditions and forages low in phosphorus (e.g.: swede – *Brassica napus*) have been contributing factors in several described outbreaks (Dittmer and Thompson, 2011; Rose, 1954; Thompson and Cook, 1987). Rickets in calves kept indoors and fed oats, sugar beet pulp, barley, hay and raw potatoes has also been described; interestingly cows on the same farm had evidence of fluorosis, which can stimulate bone formation and thus potentially exacerbate rickets (Dittmer et al., 2009; Spratling et al., 1970).

‘Stiffness in the extremities disease’ occurs in wild yaks (*Bos mutus*) grazing low phosphorus forage on the Qinghai-Tibetan Plateau; it is characterized by emaciation, lame, stiff gait, enlargement of the costochondral junctions and curvature of the long bones (Shen and Zhang, 2012). The disease primarily affects juveniles, pregnant and post-partum females and has a peak incidence between June and September; 60% of the yaks observed in one study were affected and mortality reached 27% of the animals in severely affected areas (Shen and Zhang, 2012). Involvement of the mandible was described and is suggestive of a fibrous osteodystrophy component. The presence of healed rib fractures in the adult females indicate the disease was likely chronic and recurring (Shen and Zhang, 2012). According to herders, the disease has been present for the last 10 years (Shen and Zhang, 2012). It was also noted that the affected area contained excellent autumn-winter forage until fences fragmented the pastures (Shen and Zhang, 2012). Likely related to this, female yaks have changed their habitat use over documented before heavy human exploitation of the Tibetan plateau and now graze on steeper post-glacial terrain and depend more on winter snow patches than males (Berger et al., 2015).

6. Sheep

For grazing ruminants, almost all vitamin D comes from dermal photobiogenesis, as green grasses are poor sources of Vitamin D (Smith and Wright, 1984). Pigmented sheep with heavy fleeces have reduced vitamin D biosynthesis compared to shorn sheep with white faces and legs (Mearns et al., 2008). UV radiation is adequate for photobiogenesis of vitamin D only between mid-March-mid September at latitudes greater than 55°N (Smith and Wright, 1984). One study found that while both sheep and goats are able to produce vitamin D in their skin, sheep were more dependent than goats on dietary sources, which possibly explains why there are comparatively few reports of rickets in goats (Kohler et al., 2013). With the exception of a recently described genetic mutation in Corriedale sheep with similarities to autosomal recessive hypophosphatemic rickets in humans, all reported cases of rickets in sheep are of nutritional origin (Dittmer et al., 2009; Zhao et al., 2011). A common scenario is that the affected animals were fed cereal crops, in particular lush rye grass in winter, containing high concentrations of carotene, an antagonist to the action of vitamin D in both the intestine and bone (Grant and O’Har, 1957; Mearns et al., 2008). Outbreaks of rickets in sheep have also been associated with high latitude, periods of decreased sunshine and forage low in phosphorus (Dittmer and Thompson, 2011).

Rickets is most common in recently weaned, rapidly growing lambs; however, serum vitamin D levels in sheep are lowest in late winter, when the fleece is the heaviest, and females pregnant at this time are also at risk (Dittmer and Thompson, 2011). Recently, nutritional congenital rickets was documented in the lambs of ewes grazed over the winter on fodder beet (Dittmer et al., 2017). Clinically and pathologically, the features of rickets in sheep are similar to those of other species: affected animals are in poor condition, have a stiff to lame gait, enlarged growth plates and deformation of their limbs.

7. Pigs

Under modern indoor production conditions, piglets grow rapidly and are weaned early, making them susceptible to rickets if dietary vitamin D is inadequate (Dittmer and Thompson, 2011). Barn designs that restrict exposure to sunlight have also been a factor in outbreaks, and dietary sources of vitamin D are considered a necessity for pigs housed inside (Madson et al., 2012). This means that primarily plant-based diets must be supplemented, as they do not contain vitamin D3 and only minor amounts of vitamin D2 (Madson et al., 2012). Affected piglets may have muscle tremors, are lame and reluctant to move, preferring a dog sitting or in a hunched back posture, and can die suddenly from hypocalcemia; they have soft bones, enlarged costochondral junctions and growth plates, and both acute and chronic fractures can be present (Madson et al., 2012). Lesions of fibrous osteodystrophy are often present in piglets with rickets as hypocalcemia is usually a prominent component (Dittmer and Thompson, 2011; Madson et al., 2012). The molecular basis for pseudo-vitamin D deficiency rickets (PDDR) in Hanover pigs, which is characterized by the inability to maintain ambient levels of 1,25-dihydroxyvitamin D, is due to expression of a non-functional P450C1 enzyme (CYP27B1) was found to be identical to that of PDDR in humans (Chavez et al., 2003).

8. Horses

Rickets is very rare in horses, and thus suspected cases should be thoroughly documented and fibrous osteodystrophy, which is comparatively much more common, specifically ruled out (Dittmer and Thompson, 2011). The likely reason horses are relatively resistant to rickets is because they have evolved a calcium homeostasis that is quite different than other animals. Compared to most species horses have a high intestinal absorptive capacity for calcium, high plasma calcium concentrations, high renal excretion of calcium and low plasma concentrations of vitamin D metabolites (Breidenbach et al., 1998). In fact, the normal levels of plasma vitamin D metabolites in the horse are lower than those inducing rickets in other species (Breidenbach et al., 1998). It has also been documented that intestinal calcium transport in horses is not as dependent on vitamin D as it is in other species (Wilkins et al., 2017). Interestingly, horses were more susceptible to fibrous osteodystrophy than ruminants when grazed on grasses contain oxalates, which bind calcium making it unavailable for absorption (Walthall and McKenzie, 1976). Bacterial activity can release the calcium from oxalates, but in the horse calcium absorption occurs in the small intestine while the bacterial activity is in the colon and cecum. In contrast, microbial breakdown of calcium oxalate occurs in the rumen proximal to the small intestine in ruminants.

9. Llamas, alpacas and camels

Llamas and alpacas are adapted to high altitude environments with high levels of solar radiation; consequently, they are susceptible to rickets when kept in other parts of the world, particularly during the winter and spring in temperate climates. In one study cria (juvenile
llamass) born in autumn/winter in the Northern Hemisphere had lower vitamin D levels and were more likely to develop rickets than those born in the summer (Van Saun et al., 1996). Another study from Australia found that young alpacas with dark fleeces had lower vitamin D levels than those with light fleeces in late winter (Judson et al., 2008). As in other species, crias with rickets often have angular limb deformities (McClanahan et al., 2006).

There have been few reports of rickets or osteomalacia in camels. However, studies of Bactrian camels (Camelus bactrianus) in Inner Mongolia found incidences of 32.9% for rickets and 27.8% for osteomalacia, with a marked increased incidence during drought conditions (Zongping, 2005). Outbreaks usually occurred between February and June, with pregnant females being the most affected by osteomalacia (Zongping, 2005). Young animals with rickets had stiff gait, leg deformities and enlargement of the costochondral junctions (Zongping, 2005). Adults affected with osteomalacia had fragile bones, general weakness, lameness, emaciation, stiffness and were observed chewing bones (osteoaphagia is also observed in cattle with hypophosphatemia), rocks and other objects; healed rib fractures were common, especially in adult females, indicating the condition was chronic and likely seasonally reoccurring (Zongping, 2005). Decreased phosphorus and copper levels were found in the affected camels as well as in the soil and forage; treatment with mineral supplementation and vitamin D resulted in clinical improvement (Zongping, 2005). Old herdsmen confirmed that the diseases had a long history in the area, although it had not been previously studied (Zongping, 2005). This report also mentions that the mandible was often affected and that PTH levels were elevated, which indicates fibrous osteodystrophy may also have been present.

10. Dogs and cats

There are few reports of naturally occurring rickets in dogs and cats, and fibrous osteodystrophy has not been adequately excluded as a primary cause of the lesions in all cases (Dittmer and Thompson, 2011). Unlike the herbivore diet, the carnivore diet, in particular fat, liver and blood, is rich in vitamin D. Cats and dogs have thus lost the ability to form adequate levels of vitamin D3 through dermal photosynthesis (How et al., 1994). Meat is also very high in phosphorus, making it extremely difficult to induce hypophosphatemic rickets unless no meat is fed; however, diets containing only meat and no bones will induce nutritional secondary hyperparathyroidism and fibrous osteodystrophy in both species.

Selective breeding and spontaneous gene mutations have also affected the vitamin D pathway and susceptibility to rickets in dogs and cats (Dittmer and Thompson, 2011; Grahn et al., 2012). Dogs have the highest phenotypic diversity of any mammal, there being a 200X difference in size between some of the giant and miniature breeds. This large variation in size is associated with both greatly accelerated and reduced growth rates and differences in the regulation of vitamin D, particularly at the growth plates, that can potentially impact susceptibility to rickets (Hazeuwinkel and Tryfonidou, 2002; Tryfonidou et al., 2010). The lesions of rickets in dogs and cats are similar to those described in other species: poor growth, soft bones, gait abnormalities, bowing of the legs and enlarged growth plates, most commonly in the distal limbs (Grahn et al., 2012; Malik et al., 1997). However, fibrous osteodystrophy is more common and should always be considered as a differential especially if the mandible and maxilla are involved (Fig. 3) (Dittmer and Thompson, 2011). In addition, chondrodysplasia can induce bowing of the long bones similar to that observed in rickets, and has been selected for in some dog breeds.

11. Summary and implications for animal paleopathology

Calcium/phosphorus homeostasis is critical to life and is closely regulated by a variety of mediators including vitamin D, PTH and FGF23 (Bouillon and Suda, 2014). These mediators are ancient and originally appeared in fish; however, they were repurposed through evolution as vertebrates adapted to the high gravity/low calcium conditions, a wide variety of diets and variation in solar radiation characterizing land environments (Bouillon and Suda, 2014). The ancient origin and fundamental importance of the mediators of calcium/phosphorus homeostasis mean that while all vertebrates share a basic dependence on them, their source and specifics of function differ across species because of subsequent adaptations to diet and environment. It is this evolution-produced variation that explains species differences in susceptibility to diseases of calcium/phosphorus metabolism like rickets and osteomalacia. Thus, although there are similarities in general pathogenesis, it should not be assumed that the specific factors inducing rickets in animals are exactly the same as those causing the disease in humans.

In spite of the species difference in susceptibility, the lesions of rickets and osteomalacia are generally similar same across species as they result from defective mineralization of bone: pathologically, rickets is a disease of endochondral ossification resulting in enlarged growth plates and costochondral junctions, while osteomalacia is a disease affecting re-modeling that leads to softening of the bones. Rickets can induce limb deformities, and both can predispose to pathological fractures. Rickets has been described in a variety of animals, however, not all cases in the literature have used the same pathological criteria or been confirmed histologically. In particular, fibrous osteodystrophy, which is more common in most species, has not always been ruled out. Fibrous osteodystrophy rather than rickets should be the main pathological differential for bone loss around teeth and swelling in the mandible/maxilla, as these bones form by intramembranous bone formation rather than endochondral ossification. It should also be considered that rickets and fibrous osteodystrophy often occur together in animals (Craig et al., 2016; Dittmer and Thompson, 2011). While rickets is distinctive as a disease affecting growing animals and causing leg deformities, osteomalacia, especially in adults, is probably under diagnosed in veterinary medicine (Dittmer and Thompson, 2011).

Historically, metabolic bone diseases were likely more common in animals before the advent of commercial diets, but these diseases can be difficult to definitively diagnose especially in single archaeological specimens. While several paleopathological studies have diagnosed metabolic bone diseases in animal remains (Leshchinsky, 2009; Nerlich et al., 1993; von den Driesch, 1989), a major problem in animal paleopathology is the lack of definitively documented reference parameters, which means that unlike in humans, the cut offs for normal variation versus pathological change in most animal species have not been established. For example, osteoporosis in humans is defined as bone mass 2.5 standard deviations below the young adult mean, but in most animals such precise data is not available, making the cut-off between a normal and osteoporotic bone somewhat subjective (Craig et al., 2016). In addition, selective breeding has produced great variation within domestic species, especially in dogs, which means that the parameter will likely have to be determined for individual breeds as well as for species. Diagnostic standards based upon more objective criteria are needed for animals, and a few studies to define and utilize variation in bone parameters for diagnosis of metabolic bone disease within animal populations have been performed (Horwitz and Smith, 1990; Martinakić et al., 2008). Unfortunately, metabolic bone diseases are characterized by bone loss and often markedly increased fragility, which makes affected bones less likely to be preserved.

The comparative susceptibility of domesticated animals to rickets based upon reported cases is summarized in Table 1. Bilateral bowing of the long bones of the limbs with swelling in the epiphyseal and metaphyseal regions and at the costochondral junctions is suggestive of rickets. Generalized radiolucency, thickened cortices with narrowing of the medullary cavity may also be observed radiographically, although species differences, especially in anatomy, need to be considered, as these criteria have not been confirmed in all species. In domestic animals, diet and husbandry are critical factors and have changed over...
Table 1
Summary of Rickets in Domestic Animals Based upon Reported Cases.*

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<th>Species</th>
<th>Relative Susceptibility</th>
<th>Factors/Considerations</th>
<th>Reported Causes</th>
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<tr>
<td>Poultry</td>
<td>High</td>
<td>Require UV light or vitamin D supplementation</td>
<td>Dietary vitamin D or phosphorus deficiency; dark housing</td>
</tr>
<tr>
<td>Sheep</td>
<td>Medium</td>
<td>Are more susceptible than cattle; heavy wool coat/dark pigment deases</td>
<td>Grazing plants with high carotene and/or low phosphorus; low sunlight</td>
</tr>
<tr>
<td>Cattle</td>
<td>Low</td>
<td>Affected animals have pica and eat bones</td>
<td>Dietary phosphorus deficiency most common</td>
</tr>
<tr>
<td>Pigs</td>
<td>Medium</td>
<td>Omnivore: potentially more susceptible if fed primarily a plant versus meat diet</td>
<td>Dietary vitamin D deficiency; dark housing</td>
</tr>
<tr>
<td>Llamas/Alpacas</td>
<td>High</td>
<td>Evolved at high altitudes exposed to high solar radiation, pigmentation decreases</td>
<td>Being kept in areas with low solar radiation; dietary deficiencies</td>
</tr>
<tr>
<td>Horses</td>
<td>Very Rare</td>
<td>Less dependent on vitamin D for intestinal absorption of calcium; Normally very low plasma levels of vitamin D; fibrous osteodystrophy is more common</td>
<td>Are less susceptible to vitamin D or phosphorus deficiencies</td>
</tr>
<tr>
<td>Dogs/Cats</td>
<td>Rare</td>
<td>Carnivore diet is high in vitamin D and phosphorus; lost ability to make vitamin D in the skin; fibrous osteodystrophy is more common</td>
<td>Dietary deficiency of vitamin D; gene mutations have been described</td>
</tr>
</tbody>
</table>

* Note: the lesions of rickets are the same across species (see descriptions in text).

time. Other factors that can support a diagnosis of rickets include susceptible species (see Table 1), diet (herbivores grazing cereals, low phosphorus plants) and location (Northern latitudes—many modern cases and those of the past are from Scotland (Baker and Brothwell, 1980). Information from historical documents can also be of potential use in making a retrospective diagnosis of rickets, or providing suspicion that it was likely a problem; for example: comments about an outbreak of leg deformities and increased mortality in young, growing animals during the winter/early spring months, especially following droughts, observations of osteophagia and descriptions of husbandry practices like fragmentation of pastures by fencing and dark housing.

References


