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Original Article
Livestock Diseases

Osteoporosis in swine[1]

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ABSTRACT. - Lorenzett M.P., Cecco B.S., Bianchi M.V., Cruz R.A.S., Linhares D.C.L., Guedes R.M.C., Driemeier D. & Pavarini S.P. 2022. **Osteoporosis in swine.** *Pesquisa Veterinária Brasileira 42:e07068, 2022*. Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, prédio 42505, Porto Alegre, RS 91540-000, Brazil. E-mail: sauloppvet@yahoo.com.br

Forty-six pigs presented muscle weakness, hind limb paresis and paralysis, weight loss, lateral recumbency, and death in a clinical course of 7 to 10 days. Two pigs were necropsied and exhibited bone fragility, bone callus formation, and multiple fractures in the limbs, ribs, and vertebrae. Microscopically, there was a diffuse and marked decrease in thickness and number of trabeculae. These were disconnected, with a "free-floating" appearance, while the cortex of the long bones was thinned, with an increase of the cortical porosity by enlargement of Haversian canals and endosteal erosion and decreased osteoblastic activity. Flame atomic absorption spectrometry in liver samples revealed significant zinc overload (>2300ppm) and copper deficiency (<33.1ppm). In this communication, we present the first pathologic description of an outbreak of osteoporosis in pigs, and we also provide a brief review of metabolic bone diseases in pigs.

INDEX TERMS: Swine, bone diseases, bone fragility, metabolic disorder, zinc, copper

RESUMO.- [**Osteoporose em suínos.**] Quarenta e seis suínos apresentaram fraqueza muscular, paresia e paralisia de membros pélvicos, perda de peso, decúbito lateral e morte, com um curso clínico de 7 a 10 dias. Dois suínos foram submetidos a necropsia e exibiram fragilidade óssea, formação de calo ósseo, e múltiplas fraturas em membros, costelas e vértebras. Microscopicamente notou-se difusamente um marcado decréscimo na espessura e número de trabéculas ósseas. Essas estavam desconexas, com uma aparência de flutuação, enquanto o córtex dos ossos longos estava afinado, com um aumento da porosidade pela dilatação dos canais de Haversian, erosão endosteal e diminuição da atividade osteoblástica. Espectrofotometria por chama foi realizada em amostras de fígado, e revelou um excesso de zinco (>2300ppm) e deficiência de cobre (<33.1ppm). Neste trabalho, apresentamos a primeira descrição patológica de um surto de osteoporose em suínos, além de fornecer uma breve revisão de doenças metabólicas em suínos.

TERMOS DE INDEXAÇÃO: Suínos, doenças ósseas, fragilidade óssea, desordem metabólica, zinco, cobre

INTRODUCTION

Metabolic bone diseases broadly categorize disturbances related to bone formation and remodeling (Madson et al. 2019). The bone formation and maturation process is complex and involves the direct interaction between genetic factors, local and systemic hormones, dietary nutrients, and mechanical forces (Craig et al. 2016). Metabolic bone diseases in swine are usually related to ration formulation or feed mixing errors, leading to deficiency or toxicity of specific nutrients, such as calcium, phosphorus, copper, and vitamin D or A (Fox et al. 1985). Other primary causes include parathyroid hormone disturbances, starvation, lactation, or increased stress (Spencer 1979, Doige 1982, Madson et al. 2019). The expression of a bone abnormality depends on which phase of skeletal development it occurs, the severity of the defect, the age of the affected animal, and how long persists

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(Craig et al. 2016). Metabolic bone diseases in animals include rickets, osteomalacia, fibrous osteodystrophy, and osteoporosis (Craig et al. 2016). In swine, rare osteoporosis cases have been described, usually associated with lactation (Doige 1982). We aimed to describe an outbreak of osteoporosis in growing-finishing and breeding pigs. We also provide a brief review of other metabolic bone diseases in pigs.

MATERIALS AND METHODS

The outbreak occurred in one swine farrow-to-finish farm, located in Minas Gerais, Southeastern region, Brazil (18°24'56'' S, 46°25'17'' W), from November 2014 to January 2015. We obtained clinical and epidemiological information directly from the herd veterinarians and swine farm owners. Two pigs acutely affected (Pigs 1 and 2) were selected and referred to systematic *post mortem* examination. Tissue samples were collected, fixed in 10% formalin, routinely processed for histology, and stained with hematoxylin and eosin (HE). Pigs of similar age (52 days old), without apparent clinical signs, were used as controls for bone abnormalities and further comparative analyses. Liver fragments, conserved in 10% buffered formalin solution, were sent to the "*Instituto de Biociências*" (Institute of Biosciences), "Centro de Ecologia" (Ecology Center) of the "Universidade Federal do Rio Grande do Sul" (UFRGS) to determine copper (Cu) and zinc (Zn) levels. These samples were analyzed by atomic absorption/air-acetylene flame spectrophotometry (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia), following the manufacturer's advice, and microwave-assisted nitric acid digestion (EPA 3052).

RESULTS

Piglets were weaned to nursery barns at 21 days old and transferred to the finishing barns at 60 days of age. All pigs housed had the same origin. These pigs were fed with the same ration during this period, based on corn and soybean, added with macro and micro minerals, vitamins, and lactose. Pigs had free access to water and food. The feed formulation was modified before the outbreak began, and an excessive amount of zinc oxide (ZnO) was added to the formulation.

A total of 46 growing pigs in a herd of 744 animals, including males and females, with ages between 73 and 79 days, were affected in three months. These pigs presented hind limb paresis and paralysis, weight loss, muscle weakness, recumbency, and died over a clinical course of 7 to 10 days. The mortality rate in the period was 2.4%. Grossly, both pigs had similar lesions, but these were most notable in Pig 1. In both pigs, the ribs exhibited, besides the fracture-related lesions, areas of callus formation associated with multifocal hemorrhages and edema (Fig.1). A complete fracture involved the scapula, humerus, femur (Fig.2), ribs, and vertebrae (thoracic, lumbar, and sacral). In Pig 2, complete fractures involved only the ribs, sternum, and vertebrae. Long bones exhibited marked depletion of cancellous bones and thinner cortical bones. In some areas, transverse reinforcement trabeculae were occasionally formed within these bones. The fractured bones were surrounded by moderate swelling and hemorrhage in the adjacent soft tissue. In the spine, shortening of vertebral bodies and multiple microfractures were observed in thoracic, lumbar, and sacral segments (Fig.3 and 4). No gross lesions were observed in other organs.

Histopathological analysis was performed in multiple sections of the long bones, ribs, and vertebrae. Bone trabeculae in the central part of the metaphysis were decreased, both in thickness and in numbers. The trabeculae were disconnected, with an appearance of "free-floating trabeculae" (Fig.5), when compared to normal bone (Fig.6). The cortex of long bones was severely thinned, characterized by the increase of the cortical porosity through enlargement of Haversian canals, in addition to endosteal erosion (Fig.7), also in comparison to normal bone (Fig.8). Osteoblasts were reduced in these sections, suggesting low osteoblastic activity (Fig.9), and multifocal areas of hemorrhage intermixed with eosinophilic fibrillary material deposits were seen in the medullary region. Normal bone osteoblastic activity is visualized in Figure 10. In the ribs, the callus formation exhibited focally extensive proliferation of hyaline cartilage surrounded by moderate fibrosis. Growth plaques of long bones and ribs were unremarkable. The areas of microfractures within the vertebral bodies were characterized by discontinued lamellar bone, with focally extensive areas of hemorrhage and moderate fibrosis. In the bone marrow, a moderate decrease of hematopoietic cells was observed. Fragments of the spinal cord adjacent to the vertebrae microfractures had mild compressive-related lesions, such as Wallerian degeneration. No other lesions were observed.

The determination of Zn on the liver samples showed values of 2,856ppm and 2,321ppm, more than four times higher than that described in liver samples of pigs fed excessive zinc oxide (Komatsu et al. 2020). The Cu dosage on the liver was 22.7ppm and 33.1ppm, below the reference range of 60-117ppm (Cancilla et al. 1967). After identifying dietary Zn overload in the rations, Zn levels in the diets were corrected, with a marked reduction of the clinical-pathological presentation herein described, and new cases of pathological fractures ceased to occur.

DISCUSSION

Based on the clinical and pathological findings, the diagnosis of nutritional osteoporosis was established. Osteoporosis is a skeletal disorder characterized by reduced bone mass and altered trabecular microarchitecture that leads to bone fragility and fractures (Bonucci & Ballanti 2014). This condition is one of the most frequent metabolic bone diseases in humans (Bonucci & Ballanti 2014), and can also occur in lambs (Suttle et al. 1972), pigs (Doige 1982, Craig et al. 2016), goats (Rosa et al. 2013), and dairy cows (Yoshida 2015). Osteoporosis is characterized by an imbalance between bone formation and bone resorption, resulting in a structurally normal bone with reduced strength caused by excessive resorption of cortical and cancellous bone, resulting in endosteal thinning of the trabeculae and cortices (Spencer 1979, Craig et al. 2016, Madson et al. 2019).

Osteoporotic bones are weak and easily fractured because of decreased bone mass and changes in the organic matrix. Osteoporosis in humans is frequently associated with vertebral compression fractures, hip and distal radius fractures (called Colles' fractures). Almost half of the vertebral compression fractures occur spontaneously (McCarthy & Frassica 2015), as in the cases herein presented, in which the pigs had spontaneous fractures and microfractures of the long bones, vertebrae, and ribs. The occurrence of pathologic bone fractures without evidence of excessive trauma may be the first indication that these pigs developed osteoporosis related to increased bone

fragility. In the swine industry, growing-finishing pigs have a high rate of growth and weight gain, which significantly increases the risk of fractures if concomitantly bone weakness occurs (Craig et al. 2016).

Osteoporosis is frequently caused by nutritional imbalance, such as mineral deficiency/excess or starvation (Pepper et al. 1978, Craig et al. 2016). In this investigation, we suggest that ZnO overload in the feed likely contributed to the low levels of Cu detected in the liver of the affected pigs. The possibility of a direct toxic effect of Zn on developing bones and cartilages must be considered, as other investigators described that abnormal Zn levels may prevent normal phosphorus deposition in the bones, whereas high levels of dietary Zn interferes in the calcium/phosphorus metabolism (Thompson et al. 1959). ZnO overload in pigs may reduce weight gain, cause anorexia, gastroenteritis, and lameness (Brink et al. 1959). Zn and Cu values detected in the liver samples demonstrated a Zn overload and Cu deficiency, suggestive of ZnO-induced copper deficiency caused by competitive absorption-inhibition.

Cu deficiency may be primary, caused by an inadequate diet with low levels of this compound, or secondary, resulting from an increase of dietary levels of copper antagonists, such as Zn (Craig et al. 2016). This and other divalent cations, such as iron and cadmium, compete with Cu for a common transport mechanism and, therefore, decrease copper absorption and bioavailability (Craig et al. 2016, Burrough et al. 2019, Madson et al. 2019). Cu deficiency may occur in many domestic species, usually with gross features of skeletal deformity, as Cu is required for the cross-linkage of collagen molecules, which in low levels prevents proper bone formation, skeletal mineralization, and the integrity of the connective tissue (Palacios 2006, Craig et al. 2016). Additionally, moderate depletion of hematopoietic precursors was observed in the bone marrow of the cases here presented, which is in accordance with previous studies regarding Cu deficiency in piglets, in which the main finding was severe anemia (Lahey et al. 1952).

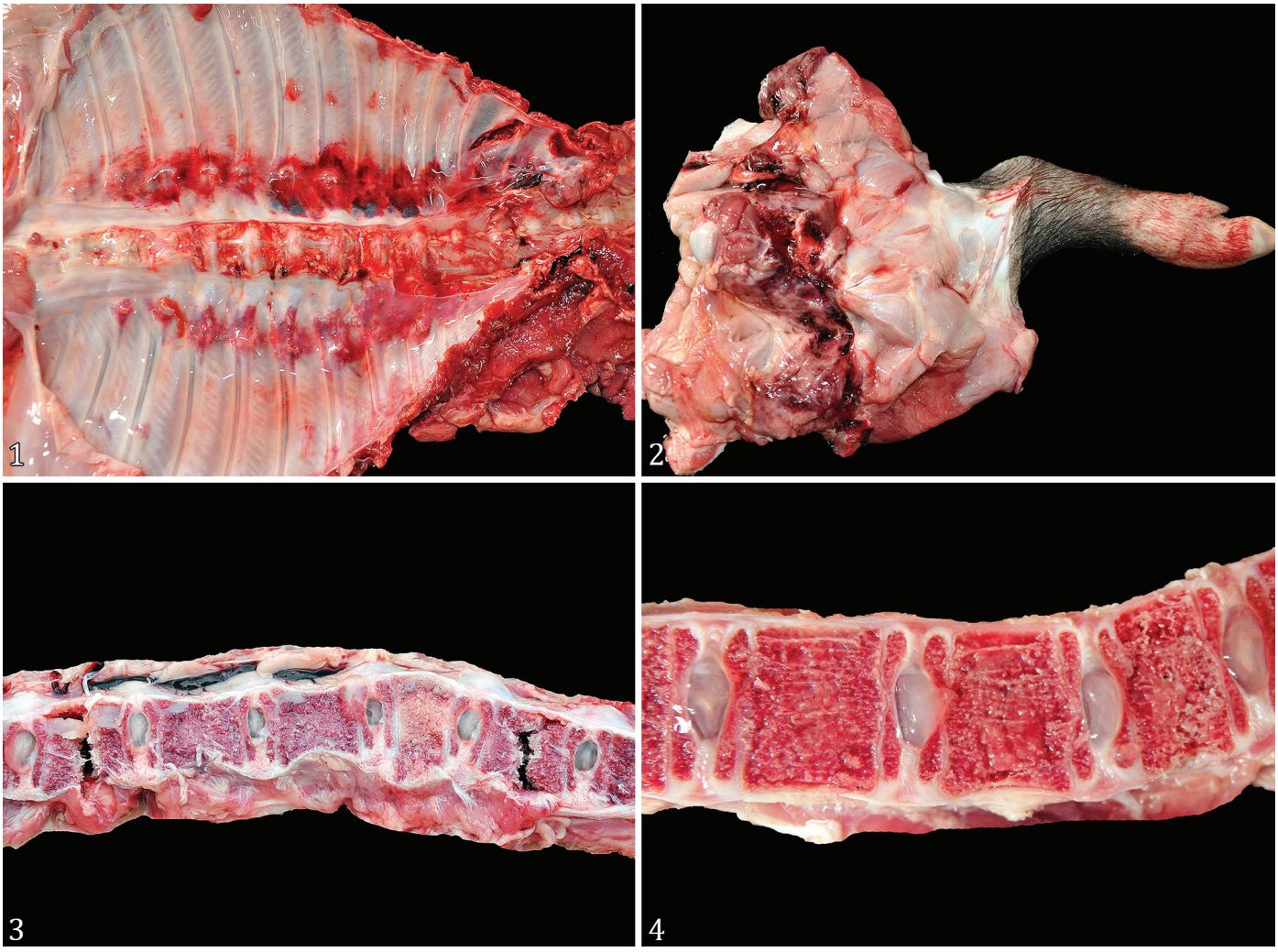

Fig.1-4. Gross features of osteoporosis in pigs associated with zinc overload and copper deficiency. (**1**) Several ribs present fractures near the costochondral junction and are surrounded by hemorrhage. (**2**) The femur exhibits a comminuted complete fracture and is surrounded by hemorrhage and edema (Pig 1). (**3**) Two vertebrae have complete transverse fractures, whereas other vertebrae are markedly shortened, indicating previous fractures. (**4**) The vertebrae show marked depletion of cancellous bone and formation of transverse reinforcement trabeculae.

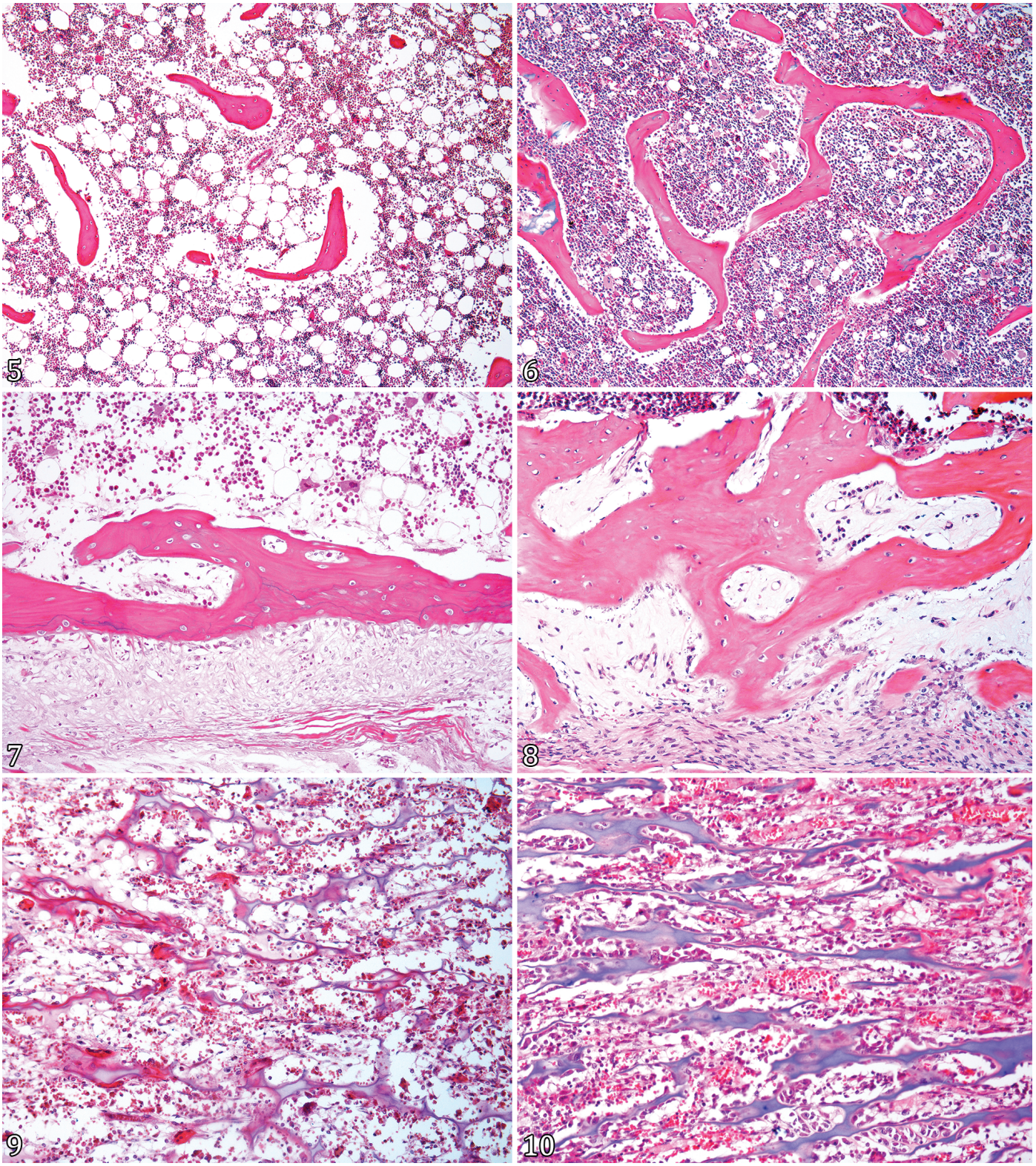

Fig.5-10. Microscopical features of osteoporosis in pigs associated with zinc overload and copper deficiency. (**5**) The bone trabeculae of the vertebrae are reduced, both in thickness and in number, and are disconnected, with the appearance of "free-floating trabeculae". There is also a moderate decrease of hematopoietic cells. HE, obj.10x. (**6**) Normal bone (vertebrae) appearance of control pig shows trabeculae connected to each other and hematopoietic cells in regular numbers. HE, obj.10x. (**7**) The cortex of long bones is severely thinned, with an increase of the cortical porosity by the enlargement of Haversian canals and by endosteal erosion. HE, obj.20x. (**8**) Normal bone (vertebrae) appearance. HE, obj.20x. (**9**) A rib section shows a moderate decrease of osteoblasts, indicating reduced osteoblastic activity. HE, obj.20x. (**10**) Normal bone (rib) appearance shows regular osteoblastic activity. HE, obj.20x.

Table 1. Metabolic disease in growing/adult pigs

Disorder	Age of affected pigs	Cause	Lesion distribution	Gross features	Histopathology
Osteoporosis (Doige 1982, Craig et al. 2016)	Growing and adult pigs	Cu/Ca deficiency, starvation, vitamin A toxicity, increased stress	Vertebrae, ribs, long bones, flat bones of the skull	Reduced breaking strength of ribs. Marked depletion of cancellous bone in the metaphyses and epiphyses. Reduced cortex thickness	Bone trabeculae decreased in thickness and number, disconnected, "free-floating trabeculae", increased porosity of the cortical bone due to enlargement of Haversian canal, low level of osteoblastic activity
Fibrous osteodystrophy (Thompson & Robinson 1989)	Growing and adult pigs	Ca and vitamin D deficiency, excess P	Skull bones, especially mandibles and maxilla. Also, scapula and vertebral column	Bilateral enlargement of the bones of the skull, movable teeth, jaw swelling. Soft, pumice-like bones, and decrease in bone density. Ribs may bend or snap with little effort	Increased osteoclastic bone resorption, marked fibroplasia, and increased osteoblastic activity with formation of immature woven bone, frequently poorly mineralized
Rickets (Pepper et al. 1978, Dittmer & Thompson 2011, Madson et al. 2012, Gris et al. 2020)	Growing pigs	Deficiency of Ca, P, vitamin D. Ca/P imbalance	Sites of rapid growth, metaphyseal and epiphyseal regions of long bones, and costochondral junctions of the large middle ribs	Weak bones that bend before breaking with a weak snap and have enlarged growth plates, giving a clinical appearance of swollen joints, increased volume of costochondral joints (rachitic rosary), kyphosis	Decreased mineralization of the growing bone, evident persistence of hypertrophic chondrocytes at sites of endochondral ossification
Osteomalacia (Craig et al. 2016)	Late finishing and adult pigs	Deficiency of Ca, P, vitamin D. Ca/P imbalance	Vertebrae, femur, pelvis, and ribs	Bones with reduced resistance to pressure and tension. In severe cases the marrow cavity is expanded, the cortex is thin, spongy, and soft	Decreased mineralization with the significant remodeling of the mature bone. Localized accumulation of osteoid at sites of mechanical stress

Cu = copper, Ca = calcium, P = phosphorus.

Commercial diets for pigs are specifically adapted for rapid growth and lean muscle mass gain, with well-established nutritional programs that provide sufficient calcium, phosphorus, and vitamin D levels for normal bone growth and mineral homeostasis (Madson et al. 2012). Therefore, metabolic bone diseases are uncommon in swine and account for 0.2-1.0% of the causes of death in these animals (Brum et al. 2013, Coelho et al. 2017). The differential diagnosis for osteoporosis in pigs must include other metabolic bone diseases, especially rickets, and fibrous osteodystrophy, although other metabolic bone diseases are described in the swine species. Clinical signs for these are usually similar and include lameness, pathological fractures, reluctance to rise, limb weakness, and, occasionally, sudden death (Madson et al. 2019). The differentiation among these conditions is best achieved through the epidemiological aspects, especially the age of the affected pig, in addition to gross and histological features (Table 1). In this study, the main lesions were characteristic of osteoporosis, with severe decrease in the thickness of the trabeculae, increased porosity of the cortical bone due to enlargement of the Haversian canal, and low level of osteoblastic activity, making it possible to differentiate from other metabolic bone diseases.

CONCLUSION

Herein, we described an outbreak of swine with lameness, associated with multiple bone fractures. Microscopically, there was a diffuse and marked decrease in thickness and number of trabeculae, characteristic of osteoporosis. Investigations of multiple spontaneous fractures, muscle weakness, paresis, and sudden death in swine should include metabolic bone diseases, such as osteoporosis, in the differential diagnosis. It is essential to perform the gross and histological evaluation of the bones and growth plates to correctly diagnose the underlying condition involved in skeletal disorders.

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Conflict of interest statement.- The author's declare no conflict of interest.

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