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Case report

Maxillofacial Fibrous Osteodystrophy in Equine: Case Report

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Abstract

A 14 year-old pregnant mare developed poor body condition (2/5) and diarrhea while grazing in kikuyu grass (*Pennisetum clandestinum*). Clinical findings included bulging of nasal and maxillary bones and mouth lacerations associated to spicules on the surface of molars and premolars. Teeth were easily fragmented and fell off. Necropsy findings included severe softening of the maxillary and mandibular bones. The mandible was easily breakable near to the masseter´s fossa. The parathyroid gland was increased in size. Histological findings were characterized by multiple irregular and very thin bone spicules in the bone tissue, most of them with microfractures, severe osteoclasts-mediated bone resorption and fibrous connective tissue proliferation. Hyperplasia and hypertrophy of parathyroid chief cells with cytoplasmic vacuolization were also observed. In conclusion, maxillofacial fibrous osteodystrophy caused by nutritional secondary hyperparathyroidism developed in a pregnant mare while grazing kikuyu grass for long periods of time without appropriate supplements. Kikuyu grass is characterized by high levels of oxalic acid that forms insoluble oxalates responsible for low calcium absorption in the small intestine and the progress of hyperparathyroidism.

Key words: nutritional hyperparathyroidism, *Pennisetum clandestinum,* oxalates, parathyroid gland, equine.

Introduction

Fibrous osteodystrophy is a disorder of the calcium metabolism of animals and humans characterized by bone resorption, conjunctive tissue proliferation, poor mineralization and immature bone appearance (20, 21). The disease is also called osteitis fibrosa, Bran disease and big head (8). In the equine species, fibrous osteodystrophy is commonly caused by nutritional secondary hyperparathyroidism, a metabolic disorder widely reported in unweaned foals and horses fed high phosphorus and low calcium (ratio ≥ 3:1) diets (19, 22). The nutritional disorder also develops in horses fed diets high in oxalates (22). In tropical regions, pastures such as blue grass (*Setaria anceps*), camolo grass (*Cenchrus* sp.), guinea grass (*Panicum máximum* var. *trichoglume*), kikuyu grass (*Pennisetum clandestinum*), pangola grass (*Digitaria eriantha,* formerly *D. decumbens*), and some *Brachiaria* species including humidicola grass (*B. humidicola*) and para grass (*B. mutica*) (14, 20, 7, 5, 4, 18) are known to accumulate oxalates that cause a negative calcium balance, chronic hypocalcemia followed by nutritional secondary hyperparathyroidism. Oxalates bind calcium to form calcium oxalate which is insoluble in typical alkaline pH of the intestine, thus interfering with calcium absorption (22). Reduced blood calcium stimulates parathormone (PTH) secretion by chief cells in the parathyroid gland and PTH induces bone resorption by increased osteoclast activity to maintain appropriate calcium levels in blood and tissues, originating fibrous osteodystrophy (1). The equine species is susceptible to develop nutritional secondary hyperparathyroidism due to low calcium absorption in the small intestine when grazing in pastures high in oxalates (19, 22).

Horses of all ages can develop the disease but those between 2 and 8 months of age are more susceptible and the lesions are more severe compared to adult horses (8), however, increasing age is not considered a factor of

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resistance. Disease predisposition by host factors such as breed or sex are no reported, although, high levels of calcium required during pregnancy may influence the development and course of the disease in mares (8, 10, 19). The prevalence and incidence of the disease may vary from 1 to 100 % and it is closely related to diet composition (8). Clinical findings of fibrous osteodystrophy include lameness, fractures, growth retardation and poor body condition, enlarged and deformed bones of the face, narrowed nasal passages and loss of lamina dura surrounding the molars. Radiographic analysis may show decreased bone and teeth density (15, 17, 21, 22).

In Colombia, metabolic disorders in horses affecting bone structure, such as fibrous osteodystrophy, are not well described in terms of epidemiology, clinical and pathology. The disease was initially suspected in a farm located in the Savannah of Bogotá some decades ago (23), however, the authors did not provide definitive evidence such as gross, microscopic lesions and potential causes. The purpose of this report was to describe the macroscopic and microscopic lesions associated with a case of maxillofacial fibrous osteodystrophy in a mare grazing kikuyu grass (*P. clandestinum*) in the savannah of Bogotá, Colombia.

Case Report

A 14-year-old mixed breed mare was subjected to clinical examination by veterinarian practitioners. The animal belonged to a herd (n=19) with 100 % of morbidity and was in the ninth month of gestation and presented poor body condition and diarrhea when grazing kikuyu grass (*P. clandestinum*), without food supplements.

The animal has poor body condition with an score of 2/5, bulging of the nasal and maxillary bones and edematous checks with lacerations associated with spicules on the lateral surface of superior premolars and molars and on the lingual surface of the lower teeth. The animal kept the tongue out of the oral cavity for prolonged periods of time and could catch up food, but was not able to chew it. The vaginal mucosa was hyperemic and showed mild mucoid discharge. When clinicians attempted to filing the teeth, they were fragmented and fell off easily. Few days later the mare appeared too weak, down fall and was sacrificed and submitted for necropsy examination.

At necropsy examination the mare had a poor body condition, conjunctival membranes were congested, and multiple lacerations on the tongue and reddened vulvar mucosa with petechial hemorrhages were also present. The skeletal system showed severe softening with increased size of the maxillary, mandible, and vomer bones that when cut showed apparent cavitations and bone lysis. The cut surface had irregular white spicules with semi-hard consistency (Figure 1). When separation of the mandibular rami was attempted, one of them was easily fractured near the masseter´s moat, the thyroid and parathyroid glands were mildly enlarged, the thoracic and abdominal cavities contained moderate transudate, the kidneys had moderate adhesion to the capsule and irregular yellow surface with dark-red spots, the tracheobronchial tree contained little foamy liquid in the lumen and the lungs were moderately congested, the fat tissue surrounding the esophagus and heart was yellow with gelatinous appearance, the stomach was moderately distended by gas and scarce pasty content, the small intestine had mild congestion and low watery content beige in color, some dark-red foci in the distal region of jejunum and first third of ileum were also present. The colon showed reddish serous layer and a mild mucoid content beige in color and few round worms (*Parascaris* sp.) were also present in the lumen. The fetus did not show pathological lesions. The lesions of the facial bones supported the clinical alterations and lead to a presumptive diagnosis of maxillofacial fibrous osteodystrophy to be confirmed by the histopathological analysis.

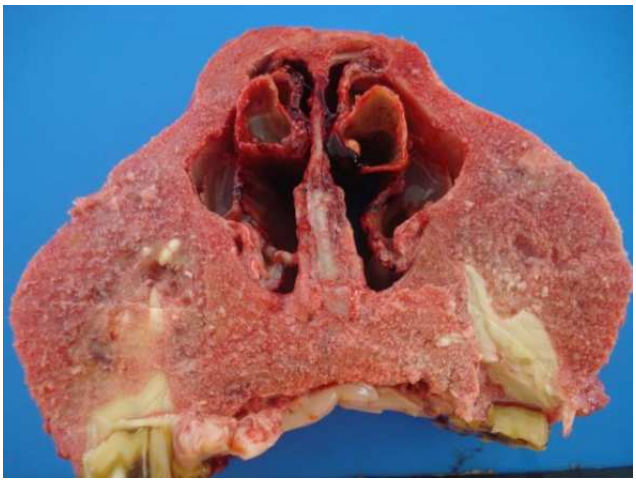


Figure 1. Maxillary and nasal bones of a mare with fibrous osteodystrophy. The bone tissues are replaced by softened tissue with granular consistency when cut. Note the hemorrhages in the nasal turbinate.

Histological sections of the vomer and mandible bones showed severely reduced bone mass, multiple irregular bone spicules surrounded by abundant connective tissue, microfracture of spicules, osteoid formation and hemorrhages (Figure 2). Masson´s trichrome staining confirmed that the bone tissue was replaced by conjunctive fibrous tissue distributed among bone spicules and trabeculae (Figure 2, Insert), various degrees of bone mineralization (various shades of bone matrix), and multiples foci of bone resorption by osteoclasts (Figure 3). Parathyroid gland showed mild hyperplasia and hypertrophy of parathyroid chief cells with severe intracytoplasmic vacuolar changes and increased cytoplasmic to nucleus ratio (Figure 4). Some chief cells also showed accumulation of brown material of unknown origin. The kidney showed slight microcirculatory changes. The liver showed severe thickening of Glisson´s capsule with multiples nodules of plasma cells,

macrophages, lymphocytes and giant cells under the capsule, mild congestion and disorganization of hepatic cords, atrophy of hepatocytes, and mild fibrosis in the portal area, moderate hyperplasia of biliary ducts and mild lymphoplasmacytic infiltrate foci. The small intestine contained mild luminal mucus, mild intestinal villus atrophy, desquamated epithelial cells, multiple cocobacilli colonies, distended lymphatic vessels, and mild lymphoplasmacytic infiltrate with few eosinophils. The spleen showed moderate congestion, severe depletion of B and T cell areas and abundant macrophages with hemosiderin pigments. Other tissues showed non-specific circulatory changes. The final diagnosis was maxillofacial fibrous osteodystrophy associated to nutritional secondary hyperparathyroidism.

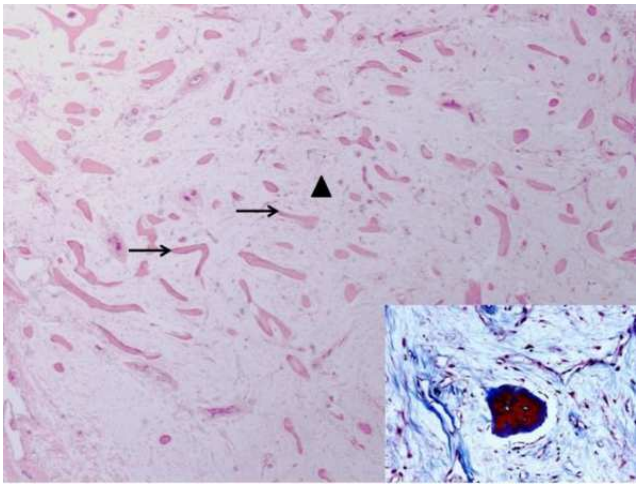


Figure 2. Maxillary bones. Multiple thin irregular bone spicules (arrows). Note the severe and extensive replacement of bone by connective tissue (arrowhead). 20X, H&E. Insert. Masson´s trichrome staining of the maxillary bone to highlight the collagen filaments (blue staining). 200X

The mare grazed on kikuyu grass with rotational system, the basal resting time of the paddock was 50 day and there were no records of chemical fertilization programs applied on the farm. Samples of kikuyu grass were submitted to the Laboratory of Toxicology at the Faculty of Veterinary Medicine, National University of Colombia, to measure total oxalates. The result indicated that oxalate in kikuyu grass was 3.03 g/kg of dry forage matter.

Discussion

Equine fibrous osteodystrophy due to nutritional secondary hyperparathyroidism is known since 400 year B.C, however, the presence of the disease and macroscopic and microscopic alterations in horses farmed in Colombia are not well documented. In the mare of the present report, gross and histological lesions associated with epidemiological and clinical data provided sufficient evidence for a diagnosis of maxillofacial fibrous osteodystrophy. In addition, the definitive etiological diagnosis was supported by the analysis of oxalate levels in a sample of kikuyu grass. The disease is poorly documented in international and national literature, although some of those reports helped to guide the diagnosis, they were: 1) predisposition to the disease when grazing in kikuyu grass for long periods of time without any supplement rich in calcium and a suitable balance of phosphorus, 2) susceptibility of the equine species due to reduced capacity to metabolize calcium oxalates, and 3) an increased risk to develop the disease in pregnant females due to its reproductive condition.

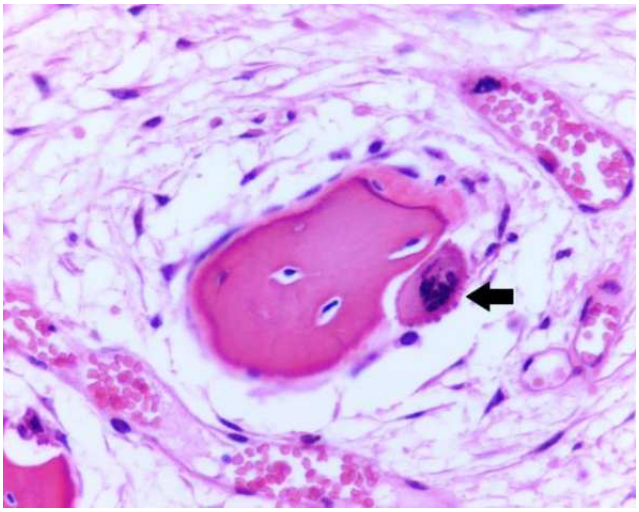


Figure 3. Osteoclast (arrow) mediated bone resorption. Note the spongy appearance of the surrounding connective tissue and dilated blood vessels. 400X, H&E.

Fibrous osteodystrophy occurred in a mare that was bred and kept for all its productive and reproductive cycle grazing kikuyu grass (*P. clandestinum*), a plant known to accumulate high levels of oxalic acid (9), and the chemical analysis of a kikuyu sample in this study showed a content of 3.03 g/kg of dry forage matter and that concentration of oxalates in kikuyu has been associated with the disease in different geographical locations. Similar findings were reported in horses grazing kikuyu for long periods of time in Australia (24). The equine species is unable to use the oxalic acid bound calcium and thus the calcium is lost with the feces and the animals develop chronic hypocalcemia. Low level of calcium in blood induces hypertrophy and hyperplasia of chief cells in the parathyroid gland that is forced to produce PTH to maintain normal calcium levels in blood (nutritional secondary hyperparathyroidism). PTH induces bone resorption and this process leads to fibrous osteodystrophy (9). Moreover, the mare was 9 months pregnant and pregnancy is an additional risk factor for triggering the disease, given the increased calcium requirements for fetal bone formation (8, 19), that could be 2-3 times (50 to 60 g of calcium/day) higher than those of adult horses (20 g)

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(22). The kikuyu grass was 50 day-old when the sample was taken for analysis, however, oxalates levels may increase in older pastures leading to a higher impact on the equine´s health. Exploration of farm´s data showed that a total of 19 animals were grazing kikuyu grass and the morbidity in the herd was 100 %, thus grazing kikuyu for long periods without appropriate supplements may cause equine fibrous osteodystrophy.

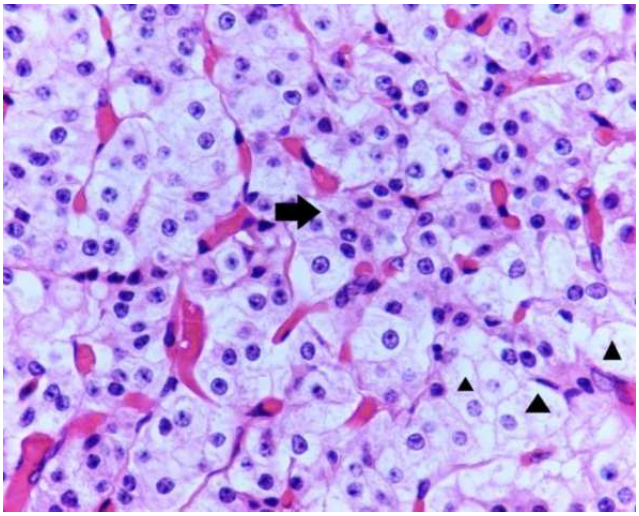


Figure 4. Parathyroid gland of a mare with maxillofacial fibrous osteodystrophy. Mild hyperplasia (arrow) and hypertrophy of chief cells, which show marked cytoplasmic vacuolar changes (arrow heads), and increased cytoplasmic to nucleus ratio. 400X, H&E.

Clinical and gross findings described in the mare of this study are consistent with fibrous osteodystrophy and complement those reported previously in natural (21) and experimental (8) cases of the disease.

The histological lesions were consistent with the gross findings in the face and head of the mare, they were severe in flat bones of the face and jaw, similar findings were reported in horses from Brazil kept for long periods grazing guinea grass (*P. maximum* var. Aruana) (5). Lesions in the trabecular bone of the skull, ribs, metaphysis of long bones, outer circumferential lamella of long bones, the haversian systems and interstitial lamellae of compact bones, are considered the most frequent sites of tissue damage (16), however, due to technical reasons, analysis of the structure and microscopic features of long bones were not conducted in this study, and their appearance and consistency during necropsy examination did not indicate significant changes. Lesions on the lamina dura surrounding the molars may explain the loss of teeth and the abnormal chewing of food that may lead to malnutrition. In this regard, nutritional deficiency is also associated with the poor body condition and mucoid degeneration of fat tissue (12) observed in this mare. Finally, hyperplasia and hypertrophy of parathyroid gland´s chief cells, with clear changes in the cytoplasmic to nucleus ratio and cytoplasmic vacuolization supported the development of this condition (3).

Nutritional secondary hyperparathyroidism develops from an imbalance in extracellular calcium-phosphorus ratio, where hypocalcemia and hyperphosphatemia predominates. The extra-cellular Ca^{2+} concentration is regulated by homeostatic mechanisms that include three body systems (renal, intestinal and bone) and three hormones (PTH, calcitonin, and calcitriol or 1,25-dihydroxyvitamin D3) (2). A decrease in the extracellular Ca^{2+} levels stimulates PTH secretion by parathyroid gland and this hormone stimulates osteoclastic bone resorption of calcium, and calcitriol synthesis in the kidney (22). The oxalic acid from kikuyu grass and other plants reacts with calcium and calcium salts to form oxalates, the main effect in monogastric animals is a reduction on the availability of calcium. When oxalic acid reacts with monovalent metals such as sodium and potassium, it forms soluble salts but the reaction with divalent cation such as calcium forms highly insoluble complex structures that are not absorbed in the intestine (9). The calcitriol hormone increases the intestinal absorption and renal reabsorption of Ca^{2+} and inorganic phosphate (Pi) to maintain homeostasis of both Ca^{2+} and Pi. Thus, an increase in Ca^{2+} concentration induces calcitonin secretion by the thyroid parafollicular C cells to inhibit osteoclast activity (2, 22), however, the persistence of reduced calcium absorption in the intestine of equines overwhelm the physiological homeostatic capacity of those tissues and hormonal systems and lead to bone tissue damage and the progress of fibrous osteodystrophy (19,22).

Fibrous osteodystrophy do not develops by the presence of 0.5% of oxalates in the forage, whereas diets with 1% oxalates may reduce about 66% the calcium absorption and increase its fecal excretion (8, 19, 22). Oxalates ranges from 3.9 to 24.4 g/kg dry matter in kikuyu grass were found to cause lesions in monogastric animals (9). Although, the value of oxalate in kikuyu grass in this study (3.03 g/kg dry forage matter) was slightly lower to the inferior limit reported by Marais (9), the long lasting ingestion of the forage (about 14 years), together with the pregnancy status of the mare and perhaps aging-related physiological changes, suggest that this pathology may develop in equines from the savannah of Bogotá at the reported concentrations of oxalates in diets ingested during considerable long periods of time and in the absence of calcium and phosphorus supplements.

Fibrous osteodystrophy may also occur by diets with excess of phosphorus that leads to hyperphosphatemia. The increase of serum phosphorus reduces intestinal calcium absorption, and diets with high P and low Ca stimulate PTH secretion, in addition, hyperphosphatemia inhibits renal synthesis of calcitriol and the later inhibits the function of parathyroid chief cells (11, 6, 13, 20, 22). This mechanism of fibrous osteodystrophy development was excluded because the mare was not supplemented on the diet. Finally, the

possibility of hyperparathyroidism of renal origin was also discarded because there were no significant gross or microscopic abnormalities in the kidney; in addition, the renal origin of this pathology in equine species is not reported (22).

Prevention measures for this calcium disorder may be to avoid the use of pastures high in oxalates levels, however, when this activity is not a practical option, supplementation with calcium and phosphorus is necessary. Animals grazing pastures high in oxalates may require about 20 mg/kg calcium and 10 mg/kg phosphorus in a daily basis diet (21).

Fibrous osteodystrophy is a disease characterized by bone resorption and replacement of the bone by connective tissue. The definitive diagnosis of maxillofacial fibrous osteodystrophy was established by a correlation of the nutritional and management data, clinical evaluation, gross and histopathological analyses. The mechanism of maxillofacial fibrous osteodystrophy development is suggested by the long period grazing kikuyu grass (*P. clandestinum*), a species that contains significant amounts of oxalic acid, which impedes calcium absorption in the intestine and leads to nutritional secondary hyperparathyroidism, responsible for increased PTH hormone production and bone resorption. The disease may develop in equines grazing kikuyu grass and other plants for long periods of time in the savannah of Bogotá, Colombia.

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