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Case Report

Spontaneous fractures and hypocuprosis in pen-backgrounded Holstein calves

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Abstract
Bone fractures are a frequent cause of limp in cattle, being pre-existing bone lesions among the predisposing factors. However, there is scant information about the natural occurrence of bone disease. This report aimed to describe an outbreak of spontaneous fractures associated with hypocuprosis in pen-backgrounded calves. Hind limp and irreversible recumbency were noticed in 9-month-old calves of a dairy farm from Córdoba province (Argentina). The issue was only evident in males fed a diet composed (DM) of 57% wet corn, 24% dry corn distiller grains, and 19% corn silage. No mineral mix was supplied. Accumulated morbidity and fatality rates were 5.8 and 100%, respectively. Two autopsies were performed, observing multiple femoral and rib fractures. Severe hypocupremia, very low copper levels in the liver and plasma, deficient dietary copper levels, normocalcemia, marginal hypophosphatemia, and high sulfate concentrations in the water supply were determined. Except for the decreased bone quantity and the multiple fractures, no bone lesion was observed, confirming osteoporosis as the pathological diagnosis, and hypocuprosis as the etiology.
Keywords: osteoporosis, bone lesion, sulphate, cattle.

Introduction

Bone fractures are a frequent cause of limp in cattle (6, 8, 14), being pre-existing bone lesions among the most important predisposing factors (3, 12, 20, 28, 29). The main etiology of bone diseases are nutritional issues such as protein-calorie deficit (17), hypocuprosis (13, 24), calcium (Ca) deficiency (12), phosphorus (P) deficiency (2, 20), and/or hypovitaminosis D (12). Before causing bone lesions, these nutritional issues produce notorious detriments in animal performance, laying here the major importance of determining the precise etiology. More than one of the aforementioned nutritional imbalances are frequently present and interrelated in the same case, making it difficult to arrive at an accurate etiological diagnosis (2, 3, 29). Additionally, there needs to be more information about natural occurrence of bone disease. This report aimed to describe an outbreak of spontaneous fractures associated with hypocuprosis in pen-backgrounded calves.

Case Description

In June 2019, the INTA Marcos Juárez Veterinary Diagnostic Service was required to assist a dairy farm located in the Unión district of Córdoba province (Argentina) due to the occurrence of irreversible paralysis of hind limbs in calves. The issue happened in the growing section, where the affected animals were about 9-month-old males. During the visit, anamnesis and population inspection were carried out. Then, a clinical examination and autopsy of two prostrated calves were performed. These animals were euthanized by rapid intravenous injection of sodium pentobarbital. During the autopsies, ribs, costochondral junction, distal metacarpal metaphysis, lung, liver, jejunum, and colon samples were collected in 10% buffered formalin for histopathological study. A metacarpus bone, liver, and feces samples were collected for radiology, Cu quantification, and parasitological studies, respectively.

For the histopathological study, tissue samples were fixed for 72 hours in 10% buffered formalin. Sections of bone

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samples were decalcified with 5% nitric acid. Then, like the other tissue samples, sections were dehydrated by immersion in increasing concentrations of ethyl alcohol, embedded in paraffin wax, 4 µm thick sectioned, and hematoxylin-eosin (H&E) stained.

A comparative radiologic study was conducted between a metacarpus from one of the autopsied calves and another metacarpus from a healthy calf, using a Rayfer ® 500 device at 54 Kv and 16 mAs.

For the parasitological study, the modified McMaster flotation technique was performed on feces samples to quantify parasite eggs and oocysts.

Asymptomatic and pre-mortem euthanized animals were bled to determine plasma total calcium (Ca), phosphorus (P), and Cu concentrations. Feed samples were collected for Cu quantification. Except for P, which was measured by UV-visible spectrophotometry, the mineral quantification was performed by atomic absorption spectrophotometry. Additionally, drinking water samples were collected to determine total dissolved solids and sulfate (SO_4) concentrations by gravimetry and turbidimetry, respectively.

The backgrounding section of the farm had 470 Holstein calves: 240 males and 230 heifers. Males were fed a diet composed (on dry basis) of 57% wet corn, 19% corn silage, and 24% dry corn distiller grains (CDG), but no mineral mix was supplied. This diet supplied about 3 Mcal ME per kg DM and 13.5% crude protein. Feed was offered daily at about 2% live weight per animal on dry matter basis. Heifers were fed a diet of 51% wet corn, 23% corn silage, 17% dry CDG, 7% lucerne hay, and 2% mineral mix. During the inspection of the pens, some animals with mild hind lameness and diarrheal stools were observed. The problem was only evident in about 9-month-old males. The affected animals were recumbent and presented hind limb paralysis with average sensitivity, mentation, and vision. Despite their condition, the animals continued feeding. Rectal temperature records were 38.5 and 39 °C. Additionally, defecation of fibrin stools with fresh blood was observed in a prostrate animal. The veterinarian assessors stated that parenteral treatments with Ca, magnesium, and thiamine had been unsuccessful, and those animals that fell into decubitus had to be sacrificed because they could not recover. Considering the animals that fell in decubitus as cases, the accumulated 20-day morbidity and fatality rates were 5.8% (14/240) and 100% (14/14), respectively.

During the autopsies, rib fractures and diaphyseal femoral fractures in both hind limbs were observed in the two autopsied animals (Fig. 1A and 1B). At opening the thoracic cavity, an unusual ease for breaking the ribs was noticed. Additionally, blood clots were observed in the lumen of the colon and rectum of one of the two autopsies.

A decrease in cancellous bone density was observed by the comparative radiological study (Fig. 1C). Reduction in the number and thickness of bone trabeculae was observed by the histopathological study (Fig. 1D). No lesions were observed in the rest of the sampled organs. The low Cu levels in the liver indicated severe Cu depletion (Table 1). Parasitological studies in feces resulted in negative for both eggs and oocysts.

Blood biochemical results indicated severe hypocupremia, normocalcemia, and marginal hypophosphatemia in asymptomatic males. Deficient Cu supply was determined in the diet fed to the males, but adequate Cu levels were quantified in that fed to the heifers. Total dissolved solids and SO_4 excess were observed in the groundwater supply from all the farm sections (Table 1).

Discussion

Spontaneous fractures in cattle are secondary to pre-existing bone lesions caused mainly by nutritional factors. Phosphorus and/or vitamin D deficiency impair normal bone mineralization producing rickets in growing animals or osteomalacia in adults (12, 20). Rickets is characterized by the permanence of unmineralized osteoid in bone and unmineralized cartilage in the metaphysis, resulting in enlargement of physis and metaphysis. Dietary low Ca:P ratio produces nutritional hyperparathyroidism, producing osteoclastic hyperactivity and fibrous replacement of bone tissue, a lesion called fibrous osteodystrophy (12). Osteopenia is a quantitative reduction of bone tissue and can be produced by hypocuprosis, chronic hypocalcinosis, protein-calorie deficit, hypovitaminosis D, and/or severe chronic gastrointestinal helminthiasis. When osteopenia reaches such severity that manifests itself in bone pain and spontaneous fractures, it is called osteoporosis (2).

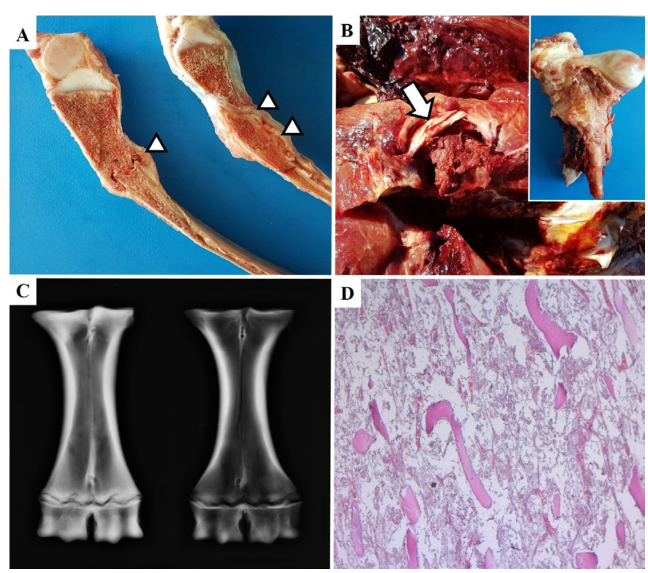

Figure 1. Osteoporosis in hypocuprotic Holstein calves. A. Multiple rib fractures (arrowheads). B. Diaphyseal femoral fracture (arrow). C. Severe radiodensity reduction in affected metacarpus (right) relative to control (left). D. Rib: severe reduction in number and thickness in bone trabeculae. H&E 10X.

Table 1. Biochemical parameters in the liver and blood from hypocuprotic Holstein calves and chemical parameters in the feed and drinking water from the dairy farm

Parameter	Units	Sampled sub-population	n	Observed value (mean ± SD)	Reference value
Dry matter liver Cu	ppm	Autopsied males	2	2.0 ± 1.1	100 - 300 (22)
Plasma Cu	µg/dL	Asimptomatic males	10	18.0 ± 10.3	60 - 120 (22)
		Autopsied males	2	40.5 ± 0.7	
Plasma Ca	mg/dL	Asimptomatic males	10	9.3 ± 1.3	8 - 12 (21)
		Autopsied males	2	8.3 ± 0.1	
Plasma P	mg/dL	Asimptomatic males	10	3.9 ± 0.6	4.5 - 5.6 (23)
		Autopsied males	2	3.3 ± 0.5	
Feed Cu	ppm	Heifers	4	9.9 ± 0.3	10 - 16 (15)
		Backgrounded males	2	4.5 ± 0.4	
Drinking water dissolved solids	mg/L	Cows	1	7445	<3000 (15)
		Rearing	1	2188	
		Backgrounded males	1	2430	
Drinking water SO_4	mg/L	Cows	1	4340	<500 (16)
		Rearing	1	555	
		Backgrounded males	1	700	

SD: standard deviation. Cu: copper. Ca: calcium. P: phosphorus. SO_4: sulfate.

In this case, osteoporosis was diagnosed because less bone tissue quantity was radiologically and histologically noticed, none of those mentioned above bone lesions were observed, and severe hypocuprosis was confirmed. Considering that the most severe damages occur with hepatic Cu values below 10 ppm and plasma Cu concentrations less than 30 µg/dL (22), it is justified to consider hypocuprosis as the cause of the bone lesions in our case since the mean values for hepatic and plasma Cu were 2 ppm and 18 µg/dL, respectively. In this sense, Cu deficiency impairs osteoblastic osteogenesis (19) and crosslinking among collagen bone fibers (13, 19, 30), reducing the breaking strength of bones. Thus, severe hypocuprosis manifests itself by bone fragility and increased incidence of spontaneous fractures, such as those observed in the present case.

Hypocuprosis is endemic in grazing cattle from this region of Argentina (south Córdoba province) because of low Cu levels in forage, S excess in drinking groundwater (from SO_4) and/or Mo excess in forage (18). The hypocuprosis, in this case, was mainly due to the omission of the mineral mix supply. In this sense, bone fractures only happened in males who were fed a Cu-deficient diet. Conversely, heifers were fed adequate Cu levels and no sign of the issue was observed among them. Additionally, SO_4 concentrations in the water supply were high enough to compromise Cu availability throughout the growth of the animals. Although feed S concentration was not determined in this case, high S concentrations have been recorded in CDG worldwide and in this region of Argentina (1). Therefore, total dietary S excess contributed to hypocuprosis development to some degree.

Fetlock enlargement is a frequent sign in severely hypocuprotic young cattle, but it was not observed in the present case. Physeal and metaphyseal swelling, mostly noticed in the distal metacarpus, distal metatarsus, and proximal phalanges, is the clinical manifestation of cartilage ossification impairment, resulting in cartilaginous tongues and islands in the metaphysis (11, 13, 24). These lesions have been reported in natural Mo-induced hypocuprosis (11) or experimental primary hypocuprosis in which calves began to feed a Cu-deficient diet at 6 to 12 weeks of age (13, 24). This could indicate the need for calves to be exposed from a very young age to develop these lesions, which did not happen in our case.

Although it was not possible to quantify the Ca concentration in the diet fed to the calves, it is well known that its ingredients (corn, corn silage, and CDG) are Ca deficient (21). Omitting the provision of mineral supplements, this diet surely had a low Ca:P ratio, predisposing to poor bone development. In addition, the SO4 excess in drinking water could reinforce the anionic dietary balance, aggravating the Ca deficiency (9, 27). The Ca status could be compromised but not manifested by hypocalcemia at the time of sampling, possibly because of the homeostatic regulation of calcemia (10, 12). Therefore, Ca deficiency was not ruled out as a possible contributor to the development of osteoporosis but could not be confirmed.

A frequent cause of osteoporosis is a protein-calorie deficiency, being metaphyseal arrest lines the pathologic indicators of this nutritional issue during the growth period (2, 3, 17). In this case, malnutrition was ruled out because no arrest lines were observed, and the supplied feed quantity and quality were more than sufficient for body maintenance.

Phosphorus deficiency produces rickets or osteomalacia and spontaneous fractures in grazing cattle (20). Less frequently, P deficiency has been associated with osteoporosis (2). Nevertheless, this deficiency is rare in cattle on concentrates-based diets because of their high P supply and ruminant availability. Therefore, additional P supplementation is not needed for cattle feeding this kind of diet (5, 4, 7). Consequently, the marginal hypophosphatemia observed in this case and the possibility of P deficiency were dismissed.

Although vitamin D status was not determined, it was unlikely that hypovitaminosis D had contributed to this case because the animals were allocated outdoors, compatible lesions were not observed, and we have not found reports in our country or latitude.

Chronic gastrointestinal helminthiasis can reduce mineral and protein deposition in bone (25, 26). In this case, its contribution was excluded because the animals had never grazed, preventing the helminthic cycle development, and the parasitological studies resulted in negative.

We support that these cases were produced by hypocuprosis, possibly favored by dietary Ca deficiency. The omission of the mineral mix supply and the SO4 excess in drinking water mainly caused these deficiencies. Although Cu deficiency is widespread worldwide, we have not found reports in the international literature about the natural occurrence of osteoporosis in cattle on concentrates-based diets. This report is helpful to remind include hypocuprosis among the differential etiological diagnoses for limp and spontaneous fractures in pen-backgrounded and/or feedlot cattle. Even in the absence of clinical signs, the inclusion of macro and micro minerals in growing and finishing diets is essential, since their deficit can cause significant subclinical losses.

Conflict of Interest

The authors declare no competing interests.

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