Vitamin D-responsive rickets in neonatal lambs

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Abstract — An unusual clinical presentation of rickets in nursing lambs is described. Two sets of twin nursing lambs were presented for enlarged joints and mild angular deformity. Rickets was suspected, based on radiographic evidence. Vitamin D deficiency was determined to be the cause, based on serum concentration and response to therapy.

Résumé — Rachitisme relié à la vitamine D chez des agneaux nouveau-nés. Un cas inhabituel de rachitisme chez des agneaux à l'allaitement est décrit. Deux couples de jumeaux à l'allaitement ont été présentés pour articulations enflées et difformité angulaire légère. Le rachitisme a été soupçonné à la suite d'observations radiographiques. La cause en a été attribuée à une déficience en vitamine D en se fiant sur la concentration sérique et sur la réponse au traitement.

(Traduit par Docteur André Blouin)

Can Vet J 2004;45:841–844

In late January 1998, 2 sets of twin lambs (3 to 4 wk of age) with enlarged joints and slightly bowed legs and their dams were admitted to the Veterinary Teaching Hospital, Oregon State University. Although lambing was not quite finished, 4 sets of twins showing similar signs had been identified by the owner. Affected lambs were first noticed because of their reluctance to move around and remaining recumbent. Closer inspection revealed slightly enlarged joints, with the carpus and tarsus being the most obviously affected. All affected lambs were greater than 21 d of age. The flock consisted of 33 Suffolk and Hampshire ewes bred to 2 unrelated, rented, purebred Suffolk rams. Lambs were raised primarily for show purposes by local 4-H participants. The owner was concerned about congenital problems, given the lambs' age. No history of any genetic abnormalities associated with the rams was identified.

The ewe flock grazed perennial ryegrass pasture year round, with a free-choice trace mineral salt block available. All ewes were in good condition, with a body condition score of 3 to 4 (1 to 5 scale). Thin ewes or pregnant ewe-lambs were supplemented with 0.23 to 0.45 kg of corn-oats-barley grain per head/d. No other supplement was provided to the ewes. No metabolic problems had been identified in the ewes during the current lambing season. Nursing lambs were provided free choice commercial creep feed (20% protein) with coccidiostat, as well as access to pasture. Shortly after birth, all lambs had their navels dipped in strong iodine and received parenteral injections of selenium (BoSe; Schering-Plough Animal Health, Union, New Jersey, USA), 0.5 mL, IM, and *Clostridium perfringens* types C + D and tetanus antitoxins. One pair of the admitted lambs (# 3 and 4) had been treated previously with a single injection of a vitamin A and D preparation. Based on history and signalment, a list of differential diagnoses for this problem list would include rickets or some form of metabolic bone disease (physical presentation), spider lamb syndrome (breed predilection), septicemia (age), and congenital deformity (age).

On physical examination, temperature, pulse, and respiratory rate for all lambs were within normal limits. Mild enlargement of all joints was seen in all lambs. In the more severely affected lambs, there was mild varus deviation in the front legs. There was no evidence of fluid, warmth, or pain in any joint. There was no obvious crepitation in any joint when it was flexed. Other than these observations, body conformation of all lambs appeared normal. All other findings on physical examination were considered normal.

Blood was collected from all 4 lambs and submitted for biochemical analyses (Table 1). Clinically, significant differences from reference ranges where observed in all lambs for creatinine (low), glucose (high), alkaline phosphatase (ALP) (high) and inorganic phosphorus (P_i) (high). The 2 lambs (# 1 and 2) not treated previously with vitamin A and D injection had elevated creatine kinase (CK) activity, which may have reflected their preference to remain recumbent. Observed hyperglycemia in these samples can be explained physiologically, as these lambs were preruminants and thus would have serum glucose concentrations more consistent with nonruminant animals. Additionally, stress might also have contributed to hyperglycemia. Measured creatinine concentrations were lower than expected in all lambs. Starvation or lack of muscle mass was not observed and, therefore, could not account for these low values. Rapid protein deposition and lower protein turnover might account for lower creatinine in young growing animals. A linear decline in serum inorganic phosphate (P_i) concentration from 2.91 to 1.94 mmol/L was observed in llamas between birth and 12 mo of age and is consistent with other observations of higher serum P_i values in young growing animals (1,2). Elevated P_i concentrations may be associated with rapid bone growth and remodeling, similar to elevations in ALP activity. Bone-specific ALP is the primary isomer accounting for elevation in

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Table 1. Serum biochemical values in 4 nursing, 3- to 4-week-old lambs exhibiting signs of metabolic bone disease

Parameter	Lamb number				Reference range[b]
	#1	#2	#3[a]	#4[a]	
Urea nitrogen	10.7	9.3	8.6	10.7	7.1 to 25 mmol/L
Creatinine	26.5	44.2	26.5	35.4	79.6 to 177 µmol/L
Glucose	5.94	6.38	6.38	7.99	2.77 to 4.72 mmol/L
Total protein	55	59	50	58	55 to 75 g/L
Albumin	35	34	32	30	25 to 39 g/L
Bilirubin, total	5.1	5.1	10.3	5.1	0 to 8.6 µmol/L
Alkaline phosphatase	975	1108	1170	545	10 to 70 IU/L
Creatine kinase	294	268	117	146	50 to 150 IU/L
γ-glutamyltranferase	85	121	93	137	30 to 94 IU/L
Aspartate aminotransferase	102	84	69	77	60 to 280 IU/L
Sodium	147	147	146	144	145 to 155 mmol/L
Potassium	5.0	5.0	4.8	4.8	4.5 to 6.0 mmol/L
Chloride	107	106	106	106	95 to 112 mmol/L
Calcium	2.5	2.7	2.8	2.5	2.1 to 3.0 mmol/L
Phosphorus	2.8	3.2	3.2	2.8	1.6 to 2.4 mmol/L
Magnesium	0.91	0.95	0.95	0.95	0.91 to 1.15 mmol/L

[a]Previously treated with commercial parenteral vitamin A and D preparation
[b]Clinical pathology laboratory, College of Veterinary Medicine, Oregon State University

activity observed in young growing animals (2). Alkaline phosphatase activity is also elevated in cases of rickets, as a result of bone remodeling (2).

Survey dorsopalmar radiographic images were made of the left carpus for all 4 lambs. A lamb from another flock of similar age and without clinical signs of swollen joints was radiographed for comparison. The radial growth plate in all affected lambs was more irregular to varying degrees and less well defined compared with that of the unaffected lamb. Other growth plates showed similar changes. The most severely affected lamb showed an altered trabecular pattern, especially in the radial and ulnar carpal bones, with multifocal punctate sclerotic areas noted. The medioproximal subchondral contour of this lamb's metacarpal bone (3 and 4) was beveled and may have contributed to the observed slight varus deformity. Based on physical and clinical findings, spider lamb syndrome, congenital deformity, and septicemia were excluded as possible diagnoses. A preliminary diagnosis of rickets, or some form of metabolic bone disease, was considered.

Because these lambs were consuming a milk-based diet and serum calcium and P_i concentrations were normal (age-based criteria), vitamin D status was evaluated. Serum from 2 lambs was submitted for determination of 25-hydroxycholecalciferol concentrations (Vitamin D_3) at the Endocrinology Laboratory, Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, Michigan, USA. This is the most abundant form of vitamin D found in serum and is routinely used in assessing vitamin D adequacy (3). Serum vitamin D concentrations were 0.4 ng/mL and 30 ng/mL (reference: $>$ 30 ng/mL), with the higher concentration coming from the lamb that had previously received a single dose of parenteral vitamin A and D. Serum vitamin D concentration less than 10 ng/mL has been associated with vitamin D-deficient rickets in sheep (4) and llamas (5). Based on these findings, a diagnosis of vitamin D deficiency and subsequent rickets was made. It was recommended that all lambs and ewes be treated with a parenteral supplement of vitamin D_3 (vitamin A and D [A = 500 000 IU/mL, D = 75 000 IU/mL]; Vedco, St. Joseph, Missouri, USA) at 1500 to 2000 IU/kg body weight (BW) and be provided with vitamin D fortified, free-choice mineral salt. One month after the initial visit, the owner reported that all lambs had recovered uneventfully and that no new cases had been identified.

Rickets is a metabolic bone disease of young growing animals resulting from a dietary deficiency of calcium, phosphorus, vitamin D, or some combination of these nutrients (6). Rickets is most commonly seen in weaned animals as a result of inappropriate mineral supplementation. In other reports of osteodystrophy in younger lambs (between 20 and 90 d of age), vitamin D inadequacy was the recognized problem (7–9). Rapid growth rate associated with weaned animals may be a predisposing factor to rickets (4). This case was unique in that it involved much younger animals, which were receiving an adequate calcium and phosphorus diet from milk, thus suggesting a deficiency in vitamin D. Although milk can contain vitamin D, it is not an adequate source without the vitamin D status in the lactating animal being sufficient.

Compounds having vitamin D activity can be derived from both diet (ergocalciferol, D_2) and endogenous synthesis in skin (cholecalciferol, D_3) (10). Both compounds require activation of their respective provitamin D structures by exposure to ultraviolet light. Vitamin D_2 and D_3 must be hydroxylated at the 25 (liver) and 1α (kidney) positions to become biologically active. The most common dietary source of vitamin D_2 is sun cured, dried forages. Green pasture contains minimal amounts of vitamin D_2 and, ultimately, contributes very little to the vitamin D status of grazing ewes. Endogenous vitamin D_3 synthesis from sunlight, when available, is the more significant contributor to overall vitamin D status in the grazing ewe (11).

Tremendous seasonal variation in vitamin D_3 status has been observed in all domestic species and humans (10). Bovine milk vitamin D concentrations also reflect a seasonal pattern, with lowest concentrations in winter (0.06 IU/g fat) compared with summer (0.23 IU/g fat)

(12). Seasonal variation in vitamin D concentration in sheep serum has been well documented, with the lowest levels occurring during the winter months (11,13,14). Inability to synthesize sufficient cholecalciferol during winter months has been attributed to the reduced angle of the sun (reduced intensity) and reduced amount of UV light in the northern and southernmost latitudes. Prolonged overcast weather conditions, indoor housing, dark skin pigmentation, and full fleece are additional confounding factors that can further reduce endogenous vitamin D production in sheep.

In the traditional once per year lambing system, gestation and lambing will coincide with a ewe's seasonal decline in vitamin D status. Vitamin D status of the neonatal lamb has been shown to be highly correlated to that of its dam (14). Supplementation of pregnant ewes resulted in improved vitamin D status of their lambs, although lamb vitamin D concentrations are only 25% of that of their dam (14). For supplementation of the pregnant ewe to influence vitamin D status of the lamb, vitamin D must cross the placenta, concentrate in colostrum, and be adequately consumed, or some combination of these processes. Data from ruminant animals are limited, but placental transfer of fat-soluble vitamins is considered minimal (15). Ewes supplemented prepartum with vitamin E had greater vitamin E concentrations in their colostrum compared with unsupplemented ewes (15). Colostrum contains more fat compared with milk and has greater concentrations of fat-soluble vitamins (1.5 versus 0.4 IU vitamin D/g fat) (16). These data suggest that supplementing the ewe will improve colostral vitamin D content and that colostral consumption by the newborn lamb is the critical determinate of postnatal vitamin D status.

Either oral or parenteral vitamin D supplementation can be used to improve the vitamin D status of the pregnant ewe (3,14,17). In contrast to the other fat-soluble vitamins, there is minimal hepatic storage of vitamin D (10). The minimal dietary vitamin D requirement to prevent rickets is 5.6 and 6.7 IU/kg BW for all classes of sheep and earlyweaned lambs, respectively (18). Oral vitamin D supplementation at 16 and 32 IU/kg BW maintained serum vitamin D at higher concentrations (35 to 40 ng/mL), which may be more appropriate during gestation and lambing (17). These levels of supplementation are well within the safety margin for vitamin D and do not approach toxic levels (19). Single parenteral doses of vitamin D_3 (5500 to 6000 IU/kg BW) raised and maintained adequate serum vitamin D concentrations for 3 mo in sheep that were previously deficient (3). If repeated doses are used, 3 doses of 1500 to 2000 IU/kg BW at 3-week intervals would be optimal (3). Pregnant ewes injected once with vitamin D_3 (5500 to 6000 IU/kg BW) at 4 or 7 wk prior to lambing, but not at 10 wk, maintained adequate vitamin D status (13).

In this case, vitamin D was not supplemented to the pregnant ewes, and a number of other factors, namely, predominately overcast weather conditions in the Pacific Northwest, dark pigmented animals, and full fleece, would all have impeded the ewe's ability to synthesize endogenous cholecalciferol. The nursing lambs had insufficient vitamin D to support normal growth as a result of the inadequate status of their dams, with subsequent low transference through milk and low intake of creep feed with marginal vitamin D content. Vitamin D-responsive osteodystrophy in young lambs has been reported in both pasture and total confinement systems (4,7–9). Total confinement systems reduce vitamin D status through lack of exposure to UV light and inadequate supplementation in the diet. Vitamin D status of conserved forage is low and will decline over time. Pasture-based nutritional systems have inherently low vitamin D without supplementation, and high intake of carotenes in pasture has been suggested to further reduce vitamin D availability (9).

Of interest in this situation was the subjective observation that twin lambs were more severely affected. One could hypothesize that the minimal vitamin D reserves of the dam were divided for twins in comparison to a singleton, resulting in lower vitamin D status in twin lambs.

Another interesting observation in comparing this case study with others describing vitamin D-deficient rickets was the inconsistent pattern of biochemical changes with calcium and phosphorus. Calcium and phosphorus concentrations were normal (age-based criteria) in this situation, whereas, previously, hypocalcemia was observed in sheep (4) and hypophosphatemia reported in llamas (5), suggesting that serum calcium and phosphorus concentrations are potentially not diagnostic. No effect on calcium and phosphorus concentrations was reported in another study (9). One possible explanation for this difference could be age differences in the affected animals. Other reports have been from weaned sheep or nursing 3- to 6-month-old llamas. In any clinical situation involving young growing animals with growth abnormalities, serum vitamin D concentrations should be considered a part of the diagnostic evaluation. CVJ

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