Hypercalcemia and hypervitaminosis D in two lambs

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- Hypervitaminosis D and hypercalcemia in neonatal lambs may be characterized by clinical signs consisting solely of unthriftiness and weakness or an inability to stand.
- Hypercalcemia should be considered as a differential diagnosis for neonatal lambs that have difficulty standing or that are continually recumbent.
- Hypervitaminosis D should be considered as a potential cause for hypercalcemia in neonatal lambs.

A 17-day-old 5-kg (11-lb) crossbred castrated male lamb (lamb 1) was examined to determine the cause of weakness and failure to thrive. Lamb 1 was housed with its twin, a 6-kg (13.2-lb) castrated male (lamb 2). The lambs were castrated and given an injection of selenium and vitamin E (0.5 mg of selenium and 34 U of DL-α-tocopheryl acetate, SC) when they were 2 days old. The lambs were not given any vaccines. Because their dam was agalactic as a result of mastitis during her previous lactation, the lambs were removed from her immediately after parturition and fed 60 ml of bovine colostrum every 3 hours for 1 week. When the lambs were 1 week old, they then were fed a milk replacer formulated for lambs in accordance with label directions. The lambs were housed at the owner's home in a confined area for the first 2 weeks after birth. Thereafter, the lambs were maintained in a wire crate, which was raised above the floor, at a research facility. They were supplied with fresh water and had ad libitum access to a textured grain mixture containing rolled oats, cracked corn, and molasses. The grain mixture was obtained from the university sheep farm where it was being fed to other lambs.

Three days after being moved to the wire crate in the research facility, lamb 1 became progressively weaker. At that time, lamb 1 could stand on its hind limbs but could not stand on its forelimbs. Although both lambs continued to suckle well from a bottle, neither lamb was growing as expected.

Initial physical examination of lamb 1 (day 1) revealed a rectal temperature of 40 C (104 F), heart rate of 180 beats/min, and respiratory rate of 30 breaths/min. The lamb was bright, alert, and responsive but recumbent. The lamb had a normal suckle reflex and was able to stand briefly with assistance; however, when lamb 1 attempted to move, it would fall. This action appeared to be caused by weakness rather than a neurologic deficit. Other abnormalities were not detected during physical examination or abdominal palpation. Neurologic examination did not reveal abnormalities.

Lamb 1 urinated normally during the physical examination. Urine was collected, and results of urinalysis performed by use of a commercial dipstick were considered within acceptable limits.

Lamb 2 was evaluated at the same time, because it had been raised in an identical manner. Physical examination of lamb 2 revealed a rectal temperature of 40.3 C (104.6 F), heart rate of 200 beats/min, and respiratory rate of 40 breaths/min. Lamb 2 was alert, responsive, and had a normal suckle reflex. The lamb was unthrifty but could stand and walk normally. Physical examination did not reveal other abnormalities.

The major problems identified in the 2 lambs were weakness, unthriftiness, and tachycardia. Differential diagnoses for weakness and unthriftiness in lambs included malnutrition, complete or partial failure of passive transfer, bacterial infection (eg, septicemia), and selenium deficiency. Internal parasitism was considered unlikely, because the lambs were only 17 days old and had been reared indoors. Anemia was unlikely because mucous membranes were pink. Anatomic congenital defects attributable to viral infection or abnormal development, trauma associated with dystocia, fractures, and botulism, were considered unlikely on the basis of the history and results of the physical examination. Tachycardia was believed to be caused by stress and excitement.

Initial data consisted of a detailed dietary history and results of hematologic and serologic analysis of samples obtained from lamb 1. It was determined that milk replacer was being mixed in accordance with label directions (0.11 kg [0.25 lb] of milk replacer/0.48 L of water). From the time they were 1 week old until time of initial examination, the lambs were fed 0.48 L of milk replacer daily. Number of feedings per day decreased from 4 when they were 1 week old to 2 at time of initial examination. Quantity of milk replacer per feeding was increased as the number of feedings per day decreased, and the amount fed in a 24-hour period remained the same. The milk replacer was considered to be of excellent quality because protein (≥ 24%) and fat (≥ 35%) content exceeded minimal limits and fiber content was less than maximal limits (≤ 0.15%).[1] The first 4 sources of protein listed on the ingredients label of the milk replacer were dried skimmed milk, dried whey, dried whey product, and dried milk protein. Adequate amounts of milk replacer were being fed for maintenance and growth (0.05 to 0.15 kg/d [0.11 to 0.33 lb/d]). Because the

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lambs' intake was appropriate and they were eating well, malnutrition was ruled out as a potential cause of the problems.

Results of a CBC for lamb 1 were within reference ranges except for an increase in hematocrit (46.2%; reference range, 26.7 to 41.6%). On the basis of these results, infectious or inflammatory disease was considered unlikely.

Abnormalities on serum biochemical analysis of samples obtained from lamb 1 at the time of initial examination included a low concentration of creatinine (0.4 mg/dl; reference range, 0.8 to 1.8 mg/dl), high concentration of total bilirubin (1 mg/dl; reference range, 0.1 to 0.4 mg/dl), hyperglycemia (165 mg/dl; reference range, 55 to 94 mg/dl), hyponatremia (132 mmol/L; reference range, 145 to 155 mmol/L), hypokalemia (3.5 mmol/L; reference range, 3.9 to 6 mmol/L), hypochloremia (98 mmol/L; reference range, 102 to 114 mmol/L), severe hypercalcemia (24.86 mg/dl; reference range, 8.83 to 10.97 mg/dl), hypermagnesemia (3.1 mg/dl; reference range, 1.4 to 3 mg/dl), and a low concentration of total protein (5.5 g/dl; reference range, 5.8 to 7.8 g/dl). The low creatinine concentration may have reflected loss of muscle mass. The high concentration of total bilirubin was considered unimportant, because it was only a mild increase and inconsistent with major causes of bilirubinemia (anemia, hepatic disease, anorexia). Hyperglycemia was attributed to stress of recumbency and handling. The low concentration of total protein may have been caused by partial failure of passive transfer. Furthermore, the total protein value actually may have been lower, considering the possibility of dehydration suggested by the increase in hematocrit. However, hyponatremia and hypochloremia were less suggestive of dehydration. Selenium deficiency was considered unlikely, because aspartate transaminase activity was within the reference range, and selenium had been administered. Calcium-induced tubular damage could have resulted in low sodium, potassium, and chloride concentrations and a high concentration of magnesium.

The most noticeable problem was hypercalcemia. Differential diagnoses for hypercalcemia of lambs include laboratory error, high-calcium diet, pseudohyperparathyroidism (neoplasia), primary hyperparathyroidism, and hypervitaminosis D (ie, vitamin D toxicosis). The best palliative treatment for hypercalcemia is volume expansion and diuresis with saline (0.9% NaCl) solution given IV. Lamb 1 was given 500 ml of saline solution (50 ml, SC, q 5 h) during a 48-hour period to promote calcium diuresis. This should have caused an increase in glomerular filtration rate and a subsequent decrease in serum calcium concentration. Diuresis may cause serum calcium concentration to decrease, but the concentration usually does not return to the reference range unless treatment also is directed against the primary cause of the hypercalcemia.

On day 2, the lambs continued to eat well, but lamb 1 was still unable to stand. Diagnostic testing for hypercalcemia included analysis of serum calcium concentration of other lambs (lamb 2, 3 lambs of similar age that were being nursed by their dams, and 2 lambs that were being fed another milk replacer). In addition, analysis of serum ionized calcium concentration and **fractional excretion (FE)** of urinary calcium was performed on lamb 1.

Laboratory error was eliminated as a reason for the hypercalcemia. Serum calcium concentrations of both twin lambs were high (lamb 1, 23.09 mg/dl; lamb 2, 23.61 mg/dl), compared with concentrations for the 3 lambs nursing their dam (11.82 to 12.46 mg/dl) and the 2 lambs being fed another milk replacer (10.45 and 11.04 mg/dl). Serum calcium concentrations of the 3 dam-nursed lambs were high when compared with values for the laboratory reference range (8.83 to 10.97 mg/dl). However, that reference range was established with samples obtained from mature ewes. Comparison with reference ranges for serum calcium listed in another source[2] suggested that serum calcium concentration of the 3 dam-nursed and the other 2 replacer-fed lambs were within acceptable limits. Serum ionized calcium concentration for lamb 1 (11.75 mg/dl) was > 2 times the concentration of ionized calcium in 2 of the clinically normal lambs (4.65 and 5.05 mg/dl). Urinary calcium concentration (13.85 mg/dl) was considered higher than normal, which was to be expected, assuming lamb 1 did not have renal insufficiency. Calculated FE of calcium was 1%. The reference ranges for calcium excretion in lambs were unable to be located, but FE of calcium in mature ewes ranged from 0.03 to 8.9% (mean, 0.48%).[3] In clinically normal foals, the FE of calcium (0 to 6%) was higher than the FE of calcium for mature horses.[4] In one study,[5] mature horses fed high-calcium diets had a high FE of calcium (3.7 to 4.6%), but even horses on a low-calcium diet had a FE of calcium of > 1%.

The cervical area of each lamb was palpated from the corner of each hemimandible to the thoracic inlet in an effort to detect enlarged thyroid or parathyroid glands. Distinguishable masses were not identified.

On day 7, the condition of lamb 1 continued to deteriorate, and lamb 2 appeared weaker, although it could still stand. Because the lambs had not improved, a serum sample was submitted for selenium analysis. Serum selenium concentrations were considered adequate (lamb 1, 108 **parts per billion [ppb]**; lamb 2, 90 ppb; reference range, > 80 ppb). Thus, selenium deficiency was ruled out.

Additionally, the milk replacer the lambs were consuming could not be eliminated as the source of excess calcium or vitamin D. Therefore, the diet was switched to a milk replacer formulated for goats, which was fed in accordance with label directions.

On day 9, conditions of the lambs were not noticeably improved. Analysis of serum calcium concentration was repeated. Serum calcium (total and ionized) concentrations had decreased in both lambs (lamb 1, 22.36 and 8.74 mg/dl; lamb 2, 19.46 and 6.66 mg/dl, respectively). Mineral analysis of the milk replacer revealed that calcium content of the milk replacer (0.81% on a dry-matter basis) was within expected limits. Excessive dietary calcium was considered an unlikely reason for hypercalcemia in these lambs. Because hypercalcemia may lead to calcification of soft tissues and bone resorption, radiography was per-

formed on lamb 1. Density of all bones appeared to be within normal limits, and evidence of soft-tissue mineralization was not detected. One reason for the normal radiographic findings may have been that hyperphosphatemia is considered more important than hypercalcemia for the development of mineralization, and serum phosphorus concentrations were within the reference range in these lambs. Pseudohyperparathyroidism was considered unlikely.

By day 10, the lambs were bright, alert, responsive, and able to stand and run without assistance. Serum calcium concentration continued to decrease slightly (lamb 1, 21 mg/dl; lamb 2, 18.45 mg/dl). Clinical recovery of the lambs may have been attributable to the reduction in ionized calcium concentration evident on day 9. Although abnormalities of serum biochemical analyses were similar to those in lamb 1 on day 1, most values were approaching reference ranges. Because the lambs' improvement coincided with switching milk replacers, primary hyperparathyroidism was considered unlikely.

On day 14, the lambs were doing well and thriving. Serum calcium and magnesium concentrations in both lambs were within reference ranges (lamb 1, 11.35 and 2.0 mg/dl; lamb 2, 10.61 and 2.0 mg/dl). The lambs were discharged to the owner. On day 45, the lambs were weaned and fed a diet consisting of only solid feed. The owner reported that they were doing well.

Serum samples of affected and clinically normal lambs were submitted (samples collected on day 8) for determination of concentrations of parathyroid hormone and **25-hydroxyvitamin D (25-OHD)**. Serum parathyroid hormone concentrations for lambs 1 and 2 were 0 and 1 pmol/L, compared with 1 pmol/L for the 2 clinically healthy lambs. The laboratory reported that they considered the parathyroid hormone concentration to be inaccurate. However, 25-OHD concentrations were more than 7 times higher in the 2 affected lambs than in the 2 clinically normal lambs (affected lambs, 154 and 174 nmol/L; clinically normal lambs, 9 and 20 nmol/L). Therefore, a diagnosis of hypervitaminosis D was made.

A sample of the milk replacer formulated for lambs was analyzed for vitamin D content. According to the label, the anticipated vitamin D content of the milk replacer was 2,273 U/kg (5,000 U/lb). Analysis at a private laboratory revealed its concentration was 2,273 U/kg. Calcium and phosphorus concentrations also were analyzed, and concentrations were as listed on the label. Therefore, the exact reason for the increased concentration of vitamin D in the lambs remained unclear; however, switching the milk replacer in the diet coincided with their recovery.

Clinical signs of hypervitaminosis D reported in sheep include bone abnormalities, cardiovascular calcinosis, renal calcinosis, hypercalcemia, loss of weight, and unwillingness to ambulate.[6,7] Several of these clinical signs were evident in the lambs reported here.

At high serum concentrations, 25-OHD can compete effectively with 1,25-dihydroxyvitamin D for receptors in the intestines and bones and can induce actions usually attributed to 1,25-dihydroxyvitamin D. Thus, 25-OHD is believed to be a critical factor in vitamin D toxicosis.[8]

Although there is no evidence of other sources of vitamin D (plants containing glycosides of vitamin D, certain rodenticides, or injections of vitamin D), it could not be ruled out completely. An accidental overdose of a vitamin D compound is plausible because vitamin D is stored in adipose tissue, and a single dose could result in systemic signs of toxicosis. The plasma half-life of vitamin D is 5 to 7 days, and the plasma half-life of 25-OHD is 20 to 30 days. The lambs' return to normalcy may have been attributable to a reduction of stored vitamin D, which may have coincided with the change in milk replacer.

The dam of the 2 affected lambs was considered an unlikely source of the hypervitaminosis D, because clinical signs were not detected until 2 weeks after they were removed from the dam. Also, the ewe was managed and fed in an identical manner to other ewes on the farm, and none of the other lambs on the farm developed clinical signs similar to those of the affected lambs.

Blood calcium concentration is regulated by parathyroid hormone, vitamin D, and calcitonin. Serum ionized calcium content is tightly regulated by parathyroid hormone. As the ionized calcium concentration decreases, parathyroid hormone secretion increases. Parathyroid hormone increases calcium reabsorption in the kidneys and bones and also increases conversion of 25-OHD to 1,25-dihydroxyvitamin D, which is the active form of vitamin D. In turn, 1,25-dihydroxyvitamin D increases intestinal uptake of calcium. As ionized calcium concentration returns to the reference range, parathyroid hormone secretion becomes minimal. Calcitonin acts to decrease calcium concentration by inhibiting parathyroid hormone-stimulated bone resorption. Calcitonin also decreases reabsorption of phosphorus from the renal tubules. Calcitonin secretion is independent of vitamin D concentration, which may partially explain the reason why the lambs reported here were not hyperphosphatemic.

Hypoadrenocorticism may cause mild hypercalcemia, but other abnormalities such as azotemia, hyperkalemia, and hyperphosphatemia usually are not evident. Renal disease is more commonly associated with normocalcemia or hypocalcemia and hyperphosphatemia.

Lack of reference values for young lambs and an appropriate test for parathyroid hormone presented difficulties. Administration of fluids for calcium diuresis or other treatments for hypercalcemia would have resulted in only a temporary cure if the primary source of vitamin D had not been altered. Although a specific cause for the hypervitaminosis D was not established, removal of the milk replacer formulated for lambs coincided with clinical recovery and serum calcium concentrations within the reference range a few days later.

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