Case Report
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Successful Detection and Removal of a Functional Parathyroid Adenoma in a Pony Using Technetium Tc 99m Sestamibi Scintigraphy

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Key words: Horse; Hypercalcemia; Hyperparathyroid; Nuclear scintigraphy.

A 20-year-old, 275-kg, Welsh pony gelding was referred to New Bolton Center for treatment of primary hyperparathyroidism. The horse was diagnosed with primary hyperparathyroidism based on persistently high total (19.5 mg/dL; reference range 10.8–13.5 mg/dL) and ionized serum calcium (9.36–13.16 mg/dL; reference range 6–7.6 mg/dL) concentrations with concurrently increased serum parathyroid hormone (PTH) concentration (Immunoradiometric assay[a]) (128.63 pmol/L; reference range 0.60–11.00 pmol/L), and lack of evidence of renal failure (serum creatinine concentration, 1.7 mg/dL; reference range 0.8–2.2 mg/dL). Hypercalcemia was first identified 19 months earlier (total serum calcium concentration, 14.6 mg/dL; reference range 10.4–12.9 mg/dL), but was not accompanied by clinical signs until 3 weeks before presentation when lethargy and inappetence were noted. The gelding had been previously diagnosed with pituitary pars intermedia dysfunction (PPID) and was being treated with pergolide[b] (1 mg PO q24h). The pony had no reported lameness problems, other than a mild bout of laminitis presumably associated with PPID. No treatment for hypercalcemia was initiated before referral.

On presentation, the pony was bright and alert, with a normal rectal temperature (38.4°C), heart rate (44 beats/min [bpm]), and respiratory rate (16 breaths/min). Body condition score was 4/9 with visible ribs and decreased musculature over the neck and back. Hypertrichosis was present. Dentition was normal for the pony's age and no loosening of the teeth was present. Visual assessment and palpation of the limbs and skull did not detect any skeletal abnormalities. The pony had intermittent weight shifting of the hind limbs, strong to bounding digital pulses, and a moderately shortened stride but did not show overt lameness at a walk. Intermittent full body fine muscle fasciculations were noted. Polyuria and polydipsia were not evident. A urine sample was not obtained.

Abbreviations:

PPID	pars pituitary intermedia dysfunction
PTH	parathyroid hormone
PTHrp	parathyroid hormone–related protein

Complete blood count disclosed mild normocytic, hypochromic anemia (Hct, 28%; reference range 34–46%; MCHC, 33.2; reference range 33.3–38.5 g/dL), moderate hyperfibrinogenemia (plasma fibrinogen concentration, 651 mg/dL; reference range 100–400 mg/dL), and a normal leukogram. A serum biochemistry profile disclosed marked hypercalcemia (total calcium concentration, 21.47 mg/dL; reference range 10.70–13.40 mg/dL; ionized calcium concentration, 10.08 mg/dL; reference range 5.48–7.04 mg/dL), mildly decreased creatine kinase activity (CK, 85 U/L; reference range 90–270 U/L), and mildly increased gamma glutamyl transferase activity (GGT, 57 U/L; reference range 12–45 U/L). Serum creatinine (1.6 mg/dL; reference range 0.6–1.8 mg/dL) and phosphorus (3.07 mg/dL; reference range 1.90–5.40 mg/dL) concentrations were within reference ranges.

Although laboratory analysis identified increased PTH, parathyroid hormone–related protein (PTHrP) was not measured, and a paraneoplastic syndrome was not ruled out. Abdominal ultrasonography was performed to identify lymphosarcoma or tumors that could produce PTHrp. No evidence of neoplasia was found. A hyperechoic medullary rim sign was detected in both kidneys, which is a nonspecific finding indicative of tubulonephrosis or nephrocalcinosis. Both kidneys were normal in size for a pony.

Technetium Tc 99m nuclear scintigraphy of the neck using 200 millicuries of technetium Tc 99m sestamibi[c] IV was performed from the ramus of the mandible to the heart base. Lateral and ventrodorsal images of the neck were acquired using a large field-of-view scintillation camera with a low-energy, all-purpose collimator[d]. Images were obtained at 10 minutes, 2.5 hours, 5.5 hours, and 24 hours after technetium injection. Uptake of radionuclide was identified in areas attributed to the salivary and thyroid glands. An abnormal circular area of uptake was identified at the thoracic inlet at the level of the point of the shoulder on

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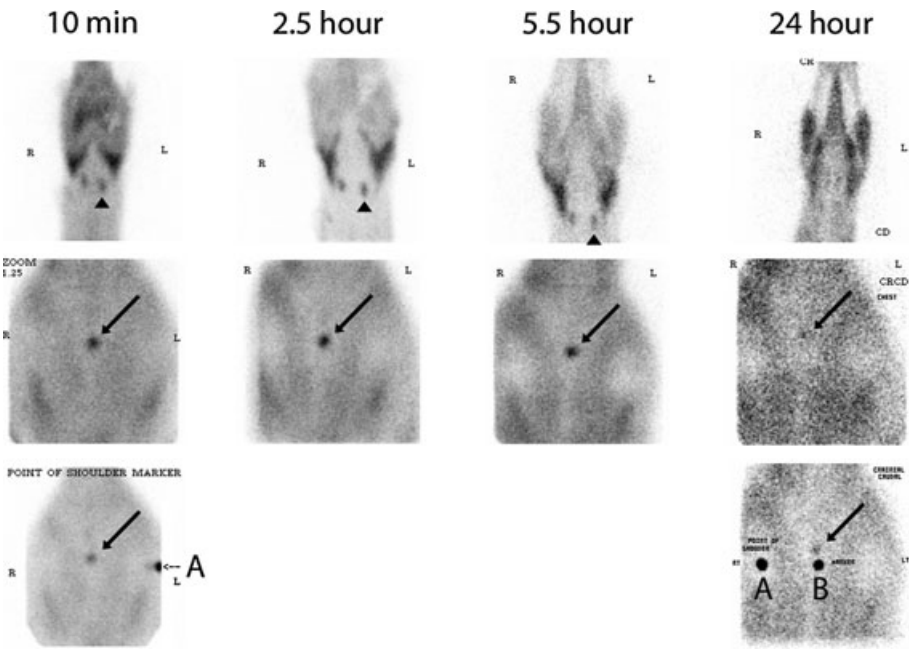

Fig 1. Serial technetium Tc 99m sestamibi scintigraphy images of the neck and thoracic inlet of a 20-year-old pony gelding with a parathyroid adenoma. Ventrodorsal view from the ramus of the mandible to the thoracic inlet and craniocaudal views of the chest are shown. Radionuclide uptake in the thyroid glands (arrowheads) was present from 10 minutes to 5.5 hours after injection. The small circular uptake at the level of the point of the shoulder at the thoracic inlet (arrows) represents the parathyroid adenoma. Radionuclide uptake persisted in this region for 24 hours after technetium injection, at which point radionuclide had washed out of the thyroid and salivary glands. Markers were used in the 10-minute and 24-hour scans to show the point of the shoulder **(A)** and the region of interest **(B)**.

midline (Fig 1). The late phase at 24 hours showed a small amount of uptake remaining at this abnormal location when the radionuclide had washed out of the thyroid tissue.

Sonography of the thoracic inlet focused on the region identified by scintigraphy identified a hypoechoic structure located between the jugular veins, measuring 1.5 × 1.5 × 1.5 cm (Fig 2).

Based on the scintigraphic and sonographic findings, and increased PTH in the presence of hypercalcemia, primary hyperparathyroidism was confirmed and single gland disease was suspected. Surgical removal was the recommended treatment of choice.

Potential complications of surgery and anesthesia were damage to the recurrent laryngeal nerve, fracture, and damage to the major vessels of the neck. Preoperative endoscopy of the upper respiratory tract demonstrated normal laryngeal function. Narrowing of the nasal passages or upper airway was not identified during endoscopy. To evaluate bone density and risk of fracture during recovery from anesthesia, radiography of the distal limbs was performed. Both forelimbs had 6 degrees of rotation of the pedal bone and small osteophytes at the tips of the pedal bones consistent with chronic mild laminitis. There was no evidence of active laminitis or abscessation in any foot. Severe generalized osteopenia and punctate osteolysis of cortical and medullary bone, with the hind limbs more affected than the forelimbs, was seen in all projections (Fig 3). The risk of fracture during recovery from general anesthesia was considered high and a standing procedure was considered. Because of the risk of damaging the carotid arteries or other vital structures during a standing procedure, general anesthesia was elected.

Diuresis to decrease the serum calcium concentration was attempted to minimize the cardiovascular and renal effects of hypercalcemia during anesthesia. Diuresis was achieved with 6 mL/kg/h isotonic crystalloid[e] IV fluids and furosemide[f] (1 mg/kg IV q6h) to promote calciuresis. This protocol decreased the ionized calcium concentration to 8.92 mg/dL (reference range 5.48–7.04 mg/dL) in 24 hours.

Parathyroidectomy was performed under general anesthesia with the pony in dorsal recumbency. Impression smears of the mass were evaluated intraoperatively and were consistent with a neuroendocrine tumor. Two doses of procaine penicillin G (22,000 IU/kg IM q12h) and a single dose of phenylbutazone (3.6 mg/kg PO) were administered perioperatively.

Parathyroid hormone concentrations measured at induction of anesthesia and 15 minutes after removal of the mass demonstrated >95% decrease in serum PTH concentration (preoperative PTH >231.00 pmol/L; 15 minutes after mass removal PTH, 11.80 pmol/L; reference range 0.60–11.00 pmol/L). Vitamin D concentration at the time of surgery was slightly increased (25-Hydroxyvitamin D, 27 nmol/L; reference range 11–24 nmol/L[a]).

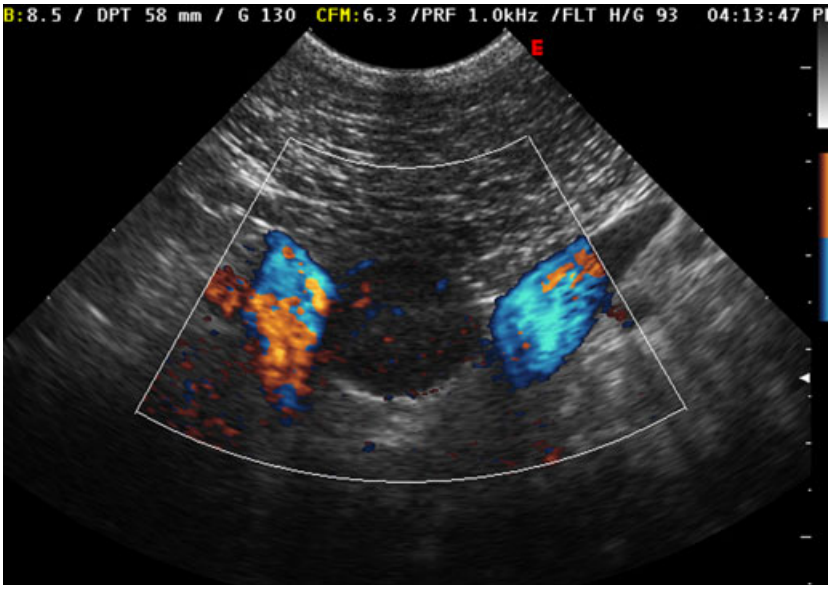

Fig 2. Sonography of a parathyroid adenoma at the thoracic inlet in a 20-year-old pony with primary hyperparathyroidism. The adenoma was identified as a homogenous hypoechoic circular structure nestled between the jugular veins, which are highlighted with Doppler color flow imaging.

Histopathology identified a well-demarcated, encapsulated neoplastic proliferation of neuroendocrine cells that formed discrete lobules divided by delicate fibrovascular septae and compressed the adjacent parathyroid glandular tissue (Fig 4a). Neoplastic cells comprised a bland population of polygonal cells with pale granular eosinophilic cytoplasm and round-to-oval nuclei containing finely stippled chromatin and 1–2 discrete nucleoli (Fig 4b). Mitoses were rare (<1 per 10 HPF). Chromogranin A indirect immunohistochemical stain[g] identified variable antigen expression within the cytoplasm of neoplastic cells, as well as within adjacent compressed parathyroid glandular epithelium (Fig 4c). Positive controls performed as expected using equine thyroid and adrenal tissue (data not shown). Immunohistochemical staining for PTH was negative in neoplastic and compressed parathyroid glandular tissue (data not shown). Positive controls for PTH staining performed as expected using canine tissue (data not shown). These features were most consistent with neuroendocrine adenoma, most likely of parathyroid cell origin.

Postoperative complications included acute kidney injury, tetany, tremors, severe electrolyte derangements, anemia, and tachycardia. Urine obtained at the time of surgery, 8 hours after the last dose of furosemide, showed a urine specific gravity of 1.022. Two hours after surgery, azotemia (serum creatinine concentration, 2.6 mg/dL; reference range 0.6–1.8 mg/dL) and isosthenuria (urine specific gravity, 1.008; reference range 1.025–1.050) were found. A complete urinalysis disclosed a pH of 8.0, rare renal tubular epithelial cells, and no casts. The increase in serum creatinine concentration and development of hyposthenuria indicated acute kidney injury. Azotemia resolved over the next 48 hours with IV fluid therapy at 4–6 mL/kg/h.

Full body fine and large muscle fasciculations and muscular stiffness with a reluctance to walk developed 24 hours after mass removal. Signs progressed to hyper-responsiveness and laryngeal spasm by 40 hours. These clinical signs were consistent with hypocalcemic tetany; however, serum ionized calcium concentration was measured every 4–6 hours after parathyroidectomy and the onset of signs occurred before hypocalcemia developed (Fig 5). At 42 hours after parathyroidectomy, ionized hypocalcemia (iCa, 5.35 mg/dL; reference range 5.48–7.04 mg/dL), ionized hypomagnesemia (iMg, 1.20 mg/dL; reference range 1.35–1.45 mg/dL), and severe hypophosphatemia (P, 0.4 mg/dL; reference range 1.90–5.40 mg/dL) were detected. At that time, blood glucose concentration was normal (114 mg/dL; reference range 72–114 mg/dL) and serum triglyceride concentration was increased (261 mg/dL; reference range 11–52 mg/dL). A combination of IV and enteral electrolyte supplementation was used to correct the electrolyte imbalances. Intravenous supplementation was initiated at 0.04 mmol/kg/h phosphorus (0.03 mL/kg/h Fleet enema; [phosphorus] = 1.38 mmol/mL of enema), 2 mg/kg/h magnesium as magnesium sulfate, and 20 mg/kg/h calcium as calcium gluconate. Fleet enema was considered safe for IV use based on prior publication describing its safe use for human dialysis.[1] Oral supplementation was initiated at 18 mg/kg calcium (30 cc of calcium chloride gel[h]) PO q12h, 0.15 g/kg potassium as potassium phosphate (K_2HPO_4) PO q12h and 0.05 g/kg magnesium as magnesium oxide (MgO) PO q12h. Intravenous supplementation was tapered over 72 hours and oral supplementation successfully maintained electrolyte concentrations within

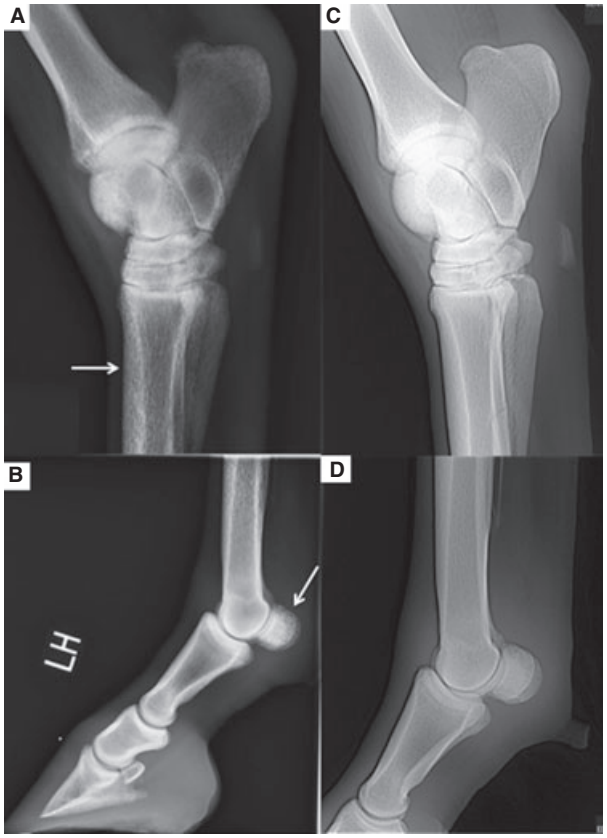

Fig 3. Lateromedial radiographic projections of the left hind tarsus and distal limb of a 20-year-old pony gelding with primary hyperparathyroidism at initial presentation **(A, B)** and 3 months after successful parathyroidectomy **(C, D)**. The radiodensity cannot be directly compared between images because different equipment was used to acquire them; however, osteolytic lesions can be seen prominently in the sesamoid and third metatarsal bones (arrows) in the initial images (A, B) and these lesions are resolved in the follow-up images (C, D).

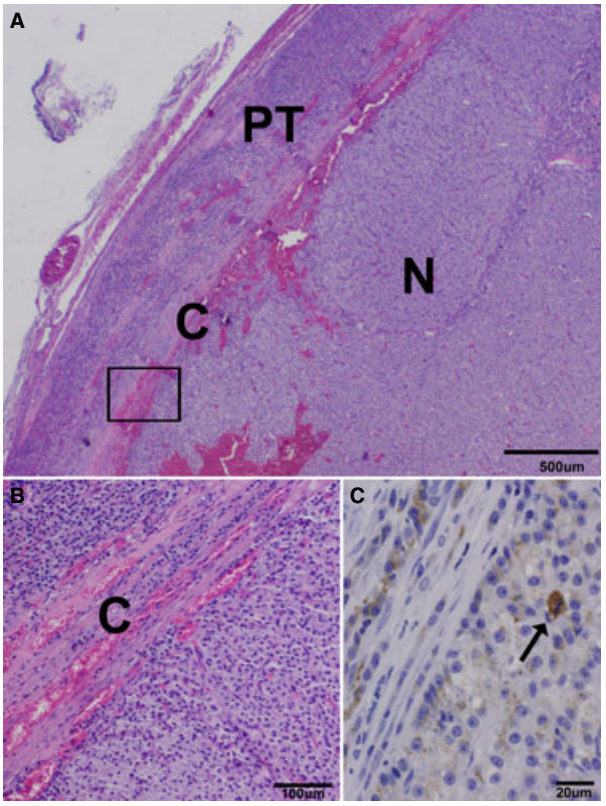

Fig 4. **(A)** Low (20X) and **(B)** high (200X) magnification H&E photomicrograph of a parathyroid adenoma excised from a 20-year-old pony gelding shows a well-demarcated neoplastic proliferation of neuroendocrine cells (A, N; B, bottom right) that compresses adjacent parathyroid glandular epithelium (A, PT; B, upper left), separated by a fibrovascular capsule (A and B, C). (B) Highlights framed region in **(A)** and shows bland polygonal cells forming nests and palisading cords with round-to-oval nuclei containing finely stippled chromatin and punctate nucleoli. **(C)** Chromogranin A indirect immunohistochemical stain shows weak-to-moderate cytoplasmic staining within neoplastic cells and adjacent parathyroid gland epithelium. Occasional strong cytoplasmic staining was seen within scattered individual neoplastic epithelium (arrow); this cell exhibits mitosis. Chromogranin A positive controls on equine neuroendocrine tissue performed as expected (not shown).

normal limits. Complete correction of the calcium concentration was achieved within 12 hours, and serum phosphorus and magnesium concentrations were corrected within 24 hours, but the tremors did not abate concurrently.

Orthopedic pain as evidenced by stiffness, reluctance to walk, and grade 4/5 left hindlimb lameness developed on the day of parathyroidectomy. Because of transient azotemia, NSAIDs were not administered. Butorphanol (0.036 mg/kg IM) resulted in no apparent improvement in attitude, heart rate, or ambulation. Gabapentin (10 mg/kg PO q8h) was administered for analgesia and was associated with mild improvement. Clinical signs resolved over the week after parathyroidectomy.

Persistent tachycardia (58–80 bpm) developed in 3 days after the surgery. Continuous ECG telemetry identified sinus tachycardia and no other arrhythmias. The heart rate was 48–52 bpm at the time of discharge.

The pony was discharged from the hospital 8 days after parathyroidectomy. Clinical signs had largely abated by that time, but intermittent muscle fasciculations and mild stiffness were reported in the weeks after discharge from the hospital. Oral electrolyte supplementation was tapered and discontinued completely after 3 weeks. Serum electrolyte concentrations remained stable, and 2 months after discharge, PTH (2.80 pmol/L; reference range 0.60–11.00 pmol/L) and serum ionized calcium concentration (6.64 mg/dL; reference range 6.32–7.60 mg/dL) were normal. Radiographs 3 months after discharge disclosed notable improvement in bone density (Fig 3).

Hypercalcemia in horses can be caused by primary or secondary hyperparathyroidism, hypervitaminosis D, or hypercalcemia of malignancy. Primary hyperparathyroidism is caused by overproduction of endogenous PTH by abnormal parathyroid tissue. It can mimic hypercalcemia of malignancy in which neoplastic tissues elsewhere in the body produce PTHrp.

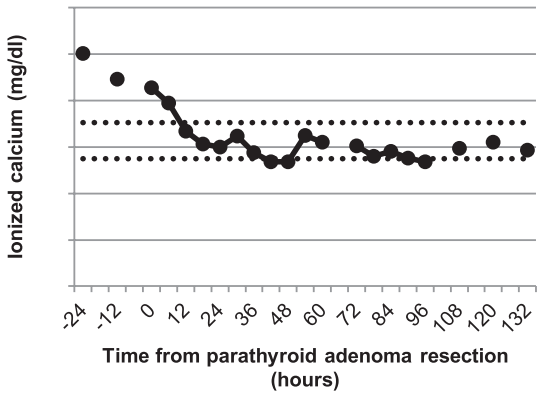

Fig 5. Ionized calcium concentrations over time in a 20-year-old pony that underwent single-gland parathyroidectomy for primary hyperparathyroidism. Horizontal lines represent the reference range for serum ionized calcium concentration. Time 0 was the measurement taken immediately after surgery. From −24 to 0 hours, the pony underwent diuresis with furosemide (1 mg/kg IV q6h) and IV polyionic fluids (6 mL/kg/h) to promote calciuresis. After parathyroidectomy, clinical signs of tetany (fasciculations and stiffness) developed by 24 hours. More severe signs of tetany, including laryngeal spasm, were present between 40 and 72 hours (denoted by *), and fasciculations persisted beyond 132 hours. Oral and IV calcium supplementation was initiated at 46 hours. Oral supplementation continued beyond 132 hours.

Increased PTH or PTHrp concentrations induce renal calcium conservation and phosphorous wasting, calcitriol synthesis, and osteoclastic bone resorption. Both situations typically result in hypercalcemia with hypophosphatemia and hypocalciuria and hyperphosphaturia. Hypercalcemia of malignancy is associated with suppression of the parathyroid glands and normal-to-low PTH concentrations. Secondary hyperparathyroidism in the horse is most commonly caused by nutritional imbalance. In other species, chronic renal failure results in accumulation of phosphorus and secondary hyperparathyroidism, but this is not a recognized finding in horses. Both hypervitaminosis D and nutritional secondary hyperparathyroidism are characterized by hyperphosphatemia. The initial hematologic findings in this pony with hypercalcemia and normal serum phosphorous concentration were suggestive of primary hyperparathyroidism. Because PTH was >10-fold above the reference range, hypercalcemia of malignancy was considered highly unlikely and PTHrp was not measured.

This is the first reported case of successful identification of a parathyroid adenoma in an equid with technetium Tc 99m sestamibi. Equids have 2 pairs of parathyroid glands, with the caudal pair located anywhere along the carotid arteries from the cranial cervical region to the thymus,[2] making detection of an adenoma by ultrasonography alone difficult. Technetium Tc 99m sestamibi has been used successfully in humans and dogs to detect parathyroid adenomas, but recent attempts in horses have not been successful.[2]

The clinical signs of weight loss, shivering or tremors, and orthopedic pain (lameness, stiffness) seen in this pony are typical of hyperparathyroidism in several species, including horses.[2–6] Studies in dogs have shown polyuria and polydipsia to be the primary presenting complaints, but weight loss, lameness, and tremors have been reported,[7,8] with up to 27% of dogs having lameness and 10% having tremors.[8] Lameness is thought to be related to ongoing osteoclastic activity and remodeling of the bones from prolonged PTH stimulation, although the effect of microfractures cannot be eliminated. In this case, radiographs were performed, but no evidence of pathologic fractures was identified. Current equine computed tomography (CT) systems have poor ability to detect microfractures and CT examination would have been unlikely to provide additional information. An alternate, and perhaps more likely, hypothesis is that bone pain results from tension on the periosteum and tearing of ligamentous insertions attributable to weakness and cartilagenous remodeling of the bones. However, there is no scientific proof of this hypothesis.[4]

A dichotomous scoring model for human hyperparathyroid patients accurately predicts single-gland primary hyperparathyroidism if ≥3 of the following 5 findings are present: (1) preoperative total serum calcium concentration ≥12 mg/dL; (2) PTH ≥ twice the upper limit of normal; (3) sestamibi scan detects a single enlarged parathyroid gland; (4) sonography detects a single enlarged parathyroid gland; and (5) scintigraphy and sonography results are concordant (identify the same single gland as enlarged).[9] Although this model is not validated in horses, this case met all criteria and a single-gland primary hyperparathyroidism was considered highly likely.

This is the first description of a functional parathyroid adenoma in an equid that had minimal Chromogranin A (CgA) reactivity and negative PTH on immunohistochemical staining. Given the clear clinical evidence that the mass removed was the source of the excessive PTH, and the histopathologic appearance consistent with neuroendocrine tissue, the diagnosis of a parathyroid adenoma was made despite the lack of PTH reactivity on immunohistochemistry. Although parathyroid adenomas in dogs that have been stained for PTH have been highly positive with either diffuse cytoplasmic or perinuclear staining,[10] in people, some parathyroid adenomas do not stain for PTH.[11,12] This is attributed to such a high rate of release of the hormone that an insufficient amount is stored in the cells to take up stain.[11] Alternatively, because normal equine parathyroid tissue was not available as a positive control, the lack of staining could have been a failure of the testing method. CgA can undergo atypical processing in neoplastic tissue, which is thought to account for the lack of staining in certain neuroendocrine neoplasms.[13] A single study in dogs showed that only 3 of 7 parathyroid adenomas were positive for CgA.[14]

Footnotes

[a] Michigan State University Diagnostic Center for Population and Animal Health, Lansing, MI.
[b] Prascend, Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO
[c] Technetium Tc 99m sestamibi, Nuclear Diagnostic Products, Cherry Hill, NJ
[d] Detector: Technicare Omega 500, Diagnostix Plus, Inc., Garden City Park, NY; Software: NuQuest by Alphanuclear, MEDX, Inc., Arlington Heights, IL
[e] Veterinary Plasma-lyte A Injection, Abbott Laboratories, North Chicago, IL
[f] Salix, Intervet International GmbH, Millsboro, DE
[g] Chromogranin A, DAKO, Carpinteria, CA
[h] High potency calcium gel, PRN, Pensacola, FL

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

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