Quantitative Pertechnetate Thyroid Scintigraphy and the Ultrasonographic Appearance of the Thyroid Gland in Clinically Normal Horses

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Abstract:

The purpose of this study was to report the scintigraphic and sonographic appearance of the thyroid gland in clinically normal horses so these modalities could be used to assess the thyroid gland in this species. Horses were divided into two age groups. Group A consisted of 8 horses between 3 and 10 years of age and Group B of 7 horses between 11 and 20 years of age. Total T_4 concentrations were within the laboratory reference interval. Thyroid to salivary (T/S) ratio, percent dose uptake of pertechnetate and thyroid lobe volume were calculated. Echogenicity of thyroid lobes and presence of nodules were documented. The two groups were compared using appropriate parametric and nonparametic tests. Total T_4 concentrations were significantly lower in the older group. Sixty minute mean ± standard deviation (SD) T/S ratios for older versus younger horses were 5.8 ± 3.0 and 5.3 ± 2.2, respectively. Sixty minute median and interquartile ranges for percent dose uptake of pertechnetate for older versus younger horses were 3.64% (1.5 to 3.98%) and 2.55% (2.33 to 2.90%), respectively. Mean ± SD thyroid lobe volumes for older versus younger horses were 18.93 ± 5.16 cm^3 and 13.55 ± 3.56 cm^3, respectively. Most thyroid lobes were hyper or isoechoic to the sternocephalicus muscle. Prevalence of thyroid nodules did not differ between groups. Older horses had trends for greater T/S ratios, percent dose uptakes and thyroid lobe volumes but had lower total T_4 concentrations. Further studies using scintigraphy and ultrasound in horses with thyroid disease are planned.

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Gregory Daniel is professor and Department head of Small Animal Clinical Sciences at the Virginia-Maryland Regional College of Veterinary Medicine. As chair of this committee he was involved in design, planning, supervision and direction of the project. Dr Daniel was primary investigator on the grant awarded by the Virginia Horse Industry Board. He performed all statistical analyses. Dr Daniel contributed to and reviewed the publication for the Journal of Veterinary Radiology and Ultrasound, and formatted images for the publication.

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1. Introduction

Establishing a diagnosis of thyroid dysfunction in horses is problematic due to limited availability and reliability of thyroid hormone assays and the influence of factors such as diet, circadian rhythm, systemic illness and drug administration on thyroid hormone concentrations. Pertechnetate thyroid scintigraphy is commonly used in human and veterinary medicine for evaluating thyroid gland morphology and function. Sonography has been used to evaluate changes in size and structure of the thyroid gland and to differentiate causes of thyroid dysfunction. The purpose of this study was to report the scintigraphic and sonographic appearance of the thyroid gland in clinically normal horses so these modalities could be used to assess the thyroid gland in this species.

2. Literature Review

Anatomy

The thyroid gland in the horse is located dorsal to the third through sixth tracheal rings and is composed of two lobes joined by a narrow fibrous isthmus. Each lobe measures approximately 2.5 by 2.5 by 5 cm.[1] The thyroid gland is not normally visible but can be palpated.[2]

Histology

A fibrous connective tissue capsule encloses the parenchyma of the thyroid gland and branches internally into very narrow septae.[3] These septae consist of a capillary network surrounded by fibrocytes and their associated extracellular matrix and separate the thyroid parenchyma into follicles. Follicular cells line each follicle and contain the various cytoplasmic organelles and enzymes needed for synthesis of thyroid hormones. The apical membrane of the follicular cell possesses numerous microvilli. The lumen (or acinus) created by the circular arrangement of follicular cells contains colloid, the main storage form of thyroid hormones.[4] Parafollicular cells lie between adjacent follicles and are involved in formation, storage and secretion of the hormone calcitonin, a peptide that helps regulate blood calcium concentrations.[4]

Physiology

Thyroid hormones are important for growth, maturation of organ systems and regulation of metabolism. Iodine is required for synthesis of thyroid hormone and is actively transported into the gland (trapped) by the sodium-iodide symporter (NIS).[5, 6] The first step in the formation of thyroid hormones is conversion of iodide ions to an oxidized form of iodine. The oxidised iodine is then capable of combining with the amino acid tyrosine on the thyroglobulin molecule. Each thyroglobulin molecule contains approximately 70 tyrosine amino acids and eventually contains up to 30 thyroxine molecules and a few triiodothyronine molecules. The binding of iodine with the thyroglobulin molecule is called organification. The thyroid gland can store large amounts of hormone in the thyroid follicles in the form of thyroglobulin. About 93% of thyroid hormone secreted by the thyroid gland is in the form of thyroxine (T_4). Approximately 7% is secreted as triiodothyronine (T_3). The main source of

tri-iodothyronine (T_3) is the conversion of T_4 to T_3 in peripheral tissues. T_3 is more metabolically active than T_4. Thyroid hormones circulate both bound to proteins and unbound (free), with the free fraction being the active fraction.[5] Secretion of thyroid hormone is dependent on thyroid-stimulating hormone (TSH), which is released from the anterior pituitary gland. Thyrotropin-releasing hormone (TRH) is released by the median eminence of the hypothalamus and stimulates release of TSH. TSH directly influences the rate of iodine trapping by the thyroid gland, primarily through NIS expression.[7] There is also some modulation of NIS expression by cytokines such as tumor necrosis factor (TNF) and transforming growth factor-ß1 (TGH-ß1).[8] Specific effects of TSH on the thyroid gland include increased proteolysis of thyroglobulin, increased iodination of tyrosine, increased size and secretory activity of thyroid cells, increased number of thyroid cells and increased activity of the iodine pump. Most of these effects result from activation of cyclic adenosine monophosphate (cAMP). cAMP is activated by the binding of TSH with specific TSH receptors on the basal membrane surfaces of the thyroid cell.[5] An increase in thyroid hormone circulating in the blood likely inhibits anterior pituitary secretion of TSH primarily by a direct effect on the anterior pituitary gland. Negative feedback on the hypothalamus is also modulated by cellular metabolism and factors such as temperature. It is the nonprotein – bound portion of thyroid hormone that interacts with the pituitary gland and hypothalamus in the feedback loop that regulates the release of TSH and TRH.[9]

Thyroid dysfunction in horses

Thyroid dysfunction in horses is incompletely understood,[10] difficult to diagnose and poorly documented in the literature.[11] Particular confusion surrounds the diagnosis and prevalence of primary hypothyroidism in adult horses. Thyroid disorders reported in the literature include nodular hyperplasia,[12] non-functional C-cell adenoma,[13-15] hyperthyroidism associated with thyroid neoplasia,[16-18] non-functional thyroid carcinoma,[19-25] idiopathic hypothyroidism[26] and Hashimoto thyroiditis-like disease.[27]

Benign adenomas are reported to comprise most thyroid gland tumors and are common in older horses. These tumors only rarely cause functional disturbances such as hypo or hyperthyroidism.[28] Adenomas derived from thyroid follicular cells can be classified into follicular and papillary types. According to one report most tumors of the thyroid gland in

horses are microfollicular adenomas[12]. Cystic adenomas, follicular nodular hyperplasia and one case of follicular adenocarcinoma were also found in this population of horses. That study examined thyroid glands obtained at post-mortem from healthy horses between 12 and 32 years of age from the North Island of New Zealand. All glands were considered of normal weight and dimensions, and the exterior of the glands appeared normal. Gross tumors were found in the thyroids of 11 of the 29 horses (37.9%), and their occurrence was reported to be strongly age related. Gross tumors ranged in size from 2 to 12 mm. They were all found in horses older than 18 years of age. Most of the lesions were solid and spherical. A small number were cystic with dark orange fluid content. The thyroid tissue surrounding the lesions lacked the histological appearance of hyperactivity or hypoactivity in the majority of cases. In some cases multiple adenomas were found. In the discussion, the authors note that some reports suggest that the incidence of thyroid tumors in domestic animals is highest in iodine-deficient areas where many animals have long-standing diffuse hyperplastic goitre.[29] Goitre is defined as an enlargement of the thyroid gland.[30] Human thyroid adenomas reach their highest incidence in iodine-deficient environments.[31] In these cases neoplastic disease is linked to chronic excessive concentrations of TSH. There was no evidence that chronic excessive thyrotropic stimulation was responsible for the high incidence of lesions in the study group from New Zealand. From this study it was concluded that benign thyroid neoplasia in horses is a common age-related abnormality and that it does not imply chronic excessive thyroid-stimulating hormone secretion. It also seemed unlikely that there was any progression from hyperplasia and adenoma to adenocarcinoma. This relationship of malignant progression has been observed in rodents but not in people.[32]

A more recent publication has described the incidence of non-functional C-cell adenomas in aged horses.[14] In this study immunohistochemical characterization of equine thyroid glands was performed using multiple primary antibodies to determine if lesions were of follicular epithelial origin or derived from parafollicular cells (C cells). Tumors derived from parafollicular cells are positive for calcitonin, calcitonin-gene-related peptide (CGRP), chromogranin A, neuron specific enolase (NSE) and synaptophysin. Thyroid glands from 38 horses, aged 10 to 29 years, that had died or been euthanatized at the Department of Veterinary Pathology, Kitasato University or the Equine Research Institute, Japan Racing Association, between 1995 and 2002, were examined histopathologically as part of the study.

Breeds included mostly thoroughbreds, 5 Anglo-arabs and one Draft horse. Four horses were of unknown breed. Nodular proliferation was found in 12 of the 38 horses (31.6%). Nine of the 12 horses (75%) that were over 20 years of age had evidence of nodular proliferation. In all cases the surface of the thyroid glands was smooth and normal in appearance. The nodules were described as white, round and medullary in location. Cystic changes were not observed. Histologic appearance of the nodules was most consistent with that of C-cell adenomas. As the previously discussed study examined only H&E-stained tissue, it was suggested by the authors that the nodules might have been misclassified and that the great majority of white nodules frequently found in the thyroid glands of older horses are C-cell adenomas. They are likely non-functional.

Hyperthyroidism has been reported in association with thyroid neoplasia but appears to be rare.[16-18] Three cases have been reported. At the time of presentation affected horses were emaciated and had experienced recent weight loss. Horses were described as having an increased appetite, polydipsia, tachycardia, and hyperactivity.[16-18] Hyperthyroidism was confirmed in two of these studies using a T_3 suppression test.[16, 18] In the remaining horse an elevated free T_4 (fT_4) was considered diagnostic even though total T_4 (tT_4) was within the normal reference interval.[17] In all cases either swelling or a mass in the cervical region was described. In one case swelling was bilateral,[18] and in the other two cases it was right-sided.[16, 17] Ultrasound was used to examine the abnormal thyroid glands in all cases. Sonographic findings were variable. In one case diagnosed with unilateral thyroid adenocarcinoma the lobe was described as homogeneous and interspersed with areas of centrally located hypoechoic foci.[17] Another report of unilateral disease described the mass as homogeneous and mildly hyperechoic. This horse was diagnosed with a right thyroid adenoma.[16] The horse with bilateral disease was described as having marked enlargement of both thyroid lobes. The left lobe was of mixed echogenicity and contained hyperechoic striations. Cystic or cavitary regions were present within the lobe. The right lobe was also of mixed echogenicity and appeared more echogenic than the left lobe. A few small cyst-like lesions were described. Histopathology of the left thyroid lobe was consistent with adenocarcinoma and was inconclusive for the right lobe. This particular horse also underwent pertechnetate thyroid scintigraphy. The left thyroid lobe was enlarged and had subjectively increased radiopharmaceutical uptake. There was only very low-intensity

radiopharmaceutical uptake in the right thyroid lobe. It was interesting that scintigraphy was the only modality able to determine relative function between the two thyroid lobes. This information would be critical if the horse had been considered a candidate for thyroidectomy.

Surgical anatomy, technique, complications and outcome of 6 horses requiring thyroidectomy for rapidly expanding thyroid tumors have been described.[33] Horses were between 10 and 22 years of age and were presented for a mass in the thyroid region with rapid expansion over 2 to 6 months. Two of the horses had clinical signs of upper respiratory noise and increased respiratory effort during exercise. All horses had visible unilateral thyroid enlargement. No evidence of metastatic disease was found on physical examination. Thoracic radiographs were not made. Sonographically, the appearance of the masses varied from heterogeneous to cystic and homogeneous. Ultrasound was useful to determine thyroid lobe size and to confirm the location of the mass. Ultrasound was not useful in determining malignancy. Thyroid hormone analysis was only performed in one horse, and total T_4 was normal both before and after surgery. Clinical signs of thyroid hormone imbalance were not seen in any of the horses. Marked deviation and compression of the trachea were present in three of the horses. Histopathologic diagnoses were adenoma, compact carcinoma and C-cell adenocarcinoma. All horses had successful surgical outcomes and returned to their intended use. Two horses had persistent upper respiratory noise during exercise, likely associated with laryngeal hemiplegia. Interestingly, rapid tumor growth was not thought to correlate with malignancy. It was noted that knowledge of T_4 status might be useful before surgery if thyroid dysfunction is suspected, as thyroid storm has been reported in a horse recovering from anesthetia post thyroidectomy.[17] Thyroid storm is a rare, life-threatening exacerbation of the hyperthyroid state in which multi-organ dysfunction occurs. Precipitating factors may include infections, parturition and surgical manipulation of the thyroid gland.[17] Importantly in this study the author discusses the potential for injury to the recurrent laryngeal nerve during surgery. Causes of laryngeal hemiplegia include direct transection of the nerve during en bloc removal of the mass, local inflammation and swelling (neuropraxia), and disruption of the local blood supply caused by surgical trauma. In one of the horses seroma formation may have caused compression of the recurrent laryngeal nerve. Thus surgical resection is not considered a benign procedure. In people, anatomic distortion by enlarging tumors is cited as an important risk factor for nerve damage.[34, 35]

Pertechnetate scintigraphy has been use in the evaluation of horses with thyroid carcinoma.[21, 22] In one case, activity in the abnormal thyroid lobe was minimal and confined to a thin peripheral rim.[22] The horse had normal T_3 and T_4 concentrations. The horse underwent unilateral thyroidectomy. A cross section of the lobe revealed numerous small cysts separated by fibrous trabeculae, with a thin rim of tissue at the periphery that appeared to correspond to the scintigraphic uptake of pertechnetate. The histopathologic diagnosis was thyroid carcinoma. Interestingly the author commented that thyroid tumors in horses were more common in areas of endemic goiter and that this might account for a higher incidence (37%) of adenomas in Minnesota (published in 1931) compared with a much lower incidence (2%) elsewhere in North America and Europe. In a more recent review of equine thyroid dysfunction it was noted that the daily iodine intake in horses in North America is typically 2 mg, which is twice the daily requirement for the average horse.[36] The congenital hypothyroidism-dysmaturity syndrome described in foals in the Pacific Northwest may be totally or in part due to iodine deficiency. It is known that mares fed an iodine-deficient diet have weak or dead foals with goiter and alopecia. It is thought that this disease of foals, now known as thyroid gland hyperplasia and musculoskeletal deformity (TH-MSD) is due to a combination of nitrate ingestion and low iodine levels in feed. Dams have normal thyroid function and are asymptomatic. There are no published reports of hypothyroidism from iodine deficiency in adult horses. However, one source recommends that this differential diagnosis still be considered when goiter is detected.[2]

A case report of work intolerance in a horse with thyroid carcinoma also describes the use of pertechnetate scintigraphy in evaluating the abnormal thyroid lobe.[21] The horse presented for deterioration of pulse and respiration recovery values. T_3 and T_4 concentrations were below the laboratory reference intervals. Results of a TSH stimulation test were within laboratory reference intervals. Pertechnetate uptake was present in both thyroid lobes. The right thyroid lobe was displaced to the right by a mass that had heterogeneous radionuclide uptake. The mass appeared to be associated with the craniomedial pole of the right thyroid lobe. Based on laboratory and clinical findings, a diagnosis of hypothyroidism caused by a thyroid carcinoma was made. The enlarged right thyroid lobe was surgically removed. Grossly the mass appeared encapsulated and was surrounded by partially compressed, pre-existing

thyroid tissue. It was concluded that the work intolerance was unlikely to be mediated through abnormal T_4 production. Scintigraphy was used primarily to determine the origin of the cervical mass and its relationship to the thyroid gland.

A recent case report descrives keratoconjunctivitis sicca attributable to parasympathetic facial nerve dysfunction associated with hypothyroidism in a horse.[26] At presentation the horse, a 6 year-old German (Saxon) Warmblood gelding was overweight and had a marked regional fat deposit over the crest of the neck. The horse also had moderate bilateral blepharospasm, bilateral hyperemic and edematous conjunctivas, and lusterless corneas. The horse was diagnosed with bilateral keratoconjunctivitis sicca and dry nasal mucous membranes, head-shaking syndrome with snorting and flehmen responses, and paresthesia and dysesthesia of the face. T_4 concentration was measured at various time points throughout the day. Values were consistently below the reference interval. TRH and TSH stimulation tests were also performed. Bovine TSH was used. Results of stimulation tests confirmed the inability of the thyroid gland to react to hormonal stimulation. Other endocrinologic diseases were ruled out. Sonographically the thyroid gland parenchyma appeared hypoechoic, and the lobulation seemed reduced. These observations were based on the author's prior experience. The author felt that the sonographic findings supported a diagnosis of hypothyroidism. To investigate the possibility of immune mediated thyroid disease the serum was anaylzed for antithyroglobulin, anti-triiodothyronine, and anti-T_4 autoantibodies. A serum sample from the horse underwent western blot analysis with equine thyroglobulin and thyrocyte-lysate. No reaction indicative of autoantibodies against thyroid hormones was detected. Facial and trigeminal nerve dysfunction were considered as peripheral neuropathies that were most likely secondary to hypothyroidism. Biopsy of the thyroid gland was not performed. The horse's clinical signs seemed to resolve 5 months after initiating treatment with L-thyroxine. This is the first report in the literature to report idiopathic primary hypothyroidism in a horse.

Primary hypothyroidism has been particularly difficult to characterize in horses due to non-specific, vague clinical signs and challenges associated with performing and interpreting thyroid function tests. Historically, clinical signs such as laminitis, obesity, recurrent myositis, anhidrosis and poor fertility were thought to be attributable to hypothyroidism in horses. None of these clinical signs have been observed in experimental models of

hypothyroidism.[37-44] Confusion arose from the fact that horses with these clinical signs were sometimes found to have low circulating thyroid hormone concentrations. Horses were not assessed using thyroid stimulation tests. When adult horses are made hypothyroid by the administration of propylthiouracil (PTU) they may show no clinical signs.[44, 45] Thyroidectomy has been used as a model of hypothyroidism and has been performed on horses from a 202-day-old fetus to an 18-year-old mare.[39-43, 46, 47] Clinical signs in thyroidectomised horses include cold intolerance, lethargy, reduced feed consumption, reduced growth rates, diminished sexual activity, thickening of the face, non-painful swelling of the eyelids, rear limb edema, a coarse hair coat, mild alopecia, and delayed shedding of hair.[39, 41, 42] Only cold intolerance and hair coat abnormalities have been observed consistently. A recent review of the literature pertaining to thyroid dysfunction in horses states that thyroidectomised horses are often clinically indistinguishable from normal healthy horses.[28]

Low thyroid hormone concentrations are sometimes found in horses diagnosed with equine metabolic syndrome (EMS).[48] Horses with this syndrome are typically obese and between 8 and 18 years of age. The most common presentation is an obese horse with regional fat deposits in the neck and tailhead regions. Horses typically develop laminitis while grazing on pasture and are reported to have insulin resistance, hypertriglyceridemia, and hyperleptinemia. Historically, clinical signs associated with this syndrome were erroneously attributed to hypothyroidism, but it is now clear that the combination of laminitis and obesity are not manifestations of insufficient thyroid hormone production. These horses respond normally when thyroid stimulation tests are performed. Low resting T_4 concentrations are likely a consequence, rather than a cause, of EMS.[48]

Goitrous autoimmune thyroiditis (Hashimoto's disease) is a recognized cause of primary hypothyroidism in people. Evidence of a Hashimoto thyroiditis-like disease has been reported in a group of horses from Eastern Europe. This study was undertaken due to the observation of certain macroscopic and microscopic alterations of the thyroid gland found at post mortem. Horses included in the study were between 8 months and 10 years of age. Pathologic glands varied in size (either larger or smaller) when compared to normal thyroid glands. Some horses had nodular changes. In animals classified as having thyroiditis there was lymphocytic infiltration and reactive fibrosis within the thyroid gland. Anti-

thyroglobulin and antimicrosomial antithyroid peroxidase (anti-TPO) autoantibodies were identified in horses with thyroiditis and were thought to account for destruction of the gland. Serum thyroglobulin concentrations were also elevated. Circulating thyroglobulin was interpreted as an indicator of thyroid gland damage. Some horses also had pituitary pathology, with accumulation of basophilic cells and initial fibrosis in the pituitary gland. Causative factors could not be determined for this population. It is uncertain if the disease is associated with clinical signs or whether it is isolated to a specific geographic region. In people, Hashimoto's thyroiditis leads to overt hypothyroidism with clinical signs of fatigue, bradycardia, arterial hypertension, myxedema and metabolic disorders such as hypercholesterolemia. Thyroglobulin and peroxidase antibodies are elevated. The disease is treated with levothyroxin.[49]

Assessing thyroid gland function in horses

Single point-in-time measurements of circulating thyroid hormones are difficult to interpret. Several drugs and certain physiologic and pathologic states are known to alter circulating thyroid hormone concentrations, particularly total T_4 concentrations. Total T_4 is the amount of total thyroxine measured in blood and includes free and protein-bound hormone. The protein-bound portion can be lowered by stress, drugs and non-thyroidal illness including chronic malnutrition, hepatic disease and renal disease.[50] In horses, most circulating thyroxine is bound to albumin.[1] In dogs and people there is a well-recognized syndrome characterized by low thyroid hormone concentrations in patients with severe non-thyroidal illness. Non-thyroidal illnesses tend to affect thyroid function by processes that are not disease specific. Although poorly documented, this syndrome likely occurs in horses.[2]

Non-thyroidal factors that affect the hypothalamic-pituitary-thyroid axis of horses, and that can therefore result in low circulating thyroid hormone concentrations include phenylbutazone administration,[51-53] high energy diets,[54] high protein diets,[55] diets high in zinc and copper,[55] diets with a high carbohydrate/roughage ratio,[56] glucocorticoid administration,[57] food deprivation,[58] level of training,[59] stage of pregnancy[60, 61] and ingestion of endophyte-infected fescue grass.[62] A more recent study showed that ingestion of endophyte-infected fescue seed had little effect on thyroid function in adult horses that were not pregnant.[63]

Thyroid gland function has been assessed in horses by measuring thyroid hormone responses to intravenous injection of TRH or TSH. These tests help determine if the hypothalamic-pituitary-thyroid axis is functioning normally. It is also recommended that TSH concentration be measured. Unfortunately these tests are rarely used in horses because of expense, limited availability, safety issues, and the potential for spurious results. Validated assays for equine TSH are not yet readily available.[28] Interestingly, a study looking at the effects of dexamethasone administration on serum thyroid hormone concentrations in clinically normal horses reported that some horses do not respond as expected to TSH administration.[57] Similar problems are encountered when assessing thyroid function in dogs with low plasma thyroxine concentration. Some dogs with non-thyroidal illness can be misclassified as having primary hypothyroidism based on a TSH stimulation test. Quantitative measurement of pertechnetate uptake by the thyroid gland is reported to have the highest discriminatory power in differentiating between primary hypothyroidism and non-thyroidal illness in dogs.[64] A review of evidence based literature pertaining to thyroid dysfunction in horses concluded that there is still a need for a reliable diagnostic test for hypothyroidism in horses.[28]

Utility of pertechnetate scintigraphy in determining thyroid gland function in people, cats and dogs

Radionuclides for thyroid imaging

Iodide uptake is a basic function of the thyroid gland and is a key step in the formation of thyroid hormones.[65] Thyroid scintigraphy can be performed using ^{131}I, ^{123}I or ^{99m}Tc-pertechnetate. Pertechnetate acts as an iodide analogue. Thyroidal uptake of these radionuclides is proportional to the expression of the sodium iodide symporter (NIS). This transporter protein is located on the basolateral membrane of the thyroid follicular cells. The movement of iodide into the cell is an active process. The energy-dependent co-transport system is driven by an inwardly directed Na^+ gradient. Under normal physiologic conditions the expression of NIS is primarily dependent on TSH concentration. Following uptake into thyroid follicular cells iodide is passively translocated via an iodide channel across the apical membrane into the colloid. The next step in iodide handling is the oxidation of iodide into

iodine and organification of the iodine into tyrosyl residues of the thyroglobulin molecule. This takes place at the luminal surface of the apical membrane of the epithelium.

Iodine-131 was the first radiopharmaceutical used for thyroid imaging. This isotope is no longer used for this purpose due to its high radioactive burden to the patient (ß emission), relatively long half-life (8.1 days) and high-energy gamma emission (364 keV). The high-energy gamma emission requires the use of a medium-energy collimator and ultimately results in poor spatial resolution of the scan.[8] However, ^{131}I is still used therapeutically. Iodine-123 is a pure gamma emitter with a shorter half-life (13.3 hours) and a lower energy gamma emission (159 keV). The main disadvantage of ^{123}I is its relatively high cost and limited availability, which can be attributed to its mode of production (cyclotron product). Apart from its use in imaging, Iodine-123 can be used to calculate iodide clearance. Thyroidal iodide clearance is defined as the amount of iodide that is cleared from plasma within a definite period of time. This parameter is considered the gold standard in the evaluation of iodide trapping function of the thyroid gland. The method is highly sophisticated and requires repeated venous blood sampling.[65]

Pertechnetate has become the most commonly used tracer for thyroid scintigraphy. Pertechnetate is produced in a ^{99}Mo/^{99}Tc generator, has a physical half-life of 6 hours and has a 140 keV gamma emission. Intravenously administered pertechnetate is loosely bound to plasma proteins and rapidly moves out of the intravascular compartment. Due to the comparable molecular sizes and valance of pertechnetate and iodine, pertechnetate is transported by the NIS into the thyroid follicular cell. Pertechnetate is not organified in the gland, and thus thyroidal uptake reflects only the 'trapping' activity of the gland. In people pertechnetate uptake increases within the first 15 minutes after intravenous injection, exhibits a plateau phase between 15 and 30 minutes when influx and efflux are balanced, and then decreases after 30 minutes. The absolute uptake of pertechnetate in people ranges from 0.3% to 3% of administered activity. In iodine deficient countries thyroidal percent dose uptake of pertechnetate ranges from 1.2% to 7%. The injected standard activity in people ranges from 37 to 74 MBq (1 mCi to 2 mCi). The recommended dose for a cat and dog is between 1 to 4 mCi and 2 to 5 mCi respectively.[66] There is a strong, linear correlation between global pertechnetate thyroid uptake and ^{123}I clearance. Thyroidal uptake of pertechnetate may

therefore be used as a more practical method for assessing the 'trapping' function of the thyroid gland. In people the correlation between thyroidal iodine clearance and thyroidal pertechnetate uptake is stronger if measurements are made during the early phase (5 to 15 minutes).[65]

Scintigraphic images are acquired with a scintillation camera integrated with a dedicated imaging computer with nuclear medicine software. The gamma camera is the radiation detector used in nuclear imaging. The camera counts gamma rays or x-rays emitted from the patient and creates an image based on distribution of the radionuclide within the patient.[67] A collimator is fitted to the surface of the camera. The collimator is composed of material that attenuates gamma rays not travelling in the direction of the collimator hole(s). The most commonly used collimator is a parallel-hole collimator that has multiple small openings extending from the outer to inner surface of the collimator. The collimator selectively allows gamma or x-rays emitted from the patient that are travelling in a certain direction to be detected by the gamma camera. It absorbs off-axis gamma rays thus maintaining geometry between the point sources of the gamma rays and their interaction with the scintillation crystal. The type of collimator used will affect the image size, orientation and overall spatial resolution. The collimator must be matched to the energy of the photons emitted by the radionuclide used for the study. Thus collimators are classified as low, medium or high energy and as to their sensitivity or resolution. A more sensitive system will inherently have lower spatial resolution. In addition, holes may be parallel, converging or diverging. A pinhole collimator is shaped like a cone and has a small single hole at the tip of the cone. The image is magnified if the patient to collimator distance is less than the length of the collimator cone. The aperture size is typically 2 to 6 mm in diameter. Thyroid imaging in people typically uses a parallel-hole, low energy, ultrahigh-resolution collimator, often with a special design to reduce the distance between the collimator face and the thyroid gland. An acquisition time of 5 to 10 minutes is recommended.[8] Imaging in small animals is typically performed with a parallel-hole, low-energy, all-purpose or high-resolution collimator. A pinhole collimator may be used to increase spatial resolution and magnify the image.[67]

Quantitative thyroid scintigraphy

In order to calculate thyroidal percent dose uptake of pertechnetate regions of interest (ROIs) are typically manually drawn around the thyroid lobes in a ventral image of the neck. The computer determines the total number of counts that are located with this ROI. The number of counts detected in the thyroidal ROI must then be corrected for soft tissue attenuation and background. Correcting for soft tissue attenuation involves correcting for activity that is coming from the thyroid gland that is attenuated/absorbed by the soft tissues of the neck on their way to the gamma camera. Correcting for background involves calculating how many counts in the thyroid region of interest are coming from other soft tissues located above and below the thyroid lobes. The background count density obtained from a nearby non-thyroidal ROI is applied to the thyroid ROI, and is then subtracted from the counts detected in the region of interest defining the thyroid gland.

Pertechnetate thyroid scintigraphy in people

Scintigraphically normal thyroid lobes in people appear as two elliptical columns slightly angled towards each other inferiorly. Lateral margins of thyroid lobes are usually straight or convex. Concave borders are considered suspicious for space-occupying lesions. The isthmus may or may not be seen. In a small percentage of patients the thyroid lobe may have a pyramidal shape. Salivary glands and gastric mucosa are seen due to NIS expression in these tissues.[8] A visual impression of thyroid activity can be established with a 5-minute anterior image. Normally activity in the thyroid gland should be equivalent to activity in the salivary gland.[6]

In adults thyroid scintigraphy is primarily used to evaluate a nodular or enlarged thyroid gland, to investigate thyrotoxicosis and to characterize ectopic tissue or a mediastinal mass.[6] In iodine deficient parts of the world scintigraphy is particularly useful in determining if thyroid nodules are functional. Quantitative pertechnetate scintigraphy is the most sensitive and specific technique for quantification of thyroid autonomy and can be used to estimate target functional thyroid volume prior to radioiodine therapy and to evaluate therapeutic success after treatment.[8]

Endemic goiter, Hashimoto's thyroiditis, dyshormonogeneis and Graves' disease can cause diffusely increased pertechnetate thyroid uptake. Focally increased uptake is most likely due

to a functional thyroid adenoma. Diffusely decreased pertechnetate thyroid uptake can be due to end-stage goiter, Hashimoto's thyroiditis, a high-iodine diet, previous administration of iodinated contrast media and thyroiditis.[6] Focally decreased thyroidal uptake can be secondary to a thyroid carcinoma or adenoma, colloid goiter, cyst, metastasis, localized thyroiditis and thyroid abscess.[6]

The clinical value of thyroidal percent dose uptake is considered limited in people without first suppressing the function of normal thyroid tissue. Global thyroidal percent dose uptake will depend on several factors including thyroid volume, iodine supply, and to a minor extent, the patient's age. It reflects iodine clearance by both normal and autonomous tissue. Only extremely high or low values are clinically useful. Extremely high values are seen with Grave's disease (thyroidal uptake > 15%), and extremely low values are present with iodine contamination (thyroidal uptake < 0.3%). Iodine contamination is the repeated administration of iodine containing drugs such as amiodarone. There is substantial overlap in percent dose uptake of pertechnetate in patient's with edemic goiter, normal thyroid function, thyrotoxic autonomy and Graves disease.[65]

Determining global pertechnetate thyroid uptake under suppression involves imaging the patient after iatrogenic suppression of normal thyroid tissue. Normally functioning tissue is not always suppressed by autonomously functioning thyroid tissue in people, particularly if concurrent iodine deficiency is present. Thyroid autonomy is the second most frequent cause of hyperthyroidism in iodine deficient areas and is typically associated with nodular goitre.[8] Thyroid suppressive therapy involves administering suppressive doses of tetraiodothyronine or triiodothyronine. Thyroid autonomy can only be accurately assessed once any suppressible thyroid tissue has been 'switched off' by TSH-suppression. Pertechnetate thyroid uptake under suppression can be used prognostically to determine which patients with thyroidal autonomy are at risk of developing hyperthyroidism. These patients would be selected for definitive treatment with radioiodine therapy.[65]

Scintigraphy is the only imaging modality that can prove the presence of autonomously functioning thyroid tissue.[8] Presence of an autonomous nodule precludes thyroid carcinoma with a very strong probability. In people eating a diet with normal iodine levels,

hyperfunctioning autonomy can be easily diagnosed on the basis of a decreased TSH concentration and ultrasound findings. A low TSH concentration implies a hyper-functioning nodule (hot nodule) is present and malignant disease is unlikely. If TSH is in the normal range and a hot nodule is present, ultrasound-guided fine needle aspirate is recommended. In iodine deficient glands the synthesis of thyroid hormones may be too low to affect the thyrotropin feedback mechanism and TSH may be in the normal range (or high), even if autonomy is present. Thus in these areas a thyroid scan is often used as a first-line investigation. In most cases thyroid carcinoma presents as a cold nodule on pertechnetate scintigraphy. There is typically a decrease, or loss, of NIS activity in malignant tissue. The presence of a cold nodule is sensitive but not specific (no higher than 10%) for malignancy. Due to limited spatial resolution, a normal pertechnetate scan does not preclude the presence of a cold nodule.[8]

Pertechnetate thyroid uptake under suppression has been used to help differentiate patients with subclinical or overt hyperthyroid autoimmune thyroiditis (AIT) and those with newly diagnosed Graves' disease. Both AIT and Graves' disease are autoimmune disorders of the thyroid gland. Although 30-40% of patients with AIT (destructive lymphocytic infiltration of the thyroid gland) are subclinical or manifest hypothyroidism, about 10 % of people initially present with transient hyperthyroidism. Graves' disease is mainly characterized by the presence of TSH receptor antibodies. Scintigraphically, patients with Graves' disease typically have high homogeneous distribution of tracer. Pertechnetate thyroid uptake under suppression is significantly lower in patients with the early hyperthyroid form of AIT than in patients with newly diagnosed Graves' disease.[8]

Pertechnetate thyroid scintigraphy in cats

Thyroid scintigraphy is most often used in veterinary medicine to evaluate for hyperthyroidism in cats.[66] The most common cause of the disease is adenomatous hyperplasia or hyperfunctioning thyroid adenoma(s). Thyroid carcinoma can also result in hyperthryoidism, but the incidence is very low (less than 3%). The feline thyroid gland is composed of two lobes without an isthmus. The normal scintigraphic appearance of the thyroid gland is characterized by homogeneous distribution of radioactivity throughout both lobes. The lobes appear as elongated ovals, symmetric in size and position.[66] Recently it has

been reported that at least some euthyroid cats have asymmetric thyroid lobes during visual inspection of pertechnetate scintigrams.[68] Margins of the thyroid lobes should be smooth and regular. Ectopic thyroid tissue is not usually seen in normal cats. The main indications for performing pertechnetate thyroid scintigraphy in hyperthyroid cats include determining unilateral versus bilateral lobe involvement and identifying sites of hyperfunctioning ectopic thyroid tissue or distant metastases. It can also be used quantitatively to assess cats with clinical signs consistent with hyperthyroidism when serum thyroid hormone concentrations are equivocal.[69]

As is reported in people, intensity of thyroid gland uptake in cats may be subjectively assessed by comparison with salivary tissue. In cats the zygomatic and molar salivary glands are used to make this comparison. The first study to report quantitative measurement of thyroid to salivary (T/S) ratio in clinically normal cats reported a reference interval of 0.6 to 1.03 with a mean (± SD) of 0.87 ± 0.13.[70] This study also reported thyroid to background ratios and concluded they were more variable than calculated T/S ratios. A total of 10 cats were examined in the study. Cats were divided into two groups, the first consisted of 5 cats classified as young adults (of unknown age), and the second consisted of 5 cats between 9 and 11 years of age. The older group was included in the study to determine if there might be differences in the appearance of the thyroid gland between the two groups and because older cats have an increased prevalence of hyperthyroidism. No difference was seen in T/S ratios between the two groups. In this study imaging was performed 10 to 15 minutes after intravenous injection of pertechnetate. This time period was likely derived from the human literature, where this early period is reported to correlate best with ^{123}I clearance (the gold-standard in evaluating thyroid trapping function).[65] In this study a converging collimator was used and each image was acquired to 100, 000 counts, rather than being based on time.

Thyroidal percent dose uptake has been reported for euthyroid and hyperthyroid cats.[69] The first study to report thyroidal percent dose uptake in cats evaluated 5 euthyroid and 37 hyperthyroid cats. Control cats were all between 2 and 5 years of age. Cats were imaged 20 minutes after intravenous injection of pertechnetate. Images were acquired using both a pinhole collimator (300 second acquisition time) and parallel-hole collimator (120 second acquisition time). Interestingly, there was no significant difference in thyroidal percent-dose

uptakes when calculated from images made with the pinhole or parallel-hole collimator. It was noted however that quantitative uptake measurements would only be reliable if pinhole geometry (distance from the pinhole collimator to the thyroid gland) could be accurately reproduced. In this particular study distance between the skin surface and the thyroid gland was standardized using a box. This method involved positioning patients in dorsal recumbency and placing the box between the skin surface and the collimator. Using this technique the neck to collimator distance was consistently 60 mm. Acquisition time was longer for images acquired with the pinhole collimator, as it is less sensitive than a parallel-hole collimator. When using a parallel-hole collimator, sensitivity is maintained despite small changes in distance between the collimator and the object, and the image is a similar size to the object. There is also uniform sensitivity across the field of view. For these reasons it is more accurate to perform quantitative analysis on images obtained with a parallel-hole collimator. In this particular study, using the parallel-hole collimator, mean thyroidal uptake of pertechnetate was 0.64% (range 0.25 to 1.55%) in the control group. Hyperthyroid cats had a mean ± standard deviation (SD) thyroidal uptake of pertechnetate of 7.0% (± 6.46%). There was no correlation between thyroidal percent-dose uptake and serum total T_4 concentration in the euthyroid cats, and it was noted that in euthyroid cats many factors other than thyroid trapping influence circulating thyroid hormone concentrations. A positive correlation was found between total T_4 concentrations in hyperthyroid cats and percent dose uptake ($R^2 = 0.67$).[69]

The first studies to describe quantitative pertechnetate thyroid scintigraphy made calculations based on images acquired between 10 and 20 minutes after injection of pertechnetate.[69, 70] These studies did not investigate the effect of time on T/S ratios or thyroidal percent dose uptake of pertechnetate. The first study to investigate optimal scan time determined thyroidal percent dose uptake in euthyroid and hyperthyroid cats up to 420 minutes after injection of pertechnetate.[71] Thirteen control cats between 2 and 17 years of age and 18 hyperthyroid cats were included in the study. Interestingly, in this study hyperthyroid cats were administered a lower dose of pertechnetate than euthyroid cats. Doses for the hyperthyroid and control cats were 0.14 mCi and 0.4 mCi, respectively. Previous studies administered doses of 0.5 to 1.2 mCi[69] and 0.7 to 1 mCi.[70] Methods of dose calculation were not discussed. Percent dose uptake was measured at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 330, 360, and 420

minutes after injection. A parallel-hole collimator was used for the study, and images were acquired for 30 seconds. The median percent dose uptake of pertechnetate in euthyroid cats was highest between 45 and 60 minutes after injection.[71] At this time uptake values ranged from 0.8 to 3.9% of the administered dose. This range is wider than previously reported for normal cats.[69] This difference may have been due to the inclusion of an increased number of control cats and the inclusion of controls with a wider age distribution.[71] Differences in technique such as dose and acquisition time may also have had an effect. Hyperthyroid cats had more rapid initial uptake of pertechnetate and higher thyroidal precent dose uptake. The median value for hyperthyroid cats peaked 60 minutes after injection. In this particular study salivary gland uptake was too low to allow for delineation of the glands or accurate calculation of salivary percent dose uptake. This was the first study to image cats without any sedation or anesthesia. The author recommended imaging cats at 60 minutes post-injection, at the time of maximal uptake.[71] People are imaged during the first 5 to 15 minutes after injection of pertechnetate. This time was selected because in people uptake increases in the first 15 minutes and exhibits a plateau phase between 15 and 30 minutes. The plateau is caused by balanced influx and efflux of pertechnetate into and out of the thyroid gland. Thus the thyroid trapping mechanism is best assessed in this early period. The early period is also known to correlate best with ^{123}I clearance studies, which are considered the gold standard for assessing 'trapping' function of the thyroid gland.[65] Although calculated percent dose uptake over time, it did not determine if there were statistically significant differences in uptake between the different time points. This was, however, addressed in later investigations.[72, 73]

The effect of methimazole (anti-thyroid medication) on thyroidal uptake of pertechnetate and ^{123}I has been evaluated in normal cats.[72] Methimazole works by blocking the incorporation of iodine into tyrosyl groups of thyroglobulin and inhibits coupling of iodotyrosines. This study calculated thyroidal percent dose uptake and rate of uptake of pertechnetate in normal cats over time. Eight, euthyroid, 1-year old, male cats were included in the study. Methimazole was administered to five of the cats and three served as controls. The three control cats and two from the treatment group were used to establish normal uptake for ^{123}I. All cats underwent pertechnetate thyroid scintigraphy prior to treatment with methimazole, and thus could serve as their own controls. This was the first study to report changes in T/S ratios over time. This parameter could not be calculated in the previously reviewed study due to

poor conspicuity of salivary tissue.[71] Cats were anesthetized for the initial 2-hour imaging period and imaged at 4 hours under sedation. A low energy, converging hole collimator was used in the study. Cats received a mean ± SD dose of 4.95 mCi (± 1.05 mCi) of pertechnetate. Images were acquired using a multiphase, dynamic frame-mode acquisition. Frames from 1 to 120 minutes after injection were acquired at a constant rate of 1 frame per minute (i.e. 60 second images). Radioiodine (^{123}I) was administered to the cats at least 48 hours after the pertechnetate scans were performed. Five-minute static images were acquired at 8 and 24 hours after radioiodine administration. This was the first study in cats to correct for soft tissue attenuation when calculating thyroidal percent dose uptake. Depth was defined as the distance from the skin surface to the middle of each thyroid lobe and was determined using ultrasound. Background correction was also performed. Gross T/S ratios were determined at 20 minutes and 1, 2 and 4 hours after administration of pertechnetate. There was no significant difference in T/S ratios between 20 minutes and 2 hours, suggesting that the time from injection to imaging is not critical if performed 20 minutes to 2 hours after injection. The thyroid percent dose uptake curves continued to increase during the 4-hour acquisition period. Thus peak thyroidal percent dose uptake was not determined. The longer reported time to peak thyroidal percent dose uptake may have been secondary to anesthesia and delayed delivery of pertechnetate to the thyroid gland. This study did not report whether thyroidal percent dose uptake of pertechnetate was significantly different between imaging time periods. Mean ± SD thyroidal percent dose uptake at 20 minutes post injection was 0.21 ± 0.06. Values for pertechnetate uptake were less than those reported in previous studies. Variation in reported quantitative parameters in normal cats likely results from variation in imaging and analysis methodologies. Some differences between studies include use of different sedation/anesthetic protocols, variable imaging time (both time from injection and duration of static acquisitions) and whether or not corrections were made for background and soft tissue attenuation. A correlation was found between the percent dose uptake of pertechnetate and the 8-hour percent dose uptake of ^{123}I (R = 0.809). A correlation was not found between percent dose uptake of pertechnetate and 24-hour uptake of ^{123}I. It was found that in normal cats iodide trapping by thyroid tissue is significantly enhanced by anti-thyroid medication and is increased for 15 days after withdrawal of the medication.[72]

There is an association between thyroidal percent dose uptake of pertechnetate and thyroid hormone concentrations in cats with hyperthyroidism.[69, 73] As thyroid hormone concentrations increased thyroidal percent dose uptake increases. A relatively recent study determined whether T/S ratio, percent thyroidal uptake of pertechnetate or rate of uptake had the best correlation with total T_4 concentration.[73] The objective was to determine which method was the best predictor of the metabolic activity of the thyroid as measured by serum total T_4 concentration and to determine optimal scanning time. Forty-five cats with hyperthyroidism (determined by elevated total T_4 concentration) and 8 clinical patients presenting for thyroid imaging but with total T_4 concentrations within the normal reference interval were included in the study. A control group of 8, 1 year-old normal male cats was also included. All cats were anesthetized for imaging and were positioned in ventral recumbency over a large field of view gamma-camera fitted with a low-energy-all-purpose parallel-hole collimator. Cats received an intravenous dose of approximately 4 mCi of pertechnetate. A multiphase, dynamic frame-mode acquisition was performed with a variable frame rate for a total dynamic image acquisition time of 20 minutes. The frame rate for the first minute was 1-frame/10 seconds (6 frames/minute), which was followed by 1 frame/minute for 19 minutes. Margins of salivary glands and thyroid lobes were easily identified. Correction for soft tissue attenuation was not performed. Thyroidal percent dose uptake was plotted at each time point to generate a thyroid uptake curve and determine rate of uptake. T/S ratios were calculated at 2, 5, 10 and 20 minutes after injection. Both average and maximum T/S ratios were calculated. Average T/S ratio was calculated using the average thyroid lobe density (averaging the count density from both thyroid lobes). Maximum T/S ratio was calculated from the most intense thyroid lobe. Thyroid percent dose uptake of pertechnetate (± SD) at 20 minutes in patients with normal total T_4 was 0.75% (± 1.38%), which was not significantly different from control cats (0.68% ± 0.90%). The 20-minute T/S ratio in control cats, using the average of both thyroid lobes, was 0.82 ± 0.05 (mean ± SD). All quantitative parameters were significantly correlated to the serum total T_4 concentration. The best correlation with total T_4 concentration was obtained using the maximum T/S ratio (calculated using only the most intense lobe) calculated at 20-minutes after injection ($R^2 = 0.83$). The difference in T/S ratio between normal and abnormal cats (as determined by total T_4 concentrations) was also greatest at 20 minutes. Rate of uptake of pertechnetate by the thyroid gland did not correlate as well with total T_4 concentration as T/S ratio. Correlation of thyroidal percent dose uptake

with total T_4 concentration at 20 minutes (R^2 = 0.66) was very similar to that reported previously (R^2 = 0.67). This study concluded that imaging 20 minutes after injection and calculating maximum T/S ratio will result in the most accurate estimate of metabolic function of the thyroid gland in cats with hyperthyroidism.[73]

Reference intervals for T/S ratio were initially established in studies that included limited numbers of cats (between 10 and 16) and using a variety of imaging protocols.[70, 72, 73] The T/S ratio has since been evaluated in a larger population of euthyroid cats.[74] Thirty-two cats between 8 and 13 years of age were included in the study. Scanning took place 20 to 40 minutes after intravenous injection of approximately 3.0 mCi of pertechnetate. Cats were sedated for scanning. A large field of view camera fitted with a low-energy all-purpose parallel-hole collimator was used for imaging. Images were acquired to 150, 000 counts, rather than for a certain length of time. The T/S ratio ranged from 0.51 to 1.65 and the 95% prediction interval for the T/S ratio was calculated to be 0.48-1.66. There was no significant relationship of T/S ratio with sex or age. The upper limit of the reference interval was higher than that reported in previous studies, possibly due to inclusion of more cats and older cats in the study.

Various sedation and anesthesic protocols have been used to restrain cats for pertechnetate thyroid scintigraphy. The effect of various sedative and anesthetic protocols on quantitative thyroid scintigraphy has been reported for a group of euthyroid cats.[75] The study calculated thyroidal percent dose uptake of pertechnetate and T/S ratio. Time activity curves were generated for thyroid gland uptake, salivary gland uptake and T/S ratio. Four sedative/anesthetic protocols were used. Anesthetic protocols were as follows: medetomidine, ketamine in combination with midazolam, ketamine in combination with midazolam and atropine, and propofol. Six healthy cats were used in the study and each cat received all 4 sedative/anesthetic protocols with a minimum washout period of one week. Thyroidal percent dose uptake increased progressively over the 45-minute imaging period for all sedative/anesthetic protocols. Salivary gland percent dose uptake also increased progressively for all sedative-anesthetic protocols over the 45-minute observation period. There was a significant difference in thyroidal percent dose uptake of pertechnetate between the ketamine-midazolam protocol (20 minute mean ± SD of 0.51 ± 0.16) and the propofol

protocol (20 minute mean ± SD of 0.85 ± 0.20) at both 20 minutes and 40 minutes after injection. There was a significant difference in salivary percent dose uptake between the ketamine-midazolam protocol and the ketamine-midazolam-atropine protocol at 40 minutes after injection. Twenty minutes after injection there was no significant difference in T/S ratios between the different sedative/anesthetic protocols. At 40 minutes after injection there were differences found between the ketamine-midazolam protocol (mean ± SD of 1.06 ± 0.22) and the propofol protocol (mean ± SD of 0.67 ± 0.10), where cats receiving the ketamine-midazolam protocol had significantly higher T/S ratios. There were also differences at 40 minutes between the ketamine-midazolam protocol and the ketamine-midazolam-atropine (mean ± SD of 0.63 ± 0.19) protocol, where significantly higher T/S ratios were seen in cats receiving ketamine-midazolam. Salivary gland uptake of pertechnetate increased when atropine was added to the ketamine-midazolam protocol as atropine prevents salivation. Overall thyroidal percent dose uptake was higher with ketamine-midazolam and ketamine-midazolam-atropine protocols than with medetomidine and propofol. These differences were likely due to increased cardiovascular suppression caused by medetomidine and propofol. Cardiovascular suppression may delay delivery of pertechnetate to the thyroid gland. Sedation and anesthesia protocols can have a significant effect on both thyroidal percent dose uptake and T/S ratio and these effects should be taken into account when interpreting results. Ideally a standardized protocol would be established.[75]

Pertechnetate thyroid scintigraphy in dogs

Pertechnetate scintigraphy is most commonly used in dogs to determine the origin and extent of a cervical mass. More recently it has been used quantitatively to evaluate thyroid gland function in patients with low circulating thyroid hormone concentrations secondary to primary hypothyroidism and non-thyroidal illness syndrome (euthyroid sick syndrome).[76]

Most acquired canine hypothyroidism is the result of lymphocytic thyroiditis or idiopathic thyroid atrophy.[77] Antithyroglobulin antibodies are present in 36 to 50% of hypothyroid dogs. Less commonly hypothyroidism may a result from thyroid neoplasia or invasion of the thyroid by metastatic neoplasia.[77] Low circulating thyroid hormone concentrations may also be found in dogs receiving certain drugs and those with non-thyroidal illness. Drugs known

to affect thyroid hormone concentrations in dogs include glucocorticoids, sulfonamides, phenobarbital, clomipramine, heparin, non-steroidal antiinflammatories, propranolol, amiodarone, iodinated contrast agents and iodide.[77]

Normal thyroid scintigraphy in dogs is characterized by uniform, symmetric, approximately equal radionuclide localization of pertechnetate within the thyroid lobes.[78] Reference intervals for quantitative scintigraphic parameters in normal dogs were determined in a study that evaluated 13 sexually intact beagles (8 male, 5 female). [78] The beagles were between 7 and 66 months of age. Dogs were determined to have normal thyroid function based on a TSH stimulation test and normal thyroid gland histopathology. Sonography was used to measure thyroid lobe depth and was performed via a ventral cervical window in both sagittal and transaxial planes. Depth was defined as the average distance from the skin surface to the ventral margin of the thyroid lobe. Sagittal and transaxial depth measurements were averaged. The depth measurement was used to correct for soft tissue attenuation when calculating thyroidal percent dose uptake. Each dog was scanned twice, 14 days apart, to assess the reliability of the quantitative scintigraphic parameters that were calculated. Dogs were anesthetized for the initial hour of imaging and were then manually restrained for imaging at 2, 3 and 4 hours. In order to determine camera counting efficiency a known-standard dose was positioned in the image field of view, adjacent to the dog. Activity injected into the patient was determined by measuring activity of pertechnetate within the syringe before and after injection. Dogs were placed in ventral recumbency for scanning. A low energy, converging-hole collimator was fixed to the gamma-camera for imaging. The converging-hole collimator was used to enlarge the image to facilitate ROI placement. A multi-phase, dynamic frame-mode study was acquired using a dedicated imaging computer. Following the dynamic phase of the study, serial 60-second, static ventral views of the cervical region were acquired at 2, 3 and 4 hours post-injection. Regions of interest (ROIs) were hand drawn around thyroid lobes and parotid salivary glands. The T/S ratio was calculated by dividing the summed count density of both thyroid lobes by the summed count density of both parotid salivary glands. Thyroid to background ratio was also calculated by dividing the count density of both thyroid lobes by the count density of the background ROI. The background ROI was drawn caudal to the thyroid gland. Thyroidal percent dose uptake was calculated for each image of the dynamic and static acquisitions. Percent dose uptake by

the salivary glands was also calculated. Total counts were decay corrected to the time of injection. Counts in the salivary and thyroid ROIs were corrected for background. Thyroid gland uptake was also corrected for soft tissue attenuation, using the linear attenuation coefficient for soft tissue, 0.153 cm^{-1}. Activity (mCi) of the radionuclide standard was measured during imaging. This allowed for calculation of the counts/minute detected by the camera by a known dose and calculation of camera counting efficiency. The injected dose was then converted from activity (mCi) into counts/minute. Percent dose uptake was calculated by dividing counts/minute in the thyroid gland by counts/minute injected. Counts from the injection apparatus were not measured (only injection and post-injection syringe counts were accounted for).[78]

There was no significant difference between data acquired from the first and second pertechnetate scans, indicating results were repeatable over time (precise). Mean thyroidal percent dose uptake within the thyroid gland progressively increased between 20 minutes and 1 hour. Values peaked between 2 and 3 hours and then declined at 4 hours. Mean (± SD) percent dose uptake by the thyroid gland at 20 minutes and 1 hour were 0.23% (± 0.09%) and 0.36% (± 0.10%) respectively. Percent dose uptake by the parotid salivary glands progressively increased over the course of the study. Mean (± SD) and median T/S ratios of 1.2 (± 0.3) and 1.1 were essentially identical at 20 minutes and 1 hour after injection. T/S ratios progressively decreased over subsequent time intervals. Thyroid/background ratios progressively increased over the course of the study.[78]

Maximum percent dose uptake occurred between 31 and 240 minutes. Variation in time to maximum thyroidal uptake of pertechnetate was high, in some animals occurring as early as 31 minutes after injection. T/S ratios in normal dogs ranged from 0.9 to 2.2 at 20 minutes and 0.8 to 2.4 at 1 hour. There is greater variability in the normal canine thyroid scan than is reported in cats. The optimal time for evaluation of T/S ratio in dogs is likely within a time period between 20 minutes and 1 hour following radionuclide injection. During this period there was minimal change in T/S ratio.[78] There was no attempt in the study to correlate T/S ratio or thyroidal percent dose uptake of pertechnetate with thyroid hormone concentrations. One of the disadvantages was the use of general anesthesia for only part of the imaging protocol.

Recent studies have reported the use of quantitative pertechnetate thyroid scintigraphy to evaluate thyroid gland function in dogs with low circulating concentrations of thyroid hormones.[64, 79] One of these studies focused on using scintigraphy to differentiate between dogs with primary hypothyroidism and those with low thyroid hormone concentrations secondary to non-thyroidal illness.[64] Medical conditions reported to decrease total T_4 concentrations in dogs include hyperadrenocorticism, diabetic ketoacidosis, hypoadrenocorticism, renal failure, hepatic disease, peripheral neuropathy, megaesophagus, heart failure, critical illness or infection and surgery or anesthesia.[77] The term euthyroid sick syndrome is sometimes used to characterize abnormalities in thyroid function tests that are observed in patients with systemic non-thyroidal illness. This condition has been well documented in people and is also referred to as nonthyroidal illness syndrome.[80] Thyroid stimulation tests have been considered the gold standard for differentiating between dogs with primary hypothyroidism and those with nonthyroidal illness. In cases where the thyroid gland is able to function normally the gland should respond appropriately to administration of TSH. However, in some dogs with non-thyroidal illness syndrome the results of the TSH simulation test may mimic those of dogs with primary hypothyroidism, which can lead to misclassification of these patients. In the study investigating the use of pertechnetate thyroid scintigraphy in dogs with low thyroid hormone concentrations bovine TSH was used to perform TSH stimulation tests.[64] Due to limited availability of bovine TSH in many cases primary hypothyroidism is diagnosed based on low total T_4 and high TSH. Interpreting these results may be challenging, as up to a third of dogs with primary hypothyroidism have plasma TSH concentrations within the normal reference interval.[64] It has also been reported that approximately 12% of people with non-thyroidal illness syndrome have supranormal TSH concentrations.[80]

Prior to imaging, dogs were injected intravenously with between 3 and 4.5 mCi (111 to 166 MBq) of pertechnetate.[64] A parallel-hole collimator was used for imaging. Sixty-second static acquisitions were made 5, 10, 20, 30, 45, 60, 90, and 120 minutes after injection of radionuclide. Dogs were manually restrained. In this particular study, pertechnetate scintigraphy (calculation of thyroidal percent dose uptake) had the highest discriminatory power to differentiate between dogs with non-thyroidal illness and those with primary

hypothyroidism. In dogs with hypothyroidism, thyroidal pertechnetate uptake at 60 minutes ranged from 0.03 to 0.26%. In dogs with nonthyroidal illness thyroidal pertechnetate uptake at 60 minutes ranged from 0.39 to 1.86%. There was no overlap between dogs with primary hypothyroidism and those with nonthyroidal illness. Plasma thyroid hormone concentrations overlapped in dogs with primary hypothyroidism and those with non-thyroidal illness, and some of the dogs with nonthyroidal illness had only a minor increase in plasma total T_4 after administration of TSH. The authors conclude that there is potential to misclassify patients with nonthyroidal illness as being hypothyroid if TSH and TRH stimulation tests are used as the gold standards.[64]

Quantitative pertechnetate thyroid scintigraphy has been used to assess thyroid gland function in greyhounds suspected of having primary hypothyroidism.[79] Dogs were suspected to be hypothyroid based on clinical signs (bald thigh syndrome) or low total T_4 concentrations. Ninety percent of the study dogs had low total T_4 and 10% of dogs had low free T_4. One dog had a total T_3 concentration below the laboratory reference interval. Scintigraphy was performed under sedation (acepromazine, midazolam and butorophanol). Images were acquired 45 minutes after administration of pertechnetate. Ventral images of the head and neck were acquired to 200,000 counts rather than for time. The exact duration of image acquisition was recorded for each case. The gross counts from each thyroid lobe ROI were corrected for background activity and soft tissue attenuation. Ultrasound was performed to measure thyroid gland depth. Depth was defined as the distance from the skin surface to the most superficial surface of each thyroid lobe. Thyroidal percent dose uptake of pertechnetate, T/S ratios and thyroid to background ratios were calculated. Total thyroidal uptake of pertechnetate ranged from 0.4% to 1.49% of the injected dose. Interestingly, uptake of pertechnetate in most of the dogs was asymmetric, with the left lobe having a mean uptake of 0.43% and the right lobe having a mean uptake of 0.33%. The significance of this finding is unclear but may represent a normal finding in this breed.[79] Salivary percent dose uptake was between 0.45% and 2.1%. T/S ratios were between 0.65 and 2.36. In some cases with similar thyroid uptake of pertechnetate there was a marked difference in the T/S ratio due to differences in salivary uptake of pertechnetate. There was no association between thyroid hormone concentrations and scintigraphic measurements. There were also no significant differences when comparing data between dogs with bald thigh syndrome and those

without.[79] Thyroidal percent dose uptakes for the greyhounds in this study were higher than those previously reported in the literature for dogs with primary hypothyroidism (0.03-0.26%),[64] suggesting the greyhounds included in the study were not hypothyroid. Hypothyroidism is unlikely the cause of bald thigh syndrome in this breed. Due to variability in salivary uptake it would be more reliable to use thyroidal percent dose uptake than T/S ratio when assessing thyroid gland function in dogs.[79]

Sonography in evaluation of the thyroid gland

Thyroid sonography in people

Some of the major indications for thyroid sonography in people include characterizing the nature of a thyroid nodule, providing real time imaging guidance for fine needle aspiration cytology or biopsy and follow-up in patients post-operatively to exclude local or regional tumor recurrence.[81] Sonography can be an important tool in determining the origin of a neck mass and can assist in the diagnosis of multinodular disease.[31]

Thyroid nodules are very common in people, and their prevalence is dramatically increased in regions of iodine deficiency[82]. Use of sensitive imaging techniques is increasing the proportion of thyroid nodules detected. The prevalence of thyroid nodules in people increases with age. Histologically most thyroid nodules are cystic or solid adenomas or colloid nodules. Clinically significant thyroid cancer is rare.[31] Current guidelines of the American Association of Clinical Endocrinologists recommend thyroid ultrasound for any patient with a palpable thyroid nodule.[82] Sonographic characteristics suggestive of malignancy include hypoechogencity of a nodule, microcalcifications, intranodal vascularization and the absence of halo sign (a rim of decreased echogenicity surrounding the lesion).[31, 83] However, single ultrasound characteristics of thyroid nodules are of limited sensitivity and specificity in determining malignancy.[82] In one study absence of a halo sign plus microcalcifications combined with intranodal blood flow was found to be highly specific for malignancy but the high specificity resulted in very low sensitivity.[83] Fine needle aspiration is the single best test for differentiating malignant from benign lesions.[84] Ultrasound-guided fine needle aspiration is recommended for the following: any size nodule with a history of radiation or family history of RET (a proto-oncogene that encodes a receptor tyrosine kinase), any nodule with

suspicious ultrasound features, nodules with extracapsular growth or cervical nodules, and non-palpable or small (<1 cm) nodules.[82]

Quantitative analysis of thyroid echogenicity has been performed in people with thyroid dysfunction, particularly in people with different forms of autoimmune thyroiditis.[49, 85-87] Diffuse reduction in thyroid echogenicity has been documented as a valid predictor of autoimmune thyroid disease in people.[86] This study prospectively examined 3,077 patients referred for thyroid ultrasound and documented reduced versus normal thyroid echogenicity. Four hundred and fifty-two patients were found to have reduced thyroid echogenicity. In 90.9% of these patients at least one laboratory finding was consistent with possible autoimmune thyroid disease (AITD). Final diagnoses included chronic autoimmune (Hashimoto's) thyroiditis, Graves' disease, subacute thyroiditis, toxic nodular goiter and toxic adenoma. The majority of patients (352) were diagnosed with Hashimoto's thyroiditis. The corresponding positive and negative predictive values of reduced thyroid echogenicity as an indicator of autoimmune thyroid disease were 88.3% and 93%.[86]

A subsequent study focused on determining if clinical features and laboratory parameters were associated with thyroid echogenicity in a group of patients with Hashimoto's thyroiditis (chronic lymphocytic thyroiditis).[49] Hashimoto's thyroiditis is an autoimmune disease that leads to hypothyroidism. Concentrations of thyroglobulin and peroxidase (TPO) antibodies are typically elevated in patients with this disease. Typical sonographic findings in patients with this disease include diffuse hypoechogenicity of the thyroid gland and goiter with fibrosis. Fifty-two patients being treated for Hashimoto's thyroiditis with levothyroxine and 100 euthyroid volunteers were included in the study. The euthyroid group served as a control group to define normal tissue echogenicity. Thyroid sonography was performed using a 7.5 MHz linear real-time transducer. Ultrasound power level, brightness gain, depth range and frame rate were standardized. Measurement of thyroid echogenicity was carried out by grey scale histograph analysis under standardized conditions. Total number of grey scales, mean density and density with highest frequency of occurrence and its frequency were recorded. Thyroid echogenicity can also be compared to the hypoechoic neck muscles, but this form of assessment is inherently subjective. Patients with Hashimoto's thyroiditis had significantly lower thyroid echogenicity as compared with controls. Large goiters were associated with

lower echogenicity. There were no associations between thyroid echogenicity with age or gender. Patients with persistently elevated TSH levels and highly elevated peroxidase (TPO) antibodies had lower thyroid echogenicities than patients with normalized TSH and lower concentrations of antibodies. Reduced thyroidal echogenicity in patients with Hashimoto's thyroiditis may be secondary to reduction of colloid content, increased intrathyroidal blood flow or lymphocytic tissue infiltration.[49]

An association between hypoechogenicity and development of hypothyroidism in people with diffuse lymphocytic thyroiditis (Hashimoto's thyroiditis) has been reported.[88] In this study diffuse thyroid hypoechogenicity was found in 44 of 238 patients with autoimmune thyroiditis. Degree of hypoechogenicity was significantly correlated with concentrations of circulating thyroid autoantibodies. Thyroid function was normal in autoimmune patients with normal thyroid echogenicity. Hypothyroidism was found in 28 of 44 patients with reduced thyroidal echogenicity. Interestingly, 8 of the patients with reduced thyroid gland echogenicity but normal thyroid function at presentation went on the develop hypothyroidism over the 18 month follow-up period. The authors suggest from their data that hypoechogenicity of the thyroid gland may help identify patients with lymphocytic thyroiditis that are at risk of becoming hypothyroid.[88]

An association between thyroid echogenicity and autoimmune activity in Graves' disease has been documented using standardized grey scale ultrasonography.[87] Graves' disease is the most common cause of hyperthyroidism in people younger than 40 years of age. Ultrasound characteristics of this disease include diffuse thyroidal hypoechogenicity and goiter with fibrosis.[87] Thyroid echogenicity has been reported to predict outcome of radioiodine therapy in patients with Grave's disease.[85] Antithyroid drugs are typically effective for acute control of the disease, but long-term remissions are only achieved in 30-50% of patients. Radioiodine therapy can be used in patients that relapse or as a first line treatment. This study included patients who had been treated with antithyroid medications for at least one year but were still suffering from active disease. Thyroid volume was calculated from linear sonographic measurements. Echogenicity was graded as either normoechoic to mildly hypoechoic or moderately to markedly hypoechoic. Grading was performed by one experienced clinician and was subjective. Both normoechoic and large thyroid glands were

more radioresistant than hypoechocoic and small glands. Normoechogenicity tripled the risk of treatment failure, whereas a large gland doubled it when compared with a hypoechoic or a small gland respectively.[85]

Apart from changes in echogenicity, patients with Grave's disease are also reported to have diffuse thyroid gland hypervascularity.[89] In this study a color video printer was used to record representative color and power Doppler sonograms on film. One radiologist retrospectively reviewed the images. Interobserver variation was evaluated by having the images graded by a second investigator. A subjective grading scheme was used to categorize vascularization in power Doppler images. Normal volunteers were included in the study for comparison with the clinical group. Clinical patients had glands with diffuse enlargement, homogeneous or heterogeneous parenchyma and hypoechogenicity compared with musculature. A diffuse pulsatile flow pattern was seen with color Doppler in all patients with Grave's disease. Flow was characterized by multiple tiny areas of pulsatile flow that varied with the cardiac cycle. This pattern is known as the 'thyroid inferno'. Power Doppler exams revealed diffuse hypervascularity in all patients. The flow covered most of the thyroid parenchyma. Intensity of power Doppler flow pattern was not associated with severity of disease.

Thyroid sonography in cats

Ultrasound has been used in a limited fashion to evaluate cats with hyperthyroidism.[90, 91] In one study sonography was compared to pertechnetate thyroid scintigraphy in a population of cats with hyperthyroidism.[91] The study included 14 hyperthyroid cats and six normal cats as controls. Control cats did not undergo scintigraphy. Instead data from a previous investigation was used as a historical control for thyroid scintigraphy. A 10.0 MHz multifrequency mechanical sector transducer was used for sonographic examinations. Measurements were made on frozen images using electronic calipers. Each thyroid lobe was evaluated using a cineloop freature until the largest value for each linear measurement was identified. Length, height and width were measured for each lobe. Low contrast resolution prevented width measurements being made in control cats and one hyperthyroid cat. In situations where width could not be measured a default measurement of 2.5 mm was given. The default value of 2.5 mm was based on measurements made at necropsy in cats that were euthanized for non-thyroidal disease. Thyroid glands were subjectively evaluated for

echogenicity, margination and parenchymal characteristics. Volume was estimated using the equation for a prolate ellipse (volume (mm^3) = length (mm) x width (mm) x height (mm) x $\pi/6$). An attempt was made to correlate total T_4 concentrations with total thyroid gland volume, as estimated by sonographic measurements. The normal thyroid lobes were thin, moderately and uniformly echogenic, fusiform in shape and were surrounded by thin hyperechoic fascia. The cranial and caudal margins were sometimes difficult to detect due to the tapering shape, and it was difficult to identify the normal gland in the transverse plane. Based on the scintigraphic studies, 6 cats had unilateral hyperthyroidism, and 8 had bilateral hyperthyroidism. There was a significant difference in mean estimated total thyroid volume between normal and hyperthyroid cats. In cats where a thyroid lobe could not be identified sonographically (n = 8) two of these lobes were identified as being hyperfunctional on scintigraphy, and the remainder of the lobes had normal, reduced or absent scintigraphic activity. In two cats the volume was thought to be normal based on ultrasound, but these cats were abnormal scintigraphically. In 13 of 14 hyperthyroid cats the calculated volume was considered increased (thyroid lobes of hyperthyroid cats were classified as abnormal if estimated volume exceeded the 99% confidence interval determined by the control group). There was 85.7% agreement between the two techniques (scintigraphy and sonography) in defining normal and abnormal thyroid lobes. Abnormal lobes were typically uniformly enlarged, had lobulated outer margins and were mostly reduced in echogenicity. Three thyroid lobes from two of the cats contained large anechoic cysts. Histopathology was not possible in this study, thus true thyroid lobe volumes could not be determined by water displacement. There was a fair correlation ($R^2 = 0.449$) between estimated total thyroid lobe volumes of hyperthyroid cats with pre-therapy serum thyroxine values. The study concluded that sonography should not replace scintigraphy as the diagnostic examination of choice but could be used in situations where access to scintigraphy was limited. The authors also noted that sonography could potentially be used to determine gland volume when planning to treat hyperthyroid cats with radioiodine.[91]

The sonographic appearance of the thyroid gland in a group of hyperthyroid cats before and after treatment with ^{131}I has recently been reported.[90] Fifteen client-owned cats were used in the study. At first presentation mean serum total T_4 was 125.9 nmol/l with concentrations all concentrations being between 52.6 and 193.5 nmol/l (normal reference interval: 10-50

nmol/l). Two cats had previously been treated with methimazole and four with carbimazole. The second ultrasound examination was performed 6 months after initial ultrasound and treatment with ^{131}I. A multifrequency (7-14 MHz) linear matrix transducer with the frequency set at 12 MHz was used for sonographic examinations. Image presets were identical for all patients. Overall gain and time gain compensation were adjusted for each patient. Maximum length, height and width were measured using electronic calipers. Homogeneity and shape of the thyroid lobes were assessed. Shape was classified as abnormal if the lobe had a round instead of triangular shape in short axis or an ovoid instead of fusiform shape in long axis. Vascularization was recorded as absent, mild, moderate or strong using Power Doppler. Lobe volume was calculated using the formula for a prolate ellipse. Some of the lobe measurements could not be obtained in two lobes pre-treatment and in seven lobes post-treatment. Scintigraphy was used to document unilateral or bilateral lobe involvement and the presence of ectopic tissue. T/S ratios were calculated. Forty percent of cats had bilateral uptake and 53% had unilateral uptake. One cat had only ectopic hyperfunctioning tissue. Ectopic tissue was detected in a total of six cats. The mean T/S ratio of abnormal lobes was 5.85:1 (all values were between 1.4:1 and 11.3:1) for the left lobe and 4.64:1 (all values were between 1.2:1 and 12.1:1) for the right lobe. The mean injected dose of radioactive iodine was 3.1 mCi (range 2.4-5 mCi) or 114.7 MBq (range: 88.8-185 MBq). Dose was determined based on total T4 concentrations and results of the pertechnetate study. Sonographic findings at presentation included parenchymal heterogeneity, hypoechoic to anechoic areas, foci of mineralization, rounded shape and variable vascularity. There was no difference in the appearance of thyroid lobes between cats that did or did not have medical treatment. Out of 10 cats with bilaterally abnormal lobes on ultrasonography, six of them had bilateral involvement scintigraphically. Of the four cats with unilaterally abnormal glands on ultrasound all cats had unilateral scintigraphic abnormalities in the same lobe. One cat had one normal and one non-detectable lobe on ultrasound and bilaterally abnormal uptake scintigraphically.

The mean follow-up (post-treatment) total T_4 concentration was 17.0 nmol/l (all total T_4 concentrations were between 6.5 and 28.4 nmol/l).[90] There was a significant reduction in mean thyroid lobe volume over time (from 819 to 210 mm^3). This corresponded to a 75% reduction in volume. The volume of all individual thyroid lobes decreased after treatment.

Generally at follow-up the thyroid lobes were smaller, more homogeneous and less round. Thyroid glands generally had decreased or absent vascularity compared with the first examination. There was no significant difference in the presence of hypoechoic/anechoic areas after treatment. The higher transducer frequency used in this study, in comparison to the previously reviewed study,[91] was thought to be an advantage in being able to determine measurements of thyroid lobes in cross section and detect smaller (atrophic) thyroid lobes. Decreased vascularity in post-treatment cats may be due to initial hypervascularity in cats with hyperthyroidism. In people with Grave's disease thyroid glands can exhibit hypervascularity before treatment, and a direct correlation has been found between pre-treatment thyroid vascularity in the periphery of autonomous nodules and laboratory parameters of hyperthyroidism.[92] It is unknown whether results from this descriptive study may be used in the future to try to predict response to radioiodine therapy and potentially modify the dose used to treat cats with hyperthyroidism.

Thyroid sonography in dogs:

The normal thyroid gland appears as a homogeneous, well-delineated structure with a hyperechoic capsule. The thyroid parenchyma is most often hyperechoic compared with surrounding musculature. Size of the thyroid gland has been correlated to the size of the dog.[76] The shape of each thyroid lobe is typically triangular in the transverse plane and fusiform in a longitudinal plane. Songraphically thyroid lobes are typically found by identifying the more medially located tracheal rings and the laterally located common carotid arteries. The esophagus is located dorsal to the left thyroid lobe.[93, 94]

Effects of intra and interobserver variability of sonographic measurements of the thyroid gland have been reported for the dog.[95] Five neutered female beagles of three years of age were used for the study. Dogs were all euthyroid based on clinical exam, hematology, serum biochemistry, total T_4 serum concentration, TSH serum concentration and absence of antithyroglobulin antibodies. Three observers trained in sonography of the neck, including two European College of Veterinary Diagnostic Imaging diplomates and one European College of Veterinary Diagnostic Imaging resident, scanned both thyroid lobes of each dog three separate times. A multifrequency linear array probe of 7-12 MHz was used for scanning. Only the highest probe frequency was used during the study. External parathyroid

glands were not included in measurement of length. Embedded internal parathyroid glands were not identified. Maximum length was measured in the longitudinal plane, and width and height were measured in the transverse plane. The volume of the thyroid lobe was calculated using the formula for a rotation ellipse (volume = length x width x height x 0.479). The correction factor of 0.479 was reported in the human literature to be more accurate than the correction factor of 0.524 ($\pi/6$), which is used in the volume calculation for a prolate ellipse.[96] A more recent study reported acceptable correction factors to be between 0.494 and 0.554.[97] In this study length measurements had the highest intra- and interobserver variability. The smallest intraobserver variability was found for height. Interobserver variability for height made a substantial contribution to the overall variability. Intraobserver variability for width was higher than for height, but there was less variability in measurements made between observers. Volume of the thyroid gland, based on length, width and height had the smallest intraobserver variability and smaller interobserver variability than the height. The reason for variability in measured length was reportedly due to difficulty in identifying the caudal end of the lobes. A second reason could include the possibility of having included the parathyroid glands in the length measurement in cases where the parathyroid glands were small and not easily identified. It was also noted that variation in measurements might result from technique, particularly in regards to how maximum dimensions are identified. In some cases height and width were measured on the same frozen image. This approach may fail to accurately determine maximum width, as maximum width and height may not occur in the same imaging plane. This study concluded that due to high interobserver variability the value of making a diagnosis of hypothyroidism based on sonographic measurements was questionable. Height and volume seemed the most promising parameters with which to evaluate thyroid lobe size in dogs.[95]

The sonographic appearance of the thyroid gland has been reported for clinically normal, hypothyroid and euthyroid (dogs with nonthyroidal illness) golden retrievers.[98] Thirty-six normal, 11 hypothyroid and 35 euthyroid dogs were included in the study. A single breed was chosen for investigation to negate possible effects of breed and thyroid gland size as a potential confounding factor when analysing results. In the majority of healthy dogs the thyroid lobes were classified as having a fusiform shape (72%). The remainder of the lobes in healthy dogs were described as elliptical. The majority of dogs with hypothyroidism and

non-thyroidal illness also had thyroid lobes with a fusiform shape. It was noted that when the thyroid was examined in the transverse plane, hypothyroid animals were more likely to have thyroid lobes with a round to oval rather than a triangular shape. In both healthy dogs and those with nonthyroidal illness most thyroid lobes were hyperechoic or isoechoic when compared with surrounding musculature. Hypoechogenicity was more prevalent in the hypothyroid group. Six percent of healthy dogs had hypoechoic thyroid glands, compared with 41% of hypothyroid dogs. Hypothyroid dogs were also more likely to have asymmetry in echogenicity between the two lobes. There were also differences seen in the texture of the parenchyma between healthy and hypothyroid dogs. Eighty-five percent of healthy dogs had homogeneous appearing parenchyma, whereas only 55 % of hypothyroid dogs had homogeneous appearing thyroid lobes. Generally there was no significant difference in the appearance of the thyroid gland between healthy dogs and euthyroid dogs with nonthyroidal illness. Thyroid lobe volume was calculated using the formula for an ellipsoid (length x height x width x $\pi/6$). The volumes of the right and left thyroid lobes and total thyroid gland volume were significantly less in hypothyroid dogs. Mean length, height and width of the right and left lobes in euthyroid dogs with nonthyroidal illness were similar to measurements in healthy dogs and were greater than measurements obtained in hypothyroid dogs. When using the mean thyroid gland volume in healthy dogs ± 2 SD, the reference interval for thyroid gland volume was 324 - 1,571 mm^3. This reference interval was specific but not sensitive for hypothyroidism as only 4 of 11 hypothyroid dogs had a thyroid gland volume below the reference interval. Thus there was considerable overlap in thyroid gland volume between healthy and hypothyroid golden retrievers. Only one of 35 euthyroid dogs with nonthyroidal illness had a thyroid gland volume below this reference interval. In summary this study found that ultrasound findings, including thyroid lobe size and volume were similar for healthy dogs and euthyroid dogs with nonthyroidal illness. These findings suggest that morphologic changes of the thyroid gland do not occur with nonthyroidal illness. It was noted that the effect of nonthyroidal illness on thyroid gland function is dependent on severity, systemic nature, and duration of illness. Additional studies may therefore be indicated to evaluate dogs with more severe or chronic illnesses before definitive statements can be made regarding the impact of nonthyroidal illness on the ultrasound characteristics of the thyroid lobe in these patients. Ultrasound findings in the hypothyroid group were more variable than for the other groups of dogs. Although hypoechogenicity was more prevalent in

the hypothyroid group, it was not a specific finding. Results indicate that sonography may be helpful to confirm hypothyroidism in a patient with a thyroid gland volume below the suggested reference interval but would not rule it out if thyroid volume measured within the reference interval.[98]

A follow up to the previously described study was performed to compare the sonographic characteristics of the thyroid gland in healthy small, medium and large-breed dogs.[99] The objective was primarily to examine differences in thyroid gland size and volume among different breeds and sizes of dog. Miniature and toy poodles were included as small-breed dogs, beagles were included as medium-sized dogs, and akitas and golden retrievers were included as large-breed dogs. Size, shape, echogenicity and homogeneity of each thyroid lobe were evaluated. Thyroid lobe echogenicity was assessed subjectively by comparison with surrounding musculature. Measurements included the maximal longitudinal length, width and height. All measurements were performed on 3 separately obtained images. Mean values of the three measurements were used in the calculation of thyroid lobe volume. Volumes were estimated using the equation for an ellipsoid. Mean thyroid gland volume was similar in akitas and golden retrievers. The mean total thyroid gland volume in akitas and golden retrievers was higher than the mean total thyroid gland volume in beagles and miniature and toy poodles. There was overlap in total thyroid gland volume between individual small-breed and medium-size dogs and between medium-size and large-breed dogs, but not between small-breed and large-breed dogs. There were weak but significant negative correlations between age and thyroid lobe length, width and total thyroid gland volume. In most of the dogs the thyroid capsule had a smooth surface, the thyroid parenchyma had a homogeneous echogenic pattern, and parenchyma was most commonly hyperechoic or isoechoic compared with surrounding musculature. Thyroid hypoechogenicity was an uncommon finding except in beagles, where it was found in 5/24 dogs. Shapes and echogenicities of left and right lobes were similar. Thyroid lobe shapes were quite variable. This study concluded there was marked variation and overlap in thyroid lobe size and volume in dogs within and between breeds. When data from all the dogs were analyzed together, there were significant and strong positive correlations for thyroid gland size and volume with body weight and body surface area (BSA). Similarly, positive correlations between thyroid gland volume and BSA have been identified in people.[100-102]

Body weight and BSA had equally strong relationships with total thyroid gland size and volume.[99]

A study was performed comparing sonographic characteristics of the thyroid gland in thyroglobulin auto-antibody (TgAA) positive hypothyroid dogs, TgAA-negative hypothyroid dogs and dogs with nonthyroidal illness syndrome.[103] Healthy dogs were also included in the study as controls. Thyroid glands were assessed for size, echogenicity, and homogeneity. This study was the first to relate calculated thyroid lobe volume to metabolic body weight in dogs. Maximal cross sectional area of the thyroid lobes was measured as a second indicator of thyroid lobe size. The sternothyroid muscle was used as a standard with which to compare echogenicity of thyroid lobes. This is the only study in dogs to report the use of image analysis software to quantify mean density of thyroid lobes and muscle to calculate relative echogenicity. Sensitivity, specificity, and accuracy were determined for different sonographic variables to help identify the potential diagnostic value of individual sonographic characteristics (e.g. thyroid lobe hypoechogenicity). In both TgAA-positive and TgAA-negative hypothyroid dogs relative thyroid echogenicity was significantly lower than for euthyroid dogs. TgAA-positive hypothyroid dogs had homogeneously hypoechoic thyroid lobes, whereas the thyroid parenchyma in TgAA-negative hypothyroid dogs appeared more heterogeneous. Thyroid lobe echotexture was characterized by dark background interrupted by hyperechoic spots and lines. In this study several limitations regarding calculation of thyroid lobe volume were discussed. These limitations included interobserver variability and difficulty in imaging the entire length of the thyroid lobe in the longitudinal plane. Despite these limitations, results of the study indicated that thyroid lobe volume might be significantly reduced in hypothyroid patients. No differences in sonographic appearance were seen between euthyroid dogs and those with nonthyroidal illness syndrome. Cut-off values were chosen by trial and error to analyze the predictive value and maximise sensititvity and specificity. Using these values (<0.05 $mL/kg^{0.75}$ and <3.3 $mm^2/kg^{0.75}$ for relative volume and relative maximal cross sectional area respectively) relative thyroid volume and maximal cross sectional area revealed high specificity (96 and 96%) and accuracy (91 and 90%) but lower sensitivity (81 and 77%) for detection of hypothyroid dogs. Variability in echotexture and echogenicity of thyroid glands of hypothyroid dogs resulted in lower sensitivity and specificity for these characteristics. These variables could however be

useful when assessed in combination with other variables. When combining relative thyroid volume and relative thyroid echogencity sonography resulted in a high sensitivity (98%) for canine hypothyroidism.[103]

A recent report describes the sonographic appearance of the thyroid gland in hypothyroid dogs both before and after treatment.[104] The purpose of the study was to determine if sonographic characteristics would change after treatment of hypothyroidism. The study included 18 hypothyroid dogs from 11 different breeds. Dogs were included in the study only if they had a positive response (resolution of clinical signs and normalization of total T_4 values) to treatment with levothyroxine. Thyroid lobe length, width and height were measured. A control group was also included. Echogenicity was assessed subjectively by comparison with the overlying sternothyroid muscle. Capsule delineation and overall homogeneity were assessed. Shapes of the lobes were assessed on transverse images. A lobe was considered normal in shape if it was triangular to polygonal in cross section. Round, ovoid and amorphously shaped lobes were considered abnormal. In this study maximum height, length and width were used to calculate lobe volume. As previously reported, thyroid lobe volume was calculated using the formula for an ellipse. Thyroid gland volume was then divided by the metabolic body weight ($BW^{0.75}$) of the dog to determine relative volume. This technique was used in the previously reviewed study.[103] Relative thyroid gland volume was considered small when calculated to be less than 0.05 $ml/kg^{0.75}$. The original study to relate thyroid gland volume to metabolic body weight in dogs suggested this cut-off value as a relatively specific test for hypothyroidism.[103] Reported median relative thyroid volumes for controls, euthyroid sick dogs, TgAA-pos hypothyroid dogs and TgAA-neg hypothyroid dogs were 0.08, 0.07, 0.04 and 0.04 $ml/kg^{0.75}$, respectively. Ranges reported for controls, euthyroid sick dogs, TgAA-pos hypothyroid dogs and TgAA-neg hypothyroid dogs were 0.03-0.13, 0.04-0.12, 0.01-0.12 and 0.01-0.07 $ml/kg^{0.75}$, respectively. Thus some overlap in values was present between groups.[103] When a cut-off value of less than 0.05 $mL/kg^{0.75}$ was used, relative volume indicated hypothyroidism with a sensitivity of 81%, specificity of 96% and accuracy of 91%. As reported previously, sensitivity for diagnosing hypothyroidism was increased by combining relative thyroid lobe volume with relative thyroid echogenicity.[103] In this study[76] the cut-off value was also applied to the control group (5 healthy beagles). All relative

thyroid gland volume measurements from healthy beagles were above the suggested cut-off value of 0.05 mL/kg$^{0.75}$.

In this group of hypothyroid dogs at initial presentation 65% of thyroid lobes were heterogeneous, 65% had an irregular capsule, 59% were abnormal in shape, and 76% were hypoechoic or isoechoic when compared with the overlying muscle.[76] Dogs re-presented for follow-up between 29 and 414 days after initial ultrasound evaluation. Relative lobe volume decreased significantly between visits. There was no significant change in echogenicity, homogeneity, shape or capsule delineation of the lobes over time. The author noted that length was the most difficult of the three measurements to obtain because of difficulty identifying the sharp caudal endpoint of the gland. The cut-off value of 0.05 mL/kg$^{0.75}$ resulted in low sensitivity (47.1%) when using relative volume as a test for hypothyroidism. This sensitivity was much lower than that reported for this variable in the previous study.[103] This disparity may have resulted from the relatively high inter-observer variability encountered when making ultrasound measurements of thyroid lobes.[95] The progressive decrease in the size of the thyroid lobes observed over time may have been secondary to effects of supplementation with levothyroxine. Hormone supplementation may have stimulated the negative feedback loop, thus decreasing TSH secretion. Decrease in thyroid lobe size could also be secondary to progression of lymphocytic thyroiditis and destruction of glandular tissue. In this group of hypothyroid dogs the thyroid lobes were sonographically small, hypoechoic, heterogeneous, misshapen, or ill-defined in 94% of patients. This study concluded that sonography could be used as an effective additional test for the diagnosis of canine hypothyroidism.

3. Manuscript

QUANTITATIVE PERTECHNETATE THYROID SCINTIGRAPHY AND THE ULTRASONOGRAPHIC APPEARANCE OF THE THYROID GLAND IN CLINICALLY NORMAL HORSES

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Running head: Thyroid Scintigraphy and Ultrasound in Horses

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Abstract:

The purpose of this study was to report the scintigraphic and sonographic appearance of the thyroid gland in clinically normal horses so these modalities could be used to assess the thyroid gland in this species. Horses were divided into two age groups. Group A consisted of 8 horses between 3 and 10 years of age and Group B of 7 horses between 11 and 20 years of age. Total T_4 concentrations were within the laboratory reference interval. Thyroid to salivary (T/S) ratio, percent dose uptake of pertechnetate and thyroid lobe volume were calculated. Echogenicity of thyroid lobes and presence of nodules were documented. The two groups were compared using appropriate parametric and nonparametic tests. Total T_4 concentrations were significantly lower in the older group. Sixty minute mean ± standard deviation (SD) T/S ratios for older versus younger horses were 5.8 ± 3.0 and 5.3 ± 2.2, respectively. Sixty minute median and interquartile ranges for percent dose uptake of pertechnetate for older versus younger horses were 3.64% (1.5 to 3.98%) and 2.55 (2.33 to 2.90%), respectively. Mean ± SD thyroid lobe volumes for older versus younger horses were 18.93 ± 5.16 cm^3 and 13.55 ± 3.56 cm^3, respectively. Most thyroid lobes were hyper or isoechoic to the sternocephalicus muscle. Incidence of thyroid nodules did not differ between groups. Older horses had trends for greater T/S ratios, percent dose uptakes and thyroid lobe volumes yet lower total T_4 concentrations. Further studies using scintigraphy and ultrasound in horses with thyroid disease are planned.

Introduction:

Thyroid dysfunction has traditionally been difficult to diagnose and is poorly understood in horses.[11] Thyroid hormone assays are currently the only objective method available to evaluate equine thyroid gland function. Thyroid hormone assays are limited by several factors that include limited availability of appropriate hormone assays, non-thyroidal factors that influence thyroid hormone levels, and large variations in hormone levels between clinically normal individuals. Thyroid stimulation tests have been suggested as the best way of assessing thyroid gland function in horses.[26, 28, 45] Thyroid stimulation tests can be expensive, time-consuming and currently have limited availability. Thus, there is a critical need to have other, reliable diagnostic methods to evaluate equine thyroid gland function. These new thyroid tests would not replace hormone assays but could be useful in conjunction with thyroid hormone analysis.

Reported diseases affecting the thyroid gland of adult horses include hyperthyroidism, Hashimoto's thyroiditis-like disease, hypothyroidism, thyroid carcinomas and thyroid adenomas.[12-27, 33, 63, 105, 106] Thyroid nodules are reportedly common in older horses, with incidences of 31.6 % and 75.0 % in a population of horses greater than 10 and 20 years of age, respectively.[14] In small animals and people, diseases affecting the thyroid gland are routinely investigated using a combination of hormone analysis, scintigraphy and ultrasound. The ability to use these modalities to evaluate the thyroid gland in horses requires knowledge of normal appearance and variation. A review of the literature failed to identify a description of the scintigraphic and ultrasonographic appearance of the thyroid gland in clinically normal horses.

In dogs, cats and people, scintigraphy and ultrasound are used to assess thyroid gland function and morphology. The quantitative scintigraphic parameters most commonly used to estimate metabolic function of the thyroid gland are thyroid to salivary ratio (T/S ratio) and percent dose uptake of pertechnetate. In cats, T/S ratio is a good predictor of the metabolic status of the thyroid gland.[73] Scintigraphy is primarily performed in cats to identify hyperfunctioning glandular tissue. In dogs, percent dose uptake of pertechnetate by the thyroid gland is thought to be a more reliable parameter than T/S ratio when assessing thyroid function.[79] Percent dose uptake of pertechnetate is a more difficult parameter to calculate and is not affected by salivary uptake of pertechnetate or secretion of saliva. Pertechnetate scintigraphy is used in dogs to investigate the origin of cervical masses and can be used to

diagnose hypothyroidism. Scintigraphy is reported to have the highest discriminatory power to differentiate between hypothyroid dogs and those with low thyroid hormones due to non-thyroidal illness (euthyroid sick).[64, 79, 107] Ultrasound can also effectively differentiate between euthyroid sick dogs and truly hypothyroid patients.[98, 103]

There has been limited use of pertechnetate thyroid scintigraphy and ultrasound in the evaluation of the thyroid gland in horses. These modalities have only been used in a non-quantitative fashion to evaluate patients with cervical masses or clinical signs of hyperthyroidism.[18, 21, 22, 24] Neither scintigraphy nor ultrasound have been used quantitatively to assess thyroid gland function in horses. The purpose of this study was to report the scintigraphic and ultrasonographic appearances of the equine thyroid gland in a group of clinically normal horses and to provide reference intervals for T/S ratio and percent dose uptake of pertechnetate. Because of the reported increase in the incidence of nodular thyroidal disease with age in horses, we chose to compare the qualitative appearance and quantitative parameters of the thyroid gland between two age groups of adult horses. Our null hypotheses were that there would be no significant differences in T/S ratios, percent dose uptakes, thyroid lobe size or incidence of thyroid gland nodules between age groups.

Materials and Methods:

Fifteen adult horses between 3 and 20 years of age were used in the study. The Animal Care and Use Committee of the Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech approved the experimental protocol. Inclusion criteria for the study included normal physical examinations, total thyroxine (T_4) concentrations, complete blood counts and serum biochemistries. Serum samples were analyzed for total T_4 concentrations at the Animal Health Diagnostic Center of Cornell University. All horses were fed a pasture diet and received no supplements or medications for a period of at least 6 months before the study. Studies were all conducted between July and September to account for seasonal variations in total T_4[108] and all blood samples were obtained in the morning to account for diurnal variation in T_4.[52] The horses were separated into two age groups. Group A consisted of 8 horses between 3 and 10 years of age (3 geldings, 5 mares). Group B consisted of 7 horses between 11 and 20 years of age (2 geldings, 5 mares).

Nuclear medicine

Horses were sedated for scintigraphy with 0.02 mg/kg of detomidine hydrochloride (Pfizer Animal Health, New York, NY) administered intravenously through a jugular catheter. Sedation was repeated at the same dose 40 minutes after injection of pertechnetate (Cardinal Health, Nuclear Pharmacy Services Location No. 223, Roanoke, VA). The dose (Mean ± SD) of pertechnetate injected was 1,020 ± 99.9 MBq (27.6 ± 2.7 mCi) and was administered into a jugular vein via a preplaced jugular catheter.

Image Acquisition:

Scintigraphic images were acquired with a large-field-of-view scintillation camera (NuCamII™; Diagnostic Services Inc., Middlesex, NJ) fitted with a low energy all-purpose parallel hole collimator and integrated to a dedicated imaging computer with motion correction software (Mirage Acquisition Application (PCI) Version 5.715f7b Copyright© 1997-2008, Segami Corporation, Columbia, MD). A 20% window was centered at a photopeak of 140 KeV, and image uniformity was software corrected. All images were stored in a 256 x 256 x 16 matrix. Prior to imaging horses, an image of a radionuclide standard of known activity, approximately 77.33 ± 6.29 MBq (2.09 ± 0.17 mCi) (Mean ± SD) was acquired using a 60 second static frame-mode sequence. Data from this image were used to calculate camera-counting efficiency. A 60 second dynamic frame-mode acquisition was used to acquire motion corrected images of the horses. Ventral, right lateral and left lateral views were made at 10, 20, 40 and 60 minutes after intravenous injection of pertechnetate. Quantification of thyroid activity was only performed using data from the ventral view to minimize activity from superimposition of adjacent tissues.

Image Analysis:

Image analysis was performed using NucLear Mac software (Scientific Imaging, Inc., Crested Butte, CO). T/S ratios were determined as follows. Using the ventral image, a single region of interest (ROI) was drawn manually around each lobe of the thyroid gland, each parotid salivary gland, and a background area of the neck that excluded thyroid, parotid and major vascular activity. A color look-up table with a standardized upper limit of the scale was used to reliably identify margins of thyroid and salivary glands. Data from salivary gland ROIs were combined. Mean count density was calculated by dividing counts recorded in each ROI by the number of pixels in the ROI. T/S ratios were calculated by dividing mean

count density within each thyroid lobe ROI by mean count density within the combined salivary gland region. T/S ratios were determined for each imaging time period.

Percent dose uptake of pertechnetate by the thyroid gland was determined as follows. The radionuclide standard was imaged by the scintillation camera using fixed geometry to determine camera counting efficiency (counts per minute/MBq). The injection apparatus (syringe, extension tubing and catheter) was imaged following injection to correct for residual activity. All measured activities were corrected for decay. Counts acquired from the ROIs of thyroid lobes in the ventral views from each time point were corrected for background using the formula:

$$\text{Net Counts} = \text{Gross counts} - \left(\left(\frac{\text{BKD ROI counts}}{\text{Number of Pixels in BKD ROI}} \right) x \text{ number of pixels in thyroid ROI} \right)$$

Thyroid gland uptake was depth corrected using thyroid lobe depth measurements obtained using ultrasound and the linear attenuation coefficient for soft tissues (0.153 cm^{-1}) using the following formula

$$\text{Depth Corrected Thyroid Counts} = \frac{\text{Thyroid Counts}}{e^{-0.153\,x}}$$

where x = thyroid depth (cm).

The following formula was used to determine percent dose uptake of pertechnetate by the thyroid gland at each time interval.

$$\text{Percent Dose Uptake} = \frac{\text{Corrected Counts (cpm) in Thyroid}}{\text{Radioisotope Dose in cpm}} \text{ x } 100$$

Ultrasound

Ultrasound was performed immediately prior to scintigraphy. Prior to ultrasound examination, two areas (approximately 6 x 6 cm) were shaved on either side of the neck, just caudal to the larynx. Sonographic examination was performed by a resident in training (SD) under the supervision of a board certified radiologist (ML). A 14-MHz multifrequency linear-array transducer (Sequoia™; Siemens Medical Systems, Malvern, PA) was used to examine the thyroid gland. Thyroid lobes were scanned in longitudinal and transverse

planes. Longitudinal images were produced by maximizing visible thyroid lobe length. Transverse images were obtained by rotating the transducer 90° at the widest portion of each thyroid lobe. The maximum width, height and length of the lobes were measured and recorded. The volume of each thyroid lobe was estimated by use of the equation for an ellipsoid: $\pi/6$ (length × height × width).[91, 99, 109, 110] Distance from the skin surface to the capsular surface of the thyroid lobe was also measured for soft tissue attenuation correction needed for the scintigraphic analysis. Focal lesions such as cystic or nodular structures within the parenchyma were described. Thyroid gland echogenicity was recorded as being hypoechoic, isoechoic or hyperechoic by subjective comparison with the adjacent sternocephalicus muscle.

Statistical Analysis

Statistical analysis was performed using a commercial statistical software program (SigmaStat® Ver 3.1.1, Systat Software, Chicago, IL). All data were assessed for normal distribution using the Kolmogorov-Smirnov test. Total T_4 concentrations were assessed for an association with age and scintigraphic measurements using Pearson product moment correlation. T-test and Mann-Whitney rank sum test were used for comparison of 2 groups of parametric or non-parametric data, respectively. A paired t-test was used to determine if there was a difference between left and right thyroid lobe volumes. One way repeated measures analysis of variance was used to compare T/S ratio and thyroidal percent dose uptake between the two groups of horses over time. All pairwise multiple comparison procedures was used to isolate the groups that differed from each other in the one way repeated measures analysis of variance. Significance for all tests was set at $p \leq 0.05$.

Results:

The mean age (± standard deviation) for horses in Group A and B were 5.2 (± 2.7) and 15.6 (± 3.9) years respectively. All horses had normal physical examinations, serum biochemistry profiles and complete blood counts. Breeds represented included seven Thoroughbreds, six Quarter Horses, one Thoroughbred Warmblood cross breed and one Oldenburg. Individual total T_4 concentrations were all within the laboratory reference interval (1.5 - 4.5 ug/dL). Summary data for total T_4 concentrations are presented in Table 1. Mean and standard deviations for total T_4 concentrations for the younger (Group A) and older

(Group B) horses were 2.97 ± 0.35 ug/dL and 2.34 ± 0.47 ug/dL, respectively. The older horses had significantly lower total T_4 concentrations. The Pearson product moment correlation demonstrated a negative correlation between age and total T_4 (correlation coefficient = 0.653).

Scintigraphically, margins of the thyroid lobes were easy to identify which facilitated ROI placement. Margins of the parotid salivary glands were less well defined. Since size of ROIs could have affected count density measurements used for T/S ratios, and to decrease variation in techniques, all ROIs were drawn by one person (GBD) after collection of all data. Sixty minute summary data for T/S ratio and percent dose uptake are presented in Tables 2. and 3. Changes in mean T/S ratios and median percent dose uptakes over time are shown in Figures 1 and 2. Ventral images of a 13 year-old horse acquired over time are presented in Figure 3. There was no significant difference between T/S ratios over time. The percent dose uptake at 60 minutes was significantly different from the 10 and 20 minute values and the 40 minute value was also different from the 10 minute value. There was no significant difference between the 60 minute and 40 minute values. There was a trend for both increased T/S ratio and percent dose uptake in the older group of horses, although this difference was not statistically significant. There was greater variation in T/S ratio data compared to the percent dose uptake data and also increased variation in the older group of horses (Tables 2 and 3). Overall, salivary gland activity increased over time for both groups of horses (Figure 4.). Two individuals (one horse from each group) had a decrease in salivary activity between 40 and 60 minutes. There was no correlation between total T_4 concentration and either T/S ratio or percent dose uptake at any imaging time point.

Results for thyroid lobe volume are presented in Figure 5. There was no significant difference in calculated volume between left and right lobes of the gland. Data for left and right lobes was therefore combined for the remaining analysis. Means and standard deviations for combined thyroid lobe volume for the older and younger groups of horses are 37.85 cm^3 (± 10.04 cm^3) and 27.10 cm^3 (± 5.27 cm^3), respectively. The older horses tended to have higher thyroid gland volume, although the difference between groups was not statistically significant. Qualitative appearance of the thyroid lobes is summarized in Table 4. A longitudinal image of the left thyroid lobe in a three year-old horse is presented in Figure 6A. This view was used to measure height and length of the left thyroid lobe. Nodules were identified in one of the thyroid lobes in 4 of 8 of the younger horses and 3 of 7

of the older horses. The oldest horse had a nodule in each thyroid lobe. Thus a total of 5 nodules were identified in the thyroid lobes of the older horses. A nodule found in the right thyroid gland of one of the younger horses is presented in Figure 6B. All nodules were less than 1cm in diameter. The incidence of thyroid nodules did not differ significantly between the two groups. The majority of the thyroid lobes were hyperechoic or isoechoic to the adjacent sternocephalicus muscle. An example of an image used to compare thyroid echogenicity with echogenicity of overlying muscle is presented in Figure 6C.

Discussion:

The older group of horses (Group B) had significantly lower total T_4 concentrations and trends towards greater T/S ratios, greater percent dose uptakes and larger thyroid lobe volumes. There was also more statistical variation in the data for the older group of horses. There was no statistically significant difference in the incidence of thyroid nodules between the two groups.

A difference in total T_4 concentration between older and younger horses has not been previously reported in the literature. One study that evaluated the relationship between T_4 concentration and age reported that newborn foals had the highest concentrations of thyroid hormones. Concentrations of hormone then declined continually to 1 month of age. The lowest observed T_4 concentrations were seen at 16 to 22 years of age, however there was no statistically significant difference in T_4 concentrations in horses between 16 and 22 years of age when compared with those at 1 month of age.[111] The same study examined differences in thyroid hormone concentrations in eight breeds, ranging in size from Miniatures to Draft horses. Differences between breeds were not significant and thyroid hormone concentrations were not related to adult body size.[111] Total T_4 concentrations were lower in clinically normal older horses than younger horses in this study. The reason for the lower total T_4 concentrations in the older group is uncertain. To the author's knowledge there are no reports documenting a negative correlation between T_4 concentration and age in people, cats or dogs. Further study of a larger population of horses is needed to determine if this is a reliable finding. It may also be helpful to include horses greater than 20 years of age in future investigations. Although all horses were clinically normal and had total T_4 concentrations within the laboratory reference interval, it is still possible that total T_4 concentrations were lower in the older horses due to subclinical thyroid gland pathology that was affecting their

ability to produce thyroid hormone. This study design was non-invasive and did not allow for histopathologic examination of the thyroid gland.

Mean T/S ratios and standard deviations for the older and younger groups of horses at 60 minutes post injection were 5.8 (± 3.0) and 5.3 (± 2.2), respectively. Mean T/S ratios increased over time, however we were unable to detect a statistically significant difference in T/S ratios between the different time points. There was a greater spread of T/S ratio data when compared with percent dose uptake data. Greater variation in T/S ratio data is likely secondary to variation in salivary uptake and excretion over time. Calculation of T/S ratio depends on salivary gland uptake, which may be affected by physiologic or pathologic conditions regardless of thyroid gland uptake.[79] In cats, calculation of the T/S ratio is preferred to percent dose uptake when investigating hyperthyroidism, as T/S ratio had the best correlation with serum T_4 concentration and is less cumbersome to calculate.[73] In this study, the variation in T/S ratio data, and the failure to detect significant changes in T/S ratio over time, suggests this parameter may not be as reliable as percent dose uptake when assessing thyroid gland function in horses. A similar finding has been reported in dogs, where percent dose uptake is reported to be a more reliable parameter than T/S ratio.[79] Discrepancy in reliability of the T/S ratio between species may partly be due to variation in salivary gland physiology for herbivores versus carnivores and differences in head morphology resulting in dissimilar tissue attenuation affecting scintigraphic quantification. There may also be some superimposition of salivary and thyroid activity on the ventral view. The dog and cat differ from the horse in the amount and type of tissue situated between the salivary gland and gamma camera.

Medians and interquartile ranges (IQR) for percent dose uptake of pertechnetate in the older and younger groups of horses were 3.64% (IQR 1.5 to 3.98%) and 2.55% (IQR 2.33 to 2.90%), respectively. Mean and median percent dose uptakes were greater in the older group of horses at 20, 40 and 60 minutes post injection. Overall, percent dose uptake of pertechnetate by the thyroid gland increased over time. The youngest horse in this study had a slight decrease in thyroid gland activity between 40 and 60 minutes. The optimal time for evaluation of percent dose uptake of pertechnetate in horses is likely between 40 minutes and 60 minutes after injection of pertechnetate, as there was no significant difference in median percent dose uptake between 40 and 60-minutes.

Variation in percent dose uptake in these horses is greater than that reported in cats

and dogs (Figure 7). Variation was greatest in the older group of horses. Dogs used to establish normal reference intervals for T/S ratio and percent dose uptake for pertechnetate scintigraphy were all between 6 months and 6 years of age[78]. Reference intervals for T/S ratio in cats were initially based on a study that evaluated a group of 10 young adults (males and females up to 11 years of age).[70] Two subsequent studies, that have included normal control groups and also calculated percent dose uptake, used only 1-year-old male cats.[72, 73] A study including a larger number of cats with a wider age range reported a higher range of T/S ratios than previous studies had suggested.[74] Some of the greater variation in our data may be due to sampling a larger age range of animals. Increased variation may also be inherent in horses or secondary to subclinical disease.

The authors are unaware of previous studies that have evaluated the effect of sedation on thyroidal uptake of pertechnetate in the horse. All horses were sedated with the same protocol to reduce effects of motion for the 60-second acquisition time. Sedation protocol is known to have a significant impact on thyroidal and salivary pertechnetate uptake in cats.[75] It was concluded that changes in salivary and thyroidal uptake with different sedation protocols could lead to an erroneous diagnosis of hyperthyroidism in cats. The recommendation is to use reference intervals established with the same sedative protocol to eliminate potential bias.[75]

No correlation was found between total T_4 concentrations and either T/S ratios or percent dose uptakes in these horses. In cats, T/S ratio and percent dose uptake were shown to correlate well with serum T_4 concentration.[73] However, this study included cats with a wide range of total T_4 concentrations and that were both euthyroid and hyperthyroid. Similar to our findings, in a study looking at healthy cats with normal total T_4 concentrations, there was a lack of correlation between total T_4 concentrations and percent dose uptake of pertechnetate.[69, 79] The lack of correlation was attributed to the fact that a number of physiological factors, other than thyroid trapping, can influence circulating thyroid hormone concentrations.[69] A lack of correlation between total T_4 concentration and percent dose uptake was also seen in a group of greyhounds with uniformly low T_4 concentrations.[79] The small range of normal total T_4 concentrations in this study likely affected our ability to find a correlation with percent dose uptake. It is also possible that percent dose uptake of pertechnetate does not accurately reflect the metabolic status of the thyroid gland in clinically normal horses. Performing pertechnetate scintigraphy in horses with abnormal thyroid

function may help determine if total T_4 concentrations can be correlated with percent dose uptake in horses and whether or not this parameter accurately reflects metabolic activity of the thyroid gland.

It was interesting to find that although the older group of horses had significantly lower total T_4 concentrations, this group had a trend towards higher percent dose uptakes and T/S ratios. This is the inverse relationship of what one would expect if the scintigraphic parameters accurately reflected the metabolic activity of the thyroid gland. As previously mentioned, pertechnetate uptake by the thyroid gland is primarily a function of thyroid trapping. Pertechnetate is not organified to form thyroid hormone. At the molecular level, the uptake (trapping) of pertechnetate is proportional to the expression of the thyroidal sodium/iodine symporter (NIS). TSH is known to induce NIS expression in the thyroid gland and stimulates activity of the iodide pump in thyroid cells.[8] TSH increases all known secretory activities of thyroid glandular cells including release of thyroid hormones into circulating blood.[5] Thus it would be expected that thyroidal uptake of pertechnetate is proportional to NIS expression and TSH concentration, and that increases in TSH concentration would translate to increased serum concentrations of total T_4, the main secretory product of the thyroid gland. In people, diffusely increased uptake of pertechnetate and low thyroid hormone concentrations can occur with endemic colloid goiter caused by dietary iodine deficiency and Hashimoto's thyroiditis.[6] Although the older group of horses in this study had total T_4 concentrations within the laboratory reference interval and were clinically normal, it is possible that they were suffering from a subclinical disorder causing TSH to be elevated relative to thyroid hormone production. There are no published reports of hypothyroidism resulting from iodine deficiency in adult horses, but it has been suggested that this differential should still be considered when goiter is detected.[2] Iodine deficiency would also be expected to increase the volume of the thyroid gland. Hashimoto's thyroiditis-like disease has been reported as a post-mortem finding in horses in Eastern Europe. This study found up to 25% of slaughtered horses had microscopic alterations in their thyroid glands that were consistent with immune-mediated thyroiditis.[27] It is not known if this disease causes clinical signs in horses or if it is isolated to a specific geographic region.[28] There are no documented cases of the disease in the United States.

Non-thyroidal factors are thought to affect the hypothalamic-pituitary–thyroid axis in horses, resulting in low thyroid hormone concentrations. These factors include

phenylbutazone administration, high energy diets, high-protein diets, diets high in zinc and copper, diets with a high carbohydrate/roughage ratio, glucocorticoid administration, food deprivation, level of training, stage of pregnancy and ingestion of endophyte-infected fescue grass.[28] The effect these factors would have on thyroidal uptake of pertechnetate is currently unknown. Horses were fed a pasture diet and received no supplements or medications for a period of at least 6 weeks prior to the study. None of the mares were pregnant. Thus factors cited above are unlikely sources of lower total T_4 concentrations in the older group. Non-thyroidal illness can also cause low total T_4 but would be an unlikely cause in this study, because all the horses were clinically normal. Lower thyroid hormone concentrations may be found in horses affected by equine metabolic syndrome (EMS). Horses diagnosed with EMS tend to be older, typically between 8 and 18 years of age and are in a state of insulin refractoriness. Horses are characteristically obese and may develop 'obesity associated' laminitis.[112] Although all study horses were clinically normal, it is possible that some of the older horses had subclinical or early metabolic syndrome, as not all horses affected with this disease are grossly obese. The cause of low thyroid hormone concentrations in horses with EMS is currently unknown; as is the affect this disease might have on thyroidal uptake of pertechnetate.

The older horses had a trend for increased thyroid lobe volume, although the difference between groups was not statistically significant. There was more variation in thyroid lobe volume in the older horses. The cause of increased thyroid lobe volume in the older horses is unknown. Interestingly, there was no significant difference in the incidence of thyroid gland nodules between the two groups. The youngest horse with a thyroid lobe nodule was 3 years of age. Overall the prevalence of thyroid nodules in this population of horses was 53%. The oldest horse (20 years of age) was the only horse to have bilateral thyroid lobe nodules. These results support previous reports documenting a high prevalence of nodular foci in the thyroid gland in horses, although our results suggest that younger horses may be affected with similar frequency. Benign adenomas are reported to comprise most thyroid gland tumors. Although glands may be enlarged the nodules rarely result in hypothyroidism or hyperthyroidism. Both follicular and parafollicular (C cell) adenomas have been reported to occur.[12, 14] Nodules identified with ultrasound were not apparent in the scintigraphic images, suggesting they were not metabolically hyperactive. Previous studies have reported the successful use of pertechnetate scintigraphy to identify hyperfunctioning

thyroid tissue in horses with thyroid carcinomas.[21, 22]

One of the limitations of this study was the low number of horses included in each group. Due to variation in the data, a larger number of horses would be needed to improve the chance of detecting statistical significance. Including horses greater than 20 years of age may also have been helpful. The number of horses included in the study was limited by funding. Ideally, normal thyroid function would have been confirmed in the study horses. However, assessing thyroid function in horses is inherently difficult. It has been suggested that the most accurate test currently available is a thyroid stimulation test. At the time of the study this test could not be performed due to lack of availability of TRH and TSH, which are administered intravenously to test responsiveness of the thyroid gland to these hormones.

Further work is needed to assess scintigraphic and ultrasonographic changes in horses with confirmed thyroid gland dysfunction. This may help clarify if percent dose uptake of pertechnetate accurately reflects metabolic activity of the gland. TSH concentration was not measured in this study because further research is need to validate equine TSH testing.[113] Once this test is validated, we predict that TSH concentrations would provide the best correlate with percent dose uptake in clinically normal horses.

Conclusion:

In summary, this study provides initial estimates of the reference intervals for thyroidal percent dose uptake of pertechnetate in two age groups of clinically normal horses. The older group of horses had trends for higher percent dose uptakes, higher T/S ratios, larger thyroid lobe volumes and more variation than the younger group of horses. The older horses also had significantly lower total T_4 concentrations. The cause of the inverse relationship between total T_4 concentration and percent dose uptake in the older group of horses is uncertain. Explanations could include early or subclinical thyroid disease or a true lack of correlation between total T_4 concentration and percent dose uptake of pertechnetate in this species. Further study is needed to determine if scintigraphy may be used in a quantitative fashion to accurately assess thyroid gland function in horses.

4. Supplemental Materials

A: Additional Results

A total of fifteen horses were included in the study, 8 in the younger group (group A) and 7 in the older group (group B). Age, breed and gender of each horse are documented (Table 5).

The older group of horses had significantly lower total T_4 concentrations than the younger group. The Pearson product moment correlation demonstrated a moderate association (correlation coefficient = 0.653) between age and total T_4 concentration (Figure 8). As age increases total T_4 concentration decreases. $R^2 = 0.4$ indicating 40% of the variation in total T_4 can be attributed to its linear relationship with age.

Although horses were sedated for scintigraphy it was sometimes difficult to restrain them adequately for the 60-second image acquisition time. Dynamic acquisitions were performed so that scintigraphic images could be corrected for motion using computer software that realigned the frames. Motion correction software matched 98% of the frames for each image acquired.

Thyroidal percent dose uptake at the various imaging time points for each horse are presented in Figures 9 and 10. At 60 minutes post injection of pertechnetate most of the horses in the younger group had thyroidal percent dose uptakes between 2.3 and 3.1% (Figure 9). An outlier had a thyroidal percent dose uptake of 1.4%. This horse was a 3.7 year-old Thoroughbred with no remarkable sonographic abnormalities. His combined (total) thyroid lobe volume calculated using sonographic measurements was 23.37 cm^3. Total thyroid lobe volumes for the all horses were between 18.72 to 52.72 cm^3. Therefore this horse's total thyroid lobe volume was small compared with most other horses. The youngest horse included in the study (3.2 years of age) had a slight decrease in thyroidal percent dose uptake between 40 and 60 minute imaging time points. This was the only horse to have decreased thyroidal percent dose uptake between 40 and 60 minutes after injection. For most of the younger horses the rate of uptake of pertechnetate decreased between 40 and 60 minute time points.

There was a wider spread of values for thyroidal percent dose uptake in the older group of horses than in the younger group (Figure 10). For the older group of horses thyroidal percent dose uptake was between 0.97 and 5.0% 60 minutes after injection of pertechnetate. Rate of uptake of pertechnetate by the thyroid gland decreased between 40 and 60 minutes post injection, as seen in the younger group. None of the horses in the older group had decreased thyroidal percent dose uptake between the 40 and 60 minute imaging time points. For the older group, variation in thyroidal percent dose uptake between individuals increased over time. At 10 minutes post injection thyroidal percent dose uptakes were between 0.16 and 1.4% (range = 1.24) whereas at 60 minutes post-injection thyroidal percent dose uptakes were between 0.97 and 5.00% (range = 4.03). Two horses appear to be outliers having thyroidal percent dose uptakes of 1.0% at the 60-minute time point. Both these horses had small nodules in their right thyroid lobes on sonographic examination. These horses had total thyroid gland volumes of 22.3 and 30.24 cm^3 (all horses had thyroid gland volumes between 18.72 to 52.72 cm^3). Subjectively, both horses had mild thyroid lobe asymmetry when examined scintigraphically.

There was a trend for greater thyroidal percent dose uptakes and T/S ratios in the older group of horses. Although median thyroidal percent dose uptake was greater in the older group, there was no association between thyroidal percent dose uptake and age (Figure 11). Similarly, although mean T/S ratios were greater for the older group there was no association between T/S ratio and age (Figure 12). Figures depict percent dose uptake and T/S ratio 60 minutes post injection.

When all horses were included in the analysis there was no association between total T_4 concentration and percent dose uptake or T/S ratio at any imaging time point. Total T_4 concentration versus thyroidal percent dose uptake at 60 minutes is shown in Figure 13. As previously noted the older group of horses had greater variability in thyroidal percent dose uptake than the younger group. The relationship between total T_4 concentration and thyroidal percent dose uptake for just the younger group of horses at 60 minutes post injection is shown in Figure 14. There is an association between total T_4 concentration and percent dose uptake for the younger group. As total T_4 concentration decreased thyroidal percent dose

uptake increased. $R^2 = 0.46$ indicating that 46% of the variation in thyroidal percent dose uptake can be attributed to its linear relationship with total T_4 concentration.

Individual thyroid lobe volumes were calculated using length, height and width measurements obtained using ultrasound. The number of pixels in each thyroid ROI was also recorded from scintigraphic images. The number of pixels in each ROI is an estimate of a dorsal cross-sectional area of the thyroid gland. There is a moderate correlation ($R^2 = 0.29$) between the numbers of pixels in individual thyroid ROIs and individual thyroid lobe volumes (Figure 15). A slightly stronger linear relationship ($R^2 = 0.34$) was present between the number of pixels in individual thyroid ROIs and individual thyroid lobe lengths (Figure 16). No gold standard was available to determine thyroid lobe volume as this was a non-invasive study and water-displacement could not be performed.

The older group of horses tended to have greater thyroid lobe volumes than the younger group of horses. Increased thyroid lobe volume with increasing age is shown graphically in Figure 17. This graph also depicts increased variability in calculated thyroid lobe volumes for the older group of horses. When thyroid lobe symmetry was assessed using calculated volume and symmetry was defined as less than 10% difference in volume between the thyroid lobes only one horse was asymmetric (Table 6). When thyroid ROI size was used to assess symmetry (by the same definition), asymmetry was present in four horses from the younger group and one horse from the older group. All thyroid nodules were small and none caused distortion of the thyroid lobe capsule.

There was a moderate association ($R^2 = 0.50$) between total (combined) thyroid lobe volume and thyroidal percent dose uptake (Figure 18). As thyroid volume increased so did thyroidal percent dose uptake. Apart from two outliers, horses in the older group had greater total thyroid lobe volumes and global thyroidal percent dose uptakes. A slightly weaker linear correlation ($R^2 = 0.39$) was present between individual thyroid lobe volumes and individual (right and left) thyroidal lobe percent dose uptakes (Figure 19).

There was a wider spread of T/S ratio values when compared with percent dose uptake. Sixty-minute T/S ratios were between 3.18 to 10.33 for the younger group of horses and 2.23

to 11.06 for the older group. When individual T/S ratios were graphed over time the oldest horse (10 years old) included in the younger group appears to be an outlier, having a particularly high T/S ratio when compared to other horses in this group (Figure 20). This horse had a small right thyroid nodule and subjectively symmetric thyroid lobes in scintigraphic images. This horse had relatively low average salivary pixel density compared with the other horses and was one of only two horses that had a decrease in average salivary pixel density between 40 and 60 minutes after injection (Figure 21). Differences in average salivary pixel density between horses increased over time (Figure 21). Average salivary pixel density varied between 12.42 and 45.99 (range = 33.57) at 10 minutes after injection and between 42.96 and 120.01 (range = 77.05) at 60 minutes after injection. One of the horses excreted pertechnetate from the parotid salivary gland into the parotid duct between the 40 and 60 minute imaging time points (Figure 22). When thyroidal percent dose uptake is compared to T/S ratio at 60 minutes (Figure 23) two horses with very similar thyroidal percent dose uptake values (2.56 and 2.54) have dissimilar T/S ratios (4.72 and 10.34). The horse with a higher T/S ratio (10. 34) had very low 60 minute salivary count density when compared to the other horses. This resulted in an elevated T/S ratio.

B: Additional Discussion

The increased variability in thyroidal percent dose uptake in the older group of horses might be in part due to the greater variation in thyroid lobe volume found in the older group. A moderate correlation was found between thyroidal percent dose uptake and thyroid lobe volume, and it is intuitive that thyroid gland volume might influence thyroidal percent dose uptake. In a normally functioning thyroid gland increased volume of tissue would be expected to translate into increased percent dose uptake of pertechnetate. Between 39 and 50% of variation in thyroidal percent dose uptake could be attributed to its linear relationship with volume. There was a marginal linear relationship between age and thyroid lobe volume in the study, with older horses tending to have larger thyroid lobes. The trend for greater thyroid lobe volumes and greater variation in thyroid lobe volume likely influenced the finding of greater thyroidal percent dose uptake and increased variation in thyroidal percent dose uptake for the older group. The reason for increasing thyroid gland volume and more variation in volume with increasing age is uncertain, but may be secondary to early subclinical disease in this population.

It is interesting that for the younger group of horses there was a moderate negative linear correlation between thyroidal percent dose uptake and total T_4 concentration. A negative linear relationship between percent dose uptake and thyroidal hormone concentration has not been described in clinically normal dogs or cats. The reason for this association is uncertain. It is possible that in horses with normally functioning thyroid tissue, those with higher total T_4 concentrations have lower thyroidal percent dose uptake of pertechnetate due to lower circulating concentrations of TSH. The presence of higher concentrations of total T_4 would be expected to decrease secretion of TSH, which would result in reduced expression of the NIS symporter. Without knowledge of TSH concentrations and evaluation of a larger population of horses it is difficult to confirm this theory.

Due to the non-invasive nature of this study there was no gold standard for determining thyroid lobe volume. Volume was calculated based on linear sonographic measurements and the formula for a prolate ellipse. This formula has been used in most small animal studies where thyroid lobe volume was calculated. The accuracy of the formula in determining

thyroid lobe volume in animals has not been reported. As expected, a linear relationship was found between the number of pixels in the scintigraphic thyroid lobe ROIs and calculated thyroid lobe volumes. The best correlation was found between length and number of pixels. Calculated thyroid lobe volume had the next best correlation with ROI size, followed by width and depth. Based on ventral scintigraphic images the number of pixels in the thyroid lobe ROI would be affected by the length and width of the thyroid lobes. Height and length could be estimated using a lateral image of the thyroid lobe. Further studies could be performed to assess the accuracy of the formula for a prolate ellipse in determining thyroid lobe volume in horses.

T/S ratio appears unreliable in horses due to variation in average salivary pixel density. Salivary gland uptake may be influenced by physiologic processes, such as excretion into the salivary ducts, and also by sedation. Salivary gland uptake is influenced by sedation in cats.[75] Future studies should focus on using thyroidal percent dose uptake to assess the thyroid gland in horses.

5. Conclusions and further study

In conclusion, this study has described the scintigraphic and sonographic appearance of the thyroid gland in a group of clinically normal horses. Initial estimates for thyroidal percent dose uptake and thyroid lobe volume have been provided. Thyroidal percent dose uptake is a more reliable parameter than thyroid to salivary ratio in horses.

The older group of horses had significantly lower total T_4 concentrations than the younger group and had trends for higher thyroidal percent dose uptakes, T/S ratios and thyroid lobe volumes. A moderate negative linear correlation was found between total T4 concentration and age. The significance of this finding is uncertain and further study should be performed in a larger population. A marginal positive linear correlation was found between age and thyroid lobe volume. Increased thyroid lobe volume might have been secondary to subclinical disease in this older group.

There was greater variation in total T_4 concentrations, thyroidal percent dose uptakes, T/S ratios and thyroid lobe volumes in the older group of horses. Variation in thyroid lobe volume likely influenced variation in percent dose uptake. The cause of the inverse relationship between total T_4 concentration and percent dose uptake in the older group of horses is uncertain. Again, further study of a larger population of horses is needed. It was interesting that in the younger group of horses there was a moderate negative linear correlation between total T4 concentration and thyroidal percent dose uptake of pertechnetate. Further studies could investigate whether thyroidal percent dose uptake of pertechnetate is associated with TSH in normal horses. Further studies could also compare results of TSH and TRH stimulation tests with thyroidal percent dose uptake and report thyroidal percent dose uptake in horses with confirmed thyroid dysfunction.

The accuracy of linear sonographic measurements and determining thyroid lobe volume using the prolate ellipse formula could be assessed by making linear sonographic measurements in patients before euthanasia and then measuring actual size of the gland at necropsy. Thyroid lobe volume could be determined at necropsy using a water displacement technique.

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Appendix A: Tables

TABLE 1. Summary Statistics for Total T_4 Concentrations in μg/dl (Laboratory Reference Interval 1.4 – 4.5 μg/dl)

Total T_4	Mean	SD*	Range
Group A (younger)	2.97	0.36	2.61 - 3.23
Group B (older)	2.34	0.47	1.64 - 3.13

*Standard Deviation

TABLE 2. Summary Statistics for Gross Thyroid Salivary (T/S) Ratios at 60 Minutes

T/S Ratio	Mean	SD†	Median	25%	75%
Group A (younger)	5.3:1	2.2	4.6:1	4.1:1	5.7:1
Group B (older)	5.8:1	3.0	5.2:1	3.5:1	7.4:1

†Standard Deviation

TABLE 3. Summary Statistics for Percent of the Injected Dose of Pertechnetate within the Thyroid Gland at 60 minutes

Percent Dose Uptake	Mean	SD‡	Median	25%	75%
Group A (younger)	2.51	0.54	2.55	2.33	2.90
Group B (older)	3.06	1.54	3.64	3.64	3.98

‡Standard Deviation

TABLE 4. Number of Thyroid Lobes that were Hyper, Iso and Hypoechoic and Number of Nodules found in the Left and Right Thyroid Lobes for each Group (Total Number of Lobes for each Group shown in Brackets)

	Echogenicity (Relative to Muscle)			Nodules		
	Hyperechoic	Isoechoic	Hypoechoic	Left	Right	Total
Group A (16)	10	5	1	2	2	4
Group B (14)	10	4	0	2	3	5

TABLE 5. Age, Name, Breed and Gender of each Horse (QH = Quarter Horse, TB = Thoroughbred and WB = Warmblood)

Age (years)	Name	Breed	Gender
3.2	Morgan	QH	Mare
3.7	Alley Cat	TB	Gelding
3.7	Tequila	QH	Mare
3.7	Berkley	Oldenburg	Gelding
3.7	Oliver Pleasant	TB	Gelding
4.2	Whiskey	QH	Gelding
8.5	Mint	TB	Mare
10.5	Wendy	TB/WB	Mare
11.1	Slew	TB	Gelding
12.6	Boggie	QH	Gelding
11.0	Pearl	TB	Mare
16.5	Annie	TB	Mare
18.3	Dancer	QH	Mare
19.5	Fancy	QH	Mare
20.0	Penny	TB	Mare

TABLE 6. Symmetry of the Thyroid Lobes Determined by Calculated Sonographic Volume and Number of Pixels in each Thyroid Lobe Region of Interest (ROI)

		Ultrasound Volume		ROI Size (Pixel Number)	
Name	Age	% Right lobe	% Left lobe	% Right lobe	% Left lobe
Morgan*	3.16	55.27	44.73	64.20	35.80
Alley Cat	3.70	54.26	45.74	56.72	43.28
Tequila*	3.73	41.46	58.54	38.28	61.72
Berkeley	3.74	51.66	48.34	47.84	52.16
Oliver	3.74	50.62	49.38	45.58	54.42
Whiskey*	4.19	53.10	46.90	64.45	35.55
Mint*	8.50	32.76	67.24	25.00	75.00
Wendy	10.00	46.30	53.70	59.54	40.46
Slew	11.00	50.42	49.58	43.84	56.16
Boggie	12.00	52.91	47.09	56.18	43.82
Pearl	13.00	46.86	53.14	43.90	56.10
Annie	16.00	57.27	42.73	56.16	43.84
Dancer	18.00	53.85	46.15	47.19	52.81
Fancy	19.00	47.72	52.28	41.28	58.72
Penny*	20.00	57.89	42.11	29.89	70.11

For the purpose of the study asymmetry was defined as a difference of more than 10% between the right and left thyroid lobes. Horses with asymmetry as determined by calculated sonographic volume or scintigraphic ROI size are marked with an asterix (*).

Appendix B: Figures

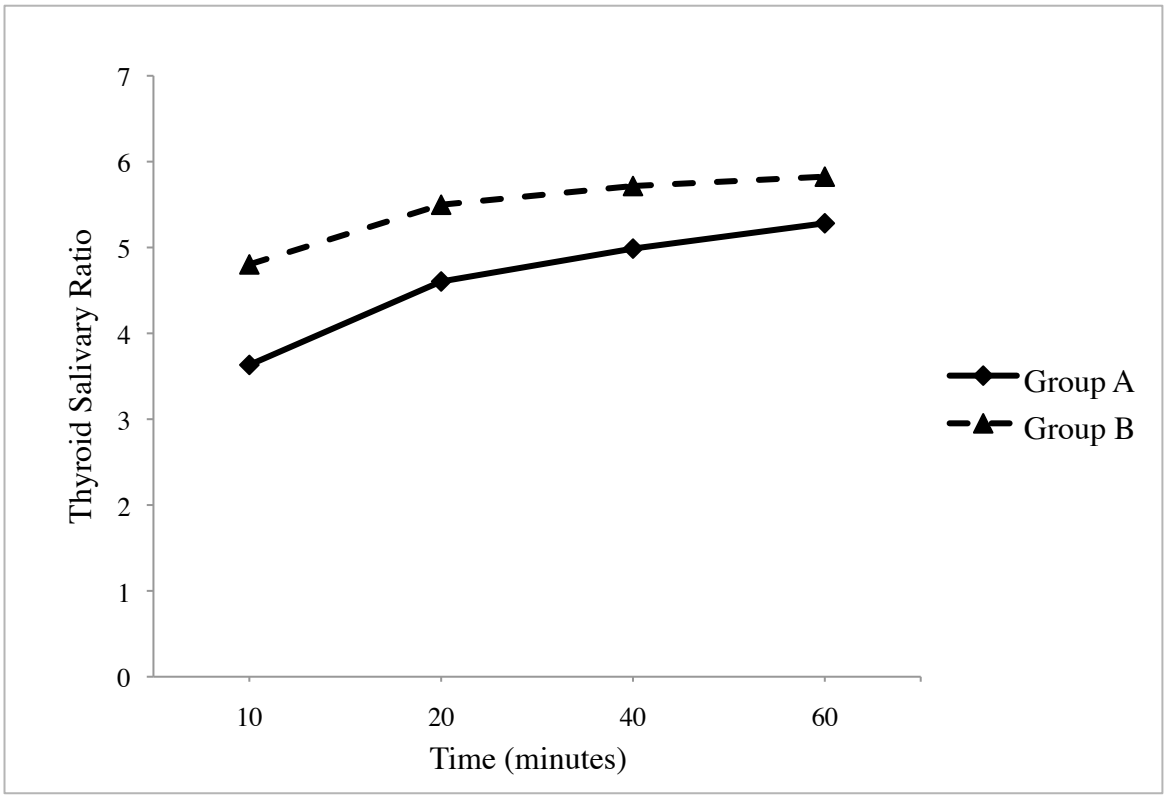

FIGURE. 1. Mean gross thyroid salivary (T/S) ratios versus time for the younger (Group A) and older (Group B) horses. There was a trend for increased T/S ratios for older horses, but this difference was not statistically significant.

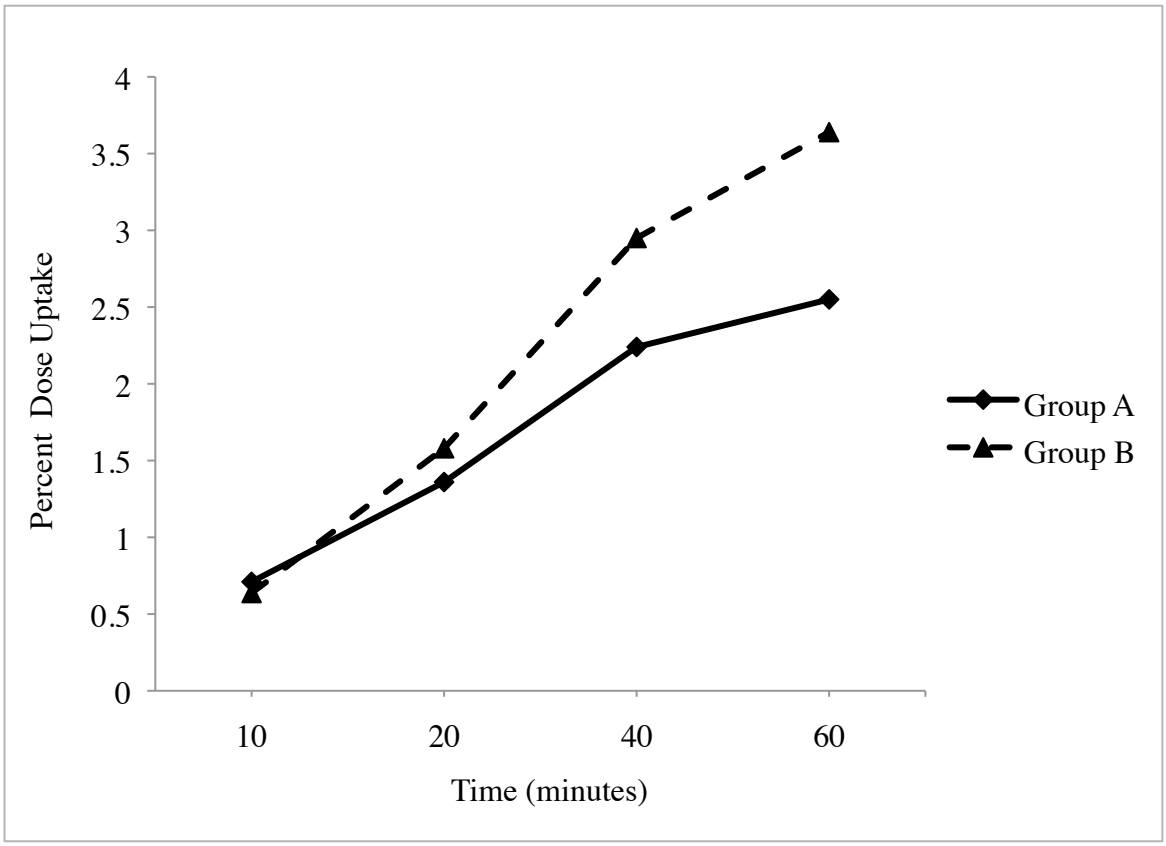

FIGURE. 2. Median percent dose uptake of pertechnetate versus time for the younger (Group A) and older (Group B) horses. The greatest difference in median percent dose uptakes between the two groups was at 60 minutes.

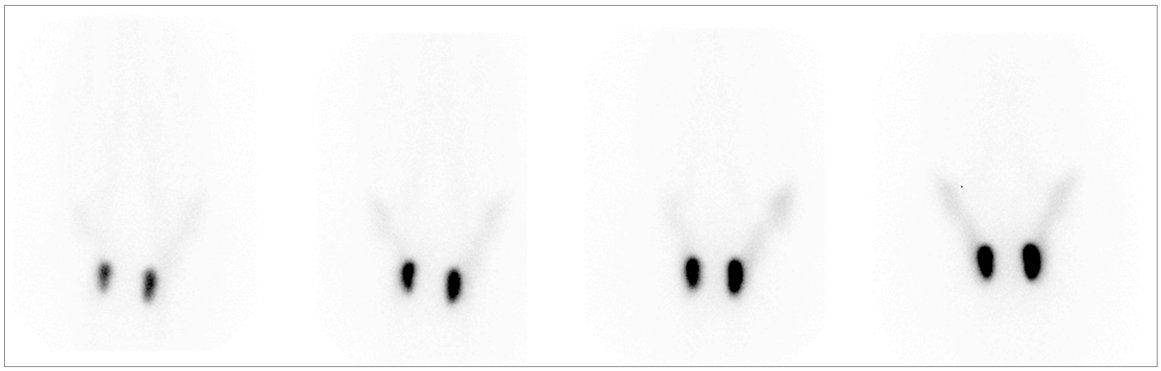

FIGURE. 3. Ventral scintigraphic images of a 13 year-old horse. There was subjectively increased uptake in the thyroid and salivary glands over time. From the left images were made at 10, 20, 40 and 60 minutes after injection.

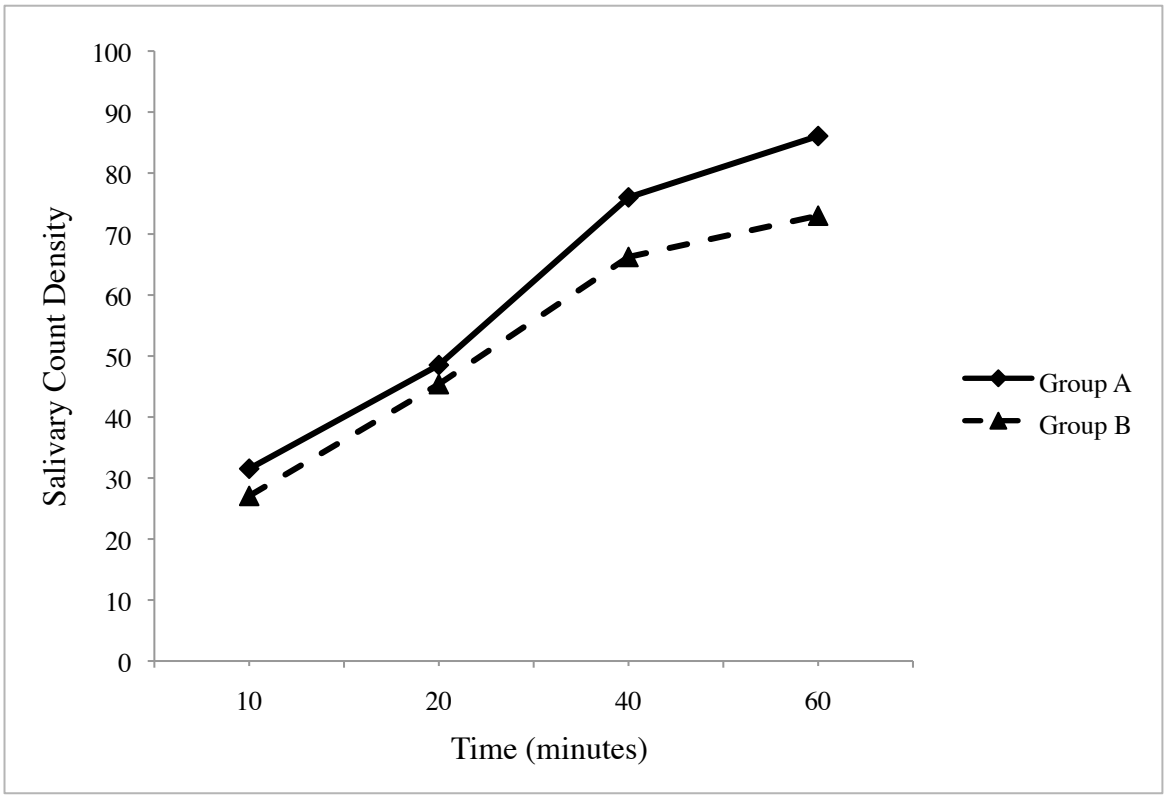

FIGURE. 4. Mean salivary count density versus time for the younger (Group A) and older (Group B) horses.

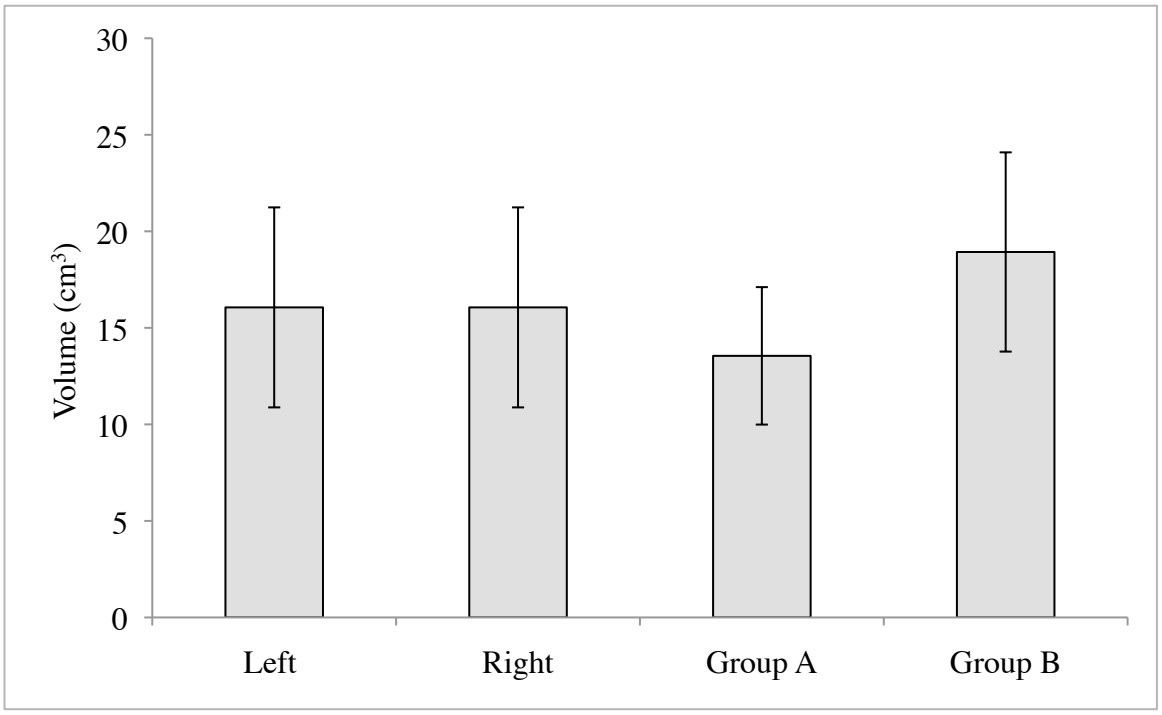

FIGURE. 5. Mean calculated volumes for the left and right thyroid lobes of all horses and mean calculated volumes for the thyroid lobes of the younger (Group A) and older (Group B) horses. Error bars indicate one standard deviation from the mean. The older group of horses had a trend for increased lobe volume, and the standard deviation was greater in this group.

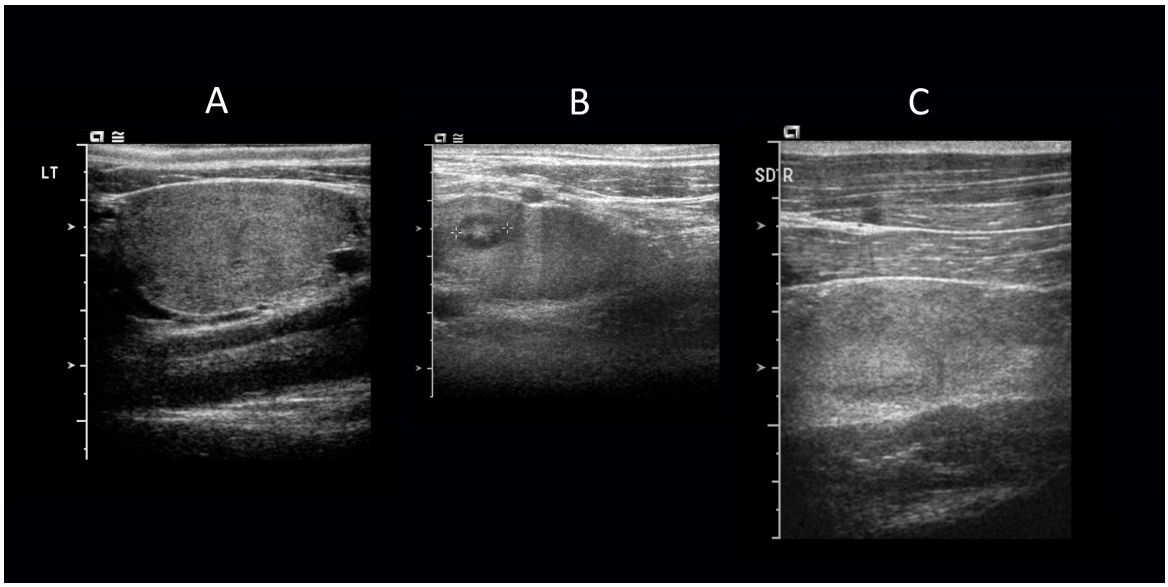

FIGURE. 6.

A. Longitudinal ultrasound image of the left thyroid lobe of a three year-old horse. The round, hypoechoic structure on the far right represents a blood vessel.

B. Image showing a mixed echogenicity nodule in the thyroid gland of an 8 year-old horse.

C. Oblique longitudinal ultrasound image from a 12 year-old horse used to compare echogenicity of the thyroid lobe with overlying musculature. This thyroid lobe was thought to be isoechoic to the overlying musculature.

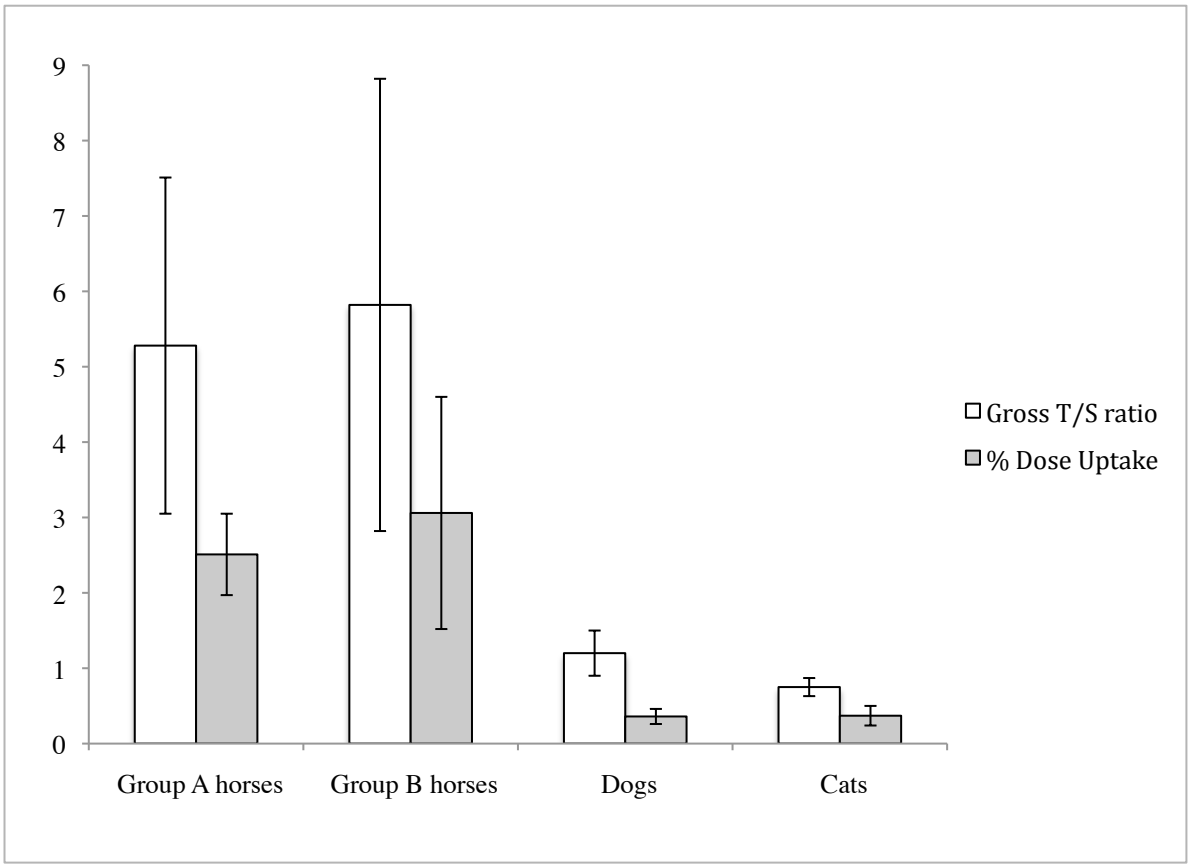

FIGURE. 7. Comparison of mean gross thyroid salivary (T/S) ratios and percent dose uptakes between the two groups of horses in our study and normal dogs[78] and cats[72] from previous studies. Error bars indicate one standard deviation from the mean. Horses had higher T/S ratios, higher percent dose uptakes and greater variance.

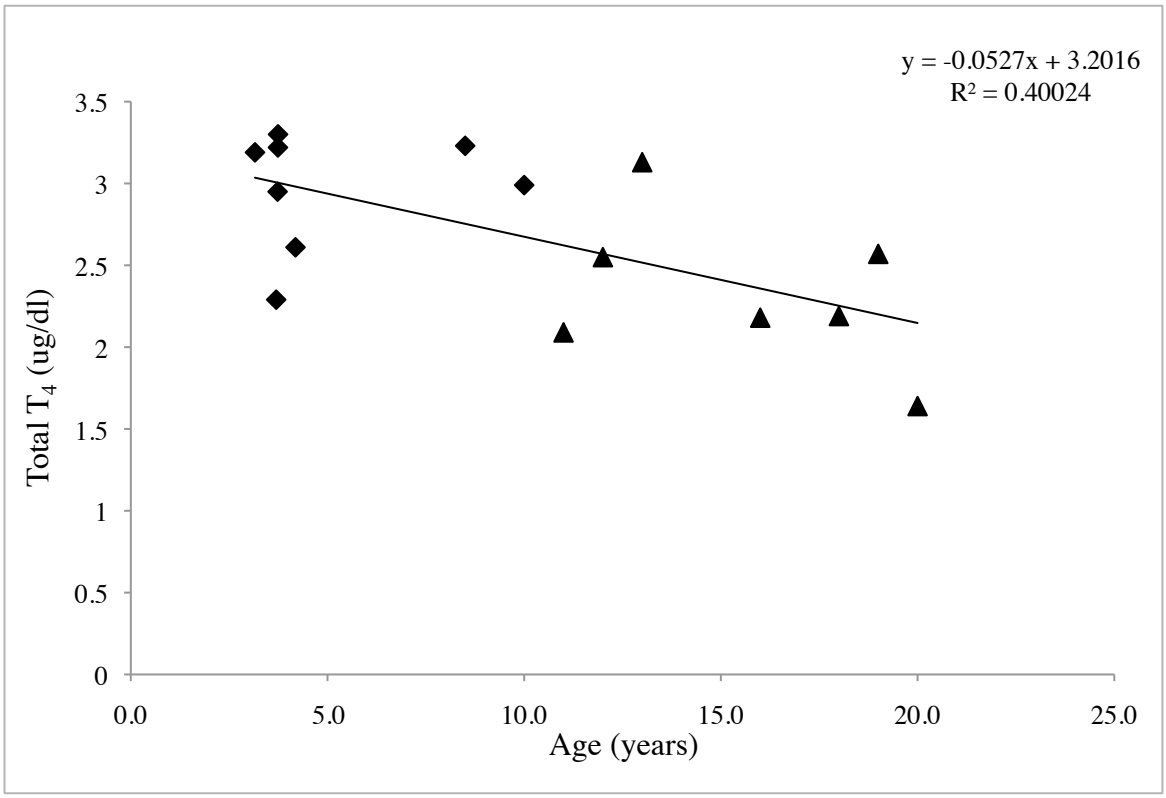

FIGURE 8. Individual total T_4 concentration versus age. There was a moderate negative linear correlation between total T_4 concentration and age such that as age increased total T_4 concentration decreased. The younger group are represented as diamonds and the older group are represented as triangles.

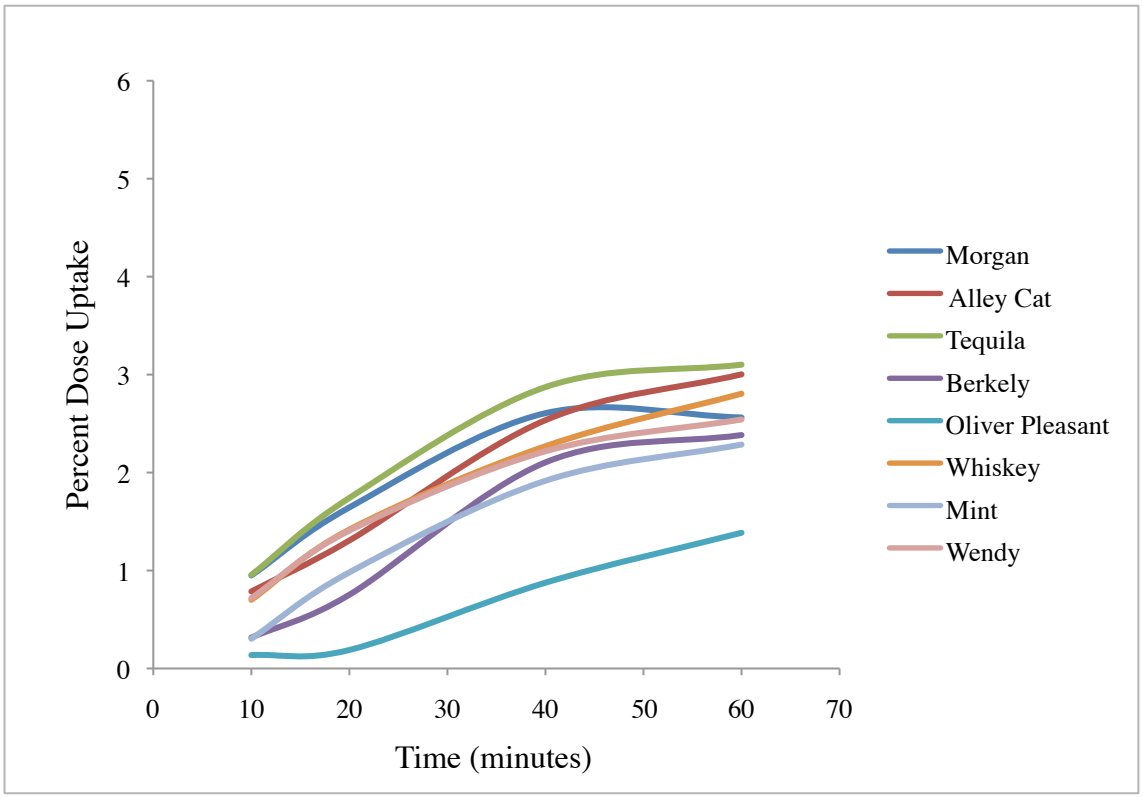

FIGURE 9. Thyroidal percent dose uptake versus time for the younger group of horses. Morgan had a slight decrease in thyroidal percent dose uptake between 40 and 60 minutes after injection. All other horses had increased thyroidal percent uptake over time.

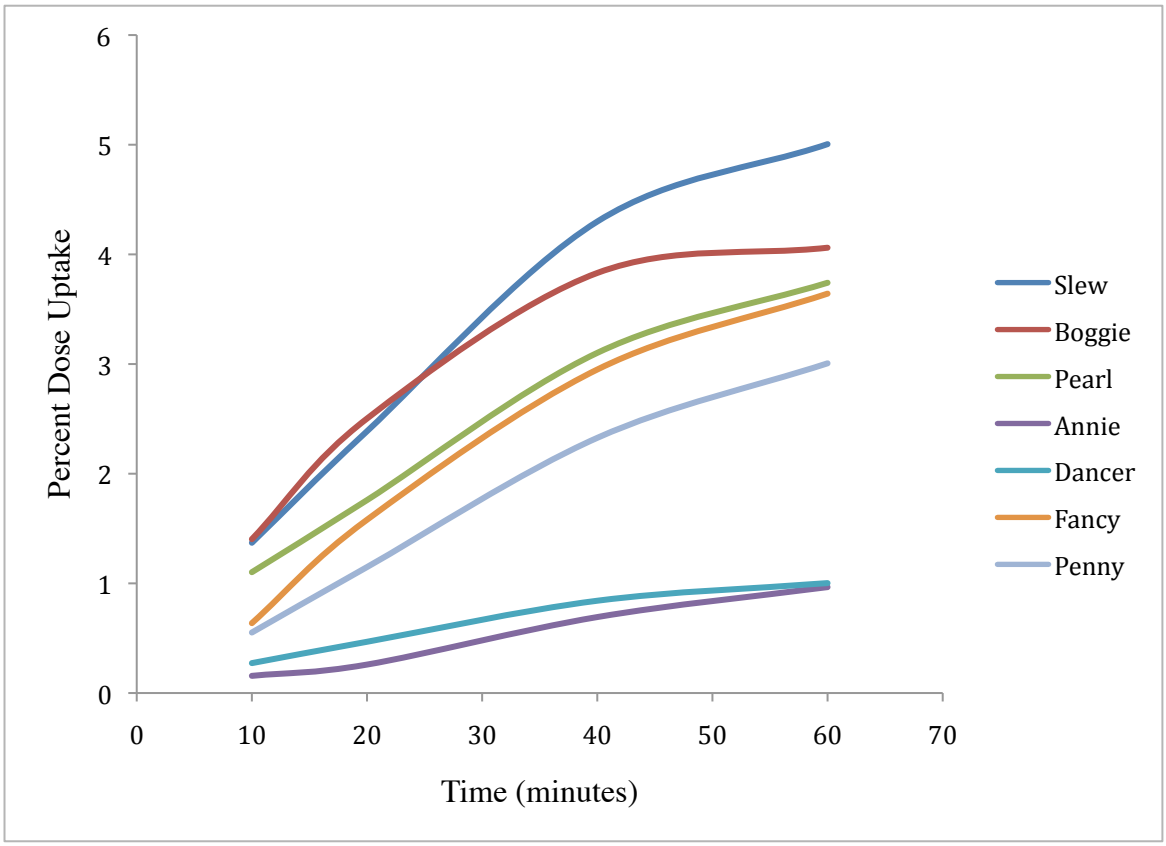

FIGURE 10. Thyroidal percent dose uptake for the older group of horses. Variation in thyroidal percent dose uptake increased over time. Overall variation in thyroidal percent dose uptake was greater for the older group of horses than for the younger group.

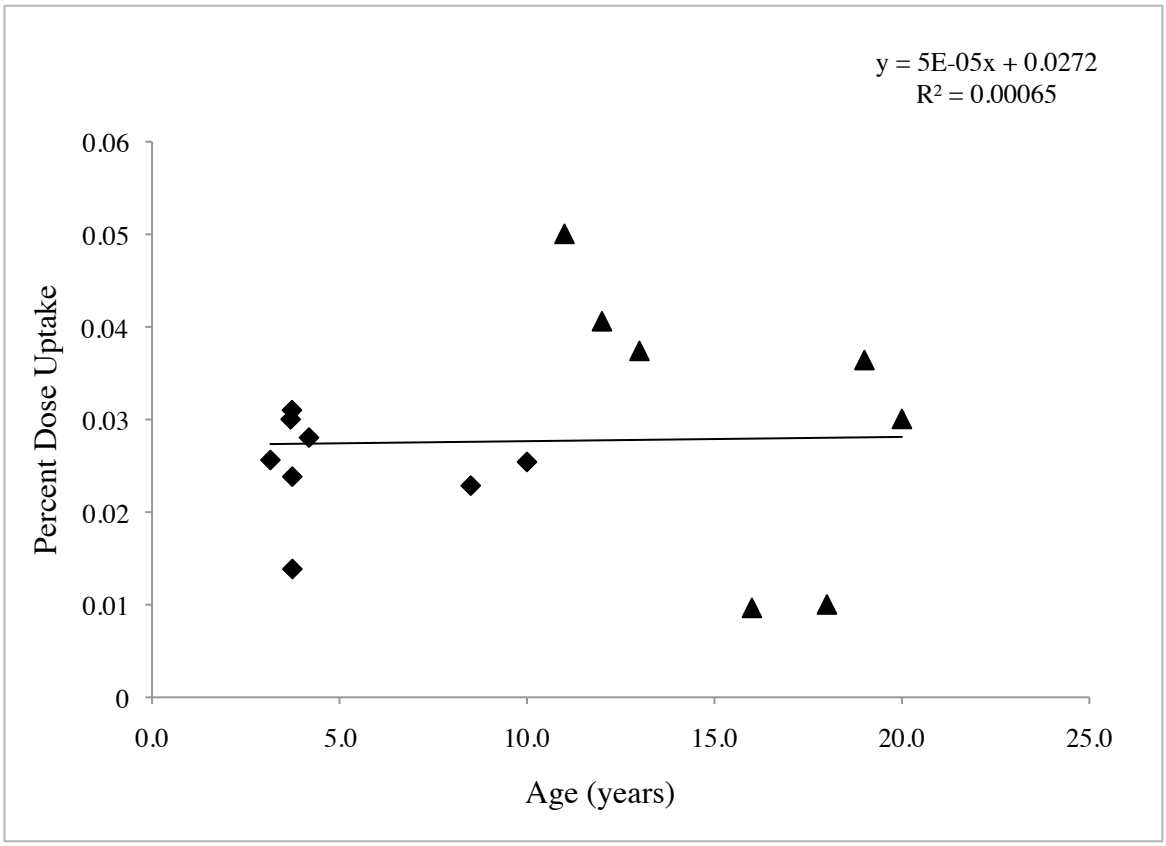

FIGURE 11. Thyroidal percent dose uptake versus age for each horse. Diamonds represent the younger group of horses and triangles represent the older group. There was no association between thyroidal percent dose uptake and age. Variation in thyroidal percent dose uptake was greater for the older horses.

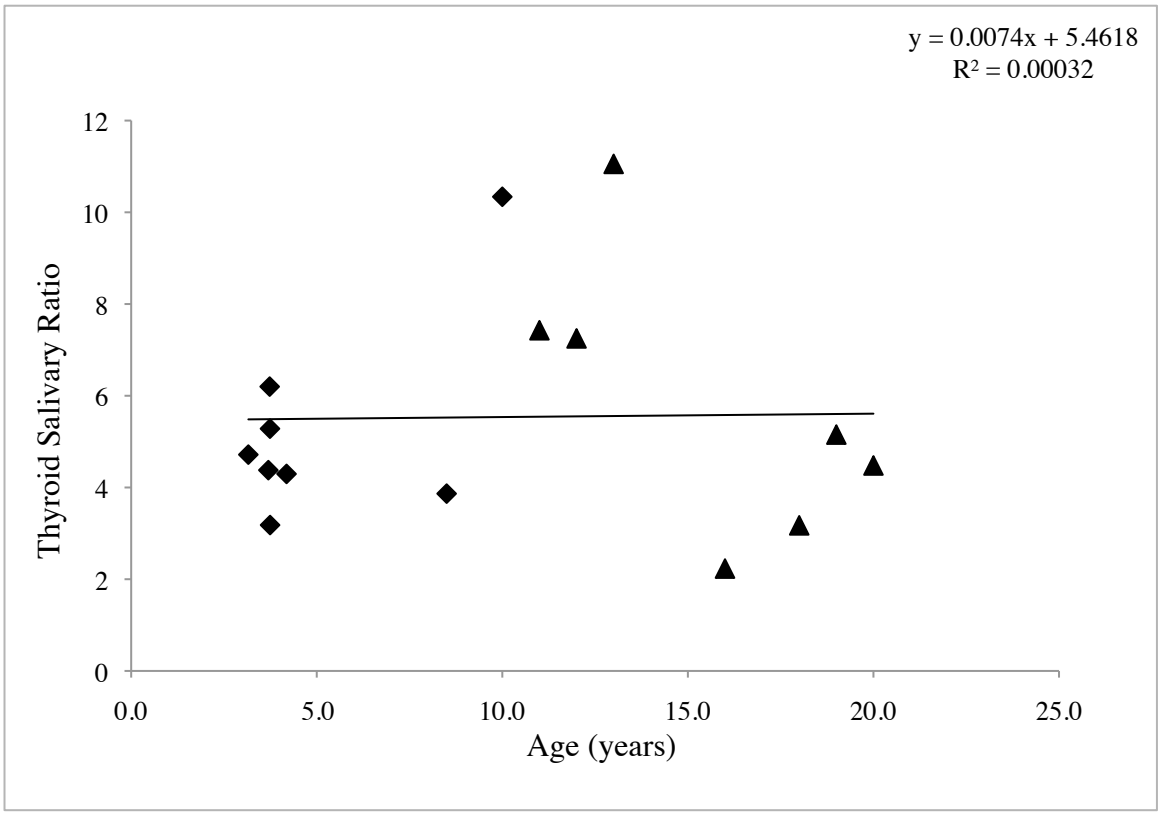

FIGURE 12. Thyroid to salivary (T/S) ratio versus age for each horse. Diamonds represent the younger group of horses and triangles represent the older group. There was no association between T/S ratio and age. Variation in T/S ratio was greater for the older group of horses.

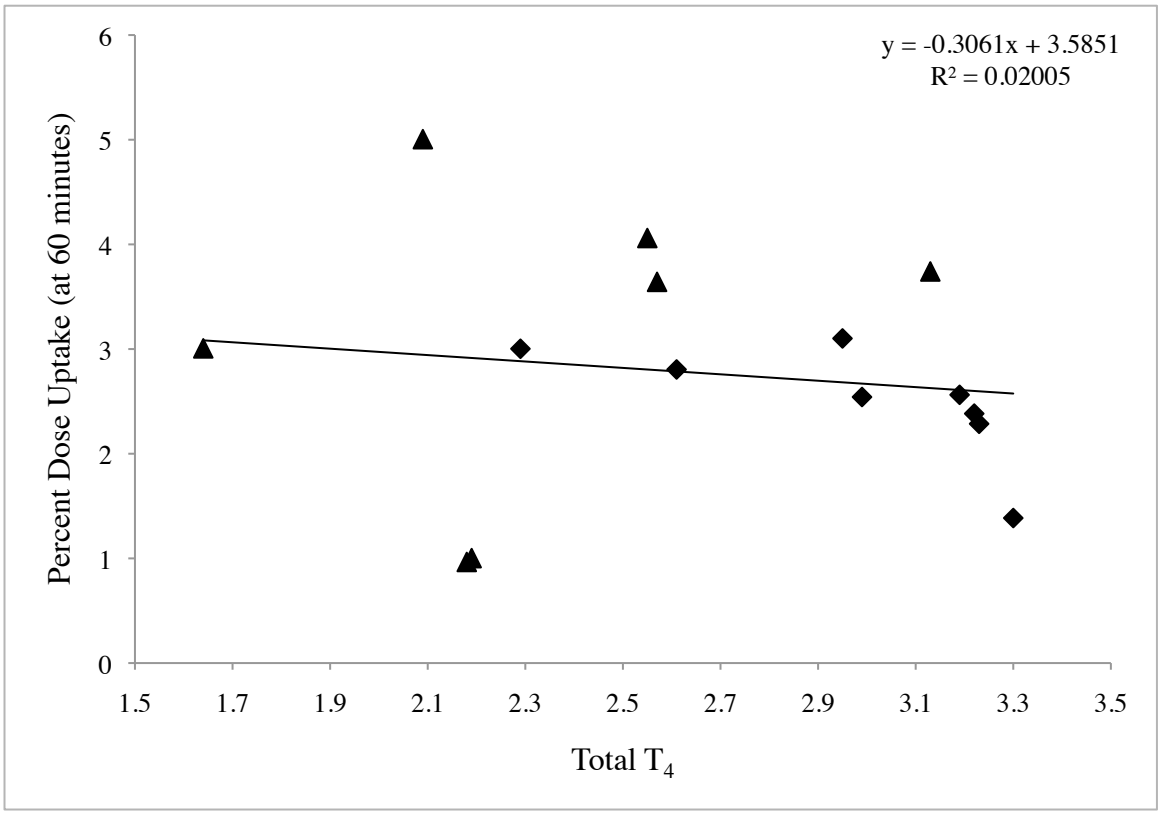

FIGURE 13. Thyroidal percent dose uptake versus total T_4 concentration for each horse. Diamonds represent the younger group of horses and triangles represent the older group. There was no association between thyroidal percent dose uptake and total T_4 concentration. Note the increased variation in both thyroidal percent dose uptake and total T_4 concentration for the older group of horses (triangles).

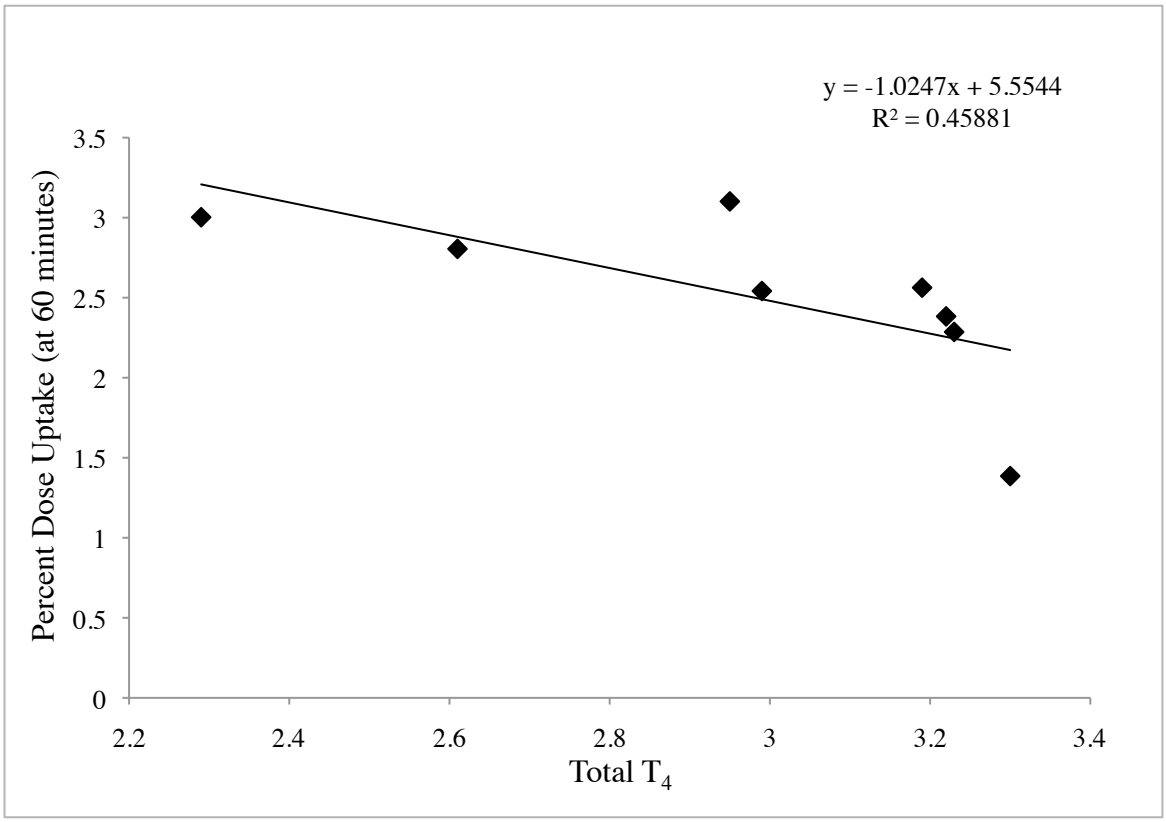

FIGURE 14. Thyroidal percent dose uptake versus total T_4 concentration for the younger group of horses. There was a moderate association between thyroidal percent dose uptake and total T_4 concentration. As total T_4 concentration increased thyroidal percent dose uptake decreased.

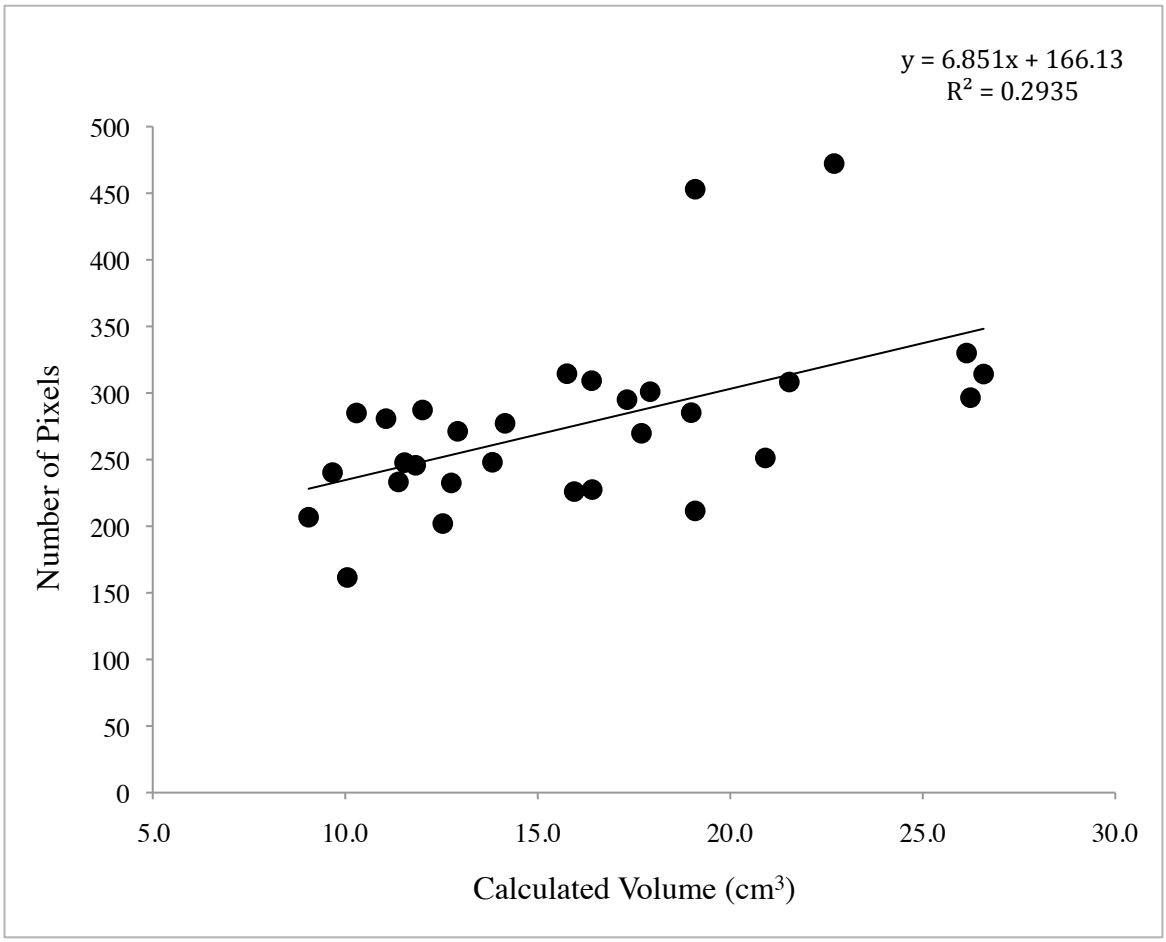

FIGURE 15. Number of pixels in each thyroid lobe region of interest (ROI size) versus calculated thyroid lobe volume for individual thyroid lobes (two data points for each horse). A mild to moderate positive linear correlation was present between ROI size and thyroid lobe volume.

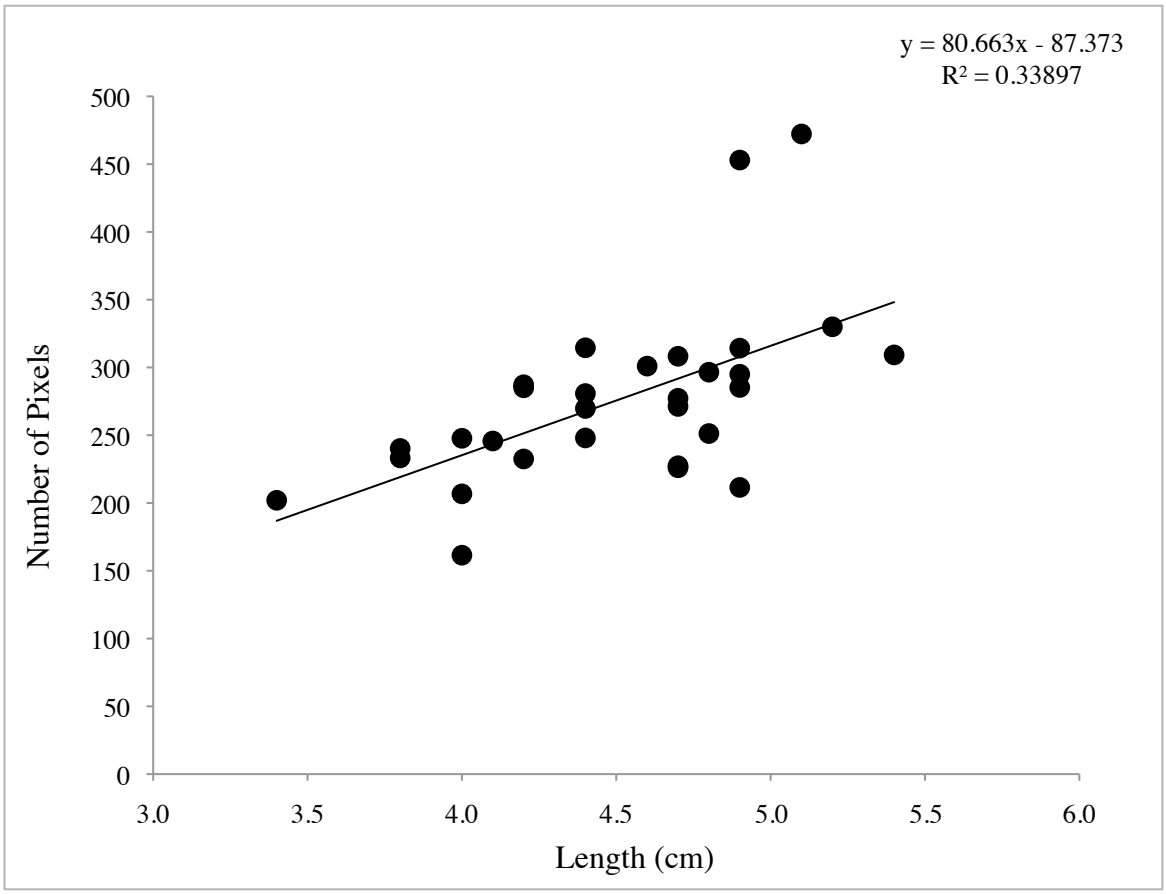

FIGURE 16. Number of pixels in the thyroid lobe region of interest (ROI size) versus sonographically measured thyroid lobe length for each lobe. There was a slightly stronger (moderate) association between ROI size and thyroid lobe length ($R^2 = 0.34$) than for ROI size and calculated thyroid lobe volume ($R^2 = 0.29$).

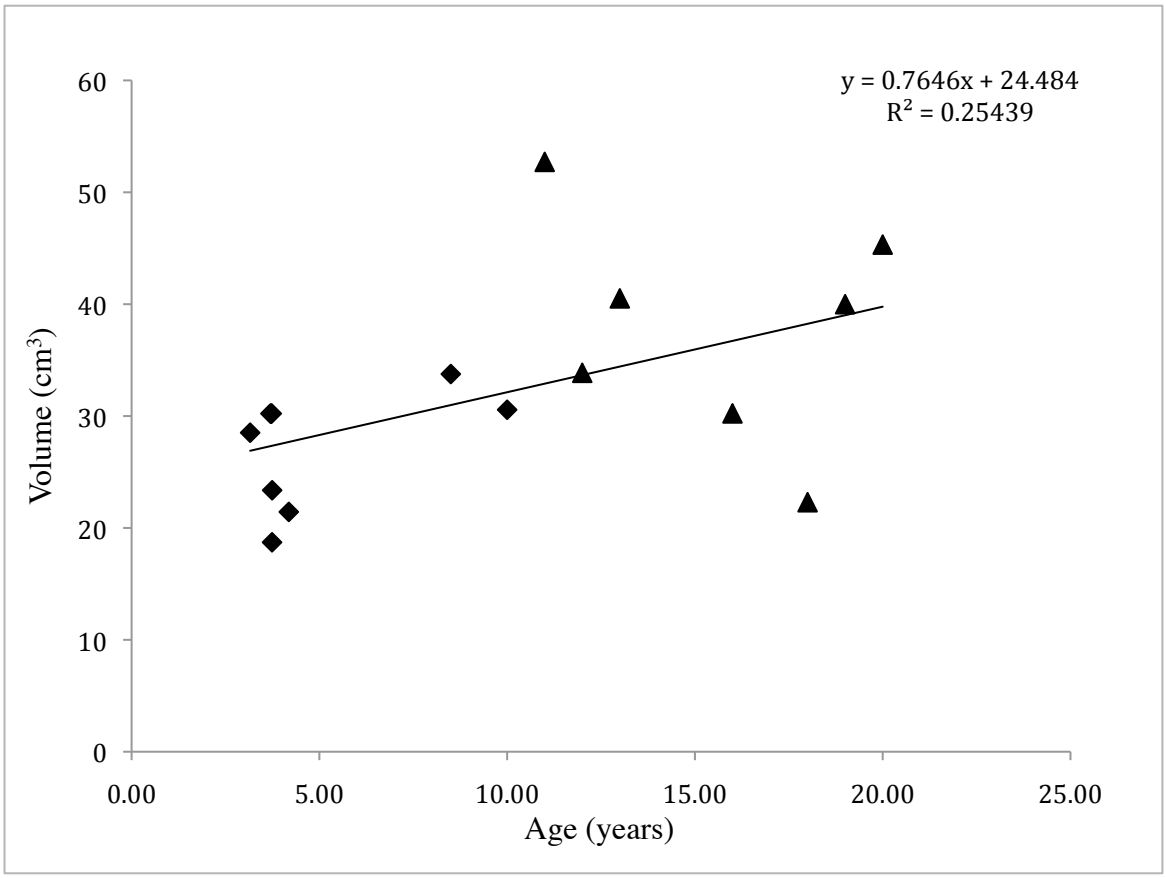

FIGURE 17. Sonographically calculated total thyroid gland volume versus age for each horse. As age increased thyroid lobe volume increased. Note the increased variation thyroid lobe volume for the older group of horses.

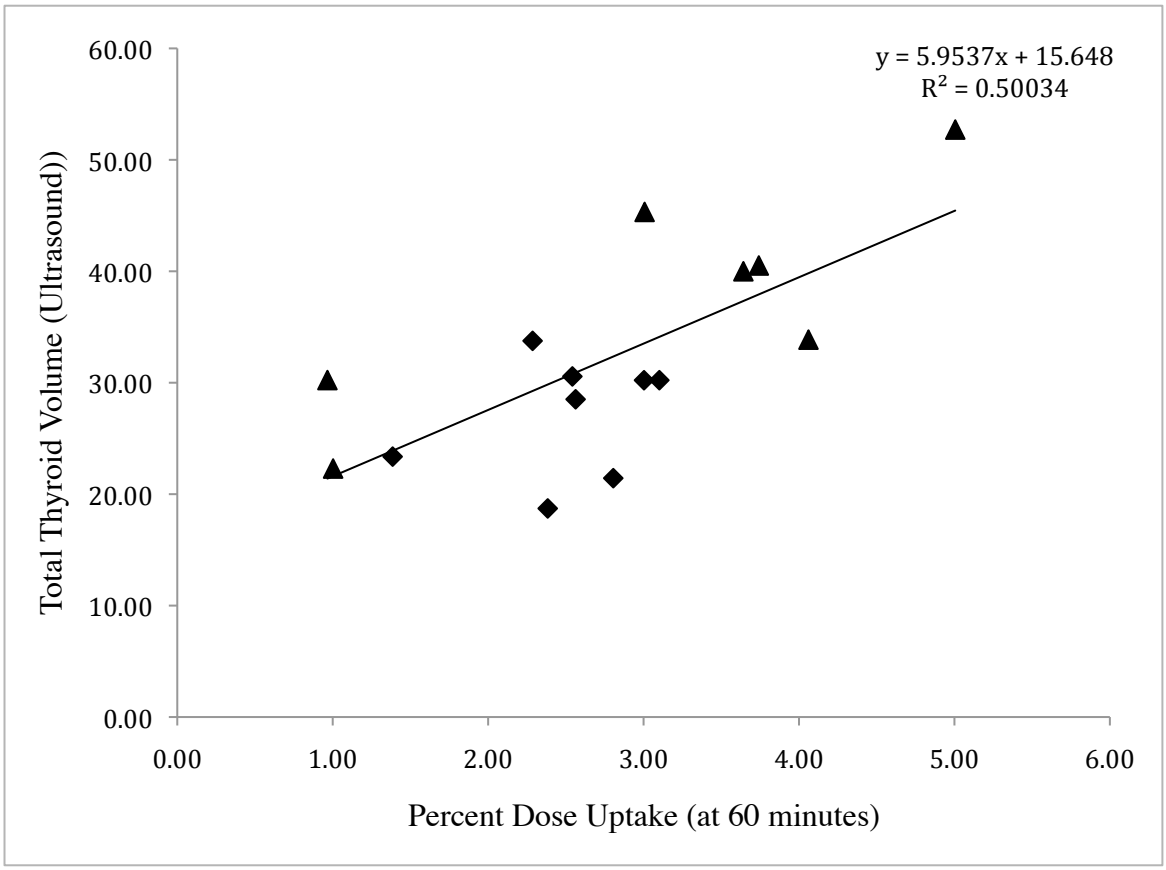

FIGURE 18. Total calculated thyroid lobe volume versus total (summed right and left) thyroidal percent dose uptake for all horses. Diamonds represent the younger group of horses and triangles represent the older group. There was a moderate association between total thyroid lobe volume and total thyroidal percent dose uptake.

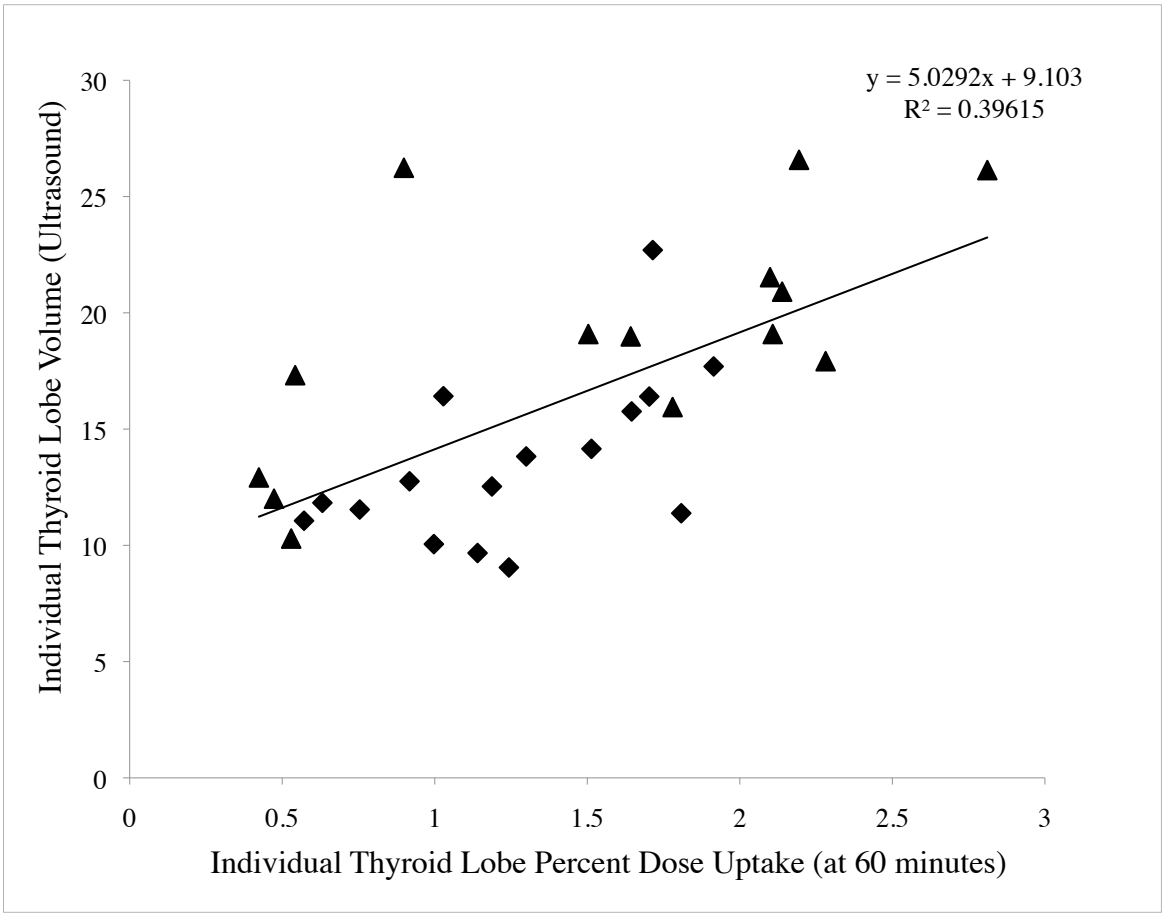

FIGURE 19. Thyroid lobe volume versus thyroidal percent dose uptake for individual thyroid lobes. The association between individual lobe volume and thyroidal percent dose uptake was not as strong ($R^2 = 0.40$) as for combined thyroid lobe volume and percent dose uptake ($R^2 = 0.50$).

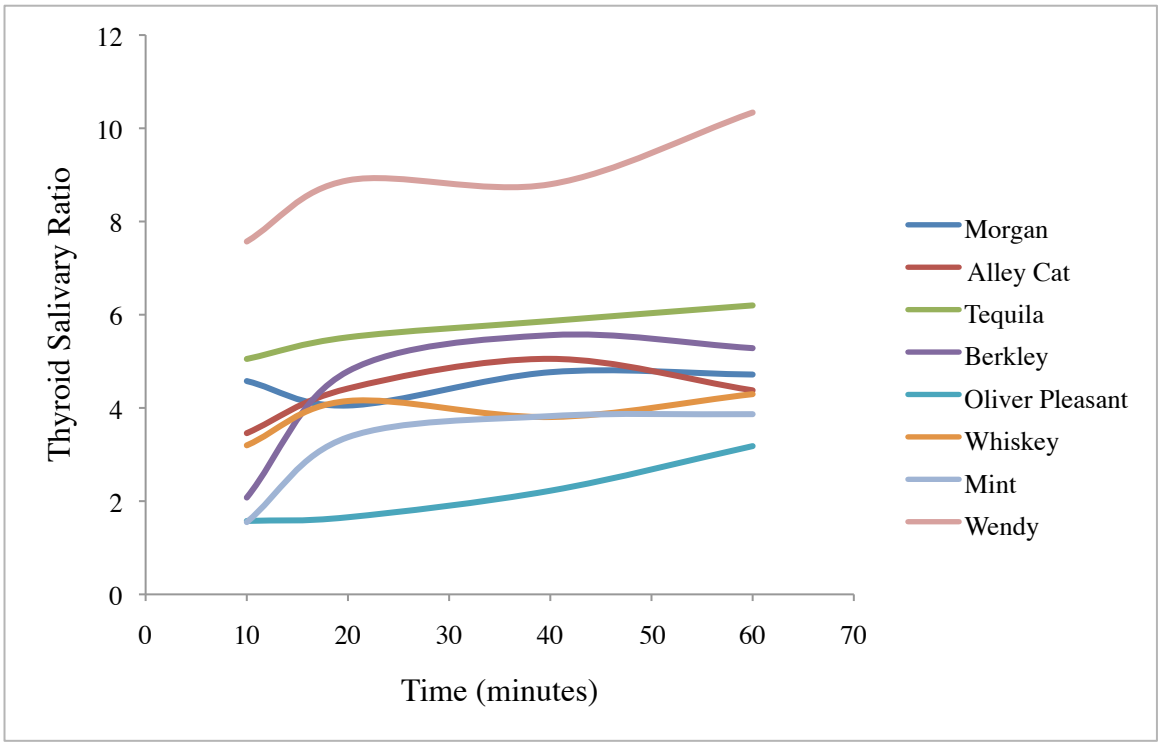

FIGURE 20. Thyroid to salivary (T/S) ratio versus time for the younger group of horses.

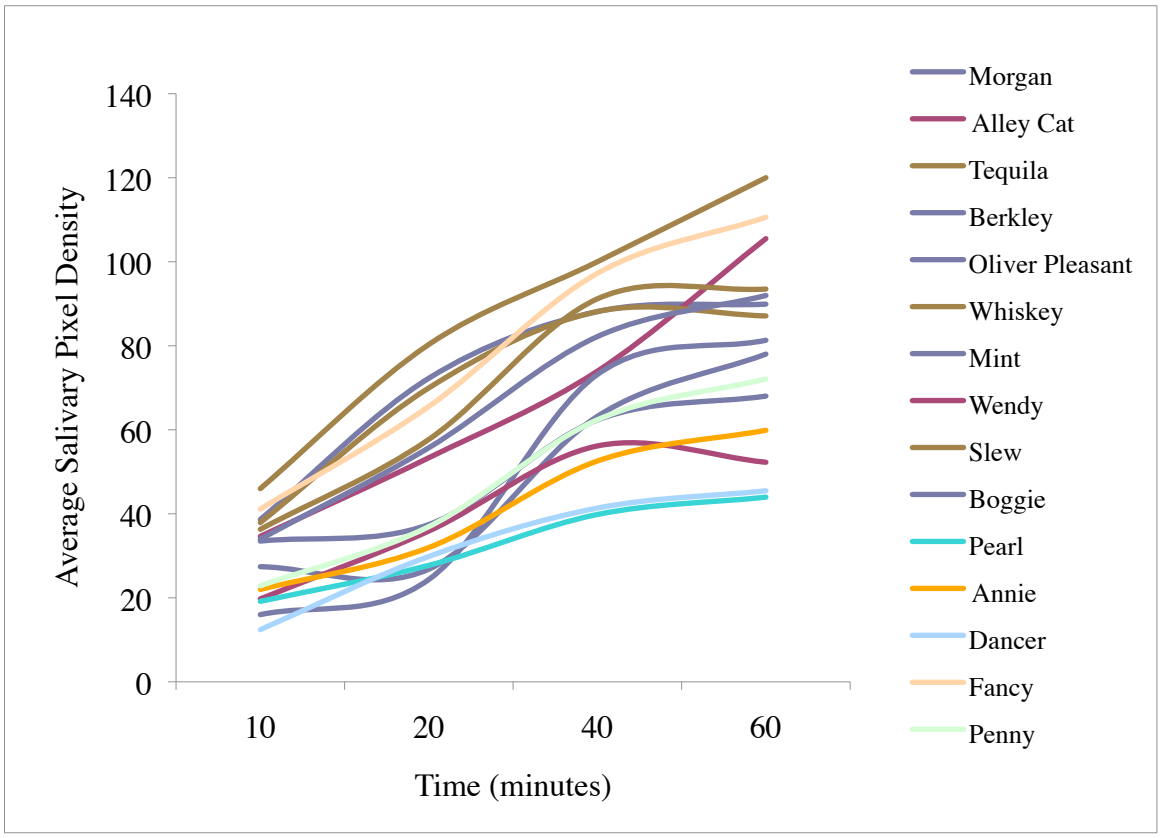

FIGURE 21. Average salivary pixel density (total salivary counts / total number of pixels in the combined salivary region of interest) versus time. Note variation in average salivary pixel density increases over time. Two horses (Wendy and Slew) had decreased average salivary pixel density between 40 and 60 minutes after injection.

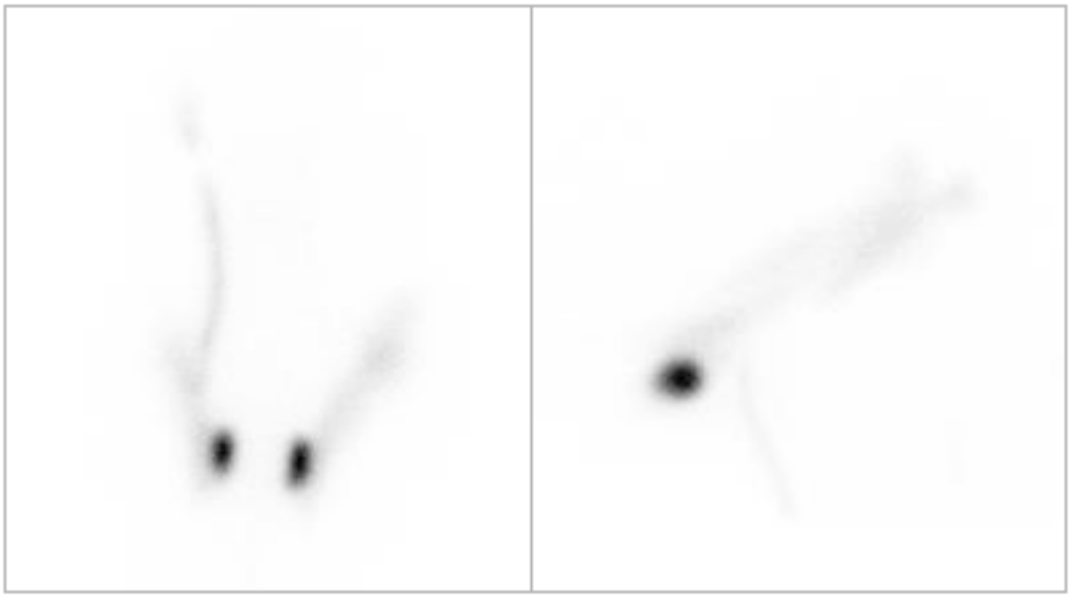

FIGURE 22. Ventral and right lateral images of a horse acquired 60 minutes after injection. Note excretion of pertechnetate into the right parotid duct.

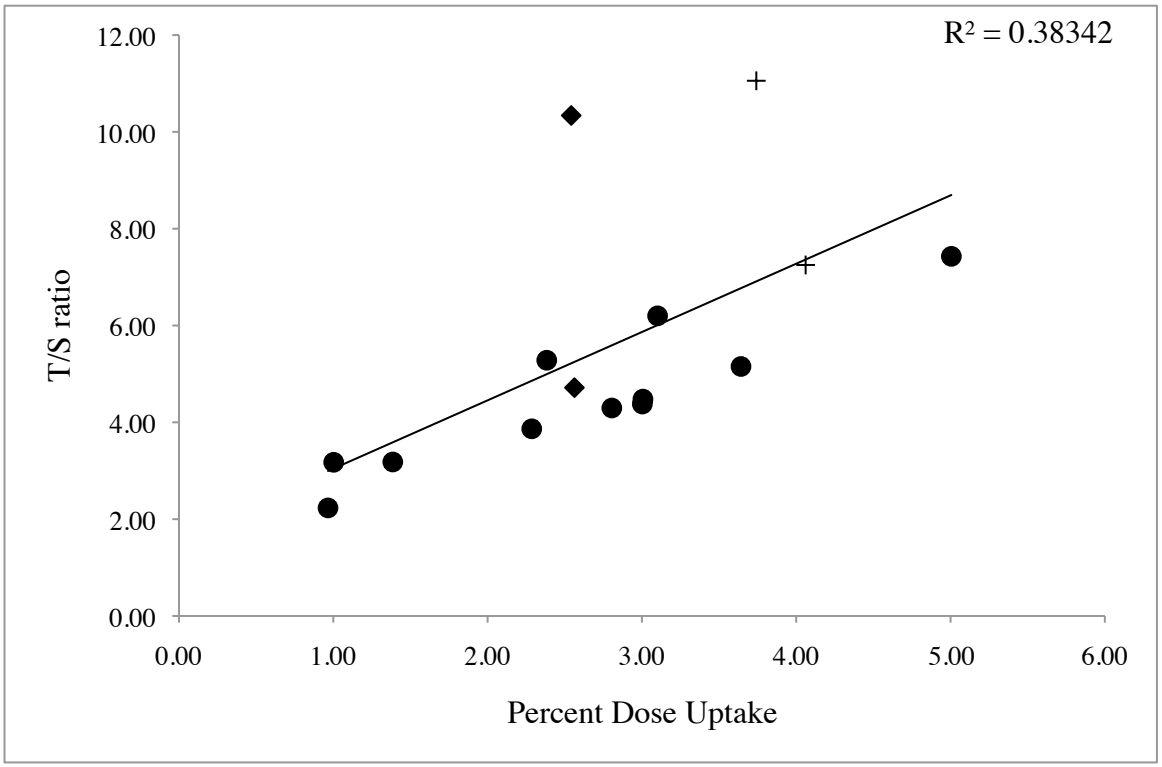

Figure 23. T/S ratio and thyroidal percent dose uptake 60 minutes after injection. There is a moderate association between the two variables. As T/S ratio increases so does thyroidal percent dose uptake. For two pairs of horses, thyroidal percent dose uptakes are very similar but there are large differences in their corresponding T/S ratios. These pairs of horses are represented as diamonds and crosses.